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Case Report

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First cases of MPV17 related mitochondrial DNA depletion syndrome with compound heterozygous mutations in p.R50Q/p.R50W: a case report

Shuichiro Umetsu¹, Ayano Inui¹, Sohya Kobayashi¹, Masaru Shimura², Tomoko Uehara³, Hajime Uchida⁴, Rie Irie⁵, Tsuyoshi Sogo¹, Haruki Komatsu⁶, Takako Yoshioka⁷, Kei Murayama², Kenjiro Kosaki³, Mureo Kasahara⁴, Tomoo Fujisawa¹

¹Department of Pediatric Hepatology and Gastroenterology, Saiseikai Yokohama City Tobu Hospital, Kanagawa 230-0012, Japan.

²Department of Metabolism, Chiba Children's Hospital, Chiba 266-0007, Japan.

³Center for Medical Genetics, Keio University School of Medicine, Tokyo 160-8582, Japan.

⁴Department of Transplantation Surgery, National Center for Child Health and Development, Tokyo 157-8535, Japan.

⁵Department of Pathology, Keio University School of Medicine, Tokyo 160-8582, Japan.

⁶Department of Pediatrics, Toho University Sakura Medical Center, Chiba 285-8741, Japan.

⁷Department of Pathology, National Center for Child Health and Development, Tokyo 157-8535, Japan.

Correspondence to: Shuichiro Umetsu, Department of Pediatric Hepatology and Gastroenterology, Saiseikai Yokohamashi Tobu Hospital, 3-6-1, Shimosueyoshi, Tsurumi Ward, Yokohama, Kanagawa 230-0012, Japan. E-mail: s_umedu@tobu.saiseikai.or.jp

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Abstract

Mutations in MPV17 lead to severe mitochondrial DNA depletion syndrome (MTDPS). All known p.R50W variants in MPV17 are lethal. The homozygous variant p.R50Q in MPV17 among patients with Navajo neurohepatopathy is known to allow longer survival, although heterozygous variants p.R50Q have not been reported. This is the first clinical report in compound heterozygosity MPV17 mutation (p.R50W/p.R50Q). Three siblings were admitted due to multiple hepatic nodules; none presented neurological abnormalities. However, they suffered from severe hypoglycemia and cyclic vomiting. The diagnosis of MPV17-related MTDPS was confirmed by detection of a compound heterozygous MPV17 mutation (p.R50W/p.R50Q), and striking reduction of hepatic mitochondrial DNA. One patient developed pediatric-onset of hepatocellular carcinoma. Notably, all patients survived for extended periods, including two patients who received liver transplantation, which contrasted the high mortality rate associated with p.R50W mutations, as previously reported. The p.R50Q mutation might be associated with longer survival and improved liver transplantation outcomes.



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Keywords: Mitochondrial DNA depletion syndrome, MPV17, compound heterozygous mutation, liver transplantation

INTRODUCTION

The causes of mitochondrial disorders are defects in mitochondrial DNA (mtDNA) or in nuclear genes that affect mtDNA biogenesis and maintenance. Defects in nuclear genes can result in the accumulation of mtDNA deletions, or with mtDNA depletion syndrome (MTDPS)^[1-3]. The latter is an autosomal recessive disease associated with decreased mtDNA copy number in clinically affected tissues^[4-6]. The disease has three known phenotypes: hepatocerebral, myopathic, and encephalomyopathy. Hepatocerebral MTDPS is linked to pathogenic variants in DNA polymerase gamma^[7], Twinkle (PEO1)^[8], deoxyguanosine kinase^[9], and mtDNA maintenance protein MPV17^[4].

MPV17-related MTDPS is a very rare disease. To date, MPV17-related hepatocerebral MTDPS has been reported in 96 patients^[4,5,10-30]. Disease prognosis is severe, given that ~80% of patients die from liver failure during early childhood^[30]. Hepatic cirrhosis has been diagnosed in 20 patients, while three patients had hepatocellular carcinoma (HCC)^[10,15,25]. Neurological manifestations were also reported in 91% of patients with developmental delays, and 74% of patients with generalized hypotonia. These patients also experienced MR imaging (MRI) abnormalities; metabolic manifestations, including hypoglycemia and lactic acidosis; failure to thrive; feeding difficulties; and retinal tubulopathy.

Currently, 48 pathogenic variants of MPV17 are known, occurring exclusively in a few families. Five cases with p.R50W mutations (three homozygous and two heterozygous) died of liver failure during early childhood^[4,12,25,30]. Previous case studies identified homozygous variant p.R50Q in MPV17 among patients with Navajo neurohepatopathy (NNH)^[10,15,29]. One specific homozygous mutation, p.R50Q, has been reported in several Navajo individuals from the southwestern United States and is the cause of NNH. This condition clinically manifests as severe sensory and motor neuropathy, corneal anesthesia, ulcers, cerebral leukoencephalopathy, failure to thrive, and metabolic acidosis. To date, compound heterozygous variant p.R50Q with other mutations has not been reported in MPV17-related MTDPS.

In this case report, we describe for the first time three patients with MTDPS who possessed the compound heterozygous MPV17 variant p.R50Q/p.R50W. Unlike deceased outcomes of p.R50W variants as previously reported, our three cases with p.R50Q/R50W mutations survived without signs of typical neurological manifestations.

CASE REPORT

Patients

The proband was a 6-year-old male (Case 1) and the first child of healthy, nonconsanguineous parents with no hereditary history of the disease (for family pedigree, see [Figure 1A](#)). The child was delivered via caesarian section after a dinochorionic and diamniotic pregnancy. Case 2 was the first girl of a twin of Case 1. A second girl (Case 3) was born three years after Cases 1 and 2, delivered without any complication. Cases 1-3 showed similar clinical manifestation: all patients suffered from severe hypoglycemia and cyclic vomiting. During infancy, Cases 1 and 3 presented with liver failure; although there was a mild spontaneous remission, acute liver failure was repeated through episodes of febrile infection. Case 2 presented with liver dysfunction only.

In Case 1, the patient developed recurrent vomiting with hypoglycemia at the age of six, and abdominal ultrasound found multiple liver masses. Each patient was admitted to our hospital due to multiple hepatic nodules, detected by abdominal ultrasound at age six in Case 2, and at age three in Case 3 [\[Figure 2A\]](#).

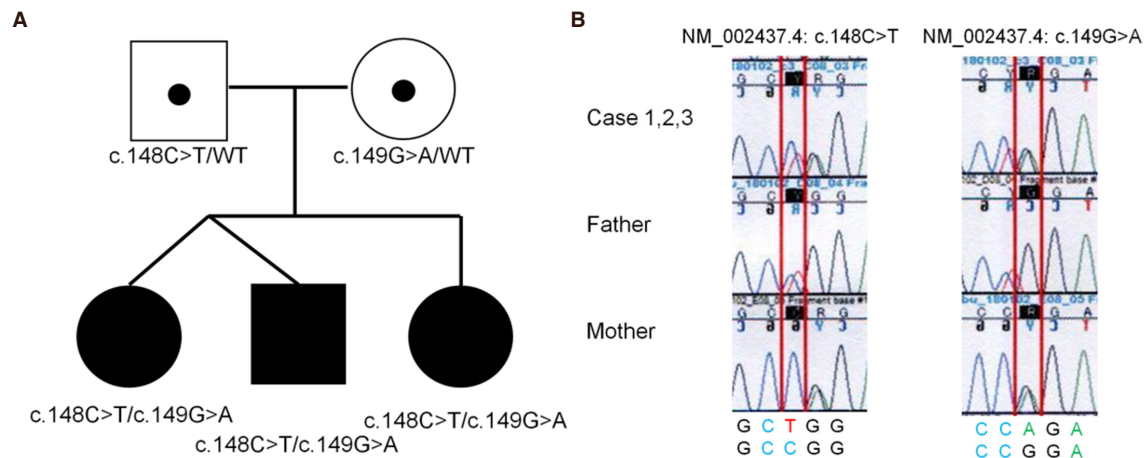


Figure 1. Family pedigree (A). Whole-exome and Sanger sequencing revealed compound heterozygous missense mutations with NM_002437.4:c.148C>T (p.R50W) and c.149G>A (R50Q) in MPV17, inherited from each of their parents (B)

Table 1 shows the clinical findings of three patients at first admission to our hospital. Case 2 and 3 showed no manifestations of neurological abnormality, but Case 1 exhibited mild intellectual disability (IQ 68). Serological tests for viral hepatitis A, B, and C, cytomegalovirus, and Epstein-Barr virus excluded the presence of these infections. Wilson's disease was excluded by measuring ceruloplasmin, serum copper, and urinary copper.

All three patients had multiple masses with hyper-echogenic occupied lesions and low-echogenic occupied lesions measuring between 0.5 and 1.0 cm, which were negatively detected by enhanced computed tomography. Transverse T2-weighted magnetic resonance (MR) image shows numerous well-defined hypointense masses in the liver, while transverse EOB-enhanced MRI demonstrated negative arterial enhancement, portal-venous phase, and liver-cell-enhance phase studies showed high intensity with masses in all three patients.

Liver biopsies revealed advanced fibrosis with regenerative nodules and mild steatosis in Cases 1 and 2 [Figure 3A]. In Case 3, liver biopsy was avoided due to hemorrhagic tendency. Electron microscopy revealed that these two children had giant mitochondria with increased inclusion-body count in hepatocytes [Figure 3B].

After obtaining approval from the institutional review board of Saiseikai Yokohama-shi Tobu Hospital and informed consent from parents, biochemical examination and molecular studies were performed.

A biochemical analysis was performed at the Department of Metabolism, Chiba Prefectural Children's Hospital. Case 1 and 2 livers were analyzed for tissue-specific enzyme activity and mtDNA levels. Hepatic respiratory chain complex I, II, III, and IV (CI-CIV) activities were evaluated as described previously^[31]. The results of this analysis showed that Case 1 had decreased CI and CIII activities, and increased citrate synthase (CS) activity (CI, 3.5%; CIII, 28.4% of CS activity; CS, 213.6% of total protein). Similarly, Case 2 exhibited decreased CI, CIII, and CIV activities, and increased CS activity (CI, 9.8%; CIII, 17.0%; CIV, 20.0% of CS activity; CS, 263.7% of total protein). Next, a quantitative PCR was performed to evaluate the mtDNA copy number using previously reported methods^[32], the analysis of which revealed a striking reduction of hepatic mtDNA in both patients (Case 1, 0.7%; Case 2, 0.6%; normal range 40%-150%).

DNA was extracted from peripheral blood samples of the three patients and their parents. A whole-exome analysis using Hiseq 2500 (Illumina, Sana Clara, USA) identified compound heterozygous missense

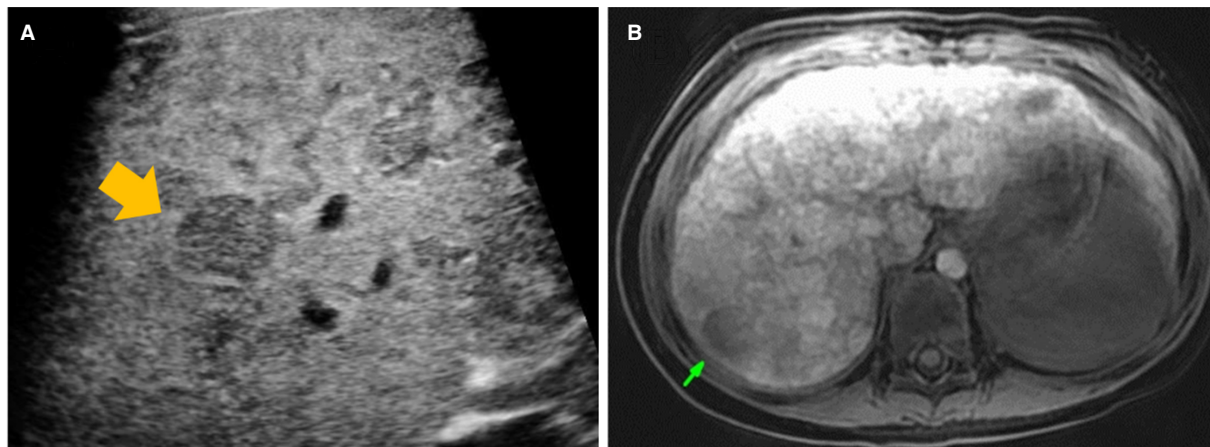


Figure 2. Abdominal ultrasound showed the mass in the liver (A). EOB-enhanced magnetic resonance images indicated the washed-out portal-venous phase and liver-cell-enhance phase in S7 hepatic mass (B)

mutations with NM_002437.4:c.148C>T (p.R50W) and c.149G>A (p.R50Q) in MPV17, respectively, inherited from each of their parents [Figure 1B]. Sanger sequencing confirmed these results.

These results confirmed a diagnosis of MPV17-related MTDPS.

Clinical treatment

Patients were given ubiquinone carnitine as medication for mitochondrial rescue supplementation. A lipid-rich diet efficiently controlled hypoglycemia and normalized liver function for a previous case study^[14]. However, a lipid-rich, carbohydrate-poor diet (lipid 45%, carbohydrate 45%, and protein 10%) caused severe hypoglycemia with vomiting symptoms in Cases 1-3. Episodic hypoglycemia and vomiting were dramatically reduced with the avoidance of fasting and uncooked cornstarch, along with the provision of a carbohydrate-rich diet with medium-chain triglyceride powder (lipid 30%, carbohydrate 60%, and protein 10%) six times per day.

Six months later, Case 2 was readmitted to our hospital because of an increasing mass measuring 2.9 cm located in segment 7, as revealed by abdominal ultrasound imaging. It showed no evidence of vascular invasion or metastasis. EOB-enhanced MR images revealed early arterial enhancement, as well as washed-out portal-venous and liver-cell-enhance phases [Figure 2B]. We diagnosed HCC. At age 7, Case 2 underwent living-donor-liver transplantation from her father. The resected liver was completely cirrhotic, and histology indicated well-differentiated HCC [Figure 4]. After liver transplantation, Case 2 did not experience post-transplant complications. Case 1 also developed end-stage liver disease and obtained a liver transplant from his grandfather five months after Case 2's operation. Case 1's resected liver was also completely cirrhotic but without neoplasm stigma. As with Case 2, Case 1 did not experience post-surgery complications. We also observed Case 3 to detect any increases in hepatic masses. At the time of writing, all three patients exhibited normal cognition and neurological function.

DISCUSSION

This is the first clinical report in compound heterozygous mutation (p.R50Q/p.R50W) of MPV17, and three cases were diagnosed with hepatocerebral MTDPS. In contrast with deceased outcomes of p.R50W variants previously reported, our three cases with p.R50Q/R50W mutations survived for a more extended period without neurological manifestations. One case developed HCC. Two of our cases underwent liver transplantation, and both showed positive post-transplant outcomes at the time of writing.

Table 1. Clinical findings of three patients with MPV17-related mitochondria hepatopathy at the first visit to our hospital

	Case1	Case2	Case3
Sex	Male	Female	Female
Height (cm) (SD)	109.9 (-1.3 SD)	110 (-0.9 SD)	88.3 (-1.8 SD)
Weight (kg) (SD)	17.3 (-1.1 SD)	19.4 (-0.3 SD)	13.6 (-0.1 SD)
Neurology	Normal	Normal	Normal
IQ	68	92	113
Metabolism	Hypoglycemia	Hypoglycemia	Hypoglycemia
AST (U/l)	53	47	82
ALT (U/l)	30	28	36
GGT (U/l)	201	99	143
Alb (mg/dL)	2.7	3.1	3.1
Plt (10 ⁴ /μL)	21.3	12.3	28.1
PT (%)	67.4	61.5	55.3
Lactic acid (mg/dL)	22.0	19.2	18.6
Pyruvic acid (mg/dL)	1.2	1.0	1.0
AFP (ng/mL)	413	1332	3078
Abdominal MRI	Multifocal masses	Multifocal masses	Multifocal masses
Liver histology	Cirrhosis/steatosis	Cirrhosis/steatosis	Not done
Head MRI	Normal	Normal	Normal
Child-Pugh score	6	6	6

IQ: intelligence quotient; AST: aspartate alanine aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyl transferase; Alb: albumin; Plt: platelet; AFP: alpha-feto protein; MRI: magnetic resonance imaging; PT: prothrombin time

Table 2 shows previous patients who had p.R50Q homozygous mutations, and p.R50W homozygous or heterozygous mutations, and compared with our cases. All previous cases with p.R50W mutations (three homozygous and two heterozygous) died prematurely during early childhood (age of death: infancy; 1, 1, and 19 months old; and 10 years old)^[4,12,25,30]. Although all our patients survived, one p.R50W homozygous patient died at 10 years old after liver transplantation and showed longer survival. However, while the patient progressed to end-stage liver disease, she was affected by severe ascites, malnutrition, and jaundice during pre-liver transplantation period^[25]. In comparison to this case, our patients lacked severe hepatic manifestations. Regarding p.R50Q mutation, currently there are 11 known cases of homozygous p.R50Q mutation^[4,10,11,25,29]; eight of them presented with NNH. It is known that patients possessing the p.R50Q homozygous mutation have higher survival rate (45%; 5 out of 11 patients), although some cases resulted in death by infantile-onset liver failure (age of death: 7 months old and 2 and 5 years old). It was surprising that none of the three cases with p.R50Q/R50W mutations died during early childhood (age of follow-up: 8, 8, and 5 years old, respectively), in contrast to 100% mortality observed in cases with p.R50W variants (age of death: infancy; 1, 1, and 19 months old; and 10 years old, respectively) and 55% in cases with p.R50Q homozygous variants (age of death: 6 months old and 2, 5, 15, 16, and 20 years old, respectively).

Additionally, our cases with a compound heterozygous p.R50Q/p.R50W mutation were free from neurological manifestations compared with high occurrence of neurological manifestations in p.R50W and p.R50Q mutations previously reported. Regarding neurological manifestations, in previous cases, 90% of p.R50Q homozygous variants and 80% of p.R50W homozygous variants had neurological symptoms such as ataxia, corneal ulcers, developmental delay, dystonia, hypotonia, peripheral neuropathy, and seizures. MRI abnormality was also observed in 40% patients who had p.R50Q homo and p.R50W cases, respectively. Compared with earlier cases, p.R50Q/p.R50W lacked neurological symptoms except mild intellectual disability observed in Case 1. Taken together, the fact that none of our cases showed mortality even though they had heterozygous p.R50W mutation and that they had no visible neurological manifestations suggests the p.R50Q mutation might be associated with longer survival compared with other mutations linked to severe outcomes of MPV17-related MTDPS, such as p.R50W.

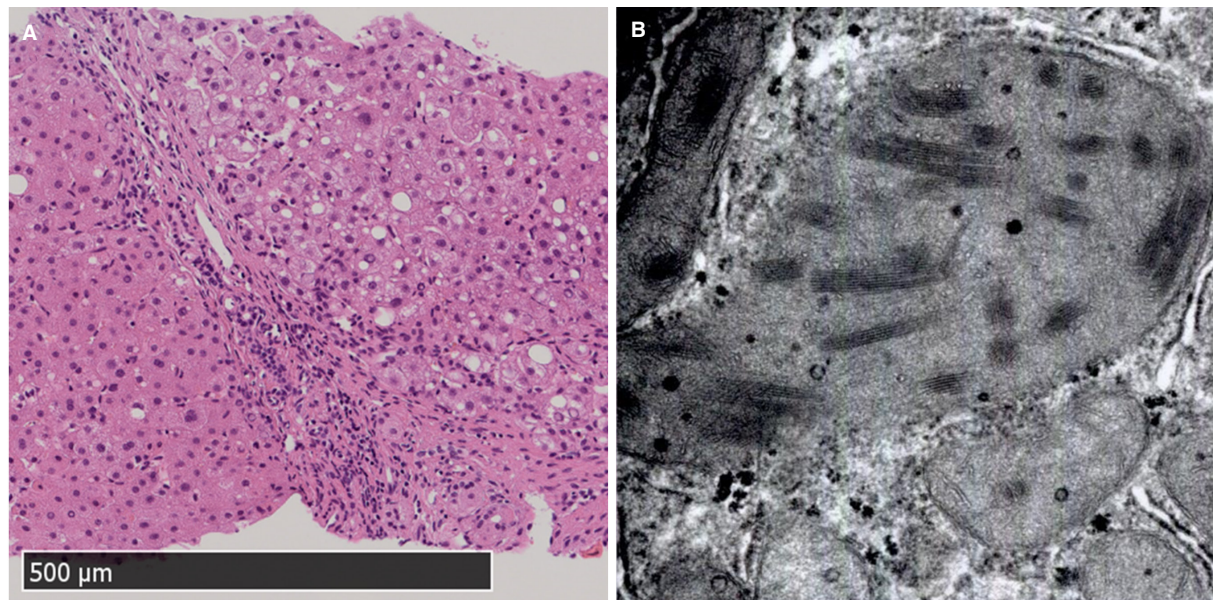


Figure 3. Liver biopsies at age six showed advanced fibrosis with regenerative nodules and mild steatosis: (A) hematoxylin-eosin staining (magnification, low power field); and (B) electron microscopy (magnification, $\times 25,000$) revealed giant mitochondria with increased inclusion bodies in hepatocytes

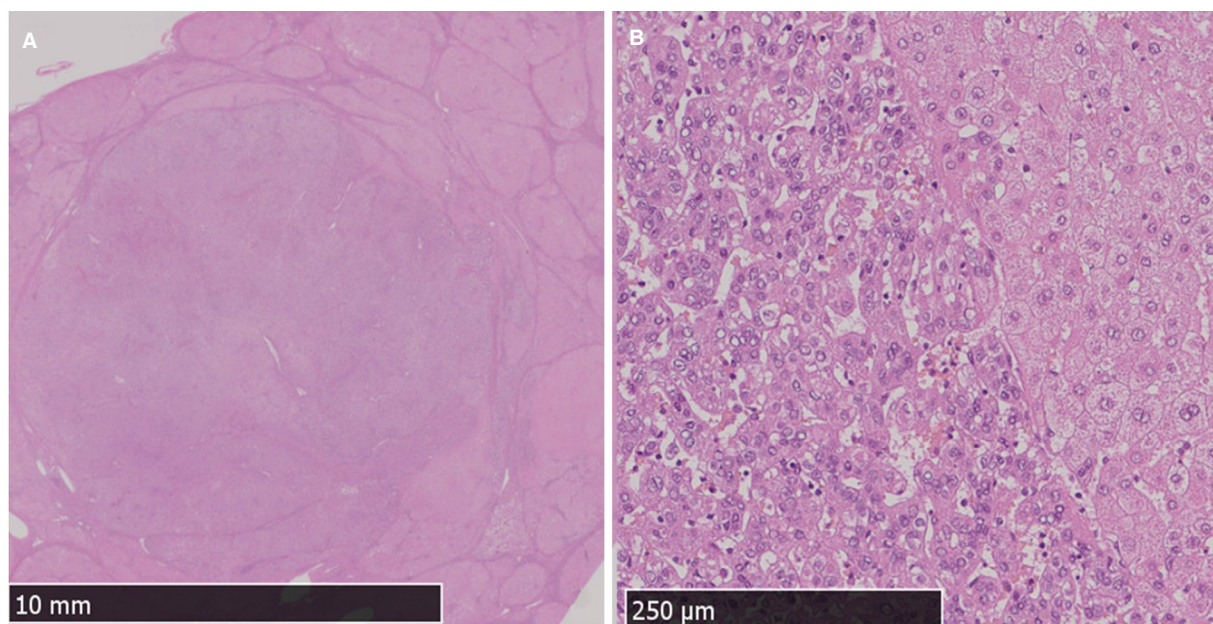


Figure 4. Explanted Case 2 liver with multiple large nodules. A histological analysis (hematoxylin-eosin staining) of the Case 2 S7 mass revealed: (A) a high nucleocytoplasmic ratio (magnification, low power field); and (B) sharply delineated small aggregates of highly pleomorphic small hepatocytes, which are typical of well-differentiated hepatocellular carcinoma (magnification, high power field)

Interestingly, one of our cases developed pediatric-onset HCC, increasing the number to four known patients with MPV17-related MTDPS who had this cancer [Table 3]. Out of four patients with HCC in MPV17-related MTDPS, no compound heterozygous mutation was reported. In contrast, two patients had homozygous mutations (another patient was not identified in one MPV17 mutation). Regarding treatment, all four patients with HCC had liver transplantation (LT), although post-LT course differed widely according to the types of genotypes. p.R50W homozygous patients died at 10 years old, one year after she had liver transplantation, while three patients showed extended survival with good post-LT course.

Table 2. Clinical phenotype of patients with MPV-17-related mitochondria hepatopathy with p.R50Q, or p.R50W mutations

		p.R50Q/p.R50W <i>n</i> = 3		p.R50W reported <i>n</i> = 5		p.R50Q reported <i>n</i> = 11	
Sex	Female	2	(67)	4	(80)	4	(36)
Outcome	Alive	3	(100)	0	(0)	5	(46)
	Liver transplantation	2	(66)	1	(20)	5	(46)
Hepatic symptoms		3	(100)	5	(100)	11	(100)
	HCC	1	(33)	1	(20)	1	(9)
	Hepatomegaly	3	(100)	3	(60)	3	(27)
	Cirrhosis	2	(67)	1	(20)	6	(55)
	Liver dysfunction	3	(100)	5	(100)	11	(100)
	Liver failure	2	(67)	5	(100)	9	(82)
	Cholestasis	0	(0)	3	(60)	8	(73)
	Steatosis	0	(0)	4	(80)	7	(64)
Neurological symptoms		1	(33)	4	(80)	10	(91)
	Ataxia	0	(0)	0	(0)	2	(18)
	Corneal ulcers	0	(0)	0	(0)	3	(27)
	Developmental delay	1	(33)	2	(40)	9	(82)
	Dystonia	0	(0)	2	(40)	0	(0)
	Hypotonia	0	(0)	2	(40)	3	(27)
	Peripheral neuropathy	0	(0)	0	(0)	8	(73)
	Seizures	0	(0)	1	(20)	1	(9)
MRI abnormality							
	Basal ganglia	0	(0)	1	(20)	0	(0)
	White matter	0	(0)	2	(40)	5	(46)
Metabolic symptoms		3	(100)	3	(60)	10	(91)
	Lactic acidemia	0	(0)	3	(60)	8	(73)
	Hypoglycemia	3	(100)	2	(40)	7	(64)
GI symptoms		3	(100)	4	(80)	8	(73)
	Feeding difficulties	0	(0)	2	(40)	0	(0)
	Failure to thrive	3	(100)	4	(80)	8	(73)

The data are numbers (percentages). HCC: hepatocellular carcinoma; GI: gastrointestinal; MRI: magnetic resonance imaging

Table 3. Clinical manifestations of patients with MPV17-related hepatocerebral mitochondrial DNA depletion syndrome who developed hepatocellular carcinoma

	Case 2	Patient 1	Patient 2	Patient 3
Sex	Female	Male	Male	Female
Ethnicity	Japan	Navajo	Caucasian	India
MPV17				
Allele 1	p.R50Q	p.R50Q	c.22insC	p.R50W
Allele 2	p.R50W	p.R50Q	NA	p.R50W
Onset age	4 years	Infancy	Infancy	5 years
LT (age)	Done (7 years)	Done (11 years)	Done (NA)	Done (9 years)
Outcome (age)	Alive (8 years)	Alive (21 years)	Alive (9 years)	Died (10 years)
Hepatic manifestation	Cirrhosis, HCC	LF, Cirrhosis, Steatosis, HCC	LF, Cirrhosis, HCC	LF, Cirrhosis, Steatosis, HCC
Other manifestation	Hypoglycemia, FTT	DD, Peripheral neuropathy, MRI abnormalities, Hypoglycemia, FTT	DD, Hypotonia, Seizures, FTT	Seizures, Dystonia, MRI abnormalities, FTT
Ref.	This report	[10]	[15]	[25]

HCC: hepatocellular carcinoma; DD: developmental delay; FTT: failure to thrive; GI: gastrointestinal; LF: liver failure; LT: liver transplantation; NA: not accessed; MRI: magnetic resonance imaging

Liver transplantation is one of the best treatments for HCC-induced cirrhotic liver. Although a recent study showed that liver transplantation for pediatric HCC patients with inherited metabolic disease has better chances of survival compared with pediatric HCC patients with non-inherited metabolic disease^[33], its efficacy in MPV17-MTDPS remains controversial. The reason behind this is that the organ's systemic complexity results in high morbidity during post-transplantation. Of the 17 known MPV17-related MTDPS patients who received liver transplantation, seven (41%) died during the post-transplantation period^[30]. Of

the five known MPV17-related MTDPS patients with the p.R50Q homozygous mutation who received liver transplantation, three (60%) survived^[4,10,11,29]. In contrast, four patients with other mutations out of 12 (33%) survived liver transplantation^[12-15,20,25,30]. Here, Case 1 received liver transplantation to treat end-stage liver disease, and Case 2 to treat hepatic cell carcinoma. At the time of writing this report, their post-transplant outcome was good. None of the patients had serious systemic organ complications. LT indication for MPV17-related MTDPS is still controversial, but LT may be an optional treatment for HCC with MPV17 related MTDPS with p.R50W mutation. In such cases, careful surveillance for systemic organ involvement should be applied, because long-term outcomes of post-LT course are still unknown.

As with previous studies of MTDPS, this case report describes a small sample size and short follow-up period. Therefore, we need to perform further studies on large patient cohorts, to determine the effectiveness and outcomes of liver transplantation in MPV17-related MTDPS. Long-term follow up should be performed, including regular neurological assessment.

This case study reported the first compound heterozygous p.R50Q/p.R50W mutation of MPV17. All the three siblings survived without neurological manifestations, in contrast with total mortality accompanied by systemic organ involvements in previous MPV17 mutation p.R50W. Two of our cases underwent liver transplantation, and both showed positive post-transplant outcomes at the time of writing. The p.R50Q mutation might be associated with more prolonged survival including liver transplantation outcomes, as compared with other previously described mutations linked to severe outcomes of MPV17-related MTDPS. Screens of the *MPV17* gene should be performed in all cases of unknown, severe hepatic dysfunction in children.

DECLARATIONS

Acknowledgments

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Authors' contributions

Drafted the manuscript: Umetsu S

Made a special support from the metabolic evaluation: Murayama K, Shimura M

Made a special support from genetic evaluation: Uehara T, Kosaki K

Made a special support from Liver transplantation: Uchida H, Kasahara M

Assisted with liver histopathology: Irie R, Yoshioka T

Supervised diagnosis and treatment: Kobayashi S, Sogo T, Komatsu K, Inui A, Fujisawa T

Read and approved the final manuscript: Umetsu S, Inui A, Kobayashi S, Shimura M, Uehara T, Uchida H, Irie R, Sogo T, Komatsu H, Yoshioka T, Murayama K, Kosaki K, Kasahara M, Fujisawa T

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The institutional review board of Saiseikai Yokohama-shi Tobu Hospital approved, and informed consent was obtained from parent.

Consent to participate

Written informed consent was obtained from the guardian of the patients for publication of this Case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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REFERENCES

1. Skladal D, Halliday J, Thorburn DR. Minimum birth prevalence of mitochondrial respiratory chain disorders in children. *Brain* 2003;126:1905-12.
2. Spinazzola A, Invernizzi F, Carrara F, Lamantea E, Donati A, et al. Clinical and molecular features of mitochondrial DNA depletion syndromes. *J Inher Metab Dis* 2009;32:143-58.
3. Wallace DC. Mitochondrial diseases in man and mouse. *Science* 1999;283:1482-8.
4. Spinazzola A, Viscomi C, Fernandez-Vizarra E, Carrara F, D'Adamo P, et al. MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. *Nat Genet* 2006;38:570-5.
5. Spinazzola A, Santer R, Akman OH, Tsiakas K, Schaefer H, et al. Hepatocerebral form of mitochondrial DNA depletion syndrome: novel MPV17 mutations. *Arch Neurol* 2008;65:1108-13.
6. Munnich A, Rustin P. Clinical spectrum and diagnosis of mitochondrial disorders. *Am J Med Genet* 2001;106:4-17.
7. Ferrari G, Lamantea E, Donati A, Filosto M, Briem E, et al. Infantile hepatocerebral syndromes associated with mutations in the mitochondrial DNA polymerase-gammaA. *Brain* 2005;128:723-31.
8. Hakonen AH, Isohanni P, Paetau A, Herva R, Suomalainen A, et al. Recessive Twinkle mutations in early onset encephalopathy with mtDNA depletion. *Brain* 2007;130:3032-40.
9. Mandel H, Szargel R, Labay V, Elpeleg O, Saada A, et al. The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nat Genet* 2001;29:337-41.
10. Karadimas CL, Vu TH, Holve SA, Chronopoulou P, Quinzii C, et al. Navajo neurohepatopathy is caused by a mutation in the MPV17 gene. *Am J Hum Genet* 2006;79:544-8.
11. Parini R, Furlan F, Notarangelo L, Spinazzola A, Uziel G, et al. Glucose metabolism and diet-based prevention of liver dysfunction in MPV17 mutant patients. *J Hepatol* 2009;50:215-21.
12. Wong LJ, Brunetti-Pierri N, Zhang Q, Yazigi N, Bove KE, et al. Mutations in the MPV17 gene are responsible for rapidly progressive liver failure in infancy. *Hepatology* 2007;46:1218-27.
13. Navarro-Sastre A, Martin-Hernandez E, Campos Y, Quintana E, Medina E, et al. Lethal hepatopathy and leukodystrophy caused by a novel mutation in MPV17 gene: description of an alternative MPV17 spliced form. *Mol Genet Metab* 2008;94:234-9.
14. Kaji S, Murayama K, Nagata I, Nagasaka H, Takayanagi M, et al. Fluctuating liver functions in siblings with MPV17 mutations and possible improvement associated with dietary and pharmaceutical treatments targeting respiratory chain complex II. *Mol Genet Metab* 2009;97:292-6.
15. El-Hattab AW, Li FY, Schmitt E, Zhang S, Craigen WJ, et al. MPV17-associated hepatocerebral mitochondrial DNA depletion syndrome: new patients and novel mutations. *Mol Genet Metab* 2010;99:300-8.
16. Merkle AN, Nascene DR, McKinney AM. MR imaging findings in the reticular formation in siblings with MPV17-related mitochondrial depletion syndrome. *AJNR Am J Neuroradiol* 2012;33:E34-5.
17. Al-Jasmi F, Penefsky HS, Souid AK. The phosphorescence oxygen analyzer as a screening tool for disorders with impaired lymphocyte bioenergetics. *Mol Genet Metab* 2011;104:529-36.
18. AlSaman A, Tomoum H, Invernizzi F, Zeviani M. Hepatocerebral form of mitochondrial DNA depletion syndrome due to mutation in MPV17 gene. *Saudi J Gastroenterol* 2012;18:285-9.
19. Nogueira C, de Souza CF, Husny A, Derks TG, Santorelli FM, et al. MPV17: fatal hepatocerebral presentation in a Brazilian infant. *Mol Genet Metab* 2012;107:764.
20. Uusimaa J, Evans J, Smith C, Butterworth A, Craig K, et al. Clinical, biochemical, cellular and molecular characterization of mitochondrial DNA depletion syndrome due to novel mutations in the MPV17 gene. *Eur J Hum Genet* 2014;22:184-91.
21. Piekutowska-Abramczuk D, Pronicki M, Strawa K, Karkucinska-Wieckowska A, Szymanska-Debinska T, et al. Novel c.191C>G (p.Pro64Arg) MPV17 mutation identified in two pairs of unrelated Polish siblings with mitochondrial hepatocerebralopathy. *Clin Genet* 2014;85:573-7.
22. Mendelsohn BA, Mehta N, Hameed B, Pekmezci M, Packman S, et al. Adult-onset fatal neurohepatopathy in a woman caused by MPV17 mutation. *JIMD Rep* 2014;13:37-41.
23. Al-Hussaini A, Faqeih E, El-Hattab AW, Alfadhel M, Asery A, et al. Clinical and molecular characteristics of mitochondrial DNA depletion syndrome associated with neonatal cholestasis and liver failure. *J Pediatr* 2014;164:553-9.e1-2.

24. Sarkhy AA, Al-Sunaid A, Abdullah A, AlFadhel M, Eiyad W. A novel MPV17 gene mutation in a Saudi infant causing fatal progressive liver failure. *Ann Saudi Med* 2014;34:175-8.
25. Vilarinho S, Choi M, Jain D, Malhotra A, Kulkarni S, et al. Individual exome analysis in diagnosis and management of paediatric liver failure of indeterminate aetiology. *J Hepatol* 2014;61:1056-63.
26. Bijarnia-Mahay S, Mohan N, Goyal D, Verma IC. Mitochondrial DNA depletion syndrome causing liver failure. *Indian Pediatr* 2014;51:666-8.
27. McKiernan P, Ball S, Santra S, Foster K, Fratter C, et al. Incidence of primary mitochondrial disease in children younger than 2 years presenting with acute liver failure. *J Pediatr Gastroenterol Nutr* 2016;63:592-7.
28. Kim J, Kang E, Kim Y, Kim JM, Lee BH, et al. MPV17 mutations in patients with hepatocerebral mitochondrial DNA depletion syndrome. *Mol Genet Metab Rep* 2016;8:74-6.
29. Bitting CP, Hanson JA. Navajo neurohepatopathy: a case report and literature review emphasizing clinicopathologic diagnosis. *Acta Gastroenterol Belg* 2016;79:463-9.
30. El-Hattab AW, Wang J, Dai H, Almannai M, Staufner C, et al. MPV17-related mitochondrial DNA maintenance defect: new cases and review of clinical, biochemical, and molecular aspects. *Hum Mutat* 2018;39:461-70.
31. Kirby DM, Crawford M, Cleary MA, Dahl HH, Dennett X, et al. Respiratory chain complex I deficiency: an underdiagnosed energy generation disorder. *Neurology* 1999;52:1255-64.
32. He L, Chinnery PF, Durham SE, Blakely EL, Wardell TM, et al. Detection and quantification of mitochondrial DNA deletions in individual cells by real-time PCR. *Nucleic Acids Res* 2002;30:e68.
33. Baumann U, Adam R, Duvoux C, Mikolajczyk R, Karam V, et al. Survival of children after liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2018;24:246-55.

Original Article

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Interim results from ongoing Phase III placebo-controlled, randomized trial of hepcortespenlisimut-L for advanced hepatocellular carcinoma indication

Aldar S. Bourinbaier¹, Jiggjidsuren Chinburen², Purev Batchuluun², Chogsom Munkhzaya², Dandii Oyungerel³, Dorjiin Dandii³, Galyna A. Kutsyna⁴, Hulan Tsogkhuu⁵, Marina G. Tarakanovskaya⁵, Alan A. Reid⁶, Vika Borisova⁶, Allen I. Bain¹, Vichai Jirathitikal¹

¹Immunitor Inc., Vancouver, BC, V6K 2G8, Canada.

²National Cancer Center, Ulaanbaatar 14000, Mongolia.

³National Center for Public Health, Ulaanbaatar 13000, Mongolia.

⁴National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

⁵Ekomed LLC, Ulaanbaatar 13381, Mongolia.

⁶Immunitor China Ltd., Chaoyang, Beijing 100027, China.

Correspondence to: Dr. Aldar Bourinbaier, Immunitor Inc., 365-2912 West Broadway, BC, V6K 2G6, Canada.
E-mail: aldar@immunitor.com

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Abstract

Aim: We aimed to further investigate the role of hepcortespenlisimut-L (Hepko-V5 or V5), a new oral immunotherapy developed by us, for hepatocellular carcinoma (HCC) indication.

Methods: The interim data from ongoing Phase III placebo-controlled, randomized trial were evaluated on the initial group of patients in advanced stage of HCC with emphasis on liver function and tumor marker alpha-fetoprotein levels. Additionally, an *in vitro* study was undertaken to elucidate the mechanism of action of V5 by measuring with flow cytometry the expression of cytokines such as IL-2, INF- γ , and TNF- α and cell activation markers CD69 and Ki67 on CD4- and CD8-positive lymphocytes isolated from peripheral blood of healthy volunteers.

Results: As early as one month after treatment initiation, there was a clear improvement in alanine transaminase, aspartate transaminase, alkaline phosphatase, and bilirubin levels among HCC patients who received daily dose of V5, but not in the placebo group. Additionally, alpha-fetoprotein (AFP) levels among V5 recipients decreased, while in the



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placebo group they rose. Clinical results are in line with *in vitro* observations indicating immune activation, as evidenced by many-fold enhancement of CD69, Ki67, and INF- γ expression and at the same time marked anti-inflammatory effect resulting in 10-fold decrease in TNF- α output and lack of influence on IL-2 production.

Conclusion: Hepcortespensilisimut-L, a tableted oral formulation derived from heat-inactivated pooled blood of patients with HCC and viral hepatitis shows beneficial clinical effect, as demonstrated by improvement in liver function and reduction of tumor marker AFP levels. These correlate with *in vitro* observations showing potent activation of the immune response and pronounced oral tolerance effect.

Keywords: Hepatocellular carcinoma, alpha-fetoprotein, alanine transaminase, aspartate transaminase, IL-2, INF- γ , TNF- α , CD69, Ki67, CD4, CD8, T lymphocytes

INTRODUCTION

Primary liver tumors originate from hepatocytes, cholangiocytes, and mesenchymal cells. Hepatoma is generally defined as a cancer originating from liver cells. It is often called hepatocarcinoma or hepatocellular carcinoma (HCC). It is the most common cancer of the liver, ranking as the second leading cause of death from all types of cancers worldwide^[1]. Multiple risk factors have been identified including chronic viral hepatitis, liver cirrhosis, non-alcoholic fatty liver disease (NASH), lifestyle factors comprising alcohol abuse and smoking, metabolic diseases such as diabetes and obesity, environmental toxins such as aflatoxin, and occasionally genetic and hereditary disorders^[2]. While the development of HCC is complex, there is a consensus that the underlying cause is chronic inflammatory damage^[3]. Conventional treatment with chemotherapy including sorafenib, regorafenib, lenvatinib, and cabozantinib is usually directed at killing cancer cells - an approach that is quite toxic, leading to liver damage^[4]. This in turn leads to hepatoma recurrence. Ideally, an effective HCC treatment must be two-pronged: in addition to destroying malignant liver cells, normal liver cells must be spared from hepatotoxicity caused by treatment. Immunotherapies are generally regarded as less toxic, although these toxicities can be life-threatening if not managed promptly^[5]. The FDA has recently approved three antibody-based drugs: nivolumab, pembrolizumab, and ramucirumab. Additional immune interventions from the checkpoint inhibitor PD family, i.e., durvalumab, atezolizumab, and bevacizumab, as well as tremelimumab, which belongs to the anti-CTLA4 class, are being investigated^[6]. Experience has shown that none of the currently approved HCC drugs are free from adverse side effects, albeit with varying degrees of severity.

Hepcortespensilisimut-L (also known as Hepko-V5 or V5) is the immunotherapeutic drug from a novel class that has been through Phase II trial^[7]. Anti-HCC property of V5 was discovered accidentally about ten years ago during clinical use for original primary indications, which are chronic viral hepatitis and liver cirrhosis^[8,9]. V5 has been commercially available since 2002, has been used by over 30,000 individuals, and has never been reported of causing any serious adverse reactions. The US FDA granted Hepko-V5 orphan drug designation status in 2014. The present paper provides preliminary data from the currently ongoing Phase III trial and summarizes some of unpublished studies.

METHODS

Patients and treatment regimen

This Phase III placebo-controlled, 1:1 randomized trial was initiated with the goal of recruiting a total of 120 patients with advanced HCC. The preliminary data from the initial batch of 30 enrolled patients are provided to evaluate whether observed results confirm the data from an earlier published Phase II open-label trial^[7]. All patients were in advanced stage of HCC and unfit for standard therapy, i.e., surgical intervention, due to tumor size, its location, or multiplicity. Many patients had single or multiple events of recurrent lesions after surgical intervention, such as resection, transarterial embolization, radiofrequency

ablation, percutaneous ethanol injection, or their combinations. At the presentation, all patients were either under palliative care or pronounced incurable without available treatment options - underlining the disease gravity. As the overwhelming majority of our HCC patients were in terminal stage of the disease, they often presented with a variety of symptoms related to decompensated cirrhosis including ascites, edema, variceal bleeding, portal thrombi, and hepatic encephalopathy. Besides these symptoms, abdominal discomfort and less frequently pain were common. Other encountered complaints were fatigue or weakness, cachexia, anorexia, skin itch, bleeding from gums and nose, vomiting, and jaundice. As no other intervention options except palliative care were available, patients consented to receive a once-per-day pill of Hepko-V5 ($n=14$) or identically appearing placebo pill ($n=16$) with follow-up lab tests at Months 1 and 2. Patients were instructed to take one pill before bedtime and come back after running out of pills after 30 days. Patients had baseline measurement for alpha-fetoprotein and standard liver function tests such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase, and total bilirubin. Additional tests included creatinine and prothrombin time. In such a way, we had a cohort representative of the real-life situation in Mongolia, where almost every patient is diagnosed only after symptoms of the disease become apparent or when tumors recur.

V5

V5 is derived from pooled blood of patients with HCC who are often viral hepatitis C, hepatitis B, and Delta virus carriers by employing proprietary technology developed by us. The process of manufacturing has been described in detail previously: it involves heat and chemical inactivation with subsequent formulation into a tablet capable of withstanding digestive degradation in the stomach^[9]. The principle for mechanism of action of V5 is not much different from established principles with old-fashioned killed vaccines, e.g., hepatitis B vaccine made from pooled plasma. V5 has been approved in Mongolia as a biologically active product since 2008. V5 Immunitor is presented as an 850 mg coated pink pill, with 30 pills per one package. The recommended dose is one to two pills per day, and the dose being used in this study is one pill per day. The preparation is stable at ambient room temperature for at least three years.

Flow cytometry analysis

Peripheral blood mononuclear cells (PBMCs) from the blood of healthy donors were obtained by density gradient centrifugation. Lymphocytes were isolated from PBMCs using anti-human CD4 or CD8 magnetic MACS microbeads following the manufacturer's protocol (Miltenyi Biotec, Somerville, MA 02143, USA). Obtained cells were incubated with a crushed pill of V5 diluted one million-fold (10^{-6}) in saline for 48 h and then subjected to fluorescence-activated cell sorter (FACS) analysis using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). V5-exposed and control cells were stained with antibodies against CD3, CD4, CD8, IFN- γ , TNF- α , and IL-2 obtained from BD Biosciences. Briefly, cells (1×10^6) were stained either with anti-CD4 or -CD8 and anti-CD69 or -Ki67 (BD Pharmingen). Labeled cells were fixed with 0.5% paraformaldehyde and analyzed using FlowJo software (Tree Star Inc., Ashland, OR).

Statistical analysis

The primary endpoint for this study was the effect of Hepko-V5 on serum AFP levels as a surrogate marker of tumor burden changes. Secondary objectives were safety, adverse effects, and changes in liver function parameters, creatine levels, and pro-thrombin time. The difference between pre- and post-treatment parametric values was assessed by paired Student's *t*-test and linear regression analysis. Wilcoxon matched pairs ranking test and contingency table analysis were employed for categorical data using the statistical calculator GraphPad, which is freely available online (GraphPad Software, 2365 Northside Dr., San Diego, CA 92108, USA). The significance level for all tests was set at $P \leq 0.05$.

Ethics approval and written consent

All patients were explained benefits and risks of the intervention and provided informed written consent agreeing to participate. The study was approved by the Institutional Review Board of Immunitor LLC

Table 1. Interim analysis of select data from ongoing Phase III trial in 30 advanced-stage HCC patients in Mongolia

Parameters	Hepcortespensimut-L (<i>n</i> = 14)			Placebo (<i>n</i> = 16)		
	Entry level	Month 1 (<i>P</i>)	Month 2 (<i>P</i>)	Entry level	Month 1 (<i>P</i>)	Month 2 (<i>P</i>)
ALT (IU/L)	68.1	36.2 (0.01)	34.4 (0.002)	51.2	44.8 (0.51)	50.3 (0.93)
AST (IU/L)	89.3	52.7 (0.002)	48.9 (0.006)	72.5	77.9 (0.71)	76.6 (0.79)
ALP (IU/L)	151.2	111.3 (0.15)	89.4 (0.06)	160.1	184.7 (0.49)	221.7 (0.23)
Bilirubin (μ M/L)	30.9	15.4 (0.002)	13.1 (0.0008)	28.1	17.8 (0.1)	25.3 (0.8)
AFP (IU/mL)	9,619.4	7,649.3 (0.65)	6,994.9 (0.69)	8,285.4	11,340.9 (0.55)	23,157.8 (0.6)

The values are expressed as means with *P* values shown in parentheses, where appropriate. *P* values shown for these parameters were derived from Student-*t* test calculation. *P* values for this parameter were derived from Wilcoxon ranking test; the difference between outcomes in AFP levels at study conclusion between two treatment arms at Month 2, i.e., 6994.9 vs. 23,157.8, is highly significant ($P < 0.0001$; Chi-square). HCC: hepatocellular carcinoma; AFP: alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase

(#IRB00010671) and conducted in accordance with the declaration of Helsinki in conformity with good clinical practice as defined by the International Conference on Harmonization. This study is registered at ClinicalTrials.gov site with ID: NCT02232490.

RESULTS

The interim analysis of data from Phase III placebo-controlled study is summarized in Table 1. At the time of interim analysis, only 30 patients randomized into two arms, with 14 patients in V5 arm and 16 patients in the placebo arm, were available for statistical analysis. The total planned number of patients to be enrolled into this trial is 120. As the study is ongoing, the codes were not broken open; thus, the statistician had no access to information as to which arm received experimental drug or placebo. Nevertheless, there is a clear difference between arms starting as early as one-month post-treatment. Pills that were stamped with “1” (experimental drug; V5) had highly significant effect on measured liver function parameters, i.e., ALT, AST, total bilirubin, and alkaline phosphatase. In contrast, pills stamped with “5” (placebo) had no significant effect. Pills “1” had a very distinct effect on liver function improvement and this trend is consistent with AFP changes, while 5-labeled pills were not able to arrest AFP accrual, which increased almost three-fold, i.e., from 8285 to 23,158 IU/mL. The patients who received hepcortespensimut-L experienced a decline in AFP levels, from 9619.4 to 6994.9 IU/mL at the end of the second month and the difference between the outcomes of the two arms was statistically significant ($P < 0.0001$ by Chi-Square). Nevertheless, *P* values for AFP within both arms did not reach significance levels due to wide inter-individual fluctuation of AFP levels at baseline ranging three or more orders of magnitude. Thus, the Wilcoxon test had insufficient power. However, when the evolution in AFP trend was compared in V5 vs. placebo arms, this discrepancy became highly statistically significant, which makes us believe that the outcome of the trial will be successful, in line with numerous other V5 trials for unrelated clinical indications. Data were analyzed on intent-to-treat fashion without excluding missing values, deaths, and non-compliance. These preliminary findings indicate that the batch of pills labeled as 5 (placebo) pills used in the trial had no desired biological activity and the V5 pills appeared to have clear benefit in terms of ameliorating baseline values. The trial is currently ongoing at the National Cancer Center, Ulaanbaatar, Mongolia and, provided additional funding is forthcoming, is expected to be completed by the end of 2021.

Effect of V5 on T cells from healthy donors

This *in vitro* study was undertaken to elucidate the effect of short exposure (48 h) of a physiologically relevant dose of V5 (diluted one million-fold, i.e., 0.1 μ g/mL of cell culture medium) on effector T cells, either CD4- or CD8-positive T lymphocytes. The cells from the same donor were incubated in an identical culture medium without any V5 and they served as controls. This experiment was repeated three times on venous blood samples from unrelated healthy donors. The results from a representative flow cytometry analysis are shown in Figure 1. While limited, the data reveal an effect that has never been reported before.

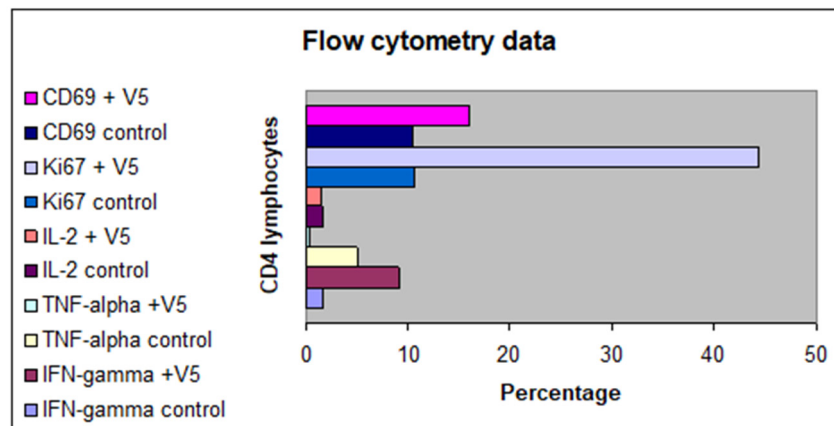


Figure 1. Effect of 48-h *in vitro* incubation of CD4 T lymphocytes from healthy volunteers with V5 (10^{-6} dilution) on expression of cell activation/proliferation markers, i.e., CD69 and Ki-67, and IL-2, TNF- α , and IFN- γ . Controls are unstimulated T cells as analyzed by flow cytometry at the same time. Except IL-2 expression, all other parameters were statistically distinct in V5-treated cells *vs.* controls

It appears that V5, which contains pooled antigens, including numerous tumor antigens and neoantigens, hepatitis virus fragments, and alloantigens, all circulating in the blood of patients with HCC, produces potent immune activation, while also inducing the anti-inflammatory effect. Markers of activation, i.e., CD69 and Ki67, rose on average 1.5- and 4-fold, respectively, compared to unstimulated CD4 and CD8 cells. The levels of IL-2 expression did not change, but IFN- γ expressing cells increased six-fold. Remarkably, the inflammation marker TNF- α decreased almost 10-fold compared to control. The response of CD8 cells to V5 followed the same pattern as seen with CD4 cells, except expression of IFN- γ , which did not change compared to controls.

DISCUSSION

This paper provides interim, select data from an ongoing placebo-controlled, randomized Phase III trial of hepcortespenlisimut-L (Hepko-V5 or V5), which has successfully passed an open label, one-arm Phase II, 75-patient retrospective trial published in 2017^[7]. Extensive preclinical studies started in 2002 have shown that V5 does not contain harmful chemical ingredients or live virus; does not cause cytotoxicity, mutagenicity, teratogenicity, or genotoxicity; and is not toxic to animals after acute or chronic exposure. V5 is very safe and remarkably well tolerated by healthy individuals as well as those with hepatitis or tuberculosis^[8-10]. V5 intake increases body weight, improves the quality of life, and produces desired clinical benefit with improved liver function. The latest studies have shown that the beneficial effect is also observed in patients with HCC. As a rule, patients who received V5 experienced improvement in their baseline symptoms in as short as one week. Nevertheless, V5 has to be subjected to well-controlled randomized clinical study, such as outlined in this manuscript, to confirm that this intervention holds promise as a safe and effective immunotherapeutic modality for HCC indication.

Liver cancer is the fifth most commonly occurring cancer in men and the ninth most commonly occurring cancer in women. There were over 840,000 new cases in 2018 and prognosis is very poor. Mongolia firmly occupies the first place among all countries when it comes to incidence of HCC, while China has the largest number of patients with HCC, over 50% of all patients with HCC^[11]. The incidence of HCC is expected to rise in the United States and across the world, given the increasing prevalence of hepatitis C and B infections, alcohol consumption, NASH, diabetes, toxin exposure, and obesity. By the time diagnosis of HCC is made, various surgical interventions or liver transplantation are often not feasible. While we have witnessed a surge of systemic therapies in recent years, the need for safer and more effective treatment remains.

Hepcortespensimut-L represents an entirely novel class of immunotherapy that has no analogs among approaches being developed currently^[12,13]. This approach is radically different from the checkpoint inhibitor class of immunotherapies, aimed to revive and keep the “weakened” immune system active. This approach is also different from classical injectable cancer vaccines that incorporate tumor antigens common in this type of cancer, such as AFP^[14], Glypican-3 expressed in > 80% of HCC^[15], or other multi-epitope tumor peptides^[16]. Considering that checkpoint inhibitors have low clinical benefit and single- or few-antigen vaccines have not delivered the expected success, it is clear that new approaches are still needed.

Scientists at Arizona State University recently identified more than 200,000 cancer neoantigens, which could lead to the development of broad-spectrum cancer vaccines^[17]. V5 inherently incorporates all circulating antigens from pooled blood of HCC patients, including tumor unrelated immunogens such as viral hepatitis antigens and very large number of alloantigens, which are not necessarily pathogenic. It is clear that the identification of these antigens is a task posing enormous challenges. Taking aside the academic pursuit of finding the mechanism of action, this challenge is not very important to a patient who needs to be treated immediately, not after the putative antigen(s) are identified.

It is clear that oral delivery route is advantageous since this route eliminates the undesirable cross-reactivity plaguing injectable vaccines. Another advantage associated with transmucosal passage of antigens is induction of the immune tolerance - a phenomenon we experience on a daily basis when we ingest, for example, food, which is composed of non-self, foreign antigens that would normally provoke violent immune reaction if delivered parenterally^[18]. The gut immunity has evolved and differs from the systemic immunity by this critically distinct trait. We would not exist as a species if we did not develop the oral tolerance.

Our flow cytometry data, despite a limited number of studied parameters, suggest that the immune tolerance is not a passive state akin to immune anergy, but rather it is a very active process with the phenomenal activation of effector cells, as demonstrated by several-fold increase of immune activation markers such as Ki67 and CD69 in as little as 48 h. At the same, time this process displays pronounced anti-inflammatory activity, as revealed by ten-fold decrease of TNF- α , and no effect on IL-2 expression - the hallmark of T-cell activation. Increase in IFN- γ output in CD4 but not in CD8 T cells was another unanticipated result. Thus, the exposure to a pool of antigens derived from a tiny amount of peripheral blood of patients with HCC produced results that have not been previously described in the literature.

The field of immunotherapy, particularly for oncology, is evolving rapidly, bringing new surprises daily. Recently, a study was published in *Nature* by Alspach *et al.*^[19], in which they gave more weight to the role of helper CD4 lymphocytes in immuno-oncology over the usual culprit, i.e., CD8 killer cells. Whether V5 more affects CD4 rather than CD8 cells remains to be established; it is likely that both subpopulations are required, and that other subsets of immune cells are also critically involved^[20,21].

Our investigation of hepcortespensimut-L is in its early stage and we are sure we will make more surprising discoveries down the road. Besides the profound influence on the immune response, which is usually expected from cancer vaccines, we see very clear clinical benefits in terms of improved liver function and decrease in AFP levels, which we have shown to be correlated with tumor shrinkage. We have successfully used V5 since 2010 in hundreds of patients with HCC and we are confident that the outcome from this Phase III trial will be consistent with the results of the Phase II retrospective study published in 2017^[7]. In conclusion, our experience in dealing with V5 and patients with HCC receiving this simple to use immunotherapy is supported by the present observation. Further studies will shed more light as to the mechanism of action and extent of the clinical benefit.

DECLARATIONS

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Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Bourinbaiair AS, Chinburen J, Batchuluun P, Munkhzaya C, Oyungerel D, Dandii D, Tsogkhuu H, Kutsyna GA, Tarakanovskaya MG, Bain AI, Jirathitikal V

Performed data acquisition, as well as provided administrative, technical, and material support: Reid AA, Borisova V

Availability of data and materials

The data will be shared upon completion and publication of this study in full.

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Conflicts of interest

All authors, except officers of Immunitor company, i.e., Bourinbaiair AS, Bain AI, Jirathitikal V, Borisova V, Reid AA, declare that there are no conflicts of interest.

Ethical approval and consent to participate

The study was approved by the Institutional Review Board of Immunitor LLC (#IRB00010671) and conducted in accordance with the declaration of Helsinki in conformity with good clinical practice as defined by the International Conference on Harmonization. Further information about this study is available at the ClinicalTrials.gov website under ID: NCT02232490.

Consent for publication

Not applicable.

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REFERENCES

1. Patrick JL, Florio AA, Znaor A, Ruggieri D, Laversanne M, et al. International trends in hepatocellular carcinoma incidence, 1978-2012. *Int J Cancer* 2019; Epub ahead of print doi: 10.1002/ijc.32723.
2. Rawla P, Sunkara T, Muralidharan P, Raj JP. Update in global trends and aetiology of hepatocellular carcinoma. *Contemp Oncol (Pozn)* 2018;22:141-50.
3. Ringelhan M, Pfister D, O'Connor T, Polansky E, Heikenwalder M. The immunology of hepatocellular carcinoma. *Nat Immunol* 2018;19:222-32.
4. Pinter M, Peck-Radosavljevic M. Review article: systemic treatment of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2018;48:598-609.
5. Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol* 2018;15:599-616.

6. Longo V, Brunetti O, Gnoni A, Licchetta A, Delcuratolo S, et al. Emerging role of immune checkpoint inhibitors in hepatocellular carcinoma. *Medicina (Kaunas)* 2019;55:E698.
7. Tarakanovskaya MG, Chinburen J, Batchuluun P, Munkhzaya C, Purevsuren G, et al. Open-label Phase II clinical trial in 75 patients with advanced hepatocellular carcinoma receiving daily dose of tableted liver cancer vaccine, hepcortespenlisimut-L. *J Hepatocell Carcinoma* 2017;4:59-69.
8. Batdelger D, Dandii D, Jirathitikal V, Bourinbaiair AS. Open-label trial of therapeutic immunization with oral V-5 Immunitor (V5) vaccine in patients with chronic hepatitis C. *Vaccine* 2008;26:2733-7.
9. Batdelger D, Dandii D, Dahgwahdorj Y, Erdenetsogt E, Oyumbileg J, et al. Clinical experience with therapeutic vaccines designed for patients with hepatitis. *Curr Pharm Des* 2009;15:1159-71.
10. Bourinbaiair AS, Mezentsseva MV, Butov DA, Nyasulu PS, Efremenko YuV, et al. Immune approaches in tuberculosis therapy: a brief overview. *Exp Rev Anti-infect Ther* 2012;10:381-9.
11. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
12. Zongyi Y, Xiaowu L. Immunotherapy for hepatocellular carcinoma. *Cancer Lett* 2019;470:8-17.
13. Baradaran Noveiry B, Hirbod-Mobarakeh A, Khalili N, Hourshad N, Greten TF, et al. Specific immunotherapy in hepatocellular cancer: a systematic review. *J Gastroenterol Hepatol* 2017;32:339-51.
14. Butterfield LH, Economou JS, Gamblin TC, Geller DA. Alpha fetoprotein DNA prime and adenovirus boost immunization of two hepatocellular cancer patients. *J Transl Med* 2014;12:86.
15. Nobuoka D, Yoshikawa T, Sawada Y, Fujiwara T, Nakatsura T. Peptide vaccines for hepatocellular carcinoma. *Hum Vaccin Immunother* 2013;9:210-2.
16. Buonaguro L; HEPAVAC Consortium. Developments in cancer vaccines for hepatocellular carcinoma. *Cancer Immunol Immunother* 2016;65:93-9.
17. Shen L, Zhang J, Lee H, Batista MT, Johnston SA. RNA transcription and splicing errors as a source of cancer frameshift neoantigens for vaccines. *Sci Rep* 2019;9:1-3.
18. Silin DS, Lyubomska OV, Jirathitikal V, Bourinbaiair AS. Oral vaccination: where we are? *Expert Opin Drug Deliv* 2007;4:323-40.
19. Alspach E, Lussier DM, Miceli AP, Kizhvato I, DuPage M, et al. MHC-II neoantigens shape tumour immunity and response to immunotherapy. *Nature* 2019;574:696-701.
20. Hilmi M, Neuzillet C, Calderaro J, Lafdil F, Pawlotsky JM, et al. Angiogenesis and immune checkpoint inhibitors as therapies for hepatocellular carcinoma: current knowledge and future research directions. *J Immunother Cancer* 2019;7:333.
21. Rafei H, Mehta RS, Rezvani K. Editorial: cellular therapies in cancer. *Front Immunol* 2019;10:2788.

Original Article

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Response rates to direct antiviral agents among hepatitis C virus infected patients who develop hepatocellular carcinoma following direct antiviral agents treatment

Michela Emma Burlone¹, Stefano Fangazio¹, Alessandro Croce², Elisa Ceriani¹, Rachele Rapetti¹, Cristina Rigamonti^{1,2}, Carlo Smirne^{1,2}, Stelvio Tonello², Paolo Ravanini³, Rosalba Minisini², Mario Pirisi^{1,2}

¹Internal Medicine, Azienda Ospedaliero-Universitaria Maggiore della Carità, Novara 28100, Italy.

²Department of Translational Medicine, Università del Piemonte Orientale, Novara 28100, Italy.

³Laboratory of Virology, Azienda Ospedaliero-Universitaria Maggiore della Carità, Novara 28100, Italy.

Correspondence to: Prof. Mario Pirisi, Internal Medicine, Azienda Ospedaliero-Universitaria Maggiore della Carità, Corso Mazzini 18, Novara 28100, Italy. E-mail: mario.pirisi@uniupo.it

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Abstract

Aim: Patients with chronic hepatitis C virus (HCV) infection who develop hepatocellular carcinoma (HCC) soon after treatment with direct antiviral agents (DAA) may have been harboring hitherto hidden tumors. If this were true, they should have a lower sustained viral response (SVR) rate, since active HCC hampers DAA efficacy. We aimed to verify this hypothesis.

Methods: We included all patients who attended an HCV clinic, provided that they: (1) had no previous history of HCC; (2) had received at least one DAA dose; and (3) had been followed-up clinically and ultrasonographically for at least six months after concluding DAA.

Results: The study population included $n = 789$ patients (55% males, median age 62 years). A median of 9.3 months (8.8-11.9) after concluding DAA, $n = 19$ (2.4%) patients were discovered to harbor HCC. In comparison to all others, patients with HCC were more commonly male (84% vs. 54%, $P = 0.009$), obese (47% vs. 17%, $P = 0.002$), and cirrhotic (95% vs. 35%, $P < 0.001$) and had less commonly achieved an SVR (68% vs. 98%, $P < 0.001$). Moreover, they had a trend for being less commonly treatment naïve (58% vs. 67%, $P = 0.051$). Based on multivariate analysis,



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the independent predictors of HCC were male sex ($P=0.031$), cirrhosis ($P=0.004$), obesity ($P=0.006$), and failure to achieve an SVR ($P<0.001$).

Conclusion: Lack of achieving SVR is a strong independent predictor of development of HCC early after treatment of hepatitis C with DAA. Treatment failure should further alert clinicians to the possibility of this dreadful complication.

Keywords: Chronic hepatitis C, direct antiviral agent, sustained viral response, hepatocellular carcinoma, obesity, cirrhosis

INTRODUCTION

Clearance of hepatitis C virus (HCV) infection, a major health problem, is obtainable by treating infected patients with one of several combinations of direct antiviral agents (DAA)^[1]. Today, this desirable outcome can be reached so predictably and safely that HCV eradication is considered by many an achievable goal both on a local scale and on a global scale. Indeed, in 2016, the World Health Organization launched a campaign that - if successful - would eliminate viral hepatitis as a major threat to global health, with substantial economic benefits. Most importantly, putting HCV infection under control would prevent over 1.2 million deaths annually^[2]. For sure, DAA treatment allows curing HCV infection in patients with advanced liver disease, including those who had undergone curative treatments for hepatocellular carcinoma (HCC).

Soon after DAA were introduced in practice, however, surprisingly high HCC incidence and/or recurrence rates were reported, an observation that generated alarm and dismay among clinicians^[3]. In fact, among HCV-related complications, HCC is the most fearsome; furthermore, in the last few years, its incidence appears to be increasing^[4]. Doubts that viral clearance by DAA might favor emergence of HCC clones by reducing immune pressure on HCV have been dispelled^[2]. In fact, recent studies demonstrate convincingly that the opposite is true, i.e., DAA-induced sustained viral response (SVR) reduces the risk for *de novo* HCC^[5-7]. The current interpretation is that the controversy - which has had the merit of highlighting the need to continue HCC surveillance in patients with cirrhosis, despite their achievement of an SVR - might have been generated mainly by inconsistencies and methodological limitations that flawed earlier studies^[8].

Conceivably, among patients “cured” of HCC, so-called “recurrences” may actually represent prevalent tumors, whose presence is recognized only after DAA treatment is started. Could the same explanation apply to the apparent increase of HCC *de novo* diagnosed after DAA treatment? By definition, the presence of HCC foci should have been excluded to call these HCCs *de novo*; however, surveillance of HCC relies on ultrasonography, whose sensitivity is limited. A clue - if not definitive proof - in favor of the hypothesis that hidden HCC foci might have already been present when DAA were started would be to observe lower than expected SVR among DAA-treated patients later found to have an incident HCC, since patients with active HCC respond sub-optimally to DAA^[9]. In the present study, we aimed to substantiate this hypothesis.

METHODS

Patients

The study population included a cohort of consecutively recruited patients attending an academic liver clinic in Northern Italy to receive interferon-free treatment for chronic hepatitis in accordance to the European Association for Liver Diseases (EASL) guidelines^[10]. Inclusion criteria were: (1) no previous diagnosis of HCC; and (2) minimum follow-up after the end of treatment of 180 days. Figure 1 presents the flow chart of the study.

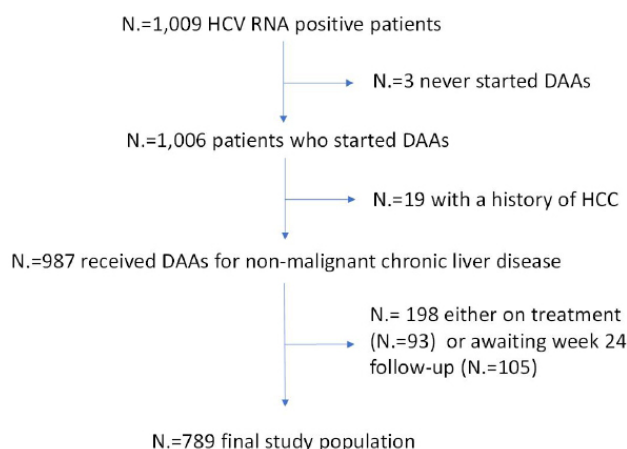


Figure 1. Flow chart of the study. HCV: chronic hepatitis C virus; DAA: direct antiviral agents; HCC: hepatocellular carcinoma

The main demographic and clinical features of the patients enrolled are presented in [Table 1](#).

The direct acting antiviral agent regimens used were sofosbuvir-based in $n = 370$ cases (47%) and protease inhibitor-based in 419 cases (53%). Among the $n = 12$ patients who were DAA-experienced, $n = 6$ (50%) had previously failed at least one interferon-based regimen.

Disease stage was assessed in all patients either invasively, with liver biopsy ($n = 22$, 2.8%), or non-invasively, with transient elastography (Fibroscan®; $n = 744$, 94.3%); in further few cases, a clinical diagnosis of liver cirrhosis was made ($n = 23$, 2.9%). Liver fibrosis in biopsies was staged from F0 to F4 according to the METAVIR staging system^[11]. A liver stiffness threshold of 12.5 kPa was indicative of cirrhosis^[12]. All patients were screened with ultrasound before starting antiviral treatment, irrespective of the presence of cirrhosis.

A clinical diagnosis of cirrhosis was reached in the presence of signs of liver decompensation and/or portal hypertension. HCC was diagnosed according to current EASL guidelines, which require a computed tomography (CT) scan or dynamic contrast-enhanced magnetic resonance imaging (MRI), showing typical hallmarks (hypervascularity in the arterial phase followed by washout in the portal or delayed phases). Focal lesions without typical hallmarks of HCC and those that developed in the absence of cirrhosis were subjected to a liver biopsy and confirmation by an expert liver pathologist^[13].

The outcomes of antiviral therapy were defined as follows:

- SVR: HCV RNA undetectable by a sensitive real-time polymerase chain reaction (PCR)-based assay, performed either after 12 or 24 weeks after the end of treatment;
- relapse: presence of detectable HCV RNA at either post-treatment week 12 or post-treatment week 24, having HCV RNA found undetectable at the end of treatment;
- dropout: patients who did not complete treatment as scheduled;
- lost to follow-up: patients who did not perform a Week 12 or Week 24 after the end of treatment visit, although they completed treatment as scheduled.

Virological methods

Circulating HCV Ribonucleic Acid (HCV-RNA) was searched with the diagnostic system of Abbott RealTime HCV (Abbott, Wiesbaden, Germany), which has a sensitivity cut-off of 12 UI/mL; and the genotyping was performed by means of Abbott RealTime HCV Genotype II (Abbott).

Table 1. Main characteristics of the study population

Variable	<i>n</i> = 789
Age, years	62 (52-74)
Male:Female, <i>n</i>	431 (55):358 (45)
Caucasian race, <i>n</i>	762 (97)
Body mass index ^A , kg/m ²	25.1 (22.6-28.4)
≥ 30 kg/m ²	136 (18)
Diabetes, <i>n</i>	112 (14)
Prediabetes, <i>n</i>	156 (20)
Cirrhosis, <i>n</i>	284 (36)
HCV Genotype, <i>n</i>	
HCV-1A	115 (15)
HCV-1B	307 (39)
HCV-2	205 (26)
HCV-3	102 (13)
HCV-4	55 (7)
HCV-5	1 (< 1)
HCV-6	1 (< 1)
Undetermined	3 (< 1)
HCV RNA, UI/mL (× 1000)	994 (276-2280)
< 400	236 (30)
400-4000	462 (59)
> 4000	91 (12)
Viral coinfections, <i>n</i>	
None	733 (93)
HIV	46 (6)
HBV (included 1 HDV positive)	10 (1)
Treatment history, <i>n</i>	
Naïve	530 (67)
Experienced, interferon based regimens	247 (31)
Experienced, direct antiviral agents	12 (2)

^ABody mass index missing in *n* = 18/790 (2%) patients. Continuous variables are presented as medians (interquartile range), categorical variables as frequencies (*n*) and percentages (%). HCV: hepatitis C virus; HIV: human immunodeficiency virus; HBV: hepatitis B virus; HDV: hepatitis D virus

Statistical analysis

Statistical analysis was performed using Stata Rel. 15.1 (StataCorp LLC, College Station TX, USA). As for continuous variables, the measures of centrality and dispersion of data were median and interquartile range, respectively, while comparisons between groups were carried out by the Mann-Whitney test. With regard to categorical variables, data are presented as frequencies (%), while the associations between groups were verified by the Fisher's exact test or the Pearson chi square test, as appropriate. Logistic regression analysis was conducted to identify predictor(s) of *de novo* HCC among a set of independent variables. The threshold for statistical significance was 0.05 (two tails) for all tests used.

RESULTS

Virologic outcomes

When analyzed with an intention-to-treat approach, SVR was 770/789 (97.6%). In detail, among the 19 patients who did not reach a SVR, 14/19 (74%) patients had a relapse, while 5/19 (26%) did not complete treatment (*n* = 1), died before reaching the 12 week post-treatment (*n* = 2), or performed neither the Post-Treatment Week 12 nor the Post-Treatment Week 24 visits (*n* = 2). Thus, the rate of virologic failure in this study was 1.8%. SVR was similar in patients who received a sofosbuvir-based regimen (359/370, 97%) *vs.* patients who received a protease inhibitor-based regimen (411/419, 98%) (*P* = 0.360), and in HCV-3 infected (98/102, 96%) *vs.* non-HCV-3 infected patients (672/688, 98%) (*P* = 0.312). There was a non-significant trend for lower SVR in patients with cirrhosis (273/284, 96%) *vs.* non-cirrhotic patients (497/505, 98%) (*P* = 0.054).

Table 2. Comparison between patients with and without de novo HCC at the end of follow-up

Variable	HCC <i>de novo</i> <i>n</i> = 19 (2.4%)	No HCC <i>n</i> = 770 (97.6%)	<i>P</i>
Age, years	68 (58-73)	62 (52-74)	0.248
Male sex, <i>n</i>	16 (84)	415 (54)	0.009
Caucasian race, <i>n</i>	19 (100)	743 (96) ^A	1.000
Body mass index ^A , kg/m ²	29.4 (21.3-31.6)	25.1 (22.6-28.3)	0.060
≥ 30 kg/m ²	9 (47)	127/752 (17)	0.002
Either diabetes or prediabetes, <i>n</i>	9 (47)	259 (34)	0.226
Cirrhosis, <i>n</i>	18 (95)	266 (35)	< 0.001
HCV-3 Genotype, <i>n</i>	2 (11)	100 (13)	1.000
HCV RNA, UI/mL (× 1000)	895 (255-1700)	1001 (276-2281)	0.763
> 4000	2 (11)	89 (12)	1.000
Coinfected, <i>n</i>	0 (0)	56 (7)	0.389
Treatment history, <i>n</i>			
Naïve	6 (32)	524 (68)	
Experienced, IFN and/or DAA	13 (68)	246 (32)	0.002
SVR, <i>n</i>	13 (68)	757 (98)	< 0.001

^ABody mass index missing in *n* = 18/790 (2%) patients. Continuous variables are presented as medians (interquartile range), categorical variables as frequencies (*n*) and percentages (%). HCV: hepatitis C virus; IFN: interferon-based regimens; DAA: direct antiviral agents; SVR: sustained viral response

Table 3. Multivariate analysis of factors associated with de novo HCC development at the end of follow-up

Variable	Odds ratio	95%CI	<i>P</i>
Age	1.03	0.99-1.08	0.131
Male sex	4.45	1.14-17.3	0.031
Obesity	4.68	1.55-14.1	0.006
Cirrhosis	21.1	2.68-166.1	0.004
Treatment-experienced	1.61	0.52-5.02	0.410
Failure to achieve SVR	24.2	5.76-101.8	< 0.001

All dependent variables were categorical except age (*n* = 771; pseudo R² = 0.350). Codes: male sex = 1, female sex = 0; obese = 1, not obese = 0; cirrhosis = 1, not cirrhosis = 0; treatment experienced (either interferon-based or DAA regimens): no = 0, yes = 1; failure to achieve SVR: SVR achieved = 0, SVR not achieved = 1. SVR: sustained viral response; DAA: direct antiviral agents; HCC: hepatocellular carcinoma

Development of *de novo* HCC

Along a median follow-up of 9.3 (interquartile range, 8.8-11.9) months, *n* = 19/789 (2.4%) patients were discovered to harbor HCC. The diagnosis was based on radiological criteria in 18/19 of patients (95%). Table 2 presents the main characteristics of these patients in comparison to all other patients.

Among patients who developed *de novo* HCC after antiviral therapy, 15/19 (79%) had either one or two nodules at the diagnosis, 3/19 (16%) had three or more nodules, and one patient had a diffuse infiltrative pattern (5%). Moreover, 7/19 (37%) had portal vein thrombosis (including complete or partial and segmental or sub-segmental thrombosis). Twelve patients (63%) fulfilled Milan Criteria^[14].

Based on multivariate analysis, conducted having *de novo* HCC as dependent variable and age, male sex, obesity, cirrhosis, previous treatment history, and SVR as independent variables, the only independent predictors were male sex, obesity, cirrhosis, and SVR. The logistic regression model is summarized in Table 3.

DISCUSSION

The present study documented that, in the experience of a single center, the strongest predictor of HCC development following treatment of HCV infection was the lack of achieving SVR; other important pre-treatment factors were presence of cirrhosis, male gender, and obesity. These data confirm findings in other clinical and experimental studies, but they also have some novel practical implications that, in our opinion, may be worth considering.

It is well known that male gender represents a risk factor to develop HCC^[15], although the reasons for the strong gender difference in HCC remain unclear. In the Italian population, the male to female ratio of HCC from any cause is 2.2 to 1, similar to what is observed in other western countries. In our cohort, the male to female ratio was higher, 5.3 to 1, possibly reflecting in part the age-specific sex difference in the incidence of HCC, which peaks at a slightly younger age than the one we observed in our study population^[16]. The highest incidence of HCC in men could be related to the higher prevalence of cirrhosis in males due to more rapid disease progression before age 50 years. In fact, women during their reproductive years have a better control of HCV replication, possibly due to estrogens, and this fact leads to less necroinflammatory response and less fibrosis progression^[17]. Others have suggested that estrogens have a direct putative antifibrogenic activity, or an interference with metabolic parameters and oxidative stress^[18-20]. Finally, higher, genetically determined expression of interleukin-6 in males may also be a factor^[21].

Most experts would agree that cirrhosis of any etiology is the strongest predictor of HCC. In fact, cirrhosis can be considered a premalignant condition, independently from the underlying liver disease^[22-24]. It is worth mentioning that we staged liver disease mainly by transient elastography; while this is consistent with what is recommended by current European guidelines on hepatitis C^[1], consideration must be given to the fact that the performance of this test may be suboptimal in obese patients. Hepatocarcinogenesis represents a multistep process, leading to chronic liver damage through persistent inflammatory damage that promotes malignant transformation^[25-27]. The annual risk of HCC is as high as 3% in patients with cirrhosis and active HCV infection^[28]. Viral hepatocarcinogenesis can be due to direct or indirect mechanisms, and is affected by host and environmental factors, such as alcohol intake, smoking, and HBV or HIV co-infections, which also increase the risk of cirrhosis. Indeed, although the estimated risk of HCC is increased 15-20-fold among persons infected with HCV in comparison to those who are not infected, most of the excess risk is limited to those with advanced hepatic fibrosis or cirrhosis^[29].

In the present study, obesity was a major independent predictor of HCC. This observation is fully consistent with current literature that suggests the existence of a vicious circle linking cirrhosis/fibrosis, HCV infection, and lipid metabolism derangement. In the obese, the inactivation of negative regulators of STAT-1 and STAT-3 signaling drives the development of non-alcoholic steatohepatitis and HCC, not only in cirrhotic patients, but also in patients with chronic hepatitis^[30,31]. Moreover, there is evidence that HCV-infected patients are prone to develop features of metabolic syndrome (MetS), probably due to the fact that the replication cycle of HCV depends heavily on the pathways of lipid metabolism in hepatocytes and considerably alters host lipid hemostasis^[32,33]. Interestingly, two large population cohort studies from Taiwan showed that HCV infection was strongly associated with MetS. The prevalence of MetS in these patients ranged from 13% to 32%; they had an aggressive and severe liver disease, developing more severe fibrosis than those without MetS, which contributed to cancer development^[34-36]. However, MetS did not affect SVR achievement after DAA^[37].

The major novelty of the present study lies in the strong association observed between lack of SVR and identification of HCC soon after concluding DAA. Overall, the rates of SVR and virologic failure in our cohort (97.6% and 1.8%, respectively) were comparable with what has been observed in registration trials^[38-40] and in real-life cohorts^[25,41,42]. While some authors affirmed in several retrospective studies that DAA increased the rate of early recurrence/occurrence of HCC, the short follow-up, the small number of patients, and the study design did not allow definite conclusions^[3,43,44]. In contrast, multiple large cohort studies and meta-analyses have since demonstrated that DAA-induced SVR is associated with reduced risk of HCC occurrence^[45-49].

One possible explanation for the low SVR in those who develop HCC is that it derives from the sum of risk factors for HCC, such as older age, high alcohol intake, more severe fibrosis, and co-infections^[50-53]. Although the difference did not reach statistical significance, we did observe a numerically lower SVR rate

in cirrhotic vs. non-cirrhotic patients, which - combined with the low number of cirrhotic patients who develop HCC - may create a bias. However, the results logistic regression analysis strongly support the independence of these two variables in predicting the development of HCC (it should also be noted that the test is not designed to compare the relative strength of each variable in the model).

A different way to reconcile these findings is to hypothesize that the apparent increase of HCC incidence/recurrence rates might be due to the difficulties of identifying small HCC foci by current screening methods. Not surprisingly, compared to explant pathology, ultrasound is insufficiently sensitive in detecting HCC in obese patients^[54] and obesity hampers the quality of HCC surveillance^[55-57]. Thus, one may speculate that, especially in obese patients, what is observed as *de novo* HCC is in fact missed HCC, hence the low SVR rate among those who “develop” HCC in our cohort. Most of the DAA failures in patients with previous HCC diagnosis occurred among patients with active cancers, where DAA failed in almost half of the cases^[9], possibly because HCC may serve as a sanctuary for HCV. In agreement with these findings, in a preliminary report from our group, we did observe unusually low SVR rates among *de novo* and recurrent HCC cases, leading us to suggest that treatment failure should be considered a clue of a yet undetected HCC^[58,59].

We must acknowledge several limitations of our work. First, it is a single-center study, with a short follow-up, during which - luckily enough - only a relatively small number of patients went on to develop HCC. Being a retrospective analysis of data generated in clinical practice, we screened our patients before and after DAA treatment with ultrasound, thus we are unable to provide pre-treatment data on higher level dynamic imaging (CT or MRI), which was performed only in the presence of suspicious focal liver lesions. Finally, we do not have reliable data about current and past alcohol intake in our study population, which are traditionally quite difficult to obtain. Nevertheless, at least in our opinion, the study conveys two messages worth considering: (1) given the extremely high SVR rates obtainable today in all subgroups of HCV infected patients, when DAA treatment fails, the possibility that the patient harbors HCC should come to mind; and (2) in male, obese, cirrhotic HCV-infected patients, a second level imaging technique should confirm that they are free of HCC before starting a DAA regimen.

In conclusion, the present study indicates virologic failure as a strong independent predictor for *de novo* HCC identification early after treatment of hepatitis C with DAA. Clearly, all patients with cirrhosis regardless of SVR response should be monitored at regular six-month intervals, since cirrhosis - either in the presence or in the absence of HCV - is the dominant risk factor for HCC. However, lack of achieving SVR should further alert clinicians to the possibility of this dreadful complication, especially among HCV carriers who are male, obese, and cirrhotic.

DECLARATIONS

Authors' contributions

Conceptualization, data curation, formal analysis, supervision, investigation, validation, visualization, writing - original draft, writing - review & editing: Burlone ME

Conceptualization, data curation, formal analysis, supervision, validation, visualization, writing -review & editing: Fangazio S

Data curation, investigation, validation, visualization: Croce A, Ceriani E, Rapetti R, Rigamonti C, Smirne C

Data curation, investigation, supervision, validation, visualization: Tonello S

Supervision, validation, visualization: Ravanini P

Data curation, investigation, supervision, validation, visualization: Minisini R

Conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, visualization, writing - review & editing: Pirisi M

Availability of data and materials

Raw data are available upon request.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Being a retrospective analysis of anonymized data of patients treated in standard clinical practice, no ethical committee approval was required. However, all patients gave written informed consent for their participation to the study, which was conducted in strict accordance to the Principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

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REFERENCES

1. Pawlowsky JM, Negro F, Aghemo A, Berenguer M, Dalgard O, et al. EASL recommendations on treatment of hepatitis C 2018. *J Hepatol* 2018;69:461-511.
2. World Health Organization. Global hepatitis report, 2017. 2017.
3. Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016;65:719-26.
4. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayho MA, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level results from the global burden of disease study 2015. *JAMA Oncol* 2017;3:1683-91.
5. Mashiba T, Joko K, Kurosaki M, Ochi H, Osaki Y, et al. Does interferon-free direct-acting antiviral therapy for hepatitis c after curative treatment for hepatocellular carcinoma lead to unexpected recurrences of hcc? A multicenter study by the Japanese red cross hospital liver study group. *PLoS One* 2018;13:e0194704.
6. Nagata H, Nakagawa M, Asahina Y, Sato A, Asano Y, et al. Effect of interferon-based and -free therapy on early occurrence and recurrence of hepatocellular carcinoma in chronic hepatitis C. *J Hepatol* 2017;67:933-9.
7. Virlogeux V, Pradat P, Hartig-Lavie K, Bailly F, Maynard M, et al. Direct-acting antiviral therapy decreases hepatocellular carcinoma recurrence rate in cirrhotic patients with chronic hepatitis C. *Liver Int* 2017;37:1122-7.
8. Su F, Ioannou GN. Hepatocellular carcinoma risk after direct-acting antiviral therapy. *Clin Liver Dis* 2019;13:6-12.
9. Prensner SB, VanWagner LB, Flamm SL, Salem R, Lewandowski RJ, et al. Hepatocellular carcinoma decreases the chance of successful hepatitis C virus therapy with direct-acting antivirals. *J Hepatol* 2017;66:1173-81.
10. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. *J Hepatol* 2017;66:153-94.
11. Bedossa P. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994;20:15-20.
12. Castéra L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008;48:835-47.
13. Galle PR, Forner A, Llovet JM, Mazzaferro V, Piscaglia F, et al. EASL clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236.
14. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996;334:693-700.
15. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. Global cancer statistics. *CA Cancer J Clin* 2011;61:69-90.
16. Liu P, Xie SH, Hu S, Cheng X, Gao T, et al. Age-specific sex difference in the incidence of hepatocellular carcinoma in the United States. *Oncotarget* 2017;8:68131-7.
17. Burlone ME, Pedrinelli AR, Giarda P, Minisini R, Pirisi M. Influence of age on sex-related differences among patients with hepatitis C. *Eur J Gastroenterol Hepatol* 2016;28:1100-1.
18. Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* 2010;138:513-21.
19. Nasta P. "Immune activation, aging and gender" and progression of liver disease. *Acta Biomed* 2011;82:115-23.

20. Prieto J. Inflammation, HCC and sex: IL-6 in the centre of the triangle. *J Hepatol* 2008;48:380-1.
21. Cussigh A, Falletti E, Fabris C, Bitetto D, Cmet S, et al. Interleukin 6 promoter polymorphisms influence the outcome of chronic hepatitis C. *Immunogenetics* 2011;63:33-41.
22. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004;127 (5 Suppl 1):S35-50.
23. Simonetti RG, Cammà C, Fiorello F, Politi F, D'Amico G, et al. Hepatocellular carcinoma - a worldwide problem and the major risk factors. *Dig Dis Sci* 1991;36:962-72.
24. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993;328:1797-1801.
25. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level. *JAMA Oncol* 2017;3:1683-91.
26. Degos F, Christidis C, Ganne-Carrie N, Farmachidi JP, Degott C, et al. Hepatitis C virus related cirrhosis: time to occurrence of hepatocellular carcinoma and death. *Gut* 2000;47:131-6.
27. West J, Card TR, Aithal GP, Fleming KM. Risk of hepatocellular carcinoma among individuals with different aetiologies of cirrhosis: a population-based cohort study. *Aliment Pharmacol Ther* 2017;45:983-90.
28. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011;365:1118-27.
29. Fabris C, Smirne C, Toniutto P, Colletta C, Rapetti R, et al. Assessment of liver fibrosis progression in patients with chronic hepatitis C and normal alanine aminotransferase values: the role of AST to the platelet ratio index. *Clin Biochem* 2006;39:339-43.
30. Alexander J, Torbenson M, Wu TT, Yeh MM. Non-alcoholic fatty liver disease contributes to hepatocarcinogenesis in non-cirrhotic liver: a clinical and pathological study. *J Gastroenterol Hepatol* 2013;28:848-54.
31. Grohmann M, Wiede F, Dodd GT, Gurusoz EN, Ooi GJ, et al. Obesity drives STAT-1-dependent NASH and STAT-3-dependent HCC. *Cell* 2018;175:1289-306.
32. Burlone ME, Budkowska A. Hepatitis C virus cell entry: role of lipoproteins and cellular receptors. *J Gen Virol* 2009;90:1055-70.
33. Schaefer EA, Chung RT. HCV and host lipids: an intimate connection. *Semin Liver Dis* 2013;33:358-68.
34. Kuo YH, Kee KM, Wang JH, Hsu NT, Hsiao CC, et al. Association between chronic viral hepatitis and metabolic syndrome in southern Taiwan: a large population-based study. *Aliment Pharmacol Ther* 2018;48:993-1002.
35. Perz JF, Armstrong GL, Farrington LA, Hutin YJF, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006;45:529-38.
36. Yoon H, Lee JG, Yoo JH, Son MS, Kim DY, et al. Effects of metabolic syndrome on fibrosis in chronic viral hepatitis. *Gut Liver* 2013;7:469-74.
37. Dong TS, Aby ES, Benhammou JN, Kawamoto J, Han SH, et al. Metabolic syndrome does not affect sustained virologic response of direct-acting antivirals while hepatitis C clearance improves hemoglobin A1c. *World J Hepatol* 2018;10:612-21.
38. Dore GJ, Conway B, Luo Y, Janczewska E, Knysz B, et al. Efficacy and safety of ombitasvir/paritaprevir/r and dasabuvir compared to IFN-containing regimens in genotype 1 HCV patients: the MALACHITE-I/II trials. *J Hepatol* 2016;64:19-28.
39. Zeuzem S, Foster GR, Wang S, Asatryan A, Gane E, et al. Glecaprevir-pibrentasvir for 8 or 12 weeks in HCV genotype 1 or 3 infection. *N Engl J Med* 2018;378:354-69.
40. Zeuzem S, Ghalib R, Reddy KR, Pockros PJ, Ari ZB, et al. Grazoprevir-Elbasvir combination therapy for treatment-naïve cirrhotic and noncirrhotic patients with chronic hepatitis C virus genotype 1, 4, or 6 infection: a randomized trial. *Ann Int Med* 2015;163:1-13.
41. Buggisch P, Vermehren J, Mauss S, Günther R, Schott E, et al. Real-world effectiveness of 8-week treatment with ledipasvir/sofosbuvir in chronic hepatitis C. *J Hepatol* 2018;68:663-71.
42. Londoño MC, Garcia-Buey L, Mallolas J, Barril G, Ahumada A, et al. Effectiveness, safety/tolerability of OBV/PTV/r ± DSV in patients with HCV genotype 1 or 4 with/without HIV-1 co-infection, chronic kidney disease (CKD) stage IIIb/V and dialysis in Spanish clinical practice - preliminary data Vie-KinD study. *J Hepatol* 2017;66:S718-9.
43. Cardoso H, Vale AM, Rodrigues S, Gonçalves R, Albuquerque A, et al. High incidence of hepatocellular carcinoma following successful interferon-free antiviral therapy for hepatitis C associated cirrhosis. *J Hepatol* 2016;65:1070-1.
44. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016;65:727-33.
45. ANRS collaborative study group on hepatocellular carcinoma. Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: data from three ANRS cohorts. *J Hepatol* 2016;65:734-40.
46. Baumert TF, Berg T, Lim JK, Nelson DR. Status of direct-acting antiviral therapy for hepatitis C virus infection and remaining challenges. *Gastroenterology* 2019;156:431-45.
47. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol* 2018.
48. Li DK, Ren Y, Fierer DS, Rutledge S, Shaikh OS, et al. The short-term incidence of hepatocellular carcinoma is not increased after hepatitis C treatment with direct-acting antivirals: an ERCHIVES study. *Hepatology* 2018;67:2244-53.
49. Waziry R, Hajarizadeh B, Grebely J, Amin J, Law M, et al. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: a systematic review, meta-analyses, and meta-regression. *J Hepatol* 2017;67:1204-12.
50. Bagella P, Fiore V, Caruana G, Madeddu G. Editorial - non AIDS-defining malignancies: a new epidemic in HIV-infected population for the upcoming decades? European review for medical and pharmacological sciences 2017. *Eur Rev Med Pharmacol Sci* 2017;21:4744-5.
51. Di Benedetto F, Tarantino G, Ercolani G, Baccarani U, Montalti R, et al. Multicenter Italian experience in liver transplantation for

- hepatocellular carcinoma in HIV-infected patients. *Oncologist* 2013;18:592-9.
52. Yang JD, Altekruse SF, Nguyen MH, Gores GJ, Roberts LR. Impact of country of birth on age at the time of diagnosis of hepatocellular carcinoma in the United States. *Cancer* 2017;123:81-9.
 53. Yang JD, Mohamed HA, Cvinar JL, Gores GJ, Roberts LR, et al. Diabetes mellitus heightens the risk of hepatocellular carcinoma except in patients with hepatitis C cirrhosis. *Am J Gastroenterol* 2016;111:1573-80.
 54. Esfeh JM, Hajifathalian K, Ansari-Gilani K. Sensitivity of ultrasound in detecting hepatocellular carcinoma in obese patients compared to explant pathology as the gold standard. *Clin Mol Hepatol* 2019;26:54-9.
 55. Bolondi L. Screening for hepatocellular carcinoma in cirrhosis. *J Hepatol* 2003;39:1076-84.
 56. Simmons O, Fetzter DT, Yokoo T, Marrero JA, Yopp A, et al. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. *Aliment Pharmacol Ther* 2017;45:169-77.
 57. Singal A, Volk ML, Waljee A, Salgia R, Higgins P, et al. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009;30:37-47.
 58. Fangazio S, Camatta D, Tran Minh M, Ceriani E, Minisini R, et al. Treatment failure after interferon-free treatment of hepatitis C as a clue of a yet undetected hepatocellular carcinoma. *J Hepatol* 2017.
 59. Yen YH, Chen CH, Hung CH, Wang JH, Lu SN, et al. Active hepatocellular carcinoma is an independent risk factor of direct-acting antiviral treatment failure: a retrospective study with prospectively collected data. *PLoS One* 2019;14:e0222605.

Review

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Frailty and Liver resection: where do we stand?

Georgios S. Sioutas^{1, #}, Ioannis A. Ziogas^{2, #}, Georgios Tsoulfas²

¹Department of Medicine, School of Health Sciences, Democritus University of Thrace, Alexandroupolis 68100, Greece.

²First Department of Surgery, Papageorgiou University Hospital, Aristotle University of Thessaloniki, Thessaloniki 54622, Greece.

[#]Authors contributed equally.

Correspondence to: Dr. Georgios Tsoulfas, FICS, FACS, Associate Professor of Surgery, First Department of Surgery, Papageorgiou University Hospital, Aristotle University of Thessaloniki, 66 Tsimiski Street, Thessaloniki 54622, Greece. E-mail: tsoulfasg@gmail.com

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Abstract

As the world population is continuously aging, the number of older patients requiring liver surgery is also on the rise. Data have shown that age should not be a limiting factor for liver resection, as it cannot accurately predict postoperative outcomes. Instead, frailty can serve as a more reliable measure of the patient's overall health and functional reserves. Several frailty assessment tools have been implemented for preoperative risk stratification before liver surgery, and higher scores have commonly been associated with postoperative morbidity, mortality, and length of hospital stay. However, no consensus has been reached on the most useful screening tool. Future studies should focus on comparing the currently available assessment tools, constructing a liver resection-specific tool, and assessing the role of frailty assessment tools in preoperative patient optimization.

Keywords: Frailty, age, elderly, liver resection, liver surgery, morbidity, morbidity, complications

INTRODUCTION

Liver resection is the current standard of care for most patients with benign or malignant liver lesions and adequate liver function^[1,2]. Advances in healthcare have led to a continually increasing life-expectancy, which consequently leads to a higher number of elderly patients (> 60 years) being offered liver surgery^[2,3]. Several studies sought to compare liver resection in younger vs. older surgical candidates and reported varying yet acceptable outcomes in appropriately selected older individuals^[2,4-8]. In fact, morbidity and mortality rates in patients undergoing liver resection for hepatocellular carcinoma (HCC) seem to range



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9%-51% and 0%-42.9%, respectively^[9]. Normal aging is associated with a gradual decline in the function of most organ systems, including the liver. The liver synthetic and metabolic activity, liver volume, and blood flow to the liver seem to be significantly affected in the elderly^[10]. It is well known that the liver remnant regenerates after liver resection, and the final liver volume after regeneration does not seem to differ between younger and older individuals^[9,11]. However, this process might be delayed in the elderly due to the liver's decreased proliferative capacity in the early period after the loss of the liver mass^[12]. Data have shown that liver regeneration after living donor liver transplantation can be delayed in older donors when compared to younger donors^[13]. These findings indicate that physiological deconditioning and remaining organ function might have a more significant effect on clinical outcomes than the actual chronological age^[14,15].

Frailty syndrome is defined as the increased vulnerability to stressors, loss of ability to adapt, and diminished resiliency secondary to an age-related decline in the physiological reserves and function of multiple organ systems^[16,17]. It is essential to distinguish frailty from "comorbidity" and "disability"; although these three terms overlap to some extent and are often used interchangeably to predict patient outcomes, they represent entirely different entities^[18]. As described by Feinstein, "comorbidity" is "any distinct additional entity that has existed or may occur during the clinical course of a patient who has the index disease under study"^[19]. The term "disability" refers to the abnormal biological functioning or the defect that renders individuals inferior to the "normal" species around them leading to loss of social stability and survival^[20]. Frailty should also not be considered synonymous to aging^[21], but rather an intermediate clinical state between normal and pathological aging^[22]. Frail patients commonly fail to return to their prior homeostasis after a stressor, resulting in adverse clinical outcomes^[23,24]. Therefore, the need for developing accurate risk-stratification tools that can potentially identify older patients at risk for postoperative complications is apparent^[25].

The aim of the present review is to summarize the impact of age on patient outcomes after liver surgery, describe the available frailty assessment tools, and discuss the impact of frailty on postoperative outcomes in patients undergoing liver resection.

LIVER RESECTION AND AGE

Several studies sought to investigate the outcomes of liver resection in young vs. old patients. Fong *et al.*^[26] published one of the first studies examining the effect of age on liver surgery. Their study included 133 patients older than 65 years undergoing liver resection for colorectal liver metastases, and they found that age was an independent risk factor for increased risk of morbidity. According to the authors, major hepatic resection may be safely performed and result in favorable functional outcomes on appropriately selected older patients^[26]. Cho *et al.*^[2] investigated the safety of liver resection in the elderly and reported favorable outcomes in patients ≥ 70 years. Although most elderly patients were transferred to rehabilitation facilities postoperatively, there was no difference in terms of severe postoperative complications. The authors also performed a literature review and included 14 previous studies; only two (14.3%)^[27,28] of them reported a statistically significant difference in severe postoperative complications and only two (14.3%)^[28,29] reported a statistically significant difference in mortality between old and young patients. Additionally, a large single-center study from France showed that age ≥ 75 years is a risk factor of mortality after liver resection^[30], while a multicenter study from the US showed that increasing age is associated with increased postoperative sepsis and overall mortality, but not overall morbidity^[31].

As liver resection represents the mainstay of treatment in non-metastatic HCC^[32-34], several studies aimed to investigate the difference in outcomes between young and old HCC patients. Therefore, data have proven the safety and feasibility of liver resection in appropriately selected patients aged not only more than 70 years, but, in some cases, even more than 80 years^[35-38]. A meta-analysis^[39] reported that the morbidity and mortality

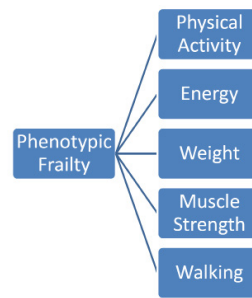


Figure 1. “Physical Frailty Phenotype” model by Fried *et al.*^[14]

rates did not differ significantly between older and younger patients undergoing hepatic resection for HCC, while the risk of mortality for younger patients was 2.7 times lower when compared to the elderly for colorectal liver metastases^[39]. Interestingly, another meta-analysis reported that hepatic resection for liver malignancies is associated with a higher risk of postoperative renal failure, infection, and mortality in older vs. younger patients, while the length of stay (LOS) in the hospital, transfusions, and disease-free survival did not differ significantly between the two groups^[40]. Nevertheless, the considerable variability in patient outcomes after liver resection in the elderly underlines the inability of age alone to predict postoperative outcomes accurately. Instead, factors that reflect the overall health status of the patient, such as frailty, may serve as more accurate predictors of postoperative outcomes. On that grounds, several frailty assessment tools have been developed in order to preoperatively determine patients at risk of adverse postoperative outcomes.

FRAILITY ASSESSMENT TOOLS

Numerous frailty screening tools have been described over the years^[17,18,41]. The most commonly implemented one is the “Physical Frailty Phenotype” model by Fried *et al.*^[14], which describes frailty as the decrease in physiological reserve secondary to a multisystem functional decline. This tool assesses the following criteria to identify frail patients: (1) walking speed; (2) grip strength; (3) weight loss; (4) physical activity; and (5) exhaustion [Figure 1]. Patients meeting one or two of these criteria are deemed “pre-frail”, while those meeting at least three criteria are categorized as frail^[14]. Makary *et al.*^[42] further validated this definition, and at the same time defined as “pre-frail” those fulfilling two or three of the above-mentioned criteria. The Phenotypic frailty tool requires only a questionnaire, a stopwatch, and a dynamometer, and thus can be completed in only 10-15 min^[17]. It is also recognized by the American College of Surgeons and the American Geriatric Society for the assessment of the elderly preoperatively^[43]. Nevertheless, the inherent drawback of this assessment method is the lack of psychosocial evaluation of the older patient^[44].

The second most commonly used frailty assessment tool is the “Deficit Accumulation Index” by Rockwood *et al.*^[15] [Figure 2]. It defines frailty using a frailty index (FI) with the number of deficits or abnormal characteristics accumulated over several areas (i.e., physical, social, functional, and cognitive) on the numerator and the total number of characteristics assessed on the denominator^[15,45,46]. Higher index values have been associated with an increased likelihood of frailty, adverse patient outcomes, disability, hospitalization, and death^[45]. Although it is considered more sensitive than the Phenotypic frailty tool^[16], its downsides include the fact that it is time-consuming (up to 70 characteristics assessed sometimes) and its extensive focus on comorbidities (symptoms, diagnoses, abnormal values on laboratory tests, *etc.*) rather than on functional decline^[18]. With the aim to assess frailty in a timely fashion and in a more efficient way, several modified FIs (mFIs), which may measure as few as five factors, have been generated^[47]. In fact, 11-point mFIs have already been used to evaluate patients undergoing liver resection^[48,49].

Comprehensive geriatric assessment (CGA) is another well-established approach implemented to evaluate frailty in older patients^[50,51]. It utilizes assessment tools and laboratory values to assess patients from several

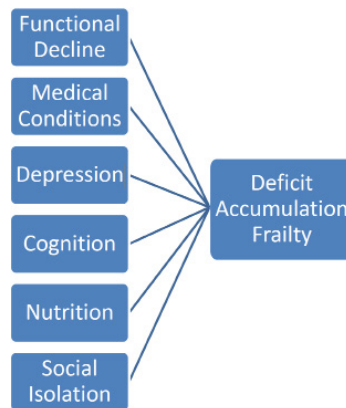


Figure 2. “Deficit Accumulation Index” by Rockwood *et al.* [15]

standpoints, including physical medical comorbidities, nutritional status, mental and cognitive status, physical functioning and fitness, social networking, and environmental status^[50,51]. A downside that CGA shares with FI is that it is time-consuming, mostly due to its complexity^[51]. Two screening tools that can help identify elderly patients who will benefit from a more comprehensive assessment with the CGA are the Geriatric-8 (G8) and the Vulnerable Elders Survey-13 (VES-13)^[52]. Those take into consideration age, self-rated health, and physical function along with other factors^[53], and both of them take only 5 min to complete^[52]. The first one is specifically designed to identify vulnerable older patients with cancer, while the latter is designed to detect vulnerable older patients in the community. Hence, G8 was found to be predictive of postoperative complications in elderly patients with HCC, while VES-13 was not shown to have a predictive role^[53]. The abbreviated CGA is another screening tool for elderly cancer patients that incorporates only the 15 most important items of the full assessment tool and requires only 5 min to complete^[54,55].

The FRAIL (Fatigue, Resistance, Ambulation, Illness, and weight Loss) index is another easy to complete screening tool that deems patients frail if three or more of its components are present^[56]. A great advantage of this scale is that it has been validated not only in the elderly but also in middle-aged individuals^[56,57]. On the other hand, the Edmonton Frail Scale (EFS) assesses the patients' health over ten frailty domains (including cognition, general health status, mood, continence, functional performance and independence, social support, polypharmacy, and nutrition)^[58]. A score below 5 indicates the “no frailty” patients, a score between 6 and 11 identifies the “apparently vulnerable” individuals, and a score between 12 and 17 distinguishes the “severe frailty” patients^[59]. Studies have shown that EFS is a valid and accurate tool that can be efficiently administered by non-geriatricians to assess and preoperatively optimize geriatric patients with or without cancer^[58,60,61]. The Clinical Frailty Scale (CFS) is another quick frailty assessment tool based on clinical judgment^[62]. Data suggest that it can accurately identify patients who may need institutional care, as well as those less likely to survive^[62]. Data have shown that CFS can reliably distinguish patients who are going to have a complicated course and prolonged LOS while in the acute medical ward^[63]. A screening tool for frailty broadly used in Japan is the Kihon Checklist (KCL)^[64]. KCL is a self-reporting “yes/no” survey that can help clinicians assess the status of older individuals in several frailty domains (functional, physical, psychological, and social)^[65].

Moreover, there are some single item tools that are less comprehensive but can serve as quick and easy to apply screening tools. The most common tool falling into this category is the handgrip test, which utilizes a hydraulic hand dynamometer to evaluate the maximum strength of the dominant hand^[52]. Its role in distinguishing “fit” from “frail” older individuals has been validated not only in the general population but also in cancer patients, as handgrip strength is highly associated with survival outcomes^[52,66]. Although the

Table 1. Frailty assessment tools

Tools	Ref.
Physical frailty phenotype	[14,42]
Deficit accumulation index	[15]
Comprehensive geriatric assessment	[50,51]
Abbreviated comprehensive geriatric assessment	[54,55]
Geriatric-8	[52,53]
Vulnerable elders survey-13	[52,53]
FRAIL index	[56]
Edmonton frail scale	[58,59]
Clinical frailty scale	[62]
Kihon checklist	[64,65]
The handgrip test	[52]
The “Up & Go” test	[52,67]

FRAIL: fatigue, resistance, ambulation, illness, and weight loss

handgrip test can be applied in multiple settings, another tool particularly useful for hospitalized patients is the timed “Up & Go” test^[67]. This test requires the patient to stand up and walk three meters, then to turn, walk back and sit down and can accurately assess balance and functional mobility^[52]. Data suggest that it is particularly useful in identifying cancer patients at risk of postoperative complications^[68]. A comprehensive list of the various tools used for the assessment of frailty is shown in Table 1.

LIVER RESECTION AND FRAILTY

There is a growing body of evidence that frailty assessment tools are useful in identifying frail patients at higher risk of postoperative morbidity and mortality, as well as extended LOS in the hospital. In fact, Kaibori *et al.*^[53] evaluated the utilization of the G8 CGA tool in patients ≥ 70 years undergoing liver resection for HCC. Patients with a score lower than 14 demonstrated higher postoperative morbidity rate and extended LOS, but no difference in mortality when compared to patients with scores ≥ 14 ^[53]. Notably, on multivariate analysis, G8 score < 14 was significantly associated with postoperative morbidity, while age ≥ 77 years was not found to be a significant risk factor^[53]. It is worth mentioning that patients with HCC arising on a background of cirrhosis demonstrated a tendency towards inferior outcomes after liver resection^[53]; however, further research is warranted in order to deduce meaningful conclusions.

Louwens *et al.*^[48] investigated the impact of frailty, assessed by the 11-point mFI tool, on morbidity and mortality after open hepatectomy in 10,300 patients from the National Surgical Quality Improvement Project (NSQIP) database. As the mFI score increased, a statistically significant increase was associated with Clavien 4 complications, mortality, and extended LOS. Notably, this statistical significance was maintained in all types of hepatectomy (partial, right, left, and extended). Although this study highlighted the importance of mFI in preoperative planning and risk stratification, the authors stressed the need for simpler hepatectomy-specific frailty assessment tools^[48]. Another study utilizing NSQIP hepatectomy data described the revised FI (rFI) on a “training set” of patients and compared it with the 11-point mFI (“validation set”)^[49]. rFI incorporates several variables, such as preoperative serum albumin and hematocrit, American Society of Anesthesiologists score, BMI, the extent of liver resection, and underlying pathology. Higher rFI scores were significantly associated with postoperative complications, prolonged LOS, and mortality, while higher mFI scores were linked only to a higher risk of morbidity but neither mortality nor LOS^[49]. Chen *et al.*^[69] evaluated the use of a five-item mFI to assess the effect of frailty on outcomes in patients undergoing combined colorectal and liver resection for colorectal cancer and liver metastases. Patients with higher mFI scores exhibited a higher incidence of mortality, overall and severe morbidity, as well as prolonged LOS. On multivariate analysis, higher mFI scores were found to be independent risk factors for overall and severe morbidity, while age was not found to be a significant factor that affects morbidity.

Table 2. Cohort studies on the association between frailty and postoperative adverse outcomes after hepatic resection

Year	Authors	Country	Number of patients	Age (years)*	Frailty assessment tool	Diagnosis (%)			Morbidity	Mortality	LOS
						Primary cancer (HCC)	Metastatic disease	Other			
2016	Kalibori et al. ^[133]	Japan	71	78.3 ± 3.2	G8	100% (100%)	0%	0%	$P < 0.0001$	$P = 0.06$	$P = 0.01$
2016	Louwers et al. ^[68]	USA	10,300	58 ± 12.2	11-point mFI	18.1% (N/A)	45.9%	36%	$P < 0.001$	$P < 0.001$	$P < 0.001$
2017	Gani et al. ^[69]	USA	1900	60 ± 14.1	rFI	80.4%		19.6%	$P < 0.001$	$P = 0.048$	$P < 0.001$
2018	Chen et al. ^[69]	USA	814	59.3 ± 13.4	11-point mFI	79%		21%	$P = 0.02$	$P = 0.29$	$P = 0.08$
2019	Itoh et al. ^[70]	Japan	154	58.8 ± 12.3	5-point mFI	0% (0%)	100%	0%	$P < 0.001$	$P = 0.006$	$P = 0.007$
2018	Tanaka et al. ^[64]	Japan	217	64.6 ± 9.4	Gait speed	69.5% (61.7%)	24.7%	5.8%	$P = 0.002$	N/A	$P = 0.03$
2019	Okabe et al. ^[71]	Japan	143	74.7 ± 4.5	KCL	78.8% (69.1%)	20.3%	0.9%	$P = 0.18$	$P = 0.02$	$P = 0.11$
				75.4 ± 4	CFS	0% (0%)	100%	0%	$P = 0.02$	N/A	$P = 0.01$

*Values converted to mean and standard deviation based on Hojo et al.^[72]. CFS: clinical frailty scale; FI: frailty index; G8: geriatric-8; HCC: hepatocellular carcinoma; KCL: kinon checklist; LOS: length of stay; mFI: modified frailty index; N/A: not available; rFI: revised frailty index

Gait speed, which is a component of the Fried's Phenotype tool, has also been utilized to determine postoperative outcomes in patients undergoing liver resection at a single-center in Japan^[70]. The authors utilized receiver operating characteristic curves and determined that a cutoff of 1.1 m/s can accurately identify patients at risk of postoperative complications, based on multivariate analysis. Patients with a gait speed at or below 1.1 m/s had longer LOS and a higher rate of complications compared to individuals with a gait speed > 1.1 m/s, while both groups had a mortality rate of 0%. Although patients with complications were not significantly older than those without complications, individuals with a gait speed of ≤ 1.1 m/s were significantly older than those walking with a speed > 1.1 m/s. As age was not included in the multivariate model, meaningful conclusions cannot be easily deduced. On another note, low serum albumin (≤ 4.0 g/dL) was also found to be an independent risk factor for postoperative complications^[70]. Another Japanese study^[71] assessed the impact of frailty on morbidity after liver resection for colorectal liver metastases using the CFS (frailty: CFS > 4). Multivariate analysis showed that frailty is an independent risk factor for severe postoperative complications, while age was not found to be a significant risk factor. In addition, the incidence of severe postoperative complications, as well as LOS, were significantly higher in frail compared to non-frail patients. Nonetheless, mortality rates were 0% in both study groups. A prospective multicenter study from Japan sought to evaluate the impact of frailty, as measured by the KCL, on age-related morbidity after hepatic resection^[64]. According to this phenotypic FI, patients are deemed frail when KCL is 8 or higher. Based on their multivariate analysis, frailty was found to be an independent risk factor of age-related events after liver resection, including major cardiopulmonary complications, delirium requiring medical treatment, transfer to a rehabilitation facility, and dependency. However, the incidence of overall and major complications, 30-day mortality, and LOS did not differ between frail and non-frail patients, while the 90-day mortality rate was significantly higher in the frailty group^[64]. Details of the studies utilizing frailty assessment tools to evaluate postoperative outcomes after liver resection are summarized in Table 2^[72].

CONCLUSION

Overall, several studies have proven the feasibility and safety of liver resection in the elderly; however, data do not unanimously support the concept that chronological age constitutes a valid predictor of postoperative outcomes. Instead, frailty assessed by numerous tools seems to better predict postoperative

morbidity, mortality, and LOS after liver resection. However, deducing meaningful conclusions remains challenging due to the lack of consensus regarding frailty definition, assessment tools, and score cutoffs. Future studies should focus on performing between-tool comparisons, developing a hepatectomy-specific frailty assessment tool, and evaluating the role of frailty assessment tools in preoperative patient optimization.

DECLARATIONS

Authors' contributions

Study concept, data analysis and interpretation, critical revision of the manuscript, final approval of the manuscript: Ziogas IA, Sioutas GS, Tsoulfas G

Data acquisition, drafting of the manuscript: Sioutas GS, Ziogas IA

Availability of data and materials

Not applicable.

Financial support and sponsorship

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

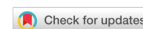
1. Tsukioka G, Kakizaki S, Sohara N, Sato K, Takagi H, et al. Hepatocellular carcinoma in extremely elderly patients: an analysis of clinical characteristics, prognosis and patient survival. *World J Gastroenterol* 2006;12:48-53.
2. Cho SW, Steel J, Tsung A, Marsh JW, Geller DA, et al. Safety of liver resection in the elderly: how important is age? *Ann Surg Oncol* 2011;18:1088-95.
3. Nanashima A, Abo T, Nonaka T, Fukuoka H, Hidaka S, et al. Prognosis of patients with hepatocellular carcinoma after hepatic resection: are elderly patients suitable for surgery? *J Surg Oncol* 2011;104:284-91.
4. Shirabe K, Kajiyama K, Harimoto N, Gion T, Tsujita E, et al. Early outcome following hepatic resection in patients older than 80 years of age. *World J Surg* 2009;33:1927-32.
5. Oishi K, Itamoto T, Kobayashi T, Oshita A, Amano H, et al. Hepatectomy for hepatocellular carcinoma in elderly patients aged 75 years or more. *J Gastrointest Surg* 2009;13:695-701.
6. Huang J, Li BK, Chen GH, Li JQ, Zhang YQ, et al. Long-term outcomes and prognostic factors of elderly patients with hepatocellular carcinoma undergoing hepatectomy. *J Gastrointest Surg* 2009;13:1627-35.
7. Lee CW, Chan KM, Tsai HI, Hsieh YC, Lin CY, et al. Hepatic resection for hepatocellular carcinoma in the octogenarian: is it justified? *Aging (Albany NY)* 2019;11:1537-50.
8. Wu FH, Shen CH, Luo SC, Hwang JI, Chao WS, et al. Liver resection for hepatocellular carcinoma in oldest old patients. *World J Surg Oncol* 2019;17:1.
9. Oishi K, Itamoto T, Kohashi T, Matsugu Y, Nakahara H, et al. Safety of hepatectomy for elderly patients with hepatocellular carcinoma. *World J Gastroenterol* 2014;20:15028-36.
10. Jansen PLM. Liver disease in the elderly. *Best Pract Res Clin Gastroenterol* 2002;16:149-58.
11. Michalopoulos GK. Liver regeneration. *J Cell Physiol* 2007;213:286-300.
12. Timchenko NA. Aging and liver regeneration. *Trends Endocrinol Metab* 2009;20:171-6.
13. Taki-Eldin A, Zhou L, Xie HY, Zheng SS. Liver regeneration after liver transplantation. *Eur Surg Res* 2012;48:139-53.
14. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci*

- Med Sci 2001;56:M146-56.
15. Rockwood K, Howlett SE, MacKnight C, Beattie BL, Bergman H, et al. Prevalence, attributes, and outcomes of fitness and frailty in community-dwelling older adults: report from the Canadian study of health and aging. *J Gerontol A Biol Sci Med Sci* 2004;59:1310-7.
 16. Chen X, Mao G, Leng SX. Frailty syndrome: an overview. *Clin Interv Aging* 2014;9:433-41.
 17. Robinson TN, Walston JD, Brummel NE, Deiner S, Brown CH, et al. Frailty for surgeons: review of a national institute on aging conference on frailty for specialists. *J Am Coll Surg* 2015;221:1083-92.
 18. Richards SJG, Frizelle FA, Geddes JA, Eglinton TW, Hampton MB. Frailty in surgical patients. *Int J Colorectal Dis* 2018;33:1657-66.
 19. Feinstein AR. The pre-therapeutic classification of co-morbidity in chronic disease. *J Chronic Dis* 1970;23:455-68.
 20. Francis L, Silvers A. Perspectives on the Meaning of "Disability". *AMA J Ethics* 2016;18:1025-33.
 21. Beggs T, Sepehri A, Szwajcer A, Tangri N, Arora RC. Frailty and perioperative outcomes: a narrative review. *Can J Anaesth* 2015;62:143-57.
 22. Fulop T, Larbi A, Witkowski JM, McElhaney J, Loeb M, et al. Aging, frailty and age-related diseases. *Biogerontology* 2010;11:547-63.
 23. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. *Lancet* 2013;381:752-62.
 24. Walston J. Frailty--the search for underlying causes. *Sci Aging Knowledge Environ* 2004;2004:pe4.
 25. Ko FC. Preoperative frailty evaluation: a promising risk-stratification tool in older adults undergoing general surgery. *Clin Ther* 2019;41:387-99.
 26. Fong Y, Cohen AM, Fortner JG, Enker WE, Turnbull AD, et al. Liver resection for colorectal metastases. *J Clin Oncol* 1997;15:938-46.
 27. Ferrero A, Viganò L, Polastri R, Ribero D, Tesoriere R Lo, et al. Hepatectomy as treatment of choice for hepatocellular carcinoma in elderly cirrhotic patients. *World J Surg* 2005;29:1101-5.
 28. Ijtsma AJC, Boevé LMS, van der Hilst CS, de Boer MT, de Jong KP, et al. The survival paradox of elderly patients after major liver resections. *J Gastrointest Surg* 2008;12:2196.
 29. Adam R, Frilling A, Elias D, Laurent C, Ramos E, et al. Liver resection of colorectal metastases in elderly patients. *Br J Surg* 2010;97:366-76.
 30. Sulpice L, Rayar M, Campillo B, Pery C, Guillaud A, et al. Advanced age remains an achilles heel for liver resections. *World J Surg* 2014;38:918-26.
 31. Reddy SK, Barbas AS, Turley RS, Gamblin TC, Geller DA, et al. Major liver resection in elderly patients: a multi-institutional analysis. *J Am Coll Surg* 2011;212:787-95.
 32. Ziogas IA, Tsoulfas G. Evolving role of Sorafenib in the management of hepatocellular carcinoma. *World J Clin Oncol* 2017;8:203-13.
 33. Brunot A, Le Sourd S, Pracht M, Edeline J. Hepatocellular carcinoma in elderly patients: challenges and solutions. *J Hepatocell Carcinoma* 2016;3:9-18.
 34. Ziogas IA, Tsoulfas G. Advances and challenges in laparoscopic surgery in the management of hepatocellular carcinoma. *World J Gastrointest Surg* 2017;9:233-45.
 35. Giovanardi F, Lai Q, Bertacco A, Vitale A. Resection for hepatocellular cancer: overpassing old barriers. *Transl Gastroenterol Hepatol* 2018;3:64.
 36. Kondo K, Chijiwa K, Funagayama M, Kai M, Otani K, et al. Hepatic resection is justified for elderly patients with hepatocellular carcinoma. *World J Surg* 2008;32:2223-9.
 37. Hamaoka M, Kobayashi T, Ishiyama K, Ohira M, Tahara H, et al. Evaluation of the risk factors and prognostic factors of hepatectomy for hepatocellular carcinoma in patients aged 80 years or more. *J Hepatobiliary Pancreat Sci* 2017;24:58-64.
 38. Portolani N, Baiocchi GL, Coniglio A, Tiberio GAM, Prestini K, et al. Limited liver resection: a good indication for the treatment of hepatocellular carcinoma in elderly patients. *Jpn J Clin Oncol* 2011;41:1358-65.
 39. Mizuguchi T, Kawamoto M, Meguro M, Okita K, Ota S, et al. Impact of aging on morbidity and mortality after liver resection: a systematic review and meta-analysis. *Surg Today* 2015;45:259-70.
 40. Phan K, An VVG, Ha H, Phan S, Lam V, et al. Hepatic resection for malignant liver tumours in the elderly: a systematic review and meta-analysis. *ANZ J Surg* 2015;85:815-22.
 41. Dent E, Kowal P, Hoogendijk EO. Frailty measurement in research and clinical practice: a review. *Eur J Intern Med* 2016;31:3-10.
 42. Makary MA, Segev DL, Pronovost PJ, Syin D, Bandeen-Roche K, et al. Frailty as a predictor of surgical outcomes in older patients. *J Am Coll Surg* 2010;210:901-8.
 43. Chow WB, Rosenthal RA, Merkow RP, Ko CY, Esnaola NF. Optimal preoperative assessment of the geriatric surgical patient: a best practices guideline from the American College of Surgeons National Surgical Quality Improvement Program and the American Geriatrics Society. *J Am Coll Surg* 2012;215:453-66.
 44. Hubbard R, Ng K. Australian and New Zealand Society for Geriatric Medicine: position statement - frailty in older people. *Australas J Ageing* 2015;34:68-73.
 45. Rockwood K, Mitnitski A. Frailty in relation to the accumulation of deficits. *J Gerontol A Biol Sci Med Sci* 2007;62:722-7.
 46. Rockwood K, Stolee P, McDowell I. Factors associated with institutionalization of older people in Canada: testing a multifactorial definition of frailty. *J Am Geriatr Soc* 1996;44:578-82.
 47. Subramaniam S, Aalberg JJ, Soriano RP, Divino CM. New 5-factor modified frailty index using American College of Surgeons NSQIP Data. *J Am Coll Surg* 2018;226:173-81.e8.
 48. Louwers L, Schnickel G, Rubinfeld I. Use of a simplified frailty index to predict Clavien 4 complications and mortality after hepatectomy: analysis of the National Surgical Quality Improvement Project database. *Am J Surg* 2016;211:1071-6.
 49. Gani F, Cerullo M, Amini N, Buettner S, Margonis GA, et al. Frailty as a risk predictor of morbidity and mortality following liver surgery. *J Gastrointest Surg* 2017;21:822-30.
 50. Welsh TJ, Gordon AL, Gladman JR. Comprehensive geriatric assessment--a guide for the non-specialist. *Int J Clin Pract* 2014;68:290-3.
 51. Feng MA, McMillan DT, Crowell K, Muss H, Nielsen ME, et al. Geriatric assessment in surgical oncology: a systematic review. *J*

- Surg Res 2015;193:265-72.
52. Decoster L, Van Puyvelde K, Mohile S, Wedding U, Basso U, et al. Screening tools for multidimensional health problems warranting a geriatric assessment in older cancer patients: An update on SIOG recommendations. *Ann Oncol* 2015;26:288-300.
 53. Kaibori M, Ishizaki M, Matsui K, Iida H, Inoue K, et al. Geriatric assessment as a predictor of postoperative complications in elderly patients with hepatocellular carcinoma. *Langenbeck's Arch Surg* 2016;401:205-14.
 54. Overcash JA, Beckstead J, Extermann M, Cobb S. The abbreviated comprehensive geriatric assessment (aCGA): a retrospective analysis. *Crit Rev Oncol Hematol* 2005;54:129-36.
 55. Overcash JA, Beckstead J, Moody L, Extermann M, Cobb S. The abbreviated comprehensive geriatric assessment (aCGA) for use in the older cancer patient as a prescreen: scoring and interpretation. *Crit Rev Oncol Hematol* 2006;59:205-10.
 56. Morley JE, Malmstrom TK, Miller DK. A simple frailty questionnaire (FRAIL) predicts outcomes in middle aged African Americans. *J Nutr Health Aging* 2012;16:601-8.
 57. Woo J, Yu R, Wong M, Yeung F, Wong M, Lum C. Frailty screening in the community using the FRAIL scale. *J Am Med Dir Assoc* 2015;16:412-9.
 58. Rolfson DB, Majumdar SR, Tsuyuki RT, Tahir A, Rockwood K. Validity and reliability of the Edmonton Frail Scale. *Age Ageing* 2006;35:526-9.
 59. Perna S, Francis MD, Bologna C, Moncaglieri F, Riva A, et al. Performance of edmonton frail scale on frailty assessment: its association with multi-dimensional geriatric conditions assessed with specific screening tools. *BMC Geriatr* 2017;17:2.
 60. Meyers BM, Al-Shamsi HO, Rask S, Yelamanchili R, Phillips CM, et al. Utility of the edmonton frail scale in identifying frail elderly patients during treatment of colorectal cancer. *J Gastrointest Oncol* 2017;8:32-8.
 61. Partridge JSL, Harari D, Dhesis JK. Frailty in the older surgical patient: a review. *Age Ageing* 2012;41:142-7.
 62. Rockwood K, Song X, MacKnight C, Bergman H, Hogan DB, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ* 2005;173:489-95.
 63. Juma S, Taabazuing MM, Montero-Odasso M. Clinical frailty scale in an acute medicine unit: a simple tool that predicts length of stay. *Can Geriatr J* 2016;19:34-9.
 64. Tanaka S, Ueno M, Iida H, Kaibori M, Nomi T, et al. Preoperative assessment of frailty predicts age-related events after hepatic resection: a prospective multicenter study. *J Hepatobiliary Pancreat Sci* 2018;25:377-87.
 65. Satake S, Senda K, Hong YJ, Miura H, Endo H, et al. Validity of the kihon checklist for assessing frailty status. *Geriatr Gerontol Int* 2016;16:709-15.
 66. García-Peña C, García-Fabela LC, Gutiérrez-Robledo LM, García-González JJ, Arango-Lopera VE, Pérez-Zepeda MU. Handgrip strength predicts functional decline at discharge in hospitalized male elderly: a hospital cohort study. *PLoS One* 2013;8:e69849.
 67. Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. *J Am Geriatr Soc* 1991;39:142-8.
 68. Huisman MG, van Leeuwen BL, Ugolini G, Montroni I, Spiliotis J, et al. "Timed Up & Go": a screening tool for predicting 30-day morbidity in onco-geriatric surgical patients? A multicenter cohort study. *PLoS One* 2014;9:e86863.
 69. Chen SY, Stem M, Cerullo M, Gearhart SL, Safar B, et al. The effect of frailty index on early outcomes after combined colorectal and liver resections. *J Gastrointest Surg* 2018;22:640-9.
 70. Itoh S, Yoshizumi T, Sakata K, Motomura T, Mano Y, et al. Slow gait speed is a risk factor for complications after hepatic resection. *J Gastrointest Surg* 2019;23:1810-6.
 71. Okabe H, Hayashi H, Higashi T, Nitta H, Ikuta Y, et al. Frailty predicts severe postoperative complication after elective hepatic resection. *Gastrointest Tumors* 2019;6:28-35.
 72. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol* 2005;5:13.

Review

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The mechanism of dysbiosis in alcoholic liver disease leading to liver cancer

Nahum Méndez-Sánchez^{1,2}, Alejandro Valencia-Rodríguez¹, Alfonso Vera-Barajas¹, Ludovico Abenavoli³, Emidio Scarpellini⁴, Guadalupe Ponciano-Rodríguez⁵, David Q.-H. Wang⁶

¹Liver Research Unit, Medica Sur Clinic & Foundation, Mexico City 14050, Mexico.

²Faculty of Medicine, National Autonomous University of Mexico, Mexico City 04510, Mexico.

³Department of Health Sciences, University "Magna Graecia" Viale Europa, Catanzaro 88100, Italy.

⁴Clinical Nutrition Unit, and Internal Medicine Unit, "Madonna del Soccorso" General Hospital, Via Luciano Manara 7, San Benedetto del Tronto (AP) 63074, Italy.

⁵Public Health Department, Faculty of Medicine, National Autonomous University of Mexico, Mexico City 04510, Mexico.

⁶Department of Medicine and Genetics, Division of Gastroenterology and Liver Diseases, Marion Bessin Liver Research Center, Einstein-Mount Sinai Diabetes Research Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

Correspondence to: Prof. Nahum Méndez-Sánchez, FACG, AGAF, Liver Research Unit, Medica Sur Clinic and Foundation, National Autonomous University of Mexico, Puente de Piedra 150, Col. Toriello Guerra, México City 14050, México.
E-mail: nmendez@medicasur.org.mx; nah@unam.mx

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Abstract

Currently, alcoholic liver disease (ALD) is one of the most prevalent chronic liver diseases worldwide, representing one of the main etiologies of cirrhosis and hepatocellular carcinoma (HCC). Although we do not know the exact mechanisms by which only a selected group of patients with ALD progress to the final stage of HCC, the role of the gut microbiota within the progression to HCC has been intensively studied in recent years. To date, we know that alcohol-induced gut dysbiosis is an important feature of ALD with important repercussions on the severity of this disease. In essence, an increased metabolism of ethanol in the gut induced by an excessive alcohol consumption promotes gut dysfunction and bacterial overgrowth, setting a leaky gut. This causes the translocation of bacteria, endotoxins, and ethanol metabolites across the enterohepatic circulation reaching the liver, where the recognition of the pathogen-associated molecular patterns via specific Toll-like receptors of liver cells will induce the activation of the nuclear factor kappa-B pathway, which releases pro-inflammatory cytokines and chemokines. In addition, the mitogenic activity of hepatocytes will be promoted and cellular apoptosis will be inhibited, resulting in the development of HCC. In this context, it is not surprising that microbiota-regulating drugs have proven effectiveness in prolonging the overall survival of patients with HCC, making attractive the implementation of these drugs as co-adjuvant for HCC treatment.



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Keywords: Alcoholic liver disease, gut microbiota, dysbiosis, hepatocellular carcinoma

INTRODUCTION

The metabolic effects of alcohol in humans has been a topic of great interest for many years due to the important relationship between excessive alcohol consumption and disease even reaching to cancer development. In this context, The Global Burden of Disease Study 2015 reported the primary liver cancer incidence, mortality, and disability-adjusted life-years of 195 countries from 1990 to 2015. Surprisingly, the cases of incident liver cancer increased by 75% between 1990 and 2015. Alcohol-induced liver cancer globally accounted for 245,000 (30%) deaths with important variations between countries and sex. Only in 2015, alcohol caused 204,000 [95% uncertainty interval (UI), 177,000-240,000] liver cancer cases in men and 45,000 (95% UI, 38,000-54,000) cases among women. Eastern Europe was the geographical region which contributed with the most alcohol-induced liver cancer cases in the world, accounting for 53% of them^[1]. According to WHO statistics, alcohol is involved in more than 200 different diseases^[2]. Among them, gastrointestinal (GI) disorders (mainly cirrhosis) represent the third cause in mortality secondary to excessive alcohol consumption^[3]. Interestingly, the metabolism of alcohol goes beyond the liver; in recent years, the role of the gut-liver axis in the development and aggravation of alcoholic liver disease (ALD) has emerged as an important element to consider^[4,5]. The gut microbiota and a selected group of catalytic enzymes of the GI tract are key elements in ethanol metabolism and its passage to systemic circulation. Furthermore, evidence has shown carcinogenic effects of different alcohol and gut metabolites in ALD patients, bringing new perspectives in the development of hepatocellular carcinoma (HCC) in this group of subjects. For this reason, this review discusses in a systematic way the role of alcohol-induced dysbiosis in the development of ALD and its progression to HCC, starting with the different metabolic pathways of ethanol within the human body and its deregulation in chronic alcohol consumption. Then, the mechanisms of alcohol-induced dysbiosis with the consequent liver injury and hepatocarcinogenesis are addressed and finally the future perspectives of microbiota-regulating drugs as adjuvants for HCC treatment are assessed.

ALCOHOLIC LIVER DISEASE AND HCC

For the development of ALD, the fulfillment of two factors is generally necessary; one is an excessive alcohol consumption, defined as ingestion of > 20 g/day in females and > 30 g/day in males, and the second one is the chronicity of this consumption^[3]. On its own, ALD is one of the less frequent etiologies that progress to HCC^[6,7]; however, its high prevalence continues to position it as one of the most important chronic liver diseases (CLDs) for public health^[8]. Recently, our group of work conducted a study to determine the main etiologies of cirrhosis worldwide [Figure 1] finding interesting results among countries^[9].

In a healthy person, alcohol is metabolized to acetaldehyde mainly in the liver by the alcohol dehydrogenase (ADH) and the microsomal ethanol-oxidizing system (MEOS), and to a lesser extent it is also metabolized in the GI tract through ADH, MEOS, and the gut microbiota^[10]. Several factors predispose the development and progression of ALD to its final stage of HCC, the most important being genetic predisposition, age, female sex, pre-existing liver disease, and daily alcohol consumption^[5]. Similarly, the GI tract has its own factors that predispose the metabolism and systemic absorption of ethanol and therefore the severity of ALD. An example of this is the diminished enzymatic activity of ADH in the stomach commonly seen in young women, elderly, alcoholics, when fasting, and after treatment with H₂-receptor antagonists. Other situations that favor systemic absorption of ethanol are delayed gastric emptying, chronic atrophic gastritis, and gastric lesion associated with *Helicobacter pylori*^[10]. Nonetheless, in ALD, there is an increase in the metabolization of ethanol to acetaldehyde by the cytosolic enzyme

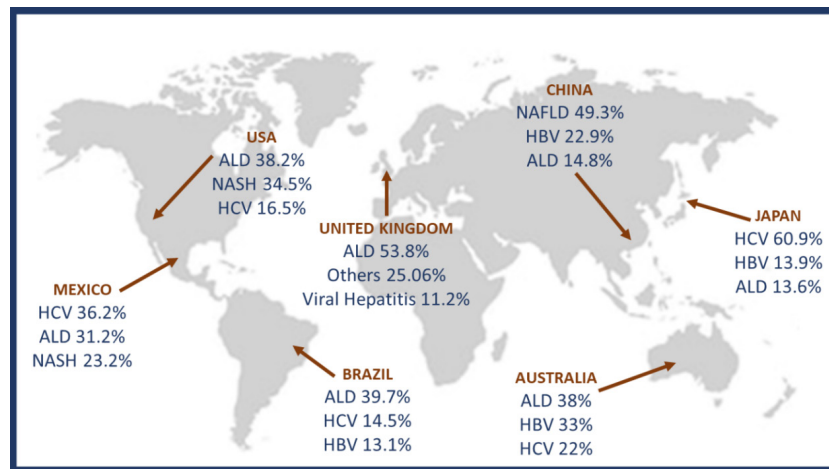


Figure 1. Worldwide prevalence of cirrhosis secondary to alcohol abuse compared with other cirrhosis etiologies. Modified from Méndez-Sánchez *et al.*^[9]. HCV: hepatitis C virus; ALD: alcoholic liver disease; NASH: non-alcoholic steatohepatitis; HBV: hepatitis B virus; NAFLD: non-alcoholic fatty liver disease

ADH and then from acetaldehyde to acetate by the mitochondrial enzyme aldehyde dehydrogenase^[11]. In the long run, this will generate mitochondrial dysfunction, which is considered a critical step for the onset and progression of ALD^[12]. Dysfunctional mitochondria can undergo a fragmentation pathway to further be cleared by autophagy or promote the apoptotic cascade in severe liver injury by a multi-step process called “mitochondrial dynamics” controlled by the activity of the mitochondria shaping proteins (MSP)^[13]. In a recent study, Palma *et al.*^[14] demonstrated that mitochondrial dynamics showed important changes in alcoholic steatohepatitis (ASH) patients by finding an increased expression of the MSP protein dynamin-related protein 1 (DRP1) compared with controls. They also found a direct correlation between DRP1 mRNA levels and blood concentration of aspartate aminotransferase in those patients. Interestingly, this was only seen in advanced ALD subjects, suggesting the study of mitochondrial deregulation in ALD progression is an important issue.

On the other hand, high alcohol consumption has been related with increased MEOS activity and its first constituent, the cytochrome P-450 2E1 (CYP2E1)^[15,16]. This has a great impact since, unlike the usual dehydrogenation process, the oxidation of ethanol by MEOS is carried out through several reactive intermediates known as reactive oxygen species (ROS) via CYP2E1^[17]. An increase in alcohol consumption upregulates the activity of intestinal MEOS, leading to an increase in ROS production, which interferes with the barrier function of the gut^[17].

MICROBIOTA AND ITS INTERACTION WITH THE INTESTINAL ENVIRONMENT

The GI tract is the natural habitat for several microorganisms, including bacteria, archaea, viruses, and parasites. In a healthy gut microenvironment, there is a predominant diversity of seven large groups: Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, and Cyanobacteria^[18]. The gut microbiome, which refers to the collective genomes of all the microorganisms that compose the gut microbiota, contains 150 times more genes than the human genome^[18]. In addition, gut bacteria has been appreciated for the benefits they can provide to the host (symbiosis) as they supply essential nutrients such as vitamins, metabolize non-digestible compounds, and even defend against pathogenic microorganisms^[19,20].

The colonization of the healthy gut environment contributes to the development of the intestinal architecture and the proper functioning of the immune system. Colon bacteria can ferment nutrients and

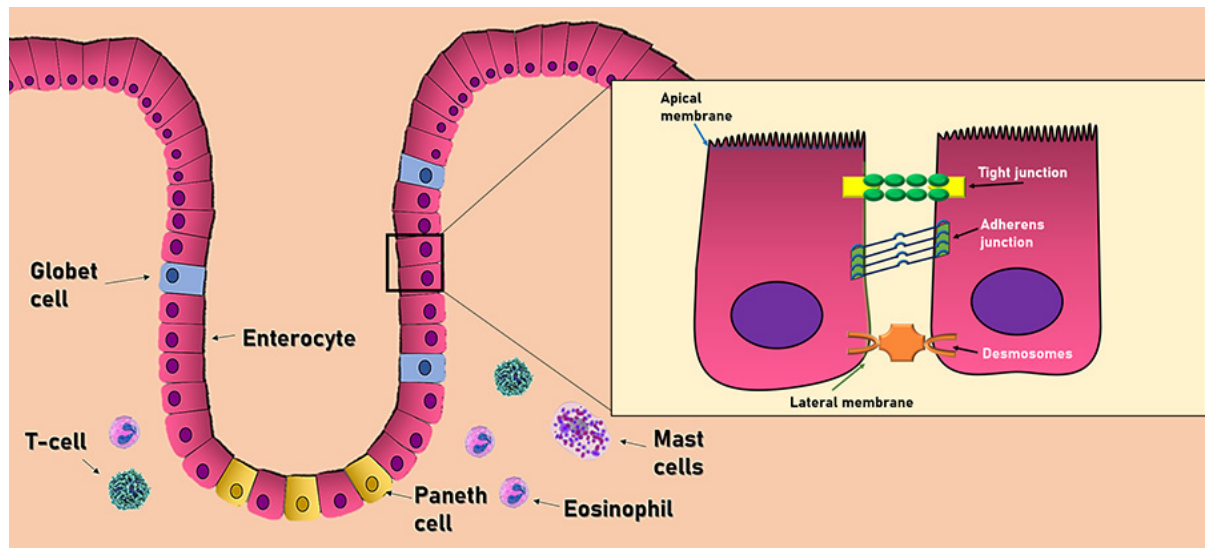


Figure 2. Cell composition of the intestinal barrier. The intestinal epithelium consists of a single layer of epithelial cells. Adjacent cells are connected by three main junctional complexes: desmosomes, adherens junctions, and tight junctions. The main immune cells of the intestinal barrier consist of T-cells, mast cells, and eosinophils

endogenous substrates derived from the host, such as mucus and pancreatic enzymes, as well as dietary components that are not absorbed in the first portions of the GI tract. Thus, the gut microbiota produce and transform a wide variety of metabolites that are absorbed in the small intestine, which can then travel through the bloodstream and reach the systemic circulation, especially the brain and liver, where they can trigger or influence important signaling pathways^[21].

The gut is a large territory occupied by both commensal and pathogenic microorganisms; therefore, it has the important protective mechanism of selectively choosing which molecules may pass to the systemic bloodstream. This mechanism is established by a multi-layer intestinal barrier covered by a mucus layer that provides a physical barrier between the underlying epithelium and the GI tract. This intestinal barrier consists in two separate sub-layers: an inner layer attached to epithelial cells lacking bacteria and an outer layer colonized by commensal microorganism. In addition to protecting against harmful agents, it acts as a selective filter for the correct translocation of nutrients, electrolytes, and water from the intestinal lumen to the circulation^[22,23].

Cell composition of the intestinal barrier

The intestinal barrier has three main cell types aimed to protect the host against external aggressions. This group includes the epithelial cells, intestinal goblet cells, and Paneth cells^[24]. Epithelial cells form a physical barrier connected by many transmembrane proteins called tight junctions (TJ), adhesion junctions (AJ), and desmosomes, each located in the basolateral membrane of epithelial cells. The TJ (also called zonula adherens) are located in the most apical part, formed by the cadherin-catenin protein junction. Below this zone, in almost the entire extension of the basolateral membrane, we can find the AJ (also known as zonula occludens), formed by the union of three main proteins: occludins, claudins, and the junctional adhesion molecules (JAM). Occludin and claudins are responsible for biochemical permeability and cell adhesion, while JAM bind cells by anchoring to the actin cytoskeleton of each cell. Finally, desmosomes can be found in the lower area of the epithelial cells, which also provide junction points by using keratin filaments^[23] [Figure 2].

Intestinal goblet cells produce different types of mucins (Muc2, Muc5AC, and Muc6), contributing to the viscous properties of the intestinal mucus layer and the protection against the pathogens that penetrate

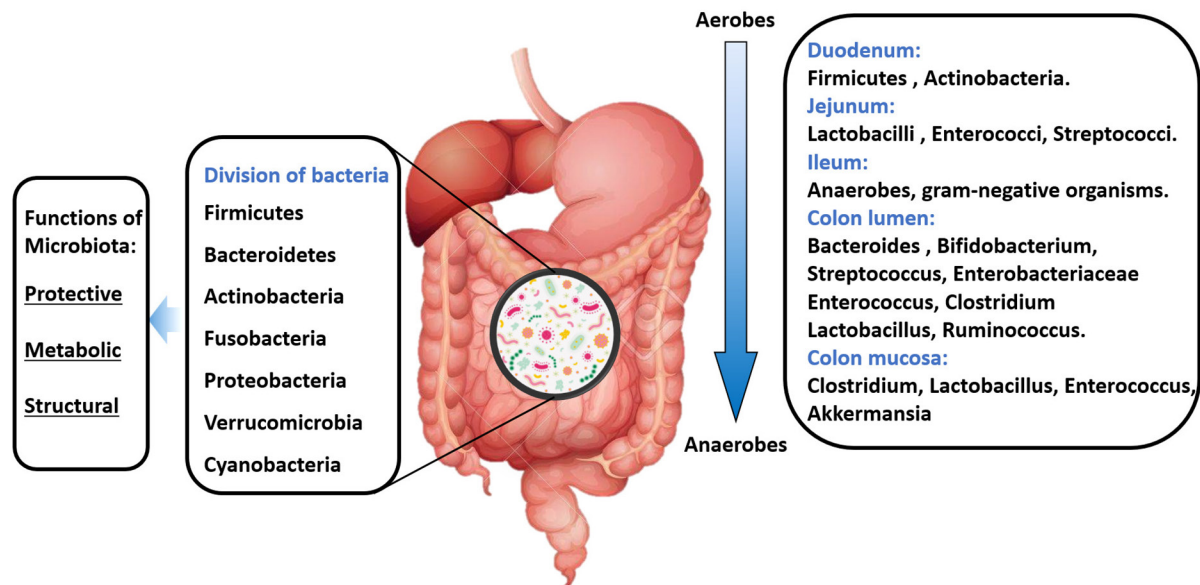


Figure 3. Composition of a “healthy” gut microbiota

this layer. Similarly, Paneth cells secrete the derived regenerating islet (Reg) 3 β in the mucus layer. These molecules are involved in gut homeostasis and exhibit antimicrobial activity that shapes the composition of the intestinal microbiome^[22]. All these gut defense mechanisms are reinforced by numerous immune cells in the *lamina propria* that play an essential role in the protection of the intestinal mucosa against the invasion of bacteria. Of this large number of immune cells, it is worth highlighting T cells, mast cells, and eosinophils due to their important contributions^[23]. First, T cells regulate cell permeability through Na⁺/K⁺ ATPase pumps, as well as the release of proinflammatory cytokines such as interferon-gamma (IFN γ), tumor necrosis factor-alpha (TNF- α), and delta-positive intestinal intraepithelial lymphocytes (iIEL $\gamma\delta$), which are also found in the basolateral membrane of epithelial cells, involved in the maintenance of its function. Mast cells release different proinflammatory mediators such as histamine, leukotrienes, platelet-activating factor, and cytokines, with important immune-mediated functions throughout the entire GI tract. Ultimately, eosinophils increase intestinal permeability through different mediators such as histamine, prostaglandins, and TNF- α ^[22].

Composition of a “healthy” gut microbiota

In the small intestine, food and nutrients absorption is mainly done in the duodenum through the release of digestive enzymes. At this site, food transit is faster, and the presence of oxygen limits bacterial density [10^{3-4} Colony-forming unit (CFU)/mL]. Firmicutes and Actinobacteria predominate in this site with an important growth of Gram-positive aerobes and facultative anaerobes, including Lactobacilli, Enterococci, and Streptococci with a progressive increase in bacterial density (10^{3-7} CFU/mL) in the jejunum^[25]. In the first part of the ileum, the bacterial density increases with a predominance of aerobic species (10^9 CFU/mL). In contrast, the distal part of the ileum (near the ileocecal valve) is inhabited by anaerobes and Gram-negative microorganisms similar to those found in the colon (characterized by a slower transit and its anaerobic condition). In the colon, the number of anaerobes exceeds aerobes microorganisms with a bacterial density of 10^{12} CFU/mL and an important predominance of Firmicutes and Bacteroidetes. Moreover, in the GI lumen, *Bacteroides*, *Bifidobacterium*, *Streptococcus*, *Enterobacteriaceae*, *Enterococcus*, *Clostridium*, *Lactobacillus* and *Ruminococcus* spp. are the bacterial genera that predominate, while *Clostridium*, *Lactobacillus*, *Enterococcus*, and *Akkermansia* spp. are more frequent in the mucosa [Figure 3]. In addition, some pathogenic bacteria including *Campylobacter jejuni*, *Salmonella enterica*, *Vibrio cholera*,

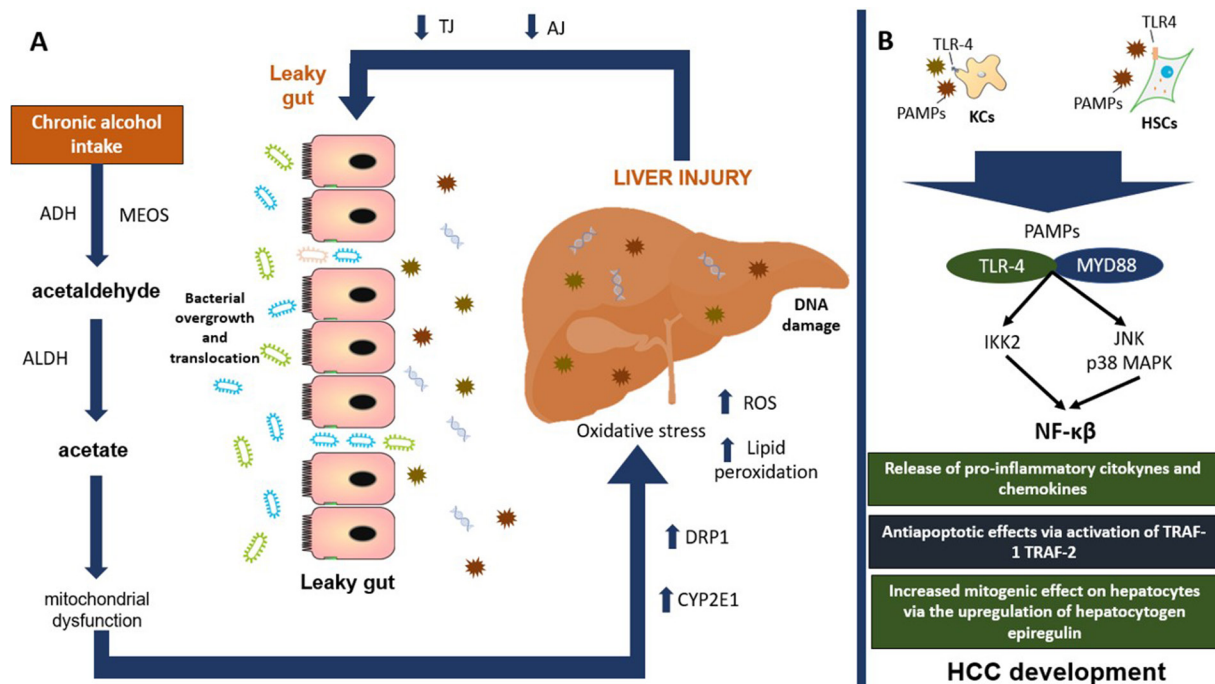


Figure 4. Main mechanisms involved in the development of HCC. A: chronic alcohol consumption increases the production of its main toxic metabolite acetaldehyde, favoring mitochondrial dysfunction and oxidative stress perpetuating liver injury. In the long run, this will generate a decreased function of TJ and AJ, interfering with the protective barrier of the intestine, developing a leaky gut. B: we can see how the bacterial overgrowth and translocation of its metabolites to the liver will increase liver injury and the recognition of PAMPs by specific TLRs such as TLR-4 binding with its ligand MYD88 and with the final activation of NF-κβ pathway with important repercussion for systemic inflammation and HCC development. ADH: alcohol dehydrogenase; MEOS: microsomal ethanol oxidizing system; ALDH: aldehyde dehydrogenase; DRP1: dynamin-related protein 1; CYP2E1: cytochrome P450 2E1; ROS: reactive oxygen species; TJ: tight junction proteins; AJ: adhesion junction proteins; PAMPs: pathogen-associated molecular patterns; TLR4: toll-like receptor-4; KCs: kupffer cells; HSCs: hepatic stellate cells; MYD88: myeloid differentiation primary response 88; JNK: c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; IKK2: inhibitor of nuclear factor kappa-B kinase 2; NF-κβ: nuclear-factor κβ; TNF: tumor necrosis factor; TRAF-1: TNF receptor associated factor-1; TRAF-2: TNF receptor associated factor-2; HCC: hepatocellular carcinoma

Escherichia coli, and *Bacteroides fragilis* can be found in smaller amounts within the GI tract^[25].

OXIDATIVE STRESS AND INTESTINAL PERMEABILITY IN ALD

When there is an increase in alcohol consumption, an upregulation of the CYP2E1-dependent ROS products such as hydroxyethyl, superoxide anion, hydroxyl radicals and numerous free radicals will accumulate in the liver, developing oxidative stress. An accumulation of ROS produces structural and functional changes in the DNA that interfere with the cell cycle, playing an important role in carcinogenesis^[11]. One of these changes induced by acetaldehyde and ROS is related to epigenetic regulations by interfering with the folate metabolism (important for DNA synthesis and methylation)^[26]. ALD patients have been found with polymorphisms in the methylene tetrahydrofolate reductase gene, leading to an alteration in folate metabolism and HCC development^[27,28]. Alcohol also has the capacity to inhibit the synthesis of S-adenosyl-L-methionine (S-AdoMet), an important methyl-donor molecule, by a diminished activity of methionine adenosyltransferase. The consequence of chronic S-AdoMet depletion seems to be associated with liver injury by interfering with the regenerative capacity of the liver^[29]. Furthermore, oxidative stress induces lipid peroxidation products such as malondialdehyde and 4-hydroxy-2-nonenal with the capacity to modify the gut microbiome, enhancing the creation of endotoxins by gut bacteria^[30], as well as induce mutations in the p53 gene, promoting HCC development^[31].

In the same way, evidence suggests that intestinal MEOS plays a permissive role in the gut, probably by the integrity disruption of the narrow epithelial junctions, which induces a decreased expression of binding

proteins (mainly claudins) with the consequent dysfunction of the AJ, establishing a leaky gut^[32]. Rodent studies have also demonstrated that alcohol-associated intestinal permeability is favored by a reduction in the intestinal hypoxia-induced factor 1- α (HIF-1 α) activity, a condition reversed by probiotic *Lactobacillus rhamnosus* GG supplementation^[33,34]. Moreover, ALD patients show a decreased bacterial diversity associated with an increase of endotoxin-producing Enterobacteriaceae and Proteobacteriaceae and a reduction in taxa that produce short-chain fatty acids such as Lachnospiraceae, Bacteroidaceae, and Ruminococcaceae^[35-37]. Interestingly, a reduced expression of lectins Reg3 β and regenerating islet-derived protein 3 gamma (Reg3 γ) is another important characteristic commonly seen in ALD, associated with bacterial overgrowth and translocation^[38]. All these factors will induce endotoxins formation such as lipopolysaccharides (LPS), peptidoglycans, and bacterial DNA. This favors intestinal inflammation and the activation of the TNF- α receptor I signaling in intestinal epithelial cells associated with increased intestinal permeability of endotoxins to the liver, boosting systemic inflammation via recognition of specific toll-like receptors (TLRs)^[39,40], as discussed below in more detail. Moreover, commensal fungi such as *Candida spp.*, *Saccharomyces cerevisiae*, and *Malassezia spp.* will develop tolerance from the host immune system during chronic alcohol consumption, fomenting an increase in these fungal species^[32]. Interestingly, studies in ALD patients have also shown higher systemic endotoxemia levels in subjects with an increased alcohol consumption regardless of the stage of liver disease, demonstrating that alcohol consumption is an independent factor for systemic endotoxemia^[41,42].

MECHANISMS INVOLVED IN HCC DEVELOPMENT

In the liver, Kupffer cells and bone-marrow derived macrophages will recognize small sequences of molecules formally called pathogen-associated molecular patterns (PAMPs) from endotoxins coming from enterohepatic circulation via Toll-like receptor-4 (TLR4). The upregulation of TLR4 will promote binding with its ligand, myeloid differentiation primary response 88, resulting in the activation of c-Jun N-terminal kinase, the inhibitor of nuclear factor kappa-B kinase 2, and mitogen-activated protein kinase (MAPK) p38, with the consequent activation of the nuclear factor kappa-B (NF- κ B) pathway. This favors the release of TNF- α , IFN- γ , prostaglandin-2, chemokine C-C motif ligand, IL-1 α , IL-1 β , IL-6, ROS, and nitric oxide, perpetuating liver inflammation^[43]. NF- κ B can also induce the antiapoptotic genes (TRAF-1 and TRAF-2) with important carcinogenic effects^[4]. Increased TNF- α production has been shown to deregulate TJ, causing disruption of the intestinal barrier. Interestingly, high levels of TNF- α and IL-6 have been found in duodenal biopsies of alcohol-dependent subjects, which tend to confirm data obtained in animal models^[28]. In another study carried out in 52 subjects diagnosed with alcohol dependence according to the DSM-IV criteria, a biochemical panel measuring LPS, TNF α , IL-6, IL-10, and high C reactive protein sensitivity showed an important elevation of these biochemical markers^[44]. On the other hand, IL-37 has been associated with anti-inflammatory effects via IL-18R α and IL-1R8 expression. In liver samples of ASH subjects, IL-37 expression was substantially reduced when compared to non-alcoholic fatty liver disease subjects^[45]. An *in vivo* system in wild-type mice suggested that hepatic IL-37 expression was suppressed by ethanol through the administration of human recombinant IL-37 followed by oral gavage of an ethanol shot in those animals^[45]. This is important since HCC clinical specimens have shown that decreased expression of IL-37 is negatively correlated with tumor size and positively associated with better overall survival and disease-free survival via the induction of tumor-infiltrating CD571 natural killer cells^[46].

In the liver, TLR4 can also be expressed in hepatic stellate cells (HSCs), endothelial cells, and hepatocytes^[47]. In HSCs, this molecule is involved in the upregulation of hepatocytogen epiregulin^[48], an epidermoid growth factor with a potent mitogenic effect on hepatocytes^[49]. In conjunction with the antiapoptotic effect of NF- κ B, it significantly promotes the hepatocarcinogenesis process. Knock-out mice studies with TLR-4 deficiency and intestinal sterilization with non-absorbable antibiotics have found a reduction in steatosis, oxidative stress, and liver inflammation with a consequent decrease in HCC risk development^[50,51], although the risk for liver injury increased, probably due to a deficiency in the innate

immunity caused by the suppression of TLR-4. In addition, chronic alcohol consumption has been associated with immunosuppression through a reduced recruitment of CD8⁺ T cells, an important group of cells responsible for the anti-tumor response in the human body^[52].

CHANGES IN THE GUT MICROBIOTA OF HCC PATIENTS

The gut microbiota undergoes an important change in the guests with early HCC. In obesity-induced mouse models, a greater number of *Clostridium* species has been found^[53,54], while in humans an important growth of *Escherichia coli*^[55], *Actinobacteria*, *Gemmiger*, and *Parabacteroides* species^[56] has been reported. In addition, due to the large number of bacteria that coexists in the body and the bacterial translocation caused by a leaky gut, it is not uncommon to find metabolically active bacteria within richly vascularized tumors attracted through a chemotactic gradient of the necrotic cell debris^[57]. In the case of HCC, *Helicobacter* species have been found with some frequency in this type of tumor tissue^[58-60]. In fact, this relationship is so important that an influence of the gut microbiota in the effectiveness and toxicity of certain chemotherapeutic agents has been pointed out, especially with the immune checkpoint inhibitors through the interaction among PAMPs, antigen-presenting cells, and TLRs, which leads to an adaptive immune response that modifies the pharmacodynamics of these types of agents^[57]. Moreover, both animal and human studies have found a significant correlation between alcohol consumption and a disturbance in the *Lactobacillus* to *Bifidobacterium* ratio, with an increase in pathogenic bacteria (namely, *Proteobacteria* and *Bacilli*). Interestingly, this ratio derangement has different presentations according to alcohol consumption habits, duration, and liver disease stage^[61,62].

Looking at other examples of HCC development related to microbiota imbalances in hepatology, we can describe the evidence regarding chronic viral hepatitis B and C. Chronic hepatitis B (CHB) patients show lower bacterial diversity (namely, an increase of *Firmicutes* and a decrease of *Bacteroidetes*). There is an increased concentration of H₂S- and CH₃SH- producing phylotypes (*Fusobacterium*, *Filifactor*, *Eubacterium*, *Parvimonas*, and *Treponema*) that may produce small bowel bacterial overgrowth, potentially involved in cirrhosis and HCC development^[63]. However, the impact of gut microbiota derangements in CHB patients on hepatocytes neoplastic transformation is different from that of chronic hepatitis C patients^[64]. In fact, obesity and/or diabetes stimulate cellular oncogenesis via gut microbiota derangement (i.e., an abundance of *Bacteroidetes* and, at a genus level, *Prevotella*, *Acinetobacter*, *Veillonella*, *Phascolarctobacterium*, and *Faecalibacterium* abundance) in HCC patients^[65-67].

Moreover, both interferon and new interferon-free direct antivirals successfully treated HCC patients presenting a permanent chronic inflammatory state triggered by an altered gut microbiota with potential HCC promotion^[68-70].

MOLECULAR INVOLVEMENT OF THE BILE ACIDS

Bile acids (BAs) are amphipathic molecules obtained from cholesterol synthesized in the liver, which play an important role in the emulsification of fats obtained from the diet to facilitate their absorption, in addition to important regulatory effects on the signaling pathways of glucose, lipids, and amino acids^[71]. In a healthy host, most of the BAs' pool is reabsorbed by active transport in the terminal ileum, while the rest is dehydroxylated by the intestinal microbiota, such as the secondary BAs deoxycholic acid (DCA) and lithocholic acid^[72].

The disruption in bacterial diversity of the host induced by ALD brings with it an important change in the BAs' pool by upregulating bacterial dehydroxylation, resulting in an increase in DCA synthesis, known for its important cytotoxic and carcinogenic effects. It is known that, under conditions of accumulation of BAs, activation of farnesoid X receptor (FXR) induces the expression of the bile salt export pump,

organic solute transporter alpha, and organic solute transporter beta, promoting the efflux of hepatic and intestinal BAs to systemic circulation^[73]. However, in CLDs, a decrease in these transporters has been observed due to an inhibition in FXR signaling by the subunit NF- κ B p65 binding directly to FXR, which inhibits its transcriptional activity, thus maintaining liver inflammation and the probable development of HCC^[74]. In addition, DCA can disrupt the plasma membrane, causing activation of protein kinase C, which in turn activates p38 MAPK, increasing the activation of NF- κ B pathway and resulting in sustained inflammation^[75]. Furthermore, the NF- κ B pathway transcribes genes encoding pro-inflammatory cytokines such as IL-6 related to the activation of the signal transducer and activator of transcription 3 pathway, which leads to decreased apoptosis^[76], and IL-1 β related to the activation of phosphoinositide 3 Kinase-MDM2 pathway, which negatively regulates p53, thus increasing the survival of DNA-damaged cells and leading to the development of HCC^[77].

Finally, recent findings have suggested an important role of DCA and cellular senescence in the development of HCC^[53]. Cellular senescence is a protective cell response to telomere erosion or oncogene activation with the final objective of bringing to an end the compromised cell cycle to prevent the development of any neoplasm^[78]. Interestingly, senescent cells develop a secretory proinflammatory profile known as senescence-associated secretory phenotype (SASP)^[79]. An experimental model in mice found that DCA induces SASP phenotype in HSCs, which in turns favors the secretion of proinflammatory cytokines and tumor-promoting factors associated with HCC development^[53]. It should be noted that this was an obesity-induced mice model; nonetheless, the results of this study could be replicated in an animal model of high-alcohol consumption to determine if there is any important variation between models^[53].

MICROBIOTA-REGULATORS AS A THERAPEUTIC OPTION FOR HCC

Due to the close relationship between dysbiosis and HCC, it is not difficult to imagine that certain microbiota-regulating agents have been used in several experimental studies in both humans and animals showing encouraging results. In this context, the drugs that have shown greater efficacy are the non-absorbable antibiotics rifaximin^[80-84] and norfloxacin^[85-87] by presenting an increase in the survival of patients with cirrhosis and HCC, in addition to preventing associated complications such as hepatic encephalopathy, portal hypertension, and spontaneous bacterial peritonitis. Other drugs included in this therapeutic arsenal are probiotics due to their modulating effects on the gut microbiota, by trying to restore bacterial diversity^[88]. Unfortunately, many pharmaceutical and food companies have made significant profits with them, which is why many so-called “healthy bacterial compounds” can be found in both pharmacies and supermarkets, making it difficult for health authorities to regulate them. Another important option that has not proven its efficacy in cancer but has in other GI conditions such as *Clostridium difficile* infection is fecal microbiota transplantation, promising to “reset” the altered microbiota, thus improving the anti-cancer immune response and preventing its development^[89]. Unfortunately, all these therapeutic options are still not included in the guidelines for the management of HCC due to the lack of standardization in different populations; thus, new clinical studies that focus on the resolution of intestinal dysbiosis for the management of HCC are necessary to increase its therapeutic options.

CONCLUSION

ALD is one of the most prevalent CLDs worldwide, representing a major health problem for most countries. Although it has a low potential for malignancy compared to other CLDs, its wide prevalence represents a major health problem for most countries. In recent years, great advances have been made in this field. To date, we know that alcohol metabolites interfere with the mitochondrial regulation pathways via increased expression of MSP, representing an attractive research field for understanding ALD pathogenesis. In addition, alcohol has the capacity to disturb gut microbiota, favoring the expansion

of endotoxin-producing bacteria and intestinal permeability, with the final translocation of bacteria and bacteria metabolites to the liver, inducing liver injury and carcinogenesis via the recognition of TLR-4 and the activation of NF- κ B pathway. Microbiota-regulating drugs have proven an important efficacy in the survival of patients with cirrhosis and HCC. However, alcohol abstinence will always be the best option for these patients; thus, emphasis should be placed on dissemination programs that teach the population about the important complications derived from alcohol consumption.

DECLARATIONS

Authors' contributions

Study concept and design: Méndez-Sánchez N

Literature research: Valencia-Rodríguez A, Vera-Barajas A, Abenavoli L, Scarpellini E, Ponciano-Rodríguez G

Drafting of the manuscript: Méndez-Sánchez N, Valencia-Rodríguez A, Vera-Barajas A, Abenavoli L, Scarpellini E, Ponciano-Rodríguez G

Critical revision of the manuscript for important intellectual content: Méndez-Sánchez N, Wang DQH

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al; Global Burden of Disease Liver Cancer Collaboration. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. *JAMA Oncol* 2017;3:1683-91.
2. WHO. Global status report on alcohol and health 2018. Available from: http://www.who.int/substance_abuse/publications/global_alcohol_report/gsr_2018/en/ [Last accessed on 18 Feb 2020]
3. Thursz M, Gual A, Lackner C, Mathurin P, Moreno C, et al. EASL clinical practice guidelines: management of alcohol-related liver disease. *J Hepatol* 2018;69:154-81.
4. Teschke R, Zhu Y. "Opinion: intestinal microbiome, endotoxins, cytochrome P450 2E1, and the gut-liver axis in alcoholic liver disease". *EC Gastroenterol Dig Syst* 2019;66-75.
5. Teschke R. Alcoholic liver disease: current mechanistic aspects with focus on their clinical relevance. *Biomedicines* 2019;7.
6. Méndez-Sánchez N, Villa AR, Chávez-Tapia NC, Ponciano-Rodríguez G, Almeda-Valdés P, et al. Trends in liver disease prevalence in Mexico from 2005 to 2050 through mortality data. *Ann Hepatol* 2005;4:52-5.
7. Teschke R. Hepatocellular carcinoma in alcoholic liver disease: mechanistic considerations and clinical facts. *Hepatoma Res* 2019;5:40.
8. Motola-Kuba D, Zamora-Valdés D, Uribe M, Méndez-Sánchez N. Hepatocellular carcinoma. An overview. *Ann Hepatol* 2006;5:16-24.
9. Méndez-Sánchez N, Zamarripa-Dorsey F, Panduro A, Purón-González E, Coronado-Alejandro EU, et al. Current trends of liver cirrhosis in Mexico: similitudes and differences with other world regions. *World J Clin Cases* 2018;6:922-30.
10. Seitz HK, Gärtner U, Egerer G, Simanowski UA. Ethanol metabolism in the gastrointestinal tract and its possible consequences.

- Alcohol Alcohol Suppl 1994;2:157-62.
11. Ganne-Carrié N, Nahon P. Hepatocellular carcinoma in the setting of alcohol-related liver disease. *J Hepatol* 2019;70:284-93.
 12. Zhong Z, Lemasters JJ. A unifying hypothesis linking hepatic adaptations for ethanol metabolism to the proinflammatory and profibrotic events of alcoholic liver disease. *Alcohol Clin Exp Res* 2018;42:2072-89.
 13. Frank S, Gaume B, Bergmann-Leitner ES, Leitner WW, Robert EG, et al. The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. *Dev Cell* 2001;1:515-25.
 14. Palma E, Riva A, Moreno C, Odena G, Mudan S, et al. Perturbations in mitochondrial dynamics are closely involved in the progression of alcoholic liver disease. *Alcohol Clin Exp Res* 2020; Epub ahead of print [PMID: 32020641 DOI: 10.1111/acer.14299]
 15. Seitz HK, Korsten MA, Lieber CS. Ethanol oxidation by intestinal microsomes: increased activity after chronic ethanol administration. *Life Sci* 1979;25:1443-8.
 16. Pronko P, Bardina L, Satanovskaya V, Kuzmich A, Zimatkin S. Effect of chronic alcohol consumption on the ethanol- and acetaldehyde-metabolizing systems in the rat gastrointestinal tract. *Alcohol Alcohol* 2002;37:229-35.
 17. Teschke R. Review article. Biochemical aspects of the hepatic microsomal ethanol-oxidizing system (MEOS): resolved initial controversies and updated molecular views. *Biochem Pharmacol (Los Angel)* 2019;8:267.
 18. Lozupone CA, Stombaugh JJ, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature* 2012;489:220-30.
 19. Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* 2016;164:337-40.
 20. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010;464:59-65.
 21. Cardona F, Andrés-Lacueva C, Tulipani S, Tinahones FJ, Queipo-Ortuño MI. Benefits of polyphenols on gut microbiota and implications in human health. *J Nutr Biochem* 2013;24:1415-22.
 22. Stärkel P, Leclercq S, de Timary P, Schnabl B. Intestinal dysbiosis and permeability: the yin and yang in alcohol dependence and alcoholic liver disease. *Clin Sci* 2018;132:199-212.
 23. Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 2009;124:3-20; quiz 21-2.
 24. Kim YS, Ho SB. Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr Gastroenterol Rep* 2010;12:319-30.
 25. Adak A, Khan MR. An insight into gut microbiota and its functionalities. *Cell Mol Life Sci* 2019;76:473-93.
 26. Varela-Rey M, Woodhoo A, Martinez-Chantar ML, Mato JM, Lu SC. Alcohol, DNA methylation, and cancer. *Alcohol Res* 2013;35:25-35.
 27. Fabris C, Toniutto P, Falletti E, Fontanini E, Cussigh A, et al. MTHFR C677T polymorphism and risk of hcc in patients with liver cirrhosis: role of male gender and alcohol consumption. *Alcohol Clin Exp Res* 2009;33:102-7.
 28. Saffroy R, Pham P, Chiappini F, Gross-Goupil M, Castera L, et al. The MTHFR 677C > T polymorphism is associated with an increased risk of hepatocellular carcinoma in patients with alcoholic cirrhosis. *Carcinogenesis* 2004;25:1443-8.
 29. Lu SC, Mato JM. Role of methionine adenosyltransferase and S-adenosylmethionine in alcohol-associated liver cancer. *Alcohol* 2005;35:227-34.
 30. Teschke R. Alcoholic liver disease: alcohol metabolism, cascade of molecular mechanisms, cellular targets, and clinical aspects. *Biomedicine* 2018;6.
 31. Hu W, Feng Z, Eveleigh J, Iyer G, Pan J, et al. The major lipid peroxidation product, trans-4-hydroxy-2-nonenal, preferentially forms DNA adducts at codon 249 of human p53 gene, a unique mutational hotspot in hepatocellular carcinoma. *Carcinogenesis* 2002;23:1781-9.
 32. Wang Y, Tong J, Chang B, Wang B, Zhang D, et al. Effects of alcohol on intestinal epithelial barrier permeability and expression of tight junction-associated proteins. *Mol Med Rep* 2014;9:2352-6.
 33. Bull-Otterson L, Feng W, Kirpich I, Wang Y, Qin X, et al. Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of *Lactobacillus rhamnosus* GG treatment. *PLoS One* 2013;8:e53028.
 34. Wang Y, Kirpich I, Liu Y, Ma Z, Barve S, et al. *Lactobacillus rhamnosus* GG treatment potentiates intestinal hypoxia-inducible factor, promotes intestinal integrity and ameliorates alcohol-induced liver injury. *Am J Pathol* 2011;179:2866-75.
 35. Bajaj JS, Kakiyama G, Zhao D, Takei H, Fagan A, et al. Continued alcohol misuse in human cirrhosis is associated with an impaired gut-liver axis. *Alcohol Clin Exp Res* 2017;41:1857-65.
 36. Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014;60:940-7.
 37. Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, et al. Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G966-78.
 38. Wang L, Fouts DE, Stärkel P, Hartmann P, Chen P, et al. Intestinal REG3 lectins protect against alcoholic steatohepatitis by reducing mucosa-associated microbiota and preventing bacterial translocation. *Cell Host Microbe* 2016;19:227-39.
 39. Chen P, Stärkel P, Turner JR, Ho SB, Schnabl B. Dysbiosis-induced intestinal inflammation activates TNFRI and mediates alcoholic liver disease in mice. *Hepatology* 2015;61:883-94.
 40. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 2009;58:1091-103.
 41. Bode C, Kugler V, Bode JC. Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute alcohol excess. *J Hepatol* 1987;4:8-14.
 42. Fukui H, Brauner B, Bode JCh, Bode Ch. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. *J Hepatol* 1991;12:162-9.

43. Duque Correa MA, Rojas López M. Activación alternativa del macrófago: La diversidad en las respuestas de una célula de la inmunidad innata ante la complejidad de los eventos de su ambiente. *Inmunologia* 2007;26:73-86. (in Spanish)
44. Leclercq S, Cani PD, Neyrinck AM, Stärkel P, Jamar F, et al. Role of intestinal permeability and inflammation in the biological and behavioral control of alcohol-dependent subjects. *Brain Behav Immun* 2012;26:911-8.
45. Grabherr F, Grander C, Adolph TE, Wieser V, Mayr L, et al. Ethanol-mediated suppression of IL-37 licenses alcoholic liver disease. *Liver Int* 2018;38:1095-101.
46. Zhao JJ, Pan QZ, Pan K, Weng DS, Wang QJ, et al. Interleukin-37 mediates the antitumor activity in hepatocellular carcinoma: role for CD57+ NK cells. *Sci Rep* 2015;4:5177.
47. Yu LX, Schwabe RF. The gut microbiome and liver cancer: mechanisms and clinical translation. *Nat Rev Gastroenterol Hepatol* 2017;14:527-39.
48. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012;21:504-16.
49. Toyoda H, Komurasaki T, Uchida D, Takayama Y, Isobe T, et al. Epiregulin. A novel epidermal growth factor with mitogenic activity for rat primary hepatocytes. *J Biol Chem* 1995;270:7495-500.
50. Uesugi T, Froh M, Arteel GE, Bradford BU, Thurman RG. Toll-like receptor 4 is involved in the mechanism of early alcohol-induced liver injury in mice. *Hepatology* 2001;34:101-8.
51. Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. *Gastroenterology* 1995;108:218-24.
52. Yan G, Wang X, Sun C, Zheng X, Wei H, et al. Chronic alcohol consumption promotes diethylnitrosamine-induced hepatocarcinogenesis via immune disturbances. *Sci Rep* 2017;7:2567.
53. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013;499:97-101.
54. Xie G, Wang X, Liu P, Wei R, Chen W, et al. Distinctly altered gut microbiota in the progression of liver disease. *Oncotarget* 2016;7:19355-66.
55. Grąt M, Krasnodębski M, Patkowski W, Wronka KM, Masior L, et al. Relevance of pre-transplant α -fetoprotein dynamics in liver transplantation for hepatocellular cancer. *Ann Transplant* 2016;21:115-24.
56. Ren Z, Li A, Jiang J, Zhou L, Yu Z, et al. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut* 2019;68:1014-23.
57. Helmink BA, Khan MAW, Hermann A, Gopalakrishnan V, Wargo JA. The microbiome, cancer, and cancer therapy. *Nat Med* 2019;25:377-88.
58. Fox JG, Feng Y, Theve EJ, Raczynski AR, Fiala JLA, et al. Gut microbes define liver cancer risk in mice exposed to chemical and viral transgenic hepatocarcinogens. *Gut* 2010;59:88-97.
59. Rogers AB. Distance burning: how gut microbes promote extraintestinal cancers. *Gut Microbes* 2011;2:52-7.
60. Huang Y, Fan XG, Wang ZM, Zhou JH, Tian XF, et al. Identification of helicobacter species in human liver samples from patients with primary hepatocellular carcinoma. *J Clin Pathol* 2004;57:1273-7.
61. Temko JE, Bouhlal S, Farokhnia M, Lee MR, Cryan JF, et al. The microbiota, the gut and the brain in eating and alcohol use disorders: a "ménage à trois"? *Alcohol Alcohol* 2017;52:403-13.
62. Bishehsari F, Magno E, Swanson G, Desai V, Voigt RM, et al. Alcohol and gut-derived inflammation. *Alcohol Res* 2017;38:163-71.
63. Ling Z, Liu X, Cheng Y, Jiang X, Jiang H, et al. Decreased diversity of the oral microbiota of patients with hepatitis b virus-induced chronic liver disease: a pilot project. *Sci Rep* 2015;5:17098.
64. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007;132:2557-76.
65. Bajaj JS, Sterling RK, Betrapally NS, Nixon DE, Fuchs M, et al. HCV eradication does not impact gut dysbiosis or systemic inflammation in cirrhotic patients. *Aliment Pharmacol Ther* 2016;44:638-43.
66. Maggio R, Viscomi C, Andreozzi P, D'Ettorre G, Viscogliosi G, et al. Normocaloric low cholesterol diet modulates Th17/Treg balance in patients with chronic hepatitis C virus infection. *PLoS One* 2014;9:e112346.
67. Preveden T, Scarpellini E, Milić N, Luzzza F, Abenavoli L. Gut microbiota changes and chronic hepatitis C virus infection. *Expert Rev Gastroenterol Hepatol* 2017;11:813-9.
68. Aly AM, Adel A, El-Gendy AO, Essam TM, Aziz RK. Gut microbiome alterations in patients with stage 4 hepatitis C. *Gut Pathog* 2016;8:42.
69. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *Elife* 2013;2:e01202.
70. Sousa GM, Oliveira IS, Andrade LJO, Sousa-Atta MLB, Paraná R, et al. Serum levels of Th17 associated cytokines in chronic hepatitis C virus infection. *Cytokine*. 2012;60:138-42.
71. Kuipers F, Bloks VW, Groen AK. Beyond intestinal soap--bile acids in metabolic control. *Nat Rev Endocrinol* 2014;10:488-98.
72. Jia W, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nat Rev Gastroenterol Hepatol* 2018;15:111-28.
73. Boyer JL, Trauner M, Mennone A, Soroka CJ, Cai SY, et al. Upregulation of a basolateral FXR-dependent bile acid efflux transporter OSTalpha-OSTbeta in cholestasis in humans and rodents. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G1124-30.
74. Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, Suchy FJ. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. *J Biol Chem* 2001;276:28857-65.
75. Anwer MS. Intracellular signaling by bile acids. *J Biosci (Rajshari)* 2012;20:1-23.
76. Jung IH, Choi JHK, Chung YY, Lim GL, Park YN, et al. Predominant activation of JAK/STAT3 pathway by interleukin-6 is implicated in hepatocarcinogenesis. *Neoplasia* 2015;17:586-97.

77. Nag S, Qin J, Srivenugopal KS, Wang M, Zhang R. The MDM2-p53 pathway revisited. *J Biomed Res* 2013;27:254-71.
78. Collado M, Serrano M. Senescence in tumours: evidence from mice and humans. *Nat Rev Cancer* 2010;10:51-7.
79. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* 2008;6:2853-68.
80. Nishida S, Hamada K, Nishino N, Fukushima D, Koyanagi R, et al. Efficacy of long-term rifaximin treatment for hepatic encephalopathy in the Japanese. *World J Hepatol* 2019;11:531-41.
81. Kang SH, Lee YB, Lee JH, Nam JY, Chang Y, et al. Rifaximin treatment is associated with reduced risk of cirrhotic complications and prolonged overall survival in patients experiencing hepatic encephalopathy. *Aliment Pharmacol Ther* 2017;46:845-55.
82. Elfert A, Abo Ali L, Soliman S, Ibrahim S, Abd-Elsalam S. Randomized-controlled trial of rifaximin versus norfloxacin for secondary prophylaxis of spontaneous bacterial peritonitis. *Eur J Gastroenterol Hepatol* 2016;28:1450-4.
83. Kalambokis GN, Mouzaki A, Rodi M, Pappas K, Fotopoulos A, et al. Rifaximin improves systemic hemodynamics and renal function in patients with alcohol-related cirrhosis and ascites. *Clin Gastroenterol Hepatol* 2012;10:815-8.
84. Vlachogiannakos J, Viazis N, Vasianopoulou P, Vafiadis I, Karamanolis DG, et al. Long-term administration of rifaximin improves the prognosis of patients with decompensated alcoholic cirrhosis: rifaximin improves prognosis in cirrhosis. *J Gastroenterol Hepatol* 2013;28:450-5.
85. Shariff MIF, Tognarelli JM, Lewis MR, Want EJ, Mohamed FEZ, et al. Plasma lipid profiling in a rat model of hepatocellular carcinoma: potential modulation through quinolone administration. *J Clin Exp Hepatol* 2015;5:286-94.
86. Ginés P, Rimola A, Planas R, Vargas V, Marco F, et al. Norfloxacin prevents spontaneous bacterial peritonitis recurrence in cirrhosis: results of a double-blind, placebo-controlled trial. *Hepatology* 1990;12:716-24.
87. Fernández J, Navasa M, Gómez J, Colmenero J, Vila J, et al. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology* 2002;35:140-8.
88. Li J, Sung CYJ, Lee N, Ni Y, Pihlajamäki J, et al. Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. *Proc Natl Acad Sci U S A* 2016;113:E1306-15.
89. Delaune V, Orci LA, Lacotte S, Peloso A, Schrenzel J, et al. Fecal microbiota transplantation: a promising strategy in preventing the progression of non-alcoholic steatohepatitis and improving the anti-cancer immune response. *Expert Opin Biol Ther* 2018;18:1061-71.

Review

Open Access



Coffee protection against the development of hepatocellular carcinoma: review article

Kellyane S. D. Carvalho, Helma P. Cotrim

Programa de Pós-Graduação em Medicina e Saúde, Faculdade de Medicina - Universidade Federal da Bahia, Bahia 40110-060, Brazil.

Correspondence to: Dr. Helma P. Cotrim, Programa de Pós-Graduação em Medicina e Saúde - PPGMS, Complexo Universitário Professor Edgard Santos, Rua Augusto Viana, 5o. andar; Canela, Salvador, Bahia 40110-060, Brazil. E-mail: helmacotrim@gmail.com

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Abstract

Coffee, a popular drink around the world, is composed of a complex mix of biologically active molecules, including caffeine, chlorogenic acid, and diterpenes. These compounds have antioxidant, anti-inflammatory, antifibrotic, and anticarcinogenic properties, which may explain observational data showing that coffee drinkers have lower rates of chronic liver disease, including cirrhosis and hepatocellular carcinoma (HCC). Recent studies have also shown that coffee consumption may also increase patient survival before and after liver transplantation. The mechanism by which coffee consumption protects against HCC is not clear; however, its relevant role has been demonstrated. This literature review article focuses on the role of coffee consumption in protecting against the development of HCC. Methodology: Scientific articles indexed through PubMed, including Medline, Scielo, and Lilacs, published in English were used as search methods. The terms used in English were: "hepatocellular carcinoma" or "Liver cancer" or "HCC" and "coffee". According to the study design or review article, cross-sectional, longitudinal, or descriptive investigations were included, showing site and year of publication until 2019.

Keywords: Hepatocellular carcinoma, coffee consumption protection, liver cancer

INTRODUCTION

Hepatocellular carcinoma (HCC) is the main and most frequent malignant liver tumor today. Its prevalence has been increasing steadily around the world in recent decades^[1], and its development is associated with chronic liver diseases caused by hepatitis B and C viruses, alcoholic cirrhosis, non-alcoholic steatohepatitis, and metabolic diseases such as primary hemochromatosis^[2,3].



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Coffee is one of the most consumed beverages in the world. Coffee consists of several components, such as caffeine, triacylglycerol, tocopherols, chlorogenic acid, cafestol, and kahweol, with relevant antioxidant and anti-inflammatory properties. Because of their biological properties, these substances can have beneficial effects on metabolism^[4].

For many years, coffee intake has been related to adverse effects, mainly cardiovascular, but recent studies show that daily coffee consumption, in addition to being harmful, can be beneficial and protective, especially against the development of chronic liver diseases such as HCC. This protection seems to be associated with the habit and frequency of coffee consumption^[5].

As most studies on this subject have been observational, the mechanisms of coffee protection against various diseases remain uncertain. However, some hypotheses have been suggested: polyphenols, antioxidants found in coffee, may protect cells against oxidative stress and damage to DNA molecules^[6]. The protection conferred by coffee is believed to occur through antioxidant components, which would influence the lower liver enzymatic activity. Gamma-glutamyl transpeptidase (GGT) and alanine aminotransferase (ALT) in these studies showed lower serum levels in coffee drinkers versus nondrinkers^[7].

The importance of hepatocarcinogenic pathways for the development of HCC has also been discussed, and molecular mechanisms that generate liver aggression and metabolic changes in hepatocytes related mainly to oxidative stress have been observed. The accumulation of several oxidative metabolites directly attacks DNA molecules or makes the general functioning of the cell unfeasible, generating apoptosis. Thus, it is observed that the protective mechanisms of substances present in coffee antagonize the hepatic carcinogenic pathways, which theoretically supports the findings of coffee consumption as a protector of the liver.

Even with the accumulation of evidence in favor of coffee consumption, its dietary prescription remains controversial. It is estimated that 36% of health professionals still believe that coffee consumption increases the risk of cancer. Thus, research that reinforces the benefits of coffee is still needed, especially when the outcomes are more impactful and chronic, such as the incidence of hepatocellular carcinoma^[8].

This literature review aims to evaluate the role of coffee consumption in protecting against the development of HCC.

METHODOLOGY

Scientific articles indexed through PubMed, including Medline, SciELO, and Lilacs, published in English were used as search methods.

The terms used in English were: “hepatocellular carcinoma” or “liver cancer” or “HCC and “coffee”. According to the study design or review article, cross-sectional, longitudinal, or descriptive investigations were included, showing site and year of publication until 2019.

This literature review included articles that evaluated the role of coffee consumption in protecting against the development of HCC and included the following topics: HCC pathogenesis; caffeine and HCC; amount of coffee consumed and protective effect on hepatocarcinogenesis; impact of coffee consumption on HCC mortality; and coffee consumption and recurrence of HCC after liver transplantation.

HCC PATHOGENESIS

The pathogenesis of HCC is multifactorial, which initially includes an inflammatory process mediated by Kupffer cells in the liver or macrophages, which stimulate the production of proinflammatory

cytokines [interleukin 6 (IL-6) and tumor necrosis factor (TNF- α)] and immunosuppressive cytokines^[9,10] (IL-10). The accumulation of these immunostimulating agents around the liver inflammation focus induces mitochondrial imbalance in hepatocytes (increased oxygen uptake and increased production of superoxide anions, hydroxyl radicals, and oxide), leading to the production of high levels of reactive oxygen species (ROS) and a consequent increase in oxidative stress^[11,12].

In addition, hepatic mitochondria are also essential in hepatocyte survival as the mediator of apoptosis and necrosis, controlling the balance between cell survival and death by regulating membrane permeability, and activating the intrinsic pathway of apoptosis (cytochrome C protein release, apoptosis formation, and caspase activation^[9]). In addition, increased ROS production causes oxidative damage to mitochondrial proteins (impairing ATP synthesis) and alters the induction of pore production from mitochondrial transition permeability. These changes make the inner membrane permeable to small molecules that can cause ischemia or reperfusion injury and DNA damage by activating the intrinsic apoptotic mechanism^[13].

These mechanisms can induce recurrent cycles of cell damage, repair, and regeneration in hepatocytes, leading to the formation of dysplastic nodular lesions, which are precursor lesions of HCC. Associated with these changes are genetic and epigenetic changes that are directly related to tumor progression and HCC^[14].

CAFFEINE AND HCC

Caffeine, one of the components of coffee, appears to play a central role in protecting against the development of chronic liver disease and HCC. It reduces HCC cell proliferation, and it has been observed that protection against HCC is lower for decaffeinated coffee^[15].

Other substances, such as cafestol and kahweol, tirpenoid molecules present in coffee beans, increase the activity of liver enzymes, which may improve metabolism and excretion of carcinogens^[16,17]. However, cafestol and kahweol are only present in minimal quantities in filtered coffee, and these coffee varieties are popular in countries, such as Japan and Finland, where studies have shown inverse associations with HCC^[18,19].

Specific coffee protection mechanisms may include inhibition of viral hepatitis activity and prevention of diabetes mellitus type 2. In addition, coffee has a number of health benefits, including a lower incidence of neurological diseases, various cancers, and reduced mortality from any cause^[20].

AMOUNT OF COFFEE CONSUMPTION AND PROTECTIVE EFFECT ON HEPATOCARCINOGENESIS

Several studies report not only the protective effect of coffee use on the pathogenesis of HCC but also that the protection is related to the amount of coffee ingested. Inoue *et al.*^[21] conducted a prospective cohort of 116,686 Japanese individuals to assess the influence of coffee consumption on the risk of hepatocellular carcinoma. Coffee proved to be a liver protective factor, with 51% lower risk of developing cancer by daily consumers compared to those who never drank coffee. An inverse relationship was also noted between consumption and incidence of this neoplasia, one or two cups per day (HR = 0.52), three or four cups per day (HR = 0.48), and more than five cups per day (HR = 0.24).

The study by Johnson *et al.*^[22] analyzed 63,257 over-middle-aged Chinese individuals over approximately 5 years between 1993 and 1998. They that 18.5% of individuals did not drink coffee, 11.1% were light drinkers and drank less than one cup per day, 36% were average consumers who drank between one and two cups a day, and 34.4% were heavy drinkers and drank more than two cups of coffee a day. Of the 362 patients diagnosed with HCC, an inverse relationship was found between coffee consumption and HCC risk, with a lower risk for each increase in the number of coffee cups drunk ($P = 0.05$). Heavy consumers had a 44% lower risk of HCC than those who did not drink coffee after adjusting for potential confounders.

A European study by Bamia *et al.*^[23] evaluated more than 521,000 individuals between 1992 and 2000 in 10 European countries to look for the etiologic factors of cancers and other chronic diseases. Dietary data, including coffee intake, were obtained through previously validated questionnaires. After 11 years of follow-up, 133 men and 68 women were diagnosed with HCC, and the results showed an average consumption of 354 mL per day of coffee among men and 290 mL of coffee per day among women. Seven percent of participants did not drink coffee. There was an inverse association between coffee consumption and the risk of HCC, and coffee consumption proved to be a liver protective factor in all groups. The higher the coffee intake, the lower the risk of liver carcinogenesis, with statistically significant results.

Aleksandrova *et al.*^[24] analyzed the data obtained from the European Prospective Investigation into Cancer and Nutrition, a study conducted from 1992 to 2000 on more than 520,000 individuals that aimed to establish a relationship between protection from coffee consumption and the risk of HCC in addition to serum markers of inflammation. Coffee consumption showed an inverse association with the development of HCC, with 20% more coffee consumers having an approximately 70% lower chance of HCC. Conjugate analysis of serum markers and coffee consumption showed a statistically significant reduction in all markers of liver inflammation (IL-6, glutamate dehydrogenase, alanine transaminase, aspartate transaminase, GGT, alkaline phosphatase, total bilirubin, and alpha-fetoprotein). All of these markers were directly associated with a higher risk of hepatocellular carcinoma.

IMPACT OF COFFEE CONSUMPTION ON HCC MORTALITY

Kurozawa *et al.*^[25], through a prospective cohort study from 1988 to 1999, evaluated the impact of coffee consumption on HCC mortality in a total of 110,792 individuals. In HCC mortality analyses, consumers of less than one cup of coffee per day had a lower risk of death from HCC, and this risk was even lower in consumers of at least one cup of coffee per day, showing a quantitative association between consumption and mortality from hepatocellular carcinoma. In subjects reporting a history of liver disease, coffee consumption was an even more prominent protective factor when compared to those who did not drink coffee or drinkers with no history of liver disease.

COFFEE CONSUMPTION AND RECURRENCE OF HCC AFTER LIVER TRANSPLANTATION

Orthotopic liver transplantation (OLT) is the therapeutic option with the most favorable outcome as it offers radical removal of the tumor and eliminates underlying chronic liver disease in selected patients or those with early-stage HCC. However, recurrence of HCC after OLT remains a serious problem with up to 20% risk. Recent studies have identified several risk factors for HCC recurrence, such as biological and radiological progression on the waiting list, number of tumor nodules, and poor differentiation^[26]. However, the mechanisms are still poorly understood, and potential post-OLT strategies to prevent HCC recurrence are still needed.

Recent studies show that caffeinated coffee consumption is associated with a reduced risk of HCC recurrence and longer survival following OLT. Experimental data suggest that such benefits of coffee are associated, at least in part, with the caffeine antagonist activity in promoting adenosine receptor-mediated growth by HCC cell effects^[27].

CONCLUSION

The present review suggests that a daily habit of drinking coffee seems to protect against the development of HCC. The mechanisms may induce recurrent cycles of cell damage, repair, and regeneration in hepatocytes, leading to the formation of dysplastic nodular lesions, which are precursor lesions of HCC. Genetic and epigenetic changes may also influence HCC development. Caffeine is only one of the components of coffee; however, it appears to play a central role in protecting against the development of chronic liver disease and

HCC. Some studies have also suggested an impact of coffee consumption on HCC mortality and recent studies showed that coffee consumption is associated with a reduced risk of HCC recurrence and longer survival following liver transplantation. These results are encouraging, mainly due to the increase in the incidence of HCC and its prognosis. Randomized studies are expected to confirm the results of mostly observational studies.

DECLARATIONS

Authors' contributions

Concept and design: Cotrim HP

Data acquisition: Carvalho KSD

Data analysis: Carvalho KSD, Cotrim HP

Manuscript preparation: Carvalho KSD, Cotrim HP

Critical revision and finalizing of the manuscript: Cotrim HP

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Bosetti C, Bertuccio P, Malvezzi M, Levi F, Chatenoud L, et al. Cancer mortality in Europe, 2005-2009, and an overview of trends since 1980. *Ann Oncol* 2013;24:2657-71.
2. Chuang SC, La Vecchia C, Boffetta P. Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection. *Cancer Lett* 2009;286:9-14.
3. Trichopoulos D, Bamia C, Lagiou P, Fedirko V, Trepo E, et al. Hepatocellular carcinoma risk factors and disease burden in a European cohort: a nested casecontrol study. *J Natl Cancer Inst* 2011;103:1686-95.
4. Barros RK, Cotrim HP, Daltro C, Alves E, de Freitas LRA, et al. Nonalcoholic steatohepatitis in morbid obese patients: coffee consumption vs. disease severity. *Ann Hepatol* 2016;15:350-5.
5. Wierzejska R. Coffee consumption vs. cancer risk - a review of scientific data. *Rocz Panstw Zakl Hig* 2015;66:293-8.
6. Yu X, Bao Z, Zou J, Dong J. Coffee consumption and risk of cancers: a meta-analysis of cohort studies. *BMC Cancer* 2011;11:96.
7. Ruhl CE, Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. *Gastroenterology* 2005;128:24-32.
8. Bohn SK, Blomhoff R, Paur I. Coffee and cancer risk, epidemiological evidence, and molecular mechanisms. *Mol Nutr Food Res* 2014;58:915-30.
9. Hösel M, Quasdorff M, Wiegmann K, Webb D, Zedler U, et al. Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. *Hepatology* 2009;50:1773-82.
10. Takaki A. Control of oxidative stress in hepatocellular carcinoma: helpful or harmful? *World J Hepatol* 2015;7:968.
11. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J* 2008;417:1-13.
12. Degli Esposti D, Hamelin J, Bosselut N, Saffroy R, Sebah M, et al. Mitochondrial roles and cytoprotection in chronic liver injury. *Biochem Res Int* 2012;2012:387626.

13. Pedro JMB, Kroemer G, Filigheddu N, Galluzzi L, Porporato PE. Mitochondrial metabolism and cancer. *Cell Res* 2017;28:265-80.
14. Dhanasekaran R, Bandoh S, Roberts LR. Molecular pathogenesis of hepatocellular carcinoma and impact of therapeutic advances. *F1000Res* 2016;5:879.
15. Okano J, Nagahara T, Matsumoto K, Murawaki Y. Caffeine inhibits the proliferation of liver cancer cells and activates the MEK/ERK/EGFR signalling pathway. *Basic Clin Pharmacol Toxicol* 2008;102:543-51.
16. Cavin C, Holzhaeuser D, Scharf G, Huber WW, Schilter B. Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity. *Food Chem Toxicol* 2002;40:1155-63.
17. Huber WW, Scharf G, Rossmanith W, Prustomersky S, Grasl-Kraupp B, et al. The coffee components kahweol and cafestol induce gamma-glutamylcysteine synthetase, the rate limiting enzyme of chemoprotective glutathione synthesis, in several organs of the rat. *Arch Toxicol* 2002;75:685-94.
18. Tanaka K, Hara M, Sakamoto T, Higaki Y, Mizuta T, et al. Inverse association between coffee drinking and the risk of hepatocellular carcinoma: a case-control study in Japan. *Cancer Sci* 2007;98:214-8.
19. Hu G, Tuomilehto J, Pukkala E, Hakulinen T, Antikainen R, et al. Joint effects of coffee consumption and serum gamma-glutamyltransferase on the risk of liver cancer. *Hepatology* 2008;48:129-36.
20. Cano-Marquina A, Tarín JJ, Cano A. The impact of coffee on health. *Maturitas* 2013;75:7-21.
21. Inoue M, Yoshimi I, Sobue T, Tsugane S; JPHC Study Group. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: a prospective study in Japan. *J Natl Cancer Inst* 2005;97:293-300.
22. Johnson S, Wang R. Coffee consumption and reduced risk of hepatocellular carcinoma: findings from the Singapore Chinese Health Study. *Cancer Causes Control* 2012;22:503-10.
23. Bamia C, Lagiou P, Jenab M, Trichopoulou A, Fedirko V, et al. Coffee, tea and decaffeinated coffee in relation to hepatocellular carcinoma in a European population: multicentre, prospective cohort study. *Int J Cancer* 2015;136:1899-908.
24. Aleksandrova K, Bamia C, Drogan D, Lagiou P, Trichopoulou A, et al. The association of coffee intake with liver cancer risk is mediated by biomarkers of inflammation and hepatocellular injury: data from the European Prospective Investigation into Cancer and Nutrition. *Am J Clin Nutr* 2015;102:1498-508.
25. Kurozawa Y, Ogimoto I, Shibata A, Nose T, Yoshimura T, et al. Coffee and risk of death from hepatocellular carcinoma in a large cohort study in Japan. *Br J Cancer* 2005;93:607-10.
26. Kondili LA, Lala A, Gunson B, Hubscher S, Olliff S, et al. Primary hepatocellular cancer in the explanted liver: outcome of transplantation and risk factors for HCC recurrence. *Eur J Surg Oncol* 2007;33:868-73.
27. Eini H, Frishman V, Yulzari R, Kachko L, Lewis EC, et al. Caffeine promotes anti-tumor immune response during tumor initiation: involvement of the adenosine A2A receptor. *Biochem Pharmacol* 2015;98:110-8.

Review

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Animal models for hepatocellular carcinoma arising from alcoholic and metabolic liver diseases

Ken Liu^{1,2,3}, Jinbiao Chen^{2,3}, Geoffrey W. McCaughan^{1,2,3}

¹A.W. Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Sydney, NSW 2050, Australia.

²Sydney Medical School, University of Sydney, Sydney, NSW 2050, Australia.

³Liver Injury and Cancer Program, Centenary Institute, Sydney, NSW 2050, Australia.

Correspondence to: Dr. Geoffrey W. McCaughan, MBBS PhD FRACP FAHMS, Head of Liver Injury and Cancer Program, Centenary Research Institute, A.W. Morrow Professor of Medicine, Director A.W. Morrow Gastroenterology and Liver Centre, Director Australian Liver Transplant Unit, Royal Prince Alfred Hospital, University of Sydney, Sydney, NSW 2050, Australia. E-mail: g.mccaughan@centenary.usyd.edu.au

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Abstract

Hepatocellular carcinoma (HCC) is a major and increasing cause of clinical and economic burden worldwide. Now that there are effective therapies to control or eradicate viral aetiologies, the landscape of HCC is changing with alcoholic and metabolic liver diseases becoming major catalysts. The pathogenesis of HCC is complex and incompletely understood, hampering improvements in therapy. Animal models are essential tools for advancing study on the cellular and molecular processes in HCC and for screening potential novel therapies. Many models of hepatocarcinogenesis have been established using various methods including genetic engineering, chemotoxic agents and dietary manipulation to direct implantation of tumour cells. However, none of these can accurately replicate all features found in human diseases. In this review, we provide an overview of different mouse models of HCC with a particular focus on cancer arising from alcoholic liver disease, non-alcoholic fatty liver disease and hereditary haemochromatosis. We also highlight their strengths and limitations and provide perspectives for future study.

Keywords: Hepatocellular carcinoma, animal models, mouse models, non-alcoholic fatty liver disease, alcohol, haemochromatosis



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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and ranks as the fifth most common incident cancer and the fourth most common cause of cancer-related death worldwide. Major causes for HCC include chronic liver disease such as infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD)^[1]. Over 80% of the world's HCCs are found in less developed countries due to the influence of chronic HBV infection; however, the incidence and mortality are largely decreasing in these regions due to immunisation and antiviral therapy^[2]. Instead, the burden of HCC is increasing in Western or developed countries due to the rise of NAFLD-associated HCC^[3,4]. Indeed, NAFLD has either already become or is on the verge of becoming the leading cause of HCC in most Western countries^[5-8]. Alarming, even in non-Western countries where viral hepatitis-related HCC predominates, the proportion of patients with HCC due to NAFLD is increasing at an exponential rate^[9,10]. Moreover, with no effective pharmacologic agents to date, the burden of NAFLD is expected to rise further in the future.

The epidemiology of HCC in the context of ALD is poorly captured with heterogeneous geographic distribution^[11]. However, current data show alcohol accounts for 21% of HCC cases globally, making it the third leading cause (behind HBV and HCV) and the leading cause in many regions^[12]. The age-specific incidence rates for ALD-related HCC are also increasing.

Alongside NAFLD, hereditary haemochromatosis (HH) is another metabolic liver disease impacted by HCC which deserves special mention. HCC accounts for up to 28%-45% of deaths in HH patients and the relative risk of HCC development in those with cirrhosis is greater than 200^[13]. HCC has also been described in HH patients without cirrhosis. Furthermore, iron has been implicated as a cofactor for HCC development in other liver diseases such as NAFLD^[14].

Therefore, with continuing improvements in global HBV vaccination coverage and effective therapies to control HBV and eradicate HCV, alcohol and metabolic liver diseases will take their place as the major contributors of hepatocarcinogenesis in the coming decades.

WHY DO WE NEED ANIMAL MODELS?

The biology of HCC is complex and incompletely understood with no single dominant molecular pathology. However, therapeutic approaches for primary intervention over the past ten years have resulted in numerous negative randomised controlled trials^[15,16]. The current approved therapies for advanced disease prolong survival by only 2-3 months^[17]. Thus, new targets for therapies are urgently needed.

Unlike other cancers, HCC can be diagnosed by imaging criteria alone and few patients (< 30%) are eligible for curative surgical resection or liver transplantation^[18]. This has limited the availability of human HCC tissue samples for study. Indeed, the large number of human studies that have classified human HCC at the molecular level have almost exclusively used tissue from relatively early HCC obtained at hepatic resection or transplantation. Thus, animal models of more advanced HCC have proved to be crucial for investigating the genetic alterations, signalling pathways and microenvironment interactions involved in hepatocarcinogenesis. Importantly, they also allow for the evaluation of potential novel treatment paradigms and drugs in preclinical trials.

Although many animal models of HCC exist, this review focuses on mouse (*Mus musculus*) models, which are considered some of the best animal models for studying HCC owing to their compact size, short lifespan, breeding capacity and physiologic and genetic similarities to human biology^[19]. After a brief overview of HCC mouse models, the review concentrates on mouse models for HCC arising from ALD, NAFLD and HH.

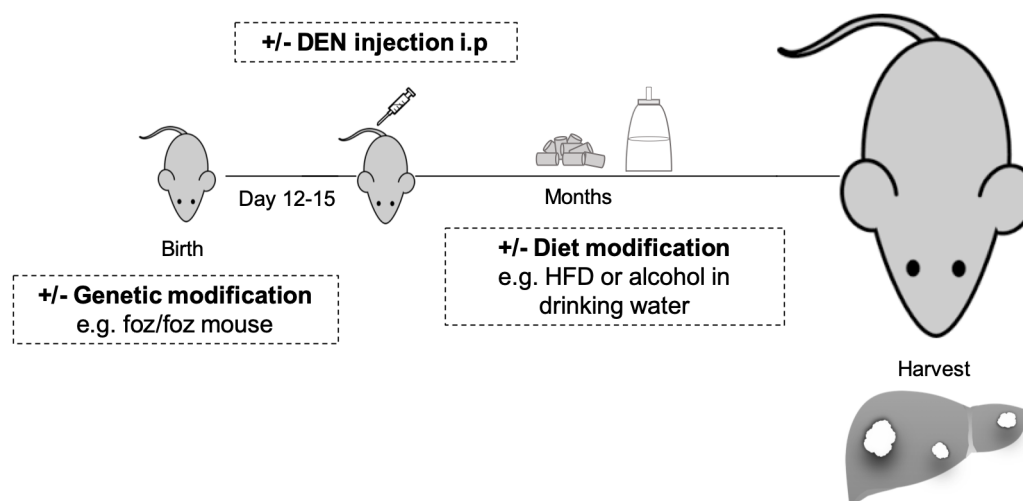


Figure 1. Examples of methodology used in mouse liver cancer models. DEN: di-ethyl-nitrosamine; HFD: high fat diet; i.p: intraperitoneal

MOUSE MODELS FOR HCC (GENERAL APPROACHES)

Hepatocarcinogenesis can be achieved through several different strategies either alone or in combination [Figure 1].

Genetically engineered mouse models

The most prevalent genetic mutations in human HCC are in the promoter region of *TERT* (60%), *TP53* (20%-30%), *CTNNB1* (15%-25%), *ARID1A* (10%-16%) and *AXIN1*, while genes commonly mutated in other solid tumours such as *EGFR*, *PIK3CA* or *KRAS* are rarely mutated in HCC (< 5%)^[20]. Various genetically engineered mouse (GEM) models have been created to reproduce these molecular features of human HCC. These models which result in activation of oncogenes or inactivation of tumour suppressor genes can be achieved via several different mechanisms including microinjection of recombinant DNA into the pronucleus of an embryo, lentiviral transduction in embryonic stem cells, homologous recombination in stem cells, conditional mutagenesis (e.g., Cre/loxP recombination system), knockdown using RNA interference and more recently genome editing with programmable endonucleases (e.g., CRISPR/Cas9 system). Liver-specific GEM models have also been created using the latter two techniques, for example with Albumin-Cre and hydrodynamic injection of plasmids, respectively^[21]. Genetic modifications can also be used to produce mouse phenotypes that represent specific aetiologies of human metabolic liver diseases such as obese mice (e.g., ob/ob, db/db and foz/foz) to study NAFLD-related HCC or *HFE* knockout mice to study HCC in the setting of HH^[22,23].

However, the use of GEM models alone cannot recapitulate human disease. Firstly, there is no single dominant molecular pathology underlying all HCCs but rather several pathways involved^[24]. Sequencing of cancer genomes had revealed that a typical cancer initiating cell accumulates at least 2-8 driver mutations^[25]. However, GEM models are generally limited to one specific driver mutation^[21,26], hence restricting models to study only specific genes or pathways in hepatocarcinogenesis. Secondly, GEM models typically lack chronic liver injury and fibrosis and HCCs develop in almost normal livers (with the notable exception of *MDR2* knockout mice). Despite this, these models have a role in providing evidence that powerful causation effects can be seen particularly following genetic ablation of key tumour suppressor genes or over amplification of oncogenic proteins. Examples of the former include liver-specific knockout of *p53* (AlfpCre⁺ Trp53^{Δ2-10/Δ2-10} mice), *PTEN* (AlbCrePten^{flox/flox}) or both^[27-29]. Conversely, overexpression of oncogenes such as *MYC* and *E2F1* alone or synergistically in combination can also drive hepatocarcinogenesis^[30,31]. Recently, Ruiz de Galarreta *et al.*^[32] were able to generate liver tumours with both *MYC* overexpression and *TP53* depletion by hydrodynamic tail-vein injections of a transposon vector expressing *MYC* and a CRISPR/Cas9 vector expressing a single-guide RNA targeting *Trp53* into C57BL/6

mice. Another method is the use of stem cell transduction, which involves retroviral infection of hepatic progenitor cells isolated from foetal livers of mice to introduce oncogenes or target tumour suppressors into a healthy liver^[21]. Manipulation of key genetic pathways in combination with liver injury models described below has now been advocated to achieve a more realistic representation of human HCC^[26].

Chemically- or diet-induced models

Several chemotoxins can induce hepatocarcinogenesis by causing direct DNA damage (genotoxic) or promoting clonal expansion of preneoplastic cells (non-genotoxic). Di-ethyl-nitrosamine (DEN) is the most widely used genotoxic drug for chemically-induced HCC. Once bioactivated by cytochrome P450, DEN becomes an alkylating agent leading to the formation of mutagenic DNA adducts while also generating reactive oxygen species (ROS) which damage DNA, overall resulting in hepatocyte death. Similar to what occurs in humans, subsequent cycles of necrosis and regeneration in the mouse liver promote mutations, neoplastic transformation and eventual HCC development^[33]. Indeed, DEN tumours have consistently exhibited high mutation rates^[34]. DEN is most effective at inducing HCC when injected intraperitoneally into young male mice (less than two weeks old) when hepatocytes are still proliferating. Commonly used non-genotoxic carcinogens include carbon tetrachloride (CCl₄) and thioacetamide (TAA). These agents act as tumour promoters by damaging cellular structures, increasing the risk of genetic error and stimulating cell malignant transformation by affecting proliferation, differentiation and apoptosis processes^[24]. CCl₄ is a potent hepatotoxin which causes centrilobular liver damage by the production of ROS and peroxidative degradation of phospholipids in plasma, lysosomal and mitochondrial membranes. Prolonged exposure (via oral, intraperitoneal or inhaled routes) leads to liver inflammation, fibrosis, cirrhosis and HCC development. TAA is another centrilobular hepatotoxin which can be administered via intraperitoneal injections or adding it to drinking water. It is bioactivated by mixed-function monooxygenases leading to its S-oxide and highly reactive S,S-dioxide, which modifies amine-lipids and proteins to initiate cellular necrosis^[24]. The carcinogenic effects of all chemically-induced HCC models vary with age, mouse strain and sex. Ethanol feeding models are discussed in the ALD-associated HCC models section.

Diet-based models are most commonly used to study fatty liver diseases, particularly NAFLD and less so ALD. Mice are usually fed *ad libitum* with one of the following diets: high-fat diet (HFD), high-fat high-cholesterol (HFHC), methionine and choline-deficient diet (MCD), choline-deficient high-fat diet (CD-HFD), choline-deficient L-amino acid-defined (CDAA) diet or a Western diet (WD). Although these models can reliably produce steatosis, inflammation and even fibrosis, not many of them will result in HCC development after a prolonged period^[22]. Furthermore, not all models reliably reproduce the accompanying metabolic features of the disease such as obesity and insulin resistance^[21]. For example, a major drawback of the MCD model is that mice exhibit the opposite of the human metabolic syndrome with weight loss, no insulin resistance and low serum glucose, triglyceride and cholesterol. The specifics of these diet-based models and their combinations are further discussed in the NAFLD-associated HCC models section.

Implantation models

Human or murine HCC cell lines can be injected into recipient mice to form orthotopic tumours (intrahepatic, intrasplenic or intraportal injection) in the liver or heterotopic tumours (subcutaneous injection) typically in the flank. The main advantages of implantation models are their quick time to develop visible tumours (weeks to months in spontaneous models) that are easy to measure (especially subcutaneous heterotopic tumours) and reproducible - making them popular models for drug screening. This is counterbalanced by disadvantages such as considerable differences between cell lines necessitating multiple cell lines to be tested, the lack of tumour-liver microenvironment interactions in heterotopic models and the need for surgical expertise for orthotopic models^[21]. Implantation of human cells (xenograft models) requires immunocompromised mice to prevent rejection of these foreign cells while murine cells can be implanted into immunocompetent mice (syngeneic/allograft models). Mouse tumour cell lines harbour mutations that are neutral or not relevant in human cancer making xenograft models more

genetically applicable to human disease^[35]. Patient-derived xenograft (PDX) models in which cells from a specific patient with HCC are transplanted into immunocompromised mice have been established^[36]. PDXs faithfully recapitulate histologic, genomic and biological characteristics of the primary tumour and have been shown to predict drug response in HCC patients. However, this model is limited by engraftment failure rates of up to 60%, long time to engraftment (several months) and high cost, which make it unsuitable for large-scale drug screening^[36,37]. Furthermore, the major drawback of xenograft models (PDX or otherwise) is the lack of a tumoural immune response, which has become increasingly important as we enter the era of immunotherapies for HCC. Attempts at overcoming this with double humanised mouse models which express human hepatocytes and haematopoietic stem cells (and hence human immune cells) are technically intensive, expensive and not yet widely adopted^[21]. Finally, xenograft of human HCC models which develop metastases (more readily than GEM models) have been established, providing the opportunity to study late-stage disease^[38].

Replicability in human disease

Human HCCs are highly complex and heterogeneous and thus cannot be adequately represented by any single mouse model. For example, gene expression profiles of tumours from the commonly-used DEN model was previously shown to be most similar to a subgroup of human HCC with poorer survival^[39,40]. Correspondingly, many poor prognostic markers in human HCC are also highly expressed in DEN-induced tumours, e.g., alpha-fetoprotein (AFP). However, the DEN model lacks other hallmarks of human HCC, particularly fibrosis in the surrounding microenvironment^[41]. In a more recent integrative genomic analysis of four separate mouse models and 987 human HCC samples, DEN tumours were found to be histologically hard to classify and least similar to human disease while Stelic Animal Model (STAM) tumours (discussed further in the NAFLD-associated HCC models section) were most molecularly similar to human HCC, especially high-grade, proliferative tumours with poor prognosis^[34]. The authors further argued that DEN models should be avoided since they are dominated by mutational mechanisms not seen in human HCC. In contrast to DEN-induced and STAM tumours, *MDR2* knockout tumours are most similar to human HCCs associated with better survival^[42]. However, the *MDR2* knockout model produces a phenotype resembling humans with primary sclerosing cholangitis or primary biliary cholangitis rather than chronic “hepatitis” diseases caused by alcohol excess and HBV or HCV infection^[43]. Very recently, experimental hepatocyte-specific activation of β -catenin also resulted the development of a phenotype that resembled the low proliferative subclass of human HCC^[32]. Interestingly these tumours also had few intratumoural immune cells and were resistant to immune checkpoint inhibitor therapy. Therefore, it is clear that different models (and their combinations) are required to simulate specific subgroups of human HCC. Furthermore, drugs with known anti-tumour activity against human HCC do not demonstrate activity in some animal models and vice versa. Indeed, the current Food and Drug Administration-approved drugs for treating advanced HCC such as sorafenib and anti-programmed cell death receptor 1 (PD1) antibodies were trialled based on success in other cancers (advanced renal cell carcinoma and melanoma, respectively) rather than positive results in HCC animal models *per se*.

SPECIFIC MOUSE MODELS FOR ALCOHOLIC AND METABOLIC LIVER DISEASE-

ASSOCIATED HCC

ALD-associated HCC models

In general, mice and other species (except the golden hamster) dislike alcohol and avoid ingestion when it is offered *ad libitum*^[21,44]. Therefore, ALD mouse models are established by one of three ways: (1) replacing the food and water source with a liquid diet in which 5% ethanol accounts for 36% of total calories (Lieber-DeCarli model); (2) binge feeding mice with ethanol via gavage in addition to chronic ingestion [National Institute of Alcohol Abuse and Alcoholism (NIAAA) model]; or (3) intragastric ethanol infusion via a surgically inserted infusion pump (Tsukamoto-French model) [Table 1].

Table 1. Examples of mouse models for hepatocellular carcinoma associated with alcoholic and metabolic liver diseases

Mouse model*	Strain	Genetic modification	Diet manipulation	Chemotoxin	HCC development	Comments
Alcohol HCC models						
Alcohol + DEN Ambade <i>et al.</i> ^[48]	C57BL/6 male	-	4% Lieber-DeCarli alcohol diet from Week 9	DEN 75 mg/kg i.p. weekly (Weeks 4-6), then 100 mg/kg weekly (Weeks 7-9)	Hepatic lesions with elevated AFP and cellular proliferation	Hepatic hyperplasia described rather than histological HCC Alcoholic hepatitis and fibrosis in non-tumour tissue
Alcohol + DEN Brandon-Warner <i>et al.</i> ^[50]	B6C3 male and female	-	10/20% (v/v) ethanol drinking water on alternate days at Week 16 or 40 for 8 weeks	DEN 1 mg/kg i.p. single dose old	No tumours at 24 weeks At 48 weeks, tumours displayed in: Males 93.7% Females 35.7%	Ethanol significantly increased tumour burden in male but not female DEN-treated mice Liver injury and fibrosis in non-tumour tissue
Alcohol + DEN + CCl ₄ Xin <i>et al.</i> ^[53]	BALB/c male	-	9% ethanol drinking water from Week 9	DEN 100 mg/kg i.p. single dose at Week 7 DEN 50 mg/kg gavage single dose at Week 9 CCl ₄ gavage 5 mL/kg twice weekly at Week 7, increased to 8 mL/kg at Week 10	100% moderate to well differentiated tumours at Day 150	7.5% mortality rate Hepatitis at Day 60, fibrosis at Day 90, 100% cirrhosis at Day 120 in non-tumour tissue
NASH HCC models						
HFD Wolf <i>et al.</i> ^[55]	C57BL/6 male and female	-	HFD from Week 4	-	1/40 (2.5%) displayed tumour at 12 months	WG, IR and steatosis but no inflammation or fibrosis HCC not described in other single diets, e.g., MCD, CD and WD
CD-HFD Wolf <i>et al.</i> ^[55]	C57BL/6 male and female	-	CD-HFD from Week 4	-	19/75 (25%) displayed tumours at 12 months	Similar rate of tumours in male and female mice WG, IR, steatosis, inflammation and fibrosis present
CDA De Minicis <i>et al.</i> ^[56]	C57BL/6J male	-	CDA from Weeks 6-8	-	30% displayed tumours at 6 months 35% displayed tumours at 9 months 89% displayed tumours at 32-52 weeks	WG, IR, steatosis, inflammation and fibrosis present 100% had well-differentiated tumour foci, 40% had poorly differentiated tumours
DIAMOND Asgharpour <i>et al.</i> ^[59]	B6/129 male	-	WD + high-fructose-glucose water from Weeks 8-12	-		WG, IR, steatosis, inflammation and fibrosis present
foz/foz mouse + DEN Arfianti <i>et al.</i> ^[69]	NOD.B10 male	foz/foz (<i>Alms1</i> mutant)		DEN 10 mg/kg i.p. single dose on Days 12-15	100% displayed tumours at 6 months (2 had lung metastases)	WG, IR and steatosis and inflammation present
HFD + DEN Park <i>et al.</i> ^[62]	C57BL/6J male and female	-	HFD from Week 6	DEN 25 mg/kg i.p. single dose at Week 2	At 9 months, tumours displayed in: Males 100% Females 80%	WG, IR and steatosis present
CD-HFD + DEN Kishida <i>et al.</i> ^[61]	C57BL/6J male	-	CD-HFD from Week 3	DEN 25 mg/kg i.p. single dose at Week 3	20% displayed tumours at 16 weeks 100% displayed tumours at 20 weeks	Mean size 2.9 mm at 24 weeks WG, IR, steatosis, inflammation and fibrosis present

HFHC + DEN Liang <i>et al.</i> ^[63]	C57BL/6J male	-	HFD or HFHC from 6 weeks	DEN 25 mg/kg i.p. single dose at Week 3	At 8 months, tumours displayed in: HFD 90% HFHC 100%	Increased size and multiplicity in HFHC- fed mice compared with HFD-fed mice WG, IR, steatosis and inflammation present
CDA + CCL ₄ De Minicis <i>et al.</i> ^[56]	C57BL/6J male	-	CDA from Weeks 6-8	CCL ₄ 0.2 xL/g i.p. weekly from Weeks 6-8	30% displayed tumours at 6 months 100% displayed tumours at 9 months	Median diameter of tumours = 9 mm WG, IR, steatosis, inflammation and fibrosis present
HFD + DEN + CCL ₄ Henderson <i>et al.</i> ^[64]	C57BL/6J male	-	HFD from Week 4	DEN 25 mg/kg i.p. single dose at Day 14 TAA 300 mg/L in drinking water from Week 4	83% displayed tumours at 24 weeks	Weight loss, steatosis, inflammation and fibrosis present
STAM Fujii <i>et al.</i> ^[66]	C57BL/6 male and female	-	HFD from Week 4	STZ 200 xg s.c. at Day 2	100% displayed tumours at 20 weeks. No female mice developed tumours	WG, diabetes (no IR), steatosis, inflammation and fibrosis present
<i>PTEN</i> knockout Horie <i>et al.</i> ^[28]	C57BL/6J male and female	Hepatocyte- specific <i>PTEN</i> knockout: AlbCre ^{fl} PTen ^{fl} /fl ^{ox}	-	-	At 74-78 weeks, tumours displayed in: Males 66% Females 30%	Insulin hypersensitivity, steatosis and inflammation but no fibrosis present Other examples of single gene knockout NASH-HCC models include: <i>Agouti</i> , <i>PPARα</i> , <i>AOX</i> and <i>MATTA</i> knockout mice
Leptin deficiency (Ob) DEN Park <i>et al.</i> ^[62]	C57BL/6J male	Leptin deficient genetically obese (Ob)	-	DEN 25 mg/kg i.p. single dose at Week 2	100% displayed tumours at 9 months	WG, IR and steatosis present.
MUP-uPA Nakagawa <i>et al.</i> ^[71]	C57BL/6 male	MUP-uPA transgene	HFD from Week 6	-	78.6% displayed had tumours > 2 mm, and 35.7% displayed tumours > 10 mm at 40 weeks	WG, IR, steatosis, inflammation and fibrosis present
Haemochromatosis HCC models						
β2-microglobulin knockout Rothenberg <i>et al.</i> ^[76]	C57BL/6 male and female	β2- microglobulin knockout	Added 10 mg ferrous sulphate/ kg normal chow from 8-15 months for 6-8 months	-	4/13 (30.7%) displayed tumours at 1-2 years	Iron overload seen in the liver with sinusoidal fibrosis
<i>FBXL5</i> knockout Muto <i>et al.</i> ^[77]	C57BL/6 male and female	Hepatocyte specific <i>FBXL5</i> knockout: Alb- Cre/ <i>Fbxl5</i> ^{fl} /fl ^{ox} mice	-	DEN 25 mg/kg i.p. single dose at Day 15	At 36 weeks, tumours displayed in: Males 95% Females 75%	Iron overload seen in the liver

*List of models presented are not intended to be exhaustive but to give representative examples of different combinations of methods to achieve hepatocarcinogenesis in alcoholic and metabolic liver diseases. AFP: alpha-fetoprotein; CCL₄: carbon tetrachloride; CD: choline deficient; CD-HFD: choline-deficient high-fat diet; CDA: choline-deficient L-amino acid-defined; DEN: di-ethyl-nitrosamine; HCC: hepatocellular carcinoma; HFD: high-fat diet; i.p.: intraperitoneal; IR: insulin resistance; MCD: methionine and choline-deficient diet; NASH: non-alcoholic steatohepatitis; s.c.: subcutaneous; STAM: stelic animal model; STZ: streptozotocin; TAA: thioacetamide; WD: Western diet; WG: weight gain

Aside from their natural aversion to alcohol, mice metabolise alcohol five times faster than humans^[45]. As a result, the aforementioned ALD mouse models tend to exhibit less liver injury than seen in human disease^[21]. The Lieber-DeCarli model induces mild steatosis with little to no inflammation or fibrosis. The technically demanding Tsukamoto-French model produces severe steatosis but only mild inflammation and mild fibrosis. Although chronic or binge ethanol feeding regimens cause minor liver changes by themselves, their combination in the NIAAA model synergistically induces more severe steatosis and inflammation, with only mild chicken-wire fibrosis^[46].

Many have studied liver injury patterns of ALD mouse models; however, few have examined hepatocarcinogenesis specifically. As described above, the mild severity of liver inflammation and fibrosis induced by standalone mouse models of ALD means HCCs do not develop spontaneously. Therefore, a “second-hit” usually consisting of a chemical hepatotoxin is required for progression of ALD to cirrhosis and/or HCC. Indeed, of the hepatotoxins, DEN-induced C57BL/6 tumours have recently been matched to most resemble alcohol-induced HCC both morphologically and by comparative genomic hybridisation in a study comparing five different HCC models with human data^[47]. Ambade *et al.*^[48] established a model of alcohol-driven HCC in adult C57BL/6 male mice. The four-week-old mice were administered six doses of DEN (or saline) intraperitoneally (75 mg/kg weekly for three weeks and then 100 mg/kg weekly for three weeks) followed by the Lieber-DeCarli diet (or calorie-matched control diet) for seven weeks before sacrifice at 15 weeks. Compared to mice fed with a control diet, alcohol-fed mice had greater liver inflammation (raised alanine aminotransferase) and fibrosis. The alcohol-fed group also exhibited numerous liver nodules of hepatic hyperplasia associated with increased AFP expression and cellular proliferation, which the authors thought represented signs of early hepatocarcinogenesis. There were no hyperplastic nodules seen in the alcohol-fed saline-injected group or the control-fed DEN-injected group, thus confirming the need for a second stressor to initiate hepatocarcinogenesis. Early precancerous lesions were also described in another model using the combination of DEN and alcohol diet^[49]. In this study, male C57BL/6 mice were injected intraperitoneally with DEN (25 mg/kg) at two weeks of age and then fed with the Lieber-DeCarli diet at eight weeks of age for 21 days. Over half of DEN-injected alcohol-fed mice developed precancerous basophilic foci compared to none in the DEN-injected control diet group. Interestingly, dietary luteolin (a flavonoid with anti-cancer properties) co-administration completely abrogated the development of precancerous lesions potentially by restoring sirtuin 1 activity and increasing downstream proliferator-activated receptor gamma coactivator 1 alpha protein expression. In a longer model, Brandon-Warner *et al.*^[50] studied DEN-injected alcohol-fed B6C3 mice for 48 weeks and observed tumours in 94% and 36% of males and females, respectively. While chronic ethanol feeding exacerbated tumour formation in DEN-injected males, fewer and smaller tumours were observed in females exposed to ethanol compared to DEN-injected control-fed mice of respective sexes. Further analysis of liver mRNA revealed elevated SMAD3 in male compared to female mice in response to liver injury from DEN and alcohol, suggesting that increased TGFβ-SMAD3 signalling may enhance HCC promotion. Indeed, gender disparity (males > females) in liver cancer both in humans and in DEN-injected mice is well-recognised and may be related to sex differences in MyD88-dependent IL-6 production mediated by the protective effect of oestrogen^[51].

The combination of alcohol and CCl₄ has also been experimented, although predominantly in rats. Weekly injections of CCl₄ and alcohol administration through drinking water led to HCC after 104 weeks in mice^[52]. The impact of chemical carcinogens on HCC formation appears to be additive. Recently, Xin *et al.*^[53] combined DEN (100 mg/kg intraperitoneal and 50 mg/kg gavage once each), CCl₄ twice weekly and 9% alcohol as drinking water together in adult (seven-week-old) BALB/c mice. Multifocal HCC was noted only five months (150 days) after DEN injection. Tumours were moderate to highly differentiated and secreted AFP, resembling human HCC. Furthermore, there was no evidence of toxicity in this model as these mice survived until sacrifice.

NAFLD-associated HCC models

Many models have been developed to represent NAFLD and non-alcoholic steatohepatitis (NASH), although, as aforementioned, not all of them exhibit features of metabolic syndrome. This is particularly important in NAFLD-related HCC since the presence of obesity and/or diabetes are themselves independent risk factors for the development of cancer^[54].

Most dietary models of NAFLD (HFD, HFHC, MCD, WD and CD diet) rarely induce HCC development alone^[22]. If spontaneous HCC does occur, it is time-consuming (e.g., 2.5% for C57BL/6 mice fed HFD for 12 months)^[55]. Combination diets such as CD-HFD and CDAA have been shown to significantly increase rates of tumour formation, although overall rates are still low: 25% after 12 months and 35% after 9 months, respectively^[55,56]. Indeed, these diets can recapitulate the key features of human NASH (including fibrosis) and metabolic syndrome more so than single diets. Susceptibility to tumour formation in dietary models also appears to be strain-dependent with DBA/2J > C57BL/6 > A/J^[57,58]. Asgharpour *et al.*^[59] generated an isogenic strain (B6/129) derived from a cross of two common mouse strains, C57BL/6J and 129S1/SvImJ, and fed them a high-fat-high-carbohydrate diet with high-fructose-glucose water - so-called DIAMOND mice. This promising model mimicked all the physiological, metabolic, histological and transcriptomic gene signature and clinical endpoints of human NASH including HCC in 89% at 32-52 weeks. These tumours had gene signatures which strongly resembled the S1 and S2 human subclasses of HCC. Interestingly, neither C57BL/6J nor 129S1/SvImJ parent strain mice fed with the same diet developed HCC.

Combining dietary models with a hepatotoxin substantially hastens and increases HCC formation (i.e., up to 100% of male C57BL/6 mice fed CDAA, HFD, CD-HFD or WD + intraperitoneal injections of DEN or CCl₄ at 6-9 months) as well as tumour size^[56,60-62]. The addition of cholesterol to a HFD (HFHC) in a DEN-induced model appears to further increase tumour burden^[63]. In another model, Henderson *et al.*^[64], treated male C57BL/6 mice with DEN (25 mg/kg once at 14 days old), TAA (300 mg/L in drinking water *ad libitum* from four weeks old) and HFD. These agents acted synergistically to develop HCCs in 83% of mice as early as 24 weeks of age, which was significantly more than control mice or those treated with DEN and TAA only. However, combining with hepatotoxins needs to be tempered by some limitations. For example, use of CCl₄ can induce liver metabolism enzymes (which may impact the use of this model for drug discovery) and also mitigate metabolic processes involved in NASH, particularly susceptibility to diet-induced obesity and insulin resistance^[65]. As mentioned above, the STAM mouse was recently shown to be the mouse model (out of four studied) that most closely resembles human HCC at a molecular level. Specifically, STAM tumours carried mutations of CTNNB1 at a rate comparable to human tumours, and (less frequently) mutations of TP53 - the most frequently altered genes in human HCC^[34]. In contrast, CTNNB1 and TP53 were rarely mutated in DEN-induced tumours, which instead carried *Hras*, *Braf* and *APC* mutations rarely seen in human HCC. The STAM combination involves first treating neonatal C57BL/6 male mice with low-dose streptozotocin (STZ) at Day 2, which induces diabetes by causing death of pancreatic β cells, resulting in lean mice with hypoinsulinaemia and hyperglycaemia, but no insulin resistance (the phenotype of type 1 diabetes)^[66]. STZ is also a DNA alkylating agent (similar to DEN) with potential carcinogenic effects^[67]. When these mice are then fed with HFD, they develop weight gain, NASH by eight weeks, cirrhosis and HCC relatively quickly by 16-20 weeks^[66]. Takakura *et al.*^[68] characterised STAM tumours at 20 weeks by clinical parameters used in human liver disease [i.e., Child-Turcotte-Pugh score and dynamic contrast-enhanced computed tomography (CT) measurements of HCCs]. Interestingly, the authors deduced that STAM mice had cirrhosis corresponding to Child-Turcotte-Pugh Class B (significant coagulopathy, occasional ascites, no encephalopathy and normal albumin and bilirubin) and tumours equivalent to Barcelona Clinic Liver Cancer Stage B (intermediate) or C (advanced) disease in humans. No HCCs develop when STZ is given alone, again pointing to the need for an additional stimulus. Female mice treated with the STAM regimen also fail to develop tumours, akin to the gender disparity seen in other models.

Genetically obese mice with metabolic syndrome such as ob/ob (leptin deficient) db/db (leptin-receptor deficient), and foz/foz (mutated *Alms1* gene) promote tumourigenesis in the presence of a secondary insult (e.g., DEN) but do not otherwise develop HCC spontaneously^[62,67,69]. Furthermore, ob/ob and db/db mice fail to develop significant liver fibrosis or NASH histology without the addition of one of the dietary models above^[21]. Park *et al.*^[62] utilised a dietary (HFD) and genetic (ob/ob) obesity model in combination with DEN to show that obesity (no matter how it was achieved) promoted the development of DEN-induced HCC in C57BL/6 mice by enhanced production of the pro-inflammatory cytokines IL-6 and TNF. Many other genetic models have been developed to study NAFLD-associated HCC including *PTEN* knockout, *PPARα* knockout, *AOX* knockout, *KK-Ay/a* (agouti gene mutation) and *MAT1A* knockout mice. While they all reliably form HCC, they fail to recapitulate NASH itself (in *KK-Ay/a* mice) or its associated aspects such as obesity and metabolic syndrome (in *PTEN*, *PPARα*, *AOX* and *MAT1A* knockout mice)^[21,62,67]. As an example, *PTEN* knockout mice (which develop tumours between 40 and 78 weeks) are hyper-responsive to insulin instead of being insulin resistant. Unsurprisingly, gene expression signatures from *PTEN* knockout mice are markedly different from that of other NASH mouse models^[28,70]. One promising genetic model of NASH-driven HCC is the MUP-uPA transgenic mouse combined with HFD^[71]. MUP-uPA mice express high amounts of urokinase plasminogen activator in hepatocytes leading to hepatocyte-specific endoplasmic reticulum stress and liver damage. These mice exhibited weight gain, insulin resistance, classic signs of NASH (steatosis, inflammation, ballooning), fibrosis and, importantly, spontaneous HCC in 80% at 40 weeks via processes dependent on TNF produced by inflammatory liver macrophages^[71]. As expected, HFD-fed wild type mice developed simple steatosis and no HCC over the same period. Furthermore, transcriptomic data from MUP-uPA mice and human NASH datasets showed signalling similarities, especially in the regulation of the immune system, innate immune response and the response to cytokine gene sets^[67]. Recently, Shalapour *et al.*^[72] used both MUP-uPA and STAM mice fed with HFD to make a landmark discovery that hepatocarcinogenesis in NASH was facilitated by immunosuppressive liver-resident IgA⁺ plasma cells, which directly inhibit anti-tumour cytotoxic CD8⁺ T lymphocyte activation.

Of the models mentioned above, it seems the MUP-uPA and DIAMOND mice (which require a combination of genetic modification and dietary manipulation) best replicate NASH-associated HCC. However, tumour formation in these models requires lengthy periods and there is considerable heterogeneity in their mutational landscapes which may limit utility and reliability in some settings, e.g., drug development studies^[67]. Although STAM mice can develop tumours more quickly than these models (20 weeks *vs.* 40 weeks), they are physiologically less similar to human NASH (lacking insulin resistance).

HH-associated HCC models

Hepatocarcinogenesis arising from iron accumulation is thought to be secondary to oxidative DNA damage from ROS generated by free hepatic iron. This leads to a cycle of cell death, and compensatory proliferation, which favours the accumulation of mutations in hepatocytes and ultimately malignant transformation^[13,73]. Recreating this in an animal model is difficult. The most common form of HH is caused by mutations in the *HFE* gene. Although *HFE* gene knockout produces the phenotype of HH in mice, spontaneous liver tumours do not develop^[74]. In a dietary model where BALB/cJ male mice were fed *ad libitum* with chow supplemented with 3% carbonyl-iron, hepatic iron concentrations at 12 months were 13-fold that of normal chow-fed controls^[75]. No liver tumours developed; however, hepatocyte nuclei changes were observed (iron-containing ferritin inclusions, enlarged nucleus, increased mitotic index and abnormal mitotic figures), which may have represented preneoplastic changes. Rothenberg *et al.*^[76] created a model of HH by knocking out β2-microglobulin (the chaperone protein for *HFE*) in C57BL/6 mice and reported that spontaneous HCCs developed in only a minority (31%) of mice. Because tumour development was not predictable and time-consuming (taking up to two years), this model has not been widely used to study HH-related HCC. Recently, Muto *et al.*^[77] developed a novel model of HCC induced by iron overload by deleting the iron-sensing ubiquitin ligase FBXL5 specifically in hepatocytes and

exposure to DEN. Alb-Cre/Fbx15^{flax/flax} mice were injected with DEN (25 mg/kg) intraperitoneally at Day 15 and tumours were significantly increased in number and size compared to DEN-injected control mice at 36 weeks in both males and females. The study demonstrated FBXL5 deficiency led to a sequence of events (iron overload, oxidative stress, liver damage and regenerative proliferation), which, with the addition of DEN, gave rise to liver tumours with high mutational load. Previously, hepatocyte-specific FBXL5 deletion without the addition of DEN was shown to cause liver inflammation but not tumours. The authors went on to analyse FBXL5 mRNA expression in five different human HCC cohorts and found that low FBXL5 expression level was indeed strongly associated with poorer prognosis in human HCC. Finally, the impact of iron on hepatocarcinogenesis has also been evaluated using a xenograft model. In this study, 3-4-week-old female BALB/c athymic mice (nu/nu) were injected subcutaneously with human HCC cell lines (Hep3B or HepG2) and followed for 21 days^[23]. The authors showed that TSC24 (a potent iron chelator) suppressed tumour growth in a dose-dependent manner by reducing available iron, and triggering cell-cycle arrest and apoptosis.

THE ROLE OF THE GUT MICROBIOME

Increasingly, the role of the gut microbiome has been implicated in alcoholic and metabolic liver diseases and HCC via the gut-liver axis, which refers to bidirectional communication between the gut (and its microbiome) and the liver^[78]. In one direction, the liver secretes bile acids and antibodies into the intestine, which influences the gut microbiome composition. Reciprocally, the microbiome and its metabolites translocate the gut to reach the liver via the portal vein (the enterohepatic circulation) and regulate metabolic functions. This gut-liver axis exists in a homeostasis, which becomes disrupted in metabolic liver diseases.

Bacterial dysbiosis has been consistently demonstrated in the gut microbiomes of patients and mice with metabolic liver diseases and HCC^[79]. Mouse model studies have already revealed several mechanisms by which the gut microbiome contributes to HCC development.

Bacterial metabolism of compounds

In a model of NASH-associated HCC, Yoshimoto *et al.*^[80] induced HCC by treatment with a chemical carcinogen [dimethylbenz (a)anthracene] and HFD. The authors found a strong increase in Gram-positive bacteria (particularly *Clostridium spp.*) as well as levels of deoxycholic acid (DCA), a secondary bile acid whose production relies on metabolism of primary bile acids by bacteria such as *Clostridium*. Significantly, DCA was shown to promote a senescence-associated secretory phenotype in hepatic stellate cells, which leads to hepatocarcinogenesis via activation of the TLR2 pathway^[79,80].

Leaky gut

Increased levels of lipopolysaccharide in the systemic circulation (due to increased intestinal permeability) and its interaction with TLR4 have been demonstrated to promote HCC formation in a CD-HFD-fed NASH model as well as a chemotoxin model with combination DEN and CCl₄^[81,82]. This process can be abrogated by gut sterilisation with oral antibiotics, especially in late-stage disease.

Immunosuppressive microenvironment

The gut microbiome also modulates tumoural adaptive immune responses. The aforementioned study by Shalapour *et al.*^[72] showed that manipulating the gut microbiome in mice with NASH-driven HCC either by knocking out their polyimmunoglobulin receptor (which regulates IgA transport into the gut lumen and maintains microbial homeostasis) or giving them broad-spectrum antibiotics (which reduces gut bacterial load) promoted and inhibited HCC development, respectively. Both these interventions modulate liver and circulating IgA levels and hence anti-tumour cytotoxic T cell activation, as discussed above.

The gut microbiome (and its associated HCC risk) can be transmissible between mice and, interestingly, this risk can also be transferred via the microbiome across generation to offspring of treated mothers^[79,83]. This opens up another avenue to induce hepatocarcinogenesis alongside GEM, hepatotoxins and dietary manipulation in future models.

Thus, as we explore the new frontier of gut microbiome, animal models will be crucial for understanding causality, pathogenesis and testing of therapeutic options targeting the microbiome (e.g., antibiotics, probiotics, synthetic bile acids and faecal microbiota transplantation). Although mouse and human gut microbiome communities are dominated by the same set of bacterial phyla, they are on the whole distinct from one another^[78]. Therefore, experimental findings from microbiome studies in mouse models need validation in human studies. The emerging use of a humanised gnotobiotic model (human donor stool transplanted into germ-free mice) may also improve the applicability of preclinical findings^[84].

CURRENT CHALLENGES AND FUTURE DIRECTIONS

Although, as described above, there are many different animal models for HCC related to alcoholic and metabolic liver diseases, a single model faithfully recapitulating all features of human disease is lacking and unlikely to exist. This is partly because human HCC is genetically heterogeneous, consisting of several subtypes that are clearly different in behaviour, prognosis and response to treatment themselves. Clearly, the identification of models that represent different human HCC subsets is required. Yan *et al.*^[26] argued for combining a chronic injury model (e.g., NASH, CCl₄ or *MDR2* knockout) with alterations in oncogenes or tumour suppressor genes found in human HCC which alone are not sufficient to cause hepatocarcinogenesis (e.g., weak activation of pathways by heterozygous deletion or targeting only a small percentage of hepatocytes) to achieve a more realistic representation of human HCC. The optimal combinations for each aetiology are yet to be determined and will be an area of further research. When achieved, this would not only help improve our understanding of the pathobiology of aetiology-specific HCC but also improve our preclinical testing of new targeted treatments as we work towards personalised medicine. Humanised mouse models may be a bridge for translating findings from mouse studies to humans and presents a promising future strategy. However, several major challenges need to be overcome not the least of which is the engraftment of a humanised immune system.

The amount of time required for tumourigenesis is another obstacle, as most models take more than nine months to produce macroscopic HCCs. Furthermore, time is also needed to establish steatosis, inflammation, fibrosis and cirrhosis^[21]. While implantation HCC models are established within weeks, they are lacking these biologically important changes in the background liver. Indeed, human liver disease typically takes decades to progress to cirrhosis and HCC. For example, patients with NASH progress at a mean rate of only 0.09-0.14 fibrosis stages per year^[54]. Thus, the models most representative of human HCC may require the most time which is suboptimal for studying response to therapy.

At present, almost all mouse studies assess tumour size and number at the one time point of sacrifice; however, in clinical practice, HCC is diagnosed and monitored regularly using imaging (CT, magnetic resonance imaging and ultrasound). Although these imaging modalities give reliable measurements that correlate with tumour size at sacrifice, they are currently time-consuming and labour intensive (requiring scanners, anaesthesia and injection of intravenous contrast agents)^[21]. Since tumour development can be lengthy and their responses to treatment (especially new immunotherapies) are dynamic over time^[85], measurement of experimental tumours on imaging will likely play an increasingly important role in the future.

Recently, three-dimensional *in vitro* cell culture systems (organoids) using cells isolated from human biopsies have been developed to study HCC. These tumour organoids (tumouroids) have been shown to

recapitulate the histological architecture, expression profile, genomic landscape and *in vivo* tumourigenesis of the parental tumour, even after long-term (> 1 year) expansion in culture^[37]. Furthermore, tumouroids could be established within 2-3 months after isolation. Therefore, tumouroids fulfil many of the criteria for a reliable cancer model which animal models could not and may represent a promising advancement for understanding tumour biology and drug efficacy testing in future studies of HCC. However, they currently lack the human immune and stromal microenvironment that is thought to be crucial in understanding tumour progression and response to treatment, particularly immune-based therapies.

CONCLUSION

Alcoholic and metabolic liver diseases will be major contributors to HCC burden in the future. Many aspects of human HCC development and progression remain unknown, negatively impacting therapeutic advancement. Animal models play a crucial role in improving our understanding of human HCC and developing novel therapeutic strategies. Currently, no animal model can faithfully replicate the complexity of the cancer and its background liver disease but mere aspects of it with varying degrees of technical demand. The careful combination of different animal models and use of novel technologies such as human organoids may help bridge this gap in the future. For the time being, the use of HCC mouse models needs to be tailored to specific experimental hypothesis or clinical testing.

DECLARATIONS

Authors' contributions

Acquisition, analysis and interpretation of data, drafting of the article, critical revision of the article: Liu K, Chen J, McCaughan GW

All authors have read and approved the final version.

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Ethical approval and consent to participate

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Consent for publication

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REFERENCES

1. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011;365:1118-27.
2. Wallace MC, Preen D, Jeffrey GP, Adams LA. The evolving epidemiology of hepatocellular carcinoma: a global perspective. *Expert Rev Gastroenterol Hepatol* 2015;9:765-79.
3. Wong MC, Jiang JY, Goggins WB, Liang M, Fang Y, et al. International incidence and mortality trends of liver cancer: a global profile. *Sci Rep* 2017;7:45846.
4. Bertuccio P, Turati F, Carioli G, Rodriguez T, La Vecchia C, et al. Global trends and predictions in hepatocellular carcinoma mortality. *J*

- Hepatol 2017;67:302-9.
5. Rinella M, Charlton M. The globalization of nonalcoholic fatty liver disease: Prevalence and impact on world health. *Hepatology* 2016;64:19-22.
6. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013;10:686-90.
7. Ertle J, Dechène A, Sowa JP, Penndorf V, Herzer K, et al. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *Int J Cancer* 2011;128:2436-43.
8. Dyson J, Jaques B, Chattopadhyay D, Lochan R, Graham J, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014;60:110-7.
9. Cho EJ, Kwack MS, Jang ES, You SJ, Lee JH, et al. Relative etiological role of prior hepatitis B virus infection and nonalcoholic fatty liver disease in the development of non-B non-C hepatocellular carcinoma in a hepatitis B-endemic area. *Digestion* 2011;84:17-22.
10. Okanoue T, Umemura A, Yasui K, Itoh Y. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in Japan. *J Gastroenterol Hepatol* 2011;26:153-62.
11. Ganne-Carrié N, Nahon P. Hepatocellular carcinoma in the setting of alcohol-related liver disease. *J Hepatol* 2019;70:284-93.
12. Global Burden of Disease Liver Cancer Collaboration. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level results from the global burden of disease study 2015. *JAMA Oncol* 2017;3:1683-91.
13. Deugnier Y, Turlin B. Iron and hepatocellular carcinoma. *J Gastroenterol Hepatol* 2001;16:491-4.
14. Sorrentino P, D'Angelo S, Ferbo U, Micheli P, Bracigliano A, et al. Liver iron excess in patients with hepatocellular carcinoma developed on non-alcoholic steato-hepatitis. *J Hepatol* 2009;50:351-7.
15. Thillai K, Ross P, Sarker D. Molecularly targeted therapy for advanced hepatocellular carcinoma - a drug development crisis? *World J Gastrointest Oncol* 2016;8:173-85.
16. Nault JC. The end of almost 10 years of negative RCTs in advanced hepatocellular carcinoma. *Lancet* 2017;389:4-6.
17. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-90.
18. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018;391:1301-14.
19. Newell P, Villanueva A, Friedman SL, Koike K, Llovet JM. Experimental models of hepatocellular carcinoma. *J Hepatol* 2008;48:858-79.
20. Bruix J, Han KH, Gores G, Llovet JM, Mazzaferro V. Liver cancer: approaching a personalized care. *J Hepatol* 2015;62:S144-56.
21. Brown ZJ, Heinrich B, Gretchen TF. Mouse models of hepatocellular carcinoma: an overview and highlights for immunotherapy research. *Nat Rev Gastroenterol Hepatol* 2018;15:536-54.
22. Lau JKC, Zhang X, Yu J. Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances. *J Pathol* 2017;241:36-44.
23. Ba Q, Hao M, Huang H, Hou J, Ge A, et al. Iron deprivation suppresses hepatocellular carcinoma growth in experimental studies. *Clin Cancer Res* 2011;17:7625-33.
24. Santos NP, Colaço AA, Oliveira PA. Animal models as a tool in hepatocellular carcinoma research: a review. *Tumour Biol* 2017;39:1-20.
25. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz Jr LA, et al. Cancer genome landscapes. *Science* 2013;339:1546-58.
26. Yan J, Caviglia JM, Schwabe RF. Animal models of HCC - when injury meets mutation. *J Hepatol* 2018;68:193-4.
27. Liu Y, Qi X, Zeng Z, Wang L, Wang J, et al. CRISPR/Cas9-mediated p53 and Pten dual mutation accelerates hepatocarcinogenesis in adult hepatitis B virus transgenic mice. *Sci Rep* 2017;7:2796.
28. Horie Y, Suzuki A, Kataoka E, Sasaki T, Hamada K, et al. Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. *J Clin Invest* 2004;113:1774-83.
29. Katz SF, Lechel A, Obenauf AC, Begus-Nahrman Y, Kraus JM, et al. Disruption of Trp53 in livers of mice induces formation of carcinomas with bilineal differentiation. *Gastroenterology* 2012;142:1229-39.e3.
30. Santoni-Rugiu E, Jensen MR, Thorgeirsson SS. Disruption of the pRb/E2F pathway and inhibition of apoptosis are major oncogenic events in liver constitutively expressing c-myc and transforming growth factor alpha. *Cancer Res* 1998;58:123-34.
31. Kent LN, Bae S, Tsai SY, Tang X, Srivastava A, et al. Dosage-dependent copy number gains in E2f1 and E2f3 drive hepatocellular carcinoma. *J Clin Invest* 2017;127:830-42.
32. Ruiz de Galarreta M, Bresnahan E, Molina-Sánchez P, Lindblad KE, Maier B, et al. β -catenin activation promotes immune escape and resistance to anti-PD-1 therapy in hepatocellular carcinoma. *Cancer Discov* 2019;9:1124-41.
33. Bakiri L, Wagner EF. Mouse models for liver cancer. *Mol Oncol* 2013;7:206-23.
34. Dow M, Pyke RM, Tsui BY, Alexandrov LB, Nakagawa H, et al. Integrative genomic analysis of mouse and human hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 2018;115:E9879-88.
35. Fidler IJ. Rationale and methods for the use of nude mice to study the biology and therapy of human cancer metastasis. *Cancer Metastasis Rev* 1986;5:29-49.
36. He S, Hu B, Li C, Lin P, Tang WG, et al. PDXliver: a database of liver cancer patient derived xenograft mouse models. *BMC Cancer* 2018;18:550.
37. Broutier L, Mastrogianni G, Versteegen MM, Francies HE, Gavarró LM, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. *Nat Med* 2017;23:1424-35.
38. Sun FX, Tang ZY, Lui KD, Ye SL, Xue Q, et al. Establishment of a metastatic model of human hepatocellular carcinoma in nude mice via orthotopic implantation of histologically intact tissues. *Int J Cancer* 1996;66:239-43.
39. Calvisi DF, Ladu S, Gorden A, Farina M, Conner EA, et al. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. *Gastroenterology* 2006;130:1117-28.
40. Lee JS, Chu IS, Mikaelyan A, Calvisi DF, Heo J, et al. Application of comparative functional genomics to identify best-fit mouse models

- to study human cancer. *Nat Genet* 2004;36:1306-11.
41. Inokuchi S, Aoyama T, Miura K, Osterreicher CH, Kodama Y, et al. Disruption of TAK1 in hepatocytes causes hepatic injury, inflammation, fibrosis, and carcinogenesis. *Proc Natl Acad Sci U S A* 2010;107:844-9.
 42. Katzenellenbogen M, Mizrahi L, Pappo O, Klopstock N, Olam D, et al. Molecular mechanisms of liver carcinogenesis in the *mdr2*-knockout mice. *Mol Cancer Res* 2007;5:1159-70.
 43. Popov Y, Patsenker E, Fickert P, Trauner M, Schuppan D. *Mdr2* (*Abcb4*)-/- mice spontaneously develop severe biliary fibrosis via massive dysregulation of pro- and antifibrogenic genes. *J Hepatol* 2005;43:1045-54.
 44. Arvola A, Forsander O. Comparison between water and alcohol consumption in six animal species in free choice experiments. *Nature* 1961;191:819-20.
 45. Holmes RS, Dudley JA, Algar EM, Mather PB, Rout UK. Biochemical and genetic studies on enzymes of alcohol metabolism: the mouse as a model organism for human studies. *Alcohol Alcohol* 1986;21:41-56.
 46. Bertola A. Rodent models of fatty liver diseases. *Liver Res* 2018;2:3-13.
 47. Friemel J, Frick L, Unger K, Egger M, Parrotta R, et al. Characterization of HCC mouse models towards an etiology-oriented subtyping approach. *Mol Cancer Res* 2019;17:1493-502.
 48. Ambade A, Satishchandran A, Gyongyosi B, Lowe P, Szabo G. Adult mouse model of early hepatocellular carcinoma promoted by alcoholic liver disease. *World J Gastroenterol* 2016;22:4091-108.
 49. Rafacho BPM, Stice CP, Liu C, Greenberg AS, Ausman LM, et al. Inhibition of diethylnitrosamine-initiated alcohol-promoted hepatic inflammation and precancerous lesions by flavonoid luteolin is associated with increased sirtuin 1 activity in mice. *Hepatobiliary Surg Nutr* 2015;4:124-34.
 50. Brandon-Warner E, Walling TL, Schrum LW, McKillop IH. Chronic ethanol feeding accelerates hepatocellular carcinoma progression in a sex-dependent manner in a mouse model of hepatocarcinogenesis. *Alcohol Clin Exp Res* 2012;36:641-53.
 51. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007;317:121-4.
 52. De Minicis S, Kisseleva T, Francis H, Baroni GS, Benedetti A, et al. Liver carcinogenesis: rodent models of hepatocarcinoma and cholangiocarcinoma. *Dig Liver Dis* 2013;45:450-9.
 53. Xin B, Cui Y, Wang Y, Wang L, Yin J, et al. Combined use of alcohol in conventional chemical-induced mouse liver cancer model improves the simulation of clinical characteristics of human hepatocellular carcinoma. *Oncol Lett* 2017;14:4722-8.
 54. Liu K, McCaughan GW. Epidemiology and etiologic associations of non-alcoholic fatty liver disease and associated HCC. *Adv Exp Med Biol* 2018;1061:3-18.
 55. Wolf MJ, Adili A, Piotrowitz K, Abdullah Z, Boege Y, et al. Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. *Cancer Cell* 2014;26:549-64.
 56. De Minicis S, Agostinelli L, Rychlicki C, Sorice GP, Saccomanno S, et al. HCC development is associated to peripheral insulin resistance in a mouse model of NASH. *PLoS One* 2014;9:e97136.
 57. Hill-Baskin AE, Markiewski MM, Buchner DA, Shao H, DeSantis D, et al. Diet-induced hepatocellular carcinoma in genetically predisposed mice. *Hum Mol Genet* 2009;18:2975-88.
 58. Kanuri G, Bergheim I. In vitro and in vivo models of non-alcoholic fatty liver disease (NAFLD). *Int J Mol Sci* 2013;14:11963-80.
 59. Asgharpour A, Cazanave SC, Pacana T, Seneshaw M, Vincent R, et al. A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. *J Hepatol* 2016;65:579-88.
 60. Tsuchida T, Lee YA, Fujiwara N, Ybanez M, Allen B, et al. A simple diet- and chemical-induced murine NASH model with rapid progression of steatohepatitis, fibrosis and liver cancer. *J Hepatol* 2018;69:385-95.
 61. Kishida N, Matsuda S, Itano O, Shinoda M, Kitago M, et al. Development of a novel mouse model of hepatocellular carcinoma with nonalcoholic steatohepatitis using a high-fat, choline-deficient diet and intraperitoneal injection of diethylnitrosamine. *BMC Gastroenterol* 2016;16:61.
 62. Park EJ, Lee JH, Yu GY, He G, Ali SR, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010;140:197-208.
 63. Liang JQ, Teoh N, Xu L, Pok S, Li X, et al. Dietary cholesterol promotes steatohepatitis related hepatocellular carcinoma through dysregulated metabolism and calcium signaling. *Nat Commun* 2018;9:4490.
 64. Henderson JM, Polak N, Chen J, Roediger B, Weninger W, et al. Multiple liver insults synergize to accelerate experimental hepatocellular carcinoma. *Sci Rep* 2018;8:10283.
 65. Castro RE, Diehl AM. Towards a definite mouse model of NAFLD. *J Hepatol* 2018;69:272-4.
 66. Fujii M, Shibasaki Y, Wakamatsu K, Honda Y, Kawauchi Y, et al. A murine model for non-alcoholic steatohepatitis showing evidence of association between diabetes and hepatocellular carcinoma. *Med Mol Morphol* 2013;46:141-52.
 67. Febbraio MA, Reibe S, Shalapour S, Ooi GJ, Watt MJ, et al. Preclinical models for studying NASH-driven HCC: how useful are they? *Cell Metab* 2019;29:18-26.
 68. Takakura K, Koido S, Fujii M, Hashiguchi T, Shibasaki Y, et al. Characterization of non-alcoholic steatohepatitis-derived hepatocellular carcinoma as a human stratification model in mice. *Anticancer Res* 2014;34:4849-55.
 69. Arfianti E, Larter CZ, Lee S, Barn V, Haigh G, et al. Obesity and diabetes accelerate hepatocarcinogenesis via hepatocyte proliferation independent of NF- κ B or Akt/mTORC1. *J Clin Transl Res* 2016;2:26-37.
 70. Teufel A, Itzel T, Erhart W, Brosch M, Wang XY, et al. Comparison of gene expression patterns between mouse models of nonalcoholic fatty liver disease and liver tissues from patients. *Gastroenterology* 2016;151:513-25.

71. Nakagawa H, Umemura A, Taniguchi K, Font-Burgada J, Dhar D, et al. ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. *Cancer Cell* 2014;26:331-43.
72. Shalapour S, Lin XJ, Bastian IN, Brain J, Burt AD, et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature* 2017;551:340-5.
73. Molina-Sánchez P, Lujambio A. Iron overload and liver cancer. *J Exp Med* 2019;216:723.
74. Zhou XY, Tomatsu S, Fleming RE, Parkkila S, Waheed A, et al. HFE gene knockout produces mouse model of hereditary hemochromatosis. *Proc Natl Acad Sci U S A* 1998;95:2492-7.
75. Pigeon C, Turlin B, Iancu TC, Leroyer P, Lan JL, et al. Carbonyl-iron supplementation induces hepatocyte nuclear changes in BALB/CJ male mice. *J Hepatol* 1999;30:926-34.
76. Rothenberg BE, Volland JR. $\beta 2$ knockout mice develop parenchymal iron overload: a putative role for class I genes of the major histocompatibility complex in iron metabolism. *Proc Natl Acad Sci U S A* 1996;93:1529-34.
77. Muto Y, Moroishi T, Ichihara K, Nishiyama M, Shimizu H, et al. Disruption of FBXL5-mediated cellular iron homeostasis promotes liver carcinogenesis. *J Exp Med* 2019;216:950-65.
78. Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, et al. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018;15:397-411.
79. Yu LX, Schwabe RF. The gut microbiome and liver cancer: mechanisms and clinical translation. *Nat Rev Gastroenterol Hepatol* 2017;14:527-39.
80. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013;499:97-101.
81. Achiwa K, Ishigami M, Ishizu Y, Kuzuya T, Honda T, et al. DSS colitis promotes tumorigenesis and fibrogenesis in a choline-deficient high-fat diet-induced NASH mouse model. *Biochem Biophys Res Commun* 2016;470:15-21.
82. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012;21:504-16.
83. Poutahidis T, Varian BJ, Levkovich T, Lakritz JR, Mirabal S, et al. Dietary microbes modulate transgenerational cancer risk. *Cancer Res* 2015;75:1197-204.
84. Llopis M, Cassard AM, Wrzosek L, Bosch L, Bruneau A, et al. Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. *Gut* 2016;65:830-9.
85. Xu W, Liu K, Chen M, Sun JY, McCaughan GW, et al. Immunotherapy for hepatocellular carcinoma: recent advances and future perspectives. *Ther Adv Med Oncol* 2019;11:1758835919862692.

Review

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Association between hereditary hemochromatosis and hepatocellular carcinoma: a comprehensive review

Aparna Jayachandran^{1,2}, Ritu Shrestha^{1,2}, Kim R. Bridle^{1,2}, Darrell H. G. Crawford^{1,2}

¹The University of Queensland, Faculty of Medicine, Brisbane, QLD 4006, Australia.

²Gallipoli Medical Research Institute, Greenslopes Private Hospital, Brisbane, QLD 4120, Australia.

Correspondence to: Prof. Darrell H. G. Crawford, Gallipoli Medical Research Institute, The University of Queensland, Faculty of Medicine, Lower Lobby Level, Administration Building, Greenslopes Private Hospital, Greenslopes, QLD 4120, Australia.
E-mail: d.crawford@uq.edu.au

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Abstract

Hepatocellular carcinoma (HCC) is a significant global health problem with high morbidity and mortality. Its incidence is increasing exponentially worldwide with a close overlap between annual incidence and death rates. Even though significant advances have been made in HCC treatment, fewer than 20% of patients with HCC are suitable for potentially curative treatment. Hereditary hemochromatosis (HH) is an important genetic risk factor for HCC. HH is an autosomal recessive disorder of iron metabolism, characterised by elevated iron deposition in most organs including the liver, leading to progressive organ dysfunction. HCC is a complication of HH, nearly always occurring in patients with cirrhosis and contributes to increased mortality rates. Identifying the susceptibility of development of HCC in HH patients has gained much traction. This review summarises the current knowledge with regard to the association of HH and HCC in order to encourage further research. In this review, we focus particularly on *HFE* gene-related HH. Herein, we highlight and discuss emerging clinical research which addresses the prevalence of HCC in HH patients and the coincidence of HH with other risk factors for HCC development. We also focus on the therapeutic tools in the management of HCC associated with HH.

Keywords: Hepatocellular carcinoma, hereditary hemochromatosis, *HFE* gene, *C282Y* mutation, *H63D* mutation, liver cirrhosis



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INTRODUCTION

Hepatocellular carcinoma (HCC) is considered to be the most frequent primary liver cancer accounting for 80%-90% of cases^[1]. HCC has become a leading cause of cancer-related death globally in the last decades, accounting for approximately 800,000 deaths annually^[2]. The incidence of HCC continues to escalate by 3%-9% cases annually worldwide with a nearly equal proportion of deaths^[3]. Surgical resection, trans-arterial chemoembolization radiofrequency ablation and liver transplantation remain the treatments of choice for HCC patients and are beneficial for patients in the early stages of the disease^[4]. The prognosis for HCC patients in the advanced stages is poor due to the limited efficacy of current therapy^[5].

Implementing HCC surveillance among at-risk populations is imperative to identify HCC at early stages amenable to curative treatments. HCC mostly develops in patients with underlying chronic hepatic disease^[6-8]. Several at-risk populations for HCC have been identified including patients with cirrhosis, hepatitis B and hepatitis C viral infection, alcoholism, aflatoxin, non-alcoholic steatohepatitis, type 2 diabetes, obesity and Wilson's Disease^[7]. Hemochromatosis is also an important risk factor for HCC.

Hereditary hemochromatosis (HH) is a common inherited iron metabolism disorder, characterised by increased deposition of iron in the liver and other organs. If left untreated, hepatic iron overload in HH patients can result in liver injury, which can progress to cirrhosis and subsequently HCC^[9,10]. Further clarification of the risk factors in individual HH patients for HCC development remains an area of unmet clinical need. Herein, we review the current literature concerning the association between HH and HCC with a focus on *HFE* gene-related HH. This review highlights and discusses clinical studies that address the prevalence of HCC in HH and risk factors linked with the development of HCC in patients with HH.

HEREDITARY HEMOCHROMATOSIS

HH comprises a number of inherited diseases of iron metabolism^[11]. Although its geographic distribution is worldwide, HH is one of the most common genetic disorders in individuals of Northern European ancestry, particularly Nordic or Celtic ancestry. In this population, the frequency of homozygous *HFE* mutation is approximately 1 in 200-250 individuals^[9,12-14]. There is considerable phenotypic diversity in HH and associated biochemical changes are more common than the clinical manifestations of iron overload-related disease. Indeed, advanced clinical expression of *HFE*-related hemochromatosis is rare^[15]. This autosomal recessive condition is characterised by excessive iron absorption by the small intestine. This leads to progressive iron loading over many years of the affected individual's adult life. As hepatocytes store most of the excess iron, the liver is the organ mostly afflicted by iron overload^[16,17]. Long-term effects of excessive iron loading include liver fibrosis, cirrhosis, HCC, cardiomyopathy, diabetes mellitus, hypogonadism and arthropathy^[10,11]. Early studies suggested that liver disease was the most frequent cause of death in HH individuals^[18]. However, more readily available genetic testing, greater public awareness and an improved understanding of the natural history of the condition means that most affected subjects are now diagnosed before significant target organ injury occurs.

HH is classified into 6 groups depending on the nature of the underlying genetic mutation^[17]. Mutations in the high iron gene (*HFE*) (Hemochromatosis Type 1, i.e., *C282Y* homozygosity, *C282Y/H63D* compound heterozygosity and other *HFE*-related genotypes, e.g., *S65C*) are responsible for the majority of hemochromatosis cases^[19]. Rarer forms of non-*HFE* associated HH have been attributed to mutations in hemojuvelin (*HJV*) (Hemochromatosis Type 2a), hepcidin (*HAMP*) (Hemochromatosis Type 2b), transferrin receptor 2 (*TFR2*) (Hemochromatosis Type 3), ferroportin (*SLC40A1*) (Hemochromatosis Type 4) and ferritin heavy chain 1 (*FTH1*) (Hemochromatosis Type 5) gene^[17]. These latter conditions are thought to account for most of the non-*HFE* forms of HH^[9].

Hemochromatosis type 1

HFE is located on chromosome 6p21.3, has seven exons and five introns and encodes for a 343-amino acid protein that is similar to human leucocytes antigen class I molecules^[20]. The *HFE* gene is important for normal iron metabolism. Feder *et al.*^[19] first identified that a mutation in this gene caused HH. The three most common mutations at exons 2 (187C→G and 193A→T) and exon 4 (845G→A) of the *HFE* gene were linked with HH^[19]. However, not all HH patients harbour these mutations, as outlined above^[21].

C282Y (845G→A) homozygotes

About 80%-85% of individuals with HH are *C282Y* (845G→A) homozygotes. This founder mutation leads to a single-base change, resulting in the substitution of tyrosine (Y) for cysteine (C) in the amino acid sequence of the *HFE* protein at position 282^[10]. *HFE*-related HH is an adult onset disorder and in expressing patients is characterised by increased transferrin saturation and serum ferritin levels compared to the healthy population^[22]. Moreover, *C282Y* homozygotes are at particular risk of cirrhosis if serum ferritin levels are greater than 1000 µg/L^[23]. Males and *C282Y* homozygotes of family members affected by HH exhibit a higher penetrance of homozygous *C282Y* gene^[22]. However, due to the variable phenotypic expression of this mutation, only some of these patients develop cirrhosis or HCC^[22]. The frequency of the *C282Y* allele is high in European populations and the reported frequency varies from 0%-3% in South Europe to 4%-10% in North Europe^[20,24].

This *HFE* mutant protein has reduced cell surface expression and undergoes rapid degradation. The mechanisms by which *HFE* protein regulates iron homeostasis at the cellular level are beginning to emerge. Earlier studies proposed that the *HFE* protein binds the transferrin receptor 1 (TFR1) to form a stable complex, which in turn decreases its binding affinity for transferrin. The *HFE* mutant protein does not form this complex, which results in transferrin binding to the transferrin receptor, leading to increased cellular uptake of iron and subsequently causing iron overload^[25,26]. Recent studies have demonstrated that the *HFE* protein is an upstream regulator of the hormone hepcidin in hepatocytes^[27,28]. Hepcidin is synthesised and secreted by hepatocytes and is a master regulator of iron homeostasis in the body. Hepcidin negatively regulates dietary iron uptake by the intestine^[29]. Under physiological conditions, hepatic hepcidin expression is regulated by proteins that are predominantly expressed in hepatocytes, including *HFE*, transferrin receptor 2 (TFR2), *HJV*, bone morphogenetic protein 6 (BMP6), matriptase-2 and transferrin^[29,30]. HH patients harbouring the *HFE* mutations have low levels of hepcidin protein allowing a slow accumulation of iron over the individual's lifetime, resulting in iron overload^[31]. Iron overload induces reactive oxygen species (ROS) formation, which causes DNA damage and somatic mutations that may play a role in the subsequent development of HCC over time^[32].

HFE compound heterozygotes

Two other mutations in the *HFE* gene, namely 187C→G (*H63D*) and 193A→T (*S65C*), have been identified^[33,34]. These additional mutations alone have not been implicated in iron overload. However, the co-occurrence of these mutations together with a *C282Y* mutation forming a compound heterozygote (*C282Y/H63D* or *C282Y/S65C*) has been implicated in iron overload^[35]. *HFE* compound heterozygotes might have increased iron indices but iron overload-related disease is most uncommon^[36,37]. Clinical disease may develop in *HFE* compound heterozygotes in conjunction with comorbid factors such as obesity, excess alcohol consumption or diabetes^[37]. The Netherlands and the Iberian Peninsula have a high frequency of the *H63D* allele that varies from 7.9% to 17.5% in the general population^[38]. The frequency of *S65C* allele is low, 0%-1% in European and Brazilian populations^[12,34,39]. Approximately 5% of individuals with a clinical diagnosis of HH are compound heterozygotes^[40,41].

THE ASSOCIATION BETWEEN HH AND HCC

HCC develops in HH individuals and contributes to the increased mortality^[42-47]. Although the biological and physiological functions of *HFE* gene within the liver are not fully understood, several case-control

and population-based studies have confirmed that *HFE* mutations confer increased risk for HCC development^[48-57]. The exact incidence and prevalence of HCC varies considerably between different studies, which is probably explained by the heterogeneity of the study cohorts. The characteristics of the study cohort is critical in the interpretation of such studies given the variable phenotype of HH and also because cirrhosis is such a critical risk factor for the development of HCC. Some studies report on cohorts where a large proportion of patients had underlying cirrhosis. Other studies have clear referral bias due to the authors' interest in disorders of iron metabolism, are retrospective in nature or are from liver transplant programs where one would expect cirrhosis and HCC to dominate the study cohort.

Population-based studies provide a more realistic assessment of the overall incidence and prevalence of HCC in HH. In one such study, the US National Centre for Health Statistics reported a close association between HH and HCC. In this study, patients who were diagnosed with HH and who died were 23-fold more likely to have HCC in comparison with individuals without a diagnosis of HH^[47]. A further study conducted in Sweden reported the risk of HCC in HH individuals to be approximately 20-fold higher than in the general population^[42]. At 10 years of follow up, the absolute risk of HCC among HH men was 6%, which was higher than the risk in women (1.5%)^[42]. Willis *et al.*^[58] found that HCC patients had a 7% prevalence of the *C282Y* homozygous mutation. In a similar study, Sánchez-Luna *et al.*^[59] found that, among 118 *C282Y* homozygotes, eight homozygotes developed HCC, representing 1.8% of patients with HCC.

Meta-analyses have recently been conducted to clarify the effect of *HFE* polymorphisms on the susceptibility to HCC. Ellervik *et al.*^[60] conducted a meta-analysis to examine associations between *C282Y* and *H63D* mutations with HCC. An odds ratio of 11 for HCC occurrence was reported for *C282Y* homozygotes (YY vs. CC). A further study conducted on 43 published articles (5758 cases and 14,741 controls) demonstrated that the *HFE C282Y* homozygous mutation was significantly associated with increased risk of HCC compared to the overall population^[61]. Another meta-analysis including nine studies based on European populations (1102 HCC cases and 3766 controls) showed an association between the Y allele of *C282Y* and HCC risk overall as well as in alcohol-related cirrhosis patients but not in viral-related cirrhosis patients^[51]. There are also contrary reports showing no association with the risk of developing HCC^[23,33,55,62,63], possibly reflecting the low penetrance of the *C282Y* mutation in the populations studied.

Cirrhosis and other risk factors for HCC development in HH

HCC accounts for 25%-45% of disease-related premature deaths in HH^[64]. In HH, the primary risk factor for the development of HCC is the presence of cirrhosis. Studies that have assessed the association of risk factors for development of HCC in HH are listed in Tables 1 and 2. Some studies have indicated that the risk of HCC in cirrhotic HH patients is higher than in patients with cirrhosis from other causes. In a meta-analysis assessment of eight studies which included follow up of cirrhotic patients, the annual incidence of HCC was 1.20% per year^[65]. One other study showed that, once cirrhosis has been established in HH patients, the annual incidence of HCC is approximately 4%^[66]. Earlier studies have revealed that risk of HCC developing in cirrhotic HH patients was 200-fold higher than non-cirrhotic control groups^[18,67]. These studies may have suffered referral bias and lacked *HFE* genetic testing with the diagnosis of HH based on clinical features and biochemical tests^[18,67]. Recent studies utilised a combination of *HFE* genotyping, clinical examination and abnormal iron indices including transferrin saturation, serum ferritin, and iron deposition in liver biopsies to confirm diagnosis of HH^[42,44]. These studies have revealed that risk of HCC developing in cirrhotic HH patients was 20-fold higher than non-cirrhotic control groups^[42,44,48]. It is worth noting that HCC has occasionally been found to occur in HH patients with no cirrhosis^[64,68,69]. In these patients, hepatic iron accumulation has been suggested to be directly involved in HCC development independently of cirrhosis^[64,69,70]. An increased risk of HCC with cirrhosis among individuals heterozygous for *HFE* gene mutations has also been reported and is discussed below^[42,51].

Table 1. Association of liver cirrhosis as a risk factors for development of HCC in HH

Author	Risk factor	Study population/country	No. of cases	HFE mutation analysis	Comments
Elmberg <i>et al.</i> ^[42]	Cirrhosis	Population study, Sweden	1847 (HH), 5973 (first-degree relatives)	<i>C282Y</i> (+/+), <i>C282Y</i> (+/-), compound heterozygotes (<i>C282Y/H63D</i>), <i>H63D</i> (+/+), <i>H63D</i> (+/-)	HH were at a 20-fold risk of developing HCC. Overall cancer risk in first-degree relatives was not increased
Cauza <i>et al.</i> ^[48]	Cirrhosis	Case-control study, Austria	162	<i>C282Y</i> (+/+), <i>H63D</i> (+/+), compound heterozygotes (<i>C282Y/H63D</i>)	<i>C282Y</i> homozygotes had a 20-fold increased risk to develop HCC in patients with cirrhosis
Allen <i>et al.</i> ^[23]	Cirrhosis	Population study, Australia	31,192	<i>C282Y</i> (+/+), <i>C282Y</i> (+/-) compound heterozygotes (<i>C282Y/H63D</i>)	Homozygotes for the <i>C282Y</i> mutation developed HCC
Asberg <i>et al.</i> ^[62]	Cirrhosis	Population study, Norway	65,238	<i>C282Y</i> (+/+), compound heterozygotes (<i>C282Y/H63D</i>)	Low prevalence of cirrhosis, 3.7% in men and none in women homozygous for the <i>C282Y</i> mutation
Nowak <i>et al.</i> ^[73]	Cirrhosis	Case-control study, Swiss	147	<i>C282Y</i> (+/+), <i>H63D</i> (+/-), compound heterozygotes (<i>C282Y/H63D</i>)	9% of <i>C282Y</i> homozygotes develop HCC and majority of the individuals had liver cirrhosis
Fargion <i>et al.</i> ^[40]	Cirrhosis	Case-control study, Italy	81 (HCC), 128 (control)	<i>C282Y</i> (+/-) and <i>H63D</i> (+/-)	<i>C282Y</i> and <i>H63D</i> heterozygotes with cirrhosis have a high risk of HCC
Lauret <i>et al.</i> ^[52]	Cirrhosis	Case-control study, Spain	554 (cirrhosis), 159 (control)	<i>C282Y</i> (+/-) and <i>H63D</i> (+/-)	20.9% patients with alcoholic cirrhosis and HCC were heterozygous for the <i>C282Y</i> mutation
Nahon <i>et al.</i> ^[53]	Cirrhosis	Case-control study, France	301	<i>C282Y</i> (+/-), compound heterozygotes (<i>C282Y/H63D</i>)	<i>C282Y</i> heterozygotes increased risk of developing HCC in patients with alcoholic but not with HCV-related cirrhosis
Blanc <i>et al.</i> ^[68]	Non-cirrhotic livers	Case-control study, France	35	<i>C282Y</i> (+/+), <i>H63D</i> (+/+), compound heterozygotes (<i>C282Y/H63D</i>)	50% of HH patients developed HCC in non-cirrhotic livers
Hlatt <i>et al.</i> ^[69]	Non-cirrhotic livers	USA	1	<i>C282Y</i> (+/+)	<i>C282Y</i> mutation increased the risk of HCC development in HH without cirrhosis

Genotypes: +/+ indicates homozygotes, +/- indicates heterozygotes. HCC: hepatocellular carcinoma; HH: hereditary hemochromatosis; HCV: hepatitis C virus

Table 2. Association of HFE mutations with other risk factors of HCC

Author	Risk factor	Study population/country	No. of cases	HFE mutation analysis	Comments
Elmberg <i>et al.</i> ^[42]	Gender	Population study, Sweden	1847 (HH), 5973 (first-degree relatives)	<i>C282Y</i> (+/+), <i>C282Y</i> (+/-), compound heterozygotes (<i>C282Y/H63D</i>), <i>H63D</i> (+/+), <i>H63D</i> (+/-)	The risk of developing HCC in HH patients was 30-fold among men and 7-fold among women
Haddow <i>et al.</i> ^[44]	Gender	Population study, USA	1,000,000	<i>C282Y</i> (+/+)	The relative risk for this cancer in <i>C282Y</i> homozygotes is 23
Ezzikouri <i>et al.</i> ^[49]	Gender	Case-control study, Morocco	222 (control), 96 (HCC)	<i>H63D</i> (+/+), <i>H63D</i> (+/-), <i>C282Y</i> (+/-)	Men carrying the <i>H63D</i> mutation had a greater risk of HCC with HCC was 1.31%-2.1% for males and zero for females
Willis <i>et al.</i> ^[71]	Gender	Population study, UK	144	<i>C282Y</i> (+/+)	The penetrance of <i>C282Y</i> homozygous genotype in HH with HCC was 1.31%-2.1% for males and zero for females
Allen <i>et al.</i> ^[23]	Gender	Population study, Australia	31,192	<i>C282Y</i> (+/+), compound heterozygotes (<i>C282Y/H63D</i>)	In <i>C282Y</i> homozygotes, HCC developed in a substantial proportion of men but in a small proportion of women
Shi <i>et al.</i> ^[56]	Chronic hepatitis B	Case-control study, China	56 (HCC), 60 (control)	<i>C282Y</i> (+/+), <i>H63D</i> (+/+)	<i>C282Y</i> mutation is associated with susceptibility to HCC after chronic hepatitis B
Fraccanzani <i>et al.</i> ^[72]	Chronic hepatitis B and Gender	Case-control study, Italy	303	<i>H63D</i> (+/-), <i>C282Y</i> (+/-)	<i>C282Y</i> heterozygous males were 3.8-fold more likely to be HBV positive in HCC patients
Nowak <i>et al.</i> ^[73]	Age	Case-control study, Swiss	147	<i>C282Y</i> (+/+), <i>H63D</i> (+/-), compound heterozygotes (<i>C282Y/H63D</i>)	Higher age at diagnosis showed the strongest association with the occurrence of HCC
Elmberg <i>et al.</i> ^[42]	Age	Population study, Sweden	1847 (HH), 5973 (first-degree relatives)	<i>C282Y</i> (+/+), <i>C282Y</i> (+/-), compound heterozygotes (<i>C282Y/H63D</i>), <i>H63D</i> (+/+), <i>H63D</i> (+/-)	The risk of developing HCC was not associated with age

Genotypes: +/+ indicates homozygotes, +/- indicates heterozygotes. HCC: hepatocellular carcinoma; HH: hereditary hemochromatosis; HBV: hepatitis B virus

Other risk factors that may synergise with cirrhosis include chronic viral hepatitis, alcohol abuse, diabetes, age and gender^[47,71] [Table 2]. Patients with HCC and diabetes mellitus were 82 times more likely to have HH^[47]. The risk of HCC was higher in males who were C282Y homozygotes when compared to C282Y homozygous females - reflecting in part the higher iron burden in men^[44,49,71]. A study reported 1.3%-2.1% penetrance of the C282Y homozygous genotype in HH patients with HCC for males and zero for females^[71]. Another study found penetrance of the C282Y homozygous genotype in male HH patients with HCC was 5.56%^[23]. Studies have also found an unequivocal relationship between risk of HCC and C282Y mutation in patients with chronic hepatitis B and male gender^[56,72]. Another study identified increased age at diagnosis as a strong predictor for the development of HCC in HH patients and the authors suggested that this is a surrogate marker of duration of exposure to iron^[73]. This latter finding has not been substantiated in other studies. Serum ferritin level of above 1000 mg/L at diagnosis confirming high iron overload was a risk factor for HCC in the study by Nowak *et al.*^[73]. A serum ferritin concentration of over 1000 mg/L is also associated with a high risk of cirrhosis, which is the likely explanation for that association. In contrast to other studies, this study found no association between alcohol consumption and HCC development^[73]. However, the level of alcohol consumption defined as “considerable” was greater than 10 g/day for women and 20 g/day for men and this may be below the oncogenic threshold.

Collectively, these data illustrate that the presence of cirrhosis is the primary risk factor for the development of HCC in patients with HH. Other risk factors, as discussed above, seem to amplify the oncogenic potential of cirrhosis. Importantly, some of these other risks can be reduced by lifestyle modifications and/or therapy of other liver diseases, particularly chronic viral hepatitis.

HFE heterozygotes and the risk of HCC in patients with cirrhosis of other causes

This area is controversial and there are conflicting data on the role of heterozygosity for *HFE* mutations in the development of HCC in patients with cirrhosis from other causes. For example, Hellerbrand *et al.*^[51] indicated that HCC patients with cirrhosis were more likely to be C282Y heterozygotes compared to cirrhotic patients without HCC or normal controls. Additionally, elevated levels of transferrin saturation, serum ferritin and liver iron deposition were reported in HCC patients harbouring the heterozygous C282Y mutation compared to those lacking this mutation, suggesting that altered hepatic iron metabolism played a pathogenic role^[51]. The prevalence of the heterozygous C282Y and H63D mutation was also observed to be higher in 81 Italian patients with cirrhosis and HCC than in 128 normal controls (8.6% vs. 1.6%)^[50]. Similarly, Lauret *et al.*^[52] found a 20.9% prevalence of the C282Y heterozygous mutation in 43 Spanish HCC patients. In 301 cirrhotic French patients prospectively followed up for six months, hepatic iron overload and the heterozygous C282Y mutation were associated with an increase in the incidence of HCC in cirrhotic patients with alcohol-related problems but not in patients with hepatitis C viral infection^[53].

In contrast, a large prospective multicentre French study compared the prevalence of *HFE* mutations in 133 cirrhotic patients with HCC and 100 cirrhotic patients without HCC with a follow up of 2.5 years^[74]. This study concluded that C282Y mutation is not linked to an increased risk of HCC in cirrhotic patients^[74]. Similarly, in another study of 162 patients with HCC and cirrhosis, the frequency of the C282Y mutation did not differ between the patients with cirrhosis or healthy controls^[48].

Initially, it was proposed that the H63D mutation has no direct association with HH^[19,33]. In line with this, several studies reported no association between the prevalence of the H63D mutation and the risk of developing HCC^[75]. Conversely, other studies have implicated occurrence of H63D mutation with an increased risk of HCC in HH patients^[55,61,63]. In a study of 196 HCC patients and 181 healthy controls, the H63D mutation was associated with an increased risk of HCC^[55]. HCC developed in HH patients exhibiting H63D mutations along with predisposing factors such as liver cirrhosis due to chronic hepatitis C virus infection and/or ethanol abuse and chronic hepatitis B virus-infection^[55]. Another large study

involving 5758 cases and 14,741 controls demonstrated that *H63D* mutation was more likely to be involved in susceptibility to HCC without cirrhosis in the African population^[61]. A positive association between compound heterozygosity for *C282Y/H63D* and the risk of HCC was also observed^[61]. Conversely, no cases of HCC were identified among the 44 compound heterozygotes examined in another study^[59]. In an Egyptian cohort study, patients with the *H63D* mutation had a higher risk of developing HCC^[63]. Additionally, the role of *S65C* in HCC remains to be elucidated. A number of other studies demonstrated that individuals harbouring *C282Y* or *H63D* mutation did not develop HCC, suggesting there was no association between *HFE* mutation and HCC^[74,76-78]. Thus, whether there is a link between these *HFE* mutations and the HCC risk remains somewhat uncertain with significant variation between different populations groups, and different underlying diseases. More studies are needed to definitively assess the influence of the *HFE* mutations on the development of HCC in HH patients.

Mechanisms of iron toxicity in HH leading to HCC

Iron is ubiquitously present in cells and a physiological optimal balance of iron is critical for the normal functioning of cells^[79,80]. Iron is essential for several important processes including the transfer of oxygen throughout the body by haemoglobin, the mitochondrial electron transport chain and as a cofactor in enzymatic reactions. However, excess iron can be very toxic to the cell due to its redox reactivity that promotes oxidative stress^[81,82]. Homeostasis of iron in the body is maintained by four major cell types: duodenal enterocytes (dietary iron absorption), erythroid precursors (iron utilisation), reticuloendothelial macrophages (iron storage and recycling) and hepatocytes (iron storage and endocrine regulation)^[83]. Duodenal enterocytes absorb dietary iron and store it in the form of ferritin. Enterocytes release iron into the circulation through the basolateral iron exporter, ferroportin. In the blood stream, iron binds to the plasma iron transport protein transferrin^[82,84]. The majority of iron in the body is found in the oxygen-carrying haemoglobin of erythrocytes. Iron is also stored in the form of ferritin in hepatocytes and reticuloendothelial macrophages. The macrophages phagocytose the senescent erythrocytes and the iron from haemoglobin is loaded onto transferrin for iron recycling^[83]. Importantly, in humans, there are no active mechanisms to eliminate excess iron from the body^[15,42].

Transferrin is highly saturated during iron overload and additional iron released into the circulation binds to low-molecular-weight compounds and is termed non-transferrin bound iron (NTBI). Excess iron in circulation enters into hepatocytes by binding the transmembrane TFR1 and TFR2 on hepatocytes^[17,83]. While both TFR1 and TFR2 are capable of iron uptake, TFR1 has a higher iron binding affinity than TFR2. TFR2 is an iron sensor that regulates body iron uptake and is sensitive to changes in transferrin saturation in the blood^[82,85]. Hepatocytes have a significant role in iron homeostasis as they also produce the hormone hepcidin, an important regulator of iron balance^[86]. Hepcidin binds ferroportin and stimulates the internalisation and subsequent degradation of ferroportin, thus decreasing the absorption of iron from the gut and release of iron into the circulation^[84]. *HFE* works in conjunction with multiple proteins including TFR2 and Hemojuvelin to induce hepcidin expression^[87]. In HH patients harbouring the *HFE* mutations, the hepcidin protein is not properly expressed, which leads to uncontrolled iron absorption, resulting in iron overload^[17]. In addition, *HFE* mutation also leads to a loss of transferrin sensitivity, suggesting that TFR2 and *HFE* complex may be involved in iron-sensing^[88]. The hepcidin-mediated increased iron absorption from the gut leads to preferential iron loading of the hepatocytes. It has been hypothesised that this in turn causes injury and subsequent malignant transformation of hepatocytes^[79,89]. The mechanisms responsible for a direct hepatocarcinogenic effect of iron have yet to be fully elucidated^[79,89].

Increase in iron absorption over time leads to iron accumulation in hepatocytes, leading to injury and subsequent malignant transformation of hepatocytes^[79,89]. The role of iron in hepatocarcinogenesis has been suggested from epidemiologic studies, animal models and *in vitro* studies^[90-93]. The carcinogenic effect of iron has been related to its ability to form mutagenic hydroxyl radicals, enhance lipid peroxidation, promote immune escape or facilitate chronic inflammation leading to cirrhosis.

One of the mechanisms by which iron accumulation in the liver may promote malignant transformation of hepatocytes is directly by the mechanism of oxidative stress^[79]. It has been proposed that the formation of free radicals by Fenton reaction causes oxidative stress, leading to the malignant transformation of hepatocytes^[94]. Although the Fenton reaction has been implicated in carcinogenic effect of iron, there is relatively little direct or experimental data to support this claim. The excess ferrous iron (Fe^{2+}) accumulates in hepatocytes and undergoes a Fenton reaction by interacting with hydrogen peroxide to form Fe^{3+} and highly reactive oxygen free radicals (ROS)^[95]. Generation of ROS causes hepatocyte injury by inducing peroxidation of membrane fatty acids followed by the production of toxic by-products that disrupt DNA and protein synthesis^[79,94,96]. In addition, ROS causes DNA damage and mutagenesis, which may lead to neoplastic transformation over time^[97-99]. Iron-generated ROS can induce mutations in *p53*, an important tumour suppressor gene^[100]. Iron-generated ROS also contributes to the production of a mutagenic and cytotoxic oxidatively DNA-damaged product, 8-hydroxy-2'-deoxyguanosine (8-OHdG)^[101,102]. 8-OHdG causes G:C to T:A transversions, DNA unwinding and strand breaks^[101,103,104]. A study has shown correlation of 8-OHdG levels with iron levels in serum in HCC patients^[101]. In liver tissue, the rate of DNA unwinding and strand breaks have been associated with 8-OHdG levels^[90]. Another study has highlighted the link between DNA unwinding and the risk of HCC in HH patients^[18]. An abnormal form of NTBI, called labile plasma iron or reactive plasma iron, also contributes to oxidative stress and the subsequent liver damage during HH^[105]. Overall, several studies support the role of iron-induced ROS formation as the main mechanism of development of HCC in HH^[32,80,99,106,107].

Iron accumulation can also lead to cirrhosis and the subsequent development of HCC, indirectly through the induction of chronic inflammation^[79]. Excess hepatic iron promotes the activation of hepatic stellate cells in HH^[92]. This can promote fibrogenesis. Iron has also been shown to induce transforming growth factor-beta, which plays an important role in the development of liver fibrosis^[108]. The combination of elevated iron levels with environmental and acquired factors such as excessive alcohol consumption, viral hepatitis and steatosis may act synergistically to precipitate the development of HCC^[109]. Iron has a direct effect on tumour growth by promoting cellular proliferation. In human HCC cell lines, iron enhances proliferation and iron deprivation leads to cell cycle arrest and increased apoptosis^[110]. It has been reported that increased iron concentration in HCC cells was associated with enhanced migration, invasion, high metastasis rate and recurrence^[111].

In addition, iron reduces immune surveillance for malignant transformation by impairing T-cell proliferation and inhibiting tumoricidal activity of macrophages^[79,103,104,112,113]. Epigenetic alterations due to iron overload have also been implicated in hepatocarcinogenesis. Epigenetic defects such as increased DNA methylation commonly occur in HCC^[114]. Lehmann *et al.*^[115] found 84% of the non-cancerous liver biopsies derived from HH patients exhibited hypermethylation of genes that are often hypermethylated in HCC. DNA hypermethylation was independent of age, cirrhosis or hepatitis infection. Several studies support the role of iron in the development of HCC in HH.

Diagnosis and treatment of HCC in HH patients

Prior to the identification of *HFE*, the diagnosis of HH was based on parameters including clinical features, increased ferritin levels, high serum transferrin saturation and characteristic findings on liver biopsy^[17]. After the discovery of the *HFE* mutations, genetic screening became the preferred diagnostic test for HH. *HFE* genetic testing together with measurements of serum transferrin saturation and ferritin levels have gained traction as the diagnostic test of choice for HH^[9-11]. A serum ferritin concentration of > 1000 µg/L in patients with HH has been associated with an increased risk of cirrhosis and HCC^[11]. Magnetic resonance imaging (MRI) has recently been applied as an imaging modality for the detection and quantification of hepatic iron in those patients where there is diagnostic uncertainty. Additionally, MRI can be utilised to evaluate HCC in HH patients^[17].

Excess iron should be removed by venesection (phlebotomy) therapy and this should eliminate the risk of progression to cirrhosis and the development of HCC in non-cirrhotic individuals. Early diagnosis and iron depletion therapy has the potential of improving the survival rate of patients^[9]. HH is readily treated by venesection therapy, which is very efficient in removing excess iron and involves two successive treatment phases^[116]. In the initial induction phase, the excess iron present at the time of diagnosis is removed by 1-2 weekly venesections (7.5 mL/kg body weight per venesection). After the removal of excess iron, maintenance therapy, the second treatment phase, prevents recurrent iron overload. Maintenance therapy involves removal of 2-4 units/year^[117].

Although venesection is the treatment of choice in hemochromatosis, other iron depletion therapies have also been tested in HH patients^[118,119]. Another iron depletion therapy involves the application of iron chelation therapy to facilitate iron mobilisation and excretion. The iron chelating drugs desferioxamine, deferiprone and deferasirox have been tested in HH patients^[118,119]. A phase I/II clinical trial for deferasirox has shown it to reduce iron burden in HH patients homozygous for the C282Y mutation^[118]. Desferioxamine is administered either by intravenous or subcutaneous route, while deferiprone and deferasirox are oral iron chelators. These iron chelators have several side effects including skin rashes, gastrointestinal disturbances and occasionally abnormal liver function tests and should only be considered in patients in whom venesection is not a possibility^[118]. Of interest, iron chelators with antitumor properties and favourable toxicity profiles have emerged^[120]. Several iron chelators with effective antitumor activities have been identified [Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone), DpT (di-2-pyridylketone thiosemicarbazone) and PKIH (di-2-pyridylketone isonicotinoyl hydrazone) analogues] but these are not routinely used in clinical practice^[120-122]. Both venesection and iron chelation therapies do not target the biological mechanisms involved in iron metabolism. HH is characterised by low hepcidin synthesis and clinical trials evaluating the role of therapeutic hepcidin by subcutaneous administration are currently underway. Patients with cirrhosis should undergo six monthly surveillance by ultrasound (with or without alpha-fetoprotein measurement) to detect HCC at an early stage when curative therapy is more likely to be successful. Of interest, liver transplantation remains an option for some patients with HCC within appropriate criteria and this procedure will also normalise hepcidin synthesis and prevent iron overload (provided the donor does not have HH)^[117,123].

DISCUSSION

Early diagnosis and treatment of HH by preventing the development of cirrhosis may reduce the incidence of HCC in the future. The American Association for the Study of Liver Diseases guidelines recommend regular surveillance for HCC in cirrhotic patients only^[9]. It has been recommended that screening for HCC be continued throughout life of the HH patients as HCC may develop years after the depletion of iron has been achieved. Whilst controversial, some recommend iron depletion therapy in patients with even minor increases in iron stores, when non-alcoholic fatty liver disease, hepatitis B or C coexist, in an attempt to reduce the risk of progressive fibrosis and subsequent HCC^[72]. It is also recommended that family screening for HH mutations and iron overload in all first-degree relatives of HH patients be performed. As *HFE* gene mutation often synergises with other risk factors of HCC, HH patients with known HCC risk factors should be regularly counselled to avoid environmental or toxic injury to the liver.

Besides HH, there are many other causes of iron overloading that result in excessive iron accumulation in the liver and other organs. It has been reported that patients with high total body iron have a higher risk of developing HCC in the absence of HH^[124-126]. As iron overload is not a benign condition, it is recommended that HCC surveillance be undertaken in patients with excess body iron, particularly in patients with cirrhosis^[127].

Further studies to identify genetic or environmental factors that could act in concert with *HFE* mutations to increase the risk of developing HCC are warranted. Investigations are underway to determine the role

of iron-regulatory proteins in abnormal iron uptake in HCC. In-depth understanding of the intricate pathways involved in HH-associated HCC needs attention and future research needs to be focused on the prevention of HCC in these patients.

CONCLUSION

Despite the controversies in the field regarding the degree of penetrance of *HFE* mutations in different patient populations and their role in hepatic iron overload, HH patients with cirrhosis are at a high risk of developing HCC. Further study in this field is needed to better understand the pathogenic process toward HCC and to prevent HCC development in HH patients, considering that there are currently no effective therapies for HCC. Furthermore, an in-depth understanding of the metabolic iron regulatory pathways in *HFE*-related HCC in HH patients will allow the discovery of novel druggable targets for effective therapeutic approaches.

DECLARATIONS

Authors' contributions

Contributed to conception and design of the study and manuscript writing: Jayachandran A, Shrestha R, Bridle KR, Crawford DHG

Final approval of manuscripts: Jayachandran A, Shrestha R, Bridle KR, Crawford DHG

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Ethical approval and consent to participate

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REFERENCES

1. Venook AP, Papandreou C, Furuse J, de Guevara LL. The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. *Oncologist* 2010;15:5-13.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017;67:7-30.
3. Galun D, Srdic-Rajic T, Bogdanovic A, Loncar Z, Zuvela M. Targeted therapy and personalized medicine in hepatocellular carcinoma: drug resistance, mechanisms, and treatment strategies. *J Hepatocell Carcinoma* 2017;4:93-103.
4. Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol* 2018;15:599-616.
5. Ikeda M, Morizane C, Ueno M, Okusaka T, Ishii H, et al. Chemotherapy for hepatocellular carcinoma: current status and future perspectives. *Jpn J Clin Oncol* 2018;48:103-14.
6. Abou-Alfa GK, Huitzil-Melendez FD, O'Reilly EM, Saltz LB. Current management of advanced hepatocellular carcinoma. *Gastrointest Cancer Res* 2008;2:64-70.
7. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;362:1907-17.
8. McGlynn KA, Petrick JL, London WT. Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional

- variability. *Clin Liver Dis* 2015;19:223-38.
9. Bacon BR, Adams PC, Kowdley KV, Powell LW, Tavill AS; Diseases AAftSoL. Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011;54:328-43.
 10. Liver EAFTSOT. EASL clinical practice guidelines for HFE hemochromatosis. *J Hepatol* 2010;53:3-22.
 11. Crawford DHG. Hereditary hemochromatosis types 1, 2, and 3. *Clin Liver Dis (Hoboken)* 2014;3:96-7.
 12. Merryweather-Clarke AT, Pointon JJ, Jouanolle AM, Rochette J, Robson KJ. Geography of HFE C282Y and H63D mutations. *Genet Test* 2000;4:183-98.
 13. Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, et al. A population-based study of the clinical expression of the hemochromatosis gene. *N Engl J Med* 1999;341:718-24.
 14. Phatak PD, Bonkovsky HL, Kowdley KV. Hereditary hemochromatosis: time for targeted screening. *Ann Intern Med* 2008;149:270-2.
 15. Beutler E. Iron storage disease: facts, fiction and progress. *Blood Cells Mol Dis* 2007;39:140-7.
 16. Gan EK, Powell LW, Olynyk JK. Natural history and management of HFE-hemochromatosis. *Semin Liver Dis* 2011;31:293-301.
 17. Golfeyz S, Lewis S, Weisberg IS. Hemochromatosis: pathophysiology, evaluation, and management of hepatic iron overload with a focus on MRI. *Expert Rev Gastroenterol Hepatol* 2018;12:767-78.
 18. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, et al. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *N Engl J Med* 1985;313:1256-62.
 19. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;13:399-408.
 20. de Campos WN, Massaro JD, Cançado ELR, Wiesel CEV, Simões AL, et al. Comprehensive analysis of. *World J Hepatol* 2019;11:186-98.
 21. Barton JC, Acton RT. HLA-A and -B alleles and haplotypes in hemochromatosis probands with HFE C282Y homozygosity in central Alabama. *BMC Med Genet* 2002;3:9.
 22. Adams PC, Barton JC. Haemochromatosis. *Lancet* 2007;370:1855-60.
 23. Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. *N Engl J Med* 2008;358:221-30.
 24. Trifa AP, Popp RA, Militaru MS, Farcaş MF, Crişan TO, et al. HFE gene C282Y, H63D and S65C mutations frequency in the Transylvania region, Romania. *J Gastrointest Liver Dis* 2012;21:177-80.
 25. Feder JN, Penny DM, Irrinki A, Lee VK, Lebrón JA, et al. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proc Natl Acad Sci U S A* 1998;95:1472-7.
 26. Giannetti AM, Björkman PJ. HFE and transferrin directly compete for transferrin receptor in solution and at the cell surface. *J Biol Chem* 2004;279:25866-75.
 27. Schmidt PJ, Toran PT, Giannetti AM, Bjorkman PJ, Andrews NC. The transferrin receptor modulates Hfe-dependent regulation of hepcidin expression. *Cell Metab* 2008;7:205-14.
 28. Vujić Spasić M, Kiss J, Herrmann T, Galy B, Martinache S, et al. Hfe acts in hepatocytes to prevent hemochromatosis. *Cell Metab* 2008;7:173-8.
 29. Zhao N, Zhang AS, Enns CA. Iron regulation by hepcidin. *J Clin Invest* 2013;123:2337-43.
 30. Zhang AS, Enns CA. Molecular mechanisms of normal iron homeostasis. *Hematology Am Soc Hematol Educ Program* 2009;207-14.
 31. Ganz T, Nemeth E. Hepcidin and iron homeostasis. *Biochim Biophys Acta* 2012;1823:1434-43.
 32. Deugnier Y. Iron and liver cancer. *Alcohol* 2003;30:145-50.
 33. Beutler E, Gelbart T, West C, Lee P, Adams M, et al. Mutation analysis in hereditary hemochromatosis. *Blood Cells Mol Dis* 1996;22:187-94.
 34. Mura C, Ragueneas O, Férec C. HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. *Blood* 1999;93:2502-5.
 35. Bacon BR, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE genotype in patients with hemochromatosis and other liver diseases. *Ann Intern Med* 1999;130:953-62.
 36. Gurrin LC, Bertalli NA, Dalton GW, Osborne NJ, Constantine CC, et al. HFE C282Y/H63D compound heterozygotes are at low risk of hemochromatosis-related morbidity. *Hepatology* 2009;50:94-101.
 37. Walsh A, Dixon JL, Ramm GA, Hewett DG, Lincoln DJ, et al. The clinical relevance of compound heterozygosity for the C282Y and H63D substitutions in hemochromatosis. *Clin Gastroenterol Hepatol* 2006;4:1403-10.
 38. Cardoso CS, de Sousa M. HFE, the MHC and hemochromatosis: paradigm for an extended function for MHC class I. *Tissue Antigens* 2003;61:263-75.
 39. Torres FR, Souza-Neiras WC, D'Almeida Couto AA, D'Almeida Couto VS, Cavasini CE, et al. Frequency of the HFE C282Y and H63D polymorphisms in Brazilian malaria patients and blood donors from the Amazon region. *Genet Mol Res* 2008;7:60-4.
 40. Fargion S, Mandelli C, Piperno A, Cesana B, Fracanzani AL, et al. Survival and prognostic factors in 212 Italian patients with genetic hemochromatosis. *Hepatology* 1992;15:655-9.
 41. Kew MD. Pathogenesis of hepatocellular carcinoma in hereditary hemochromatosis: occurrence in noncirrhotic patients. *Hepatology* 1990;11:1086-7.
 42. ElMBERG M, Hultcrantz R, Ekblom A, Brandt L, Olsson S, et al. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. *Gastroenterology* 2003;125:1733-41.
 43. Fracanzani AL, Conte D, Fraquelli M, Taioli E, Mattioli M, et al. Increased cancer risk in a cohort of 230 patients with hereditary hemochromatosis in comparison to matched control patients with non-iron-related chronic liver disease. *Hepatology* 2001;33:647-51.
 44. Haddow JE, Palomaki GE, McClain M, Craig W. Hereditary haemochromatosis and hepatocellular carcinoma in males: a strategy for estimating the potential for primary prevention. *J Med Screen* 2003;10:11-3.

45. Hsing AW, McLaughlin JK, Olsen JH, Møller J, Wacholder S, et al. Cancer risk following primary hemochromatosis: a population-based cohort study in Denmark. *Int J Cancer* 1995;60:160-2.
46. Strohmeyer G, Niederau C, Stremmel W. Survival and causes of death in hemochromatosis. Observations in 163 patients. *Ann N Y Acad Sci* 1988;526:245-57.
47. Yang Q, McDonnell SM, Khoury MJ, Cono J, Parrish RG. Hemochromatosis-associated mortality in the United States from 1979 to 1992: an analysis of Multiple-Cause Mortality Data. *Ann Intern Med* 1998;129:946-53.
48. Cauza E, Peck-Radosavljevic M, Ulrich-Pur H, Datz C, Gschwandler M, et al. Mutations of the HFE gene in patients with hepatocellular carcinoma. *Am J Gastroenterol* 2003;98:442-7.
49. Ezzikouri S, El Feydi AE, El Kihal L, Afifi R, Benazzouz M, et al. Prevalence of common HFE and SERPINA1 mutations in patients with hepatocellular carcinoma in a Moroccan population. *Arch Med Res* 2008;39:236-41.
50. Fargion S, Stazi MA, Fracanzani AL, Mattioli M, Sampietro M, et al. Mutations in the HFE gene and their interaction with exogenous risk factors in hepatocellular carcinoma. *Blood Cells Mol Dis* 2001;27:505-11.
51. Hellerbrand C, Pöppl A, Hartmann A, Schölmerich J, Lock G. HFE C282Y heterozygosity in hepatocellular carcinoma: evidence for an increased prevalence. *Clin Gastroenterol Hepatol* 2003;1:279-84.
52. Lauret E, Rodríguez M, González S, Linares A, López-Vázquez A, et al. HFE gene mutations in alcoholic and virus-related cirrhotic patients with hepatocellular carcinoma. *Am J Gastroenterol* 2002;97:1016-21.
53. Nahon P, Sutton A, Rufat P, Ziol M, Thabut G, et al. Liver iron, HFE gene mutations, and hepatocellular carcinoma occurrence in patients with cirrhosis. *Gastroenterology* 2008;134:102-10.
54. Pirisi M, Toniutto P, Uzzau A, Fabris C, Avellini C, et al. Carriage of HFE mutations and outcome of surgical resection for hepatocellular carcinoma in cirrhotic patients. *Cancer* 2000;89:297-302.
55. Ropero P, Briceño O, López-Alonso G, Agúndez JA, González Fernández FA, et al. The H63D mutation in the HFE gene is related to the risk of hepatocellular carcinoma. *Rev Esp Enferm Dig* 2007;99:376-81. (in Spanish)
56. Shi WJ, Chen H, Zhou B, Cheng J. Association of mutations of HFE gene and hepatocellular carcinoma following chronic hepatitis B. *Zhonghua Gan Zang Bing Za Zhi* 2005;13:682-4. (in Chinese)
57. Willis G, Wimperis JZ, Lonsdale R, Fellows IW, Watson MA, et al. Incidence of liver disease in people with HFE mutations. *Gut* 2000;46:401-4.
58. Willis G, Wimperis JZ, Lonsdale R, Jennings BA. Haemochromatosis gene mutation in hepatocellular cancer. *Lancet* 1997;350:565-6.
59. Sánchez-Luna SA, Brown KE. Clinical burden of liver disease from hemochromatosis at an academic medical center. *Hepatol Commun* 2017;1:453-9.
60. Ellervik C, Birgens H, Tybjaerg-Hansen A, Nordestgaard BG. Hemochromatosis genotypes and risk of 31 disease endpoints: meta-analyses including 66,000 cases and 226,000 controls. *Hepatology* 2007;46:1071-80.
61. Ye Q, Qian BX, Yin WL, Wang FM, Han T. Association between the HFE C282Y, H63D polymorphisms and the risks of non-alcoholic fatty liver disease, liver cirrhosis and hepatocellular carcinoma: an updated systematic review and meta-analysis of 5,758 cases and 14,741 controls. *PLoS One* 2016;11:e0163423.
62. Asberg A, Hveem K, Thorstensen K, Ellekjer E, Kannelønning K, et al. Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. *Scand J Gastroenterol* 2001;36:1108-15.
63. Gharib AF, Karam RA, Pasha HF, Radwan MI, Elsayy WH. Polymorphisms of hemochromatosis, and alpha-1 antitrypsin genes in Egyptian HCV patients with and without hepatocellular carcinoma. *Gene* 2011;489:98-102.
64. Harrison SA, Bacon BR. Relation of hemochromatosis with hepatocellular carcinoma: epidemiology, natural history, pathophysiology, screening, treatment, and prevention. *Med Clin North Am* 2005;89:391-409.
65. Tarao K, Nozaki A, Ikeda T, Sato A, Komatsu H, et al. Real impact of liver cirrhosis on the development of hepatocellular carcinoma in various liver diseases-meta-analytic assessment. *Cancer Med* 2019;8:1054-65.
66. Villanueva A, Newell P, Hoshida Y. Inherited hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol* 2010;24:725-34.
67. Bradbear RA, Bain C, Siskind V, Schofield FD, Webb S, et al. Cohort study of internal malignancy in genetic hemochromatosis and other chronic nonalcoholic liver diseases. *J Natl Cancer Inst* 1985;75:81-4.
68. Blanc JF, De Ledinghen V, Bernard PH, de Verneuil H, Winnock M, et al. Increased incidence of HFE C282Y mutations in patients with iron overload and hepatocellular carcinoma developed in non-cirrhotic liver. *J Hepatol* 2000;32:805-11.
69. Hiatt T, Trotter JF, Kam I. Hepatocellular carcinoma in a noncirrhotic patient with hereditary hemochromatosis. *Am J Med Sci* 2007;334:228-30.
70. Asare GA, Mossanda KS, Kew MC, Paterson AC, Kahler-Venter CP, et al. Hepatocellular carcinoma caused by iron overload: a possible mechanism of direct hepatocarcinogenicity. *Toxicology* 2006;219:41-52.
71. Willis G, Bardsley V, Fellows IW, Lonsdale R, Wimperis JZ, et al. Hepatocellular carcinoma and the penetrance of HFE C282Y mutations: a cross sectional study. *BMC Gastroenterol* 2005;5:17.
72. Fracanzani AL, Fargion S, Stazi MA, Valenti L, Amoroso P, et al. Association between heterozygosity for HFE gene mutations and hepatitis viruses in hepatocellular carcinoma. *Blood Cells Mol Dis* 2005;35:27-32.
73. Nowak A, Giger RS, Kräyenbühl PA. Higher age at diagnosis of hemochromatosis is the strongest predictor of the occurrence of hepatocellular carcinoma in the Swiss hemochromatosis cohort: a prospective longitudinal observational study. *Medicine (Baltimore)* 2018;97:e12886.
74. Boige V, Castéra L, de Roux N, Ganne-Carrié N, Ducot B, et al. Lack of association between HFE gene mutations and hepatocellular carcinoma in patients with cirrhosis. *Gut* 2003;52:1178-81.
75. Jin F, Qu LS, Shen XZ. Association between C282Y and H63D mutations of the HFE gene with hepatocellular carcinoma in European populations: a meta-analysis. *J Exp Clin Cancer Res* 2010;29:18.
76. Campo S, Restuccia T, Villari D, Raffa G, Cucinotta D, et al. Analysis of haemochromatosis gene mutations in a population from the

- Mediterranean Basin. *Liver* 2001;21:233-6.
77. Constantine CC, Gurrin LC, McLaren CE, Bahlo M, Anderson GJ, et al. SNP selection for genes of iron metabolism in a study of genetic modifiers of hemochromatosis. *BMC Med Genet* 2008;9:18.
 78. Racchi O, Mangerini R, Rapezzi D, Gaetani GF, Nobile MT, et al. Mutations of the HFE gene and the risk of hepatocellular carcinoma. *Blood Cells Mol Dis* 1999;25:350-3.
 79. Kew MC. Hepatic iron overload and hepatocellular carcinoma. *Liver Cancer* 2014;3:31-40.
 80. Shen Y, Li X, Zhao B, Xue Y, Wang S, et al. Iron metabolism gene expression and prognostic features of hepatocellular carcinoma. *J Cell Biochem* 2018;119:9178-204.
 81. Pantopoulos K. Inherited disorders of iron overload. *Front Nutr* 2018;5:103.
 82. Worthen CA, Enns CA. The role of hepatic transferrin receptor 2 in the regulation of iron homeostasis in the body. *Front Pharmacol* 2014;5:34.
 83. Andrews NC. Disorders of iron metabolism. *N Engl J Med* 1999;341:1986-95.
 84. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004;306:2090-3.
 85. West AP, Bennett MJ, Sellers VM, Andrews NC, Enns CA, et al. Comparison of the interactions of transferrin receptor and transferrin receptor 2 with transferrin and the hereditary hemochromatosis protein HFE. *J Biol Chem* 2000;275:38135-8.
 86. Lesbordes-Brion JC, Viatte L, Bennoun M, Lou DQ, Ramey G, et al. Targeted disruption of the hepcidin 1 gene results in severe hemochromatosis. *Blood* 2006;108:1402-5.
 87. Wu XG, Wang Y, Wu Q, Cheng WH, Liu W, et al. HFE interacts with the BMP type I receptor ALK3 to regulate hepcidin expression. *Blood* 2014;124:1335-43.
 88. Gao J, Chen J, Kramer M, Tsukamoto H, Zhang AS, et al. Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. *Cell Metab* 2009;9:217-27.
 89. Zacharski LR, Ornstein DL, Woloshin S, Schwartz LM. Association of age, sex, and race with body iron stores in adults: analysis of NHANES III data. *Am Heart J* 2000;140:98-104.
 90. Asare GA, Paterson AC, Kew MC, Khan S, Mossanda KS. Iron-free neoplastic nodules and hepatocellular carcinoma without cirrhosis in Wistar rats fed a diet high in iron. *J Pathol* 2006;208:82-90.
 91. Ioannou GN, Kowdley KV. Iron, HFE mutations, and hepatocellular carcinoma: is hepatic iron a carcinogen? *Clin Gastroenterol Hepatol* 2003;1:246-8.
 92. Ramm GA, Crawford DH, Powell LW, Walker NI, Fletcher LM, et al. Hepatic stellate cell activation in genetic haemochromatosis. Lobular distribution, effect of increasing hepatic iron and response to phlebotomy. *J Hepatol* 1997;26:584-92.
 93. Yu Y, Kovacevic Z, Richardson DR. Tuning cell cycle regulation with an iron key. *Cell Cycle* 2007;6:1982-94.
 94. Toyokuni S. Role of iron in carcinogenesis: cancer as a ferrotoxic disease. *Cancer Sci* 2009;100:9-16.
 95. Fargion S, Valenti L, Fracanzani AL. Role of iron in hepatocellular carcinoma. *Clin Liver Dis (Hoboken)* 2014;3:108-10.
 96. Moukhaider HM, Halawi R, Cappellini MD, Taher AT. Hepatocellular carcinoma as an emerging morbidity in the thalassemia syndromes: a comprehensive review. *Cancer* 2017;123:751-8.
 97. Marshall A, Lukk M, Kutter C, Davies S, Alexander G, Odom DT. Global gene expression profiling reveals SPINK1 as a potential hepatocellular carcinoma marker. *PLoS One* 2013;8:e59459.
 98. Miyanishi K, Tanaka S, Sakamoto H, Kato J. The role of iron in hepatic inflammation and hepatocellular carcinoma. *Free Radic Biol Med* 2019;133:200-5.
 99. Muto Y, Moroishi T, Ichihara K, Nishiyama M, Shimizu H, et al. Disruption of FBXL5-mediated cellular iron homeostasis promotes liver carcinogenesis. *J Exp Med* 2019;216:950-65.
 100. Hussain SP, Hollstein MH, Harris CC. p53 tumor suppressor gene: at the crossroads of molecular carcinogenesis, molecular epidemiology, and human risk assessment. *Ann N Y Acad Sci* 2000;919:79-85.
 101. Ichiba M, Maeta Y, Mukoyama T, Saeki T, Yasui S, et al. Expression of 8-hydroxy-2'-deoxyguanosine in chronic liver disease and hepatocellular carcinoma. *Liver Int* 2003;23:338-45.
 102. Valerio LG, Petersen DR. Characterization of hepatic iron overload following dietary administration of dicyclopentadienyl iron (Ferrocene) to mice: cellular, biochemical, and molecular aspects. *Exp Mol Pathol* 2000;68:1-12.
 103. Kowdley KV. Iron, hemochromatosis, and hepatocellular carcinoma. *Gastroenterology* 2004;127:S79-86.
 104. Olynyk JK, St Pierre TG, Britton RS, Brunt EM, Bacon BR. Duration of hepatic iron exposure increases the risk of significant fibrosis in hereditary hemochromatosis: a new role for magnetic resonance imaging. *Am J Gastroenterol* 2005;100:837-41.
 105. Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. *Best Pract Res Clin Haematol* 2005;18:277-87.
 106. Asare GA, Bronz M, Naidoo V, Kew MC. Synergistic interaction between excess hepatic iron and alcohol ingestion in hepatic mutagenesis. *Toxicology* 2008;254:11-8.
 107. Nahon P, Sutton A, Pessayre D, Rufat P, Charnaux N, et al. Do genetic variations in antioxidant enzymes influence the course of hereditary hemochromatosis? *Antioxid Redox Signal* 2011;15:31-8.
 108. Houglum K, Bedossa P, Chojkier M. TGF-beta and collagen-alpha 1 (I) gene expression are increased in hepatic acinar zone 1 of rats with iron overload. *Am J Physiol* 1994;267:G908-13.
 109. Wallace DF, Subramaniam VN. Co-factors in liver disease: the role of HFE-related hereditary hemochromatosis and iron. *Biochim Biophys Acta* 2009;1790:663-70.
 110. Hann HW, Stahlhut MW, Hann CL. Effect of iron and desferoxamine on cell growth and in vitro ferritin synthesis in human hepatoma cell lines. *Hepatology* 1990;11:566-9.
 111. Shen Y, Li X, Su Y, Badshah SA, Zhang B, et al. Downregulation contributes to aggressive hepatocellular carcinoma via mechanism

- mediated by cyclin4-dependent kinase-1/STAT3 pathway. *Diagnostics* (Basel) 2019;9.
112. Djeha A, Brock JH. Effect of transferrin, lactoferrin and chelated iron on human T-lymphocytes. *Br J Haematol* 1992;80:235-41.
 113. Green R, Esparza I, Schreiber R. Iron inhibits the nonspecific tumoricidal activity of macrophages. A possible contributory mechanism for neoplasia in hemochromatosis. *Ann N Y Acad Sci* 1988;526:301-9.
 114. Cozma A, Fodor A, Vultur R, Sitar-Tăut AV, Orășan OH, et al. DNA methylation and micro-RNAs: the most recent and relevant biomarkers in the early diagnosis of hepatocellular carcinoma. *Medicina* (Kaunas) 2019;55.
 115. Lehmann U, Wingen LU, Brakensiek K, Wedemeyer H, Becker T, et al. Epigenetic defects of hepatocellular carcinoma are already found in non-neoplastic liver cells from patients with hereditary haemochromatosis. *Hum Mol Genet* 2007;16:1335-42.
 116. Adams P, Altes A, Brissot P, Butzeck B, Cabantchik I, et al. Therapeutic recommendations in HFE hemochromatosis for p.Cys282Tyr (C282Y/C282Y) homozygous genotype. *Hepatol Int* 2018;12:83-6.
 117. Loréal O, Cavey T, Robin F, Kenawi M, Guggenbuhl P, et al. Iron as a therapeutic target in HFE-related hemochromatosis: usual and novel aspects. *Pharmaceuticals* (Basel) 2018;11.
 118. Phatak P, Brissot P, Wurster M, Adams PC, Bonkovsky HL, et al. A phase 1/2, dose-escalation trial of deferasirox for the treatment of iron overload in HFE-related hereditary hemochromatosis. *Hepatology* 2010;52:1671-779.
 119. Tauchenová L, Křížová B, Kubánek M, Fraňková S, Melenovský V, et al. Successful treatment of iron-overload cardiomyopathy in hereditary hemochromatosis with deferoxamine and deferiprone. *Can J Cardiol* 2016;32:1574.e1-e3.
 120. Whitnall M, Howard J, Ponka P, Richardson DR. A class of iron chelators with a wide spectrum of potent antitumor activity that overcomes resistance to chemotherapeutics. *Proc Natl Acad Sci U S A* 2006;103:14901-6.
 121. Richardson DR, Tran EH, Ponka P. The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents. *Blood* 1995;86:4295-306.
 122. Wadler S, Makower D, Clairmont C, Lambert P, Fehn K, et al. Phase I and pharmacokinetic study of the ribonucleotide reductase inhibitor, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone, administered by 96-hour intravenous continuous infusion. *J Clin Oncol* 2004;22:1553-63.
 123. Fung E, Sugianto P, Hsu J, Damoiseaux R, Ganz T, et al. High-throughput screening of small molecules identifies hepcidin antagonists. *Mol Pharmacol* 2013;83:681-90.
 124. MacPhail AP, Mandishona EM, Bloom PD, Paterson AC, Rouault TA, et al. Measurements of iron status and survival in African iron overload. *S Afr Med J* 1999;89:966-72.
 125. Mandishona E, MacPhail AP, Gordeuk VR, Kedda MA, Paterson AC, et al. Dietary iron overload as a risk factor for hepatocellular carcinoma in Black Africans. *Hepatology* 1998;27:1563-6.
 126. Turlin B, Juguet F, Moirand R, Le Quilleuc D, Loréal O, et al. Increased liver iron stores in patients with hepatocellular carcinoma developed on a noncirrhotic liver. *Hepatology* 1995;22:446-50.
 127. Blonski W, Kotlyar DS, Forde KA. Non-viral causes of hepatocellular carcinoma. *World J Gastroenterol* 2010;16:3603-15.

Review

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The interplay between direct-acting antivirals and hepatocellular carcinoma in chronic hepatitis C

Sun Hong Yoo, Jung Hyun Kwon

Department of Internal Medicine, Catholic University Liver Research Center, The Catholic University of Korea, Seoul 06591, Korea.

Correspondence to: Prof. Jung Hyun Kwon, Department of Internal Medicine, Catholic University Liver Research Center, The Catholic University of Korea, Seoul, Korea. E-mail: doctorkwon@catholic.ac.kr

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Abstract

Direct-acting antivirals (DAAs) have been introduced for the treatment of hepatitis C virus, and the sustained virological response rate after DAAs was reported to be over 95%. Because of the high sustained virological response rate, the risk of hepatocellular carcinoma (HCC) was expected to be reduced. However, an unexpected high risk of HCC recurrence after DAA treatment was reported, and thus the dispute about the association of DAA and HCC arose. The present article reviews the interplay between DAAs and HCC.

Keywords: Chronic hepatitis C, hepatocellular carcinoma, direct-acting antivirals

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the most common primary liver cancer. The major causes of HCC are cirrhosis of any cause, chronic hepatitis B, chronic hepatitis C (CHC), alcohol, and nonalcoholic fatty liver disease. Among the causes, the incidence of chronic viral hepatitis-related HCC is 3%-5% per year in patients with cirrhosis and < 1.5% per year in patients with both hepatitis C and stage 3 fibrosis^[1]. The sustained virological response (SVR) rate for pegylated interferon (IFN)-based therapy has been reported to be 42%-65% for genotype 1 and 74%-93% for genotype 2 virus^[2-4]. Despite the low SVR, several previous retrospective studies suggest that achieving SVR after pegylated IFN plus ribavirin therapy reduces the risk of hepatic decompensation, liver related mortality, liver transplantation, and HCC^[5-7].



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Recently, an IFN-free regimen as a treatment for CHC including NS3/4A protease inhibitor, NS5A inhibitor, and NS5B polymerase inhibitor was introduced. There have been several reports indicating that the SVR rate is up to 97.8% and the adverse event rate at all stages of CHC is lower in patients treated with direct-acting antivirals than IFN-based therapy^[8-12]. Despite expectations of a decrease in the incidence of HCC because of the high rate of SVR, Reig *et al.*^[13] reported an unexpected high rate of HCC recurrence after treating with direct-acting antivirals (DAA) in patients who experience previous HCC. Conti *et al.*^[14] also reported a high rate of HCC recurrence (28.81%) 24 weeks after DAAs. Since the two aforementioned reports were published, much debate has been raised about the recurrence and occurrence of HCC after treating DAAs.

In this article, we review the pros and cons of the effects of the DAAs on occurrence/recurrence of HCC.

THE INTERPLAY BETWEEN DIRECT-ACTING ANTIVIRALS AND OCCURRENCE OF HEPATOCELLULAR CARCINOMA

CHC is the most common cause of HCC worldwide. The incidence of HCC is below 1% per year in CHC patients without liver cirrhosis^[15]. However, the risk of HCC increases by 2%-8% in CHC with liver cirrhosis^[16].

The papers on HCC occurrence related to DAAs are listed in Table 1. A negative paper was first published on the occurrence of the HCC after the treatment of DAAs. In 2016, Conti *et al.*^[14] published the first report about early occurrence of HCC in hepatitis C virus-related cirrhosis treated with DAAs. They retrospectively analyzed 285 consecutive cirrhotic patients who completed antiviral therapy with DAA regimens and HCC developed in 9 of 285 patients (3.16%, 95%CI: 1.45-5.90) during the 24-week post-treatment evaluation. The report concluded that DAA-induced resolution of HCV infection does not seem to reduce occurrence of HCC.

Since the publication of the previous paper, several papers have been published indicating that DAAs are not associated with occurrence of HCC. A thesis against the previous study was published by Kanwal *et al.*^[17] in 2017. This retrospective cohort study included 22,500 patients who received DAA treatment; 39.0% of the patients had cirrhosis and 86.74% achieved SVR. The incidence rate of HCC was 0.90 per 100 person-year (95%CI: 0.77-1.03) in patients with SVR and 3.45 per 100 person-year (95%CI: 2.73-4.18) in patients without SVR.

A large prospective study of 2249 patients with HCV-associated cirrhosis was published in Italy by Calvaruso and his colleagues^[18]. SVR after DAA treatment was achieved in 95.2% of patients and the overall rate of HCC occurrence was 3.4%. They analyzed the HCC incidence according to achieved SVR, and HCC occurrence was 3% in SVR group and 12.8% in non-SVR group ($P < 0.001$). Although this study did not contain the analysis of control group, they found the SVR to DAA treatment decreased the incidence of HCC. A similar study in the same country including 3917 patients with fibrosis stage \geq F3 was published by Romano and colleagues^[19]. This large, prospective cohort study showed that the incidence of HCC occurrence was 0.42% in F3, 1.88% in cirrhosis, and 0.97 per 100 person-year (95%CI: 0.73-1.26) in all patients.

Nagata *et al.*^[20] compared data between IFN-based and IFN-free regimens for occurrence of HCC. This report included 1085 patients treated with IFN and 669 patients treated with DAAs. The cumulative incidence of HCC occurrence after SVR was 2.6% (five-year incidence) in IFN-based and 3.3% (three-year incidence) in IFN-free therapies. Although the incidence of HCC appears to be higher in IFN-free group than IFN-based group, there are no significant differences between the two groups after performing

Table 1. Studies about de novo HCC occurrence after receiving DAAs in patients with hepatitis C virus infection

Author	Study design	Patient number	Median follow-up period (months)	SVR rate	Outcomes	Conclusion
Conti <i>et al.</i> ^[14]	Retrospective observational cohort study	HCV-associated cirrhosis with DAAs (<i>n</i> = 285)	5.6	91.00% (3.16%)	HCC occurrence rate	DAA-induced resolution of HCV infection does not seem to reduce occurrence of HCC
Kanwal <i>et al.</i> ^[17]	Retrospective observational cohort study	Chronic hepatitis C with DAAs (<i>n</i> = 22,500)	NA	86.74% (1.2%)	HCC occurrence rate - SVR (0.90/100 PY) - non-SVR (3.45/100 PY)	Among patients treated with DAA, SVR was associated with a considerable reduction in the risk of HCC
Calvaruso <i>et al.</i> ^[18]	Prospective observational cohort study	HCV-associated cirrhosis with DAAs (<i>n</i> = 2249)	14	95.20% (1.4%)	HCC occurrence rate - CPC A + SVR (2.1%) - CPC A + no SVR (6.6%) - CPC B + SVR (7.8%) - CPC B + no SVR (12.4%)	SVR to DAA treatment decreased the incidence of HCC over a mean follow-up of 14 months
Romano <i>et al.</i> ^[19]	Prospective observational cohort study	Fibrosis stage U F3 CHC with DAAs (<i>n</i> = 3917)	17.9	93.90% (1.4%)	HCC occurrence rate	In patients with advanced hepatitis C receiving DAAs, the risk of “de novo” HCC during the first year is not higher, and might be lower, than that of untreated patients
Nagata <i>et al.</i> ^[20]	Retrospective observational cohort study	Chronic hepatitis C -IFN (<i>n</i> = 1145) -DAAs (<i>n</i> = 752)	IFN 81.6 DAAs 21.6	96.00% (3.3%)	HCC occurrence rate after viral eradication - IFN (3.3%), DAAs (1.4%)	The risks of early HCC occurrence and recurrence after viral eradication were similar between IFN-based and IFN-free therapies
Nahon <i>et al.</i> ^[21]	Prospective observational cohort study	Biopsy-proven HCV-associated cirrhosis with DAAs (<i>n</i> = 1270) -DAAs (<i>n</i> = 336) -SVR-IFN (<i>n</i> = 495) -non-SVR (<i>n</i> = 439)	67.5	HCC occurrence rate (5 year-CumI 14.7%) - DAAs 3-year CumI: 5.9% - SVR-IFN 3-year CumI: 3.1% - non-SVR 3-year CumI: 12.7%	There is no statistically significant increase in risk of HCC was associated with DAA use	
Ioannou <i>et al.</i> ^[42]	Retrospective observational cohort study	Chronic hepatitis C -IFN only (<i>n</i> = 35,871, 58%) -DAA + IFN (<i>n</i> = 4535, 7.2%) -DAA only (<i>n</i> = 21,948, 35%)	73.2	NA	HCC occurrence rate (5.2%)	DAA-induced SVR is associated with a 71% reduction in HCC risk. Treatment with DAAs is not associated with increased HCC risk compared with treatment with IFN
Yoo <i>et al.</i> ^[22]	Retrospective observational cohort study	Chronic hepatitis C -DAAs (<i>n</i> = 574) -IFN (<i>n</i> = 211)	IFN 43.6 DAAs 10.4	DAAs 95.1% IFN 75.4%	HCC occurrence rate (0.89%) - DAAs (1.05%) - IFN (0.47%)	The rate of early development of HCC did not differ between patients treated with IFN and those treated with DAAs
Singer <i>et al.</i> ^[23]	Retrospective observational cohort study	Chronic hepatitis C -DAAs (<i>n</i> = 30,183) -Untreated (<i>n</i> = 137,502) -IFN (<i>n</i> = 12,948)	DAAs 1.05 PY Untreated 1.24 PY	NA	HCC occurrence rate - DAAs 0.64/100 PY - Untreated 1.18/100 PY	DAA-based treatment was associated with a reduced risk of incident liver cancer relative to both no HCV treatment and to IFN-based treatment in the pre-DAA era
Carrat <i>et al.</i> ^[24]	Prospective observational cohort study	Chronic hepatitis C -DAA (<i>n</i> = 7344) -Untreated (<i>n</i> = 2551)	33.4	94.00% (2.54%)	HCC occurrence rate of DAA treated group	Treatment with direct-acting antivirals is associated with reduced risk for mortality and hepatocellular carcinoma
Ide <i>et al.</i> ^[25]	Prospective observational cohort study	Chronic hepatitis C with DAAs (<i>n</i> = 2552)	NA	NA	HCC occurrence rate (2.7%)	Achieving SVR by DAA treatment reduces the incidence of HCC

HCV: hepatitis C virus; DAAs: direct-acting antivirals; HCC: hepatocellular carcinoma; NA: not available; SVR: sustained virological response; PY: person-year; CPC: Child-Pugh Class; IFN: interferon; CumI: cumulative incidence

propensity score-matched analysis (three-year incidence: 3.3% in IFN-based therapy and 1.4% in IFN-free therapy; $P = 0.49$). In a study from France, Nahon *et al.*^[21] published a report about the incidence of HCC after DAA for HCV in patients with cirrhosis included in surveillance programs. The retrospective cohort study included 1270 patients with biopsy-proven cirrhosis and classified into DAA group ($n = 336$), SVR-IFN group ($n = 495$), and non-SVR group ($n = 439$). The three-year cumulative incidences of HCC were 5.9% in the DAA group, 3.1% in the SVR-IFN group, and 12.7% in the non-SVR group (HR: 2.03, 95%CI: 1.07-3.84, $P = 0.03$ for the DAA group vs. the SVR-IFN group). However, under propensity score matched analysis, there was no significant increase in risk of HCC for DAA use (HR: 0.89, 95%CI: 0.46-1.73, $P = 0.735$). The DAA group was older, and had a higher rate of diabetes or portal hypertension than SVR-IFN group. These features suggested that a more advanced liver disease, older age, and higher rates of comorbidities favor liver carcinogenesis. Yoo *et al.*^[22] published similar comparative data of *de novo* HCC occurrence in DAA group and IFN group. The cumulative incidence of HCC occurrence was not different between DAA group and IFN group ($P = 0.827$). In USA, Singer *et al.*^[23] analyzed 30,138 patients receiving DAA treatment, 137,502 patients without any treatment, and 12,948 patients receiving IFN treatment. This study revealed that DAA treatment was associated with a reduced risk of HCC compared to IFN treatment after performing inverse probability of treatment weighting (adjusted HR: 0.69, 95%CI: 0.59-0.81).

In 2019, the debate on the interplay between DAA and HCC continued, and Carrat *et al.*^[24] and Ide *et al.*^[25] published prospective cohort studies. In the former study in France, 7344 patients with DAA treatment, and 2551 patients without treatment were enrolled^[24]. DAA treatment seems to increase the risk of HCC (HR: 2.77, 95%CI: 2.07-3.71). However, after adjustment for variables, DAA treatment was associated with a decrease in HCC (adjusted HR: 0.66, 95%CI: 0.46-0.91) and all-cause mortality (adjusted HR: 0.48, 95%CI: 0.33-0.70). A prospective study from Japan by Ide *et al.*^[25] enrolled 2552 patients who were treated with DAAs and achieved a SVR. The three-year cumulative incidence of HCC was 4.9% in all patients, 10.0% in patients with cirrhosis, and 2.9% in patients without cirrhosis. They concluded that DAAs do not increase the risk of HCC occurrence after achieving SVR.

THE INTERPLAY BETWEEN DIRECT-ACTING ANTIVIRALS AND RECURRENCE OF HEPATOCELLULAR CARCINOMA

HCC is treated with curative treatment or non-curative interventions according to tumor stage, liver function, and performance status. After curative treatment such as surgical resection for HCC, the risk of recurrence is 60%-70% at five years^[26,27]. Several studies have shown that adjuvant IFN therapy after curative treatment can reduce the recurrence rate of HCC^[28-32].

The papers published on the HCC recurrence after DAA treatment are organized in Table 2. Despite expectations that achieving SVR after DAA treatment will reduce the recurrence of HCC, Reig *et al.*^[13] reported an unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing DAA therapy in 2016. The study included 58 patients with prior history of treated HCC with complete response who lacked “non-characterized nodules”. They reported unexpected high recurrence rate of 27.6% and median time from DAA start to recurrence was 3.5 months (range 1.1-8 months). Conti *et al.*^[14] published another similar report about early recurrence of HCC. In this retrospective cohort study, the recurrence rate of HCC after completing DAA therapy was 28.81% (17 of 59 patients, 95%CI: 17.76-42.07) during the 24-week post-treatment evaluation. Fifty-nine patients with a history of previous HCC included 11 patients who received transarterial chemoembolization”. This term has only been mentioned once for previous HCC. The study indicated that patients previously treated for HCC still have a high risk of tumor recurrence.

Opposite opinions to the previous paper were subsequently published. One prospective study used three French multicenter ANRS cohorts^[33]. The DAA group and untreated group were analyzed and the rate

Table 2. Studies about HCC recurrence after receiving DAAs in patients with hepatitis C virus infection

Author	Study design	Patient number	Median follow-up period (months)	SVR rate	Outcomes	Conclusion
Reig <i>et al.</i> ^[13]	Retrospective observational cohort study	Prior history of treated HCC with DAAs (<i>n</i> = 103)	5.7	97.50%	Tumor recurrence (27.6%)	The study showed an unexpected high rate and pattern of tumor recurrence coinciding with HCV clearance
Conti <i>et al.</i> ^[14]	Retrospective observational cohort study	Prior history of treated HCC in HCV-associated cirrhosis with DAAs (<i>n</i> = 59)	5.6	91.00%	HCC recurrence rate (28.81%)	DAA-induced resolution of HCV infection does not seem to reduce recurrence of HCC
ANRS ^[33]	Collaborative study Prospective observation cohort study	HEPATIER cohort Prior history of treated HCC - DAAs (<i>n</i> = 189) - Untreated (<i>n</i> = 78)	20.2	NA	HCC recurrence rate - DAA 0.73/100 person-month - Untreated 0.66/100 person-month	The study did not find an increase in HCC recurrence rate during the first 3 months of the treated period
Cabibbo <i>et al.</i> ^[34]	Prospective observational cohort study	CirVir cohort Prior history of treated HCC in HCV-associated cirrhosis - DAAs (<i>n</i> = 13) - untreated (<i>n</i> = 66)	58.6	NA	HCC recurrence rate - DAA 1.11/100 person-month - Untreated 1.73/100 person-month	There was no evidence of an increased risk of HCC recurrence in treated compared with untreated patients
Nagata <i>et al.</i> ^[20]	Retrospective observational cohort study	CUPILT cohort Liver transplanted patients for HCC with DAA (<i>n</i> = 314) Prior history of treated HCC with DAAs (<i>n</i> = 143)	70 months from LT	96.80%	HCC recurrence rate (2.2%)	The observed recurrence rate of 2.2% was lower than the expected rate according to previous studies with interferon regimen
Nishibatake Kinoshita <i>et al.</i> ^[35]	Retrospective observational cohort study	Chronic hepatitis C - IFN (<i>n</i> = 1145) - DAAs (<i>n</i> = 752)	IFN 81.6 DAAs 21.6	96.00%	HCC recurrence after viral eradication - IFN (54.2%) - DAAs DAAs (45.1%)	The risk of HCC early recurrence was comparable and not higher than that observed in DAA unexposed patients
Singal <i>et al.</i> ^[37]	Retrospective observational cohort study	RFA for HCV-related HCC - DAAs (<i>n</i> = 147) - IFN (<i>n</i> = 156)	IFN 86.4 DAAs 21.6	NA	HCC recurrence rate at 2years - DAAs (60%) - IFN (61%)	The risks of early HCC occurrence and recurrence after viral eradication were similar between IFN-based and IFN-free therapies
		Prior history of treated HCC (<i>n</i> = 793) - DAAs (<i>n</i> = 231) - Untreated (<i>n</i> = 562)	10.4	NA	HCC recurrence rate (52.5%) - DAAs (55.4%) - Untreated (51.2%)	There is no significant difference in early HCC recurrence rates and patterns between patients who received interferon-based and direct-acting antiviral therapy after HCC treatment

HCC: hepatocellular carcinoma; DAAs: direct-acting antivirals; NA: not available; HCV : hepatitis C virus; LT: liver transplantation; IFN: interferon

of HCC recurrence was not different between the two groups. This suggested that there was no evidence that DAA treatment increases the risk of HCC recurrence.

Cabibbo *et al.*^[34] reported a prospective multicenter study in Italy. The study included 143 patients with complete response after curative treatment of HCC, and the incidences of HCC recurrence were 12%, 26.6%, and 29.1% at 6-, 12-, and 18-month follow-ups. Although risk of HCC recurrence remained high, the risk was comparable between DAA group and untreated group.

Table 3. Risk factors for occurrence/recurrence of HCC

Author/Study design	Occurrence/Recurrence	Risk factors for the development of HCC
Conti <i>et al.</i> ^[14]	Occurrence	No associate factor
	Recurrence	Age, liver stiffness
Kanwal <i>et al.</i> ^[17]	Occurrence	non-SVR, alcohol use, non-African Americans, cirrhosis
Calvaruso <i>et al.</i> ^[18]	Occurrence	Albumin < 3.5 g/dL, platelet count < 120 × 10 ⁹ /L, absence of SVR
Romano <i>et al.</i> ^[19]	Occurrence	Positive for HBsAg, APRI score ≥ 2.5, CPC B, treatment failure
Nagata <i>et al.</i> ^[20]	Occurrence	IL-28 genetic polymorphism, post-treatment WFA*M2BP
	Recurrence	IL-28 genetic polymorphism, post-treatment WFA*M2BP
Nahon <i>et al.</i> ^[21]	Occurrence	non-SVR, older age, excessive alcohol consumption, lower platelet count, high GGT levels, HCV genotype 1
Ioannou <i>et al.</i> ^[42]	Occurrence	non-SVR, cirrhosis
Yoo <i>et al.</i> ^[22]	Occurrence	Alpha-fetoprotein level > 9.5 ng/mL
Singer <i>et al.</i> ^[23]	Occurrence	Older age, male gender, cirrhosis, thrombocytopenia, portal hypertension, diabetes, tobacco use, alcoholic liver disease
Carrat <i>et al.</i> ^[24]	Occurrence	Untreated, non-SVR
Ide <i>et al.</i> ^[25]	Occurrence	Age ≥ 62 years old, male gender, FIB-4 index ≥ 4.6, and GGTP level ≥ 44 IU/L
Cabibbo <i>et al.</i> ^[34]	Recurrence	Main tumor size > 2.5 cm, history of prior recurrence
Nishibatake Kinoshita <i>et al.</i> ^[35]	Recurrence	Higher lens culinaris agglutinin-reactive fraction of alpha-fetoprotein level, a history of multiple HCC treatments, and a shorter interval between HCC treatment and initiation of antiviral therapy
Singal <i>et al.</i> ^[37]	Recurrence	No associate factor

HCC: hepatocellular carcinoma; SVR: sustained virological response; CPC: Child-Pugh Class; HCV: hepatitis C virus; IFN: interferon

The retrospective cohort study from Japan by Nagata *et al.*^[20] analyzed 60 patients in the IFN-based therapy group and 83 patients in the IFN-free therapy group. The incidence of HCC recurrence after locally curative treatment was not significantly different between IFN-based and IFN-free therapy groups by propensity score-matched analysis (five-year incidence: 54.2% in IFN-based and 45.1% in IFN-free therapy, $P = 0.54$). Nishibatake Kinoshita *et al.*^[35] enrolled HCC patients previously treated with radiofrequency ablation (147 patients in DAA group and 156 patients in IFN group). The rate of HCC recurrence at one and two years was 39% and 61% in IFN group and 39% and 60% in DAA group, respectively ($P = 0.43$). There was also no significant difference between the two groups after performing matching analysis ($P = 0.68$). To compare the rate of HCC recurrence between the patients who received DAA and IFN-based therapies, Waziry *et al.*^[36] published meta-analyses study containing 17 studies. The incidence of HCC recurrence after SVR was 9.21 per 100 person-year in DAA group and 12.16 per 100 person-year in IFN group. After adjusting analysis, DAA treatment was not associated with HCC recurrence (Relative risk: 0.62, 95%CI: 0.11-3.45, $P = 0.56$). To solve this debate firmly, a large study from USA and Canada was published by Singal *et al.*^[37] in 2019. In total, 793 patients with HCV-associated HCC, including 304 patients who received DAA and 489 patients without treatment, were analyzed. HCC recurred in 42.1% patients in the DAA group and 58.9% in the untreated group. Although DAA treatment seems to decrease the risk of HCC recurrence (HR: 0.32, 95%CI: 0.25-0.41), after accounting for time-varying exposure, DAA treatment was not associated with increasing or decreasing the risk of HCC recurrence after complete response (HR: 0.90, 95%CI: 0.70-1.16).

RISK FACTORS FOR OCCURRENCE/RECURRENCE OF HEPATOCELLULAR CARCINOMA AFTER TREATING WITH DIRECT-ACTING ANTIVIRALS

In most of the studies on the interplay between DAA and HCC, non-SVR, advanced liver disease, and older age were associated with risk of HCC. Table 3 contains the risk factors for development of HCC.

In a report about the early occurrence and recurrence of HCC in HCV-related cirrhosis treated with DAA, Child-Pugh class (OR: 4.18, 95%CI: 1.17-14.8, $P = 0.03$) and history of HCC (OR: 12.0, 95%CI: 4.02-35.74, $P < 0.0001$) were associated with HCC development. There was no significant factor in patients without history of previous HCC, while age (OR: 0.82, 95%CI: 0.69-0.97, $P = 0.02$) and liver stiffness (OR: 1.19,

95%CI: 1.01-1.39, $P = 0.03$) were significant factors prone to experience HCC recurrence^[14]. A study from Japan reported on the impact of DAA on early recurrence of HCC and higher alpha-fetoprotein (AFP)-L3 level (HR: 1.47, 95%CI: 1.02-2.11, $P = 0.04$), larger number of HCC treatments (HR: 1.65, 95%CI: 1.16-2.35, $P = 0.007$), and shorter interval between the last HCC treatment and initiation of antiviral therapy ($P = 0.007$) were associated with the risk of HCC recurrence^[35]. In the comparative study for occurrence and recurrence of HCC in IFN-based and IFN-free therapies, AFP and WFA*M2BP levels were significantly associated with HCC occurrence after achieving an SVR^[20]. This study suggested that AFP > 5.4 ng/mL and WFA*M2BP > 1.8 COI could be helpful markers of HCC occurrence. A prospective cohort study including 2249 patients with HCV-associated cirrhosis reported that albumin level < 3.5 g/dL (HR: 1.77, 95%CI: 1.12-2.82, $P = 0.01$), platelet count < $120 \times 10^9/L$ (HR: 3.89, 95%CI: 2.11-7.15, $P < 0.001$), and absence of SVR (HR: 3.40, 95%CI: 1.89-6.12, $P < 0.001$) were associated with an increased risk of HCC occurrence^[18].

The retrospective cohort study using national data of 22,500 patients revealed that the patients with SVR (HR: 0.24, 95%CI: 0.19-0.31, $P < 0.0001$) and African American patients (HR: 0.56, 95%CI: 0.39-0.81, $P = 0.02$) were associated with low risk of HCC^[17]. Patients with cirrhosis (HR: 4.73, 95%CI: 3.34-6.68, $P < 0.0001$) and alcohol abuse (HR: 1.56, 95%CI: 1.11-2.18, $P = 0.01$) were associated with high incidence of HCC. A large, prospective, population-based study from Italy including 3917 patients with fibrosis stage $\geq F3$ revealed that DAA treatment failure (HR: 9.09, 95%CI: 5.2-16.1, $P = 0.0001$), HBV coinfection (HR: 3.99, 95%CI: 1.24-12.91, $P = 0.021$), and APRI score > 2.5 (HR: 2.03, 95%CI: 1.14-3.61, $P = 0.016$) were significantly associated with HCC occurrence^[19]. A comparative study including DAA group, SVR-IFN group, and non-SVR group suggested that increased age, alcohol consumption, HCV genotype 1, and impaired liver function were statistically significantly associated with risk of HCC^[21]. There was no significant association between DAA use and risk of HCC. In our study, we compared the rates of HCC between DAA group and IFN group, and alpha-fetoprotein > 9.5 ng/mL at the time of end-of treatment response was the only significant risk factor for HCC occurrence^[22]. Moreover, in a prospective study in France, exposure to DAA was strongly associated with a decrease in all-cause mortality (adjusted HR: 0.34, 95%CI: 0.22-0.55, $P < 0.0001$) and risk of HCC (adjusted HR: 0.57, 95%CI: 0.40-0.81 $P = 0.016$)^[24]. A study including HCV patients with received DAAs and who achieved SVR showed that male gender (HR: 2.40, 95%CI: 1.46-3.96, $P = 0.0006$), older age (HR: 1.51, 95%CI: 1.20-1.91, $P = 0.0005$), higher FIB-4 index (HR: 1.12, 95%CI: 1.07-1.17, $P < 0.0001$), and higher GGTP level (HR: 1.04, 95%CI: 1.02-1.06, $P < 0.0001$) were independently associated with HCC occurrence^[25].

CONCLUSION

Since the initial reports about the unexpected high rate of early recurrence of HCC were published, most recent reports showed favorable effects of DAA treatment in regard to HCC occurrence/recurrence. Several published studies have indicated that non-SVR, older age, advanced liver disease, combined liver disease (chronic hepatitis B and alcohol abuse), higher AFP, and history of previous HCC may play roles in increasing HCC risk. Accordingly, the Asian Pacific Association for the Study of the Liver guidelines suggest that surveillance be performed every six months for patients with SVR and liver cirrhosis and every four months for patients with SVR and previous history of HCC^[38]. Achieving SVR in patients with HCV improved their outcomes in terms of deaths, Child-Pugh Class, and model for end-stage liver disease of advanced liver disease, as well as the incidence of HCC. In addition, patients with previous HCC after achieving SVR had significantly better survival than untreated patients, thus patients eligible for HCC therapy should be considered for DAA treatment^[39]. However, the risk of HCC is not completely eliminated by achieving SVR after DAA treatment, and regular surveillance of HCC including biomarkers for tumor should be considered in patients with cirrhosis, combined liver disease, and previous history of HCC^[40,41].

DECLARATIONS

Authors' contributions

Study concept and design: Yoo SH, Kwon JH

Acquisition of data: Yoo SH

Drafting of the manuscript: Yoo SH

Study supervision: Kwon JH

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American association for the study of liver diseases. *Hepatology* 2018;68:723-50.
2. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001;358:958-65.
3. Zeuzem S, Hultcrantz R, Bourliere M, Goeser T, Marcellin P, et al. Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. *J Hepatol* 2004;40:993-9.
4. Lee SD, Yu ML, Cheng PN, Lai MY, Chao YC, et al. Comparison of a 6-month course peginterferon alpha-2b plus ribavirin and interferon alpha-2b plus ribavirin in treating Chinese patients with chronic hepatitis C in Taiwan. *J Viral Hepat* 2005;12:283-91.
5. Morgan TR, Ghany MG, Kim HY, Snow KK, Shiffman ML, et al; Group H-CT. Outcome of sustained virological responders with histologically advanced chronic hepatitis C. *Hepatology* 2010;52:833-44.
6. Lok AS, Everhart JE, Wright EC, Di Bisceglie AM, Kim HY, et al; Group H-CT. Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. *Gastroenterology* 2011;140:840-9.
7. van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012;308:2584-93.
8. Kowdley KV, Lawitz E, Poordad F, Cohen DE, Nelson DR, et al. Phase 2b trial of interferon-free therapy for hepatitis C virus genotype 1. *N Engl J Med* 2014;370:222-32.
9. Lawitz E, Poordad FF, Pang PS, Hyland RH, Ding X, et al. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naïve and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014;383:515-23.
10. Muir AJ, Poordad F, Lalezari J, Everson G, Dore GJ, et al. Daclatasvir in combination with asunaprevir and beclabuvir for hepatitis C virus genotype 1 infection with compensated cirrhosis. *JAMA* 2015;313:1736-44.
11. Lawitz E, Makara M, Akarca US, Thuluvath PJ, Preotescu LL, et al. Efficacy and safety of ombitasvir, paritaprevir, and ritonavir in an open-label study of patients with genotype 1b chronic hepatitis C virus infection with and without cirrhosis. *Gastroenterology* 2015;149:971-80.e1.
12. Kwon JH, Yoo SH, Nam SW, Kim HY, Kim CW, et al. Clinical outcomes after the introduction of direct antiviral agents for patients infected with genotype 1b hepatitis C virus depending on the regimens: A multicenter study in Korea. *J Med Virol* 2019;91:1104-11.
13. Reig M, Marino Z, Perello C, Inarrairaegui M, Ribeiro A, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016;65:719-26.
14. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-

- related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016;65:727-33.
15. Masuzaki R, Tateishi R, Yoshida H, Yoshida H, Sato S, et al. Risk assessment of hepatocellular carcinoma in chronic hepatitis C patients by transient elastography. *J Clin Gastroenterol* 2008;42:839-43.
 16. Goodgame B, Shaheen NJ, Galanko J, El-Serag HB. The risk of end stage liver disease and hepatocellular carcinoma among persons infected with hepatitis C virus: publication bias? *Am J Gastroenterol* 2003;98:2535-42.
 17. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, et al. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. *Gastroenterology* 2017;153:996-1005.e1.
 18. Calvaruso V, Cabibbo G, Cacciola I, Petta S, Madonia S, et al; Rete Sicilia Selezione Terapia HCV. Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. *Gastroenterology* 2018;155:411-21.e4.
 19. Romano A, Angeli P, Piovesan S, Noventa F, Anastassopoulos G, et al. Newly diagnosed hepatocellular carcinoma in patients with advanced hepatitis C treated with DAAs: A prospective population study. *J Hepatol* 2018;69:345-52.
 20. Nagata H, Nakagawa M, Asahina Y, Sato A, Asano Y, et al; Ochanomizu Liver Conference Study G. Effect of interferon-based and -free therapy on early occurrence and recurrence of hepatocellular carcinoma in chronic hepatitis C. *J Hepatol* 2017;67:933-9.
 21. Nahon P, Layese R, Bourcier V, Cagnot C, Marcellin P, et al; Group ACC. Incidence of Hepatocellular Carcinoma After Direct Antiviral Therapy for HCV in Patients With Cirrhosis Included in Surveillance Programs. *Gastroenterology* 2018;155:1436-50.e6.
 22. Yoo SH, Kwon JH, Nam SW, Kim HY, Kim CW, et al. Early development of de novo hepatocellular carcinoma after direct-acting agent therapy: comparison with pegylated interferon-based therapy in chronic hepatitis C patients. *J Viral Hepat* 2018;25:1189-96.
 23. Singer AW, Reddy KR, Telep LE, Osinusi AO, Brainard DM, et al. Direct-acting antiviral treatment for hepatitis C virus infection and risk of incident liver cancer: a retrospective cohort study. *Aliment Pharmacol Ther* 2018;47:1278-87.
 24. Carrat F, Fontaine H, Dorival C, Simony M, Diallo A, et al; French ACOHc. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. *Lancet* 2019;393:1453-64.
 25. Ide T, Koga H, Nakano M, Hashimoto S, Yatsuhashi H, et al. Direct-acting antiviral agents do not increase the incidence of hepatocellular carcinoma development: a prospective, multicenter study. *Hepatol Int* 2019;13:293-301.
 26. Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. *Ann Surg* 2002;235:373-82.
 27. Tabrizian P, Jibara G, Shrager B, Schwartz M, Roayaie S. Recurrence of hepatocellular cancer after resection: patterns, treatments, and prognosis. *Ann Surg* 2015;261:947-55.
 28. Shen YC, Hsu C, Chen LT, Cheng CC, Hu FC, et al. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. *J Hepatol* 2010;52:889-94.
 29. Ikeda K, Arase Y, Saitoh S, Kobayashi M, Suzuki Y, et al. Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor-A prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* 2000;32:228-32.
 30. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, et al. Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. *Ann Intern Med* 2001;134:963-7.
 31. Lin SM, Lin CJ, Hsu CW, Tai DI, Sheen IS, et al. Prospective randomized controlled study of interferon-alpha in preventing hepatocellular carcinoma recurrence after medical ablation therapy for primary tumors. *Cancer* 2004;100:376-82.
 32. Mazzaferro V, Romito R, Schiavo M, Mariani L, Camerini T, et al; Force HCCIT. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006;44:1543-54.
 33. ANRS collaborative study group on hepatocellular carcinoma. Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: data from three ANRS cohorts. *J Hepatol* 2016;65:734-40.
 34. Cabibbo G, Petta S, Calvaruso V, Cacciola I, Cannavo MR, et al; Rete Sicilia Selezione Terapia HCV. Is early recurrence of hepatocellular carcinoma in HCV cirrhotic patients affected by treatment with direct-acting antivirals? A prospective multicentre study. *Aliment Pharmacol Ther* 2017;46:688-95.
 35. Nishibatake Kinoshita M, Minami T, Tateishi R, Wake T, Nakagomi R, et al. Impact of direct-acting antivirals on early recurrence of HCV-related HCC: comparison with interferon-based therapy. *J Hepatol* 2019;70:78-86.
 36. Waziry R, Hajarizadeh B, Grebely J, Amin J, Law M, et al. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression. *J Hepatol* 2017;67:1204-12.
 37. Singal AG, Rich NE, Mehta N, Branch A, Pillai A, et al. Direct-Acting Antiviral Therapy Not Associated With Recurrence of Hepatocellular Carcinoma in a Multicenter North American Cohort Study. *Gastroenterology* 2019;156:1683-92.e1.
 38. Kanda T, Lau GKK, Wei L, Moriyama M, Yu ML, et al. APASL HCV guidelines of virus-eradicated patients by DAA on how to monitor HCC occurrence and HBV reactivation. *Hepatol Int* 2019;13:649-61.
 39. Dang H, Yeo YH, Yasuda S, Huang CF, Iio E, et al. Cure with Interferon Free DAA is Associated with Increased Survival in Patients with HCV related HCC from both East and West. *Hepatology* 2019;1002/hep.30988.
 40. Cheung MCM, Walker AJ, Hudson BE, Verma S, McLauchlan J, et al; UK HCVR. Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. *J Hepatol* 2016;65:741-7.
 41. Hanafy AS, Bassiony MA, Basha MAA. Management of HCV-related decompensated cirrhosis with direct-acting antiviral agents: who should be treated? *Hepatol Int* 2019;13:165-72.
 42. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol* 2018;1:25-32.

Original Article

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Microwave ablation of hepatocellular carcinomas in octogenarians

Jacob Freedman^{1,2}

¹Department of Surgery and Urology, Danderyd University Hospital, Stockholm 18288, Sweden.

²Department of clinical sciences, Karolinska Institutet, Stockholm 18288, Sweden

Correspondence to: Dr. Jacob Freedman, Department of Surgery and Urology, Danderyd University Hospital, Stockholm 18288, Sweden. E-mail: jacob.freedman@ki.se

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Abstract

Aim: To evaluate whether it is safe and meaningful to treat octogenarians with microwave ablation for hepatocellular carcinoma. With an ageing population being healthier than previous generations, old limits for treating disease founded on patient age need to be revised. One of the most common tumour related death causes is hepatocellular carcinoma (HCC). With the development of minimally invasive therapies with curative potential, new ground is being broken offering treatments to older patients in the hope of achieving prolongation and better quality of life.

Methods: In this retrospective single centre study of patients having a first microwave ablation therapy for HCC in a national referral centre for ablative liver treatments, septuagenarians ($n = 161$, age 70-80) were compared with octogenarians ($n = 32$, age 80-90).

Results: Octogenarians selected for microwave ablation of HCC at a regional multidisciplinary team conference have similar outcomes as their younger control group. Survival, complications and length of stay are not different.

Conclusion: Octogenarians who are fit for ablative treatment of HCC should not be disqualified on grounds of age, recognising that this group has an obvious immortality, or lead-time, bias as well as a probable selection bias in part explaining their good results.

Keywords: Microwave, ablation, hepatocellular carcinoma, octogenarians, survival, complications



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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the fourth most common cause of cancer death according to the 2018 WHO report^[1]. During the last 30 years, life expectancy, worldwide, has increased from 63 to 71.7 years with countries such as Japan and South Korea, as well as those in Western European and Northern America having a life expectancy of 80-84 years in 2015^[2]. At present, a 70-year-old person in Sweden is expected to live 16.3 more years and a person of 80 has on average 9.2 years left^[3].

Curative treatment of HCC is foremost surgical. Postoperative complications increase with age. In a UK study, patients over 75 had a 62% increase in risk of complications and a more than three-fold increased risk of one-year overall mortality compared to patients younger than 65. For one-year overall mortality, the risk was increased by 349% after surgery for colorectal cancer^[4]. Leal *et al.*^[5] investigated the impact of liver resection for colorectal liver metastases on octogenarians compared to younger patients in a matched cohort study and found twice the morbidity (19% vs. 9%) and a 90-day mortality of 7% vs. 0%. In a recent review by Cho *et al.*^[6], curative intended treatments for hepatocellular cancer with resection or radiofrequency ablation (RFA) was found to be safe in selected patients over the age of 75^[5], namely patients who generally had less severe underlying liver disease, were predominantly female and had more well-differentiated tumours, indicating that there was a clear selection bias when comparing the elderly with younger HCC patients. With careful selection, excellent results of resection can be achieved^[7-9]. However, with increasing age, comorbidities amass and resective surgery is often not deemed appropriate and patients can be offered local ablative treatments with RFA instead without having age or comorbidities affecting outcome^[10,11]. In a recent publication, octogenarians undergoing stereotactic RFA for primary liver tumours were compared to a younger control group using propensity score matching and no significant differences in terms of local recurrence, major complications and overall survival were found^[12].

With microwave technology (MWA) entering the scene, with quicker energy delivery and larger ablation volumes compared to RFA^[13], the present study aimed to evaluate the results of treating octogenarians with HCC using MWA in comparison with septuagenarians in a highly specialised centre in northern Europe.

METHODS

We retrospectively analysed all patients undergoing microwave ablation for HCC who were seventy years or older at first ablation in a single centre specialised in minimally invasive ablative treatments in Sweden, from June 2010 to December 2018. The collection and publication of data was approved by the regional ethics committee.

All patients were selected for ablative treatment for their HCC at the regional multidisciplinary team conference. Patients without cirrhosis or with cirrhosis without portal hypertension were typically firstly considered for resection, general condition permitting. For the others, with Child-Pugh grade below C and performance status 0-2 as well as with the possibility of curative treatment and largest tumour diameter below 30 mm, ablative treatment was the first choice. The diagnosis was based on radiological LIRADS criteria and not primarily on histology. Microwaves was the energy source of choice for ablative treatment. Tumour targeting was performed with computer-assisted technologies such as ultrasound fused with computed tomography (CT) images or with the aid of computer assisted CT-guided navigation technology (CAS-one, Cascination AG, Bern). Details on the set-up, ablation technique, energy devices and targeting technologies applied were described previously^[14].

Patients were followed-up with CT or MRI imaging every three months for one year and according to the national surveillance guidelines^[15]. Ablation site recurrence was defined as viable tumour tissue detected

on follow-up imaging within an area of 1 cm surrounding the ablation zone, applying the LIRADS criteria for HCC^[16]. In the case of ablation site recurrence or new intrahepatic lesions on follow-up, patients were retreated with minimally invasive microwave ablation whenever possible.

Data on patient and tumour characteristics were extracted from the Swedish Liver registry^[17]. Complications were classified according to the Clavien-Dindo classification^[18], with major complication defined as a grade 3b or higher within thirty days of treatment. Data on tumour recurrence were extracted from patient's medical records. Overall survival (OS) was calculated from the day of the index treatment, with all patients being followed until death or censored on 15 October 2019.

Descriptive statistics was used to describe baseline characteristics with medians and range for non-normally distributed data. Categorical variables were expressed as total and percentages. Ratios were analysed with the χ^2 -test. Overall survival was illustrated using Kaplan-Meier curves and differences in survival analysed with log-rank test. Factors influencing survival were analysed with the Cox proportional hazards method. All statistical computations were made with SigmaPlot 13.0 (Systat software, Inc, San Jose CA)

RESULTS

In total, 193 patients treated with MWA at the age of 70 or above were included in this study. Of these, 32 (17%) were 80 or above years of age and 161 (83%) were 70-80 years of age. Patient and tumour characteristics are outlined in [Table 1](#).

In the group of octogenarians, there was less underlying liver disease with cirrhosis of various reasons (59% vs. 80%, $P = 0.021$) and the proportion of females was higher (34% vs. 19%, $P = 0.08$). In the other baseline characteristics, and somewhat fewer tumours treated. The age distribution is presented in [Figure 1](#).

Major complications within one month occurred in seven (5%) of the septuagenarians and none of the octogenarians. These were one liver abscess that responded well to drainage and antibiotics, one hematoma, one patient with ascites that was drained, one with a pneumothorax that was evacuated, one patient had a coronary infarction one week after the ablation and one patient had a bleeding oral polyp a week after the ablation. The last developed progressive liver failure with intensive care needs, alas irreversible and died two months after the ablation.

There was no difference in OS between the two groups with a median survival time of 3.9 years for patients between 70 and 80 years of age and 4.3 years for octogenarians ($P = 0.416$). One-, three- and five-year OS were 89%, 59% and 38% (70-80 years of age) and 100%, 100% and 30% (octogenarians), respectively [[Figure 2](#)].

In the Cox proportional hazards model, no single analysed factor significantly influenced survival including gender, associated liver disease, ASA-score, Child-Pugh grade, number of tumours treated and largest tumour treated.

Local ablation site recurrence occurred in 19 % (36/194) and 26% (50/194) had a new tumour in another part of the liver within one year of follow-up.

The distribution of repeat treatments is shown in [Figure 3](#). Patients were retreated when there was a chance of cure, not as an upfront palliative measure.

Age distribution

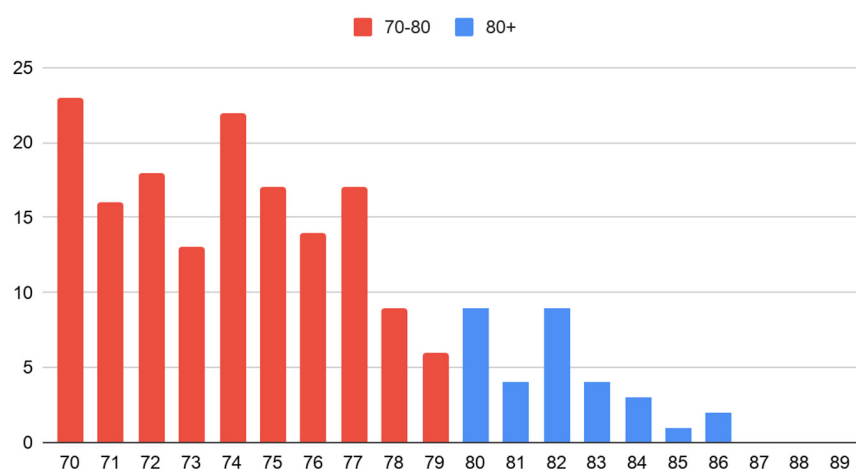


Figure 1. Age distribution of subjects in the study. The Y-axis is the number of patients per age group and the X-axis the age groups

Table 1. Baseline and outcome characteristics

	80+	%	70-80	%	P
n	32		161		
Age [median (min-max)]	74.2 (70-79.6)		82.3 (80.1-86.3)		
Male	21	66%	131	81%	0.08
Associated liver disease	19	59%	129	80%	0.021
Alcohol	8	25%	63	39%	0.189
Hemochromatosis	1	3%	5	3%	NS
Hepatitis B	0	0%	4	2%	NS
Hepatitis C	1	3%	34	21%	0.016
NASH	5	16%	25	16%	NS
Porphyria	1	3%	2	1%	NS
Child-Pugh					
A	24	75%	114	71%	NS
B	4	13%	18	11%	NS
C	0	0%	0	0%	NS
Missing	4	13%	28	17%	NS
MELD [median (min-max)]	6.58 (0.86-22.78)		6.26 (0-19.32)		NS
ASA					
1	0	0%	2	1%	NS
2	7	22%	39	24%	NS
3	21	66%	93	58%	NS
4	4	13%	25	16%	NS
Number of tumours					
1	20	63%	88	55%	NS
2	6	19%	34	21%	NS
3+	6	19%	36	22%	NS
Max diameter [median (min-max)]	22 (11-37)		20 (8-58)		NS
Clavien-Dindo class					
0-1	28	84%	142	88%	NS
2	4	13%	12	7%	NS
3a	0	0%	5	3%	NS
3b	0	0%	1	1%	NS
4a	0	0%	1	1%	NS
4b	0	0%	0	0%	NS
5	0	0%	0	0%	NS
Postop stay [median (min-max)]	1 (0-5)		1 (0-32)		NS
Number of subsequent liver treatments	1 (1-6)		2 (1-7)		NS

Baseline characteristics and complications following microwave ablation for hepatocellular carcinoma in elderly patients. Only associated liver disease reached statistical significance with a *P*-value < 0.05. NASH: Non Alcohol Steato Hepatitis; MELD: Model for Endstage Liver Disease; ASA: American Society of Anaesthetists physical status classification

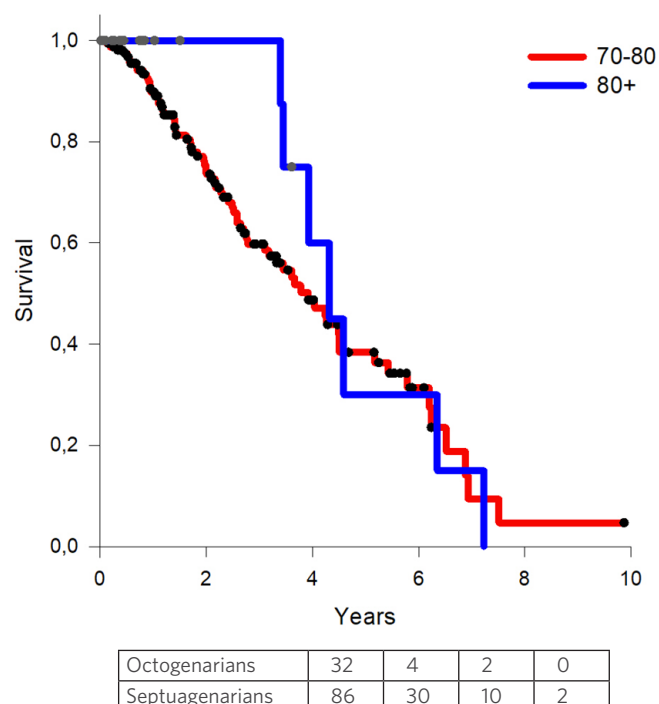


Figure 2. Overall survival after a first microwave ablation of hepatocellular carcinoma in octogenarians compared to septuagenarians

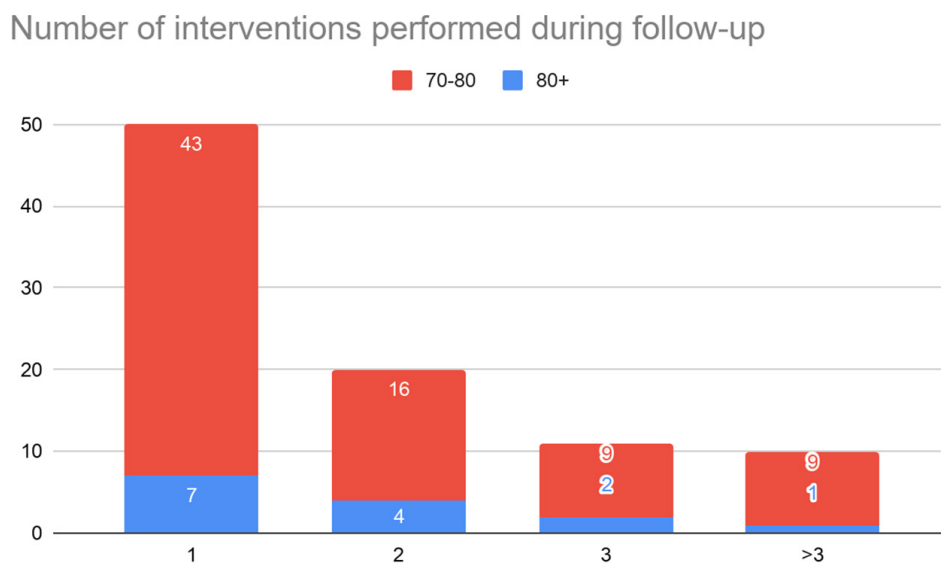


Figure 3. Number of interventions performed during follow-up of the patient cohort grouped into the two age groups. The y-axis represents the number of patients and the x-axis the different strata of interventions

DISCUSSION

The presented data show that microwave ablation of HCC in selected octogenarians can be performed with low morbidity and with results that are equal to those for septuagenarians, which was the primary aim of the study.

This is well in line with previous findings by Shen *et al.*^[10] and Zhang *et al.*^[11] offering a potentially curative treatment for octogenarians not deemed fit for surgical resection or as a first-line therapy.

Survival in the presented cohort is well comparable to results presented in a meta-analysis of elderly patients resected for HCC by Cho *et al.*^[6], but with much shorter length of stay and much fewer complications, where, typically, resected elderly patients stay for 9-18 days in hospital and with 13%-36% having postoperative complications. In a US study from 2012 on RFA^[19], the postoperative mortality was worse, as was survival with 72% surviving the first year and 39% and 34% at three and five years compared to the present study's 100%, 69% and 40%, respectively, for the octogenarian group, numbers that are somewhat inferior to what Takahashi *et al.*^[20] presented. Numbers are difficult to compare as underlying causes of HCC and expected life span varies greatly among the US, Japan and Sweden.

In the present study, the mean age in the older cohort was 82 with a median survival of 4.3 years and the younger cohort with a mean age of 74 had a median survival of 3.9 years. This is considerably shorter than the average life expectancy for a 74-year-old (13 years) or an 82-year-old (8 years) in Sweden^[21].

Selection bias is an obvious disadvantage when analysing this kind of dataset. Associated liver disease and hepatitis C was more prevalent in the younger group.

The small size of the octogenarian cohort could easily mask a Type 2 error. On the other hand, there is the obvious problem with immortality bias as the octogenarians have by necessity survived until 80 and are thus a selected group with a slightly higher life expectancy. This could perhaps in part explain the excellent three-year survival of 100% in that cohort.

In conclusion, as the population steadily has a greater life expectancy, the indications for treating tumours in older and healthier age groups becomes necessary. The results of the present study indicate that ablating hepatocellular carcinomas in octogenarians can safely be performed with good results if no obvious contraindication is present.

DECLARATIONS

Authors' contributions

The author contributed solely to the article.

Availability of data and materials

The data source is a local prospective database where all microwave ablations has been recorded since June 2010.

Financial support and sponsorship

None.

Conflicts of interest

The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

This retrospective study was approved by the regional ethics board in preparation of a previous study.

Consent for publication

Not applicable.

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REFERENCES

1. Cancer today. Available from: <http://gco.iarc.fr/today/home> [Last accessed on 6 Mar 2020]
2. Roser M. Life Expectancy. Our World in Data 2013. Available from: <https://ourworldindata.org/life-expectancy> [Last accessed on 6 Mar 2020]
3. Femårig livslängdstabell för hela riket efter kön och ålder. År 1998-2002 - 2014-2018-Statistikdatabasen. Statistikdatabasen. Available from: http://www.statistikdatabasen.scb.se/pxweb/sv/ssd/START_BE_BE0101_BE0101/LivslangdFemariga/ [Last accessed on 6 Mar 2020]
4. Aquina CT, Mohile SG, Tejani MA, Becerra AZ, Xu Z, et al. The impact of age on complications, survival, and cause of death following colon cancer surgery. *Br J Cancer* 2017;116:389-97.
5. Leal JN, Sadot E, Gonen M, Lichtman S, Kingham TP, et al. Operative morbidity and survival following hepatectomy for colorectal liver metastasis in octogenarians: a contemporary case matched series. *HPB (Oxford)* 2017;19:162-9.
6. Cho E, Cho HA, Jun CH, Kim HJ, Cho SB, et al. A review of hepatocellular carcinoma in elderly patients focused on management and outcomes. *In Vivo* 2019;33:1411-20.
7. Zarour LR, Billingsley KG, Walker BS, Kristian Enestvedt C, Orloff SL, et al. Hepatic resection of solitary HCC in the elderly: a unique disease in a growing population. *Am J Surg* 2019;217:899-905.
8. Okamura Y, Sugiura T, Ito T, Yamamoto Y, Ashida R, et al. The short- and long-term outcomes in elderly patients with hepatocellular carcinoma after curative surgery: a case-controlled study with propensity score matching. *Eur Surg Res* 2018;59:380-90.
9. Zhao LY, Huo RR, Xiang X, Torzilli G, Zheng MH, et al. Hepatic resection for elderly patients with hepatocellular carcinoma: a systematic review of more than 17,000 patients. *Expert Rev Gastroenterol Hepatol* 2018;12:1059-68.
10. Shen X, Ma S, Tang X, Wang T, Qi X, et al. Clinical outcome in elderly Chinese patients with primary hepatocellular carcinoma treated with percutaneous microwave coagulation therapy (PMCT): a Strobe-compliant observational study. *Medicine* 2018;97:e11618.
11. Zhang F, Wu G, Sun H, Ding J, Xia F, et al. Radiofrequency ablation of hepatocellular carcinoma in elderly patients fitting the Milan criteria: a single centre with 13 years experience. *Int J Hyperthermia* 2014;30:471-9.
12. Schullian P, Putzer D, Silva MA, Laimer G, Kolbitsch C, et al. Stereotactic Radiofrequency ablation of liver tumors in octogenarians. *Front Oncol* 2019;9:929.
13. Glassberg MB, Ghosh S, Clymer JW, Qadeer RA, Ferko NC, et al. Microwave ablation compared with radiofrequency ablation for treatment of hepatocellular carcinoma and liver metastases: a systematic review and meta-analysis. *Onco Targets Ther* 2019;12:6407-38.
14. Beermann M, Lindeberg J, Engstrand J, Galmén K, Karlgren S, et al. 1000 consecutive ablation sessions in the era of computer assisted image guidance - Lessons learned. *Eur J Radiol Open* 2019;6:1-8.
15. Gällande vårdprogram levercellscancer - RCC. Available from: <https://www.cancercentrum.se/samverkan/cancerdiagnoser/lever-och-galla/vardprogram/gallande-varldprogram-levercellscancer/> [Last accessed on 6 Mar 2020]
16. Purysko AS, Remer EM, Coppa CP, Leão Filho HM, Thupili CR, et al. LI-RADS: a case-based review of the new categorization of liver findings in patients with end-stage liver disease. *Radiographics* 2012;32:1977-95.
17. Kvalitetsregister - Väst. Available from: <https://www.cancercentrum.se/vast/cancerdiagnoser/lever-och-galla/kvalitetsregister/> [Last accessed on 6 Mar 2020]
18. Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, et al. The Clavien-Dindo classification of surgical complications. *Ann Surg* 2009;250:187-96.
19. Massarweh NN, Park JO, Yeung RSW, Flum DR. Comparative assessment of the safety and effectiveness of radiofrequency ablation among elderly medicare beneficiaries with hepatocellular carcinoma. *Ann Surg Oncol* 2012;19:1058-65.
20. Takahashi H, Mizuta T, Kawazoe S, Eguchi Y, Kawaguchi Y, et al. Efficacy and safety of radiofrequency ablation for elderly hepatocellular carcinoma patients. *Hepatol Res* 2010;40:997-1005.
21. Ettårig livslängdstabell för hela riket efter kön och ålder. År 1960 - 2018-Statistikdatabasen. Statistikdatabasen. Available from: http://www.statistikdatabasen.scb.se/pxweb/sv/ssd/START_BE_BE0101_BE0101/LivslangdEttariga/ [Last accessed on 6 Mar 2020]

Original Article

Open Access



Recurrence of hepatocellular carcinoma following deceased donor liver transplantation: case series

Cem Simsek¹, Amy Kim¹, Michelle Ma¹, Nilay Danis¹, Merve Gurakar², Andrew M. Cameron³, Benjamin Philosophe³, Jacqueline Garonzik-Wang³, Shane Ottmann³, Ahmet Gurakar¹, Behnam Saberi¹

¹Johns Hopkins University School of Medicine, Division of Gastroenterology and Hepatology-Transplant Hepatology, Baltimore, MD 21205, USA.

²Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21205, USA.

³Johns Hopkins University School of Medicine, Division of Transplant Surgery, Baltimore, MD 21205, USA.

Correspondence to: Dr. Ahmet Gurakar, Division of Gastroenterology and Hepatology, The Johns Hopkins University School of Medicine, 720 Rutland Avenue Ross Research Building, Suite 918 Baltimore, MD 21205, USA. E-mail: aguraka1@jhmi.edu

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Abstract

Aim: We aimed to study the clinical and pathological characteristics of liver transplant recipients with hepatocellular carcinoma recurrence.

Methods: We reviewed the data for 26 patients who had tumor recurrence after deceased donor liver transplant for hepatocellular carcinoma at the Johns Hopkins Hospital from January 2005 to December 2015.

Results: In total, 88% of recipients were males. The mean age was 59 years. On explant, poor differentiation was detected in 43%, while 73% had microvascular invasion. Overall, 62% were diagnosed to be outside of Milan criteria. Out of these, 15% met the criteria for downstaging. Twenty (77%) patients had pre-transplant alpha fetoprotein levels ≥ 20 ng/mL. In 54% of patients, the location of hepatocellular carcinoma (HCC) recurrence was extrahepatic, followed by intrahepatic in 31% and both intra- and extrahepatic in 15%. The post-transplant tumor recurrence was diagnosed at a mean of 427 days (range 34-1502). Fifty percent of HCC recurrences were diagnosed within one year following liver transplant. Twenty (77%) patients received treatment for their recurrent HCC: external radiation ($n = 10$), surgical resections ($n = 8$; brain 4, spine 2, bone 1, and Whipple surgery 1), sorafenib ($n = 7$), locoregional therapy ($n = 5$). Overall, 24 out of 26 (92%) recipients died within four years after the transplant.

Conclusion: HCC recurrence after liver transplant is infrequent. More than fifty percent of HCC recurrences following liver transplant are extrahepatic. Despite better recipient selection for liver transplant, the curative options are limited in recurrent cases and associated with extremely poor outcomes.

Keywords: Hepatocellular carcinoma, liver transplant, liver resection, locoregional therapy



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INTRODUCTION

Liver transplant (LT) has become the treatment of choice in patients with hepatocellular carcinoma (HCC) and cirrhosis who meet the Milan criteria (MC)^[1]. Although additional extended criteria models have been proposed, HCC recurrence following LT remains an unfortunate incident associated with poor survival^[2,3]. Tumor biology and alpha fetoprotein (AFP), as well as tumor size and number, have been proposed by various groups as other potentially relevant factors of tumor recurrence^[4-6].

Overall, two thirds (2/3) of patients, who develop recurrent HCC post-LT, present with extrahepatic recurrence^[7,8]. The treatment of choice in post LT HCC recurrence is determined based on the site and the extent of the recurrence^[8]. However, treatments are not standardized and mostly based on expert opinion and retrospective studies^[9]. Surgical treatment options have been proposed with promising outcomes in selected patients^[10,11]. Locoregional therapy options, transarterial chemoembolization, radiofrequency ablation, and stereotactic radiation are considered in selected cases^[9].

In a recent report, we published our experience in LT recipients with HCC at the Johns Hopkins University Comprehensive Liver Transplant Center^[12]. As a follow-up study, we aimed to study the clinicopathological features and outcomes of 26 cases with HCC recurrence following LT. In addition, we evaluated the details on the outcomes and the application of different treatment modalities in this group.

METHODS

The study was approved by the institutional review board at the Johns Hopkins Hospital. HCC-related deceased donor LT recipients between January 2005 and December 2015 were evaluated. In total, 26 patients with post-LT HCC recurrence were identified among 165 recipients who were included in the study. All recipients were listed following a standard work up and discussion at the weekly selection meeting. They were within Milan criteria or downstaged into Milan criteria. The transplant was performed by piggyback technique. Postoperative HCC surveillance consisted of contrasted cross-sectional imaging with computerized tomography or magnetic resonance imaging with AFP every three months for the first year and every six months for the second and third years. There was no set therapeutic protocol for recurrence; treatment options were discussed in a multidisciplinary fashion. The Pre-LT AFP was obtained within the past three months prior to deceased donor liver transplantation (DDLT) and immediate post-LT AFP was obtained within three months post DDLT.

Data on clinical, radiologic, pathology, HCC recurrence, and survival were collected from the records, reviewed, and analyzed. Explant pathologies were reviewed retrospectively, and the following tumor parameters were collected: size, number of lesions, microvascular invasion status, and differentiation. It was determined whether patients met the Milan or University of California San Francisco (UCSF) criteria based on the number and size of HCC lesions on explant pathology. The data collected for categorical variables were reported as percentages. Data for continuous variables were reported by the mean and standard deviation. Patient survivals were analyzed using Kaplan-Meier statistics. STATA V.13 (StataCorp college station, TX) was used to perform the statistical analyses.

RESULTS

Patient characteristics

Among the deceased donor LT recipients, HCC was the primary indication for transplantation varying from 21% to 53% of patients [Figure 1] according to the year. Clinical information on the 26 LT recipients with recurrent HCC is summarized in Table 1. Patients were predominantly male (88.5%) with a mean age of 59 years (range 47-72 years). The majority of recipients were white ($n = 17$, 65.4%), followed by African American ($n = 7$, 27.0%) and Asian ($n = 2$, 7.6%) ethnicities. Primary etiology of liver disease was chronic

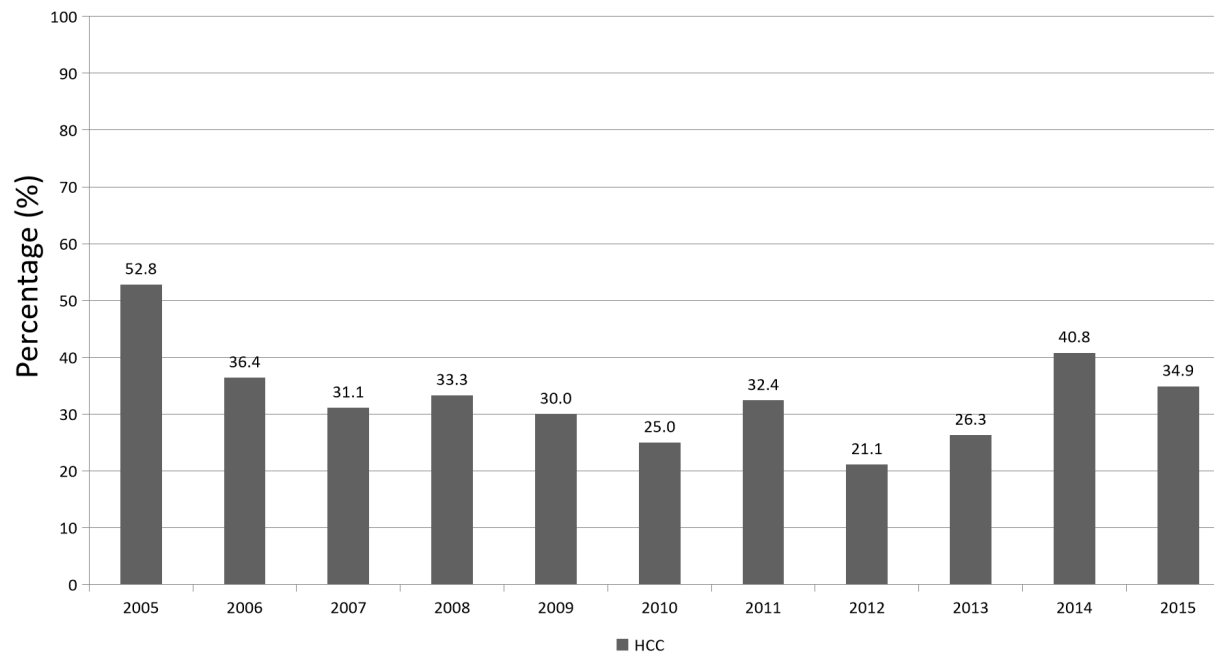


Figure 1. Overall, rate of deceased donor liver transplant for hepatocellular carcinoma indication at the Johns Hopkins Hospital from 2005 to 2015. HCC: hepatocellular carcinoma

hepatitis C (positive hepatitis C antibody and/or hepatitis C RNA) in 13 patients (50%) and hepatitis C and alcoholic liver disease in 6 (23%) patients. Chronic hepatitis B (positive hepatitis B surface antigen and/or hepatitis B DNA) was seen in three patients (11.5%), followed by alcoholic liver disease ($n = 2$, 7.7%), and non-alcoholic fatty liver disease ($n = 1$, 3.9%).

Laboratory results

The average model for end-stage liver disease (MELD) score was 13, ranging from 6 to 35. Mean AFP was 27.6 ng/mL for pre-LT vs. 23.6 ng/mL for post-LT time periods [Tables 1 and 2]. Four patients had pre-LT AFP levels of > 1000 ng/mL. The other available laboratory results are summarized in Table 1.

Immunosuppression

Overall, nine (34.6%) patients were treated with mammalian target of rapamycin (mTOR) treatment with sirolimus in eight and everolimus in one patient. Seventeen patients received Tacrolimus-based therapy.

Explant-pathology findings

In the explant pathologies of LT recipients, 9 (34.6%) patients had only one lesion and 11 (42.4%) had 4 or more lesions. The average for the largest lesion size was 4.3 cm. In total, 12 patients (46.1%) had multi-lobar tumors and 13 (50%) had tumors that were located in the right lobe. Overall, 10 patients (38.4%) were within MC criteria and 11 patients (42.3%) were within UCSF criteria. Four patients (15.4%) were downstaged to MC with locoregional treatment. Seventeen (65.4%) patients underwent locoregional therapy before transplant. None of the tumors were well-differentiated. Overall, 14 (53.8%) patients had moderately differentiated HCC. Eleven (42.3%) patients had HCC with poor differentiation. Microvascular invasion was detected in 19 of the 26 cases (73.1%) while one patient had bile duct invasion only.

Recurrence and survival

The overall rate of HCC recurrence following LT in our series was 15%. The rate of HCC recurrence has improved over the years with a recurrence rate of 10% in 2015 [Figure 2]. Mean time for diagnosis of

Table 1. Characteristics of the study population

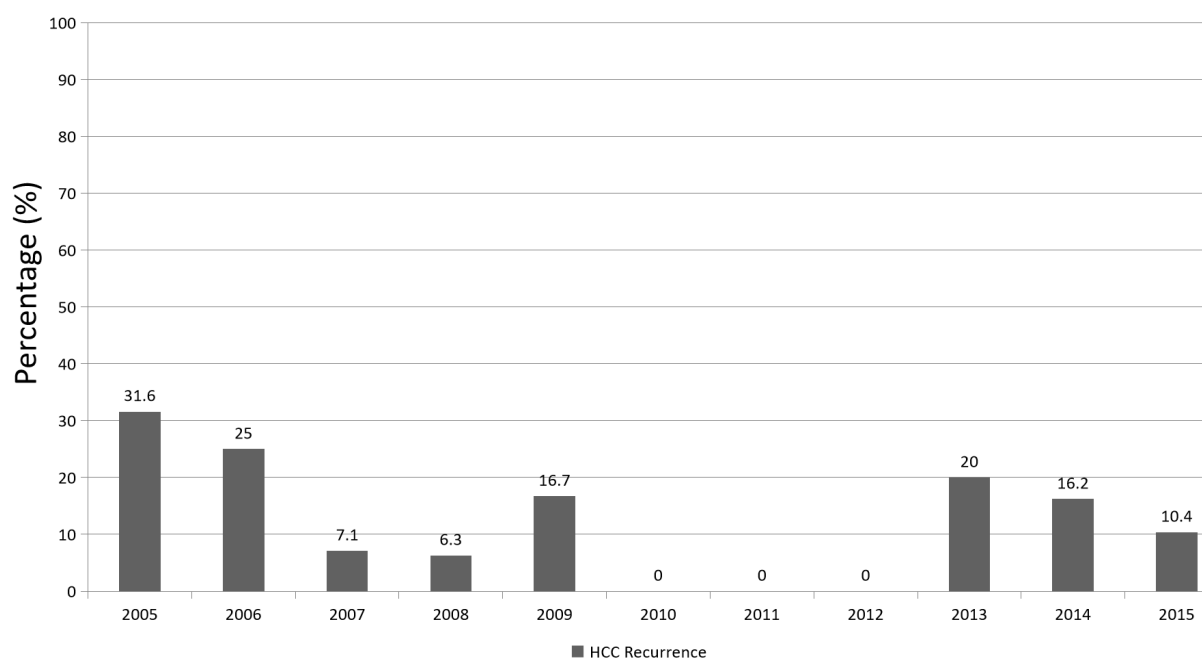
Variable	n = 26
Clinical features	
Male sex, n (%)	23 (88.5%)
Age (years)	58.9 (6.8)
Ethnicity, n (%)	
White	17 (65.4%)
African American	7 (27.0%)
Asian	2 (7.6%)
Etiology	
HCV	13 (50%)
HBV	3 (11.5%)
ALD	2 (7.7%)
NAFLD	1 (3.9%)
HCV/ALD	6 (23%)
Other	1 (3.9%)
Explant pathology	
Number of lesions, n (%)	
1	9 (34.6%)
2	3 (11.5%)
3	3 (11.5%)
> 4	11 (42.4%)
Largest lesion (cm)	4.3 (3.8)
Tumor location, n (%)	
Right lobe	13 (50%)
Left lobe	1 (3.9%)
Multi-lobar	12 (46.1%)
Tumor differentiation, n (%)	
Well	0 (0%)
Moderate	14 (53.8%)
Poor	11 (42.3%)
Unknown	1 (3.9%)
Microvascular invasion, n (%)	
Yes	19 (73.1%)
No	6 (23%)
Bile duct invasion	1 (3.9%)
Total number of loco-regional therapies, n (%)	
0	9 (34.6%)
1	9 (34.6%)
2	5 (19.2%)
> 2	3 (11.6%)
Patients with viable tumor, n (%)	
Yes	25 (96.2%)
No	1 (3.8%)
Within Milan, n (%)	
Yes	10 (38.4%)
No	16 (61.6%)
Downstaged to Milan, n (%)	4 (15.4%)
Within UCSF, n (%)	
Yes	11 (42.3%)
No	15 (57.7%)
Downstaged to UCSF, n (%)	3 (11.5%)
Laboratory	
Pre-LT AFP (ng/mL)	27,578 (133,183)
Post-LT AFP (ng/mL)	23,586 (81,707)
MELD	13 (7)
WBC (10 ⁹ /L)	6 (2.2)
Hgb (g/dL)	12.9 (2.7)
MCV (fL)	91 (6)
PLT (10 ³ /μL)	116 (67)
BUN (mg/dL)	15 (6)
Creatinine (mg/dL)	1.1 (0.6)
TP (g/dL)	7.2 (0.8)
Alb (g/dL)	3.6 (0.7)
ALP (U/L)	141 (58)
AST (U/L)	109 (167)
ALT (U/L)	71 (122)
T.Bili (mg/dL)	2.2 (2.4)
PT (sec)	14 (4.1)
INR	1.3 (0.4)

Clinical and pathological characteristics of the 26 recipients with hepatocellular carcinoma recurrence following liver transplant. Quantitative data are expressed as mean and categorical variables are reported as percentages. AFP: alpha fetoprotein; ALD: alcoholic liver disease; Alb: albumin; ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; HBV: hepatitis B virus; HCV: hepatitis C virus; Hgb: hemoglobin; INR: international normalized ratio; LT: liver transplant; MCV: mean corpuscular volume; MELD: model for end-stage liver disease; NAFLD: non-alcoholic fatty liver disease; PLT: platelet count; PT: prothrombin time; TP: total protein; T.Bili: total bilirubin; UCSF: University of California San Francisco; WBC: white blood cell count

Table 2. Alpha fetoprotein levels pre and post-liver transplant

Patient	Pre-LT AFP	Initial post-LT AFP	AFP at recurrence
1	9	7	2019
2	28,139	365,210	NA
3	135	4.1	15
4	3.6	0.6	1.7
5	27	3864	NA
6	488	57	86
7	22	2	26
8	23	12	1416
9	162	6.4	7
10	169	682	3342
11	34	3	389
12	48	4	12
13	323	76	157
14	7	21	NA
15	23	10	51
16	4659	25,154	NA
17	304	35	5.4
18	3.3	4.3	210
19	1707	100	47,304
20	34	2	3.7
21	680,000	217,576	120,848
22	22	2	17
23	4	2	NA
24	207	317	40.9
25	486	104	3677
26	5.2	5.5	4

Alpha fetoprotein levels (ng/mL) pre- and post-liver transplant in 26 liver transplant recipients with hepatocellular carcinoma recurrence. AFP: alpha fetoprotein; LT: liver transplant; NA: data not available

**Figure 2.** Overall, per year rate of hepatocellular carcinoma recurrence in deceased donor liver transplant recipients at the Johns Hopkins Hospital from 2005 to 2015. HCC: hepatocellular carcinoma

HCC recurrence after LT was 427 days, ranging from 34 to 1502 days. The site of HCC recurrence was intrahepatic in 8 (31%), extrahepatic in 14 (54%), and both intra- and extrahepatic in 4 (15%) patients. Overall, 31% of recipients had intrahepatic HCC recurrence following LT when compared to 69% with extrahepatic recurrence. Twenty-two percent of the patients who had extrahepatic involvement had concomitant liver involvement. The most common sites of extrahepatic involvement were the lungs (44.4%) and bones (44.4%) (spine, rib, pelvis, and humerus), followed by mediastinum (27.8%), brain (22.2%), portal lymph nodes (11.1%), gastro-hepatic ligament (5.6%), adrenal gland (5.6%), pleura (5.6%), and peritoneum (5.6%).

A range of different treatment modalities was used for recurrences [Table 3]. Six (21.4%) of the 26 patients were managed with supportive care. The remaining 20 cases received various treatment modalities including locoregional therapy (transarterial chemoembolization in 3, Y 90 in 1, and radiofrequency ablation in 1), external radiation in 10, and surgical resections in 8 (brain 4, spine 2, bone 1, and Whipple surgery in 1). Nine (32%) patients received combination therapies of the above-mentioned modalities. Seven patients (27%) received sorafenib. An additional two patients received chemotherapy regimens other than sorafenib [Table 3]. Recurrence-free survival and overall survival are shown in Figure 3. Patients who developed HCC recurrence following LT had an extremely poor overall survival (7.7%). In total, 19% of patients died within one year following LT. Overall, 24 out of 26 (92.3%) patients died throughout the four-year follow-up period. Timing of death relevant to the time of LT is shown in Table 3.

DISCUSSION

In this series, we report a rate of 15% HCC recurrence following deceased donor LT at our transplant program. This rate is consistent with the literature report of 15%-20% post-LT HCC recurrence^[13]. It is well known that the patients who are outside of MC prior to LT have higher rates of tumor recurrence following LT, compared to those within the MC^[1]. Although all of the patients within our series were thought to be within MC radiographically prior to LT, according to radiology findings, only 34% were within the criteria by reviewing the explant. When including an additional four (15%) patients who were downstaged, in total 49% were within MC based on pathology. This discrepancy between radiology and pathology has been previously described by other groups in the literature^[13].

Our sites of recurrence findings are very similar to the recent reports^[8]. In a systematic review of post-LT HCC recurrence, extrahepatic site was the most common site of recurrence in 67% of cases, compared to intrahepatic in 33%^[8]. The extrahepatic sites of involvement included: bone, pulmonary, adrenal, lymph nodes, and brain^[8].

Within our series, 54% of the HCC recurrences were diagnosed within 1 year post-OLT, while 81% and 96% of recurrences occurred within 2 and 3 years following OLT, respectively. The average time to HCC recurrence within our series was 427 days (range 34-1502 days). It is shown by others that early versus late recurrence is a predictor of post-LT survival^[14]. The patients with early HCC recurrence, defined as recurrence within 24 months post-LT, have a worse prognosis^[14]. There are a few potential theories for early HCC recurrence post-LT: (1) biologically rapid growing, aggressive tumors; (2) lack of high-quality pre-LT imaging or overlooking intra- or extrahepatic imaging^[8]; (3) extrahepatic microscopic viable HCC cells that could not be detected by conventional imaging prior to LT; and (4) presence of circulating tumor cells that seed to other sites. The mechanism by which the late recurrence occurs is unclear^[15]. Presence of pre-LT HCCs that are biologically slow growing, or development of *de novo* HCC recurrence in the liver allograft could be the cause. Within our series, we did not have any cases who had HCC recurrence that occurred or were diagnosed beyond five years following LT.

The selection of an ideal treatment for post LT HCC recurrence is a matter of debate, and the evidence is mainly based on expert opinion and non-randomized cohort studies^[9]. The treatment modality will vary

Table 3. Specific characteristics of the tumors, treatment, and outcomes

Patient	Number of lesions	Largest lesion (cm)	Within Milan criteria	Downstaged	MVI	Differentiation	Diagnosis of recurrence following LT (in days)	Site of recurrence	Survival	Time of death following LT (days)	Treatment of recurrence
1	5	4	No	No	Yes	Moderate	984	Gastro-hepatic ligament, mediastinal	Died	1466	Sorafenib
2	1	8	No	Yes	Yes	Poor	314	Liver	Died	369	Supportive care
3	5	8.5	No	Yes	Yes	Moderate	950	Brain	Died	1062	Brain metastasis resection
4	Infiltrative	1.1	No	No	Yes	Moderate	536	Liver	Died	780	Y90
5	1	1	Yes	No	No	Moderate	80	Porta-hepatis	Died	366	Chemotherapy with capecitabine
6	1	7.5	No	No	Yes	Poor	102	Perihilar, lung	Died	222	Supportive care
7	Infiltrative	2	No	No	Yes	Poor	224	Brain, liver, adrenal	Died	1459	Brain metastasis resection and radiation, cryoablation of adrenal metastasis, Y90 in liver
8	3	1	Yes	No	No	Moderate	490	Humerus, brain	Died	663	Bone resection and radiation, brain met resection
9	8	4.3	No	No	Yes	Poor	291	Ribs, spine	Died	602	Sorafenib
10	2	1.5	Yes	No	No	Moderate	94	Portal nodes, mediastinal, lung	Died	253	Spine surgery and radiation
11	2	1.6	Yes	No	Yes	Poor	464	Bone, lung	Died	547	Sorafenib, external radiation
12	1	5	Yes	No	Yes (bile duct)	Poor	482	Bile ducts	Died	1131	Supportive care
13	5	6	No	No	Yes	Moderate	111	Lung, peritoneal carcinomatosis	Died	332	Whipple surgery, Sorafenib
14	Infiltrative	2.8	No	No	Yes	Moderate	544	Pelvic bone	Died	1079	Supportive care
15	1	2.3	Yes	No	No	Moderate	795	Spine	Died	1319	External radiation, Sorafenib
16	1	7.2	No	No	Yes	Poor	93	Pelvic bone, lung	Died	493	Spine surgery and radiation
17	5	3.5	No	No	Yes	Poor	261	Liver	Alive	-	External radiation
18	3	3.5	No	Yes	Yes	Moderate	713	Lung, liver	Died	861	TACE
19	1	2.9	Yes	No	Yes	Poor	346	Liver	Died	407	Supportive care
20	1	2.8	Yes	No	Yes	Moderate	1502	Liver	Alive	-	RFA, TACE, Sorafenib
21	Infiltrative	19	No	No	Yes	Poor	78	Lung, bone, liver	Died	178	Chemotherapy with Gemcitabine/Cisplatin, external radiation
22	2	2.6	Yes	No	Yes	Poor	199	Liver	Died	260	Supportive care
23	5	2	No	No	No	Moderate	594	Brain	Died	1156	Brain metastasis resection
24	3	7.5	No	Yes	Yes	Moderate	147	Mediastinal lymph nodes, pleura	Died	697	Sorafenib
25	Infiltrative	4	No	No	Yes	Moderate	34	Spine, mediastinal	Died	1065	Spine radiation
26	1	2.2	Yes	No	No	No viable tissue	917	Lung, mediastinal	Died	1359	External radiation

Specific characteristics of the tumors, treatment, and outcomes in 26 liver transplant recipients with a recurrence of hepatocellular carcinoma following liver transplant. LT: liver transplant; MVI: microvascular invasion; RFA: radiofrequency ablation; TACE: transarterial chemoembolization

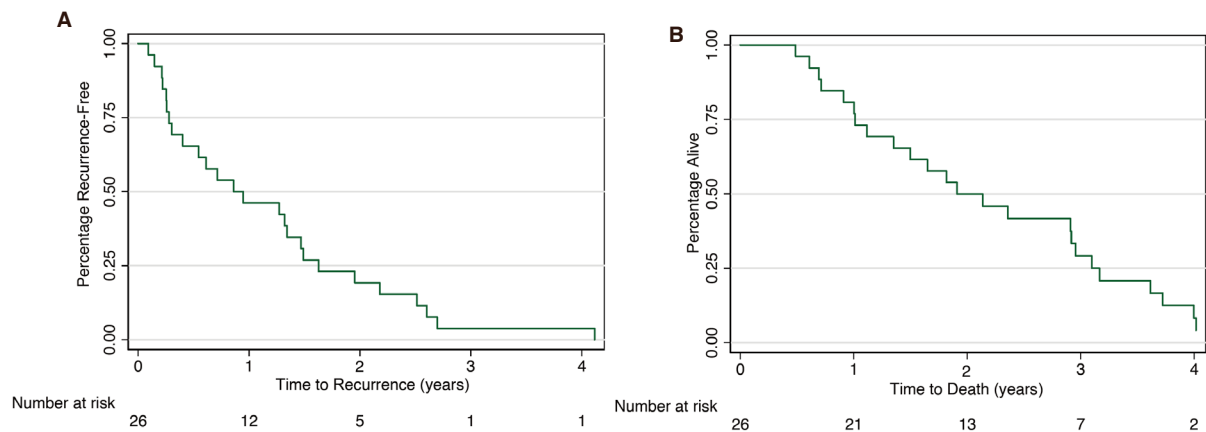


Figure 3. Survival analysis for 26 liver transplant recipients with hepatocellular carcinoma recurrence: A: Kaplan-Meier curve for recurrence-free survival; B: Kaplan-Meier curve for overall survival

based on the type of recurrence (intrahepatic versus extrahepatic), organ of involvement, and extent of involvement. This includes a wide range of surgical (intra- or extrahepatic resection and re-transplantation) and non-surgical treatments (locoregional therapies, sorafenib, other systemic chemotherapy, mTOR inhibitors, and best supportive care)^[16].

Surgical options including extrahepatic resection, liver graft resection, and liver re-transplant have also been considered for patients presenting with HCC recurrence. In 2004, the Mount Sinai group reported resection of the liver allograft in five out of 18 recipients with HCC recurrence^[11]. The authors concluded that, in selected cases with recurrent intrahepatic-HCC, liver resection improved survival^[11]. Similarly, Kornberg *et al.*^[10] reported that HCC recurrence should be treated surgically in eligible patients with good long-term outcomes. In multivariate analysis of post-LT HCC recurrence, late tumor recurrence (> 24 months) and surgical resection were the two independent predictors of survival^[10]. A systematic review in 2015 reported that the surgical approach to localized intra- or extra-hepatic recurrences are uneventful and not associated with higher mortality^[8]. Retransplantation for recurrent HCC is not a practical option^[17] due to the higher risk of recurrence with a limited organ availability.

Sorafenib, a multikinase inhibitor, has been approved as first-line treatment for the management of advanced-stage HCC following two clinical trials in 2008 and 2009^[18,19]. In a multicenter phase 2, blinded placebo-controlled, clinical trial, the efficacy of sorafenib for preventing HCC recurrence post-LT in high-risk recipients is being actively investigated [ClinicalTrials.gov identifier (NCT number): NCT01624285]. There are currently no systemic therapies that have been shown to improve survival in HCC recurrence post-LT. Recently, other tyrosine kinase inhibitors were approved as first- or second-line treatment in HCC in the non-transplant setting^[20]. The role of these agents as adjuvant therapy or post-LT HCC recurrence is unclear and deserves further investigation in the near future. Nivolumab, an anti-PD1 inhibitor, was recently approved for the treatment of HCC, as second line, in the non-transplant setting, with the objective response rate of 20%^[21]. The role of immunotherapy among post-LT recipients with HCC has not been yet established. It is possible that the immunotherapy will affect the liver allograft leading to acute cellular rejection^[22].

Mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, has been shown to be upregulated in 40%-50% of HCCs. mTOR is involved in the regulation of cell metabolism and growth^[23]. Therefore, various studies have suggested that mTOR inhibitors may have antineoplastic properties in HCC patients and mTOR inhibitors should be used after LT. In a meta-analysis of 2950 patients from five studies, sirolimus-based immunosuppression reduced the rate of tumor recurrence and improved overall survival^[24].

HCC recurrence following LT is an unfortunate event and associated with poor outcomes. In a recent meta-analysis, the median overall survival was 13 months following the diagnosis of HCC recurrence post-LT^[8]. Herein, supportive care was associated with the lowest survival rate of 3.3 months^[8]. There is no standardized protocol regarding the type and frequency of post-LT cross-sectional imaging in surveillance of HCC LT recipients. It is important to note that more than 50% of patients develop tumor recurrences that are outside of liver (extrahepatic), therefore imaging limited to the liver may not be sufficient for the diagnosis of majority of HCC recurrences. We also note that AFP is a useful marker in post-LT HCC surveillance only for high-AFP-secreting tumors. Four patients in our study had pre-AFP levels of > 1000 ng/mL. It is well known that patients with high AFP producing tumors have worse tumor biology and have worse outcomes^[12,25]. HCC candidates need to have AFP of ≤ 1000 ng/mL to receive extra points to shorten the waiting period for liver transplantation^[25]. The overall prognosis of HCC recurrence following LT is poor in the majority of cases and there are no available studies evaluating cost-effectiveness of surveillance protocols specific to this group of patients.

In conclusion, HCC recurrence post liver transplant is an unfortunate event associated with extremely poor survival. The majority of the cases are early recurrence occurring 1-2 years following liver transplantation. More than 50% of HCC recurrences are extrahepatic. Therefore, post-liver transplant imaging confined to the liver may not be enough to detect all of the recurrences. In patients with AFP producing tumors, this marker may also be helpful to diagnose the HCC recurrence. There is no general consensus on the treatment for post liver transplant hepatocellular carcinoma recurrence. The current reports are mainly based on single-center retrospective experience.

DECLARATIONS

Authors' contributions

Acquisition of data, analysis and interpretation of data, drafting of the manuscript: Simsek C

Interpretation of the data, Final Editing and Critical Review of the manuscript for important intellectual content: Kim A

Acquisition of data, analysis and interpretation of data, drafting of the manuscript: Ma M

Review of the data, drafting of the manuscript and editing: Danis N

Analysis and interpretation of data, statistical analysis: Gurakar M

Study concept and design, interpretation of data, critical revision manuscript for important intellectual content: Cameron AM

Study concept and design, interpretation of data, critical revision of the manuscript for important intellectual content: Philosophie B

Study concept and design, interpretation of data, statistical analysis, critical revision of the manuscript for important intellectual content: Garonzik-Wang J

Study concept and design, critical revision of the manuscript for important intellectual content: Ottmann S

Study concept and design, acquisition of data, drafting of the manuscript, analysis and interpretation of data, revision of the manuscript for important intellectual content study supervision: Gurakar A

Study concept and design, acquisition of data, drafting of the manuscript, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, study supervision: Saberi B

Availability of data and materials

Data source has been the Electronic Medical System. Please contact the corresponding author for unidentified data availability.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This retrospective study was approved by Johns Hopkins IRB NA_00028034.

Consent for publication

Not applicable.

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REFERENCES

- Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996;334:693-9.
- Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001;33:1394-403.
- Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009;10:35-43.
- Andreou A, Gul S, Pascher A, Schoning W, Al-Abadi H, et al. Patient and tumour biology predict survival beyond the Milan criteria in liver transplantation for hepatocellular carcinoma. *HPB (Oxford)* 2015;17:168-75.
- Marshall AE, Rushbrook SM, Vowler SL, Palmer CR, Davies RJ, et al. Tumor recurrence following liver transplantation for hepatocellular carcinoma: role of tumor proliferation status. *Liver Transpl* 2010;16:279-88.
- Llovet JM, Paradis V, Kudo M, Zucman-Rossi J. Tissue biomarkers as predictors of outcome and selection of transplant candidates with hepatocellular carcinoma. *Liver Transpl* 2011;17 Suppl 2:S67-71.
- Fernandez-Sevilla E, Allard MA, Selten J, Golse N, Vibert E, et al. Recurrence of hepatocellular carcinoma after liver transplantation: is there a place for resection? *Liver Transpl* 2017;23:440-7.
- de'Angelis N, Landi F, Carra MC, Azoulay D. Managements of recurrent hepatocellular carcinoma after liver transplantation: a systematic review. *World J Gastroenterol* 2015;21:11185-98.
- Chok K. Management of recurrent hepatocellular carcinoma after liver transplant. *World J Hepatol* 2015;7:1142-8.
- Kornberg A, Kupper B, Tannapfel A, Katenkamp K, Thrum K, et al. Long-term survival after recurrent hepatocellular carcinoma in liver transplant patients: clinical patterns and outcome variables. *Eur J Surg Oncol* 2010;36:275-80.
- Roayaie S, Schwartz JD, Sung MW, Emre SH, Miller CM, et al. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl* 2004;10:534-40.
- Gurakar A, Ma M, Garonzik-Wang J, Kim A, Anders RA, et al. Clinicopathological distinction of low-AFP-secreting vs. high-AFP-secreting hepatocellular carcinomas. *Ann Hepatol* 2018;17:1052-66.
- Guerrero-Misas M, Rodriguez-Peralvarez M, De la Mata M. Strategies to improve outcome of patients with hepatocellular carcinoma receiving a liver transplantation. *World J Hepatol* 2015;7:649-61.
- Chok KS, Chan SC, Cheung TT, Chan AC, Fan ST, et al. Late recurrence of hepatocellular carcinoma after liver transplantation. *World J Surg* 2011;35:2058-62.
- Zhang JA, Kwee SA, Wong LL. Late recurrence of hepatocellular carcinoma after liver transplantation. *Hepatoma Res* 2017;3:58-66.
- Rubin J, Ayoub N, Kaldas F, Saab S. Management of recurrent hepatocellular carcinoma in liver transplant recipients: a systematic review. *Exp Clin Transplant* 2012;10:531-43.
- Tabrizian P, Roayaie S, Schwartz ME. Current management of hepatocellular carcinoma. *World J Gastroenterol* 2014;20:10223-37.
- Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-90.
- Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009;10:25-34.
- Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol* 2018;15:599-616.
- El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
- Munker S, De Toni EN. Use of checkpoint inhibitors in liver transplant recipients. *United European Gastroenterol J* 2018;6:970-3.
- Matter MS, Decaens T, Andersen JB, Thorgeirsson SS. Targeting the mTOR pathway in hepatocellular carcinoma: current state and future trends. *J Hepatol* 2014;60:855-65.
- Liang W, Wang D, Ling X, Kao AA, Kong Y, et al. Sirolimus-based immunosuppression in liver transplantation for hepatocellular carcinoma: a meta-analysis. *Liver Transpl* 2012;18:62-9.
- Mehta N, Dodge JL, Roberts JP, Hirose R, Yao FY. Alpha-fetoprotein decrease from > 1,000 to < 500 ng/mL in patients with hepatocellular carcinoma leads to improved posttransplant outcomes. *Hepatology* 2019;69:1193-205.

Original Article

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Distinctive magnetic resonance imaging findings of hepatocellular carcinoma after hepatitis C virus eradication with direct-acting antivirals

Takao Shimizu^{1,2,#}, Kaoru Tsuchiya^{1,#}, Masayuki Kurosaki¹, Yutaka Yasui¹, Sakura Kirino¹, Keiya Watakabe¹, Leona Osawa¹, Mao Okada¹, Wan Wang¹, Mayu Higuchi¹, Kenta Takaura¹, Shun Kaneko¹, Nobuharu Tamaki¹, Hiroyuki Nakanishi¹, Jun Itakura¹, Yuka Takahashi¹, Tadakazu Hisamatsu², Namiki Izumi¹

¹Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo 180-8610, Japan.

²Department of Gastroenterology and Hepatology, Kyorin University Hospital, Tokyo 180-8610, Japan.

#Authors contributed equally.

Correspondence to: Dr. Namiki Izumi, Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino-shi, Tokyo 180-8610, Japan. E-mail: izumi012@musashino.jrc.or.jp

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Abstract

Aim: The aim of the present study was to evaluate the characteristics of the magnetic resonance imaging features of hepatocellular carcinoma (HCC) that developed early after the eradication of hepatitis C virus (HCV) by direct-acting antiviral (DAA) treatment.

Methods: This study included 26 patients who achieved sustained viral response with DAA and developed HCC thereafter within one year (DAA-SVR HCC). The radiologic characteristics of these patients were evaluated by contrast-enhanced magnetic resonance imaging, including diffusion-weighted imaging (DWI) and T2-weighted imaging (T2WI). For comparison, 80 HCC patients with positive HCV RNA (HCV-positive HCC) were included. Among 42 patients where tumor biopsy was available, histological grade and radiologic findings were compared.

Results: The rates of high intensity on DWI and T2WI were significantly higher in DAA-SVR HCC compared to HCV-positive HCC (DWI: 100% *vs.* 67.5%, $P < 0.001$; T2WI: 92.6% *vs.* 67.5%, $P = 0.01$). HCC with high intensity on DWI or T2WI was more likely to have moderately or poorly differentiated HCC compared to well-differentiated HCC (DWI: 69.7% *vs.* 30.3%, $P = 0.02$; T2WI: 66.7% *vs.* 27.3%, $P = 0.03$).



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Conclusion: High intensity on DWI and hyperintensity on T2WI were distinctive features of HCC that developed within one year after the end of DAA treatment.

Keywords: Hepatocellular carcinoma, direct-acting antivirals, sustained viral response, contrast enhanced magnetic resonance imaging

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancer types^[1] and the major cause of cancer-related deaths^[2]. Chronic liver disease and cirrhosis are closely associated with the development of HCC^[3]. In patients with chronic hepatitis C virus (HCV) infection, sustained viral response (SVR) can be observed in more than 90% of the cases by antiviral therapy using direct-acting antivirals (DAAs)^[4]. However, it remains controversial whether HCC that developed after the eradication of HCV with DAA regimens differs from those that developed during active infection with HCV. Although the incidence of HCC development may not be suppressed or enhanced by the eradication of HCV with DAA^[5], the question remains whether there are differences in the biological characteristics, such as histology or radiologic findings, or the clinical course after curative treatment. Imaging features of HCC after SVR with DAA have not been studied in detail.

For the diagnosis of HCC, contrast-enhanced magnetic resonance imaging (MRI) using gadolinium ethoxybenzyl diethylenetriamine penta-acetic acid (Gd-EOB-DTPA) is often used^[6-9]. MRI has an advantage over computer tomography (CT) in that various images, including diffusion-weighted images (DWI) or T2-weighted images (T2WI), can be obtained in addition to the vascularity information of HCC nodules. DWI is imaging associated with the restriction of water molecule movement and reflects the histopathologic features of organs and tissues by virtue of high scan speed. It is widely known that DWI is valuable for the diagnosis and differential diagnosis of neoplasm in the liver, including small (< 2 cm) HCC^[10]. In this study, we evaluated the MRI features of HCC that developed within one year after the end of DAA treatment.

METHODS

Patients

In our hospital cohort, 695 patients with chronic hepatitis C were treated with DAA regimens and achieved SVR between October 2014 and September 2016. At baseline and after SVR, the surveillance for HCC was performed using ultrasonography, contrast-enhanced CT, or EOB-MRI. Among these 695 patients, 26 patients developed HCC within one year after the end of DAA therapy (DAA-SVR HCC). All of these patients had no previous history of HCC treatment. For comparison, 80 HCC patients with positive HCV RNA (HCV-positive HCC) were selected from 208 consecutive HCC patients with HCV infection who were treated for the first time from December 2011 and February 2018 according to the following inclusion criteria: (1) HCV RNA positive at the time of HCC diagnosis; (2) Child-Pugh A; (3) tumor diameter ≤ 3 cm and number of nodules ≤ 3 ; (4) no vascular invasion and no extrahepatic metastasis; (5) EOB-MRI available; and (6) radiofrequency ablation (RFA) was performed. This study was conducted according to the ethical principles of the Declaration of Helsinki and STROBE guidelines. This study was approved by the Ethics Committees of Musashino Red Cross Hospital (approval number 28077).

HCC diagnosis

The radiologic diagnosis for HCC was based on the Japan Society of Hepatology^[11], the American Association for the Study of Liver Disease^[12], and the European Association for the Study of the Liver^[13] guidelines. Typical radiographic findings on dynamic study (CT or MRI) or needle biopsy were used for diagnosis.

Magnetic resonance examinations were performed in all patients using a 1.5 T scanner (Signa Excite HD; GE Healthcare, USA). Gd-EOB-DTPA was used as a contrast agent. The contrast material was administered as a bolus at a dose of 0.1 mL/kg body weight at a rate of 2 mL/s followed by flushing with 20 mL saline solution using a power injector. Images of the arterial phase, portal venous phase, late phase, and hepatobiliary phase (HPB) were obtained 18 s, 60 s, 150 s, and 20 min after the peak time, respectively, after contrast injection. DWI was acquired before HPB with b values of 0 and 1000 s/mm². Apparent diffusion coefficient (ADC) maps were obtained automatically by two images of b values of 0 and 1000 s/mm². Dynamic CT scan with a section thickness of 2 mm was performed. For triple-phase dynamic CT scans, arterial, portal, and equivalent phases were set at 35, 70, and 150 s, respectively, after injection of the contrast agent. Board-certified radiologists diagnosed HCC based on typical patterns, such as an early-phase hyperattenuation area and a late-phase hypoattenuation area on dynamic study. Pathologic diagnosis was confirmed by a certified pathologist who was unaware of the patient's clinical data.

RFA procedure

RFA was percutaneously performed by using local anesthesia. We used an internally water-cooled 17 G cooled-tip electrode with an impedance-controlled generator (Cosman generator, Cool-Tip System, Radionics, Burlington, MA, USA). When the target nodule was more than 20 mm in diameter, multiple needle insertions and multiple ablations of one nodule were performed.

Statistical analysis

All statistical analyses were performed using Easy R (EZR) version 3.4.1 (Saitama Medical Center, Jichi Medical University, Saitama, Japan)^[14], a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). $P < 0.005$ was considered statistically significant. Fisher's exact test, paired t test, and Mann-Whitney U test were adopted to determine the differences between the two indexes. Fisher's exact test was used to evaluate the intensity of DWI and T2WI. Survival curves were estimated using the Kaplan-Meier method. Overall survival (OS) was defined as the period between the treatment date of HCC and the patient's death or last visit, and progression-free survival (PFS) was defined as the period between the treatment date of HCC and the recurrence confirmation date of dynamic CT or EOB-MRI.

RESULTS

Comparison of demographics and laboratory data

The baseline clinical characteristics of all patients are shown in Table 1. Among them, 26 patients achieved SVR with DAAs before HCC diagnosis (DAA-SVR HCC) and HCV RNA was detected in the remaining 80 patients (HCV-positive HCC). The median age of all patients was 75 years old and the median tumor size was 18 (8-29) mm. Serum alanine aminotransferase (ALT; 18.5 vs. 53.0 IU/mL, $P = 0.001$) and α -fetoprotein (AFP; 3.42 vs. 25.3, $P < 0.001$) were significantly lower in DAA-SVR HCC patients than in HCV-positive HCC patients and the mean platelet count was higher in DAA-SVR HCC patients than in HCV-positive HCC patients ($149 \times 10^3/\mu\text{L}$ vs. $102 \times 10^3/\mu\text{L}$, $P = 0.009$). There was no significant difference in age, tumor size, tumor number, albumin, total bilirubin, and prothrombin time international normalized ratio between DAA-SVR and HCV-positive HCC patients. The median value of Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA \pm M2BP) in 25 patients with DAA-SVR HCC was 1.48.

Imaging features

The imaging features are shown in Table 2. There was no difference between DAA-SVR and HCV-positive HCC in terms of features of arterial phase, late phase, or HPB. In contrast, all DAA-SVR HCC was depicted as high intensity on DWI, whereas only 67.5% HCV-positive HCC was found ($P < 0.001$). Moreover, high intensity on T2WI was significantly higher in DAA-SVR HCC than in HCV-positive HCC (92.6% vs. 67.5%, $P = 0.01$). The typical imaging features of DAA-SVR HCC are shown in Figure 1. We also measured

Table 1. Patients' characteristics

	DAA-SVR HCC (<i>n</i> = 26)	HCV-positive HCC (<i>n</i> = 80)	<i>P</i> value
Age (years), median (range)	75 (63-83)	75 (44-93)	0.27
Sex (male/female)	10/16	43/37	0.26
Number of tumors (single/multiple)	21/5	60/20	0.61
Tumor diameter (cm), median (range)	18.0 (10-29)	17.6 (8.0-29)	0.7
BCLC stage A/B/C	26/0/0	80/0/0	1.0
AFP (ng/mL)	3.4 (2.0-6116.5)	25.3 (2.0-1660)	< 0.001
PIVKA-II (mAU/mL)	19.0 (12-94)	53.0 (13-245)	0.2
Albumin (g/dL)	4.0 (2.9-4.7)	3.7 (2.8-273)	0.6
ALT (IU/mL)	18.0 (12-94)	53 (13-245)	< 0.001
Platelet (10 ³ /μL)	149 (45-189)	102 (33-344)	0.009
Total bilirubin (mg/dL)	0.7 (0.3-2.0)	0.8 (0.3-2.6)	0.71
PT%	91 (57-113)	89 (31-120)	0.24
Child-Pugh score 5/6	25/1	68/12	0.18
Fib 4 index	3.22(0.55-11.5)	6.01(1.66-25.2)	< 0.001

BCLC: barcelona clinic liver cancer; AFP: alpha-fetoprotein; PIVKA-II: protein induced by vitamin K absence or antagonist-II; ALT: alanine aminotransferase; PT: prothrombin time; DAA: direct-acting antiviral; SVR: sustained viral response; HCC: hepatocellular carcinoma; HCV: hepatitis C virus

Table 2. Image features of EOB-MRI at HCC diagnosis

	Early high	Late low	HBP low	T2WI high	DWI high
DAA-SVR HCC (<i>n</i> = 26)	19/26 (73.0%)	24/26 (92.3%)	24/26 (92.3%)	24/26 (92.3%)	26/26 (100%)
HCV-positive HCC (<i>n</i> = 80)	63/74 (85.1%)	74/74 (100%)	70/74 (94.6%)	54/80 (67.5%)	54/80 (67.5%)

HBP: hepatobiliary phase; DWI: diffusion-weighted imaging; T2WI: T2-weighted imaging; DAA: direct-acting antiviral; SVR: sustained viral response; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; EOB: ethoxybenzyl; MRI: magnetic resonance imaging

the ADC values of HCC lesions on DWI; however, there was no significant difference between the two groups.

Tumor biopsy was performed in 9 patients with DAA-SVR HCC and 33 patients with HCV-positive HCC. In nine patients with DAA-SVR HCC, four patients showed well-differentiated HCC and five patients were diagnosed with moderately or poorly differentiated HCC. In 33 patients with HCV-positive HCC, 13 patients showed well-differentiated HCC and 20 patients were diagnosed with moderately or poorly differentiated HCC. There were significant associations with MRI and pathologic findings. HCC with high intensity on DWI or T2WI was more likely to have moderately or poorly differentiated HCC compared to well-differentiated HCC (DWI: 69.7% *vs.* 30.3%, *P* = 0.02; T2WI: 66.7% *vs.* 27.3%, *P* = 0.03).

Prognosis after curative HCC treatment

Among patients with DAA-SVR HCC, 1 patient received resection, 2 patients were treated by transcatheter arterial chemoembolization, 1 patient was not treated, and the remaining 22 patients were treated with RFA. All patients with HCV-positive HCC were treated with RFA. OS and PFS were not different between DAA-SVR and HCV-positive HCC [Figure 2].

DISCUSSION

The present study evaluated the imaging features of HCC that developed early after the eradication of HCV by DAA therapy. All of these HCC showed high intensity on MRI DWI and more than 90% showed high intensity on T2WI, which was significantly different from HCC with positive HCV RNA. The stage of HCC, such as the number of HCC nodules and the maximum diameter, was not different between these two groups, indicating that high intensity on DWI or T2WI on MRI is the characteristic imaging feature of HCC after DAA treatment.

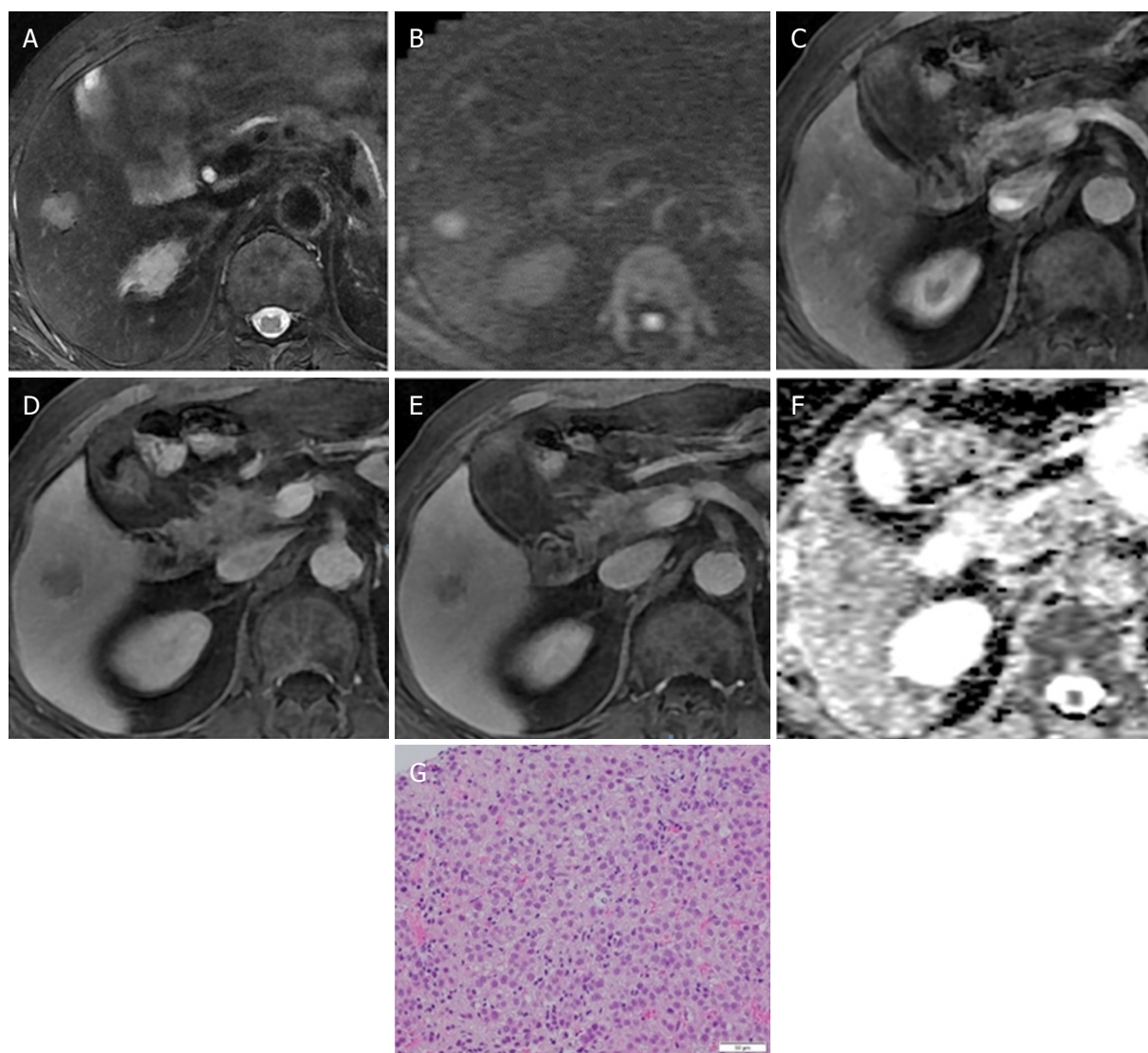
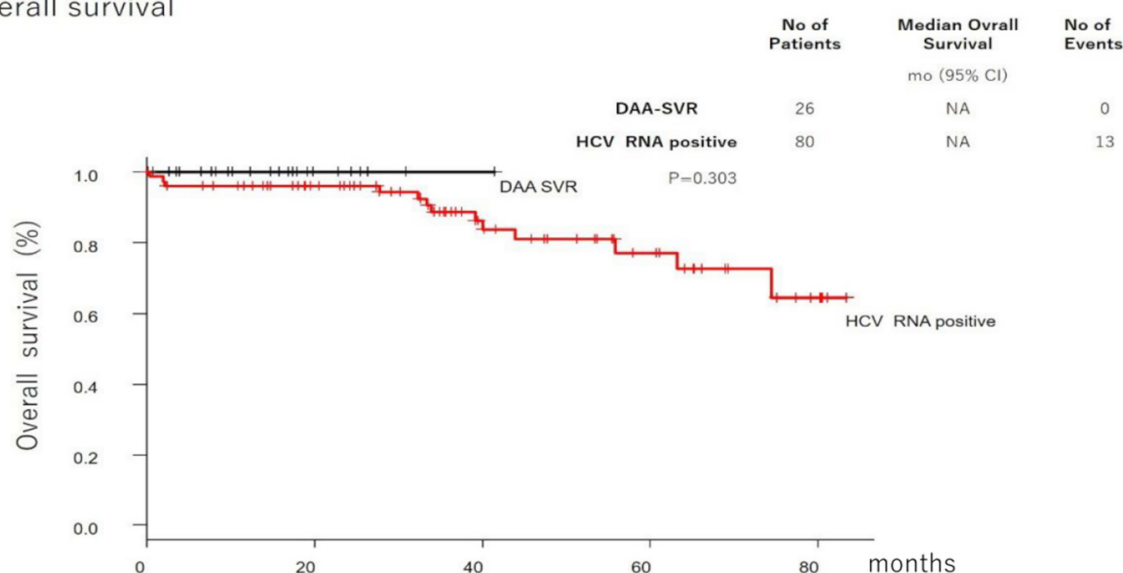


Figure 1. Hepatocellular carcinoma in an 82-year-old male. In hepatic segment VI, a 2-cm nodule shows: A: hyperintensity on axial T2-weighted imaging; B: hyperintensity on diffusion-weighted imaging (b value = 800 s/mm²); C: hyperintensity on arterial phase; D: hypointensity on late phase; E: hypointensity on hepatobiliary phase; F: hypointensity on apparent diffusion coefficient map; G: pathology was moderate

The high signal intensity on DWI or T2WI is reported to be linked to the pathology of HCC. Among small hepatic nodules with hypovascularity on arterial phase, high intensity on DWI or T2WI is the characteristic feature of HCC compared to dysplastic nodules^[15,16]. High signal intensity on T2WI correlates with increased intratumoral arterial supply and decreased intratumoral portal blood supply. Among HCC with hypovascularity on arterial phase, high signal intensity on T2WI was associated with histologic grade^[17].

In a report that classified the signal intensity of DWI into three stages (iso/slight, moderate, and obvious) in 254 resected HCC, the prevalence of well-differentiated HCC was high and that of moderately or poorly differentiated HCC was low in the iso/slight intensity imaging group compared to the obvious intensity imaging group: the pathology of well/moderate/poor HCC was 38.9%/57.4%/3.7% for the iso/slight intensity group and 5.8%/77.4%/16.8% for the obvious intensity group^[18]. Other reports showed that DWI features were linked to poor differentiation on pathology, vascular invasion, and recurrence risk factor after resection of small HCC^[19,20]. Although the number of patients with the information of pathology was small

A Overall survival



B Progression free survival

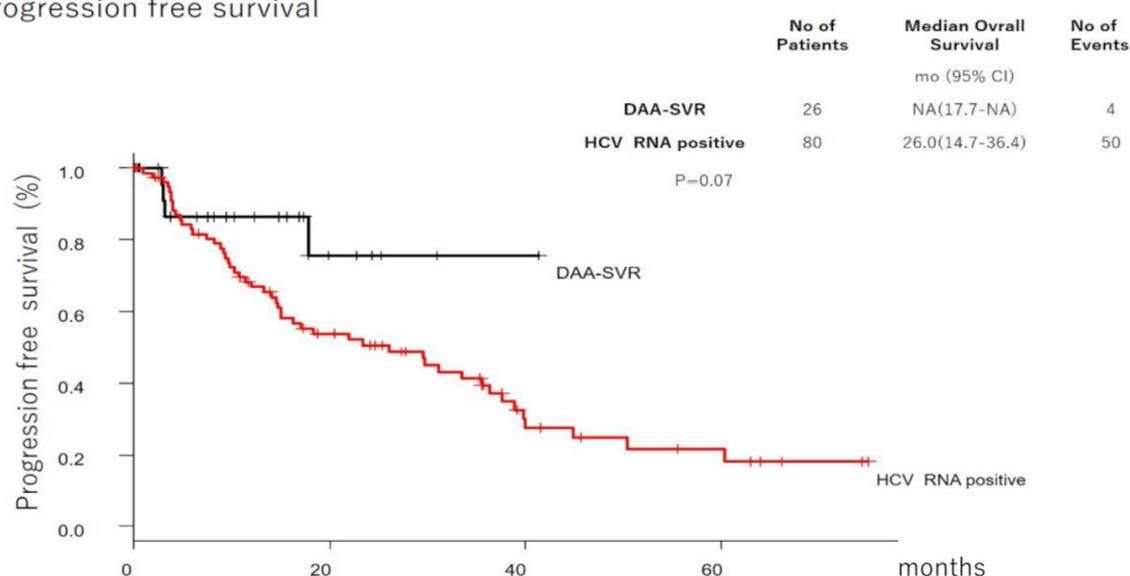


Figure 2. Kaplan-Meier analysis of overall survival and progression-free survival. DAA: direct-acting antiviral; SVR: sustained viral response; HCV: hepatitis C virus

in our study, HCC with high intensity on DWI or T2WI was more likely to have moderately or poorly differentiated HCC compared to well-differentiated HCC. Taken together, these results suggest that high intensity on DWI or T2WI, the characteristic feature of HCC that developed early after the eradication of HCV by DAA therapy, may be the hallmark of the possible malignant phenotype of HCC in general.

Despite having these imaging features of malignant potential, the prognosis of HCC after DAA treatment was comparable to HCV-positive HCC that received curative RFA therapy in terms of OS or PFS. The possible reason for this fair prognosis is that all DAA-SVR HCC was found in the early stage (mean diameter of 18 mm and 81% as single nodule), and actually 25 of 26 patients received curative therapy (resection or RFA in 23 patients and transcatheter arterial chemoembolization with curative consent in 2 patients). However, if these patients were found in a more advanced stage, the prognosis may have been worse due to rapid progression or by the inability to receive curative therapy. Mariño *et al.*^[21] recently

reported that HCC incidence was 3.73 HCC/100 person-years in 1123 cirrhotic patients treated with interferon (IFN)-free regimens and 72 patients developed HCC within a median of 10.3 months after starting antiviral treatment. Although some large-cohort studies and systemic reviews revealed that there were no significant differences in hepatocarcinogenesis after achieving SVR between patients treated with IFN and those treated with DAAs, there are still unknown mechanisms involved in the increased risk of HCC emergence in IFN-free regimens. Yoshimasu *et al.*^[22] reported that the HCC occurrence rate after DAA treatment was very low and the recurrence rate was lower than that in previous IFN reports.

The AFP level and AFP-L3% were identified as important factors in predicting the occurrence/recurrence of HCC; thus, patients with such levels are still at risk of developing cancer after SVR. Villani *et al.*^[23] reported that DAA administration induced an early increase in serum vascular endothelial growth factor (VEGF) and a change in the inflammatory pattern, coinciding with HCV clearance. Debes *et al.*^[24] identified a set of 12 immune mediators whose levels were significantly higher in serum before DAA treatment of patients who eventually developed *de novo* HCC compared to controls. A panel of nine cytokines, measured in serum before treatment (MIG, IL22, TRAIL, APRIL, VEGF, IL3, TWEAK, SCF, and IL21), identified patients who developed *de novo* HCC with an area under the receiver operating characteristic curve value higher than 0.8. Further analyses of the mechanism also provide important information about HCV-induced carcinogenesis and the effects of DAAs. In this study, we focused on the HCV status; however, HCC recurrence and overall survival are associated with multiple factors including liver fibrosis, immune status, life style, and comorbidities.

Several studies revealed that the ADC value was a predictive factor of the histological grade of HCC^[25-27]. However, in this study, we could not find a significant difference in ADC values between DAA-SVR and HCV-positive HCC. In contrast to these studies that included patients who received resection or transplantation, our patients had a smaller size of HCC, which led to inaccurate quantification values due to technical error.

There are some limitations in this study. The sample size was small and this study was conducted in a single center. DAA-SVR HCC patients received HCC screening within six months before administration of DAAs; however, the imaging modality was not uniform, including CT, MRI, or ultrasound. Therefore, we could not definitely exclude the possibility that small hepatic nodules that could be detected only by HPB of EOB-MRI were missed in some patients before DAA treatment. Tumor biopsy was performed only when the patients agreed to the procedure and the location of the tumor was not near large vessels, liver surface, and other organs. Therefore, the correlation between pathological and MRI features should be evaluated in a large cohort in which patients received liver resection.

Our study is the first to reveal the significant differences in MRI findings between DAA-SVR and HCV-positive HCC. According to our results, HCC that develop within one year after the end of DAA treatment would have unique imaging features that may be linked to malignant phenotype if not found and treated early. Today, most patients with HCV infection can achieve viral eradication with DAA therapy. However, in high-risk patients such as those with cirrhosis, the surveillance of HCC should be done at early time points after SVR to diagnose HCC at an early stage and curatively treat it with resection, RFA, or microwave ablation, which may lead to better OS in these patients.

DECLARATIONS

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Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Shimizu T, Tsuchiya K, Kurosaki M

Conceptualization: Shimizu T, Tsuchiya K, Kurosaki M

Methodology: Yasui Y, Hisamatsu T

Data collection: Kirino S, Watakabe K, Osawa L, Okada M, Wang W, Higuchi M, Takaura K, Kaneko S, Tamaki N, Nakanishi H, Itakura J, Takahashi Y

Writing - original draft preparation: Shimizu T, Tsuchiya K,

Writing - review and editing: Kurosaki M

Supervision: Hisamatsu T, Izumi N

Availability of data and materials

The original raw data used to support the findings of this study have not been made available because of the risk that will come into conflict with Personal Information Protection Law in Japan.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study was approved by the ethical committee at Musashino Red Cross Hospital (approval number 28077).

Consent for publication

Not applicable.

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REFERENCES

1. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012;379:1245-55.
2. Ferlay J, Shin, HR, Bray F, Forman D, Mathers C, et al. Estimates of worldwide burden of cancer in 2008: Globocan 2008. *Int J Cancer* 2010;127:2893-917.
3. Bruix J, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014;63:844-55.
4. Pawlotsky JM. New hepatitis C therapies: the toolbox, strategies, and challenges. *Gastroenterology* 2014;146:1176-92.
5. Toyoda H, Kumada T, Tada T, Mizuno K, Tanaka J, et al. The impact of HCV eradication by direct-acting antivirals on the transition of precancerous hepatic nodules to HCC: a prospective observational study. *Liver Int* 2019;39:448-54.
6. Huppertz A, Balzer T, Blakeborough A, Breuer J, Giovagnoni A, et al. Improved detection of focal liver lesions at MR imaging: multicenter comparison of gadoxetic acid enhanced MR images with intraoperative findings. *Radiology* 2004;230:266-75.
7. Kitao A, Matsui O, Yoneda N, Kozaka K, Shinmura R, et al. The uptake transporter OATP8 expression decreases during multistep hepatocarcinogenesis: correlation with gadoxetic acid enhanced MR imaging. *Eur Radiol* 2011;21:2056-66.
8. Kim HY, Choi JY, Kim CW, Bae SH, Yoon SK, et al. Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging predicts the histological grade of hepatocellular carcinoma only in patients with Child-Pugh class A cirrhosis. *Liver Transplant* 2012;18:850-7.
9. Kogita S, Imai Y, Okada M, Kim T, Onishi H, et al. Gd-EOB-DTPA enhanced magnetic resonance images of hepatocellular carcinoma: correlation with histological grading and portal blood flow. *Eur Radiol* 2010;20:2405-13.
10. Lim KS. Diffusion-weighted MRI of hepatocellular carcinoma in cirrhosis. *Clin Radiol* 2014;69:1-10.
11. Kokudo N, Takemura N, Hasegawa K, Takayama T, Kubo S, et al. Clinical practice guidelines for hepatocellular carcinoma: the Japan Society of Hepatology 2017 (4th JSH-HCC guidelines) 2019 update. *Hepatol Res* 2019;49:1109-13.
12. European Association for the Study of the Liver. EASL clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol*

- 2018;69:182-236.
13. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;68:723-50.
 14. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013;48:452-8.
 15. Hwang J, Kim YK, Jeong WK, Choi D, Rhim H, et al. Nonhypervascular hypointense nodules at gadoxetic acid enhanced MR imaging in chronic liver disease. *Radiology* 2015;276:137-46.
 16. Kwon HJ, Byun JH, Kim JY, Hong GS, Won HJ, et al. Differentiation of small (≤ 2 cm) hepatocellular carcinomas from small benign nodules in cirrhotic liver on gadoxetic acid-enhanced and diffusion-weighted magnetic resonance images. *Abdom Imaging* 2015;40:64-75.
 17. Inoue T, Hyodo T, Murakami T, Takayama Y, Nishie A, et al. Hypovascular hepatic nodules showing hypointense on the hepatobiliary-phase image of Gd-EOB-DTPA-enhanced MRI to develop a hypervascular hepatocellular carcinoma: A nationwide retrospective study on their natural course and risk factors. *Dig Dis* 2013;31:472-9.
 18. Chou CT, Chou JM, Chang TA, Huang SF, Chen CB, et al. Differentiation between dysplastic nodule and early-stage hepatocellular carcinoma: the utility of conventional MR imaging. *World J Gastroenterol* 2013;19:7433-9.
 19. Jiang T, Xu JH, Zou Y, Chen R, Peng LR, et al. Diffusion-weighted imaging (DWI) of hepatocellular carcinomas: a retrospective analysis of the correlation between qualitative and quantitative DWI and tumor grade. *Clin Radiol* 2017;72:465-72.
 20. Okamura S, Sumie S, Tonan T, Nakano M, Satani M, et al. Diffusion-weighted magnetic resonance imaging predicts malignant potential in small hepatocellular carcinoma. *Dig Liver Dis* 2016;48:945-52.
 21. Mariño Z, Darnell A, Lens S, Sapena V, Díaz A, et al. Time association between hepatitis C therapy and hepatocellular carcinoma emergence in cirrhosis: Relevance of non-characterized nodules. *J Hepatol* 2019;70:874-84.
 22. Yoshimasu Y, Furuichi Y, Kasai Y, Takeuchi H, Sugimoto K, et al. Predictive factors for hepatocellular carcinoma occurrence or recurrence after direct-acting antiviral agents in patients with chronic hepatitis C. *J Gastrointest Liver Dis* 2019;28:63-71.
 23. Villani R, Vendemiale G, Serviddio G. Molecular mechanisms involved in HCC recurrence after direct-acting antiviral therapy. *Int J Mol Sci* 2018;20:49.
 24. Debes JD, van Tilborg M, Groothuisink ZMA, Hansen BE, Schulze Zur Wiesch J, et al. Levels of cytokines in serum associate with development of hepatocellular carcinoma in patients with HCV infection treated with direct-acting antivirals. *Gastroenterology* 2018;154:515-7.e3.
 25. Nakanishi M, Chuma M, Hige S, Omatsu T, Yokoo H, et al. Relationship between diffusion-weighted magnetic resonance imaging and histological tumor grading of hepatocellular carcinoma. *Ann Surg Oncol* 2012;19:1302-9.
 26. Shankar S, Kalra N, Bhatia A, Srinivasan R, Singh P, et al. Role of diffusion weighted imaging (DWI) for hepatocellular carcinoma (HCC) detection and its grading on 3T MRI: a prospective study. *J Clin Exp Hepatol* 2016;6:303-10.
 27. Granata V, Fusco R, Filice S, Catalano O, Piccirillo M, et al. The current role and future perspectives of functional parameters by diffusion weighted imaging in the assessment of histologic grade of HCC. *Infect Agent Cancer* 2018;13:23.

Review

Open Access



Prehabilitation in elderly patients scheduled for liver resection and protocol for Recovery Of Surgery in Elderly

Ramkumar Mohan¹, Cheong Wei Terence Huey², Sameer Junnarkar², Jee Keem Low², Vishal G. Shelat²

¹Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

²Senior Consultant, Hepatopancreaticobiliary Surgery, Tan Tock Seng Hospital, Singapore.

Correspondence to: Ramkumar Mohan, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.
E-mail: m.ramkumar@u.nus.edu

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Abstract

Ageing population of first world economies pose unique challenges to surgical community. Enhanced recovery after surgery protocols and pathways do not attempt to optimize or enhance physical function of patients by customized program of physical activity. Increasingly, prehabilitation programs (PP) have gained momentum in orthopaedics, urology, colorectal surgery and hepatopancreaticobiliary surgery. Current evidence of PP in various elective surgical procedures have shown improved outcomes with minimal to none drawback or harm. There is emerging evidence of role of PP in elective liver resection. The aim of this paper is to review the basis of PP and share local multidisciplinary team protocol specifically customized to frail and elderly population - Recovery Of Surgery in Elderly.

Keywords: Prehabilitation, liver resection, hepatocellular carcinoma, pre-operative exercise, ageing

INTRODUCTION

Liver is the largest solid intra-abdominal organ and common site for primary and metastatic cancers. The most common liver malignancy is secondary from gastrointestinal tract with colorectal liver metastasis being the commonest. Hepatocellular carcinoma is the most common primary liver cancer and fifth most common cancer worldwide^[1]. Hepatocellular carcinoma occurs in elderly population and the incidence is expected to rise with epidemic of diabetes as well as ageing population. With increased participation in



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screening programs for Hepatitis B, increasing awareness and advances in imaging technology facilitating early diagnosis, surgery has an increasing role in management of hepatocellular carcinoma. Age-related comorbidities increase operative morbidity and impacts peri-operative outcomes. Enhanced recovery after surgery (ERAS) programs emphasize on intra-operative and post-operative care standardization with minimal guidance on pre-operative phase except nutritional optimization and carbohydrate loading. Pre-operative phase provides an opportunity for surgeons to optimize patients to meet up for impending physiologic stress imposed by surgery. In this paper, we discuss the role of prehabilitation program in elderly patients scheduled for liver resection and also discuss the current protocol of Recovery Of Surgery in Elderly (ROSE) program.

AGEING

Along with greying of hair, development of cataracts, degenerative bone disorders and declining cognition, ageing leads to a myriad of other physiologic changes in organ systems. Atherosclerosis, hypertension and decreased cardiac output are noted in the cardiovascular systems. Impaired gas exchange, reduction in vital capacity and reduced expiratory flow rates are noted in pulmonary system. Furthermore, decline in lean body mass, creatinine clearance reduction, hepatic drug metabolism impairment and gastrointestinal motility reduction are noted. This functional and metabolic alteration is compounded with polypharmacy for managing chronic illnesses. [Figure 1](#) shows age related physiologic changes in organ systems. Frailty and sarcopenia are common in elderly too^[2].

FRAILITY AND SARCOPENIA

Frailty is a pre-disability syndrome in the elderly when exposed to stressors with increased risk of disability or need for hospitalization. The European Working Group on Sarcopenia in Older People has defined sarcopenia as progressive, generalized loss of skeletal mass with associated functional losses^[3]. Low skeletal muscle mass^[4] and low functional capacity^[5] associated with sarcopenia lead to increased post-operative complications as well as reduced long term survival. As such the elderly are vulnerable, especially when faced with peri-operative stress. Morley *et al.*^[6], reported that sarcopenia and frailty are closely linked to ageing. They reported decreased hand grip strength, walking speed and weight in the elderly and concluded lack of muscle usage as one of the reasons which impacts outcomes. Prehabilitation aims to mitigate the drawbacks of frailty.

PREHABILITATION

Rehabilitation is integrated in routine clinical medicine. It focuses on recovery following a surgical stressor. Prehabilitation is defined as the process of augmenting functional capacity before surgery with aim of reducing post-operative morbidity and/or mortality. This is done through a personalized regimen of aerobic, functional and strength training. It aims to optimize pre-operative functional cardiorespiratory and nutritional reserves. As the scope of prehabilitation overlaps with concept of rehabilitation and rehabilitation is integral to orthopaedic surgery, orthopaedic teams were early adopters of prehabilitation^[7]. Rooks *et al.*^[8] reported 108 patients scheduled for total hip arthroplasty and total knee arthroplasty and showed improvements in preoperative and post-operative muscle strength and reduced need for inpatient rehabilitation. In a systemic review by Coudeyre *et al.*^[7], prehabilitation contributed to reduced hospitalization and improved discharge conditions in patients with total hip arthroplasty and total knee arthroplasty. A pilot randomized study including 30 elderly patients above 65 years old undergoing total hip arthroplasty showed that home based physical therapy was feasible to implement and it improved 6-min walk test^[9] as compared to standard care. Prehabilitation is also implemented in urology and colorectal surgery. Au *et al.*^[10] reported increased inpatient physical activity on post-operative day 1 in patients undergoing radical prostatectomy. A randomized controlled trial by Painter *et al.*^[11] included 167 patients

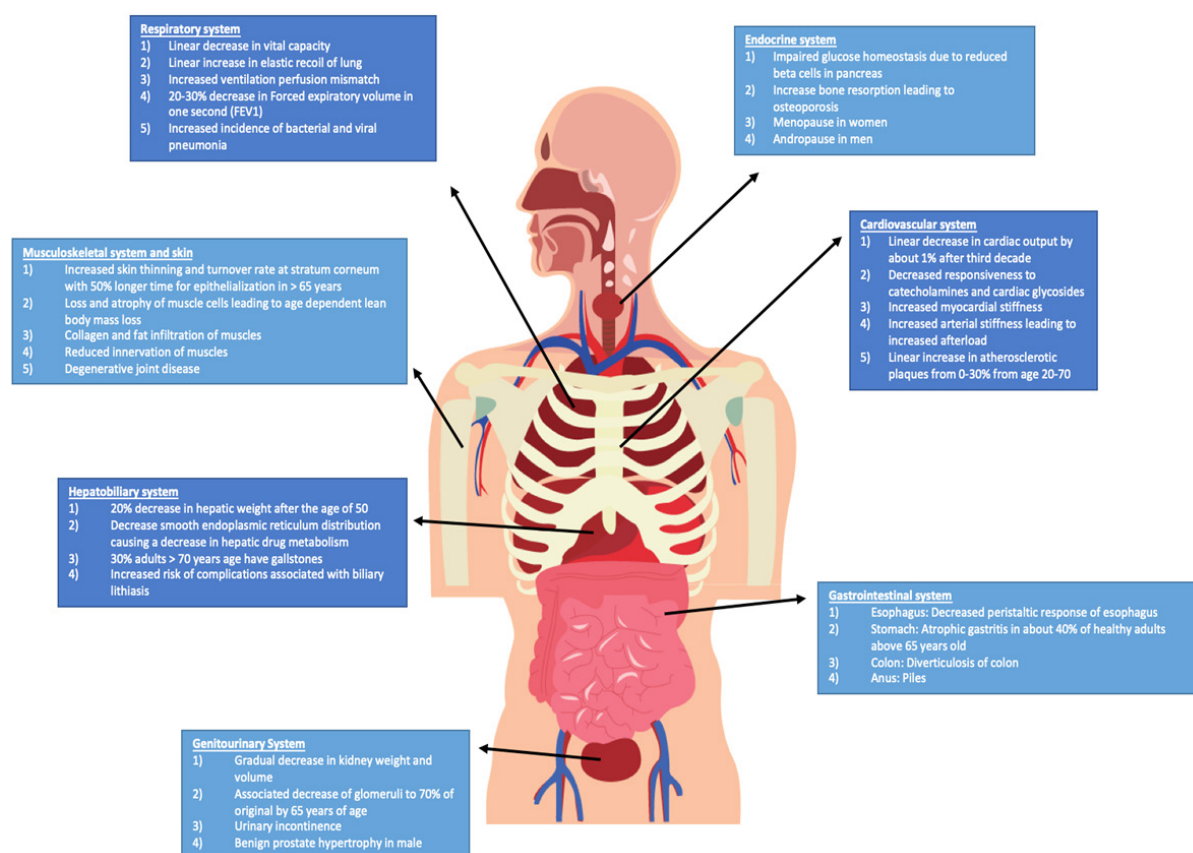


Figure 1. Age related physiological changes by system^[2]

undergoing renal transplant and showed improved graft function, aerobic fitness, quality of life and patient reported physical functionality. Similar reports were also found in reviews of patients undergoing renal transplant^[12,13]. Mayo *et al.*^[14] reported 95 patients with colorectal resections and showed increased functional outcomes such as in the 6-min walking test in 33% of patients. A recent meta-analysis by Gillis *et al.*^[15] also showed that prehabilitation could reduce post-operative admissions by two days in patients who underwent colorectal surgery. A systemic review by Cabilan *et al.*^[16], included 13 orthopedic, one colorectal, two cardiac and one foregut study. Prehabilitation did not demonstrate benefits in objective and self-reported function or reduction in inpatient rehabilitation admission. Some studies include cognitive behavioral therapy in addition to prehabilitation and this introduces heterogeneity^[17]. As such, more studies are needed to establish the role of prehabilitation and define patient selection criteria. Prehabilitation should be personalized based on various factors such as: (1) surgery: type, duration, expected blood loss, predicted morbidity and mortality; (2) patient factors: age, frailty, sarcopenia, co-morbidity, caregiver support; and (3) pathology: malignancy, burden of disease, malnutrition, cachexia, emotional impact.

PREHABILITATION IN HEPATO-PANCREATICO-BILIARY SURGERY

Hepato-pancreatico-biliary surgery is complex and many units have adopted prehabilitation. In a local study^[18] including 245 patients with liver resection the post-operative morbidity, 30-day mortality and 90-day mortality was 38.3%, 2.4% and 3.7% respectively. In a local study^[19] including 196 patients with pancreatic resection, the rate of grade B and C post-operative pancreatic fistula, 30-day mortality and 90-day mortality was 5.1%, 0.5% and 2%, respectively. Prehabilitation has potential to improve peri-operative outcomes. In a propensity-score matched study including 76 patients undergoing major hepato-pancreatico-biliary

surgery, Nakajima *et al.*^[20] reported improvements in serum albumin levels and reduction in hospital stay length but no difference in complication rates. Low serum albumin is associated with increased morbidity in elective and emergency procedures^[21]. In 40 patients undergoing pancreaticoduodenectomy, Ausania *et al.*^[22] reported improvement in delayed gastric emptying but not in post-operative complications such as post-operative pancreatic fistula. Most studies do not report significant negative outcomes even though some trials fail to demonstrate benefit.

Outcomes of success of prehabilitation program are multifaceted: (1) mortality/morbidity related outcomes: disease progression during prehabilitation, infectious morbidity during prehabilitation; (2) resource related outcomes: total cost of care, value driven outcomes; and (3) efficacy related outcomes: length of stay, admission to rehabilitation unit, morbidity/mortality post operatively, long term disease progression, acceptance of chemotherapy and adjunct therapy post operatively.

Most trials report clinical outcomes through careful patient selection and elimination of emergent cases and those in need of urgent intervention. Janssen *et al.*^[23] has reported single centre uncontrolled before and after study including 627 patients aged 70 years and older and undergoing elective abdominal surgery for colorectal carcinoma or aortic aneurysm. The prehabilitation group received interventions to improve patients' physical health, nutritional status, frailty and anaemia prior to surgery. With a mean prehabilitation of 39 days duration, they showed that the incidence of delirium was reduced significantly from 11.7% to 8.2% (OR = 0.56, 95%CI: 0.32-0.98, $P = 0.043$). Some studies such as that by Nielsen *et al.*^[24] report that even though intervention costs increase, overall costs reduce by about 15%; however, this remains to be validated for liver resection.

Prehabilitation has also shown improved outcomes in patients with liver resection. For most of these patients the effects of ageing are compounded by ongoing cirrhosis and oncological burden. A prospective study including 104 patients treated with elective liver resection showed reduced overall complication rates by up to 22.9% and median length of hospital stay by 2.5 days less^[25]. The authors also highlighted social benefits of prehabilitation. These included less social issues that may delay discharge, increased quality of life and reduced median cost by up to 16.5%^[25]. Though this study was not randomized and included small sample, it sets the precedent for positive outcomes of prehabilitation. Another trial by Dunne *et al.*^[26] on 35 randomized patients undergoing liver resection for colorectal liver metastases, reported significant preoperative score increase in both mental and physical aspects of the Short Form Health Survey 36. This supports the idea of both quality of life and physical fitness improvement due to prehabilitation. In a review by Tandon *et al.*^[27], elderly patients with cirrhosis have shown improvements in muscle mass, strength and functional capacity. They have also reported reduction in hepatic venous gradient and hepatic steatosis. These results were replicated in a review by Locklear *et al.*^[28] which showed such decreases in hepatic venous gradient and increased scores on 6-min walk tests for patients with end stage liver disease. Prehabilitation includes components of ERAS and more comprehensive preoperative strategy. ERAS has shown lower major complication rates and reduced cost in patients undergoing liver resection^[29]. A systemic review on ERAS application in liver surgery by Brustia *et al.*^[30] suggests positive outcomes such as reduced time taken for functional recovery by 2.5 days. Similar to ERAS, the American College of Surgeons have implemented a program called strong for surgery^[31], which is aimed at identifying and evaluating evidence-based practices to optimize the health of patients before surgery. It includes optimisation of nutrition, smoking cessation, pain control and prehabilitation. We have started ROSE program at Tan Tock Seng Hospital, Singapore to improve outcomes of elderly patients undergoing elective liver resection. In this paper we shall discuss our protocol of implementation of ROSE program.

ROSE

In Singapore, a population health strategy is designed to meet evolving healthcare needs for ageing society and "future ready". National Healthcare Group is the central cluster serving a population of 1.4 million

and embraces the concept of “River of Life”. This defines five segments of care - Living Well, Living with Illness, Crisis and Complex Care, Living with Frailty and Leaving Well - rooted by strong partnerships amongst care providers. Six population care streams feed into “River of Life” - Preventive Care, Primary Care, Hospital Care, Intermediate Care, Transitional and Community Care, End-of-Life and Long-Term Care. With the increasing burden of chronic diseases, primary care will take on an even bigger role in the community. Though there are reports of safe surgery with acceptable outcomes in elderly as compared to non-elderly^[32,33]; elderly do have additional needs to ensure comparable outcomes. Hence, additional resources have to be invested to achieve good outcomes. ROSE program was initiated in 2018 by a multidisciplinary group of healthcare professionals who recognized the need for our elderly patients to be optimized prior to major abdominal oncology surgery. It aims to identify elderly (> 65 years old) and frail patients and our discussion in this chapter is relevant to elective liver resection.

CURRENT IMPLEMENTATION OF ROSE

Target population

Not all elderly patients are the same and hence patient selection is essential to streamline the resources. One method of patient selection is by virtue of risk prediction. Various risk prediction models are reported in diverse pathologies and surgeries to enhance resource allocation^[18,34,35]. A prospective study on 162 patients undergoing hepatopancreaticobiliary surgery by van der Windt *et al.*^[36], showed that scoring systems such as the Risk Analysis Index, was able to accurately predict post-operative outcomes in patients. The study further elaborates on the possible use of such a scoring system to identify target groups for prehabilitation to optimise outcomes. Tan Tock Seng Hospital Nutrition Screening Tool is a locally developed tool which is validated against subjective global assessment in a cohort of elderly patients^[37]. In a local study including 281 acute admissions with age range of 61-102 years, Tan Tock Seng Hospital Nutrition Screening Tool predicted risk of malnutrition with high accuracy (area under the curve 0.87) and malnutrition predicted 6-month mortality (adjusted OR = 2.2; $P = 0.05$) and hospital length of stay^[37] ($P < 0.05$).

Currently ROSE program is piloted for patients who undergo liver, pancreas and colorectal surgeries. Initially, patients (≥ 65 years old) are screened for frailty and malnutrition. Those at risk will be enrolled into ROSE program [Figure 2].

Multidisciplinary team

Upon decision for liver resection, an assessment of frailty and nutrition is done by consultant surgeon. Patient who fulfil the criteria are then sent to allied healthcare members to be enrolled and seen as part of the ROSE program. Members of the program and their role are as follows (further details on workflow in Appendix 1).

Dietician

Nutritional counseling pre and post operatively: the role of the dietician has been studied in multimodal care teams such as in Poindessous *et al.*^[38] which demonstrates the benefit in a dietician functioning as an educator as well, with decreased recidivism and increased return to independence.

To obtain initial anthropometric and to conduct subjective global assessment: this is especially important in context of liver resection as malnutrition is very common in patients with cirrhosis^[39] and to add to this, precise evaluation of their nutrition status is difficult with the presence of ascites and edema^[40]. A review by Doherty *et al.*^[41] identifies intra hepatic fat as an independent risk factor for post-operative morbidity following hepatic resection. Dietary interventions such as calorie restriction, carbohydrate restriction or a Mediterranean diet have shown reductions in pre-operative intra hepatic fat by up to 55%^[40].

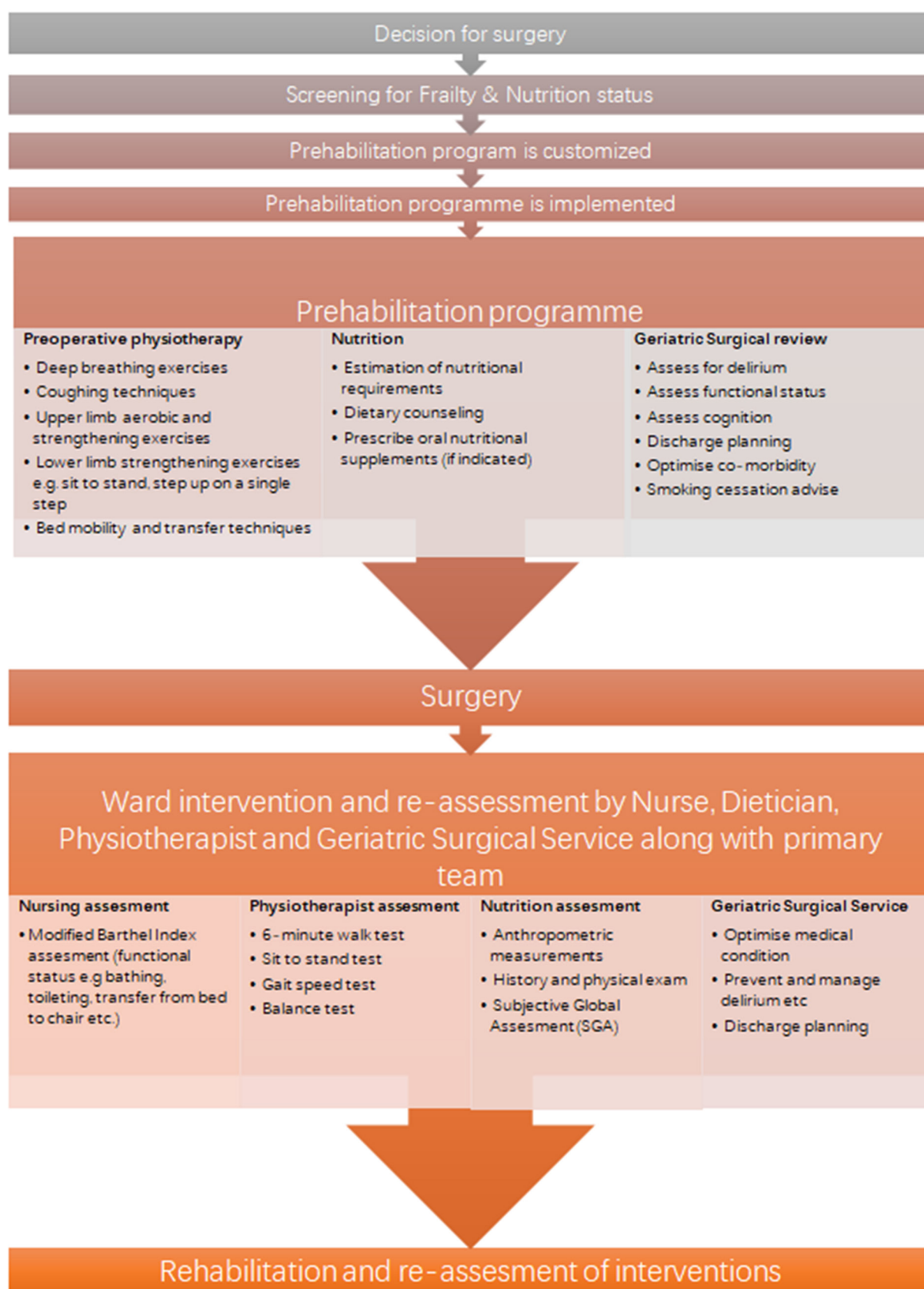


Figure 2. Recovery of surgery in elderly patient journey flowchart

Nutritional supplementation and monitoring pre and post-operatively: supplementation could include Branch chain amino acids for protein and glycogen synthesis promotion and for the regulation of immune system^[40].

Glutamine is a conditionally essential amino acid and is a fuel for neutrophils, lymphocytes and enterocytes^[42]. A Cochrane review^[43] reporting 4671 patients with critical illness or elective major surgery showed that glutamine supplementation reduced infection rates by 21% and days on mechanical ventilation by 0.69 days.

Physiotherapist

To assess objective and subjective markers of functional capacity pre and post-operatively: 6-min walk test, sit to stand test, Gait speed test and balance tests. Structured exercise program, either inpatient or outpatient is essential to prehabilitation^[44], these show improved outcomes such as in functional physical tests such as the 6MWT.

To conduct physiotherapy sessions pre operatively to increase functional capacity, respiratory function and mobility training in preparation for post-operative period: in a review by Guinan *et al.*^[45] on patients who underwent esophagectomies, it was reported that inspiratory muscle training conducted by physiotherapists may improve post-operative outcomes such as lowering hospitalization by 4.5 days and reducing the rate of high grade post-operative pulmonary complications.

To conduct physiotherapy as required to rehabilitate to baseline fitness post operatively.

Nurse

Modified Barthel Index assessment pre and post operatively: this is to ascertain patients pre and post-operative baselines and ensure return to function. In a study by Fortinsky *et al.*^[46] on 89 chronically ill patients, the Modified Barthel Index was able to reliably measure and track patient's ability to perform activities of daily living effectively. Furthermore, the Modified Barthel Index was easy to administer without much variability in reporting activities of daily living^[47].

Geriatric surgery service

Peri-operative medicine aims to provide high quality evidence based cross-specialty multidisciplinary care for surgical patients. Partridge *et al.*^[48] has reported a prospective randomized controlled study including 176 patients aged 65 years and older undergoing elective aortic aneurysm repair or lower-limb arterial surgery comparing standard preoperative assessment with preoperative comprehensive geriatric assessment and optimization. They reported lower incidence of delirium (11% vs. 24%, $P = 0.018$), cardiac complications (8% vs. 27%, $P = 0.001$) and bladder/bowel complications (33% vs. 55%, $P = 0.003$) in the intervention group. We have a geriatric surgical service capability with a consultant lead team and includes geriatric trained nursing staff. The team assists in managing diverse care needs upon referral: (1) optimize medical co-morbidity and assist with peri-operative care; (2) combined care provided by the geriatrician, anesthesiologist and surgeon is pivotal in the functioning of the prehabilitation program^[49].

Financial counselor

Finance is an important element of health care and often considered less important in context of public healthcare as treatment/intervention is "paid for" by the state in many high income countries. In Singapore, patient co-share the cost burden and hence it is imperative to appropriately counsel patients with estimated bill size and how does it vary based on different choices of therapy, e.g., open surgery vs. laparoscopic surgery, *etc.* A trained professional supplements the multidisciplinary team for this task and the role is: (1) to ensure financial security and if needed to counsel patients on financial aid programs pre operatively; (2)

financial counselors aim to allow patients to attain financial security through: inpatient bill estimates to appropriately plan, advice on available financial schemes, subsidies and aid, payment modalities available.

Anesthesiologist

To conduct pre admission anesthesia risk assessment for patient, these are done through pre-anesthetic clinics in which all pre-operative testing, specialist consultation, nursing and laboratory reviews are integrated into a one-stop clinic with minimal repeat visits. This has also been shown to reduce overall healthcare costs and manpower wastage due to cancellations^[50].

Case manager

To oversee patient journey and ensure seamless process through the ROSE program, e.g., same day appointment scheduling, *etc.*

In conclusion, age itself is not a risk factor for inferior peri-operative outcomes of elderly patient undergoing liver resection. Co-morbidities should be optimized prior to elective surgery. With an ageing society, prehabilitation has an important and integral role in surgical care. Structured programs involving multidisciplinary teams are essential to enhance peri-operative outcomes. To increase compliance and ensure direct oversight of implementation, resources are needed. Further research into the efficacy of specific prehabilitation regimes is needed to guide patient selection, define best practices and establish the value of such initiatives.

DECLARATIONS

Authors' contributions

Conceived of the presented idea: Shelat VG

Wrote the manuscript (under supervision from Shelat VG): Mohan R

Edited and provided critic to the manuscript: Shelat VG

Provided inputs and contributed to final manuscript: Mohan R, Huey CWT, Junnarkar S, Low JK

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Ahmed S, de Souza NN, Qiao W, Kasai M, Keem LJ, et al. Quality of life in hepatocellular carcinoma patients treated with transarterial chemoembolization. *HPB Surg* 2016;2016:6120143.

2. Boss GR, Seegmiller JE. Age-related physiological changes and their clinical significance. *West J Med* 1981;135:434-40.
3. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, et al.; European Working Group on Sarcopenia in Older People. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. *Age Ageing* 2010;39:412-23.
4. Otsuji H, Yokoyama Y, Ebata T, Igami T, Sugawara G, et al. Preoperative sarcopenia negatively impacts post-operative outcomes following major hepatectomy with extrahepatic bile duct resection. *World J Surg* 2015;39:1494-500.
5. Hayashi K, Yokoyama Y, Nakajima H, Nagino M, Inoue T, et al. Preoperative 6-minute walk distance accurately predicts post-operative complications after operations for hepato-pancreato-biliary cancer. *Surgery* 2017;161:525-32.
6. Morley JE. Frailty and sarcopenia in elderly. *Wien Klin Wochenschr* 2016;128:439-45.
7. Coudeyre E, Jardin C, Givron P, Ribinik P, Revel M, Rannou F. Could preoperative rehabilitation modify post-operative outcomes after total hip and knee arthroplasty? Elaboration of French clinical practice guidelines. *Annales de Réadaptation et de Médecine Physique* 2007;50:189-97.
8. Rooks DS, Huang J, Bierbaum BE, Bolus SA, Rubano J, et al. Effect of preoperative exercise on measures of functional status in men and women undergoing total hip and knee arthroplasty. *Arthritis Rheum* 2006;55:700-8.
9. Oosting E, Jans MP, Dronkers JJ, Naber RH, Dronkers-Landman CM, et al. Preoperative home-based physical therapy versus usual care to improve functional health of frail older adults scheduled for elective total hip arthroplasty: a pilot randomized controlled trial. *Arch Phys Med Rehabil* 2012;93:610-6.
10. Au D, Matthew AG, Lopez P, Hilton WJ, Awasthi R, et al. Prehabilitation and acute postoperative physical activity in patients undergoing radical prostatectomy: a secondary analysis from an RCT. *Sports Med Open* 2019;5:18.
11. Painter PL, Hector L, Ray K, Lynes L, Dibble S, et al. A randomized trial of exercise training after renal transplantation. *Transplantation* 2002;74:42-8.
12. Takahashi A, Hu SL, Bostom A. Physical activity in kidney transplant recipients: a review. *Am J Kidney Dis* 2018;72:433-43.
13. Romano G, Lorenzon E, Montanaro D. Effects of exercise in renal transplant recipients. *World J Transplant* 2012;2:46-50.
14. Mayo NE, Feldman L, Scott S, Zavorsky G, Kim DJ, et al. Impact of preoperative change in physical function on postoperative recovery: argument supporting prehabilitation for colorectal surgery. *Surgery* 2011;150:505-14.
15. Gillis C, Buhler K, Bresee L, Carli F, Gramlich L, et al. Effects of nutritional prehabilitation, with and without exercise, on outcomes of patients who undergo colorectal surgery: a systematic review and meta-analysis. *Gastroenterology* 2018;155:391-410.e4.
16. Cabilan CJ, Hines S, Munday J. The effectiveness of prehabilitation or preoperative exercise for surgical patients: a systematic review. *JBIC Database System Rev Implement Rep* 2015;13:146-87.
17. Rolving N, Nielsen CV, Christensen FB, Holm R, Bünger CE, et al. Does a preoperative cognitive-behavioral intervention affect disability, pain behavior, pain, and return to work the first year after lumbar spinal fusion surgery? *Spine* 2015;40:593-600.
18. Madhavan S, Shelat VG, Soong SL, Woon WWL, Huey T, et al. Predicting morbidity of liver resection. *Langenbecks Arch Surg* 2018;403:359-69.
19. Tang EL, Shelat VG, Junnarkar SP, Huey CWT, Low JK, et al. Predictive value of post-operative drain amylase levels for post-operative pancreatic fistula. *Ann Hepatobiliary Pancreat Surg* 2018;22:397-404.
20. Nakajima H, Yokoyama Y, Inoue T, Nagaya M, Mizuno Y, et al. Clinical benefit of preoperative exercise and nutritional therapy for patients undergoing hepato-pancreato-biliary surgeries for malignancy. *Ann Surg Oncol* 2019;26:264-72.
21. Seow JG, Lim YR, Shelat VG. Low serum albumin may predict the need for gastric resection in patients with perforated peptic ulcer. *Eur J Trauma Emerg Surg* 2017;43:293-8.
22. Ausania F, Senra P, Meléndez R, Caballeiro R, Ouviña R, et al. Prehabilitation in patients undergoing pancreaticoduodenectomy: a randomized controlled trial. *Rev Esp Enferm Dig* 2019;111:603-8.
23. Janssen TL, Steyerberg EW, Langenberg JCM, de Lepper CCHAVH, Wielders D, et al. Multimodal prehabilitation to reduce the incidence of delirium and other adverse events in elderly patients undergoing elective major abdominal surgery: an uncontrolled before-and-after study. *PLoS One* 2019;14:e0218152.
24. Nielsen PR, Jørgensen LD, Dahl B, Pedersen T, Tønnesen H. Prehabilitation and early rehabilitation after spinal surgery: randomized clinical trial. *Clin Rehabil* 2010;24:137-48.
25. Wang B, Shelat VG, Chow JLL, Huey TCW, Low JK, et al. Prehabilitation program improves outcomes of patients undergoing elective liver resection. *J Surg Res* 2020;251:119-25.
26. Dunne DF, Jack S, Jones RP, Jones L, Lythgoe DT, et al. Randomized clinical trial of prehabilitation before planned liver resection. *Br J Surg* 2016;103:504-12.
27. Tandon P, Ismond KP, Riess K, Duarte-Rojo A, Al-Judaibi B, et al. Exercise in cirrhosis: Translating evidence and experience to practice. *J Hepatol* 2018;69:1164-77.
28. Locklear CT, Golabi P, Gerber L, Younossi ZM. Exercise as an intervention for patients with end-stage liver disease: systematic review. *Medicine (Baltimore)* 2018;97:e12774.
29. Wimble K, Jones C. Enhanced recovery after surgery for liver resection surgery. *Dig Med Res* 2019;2. Available from: <http://dmr.amegroups.com/article/view/5386> [Last accessed on 20 Mar 2020]
30. Brustia R, Slim K, Scatton O. Enhanced recovery after liver surgery. *J Visc Surg* 2019;156:127-37.
31. Strong for Surgery. Available from: <https://www.facs.org/quality-programs/strong-for-surgery> [Last accessed on 20 Mar 2020]
32. Shelat VG, Chia VJ, Low J. Common bile duct exploration in an elderly Asian population. *Int Surg* 2015;100:261-7.
33. Martínez-Cecilia D, Cipriani F, Shelat V, Ratti F, Tranchart H, et al. Laparoscopic versus open liver resection for colorectal metastases

- in elderly and octogenarian patients: a multicenter propensity score based analysis of short- and long-term outcomes. *Ann Surg* 2017;265:1192-200.
34. Anbalakan K, Chua D, Pandya GJ, Shelat VG. Five year experience in management of perforated peptic ulcer and validation of common mortality risk prediction models - are existing models sufficient? A retrospective cohort study. *Int J Surg* 2015;14:38-44.
 35. Kiat TTJ, Gunasekaran SK, Junnarkar SP, Low JK, Woon W, et al. Are traditional scoring systems for severity stratification of acute pancreatitis sufficient? *Ann Hepatobiliary Pancreat Surg* 2018;22:105-15.
 36. van der Windt DJ, Bou-Samra P, Dadashzadeh ER, Chen X, Varley PR, et al. Preoperative risk analysis index for frailty predicts short-term outcomes after hepatopancreatobiliary surgery. *HPB (Oxford)* 2018;20:1181-8.
 37. Lim YP, Lim WS, Tan TL, Daniels L. Evaluating the validity of a nutritional screening tool in hospitalized older adults. *Ann Acad of Med Singapore* 2008;37:S5.
 38. Poindessous JL, Basta M, Renaud A, Vincent M, Héron A. Infirmière et diététicienne: une collaboration efficace en service de rééducation [Nurse and dietitian: effective collaboration in rehabilitation]. *Rev Infirm* 2018;67:39-41. (in French)
 39. Anastácio LR, Davisson Correia MI. Nutrition therapy: integral part of liver transplant care. *World J Gastroenterol* 2016;22:1513-22.
 40. Masuda T, Shirabe K, Yoshiya S, Matono R, Morita K, et al. Nutrition support and infections associated with hepatic resection and liver transplantation in patients with chronic liver disease. *JPEN J Parenter Enteral Nutr* 2013;37:318-26.
 41. Doherty DT, Coe PO, Rimmer L, Lapsia S, Krige A, et al. Hepatic steatosis in patients undergoing resection of colorectal liver metastases: a target for prehabilitation? A narrative review. *Surg Oncol* 2019;30:147-58.
 42. Shelat VG, Pandya GJ. Nutritional Support in dysphagia. Available from: <https://www.intechopen.com/books/seminars-in-dysphagia/nutritional-support-in-dysphagia> [Last accessed on 20 Mar 2020]
 43. Tao KM, Li XQ, Yang LQ, Yu WF, Lu ZJ, et al. Glutamine supplementation for critically ill adults. *Cochrane Database Syst Rev* 2014;2014:CD010050.
 44. Carli F, Scheede-Bergdahl C. Prehabilitation to enhance peri-operative care. *Anesthesiol Clin* 2015;33:17-33.
 45. Guinan EM, Dowds J, Donohoe C, Reynolds JV, Hussey J. The physiotherapist and the esophageal cancer patient: from prehabilitation to rehabilitation. *Dis Esophagus* 2017;30:1-12.
 46. Fortinsky RH, Granger CV, Seltzer GB. The use of functional assessment in understanding home care needs. *Med Care* 1981;19:489-97.
 47. Collin C, Wade DT, Davies S, Horne V. The Barthel ADL Index: a reliability study. *Int Disabil Stud* 1988;10:61-3.
 48. Partridge JS, Harari D, Martin FC, Peacock JL, Bell R, et al. Randomized clinical trial of comprehensive geriatric assessment and optimization in vascular surgery. *Br J Surg* 2017;104:679-87.
 49. Carli F, Ferreira V. Prehabilitation: a new area of integration between geriatricians, anesthesiologists, and exercise therapists. *Aging Clin Exp Res* 2018;30:241-4.
 50. Gupta A, Gupta N. Setting up and functioning of a preanaesthetic clinic. *Indian J Anaesth* 2010;54:504-7.

Review

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Ion channels in liver diseases and hepatocellular carcinoma: potential tools for diagnosis, prognosis, and therapy

María de Guadalupe Chávez-López¹, Andrea Cruz-Díaz², Karla Nicol Tlapalcoyoa-Apanco², Julio Isael Pérez-Carreón³, Javier Camacho¹

¹Department of Pharmacology, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City 07360, Mexico.

²Escuela de Ciencias de la Salud, Universidad del Valle de México, Mexico City 04910, Mexico.

³Instituto Nacional de Medicina Genómica, Mexico City 14610, Mexico.

Correspondence to: Dr. Javier Camacho, Department of Pharmacology, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Avenida Instituto Politécnico Nacional 2508, Mexico City 07360, Mexico.
E-mail: fcamacho@cinvestav.mx

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Abstract

Cancer is a major cause of death worldwide. Hepatocellular carcinoma (HCC) is one of the malignancies with the highest mortality-to-incidence ratio (> 0.9), and in some countries this value is up to 1. Unfortunately, many patients are diagnosed at advanced stages of the disease. Therefore, HCC early markers, as well as novel therapeutic approaches, are urgently needed. HCC is the main type of liver cancer and it is associated with different factors including alcohol use, viral infections, and fatty liver disease. A significant percentage of HCC patients previously had liver cirrhosis. Several ion channels have been proposed as novel potential markers and therapeutic targets for diverse cancers including HCC. Here, we review most of the findings associating ion channel expression with HCC and its etiological factors, as well as some possible pro-tumorigenic mechanisms of action for ion channels in HCC. Novel therapies for HCC treatment and prevention are also discussed. Ion channel targeting offers a plethora of opportunities for HCC prevention, early diagnosis, and therapy that may help to reduce the extremely high mortality-to-incidence ratio of this malignancy.

Keywords: Ion channels, hepatocellular carcinoma, hepatitis virus, cirrhosis, liver disease, alcohol



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INTRODUCTION

Cancer is a leading cause of death worldwide, despite the existence of hundreds of clinical trials testing novel therapies^[1,2]. There are several types of liver cancer including hepatoblastoma, cholangiocarcinoma, and angiosarcoma, but hepatocellular carcinoma (HCC) accounts for up to 90% of primary liver cancers^[3-7]. Liver cancer is one of the malignancies with the worst prognosis, representing the second leading cause of cancer-related deaths in the world^[3-5].

The liver plays a central role in regulating whole-body carbohydrate, lipid, and protein homeostasis, as well as playing additional very important physiological roles including the synthesis and transport of bile acids and the detoxification of endogenous and exogenous metabolites^[6,8]. This very important organ is exposed to several factors including infections by hepatitis viruses B and C, alcohol use, aflatoxin B1, and fatty diet. Several of these factors lead to liver cirrhosis, which is the major HCC-associated risk factor^[6,9-11]. In fact, a significant percentage (> 80%) of HCC patients previously had liver cirrhosis. Unfortunately, HCC is rarely detected at early stages, and is usually fatal within a few months of diagnosis. The percentage of mortality-to incidence ratio of liver cancer is very high; it is more than 90% globally, reaching up to 100% in some countries^[1]. Therefore, novel early HCC markers and therapeutic strategies are urgently needed. In this regard, ion channels have gained great interest in oncology, as novel tools for both diagnosis and treatment^[5]. Here, we summarize most of the research associating ion channels with HCC. We also discuss the potential tumorigenic mechanisms of action of ion channels in HCC, as well as ion channel-based therapies for HCC prevention and treatment. The growing research field of ion channels in cancer may lead to reduce the incidence and mortality of liver cancer.

ION CHANNELS AND CANCER

Ionic channels are pore-forming membrane proteins allowing ion flux across membranes, including the plasma membrane and those from of intracellular organelles. In most cases, these proteins selectively transport specific ions and the vast majority need special stimulus to be activated^[12]. These gating stimuli include changes in membrane potential (voltage-gated ion channels), different ligands such as hormones or neurotransmitters, temperature, mechanical forces, *etc.* Thus, the role of ion channels in human physiology comprises very important phenomena such as neural transmission, cardiac function, hormone release, sensory physiology, *etc.* Accordingly, many channelopathies exist including epilepsy, cardiac arrhythmias, renal diseases, blindness, skeletal muscle disorders, *etc.*^[12]. Cancer is a multi-factorial disease characterized by an increased cell proliferation rate; ion channels are essential for regulation of proliferation and are also involved in many relevant processes occurring during carcinogenesis, which convert these proteins into potential cancer diagnostic tools and therapeutic targets.

Ion channels associated with cancer

The roles that ion channels have during carcinogenesis depend on the step of tumor development and the tissue type^[13,14].

Calcium channels participate in pivotal functions in the body such as regulation of blood pressure, muscle contraction, secretion, metabolism, excitability, and cell proliferation^[15]. These channels are important in the cell cycle, especially to enter and accomplish the S and M phase^[14-18]; thus, their participation in cancer cell proliferation is very relevant^[14,16,17]. In addition, because these ions are very important for cell migration, they also play a very important role in cancer cell migration and metastasis^[16-19].

Potassium channels play crucial roles in every cell type and in all species. Based on their structure and function, they are categorized into three major classes: the voltage-gated (Kv), inwardly rectifying (Kir), and tandem pore domain (K2P) channels. Furthermore, various messengers can stimulate the ligand-

gated (Kligand) channels^[20]. Membrane hyperpolarization due to potassium channel activity is needed for cell cycle progression from G1 to S phase. Potassium flux is also very important for apoptosis, cell volume regulation, and cytokine release. Therefore, even though the precise molecular mechanism of K⁺ channel participation in cancer remains elusive, these channels have a significant role in the cell proliferation, migration, and angiogenesis of a variety of carcinoma cells^[14,21,22].

Different subtypes of voltage-gated sodium channels (VGSC) are differentially expressed throughout the body, and they have essential roles in the generation and propagation of action potentials in electrically excitable cells such as neurons and cardiac and skeletal muscle^[23]. Several carcinoma cells express VGSC^[14,24,25]. Interestingly, these channels are active in metastatic cells^[25]. Accordingly, sodium currents through VGSC enhance migration, invasion, and metastasis *in vivo*^[26].

Chloride channels are involved in many biological functions such as epithelial fluid secretion, cell-volume regulation, modulation of excitability, smooth-muscle relaxation, and pH regulation. Cystic fibrosis is a disease where the relevance of alterations in chloride flux has been shown^[27]. Cl⁻ channels are involved in apoptosis, and in cancer cells these proteins promote proliferation, migration, and invasion^[14,21,28-30]. These channels are over-expressed in many cancer tissues including liver compared to noncancerous tissues, and are significantly associated with tumor size, metastasis, and poor prognosis^[31].

Before going into the details of ion channels in liver diseases leading to HCC, we first review some of the channels for which expression has been reported in the normal liver.

ION CHANNELS IN HEALTHY LIVER

The importance of ion channels in different functions of the normal healthy liver has been reported by several studies. Water crosses the plasma membrane either directly through the lipid bilayer or via protein water channels [aquaporins (AQPs)]^[32]. The liver expresses at least six AQPs (AQP1, -3, -7, -8, -9, and -11). Immunohistochemical studies showed the expression of AQPs in different hepatic cell types including cholangiocytes (AQP1 and -7), endothelial cells (AQP1), Kupffer cells (AQP3), and hepatocytes, (AQP7, -8, and -9)^[33,34]. AQP8 and -9 are relevant for bile synthesis regulation, secretion, and modification^[33]. Additionally, AQP9 functions as a glycerol channel in the liver^[35].

The ATP-sensitive potassium channel (K_{ATP}) is composed of two types of subunits, namely an inwardly rectifying K⁺ channel (Kir6.x) and a sulfonylurea receptor. Kir6.x subunits form the pore, while sulfonylurea receptor subunits have regulatory activity. Depending on its localization at the plasma membrane or in organelles, these channels are classified as sarcolemmal ("sarcoK_{ATP}"), mitochondrial ("mitoK_{ATP}"), or nuclear ("nucK_{ATP}") channels^[36,37]. Interestingly, K_{ATP} channel opening has been shown to alleviate liver injury by preventing inflammation and increasing the liver tolerance to ischemia/reperfusion injury^[38,39]. Besides, DNA synthesis demonstrated that these channels play significant roles in liver growth control^[37].

Nucleotides act as extracellular signaling molecules via purinergic receptors. These receptors are separated into seven P2X ionotropic receptors and eight P2Y G protein-coupled receptors^[40,41]. For instance, the P2X4 receptor is the dominant P2X isoform expressed in cholangiocytes in the liver^[42,43]. ATP is released by hepatocytes, and it regulates hepatocyte glycogen metabolism, cell volume, bile formation, and other cell functions. When activated by ATP, P2X receptors function as cation-permeable channels that allow the influx of sodium and calcium ions^[42,43]. Interestingly the expression of P2X7 receptors is decreased in HCC Huh-7 cells^[43].

Acid sensing ion channels (ASICs) are H⁺ channels that mediate tumor cell migration and invasion^[44], and store-operated calcium entry (SOCE) controls HCC cell proliferation and migration^[45]. T-type Ca²⁺

channels participate in modulating the proliferation of some HCC cells^[46]. Because the expression levels of these channels (ASICs, SOCE, and T-type)^[44,45] are increased in HCC in comparison with the normal liver, they may be used as markers of the disease.

In the following, we describe several findings associating ion channels with HCC, beginning with some liver diseases representing important HCC etiological factors.

ION CHANNELS IN LIVER DISEASES

Several liver diseases have been identified as HCC etiological factors, and many ion channels have been found to have a role in liver diseases. Table 1 summarizes the ion channel expression changes for the most common liver diseases.

Viral hepatitis and ion channels

It is estimated that 350 million people are chronic hepatitis B virus (HBV) carriers in the world, and that up to 30% of them develop progressive chronic liver disease appearing as hepatitis, fibrosis, cirrhosis, and HCC^[47]. HBV infection produces chronic necro-inflammation with subsequent fibrosis and hepatocyte proliferation. One of the viral factors potentially involved in HBV-related hepatocarcinogenesis is the HBx protein, which promotes cell cycle progression and inactivates negative growth regulators. This protein also binds to and inhibits the expression of *p53*, as well as other tumor suppressor genes and senescence-related factors^[3,48-50]. The HBx protein regulates calcium signaling through the activation of store-operated calcium channels (SOCs), which stimulate HBV replication^[51,52]. In addition, HBx can activate SOCs by binding C-terminal of Orai protein channels^[53]. Interestingly, co-immunoprecipitation experiments and pull-down assays demonstrated the interaction between HBx and the Orai1 protein; the C-terminus of the Orai1 protein was involved in such interaction. The authors concluded that the HBx protein binds to the STIM1-Orai1 complexes regulating the activity of SOCs^[53]. In this same direction, the HBV PreS2-mutant large surface antigen activates store-operated calcium entry and promotes chromosome instability^[54].

On the other hand, miR-125b inhibits HBV expression *in vitro* by targeting the sodium channel *SCNN1A* gene^[55]. It has also been observed that P2X7 function is necessary for the infection of human hepatocytes by HBV. Because P2X7 activation is a major component of inflammatory responses, HBV may contribute to liver inflammation^[56].

In the case of hepatitis C virus (HCV) infections, it is estimated that 130 million people have chronic HCV infection and most of them develop chronic liver disease^[47]. Continuous inflammation and hepatocyte regeneration in the setting of chronic hepatitis and subsequent progression to cirrhosis are thought to lead to chromosomal damage, and possibly to initiate hepatic carcinogenesis. HCV also induces steatosis; oxidative stress causes steatohepatitis and these pathways lead to liver injury or HCC in chronic HCV infection^[3,57,58]. Interestingly, the HCV p7 protein forms a cation channel *in vitro*^[59-61], and p7 deletions and point mutations markedly reduce the production of infectious virions in cell culture^[61-63]. p7 is a proton channel required for the production of infectious virions^[64]. There are some small molecules that block the p7 channel function and virion production in culture, rendering it an attractive antiviral target^[59,65-71].

P2X4 receptors expression form part of the purinergic signaling complex in HCV-induced liver pathogenesis^[72,73]. Additionally, the modulation of the gamma-aminobutyric acid type A (GABA-A) receptor activity was observed in several chronic hepatitis failures, including hepatitis C. Increased expression of GABA-A $\alpha 1$ receptor subunit, and decreased expression of GABA-A $\beta 3$ subunit have been found in chronic hepatitis C patients. Thus, the expression of GABA-A receptor subunits may be associated with either current or previous HCV infection^[74].

Table 1. Ion channel expression in major liver diseases

Liver disease	Channel/Transporter	Gene symbol	Developed name	Transported ion(s)	Genomic mapping (chromosome in homo sapiens)	Expression change (compared to normal tissue)	Ref.
Viral hepatitis	p7	--	Hepatitis C virus p7 protein	Ca ²⁺	--	Overexpression	[59-61]
	SCNN1A	<i>SCNN1A</i>	Sodium channel non-voltage-gated 1 alpha	Na ⁺	12	Overexpression	[55]
	P2X7	<i>P2RX7</i>	Purinergic receptor P2X, ligand-gated ion channel 7	Na ⁺ , Ca ²⁺	12	Overexpression	[56]
	P2X4	<i>P2RX4</i>	Purinergic receptor P2X, ligand-gated ion channel 4	Na ⁺ , Ca ²⁺	12	Overexpression	[72,73]
	SOCs	<i>ORAI1</i>	Calcium release- activated calcium modulator	Ca ²⁺	12	Overexpression	[51-54]
NAFLD	GABA A α 1	<i>GABRA1</i>	Gamma-aminobutyric acid type A receptor alpha 1 subunit	Cl ⁻	5	Overexpression	[74]
	K _{Ca3.1}	<i>KCNN4</i>	Calcium-activated potassium channel subfamily N member 4	K ⁺	19	Overexpression	[79,80]
	P2X7	<i>P2RX7</i>	Purinergic receptor P2X, ligand-gated ion channel 7	Na ⁺ , Ca ²⁺	12	Overexpression	[81-83]
	SOCs	--	Store-operated calcium channels	Ca ²⁺	NS	Overexpression	[8,84]
	TPC2	<i>TPCN2</i>	Two-pore segment channel 2	Ca ²⁺	11	Overexpression	[21,85]
Fibrosis	TRPV1	<i>TRPV1</i>	Transient receptor potential cation channel subfamily V member 1	Non selective cation	17	Overexpression	[86,87]
	TRPV4	<i>TRPV4</i>	Transient receptor potential cation channel subfamily V member 4	Non-selective cation	12	Overexpression	[88]
	TRPC6	<i>TRPC6</i>	Transient receptor potential cation channel subfamily C member 6	Ca ²⁺	11	Overexpression	[89]
	TRPM7	<i>TRPM7</i>	Transient receptor potential cation channel subfamily M member 7	Ca ²⁺ , Mg ²⁺	15	Overexpression	[90]
	ASIC1a	<i>ASIC1</i>	Acid sensing ion channel subunit 1	Na ⁺	12	Overexpression	[91]
Cirrhosis	TRPV2	<i>TRPV2</i>	Transient receptor potential cation channel subfamily V member 2	Non-selective cation	17	Overexpression	[95]
	TRPC6	<i>TRPC6</i>	Transient receptor potential cation channel subfamily C member 6	Ca ²⁺	11	Overexpression	[96]
	Nav _{1.2}	<i>SCN2A</i>	Voltage-gated sodium channel alpha subunit 2	Na ⁺	2	Overexpression	[96]
	K _{Ca3.1}	<i>KCNN4</i>	Calcium-activated potassium channel subfamily N member 4	K ⁺	19	Overexpression	[96]
	ABCC3	<i>ABCC3</i>	ATP binding cassette subfamily C member 3	--	17	Overexpression	[96]
HCC	ITPRs	<i>ITPR</i>	Inositol 1,4,5-trisphosphate receptor	Ca ²⁺	NS	Overexpression	[97]
	AQP1	<i>AQP1</i>	Aquaporin 1	water channel	7	Overexpression	[98-100]
	K _{Ca1.1} (BK)	<i>KCNMA1</i>	Calcium-activated potassium channel subfamily M alpha 1	K ⁺	10	Overexpression	[21,101]
	NCC	<i>SLC12A3</i>	Solute carrier family 12 member 3	Na ⁺ , Cl ⁻	16	Overexpression	[102]
	K _{Ca3.1}	<i>KCNN4</i>	Calcium-activated potassium channel subfamily N member 4	K ⁺	19	Overexpression	[103,104]
HCC	KCNQ1	<i>KCNQ1</i>	Voltage-gated potassium channel subfamily Q member 1	K ⁺	11	Downregulation	[105]
	KCNJ11	<i>KCNJ11</i>	Inwardly rectifying potassium channel subfamily J member 1	K ⁺	11	Overexpression	[106]
	K _{ATP} channels	--	ATP-sensitive potassium channels	K ⁺	NS	Overexpression	[37]
	Eag1	<i>KCNH1</i>	Voltage-gated potassium channel subfamily H member 1	K ⁺	1	Overexpression	[115]

T-type Ca ²⁺ channels	<i>CACNA1G</i> <i>CACNA1H</i> <i>CACNA1I</i>	Voltage-gated calcium channels	Ca ²⁺	17 16 22	Overexpression	[117]
P2X3	<i>P2RX3</i>	Purinergic receptor P2X, ligand gated ion channel 3	Na ⁺ , Ca ²⁺	11	Overexpression	[119]
SOCs	<i>ORA1I</i>	Store-operated calcium channels	Ca ²⁺	12	Overexpression	[120,121]
CLIC-3	<i>CLCN3</i>	Voltage-gated chloride channel 3	Cl ⁻	4	Overexpression	[122]
CLIC1	<i>CLIC1</i>	Chloride intracellular channel 1	Cl ⁻	6	Overexpression	[123]
VGSCβ1	<i>SCN1B</i>	Voltage-gated sodium channel beta subunit 1	Na ⁺	19	Downregulation	[124]
Nav _{1.2}	<i>SCN2A</i>	Voltage-gated sodium channel alpha subunit 2	Na ⁺	2	Overexpression	[96]
AQP5	<i>AQP5</i>	Aquaporin 5	water channel	12	Overexpression	[125,126]
AQP9	<i>AQP9</i>	Aquaporin 9	water channel	15	Downregulation	[127,128]
TRPC6	<i>TRPC6</i>	Transient receptor potential cation channel subfamily C member 6	Ca ²⁺	11	Overexpression	[129-131]
TRPC1	<i>TRPC1</i>	Transient receptor potential cation channel subfamily C member 1	Non-selective cation	3	Overexpression	[132,133]
TRPV1	<i>TRPV1</i>	Transient receptor potential cation channel subfamily V member 1	Non-selective cation	17	Downregulation	[134,135]
TRPV2	<i>TRPV2</i>	Transient receptor potential cation channel subfamily V member 2	Non-selective cation	17	Overexpression	[136]
TRPV4	<i>TRPV4</i>	Transient receptor potential cation channel subfamily V member 4	Non-selective cation	12	Overexpression	[137]
TRPM7	<i>TRPM7</i>	Transient receptor potential cation channel subfamily M member 7	Ca ²⁺ , Mg ²⁺	15	Overexpression	[138]
ASIC1a	<i>ASIC1</i>	Acid sensing ion channel subunit 1	Na ⁺	12	Overexpression	[139]
ITPR3	<i>ITPR3</i>	Inositol 1,4,5-trisphosphate receptor type 3	Ca ²⁺	6	Overexpression	[141]

NS: no specific channel indicated in the original source; HCC: hepatocellular carcinoma; NAFLD: nonalcoholic fatty liver disease; NCC: NaCl cotransporter

Ion channels in nonalcoholic fatty liver disease and liver fibrosis

Nonalcoholic fatty liver disease (NAFLD) defines liver abnormalities ranging from simple steatosis (abnormal hepatic fat accumulation) or nonalcoholic fatty liver to nonalcoholic steatohepatitis (NASH) that have been identified as a cause of fibrosis, cirrhosis, and HCC. It is closely related to obesity and metabolic syndrome. The precise mechanism of HCC development from NAFLD has not yet been fully elucidated^[3,75-77].

K_{Ca3.1} potassium channels are expressed in non-excitabile tissues such as epithelia affecting proliferation, migration, and vascular resistance, and play an important role in the modulation of Ca²⁺ signaling^[78]. In liver disease, the K_{Ca3.1} channel inhibitor TRAM-34 downregulates fibrosis-associated gene expression and reduces portal perfusion pressure^[79]. It has also been found that the K_{Ca3.1} channel inhibitor senicapoc mitigates both steatosis and fibrosis in non-alcoholic liver disease models^[80]. P2X7 deficiency^[81] or blockage attenuates nonalcoholic steatohepatitis^[82] and liver fibrosis^[83].

Intracellular Ca²⁺ homeostasis is altered in steatotic hepatocytes. Decreased Ca²⁺ concentration in the endoplasmic reticulum may lead to endoplasmic reticulum stress, which has been identified as an important mediator of the progression from liver steatosis to nonalcoholic steatohepatitis, type 2 diabetes, and HCC. SOC are responsible for proper Ca²⁺ maintenance in the hepatocyte endoplasmic reticulum

lumen. Accordingly, SOCE is substantially inhibited in steatotic hepatocytes. This inhibition enhances lipid accumulation by positive feedback and may contribute to the development of NASH and insulin resistance^[8,84]. The antidiabetic drug exendin-4 reverses the lipid-induced inhibition of SOCE and decreases liver lipid with rapid onset^[8].

Two-pore channels (TPCs) are cation-selective intracellular ion channels, and their activation mediates calcium release from lysosomal stores. TPC2-deficient mice show hepatic cholesterol accumulation, hyperlipoproteinemia, and finally NASH^[21,85]. Interestingly, the activation of transient receptor potential type vanilloid 1 (TRPV1) by capsaicin prevents nonalcoholic fatty liver disease^[86,87]. Additionally, the TRPV4, TRPC6, TRPM7, and acid-sensing ion channels (ASIC1a) have been suggested as liver fibrosis mediators. The blockage of these channels inhibits hepatic fibrosis, positioning them as promising therapeutic targets^[88-91].

Liver cirrhosis and ion channels

Liver cirrhosis from any cause is the most important clinical risk factor for HCC with an annual incidence between 2% and 4%. The transition from chronic liver disease to cirrhosis involves inflammation and activation of hepatic stellate cells with ensuing fibrogenesis and angiogenesis. Liver cirrhosis is characterized by diffuse regenerative nodule of hepatocytes surrounded by dense fibrotic septa with subsequent parenchymal extinction and liver structure collapse. Over time, compensated cirrhosis may progress to decompensated cirrhosis that results in liver failure and death^[3,92-94].

As stated above, TRPV4, TRPC6, TRPM7, and ASIC1a channels could act as liver fibrosis mediators. Fibrosis is the prelude to cirrhosis, thus these channels might somehow also modulate cirrhosis. Other studies have also observed over-expression of TRPV2^[95], TRPC6, Nav1.2, and $K_{Ca3.1}$ channels as well as the Abcc3 transporter^[96] in liver cirrhosis, suggesting them as potential markers of the disease. Ca^{2+} signals mediate the hepatic effects of numerous hormones and growth factors. Liver Ca^{2+} signals are elicited by the intracellular Ca^{2+} channel inositol trisphosphate receptor (ITPRs). Three isoforms of this receptor have been identified, and cirrhosis affects the isoform expression^[97].

Some reports have shown an overexpression of AQP1 in liver cirrhosis^[98]; this protein contributes to microvascular resistance in cirrhosis^[99]. It has been also proposed that AQP1 polymorphism may be involved in the genetic susceptibility to develop water retention in patients with liver cirrhosis^[100]. The large conductance $K_{Ca1.1}$ K^+ channels (BK) are activated by membrane depolarization and/or elevations in intracellular Ca^{2+} concentration. Cirrhotic livers display increased activity of BK channels; accordingly, blockage of these channels increased the baseline portal perfusion pressure in cirrhotic livers^[21,101]. Liver cirrhosis is associated with enhanced renal tubular sodium retention, but the mechanism involved is unknown. Interestingly, liver cirrhosis is associated with increased renal abundance of the NaCl cotransporter^[102]. Then, diverse ion channels may serve as potential markers and drug targets for several liver diseases leading to HCC; if so, these proteins could be used as targets for HCC prevention.

Ion channels in hepatocellular carcinoma

Because the above-mentioned liver diseases may lead to HCC, and because cancer is a multi-factorial disease, a significant amount of ion channels have been studied as potential markers and therapeutic targets of this very poor prognosis malignancy.

Potassium channels play an important role in a variety of carcinoma cells. $K_{Ca3.1}$ channels are over-expressed in HCC and the channel blockade with TRAM-34 inhibits HCC cell proliferation in a time- and dose-dependent manner^[103,104]. A recent work showed that KCNQ1 was frequently downregulated in HCC cell lines and HCC tissues, and that HCC patients with low KCNQ1 expression had poor prognosis. Gain-of-

function studies showed that KCNQ1 exhibited remarkable inhibitory roles on tumor metastasis *in vitro* and *in vivo*; thus, this channel could represent a prognostic marker, as well as a promising therapeutic target for HCC^[105]. Another study found that the KCNJ11 channel was differentially expressed in HCC, and it predicted the poor prognosis in HCC patients. KCNJ11 promotes tumor progression through interaction with lactate dehydrogenase A (LDHA). Pharmacological inhibition of LDHA or knockdown of KCNJ11 expression inhibited cell proliferation, promoted apoptosis, and reduced cell invasive capacity^[106]. K_{ATP} channels regulate mitogen-induced proliferation in the human liver cell lines HepG2, which could have implications for liver growth control and serve as a potential therapeutic target^[37]. The voltage-gated potassium channel ether à-go-go-1 (Eag1) has gained enormous interest in cancer research because of its oncogenic properties^[107-109]. Eag1 channels have also been proposed as early tumor biomarkers and therapeutic targets for different types of cancers^[110,111]. Moreover, the inhibition of Eag1 reduces tumor cell proliferation *in vitro* and *in vivo*^[112-114]. We reported that HepG2 and HuH-7 HCC cells displayed Eag1 channel expression, and that the anti-histamine astemizole (a non-specific Eag1 inhibitor) decreased cell proliferation and induced apoptosis in both cell lines. In addition, an increase in Eag1 expression was found during HCC development in rats. Astemizole treatment prevented HCC development and seems to induce tumor regression in rats with HCC^[115].

T-type calcium channels play an important role in cell cycle progression in different types of cancer^[116]. The expression of the three T-type calcium channel subunits was observed in HCC cell lines and T-type channel blockage with mibefradil decreased cell proliferation in the SNU449 cell line^[117]. P2 purinergic receptors are overexpressed in certain cancer tissues; the levels of P2Y2 receptor are enhanced in HCC compared with human normal hepatocytes. These receptors are involved in ATP-induced (Ca²⁺)_i increase. Silencing P2Y2R expression inhibited ATP-induced human HCC cell proliferation and migration, and P2Y2R blockage inhibited cell growth in mice^[21,118]. In addition, high P2X3 receptor expression is associated with poor recurrence-free survival in HCC, while high P2Y13 expression is associated with improved recurrence-free survival. Moreover, extracellular nucleotide treatment induce cell cycle progression and extracellular ATP-mediated activation of P2X3 receptors promotes proliferation of HCC cells^[119]. SOCE is a major Ca²⁺ influx pathway controlling the intracellular Ca²⁺ concentration in normal hepatocytes and HCC cells, and Ca²⁺ influx has been demonstrated to be involved in liver oncogenesis. Accordingly, the blockade of SOCE inhibits hepatocarcinoma cell migration and invasion, by regulating focal adhesion turnover^[120]. The activation of SOCE channels is implicated in cancer cell chemoresistance, although the underlying molecular mechanisms are not well understood. However, inhibition of Orai1-mediated Ca²⁺ entry enhances chemosensitivity to 5-fluorouracil of HepG2 hepatocarcinoma cells^[121]. The specific roles and molecular mechanisms of calcium entry in drug response deserve further investigation.

CIC-3 chloride channels have multiple functions in tumorigenesis and tumor growth in HCC; the CIC-3 channel blocker DIDS (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid) arrests the cell at the G1 phase, inhibiting the proliferation of Hep3B HCC cells^[122]. Proteomic approaches found that the chloride intracellular channel 1 (CLIC1) is upregulated in HCC tissues, and that it participates in HCC migration and invasion by targeting maspin^[123].

The voltage-gated sodium channel β1 subunit was proposed as a cell adhesion molecule in some HCC cell lines. The analgesic-antitumor peptide (a scorpion toxin polypeptide with antitumor activity) inhibits the migration and invasion of HepG2 cells by an upregulated VGSC β1 subunit^[124]. Additionally the over-expression of Nav_{1.2} channels has been observed in an HCC *in vivo* model^[96].

AQP5 is highly expressed in HCC cell lines and its downregulation inhibits HCC cell invasion and tumor metastasis. Downregulation of AQP5 suppressed the epithelial-to-mesenchymal transition process in HCC cells^[125]. Another report found that microRNA-325-3p inhibits cell proliferation and induces apoptosis

in HBV-related hepatocellular carcinoma by downregulation of AQP5^[126]. These findings suggest that AQP5 may be a potential therapeutic target for HCC. AQP9 is the main aquaglyceroporin in the liver and its mRNA and protein levels are downregulated in HCC tissues compared to normal hepatocytes. Moreover, AQP9 over-expression inhibits hepatocellular carcinoma by upregulating FOXO1 expression, and suppresses invasion by inhibiting epithelial-to-mesenchymal transition. These findings suggest that the restoration of AQP9 expression can inhibit development of liver cancer^[127,128].

The TRP channel family has gained great relevance due to its role in several diseases. A recent study investigated the roles of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger 1 (NCX1) and the canonical transient receptor potential channel 6 (TRPC6) in regulating TGF β in human HCC. They found that TGF β induces the formation and activation of a TRPC6/NCX1 molecular complex, which mediates the effects of TGF β on the migration, invasion, and intrahepatic metastasis of HCC. These findings suggest TRPC6 and NCX1 as potential targets for HCC therapy^[129,130]. HCC develops multi-drug resistance in most cases; interestingly, multi-drug resistance regulation by TRPC6 and calcium-dependence has been shown in HCC cells^[131]. Silencing of TRPC1 channels suppressed cell proliferation while store-operated Ca^{2+} entry was significantly increased^[132,133]. On the other hand, it has been found that high expression of the vanilloid receptor-1 (TRPV1) is associated with better prognosis of HCC patients^[134].

The combined effect of static magnetic field and anti-cancer drugs has gained great interest in cancer. Static magnetic field enhances the anti-cancer effect of capsaicin on HepG2 cells through the mitochondria-dependent apoptosis pathway. This synergy may be explained if static magnetic field increased the binding efficiency of capsaicin to TRPV1 channels^[135]. TRPV2 contributes to the stemness of liver cancer and is a potential target in the treatment of human liver cancer patients^[136]. TRPV4 is over-expressed in HCC tissues when compared with non-tumoral liver. Furthermore, pharmacological inhibition of TRPV4 suppressed cell proliferation, induced apoptosis, and decreased the cell migration capability by attenuating the epithelial-to-mesenchymal transition process in HCC via modulation of the ERK signaling pathway^[137]. TRPM7 channels play a role in the migration and invasion of different types of cancer; actually, bradykinin promotes cell migration and invasion of HCC cells via TRPM7 channels^[138].

ASICs are H^+ -, Ca^{2+} -, and Na^+ -gated cation channels activated by changes in the extracellular pH, and ASIC1 α (ASIC1a) has been associated with tumor proliferation and migration. ASIC1 α is overexpressed in HCC tissues and associated with advanced clinical stage. Silencing of ASIC1 α expression inhibited the migration and invasion of HCC cells, suggesting a novel approach for HCC therapy^[139].

The R-Tf-D-LP4 cell-penetrating peptide derived from the mitochondrial multifunctional protein VDAC1 (voltage-dependent anion channel) induced apoptosis in liver cancer cell lines and inhibited liver tumor growth *in vivo*, representing a promising therapeutic approach for HCC^[140]. Inositol 1,4,5-trisphosphate receptors (ITPRs) are intracellular Ca^{2+} channels. ITPR3 is either absent or expressed at low levels in normal hepatocytes, but it is over-expressed in HCC patients; its increased expression level was associated with poor survival. Besides, cell proliferation and liver regeneration were enhanced *in vivo*, and ITPR3 deletion in human HCC cells increased apoptosis^[141].

Discussion: ion channels as potential tools for chronic liver diseases and HCC prevention, diagnosis, and therapy

HCC is a leading cause of cancer-death worldwide and is one of the most chemo-resistant tumors^[3,142]. The combination of new therapeutic targets with existing therapies may be very helpful. Several ion channels play very important roles in cancer-associated processes including inflammation, oxidative stress, cell proliferation, apoptosis, migration, invasion, angiogenesis, metastases, and drug response. These proteins are differentially expressed in HCC and liver diseases compared to their expression in the healthy

Table 2. Ion channel inhibitors as potential therapeutic agents studied in HCC

Inhibitor	Targeted ion channel	Ion channel gene symbol [*]	Ref.
TRAM-34	K _{Ca3.1}	<i>KCNN4</i>	[103,104]
ASTEMIZOLE	Eag1, Herg	<i>KCNH1, KCNH2</i>	[115]
MIBEFRADIL	T-type Ca ²⁺ channels	--	[117]
2-APB, SKF96365	SOCs	--	[21,118,121]
DIDS	CIC-3	<i>CLCN3</i>	[122]
MicroRNA-325-3P	AQP5	<i>AQP5</i>	[126]
CAPSAICIN	TRPV1	<i>TRPV1</i>	[135]
HC-067047	TRPV4	<i>TRPV4</i>	[137]

*When the specific ion channel has been reported to be targeted. HCC: hepatocellular carcinoma

Table 3. Ion channels suggested as HCC prognostic markers

Channel	Gene symbol	Expression in HCC	Association to prognosis	Ref.
KCNQ1	<i>KCNQ1</i>	Downregulated	Poor prognosis	[105]
KCNJ11	<i>KCNJ11</i>	Differentially expressed	Poor prognosis	[106]
P2X3	<i>P2RX3</i>	Overexpression	Poor recurrence-free survival	[119]
TRPV1	<i>TRPV1</i>	Overexpression	Better prognosis	[134]
ASIC1a	<i>ASIC1</i>	Overexpression	Advanced clinical stage	[139]
ITPR3	<i>ITPR3</i>	Overexpression	Poor survival	[141]

HCC: hepatocellular carcinoma

liver. Thus, patients at risk of developing some liver diseases, e.g., people infected with hepatitis viruses, patients with liver cirrhosis, or those suffering from alcoholism, might be candidates in whom ion channel expression can be studied. Nevertheless, an important issue to solve is how to detect ion channel expression in not easily accessible tissues such as the liver. An option may be ion channel detection by imaging studies. For instance, Eag1 channel expression has been detected *in vivo* with labeled antibodies and near-infrared imaging techniques, even in non-palpable tumors, in mice^[143]. Another option may be the detection of ion channels in extracellular vesicles released to the bloodstream by the liver. The investigation of ion channel expression in extracellular vesicles released by the liver in different pathological conditions is needed. These approaches should benefit patients by being diagnosed at earlier stages of the disease.

The precise molecular mechanisms involved in the association of ion channel function with cancer remain elusive. The antiproliferative effect of channel blockage on cell proliferation indicates that ion flux may play an important role. However, non-canonical functions of ion channel may also play a role, as occurs in other tissues and diseases^[144]. For instance, mutant non-conducting Kv10.1 potassium channels partially preserve their oncogenic potential^[145]. On the other hand, cleavage and translocation to the nucleus of a fragment of the carboxy-terminus of some calcium channels induce the transcription of genes associated with proliferation^[146]. Thus, the potential role of non-canonical functions of ion channels in liver diseases warrants investigation.

In accordance with the potential role of ion channels in liver diseases, blockage of over-expressed ion channels or activation of downregulated channels results in the inhibition of hepatitis virus replication, development of NAFLD, NASH, liver cirrhosis, and/or HCC [Table 2].

However, because of the relevance of ion channels in normal physiology, targeting these proteins may have non-desirable side-effects. In this direction, drug repurposing is a very good alternative to reduce costs and time for approval, as well as unknown side effects. Actually, several drugs have been suggested for repurposing in cancer, including anti-histamines such as astemizole (which also blocks potassium channels) and loratadine, as well as calcium and potassium channel blockers such as mibefradil and glibenclamide, respectively^[147,148].

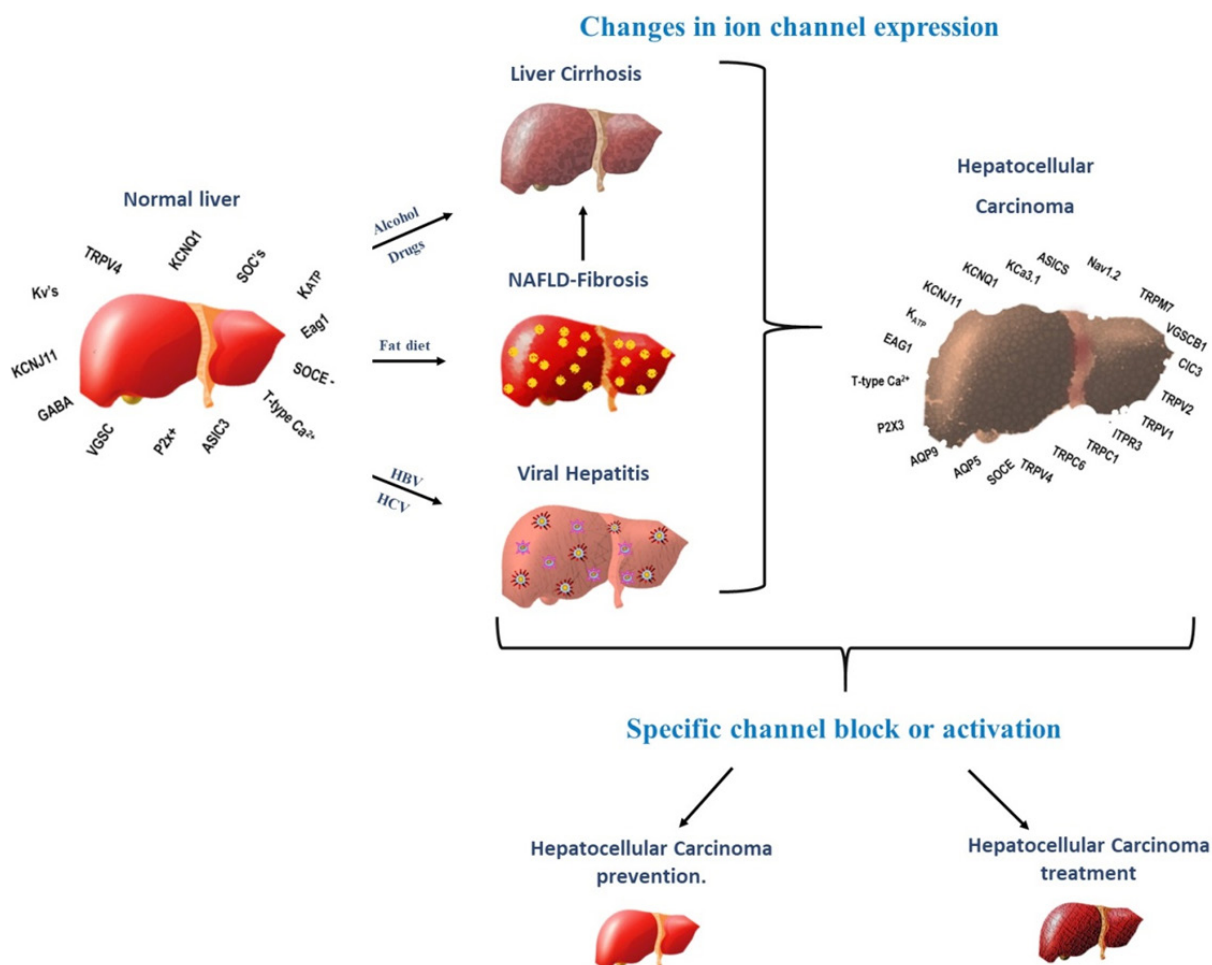


Figure 1. Ion channel-based therapy for HCC prevention and treatment. The expression of several ion channels is altered in liver disorders leading to HCC, as well as in HCC. Because of the very relevant participation of ion channels in cellular processes leading to HCC, targeting either the expression or activity of these proteins may lead to the prevention and treatment of liver diseases including HCC. HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HBV: hepatitis B virus; NAFLD: nonalcoholic fatty liver disease

CONCLUSION

Ion channels offer a plethora of opportunities for the prevention, diagnosis, and treatment of liver diseases [Figure 1], as well as represent potential tools as HCC prognostic markers [Table 3]. This ion-channel-based approach may help to reduce the mortality of this very poor prognosis disease.

DECLARATION

Authors' contributions

Contributed to the conception and design of the review, wrote the paper and revised the final draft: Chávez-López MG, Cruz-Díaz A, Tlapalcoyoa-Apanco KN, Pérez-Carreón JI, Camacho J

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All authors declared that there are not conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. American Cancer Society. Cancer Facts & Figures, 2019. Available from: <https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2019/cancer-facts-and-figures-2019.pdf> [Last accessed on 26 Mar 2020]
2. World Health Organization, Cancers, 2010. Available from: https://www.who.int/nmh/publications/fact_sheet_cancers_en.pdf [Last accessed on 26 Mar 2020]
3. Chávez-López MG, Zúñiga-García V, Pérez-Carreón JI, Avalos-Fuentes A, Escobar Y, et al. Eag1 channels as potential early-stage biomarkers of hepatocellular carcinoma. *Biologics* 2016;10:139-48.
4. Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. *J Clin Gastroenterol* 2013;47:S2-6.
5. Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. Cancer statistics, 2008. *CA Cancer J Clin* 2008;58:71-96.
6. Chuang SC, La Vecchia C, Boffetta P. Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection. *Cancer Lett* 2009;286:9-14.
7. Gomes MA, Prioli DG, Tralhão JG, Botelho MF. Hepatocellular carcinoma: epidemiology, biology, diagnosis, and therapies. *Rev Assoc Med Bras* (1992) 2013;59:514-24.
8. Ali ES, Rychkov GY, Barritt GJ. Metabolic disorders and cancer: hepatocyte store-operated Ca^{2+} channels in nonalcoholic fatty liver disease. *Adv Exp Med Biol* 2017;993:595-621.
9. Herbst DA, Reddy KR. Risk factors for hepatocellular carcinoma. *Clin Liver Dis* (Hoboken) 2012;1:180-2.
10. Sherman M, Llovet JM. Smoking, hepatitis B virus infection, and development of hepatocellular carcinoma. *J Natl Cancer Inst* 2011;103:1642-3.
11. Bruix J, Llovet JM. Hepatitis B virus and hepatocellular carcinoma. *J Hepatol* 2003;39:S59-63.
12. Barker BS, Young GT, Soubance CH, Stephens GJ, Stevens EB, et al. *Conn's Translational Neuroscience*, Chapter 2- Ion Channels. Academic Press; 2017. pp. 11-43.
13. Martial S. Involvement of ion channels and transporters in carcinoma angiogenesis and metastasis. *Am J Physiol Cell Physiol* 2016;310:C710-27.
14. Lang F, Stourmaras C. Ion channels in cancer: future perspectives and clinical potential. *Philos Trans R Soc Lond B Biol Sci* 2014;369:20130108.
15. Catterall WA. Voltage-gated calcium channels. *Cold Spring Harb Perspect Biol* 2011;3:a003947.
16. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 2003;4:517-29.
17. Prevarskaya N, Ouadid-Ahidouch H, Skryma R, Shuba Y. Remodelling of Ca^{2+} transport in cancer: how it contributes to cancer hallmarks? *Philos Trans R Soc Lond B Biol Sci* 2014;369:20130097.
18. Taylor JT, Zeng XB, Pottle JE, Lee K, Wang AR, et al. Calcium signaling and T-type calcium channels in cancer cell cycling. *World J Gastroenterol* 2008;14:4984-91.
19. Roderick HL, Cook SJ. Ca^{2+} signalling checkpoints in cancer: remodelling Ca^{2+} for cancer cell proliferation and survival. *Nat Rev Cancer* 2008;8:361-75.
20. Kuang Q, Purhonen P, Hebert H. Structure of potassium channels. *Cell Mol Life Sci* 2015;72:3677-93.
21. Ramirez A, Vazquez-Sanchez AY, Carrion-Robalino N, Camacho J. Ion channels and oxidative stress as a potential link for the diagnosis or treatment of liver diseases. *Oxid Med Cell Longev* 2016;2016:3928714.
22. Pardo LA, Stuhmer W. The roles of K^{+} channels in cancer. *Nat Rev Cancer* 2014;14:39-48.
23. Angus M, Ruben P. Voltage gated sodium channels in cancer and their potential mechanisms of action. *Channels (Austin)* 2019;13:400-9.
24. Djamgoz MBA, Onkal R. Persistent current blockers of voltage-gated sodium channels: a clinical opportunity for controlling metastatic disease. *Recent Pat Anticancer Drug Discov* 2013;8:66-84.
25. Fraser SP, Ozerlat-Gunduz I, Brackenbury WJ, Fitzgerald EM, Campbell TM, et al. Regulation of voltage-gated sodium channel expression in cancer: hormones, growth factors and auto-regulation. *Philos Trans R Soc Lond B Biol Sci* 2014;369:20130105.
26. Brackenbury WJ. Voltage-gated sodium channels and metastatic disease. *Channels (Austin)* 2012;6:352-61.
27. Verkman AS, Galletta LJ. Chloride channels as drug targets. *Nat Rev Drug Discov* 2009;8:153-71.

28. Turner KL, Sontheimer H. Cl^- and K^+ channels and their role in primary brain tumour biology. *Philos Trans R Soc Lond B Biol Sci* 2014;369:20130095.
29. Li RK, Zhang J, Zhang YH, Li ML, Wang M, et al. Chloride intracellular channel 1 is an important factor in the lymphatic metastasis of hepatocarcinoma. *Biomed Pharmacother* 2012;66:167-72.
30. Saberbaghi T, Wong R, Rutka JT, Wang GL, Feng ZP, et al. Role of Cl^- channels in primary brain tumor. *Cell Calcium* 2019;81:1-11.
31. Zhang S, Wang XM, Yin ZY, Zhao WX, Zhou JY, et al. Chloride intracellular channel 1 is overexpression in hepatic tumor and correlates with a poor prognosis. *APMIS* 2013;121:1047-53.
32. Jablonski EM, Mattocks MA, Sokolov E, Koniaris LG, Hughes FM Jr, et al. Decreased aquaporin expression leads to increased resistance to apoptosis in hepatocellular carcinoma. *Cancer Lett* 2007;250:36-46.
33. Pelagalli A, Squillacioti C, Mirabella N, Meli R. Aquaporins in health and disease: an overview focusing on the gut of different species. *Int J Mol Sci* 2016;17.
34. Gregoire F, Lucidi V, Zerrad-Saadi A, Virreira M, Bolaky N, et al. Analysis of aquaporin expression in liver with a focus on hepatocytes. *Histochem Cell Biol* 2015;144:347-63.
35. Zhu S, Ran J, Yang B, Mei Z. Aquaporins in digestive system. *Adv Exp Med Biol* 2017;969:123-30.
36. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP, Boyd AE, et al. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 1995;268:423-6.
37. Malhi H, Irani AN, Rajvanshi P, Suadcani SO, Spray DC, et al. KATP channels regulate mitogenically induced proliferation in primary rat hepatocytes and human liver cell lines. Implications for liver growth control and potential therapeutic targeting. *J Biol Chem* 2000;275:26050-7.
38. Mohamed YS, Ahmed LA, Salem HA, Agha AM. Role of nitric oxide and KATP channel in the protective effect mediated by nicorandil in bile duct ligation-induced liver fibrosis in rats. *Biochem Pharmacol* 2018;151:135-42.
39. Nogueira MA, Coelho AM, Sampietre SN, Patzina RA, Pinheiro da Silva F, et al. Beneficial effects of adenosine triphosphate-sensitive K^+ channel opener on liver ischemia/reperfusion injury. *World J Gastroenterol* 2014;20:15319-26.
40. Burnstock G, Vaughn B, Robson SC. Purinergic signalling in the liver in health and disease. *Purinergic Signal* 2014;10:51-70.
41. Burnstock G. Purinergic signalling: therapeutic developments. *Front Pharmacol* 2017;8:661.
42. Doctor RB, Matzakos T, McWilliams R, Johnson S, Feranchak AP, et al. Purinergic regulation of cholangiocyte secretion: identification of a novel role for P2X receptors. *Am J Physiol Gastrointest Liver Physiol* 2005;288:G779-86.
43. Emmett DS, Feranchak A, Kilic G, Puljak L, Miller B, et al. Characterization of ionotropic purinergic receptors in hepatocytes. *Hepatology* 2008;47:698-705.
44. Zhang Y, Zhang T, Wu C, Xia Q, Xu D. ASIC1a mediates the drug resistance of human hepatocellular carcinoma via the Ca^{2+} /PI3-kinase/AKT signaling pathway. *Lab Invest* 2017;97:53-69.
45. Ali ES, Rychkov GY, Barrit GJ. Deranged hepatocyte intracellular Ca^{2+} homeostasis and the progression of non-alcoholic fatty liver disease to hepatocellular carcinoma. *Cell Calcium* 2019;82:102057.
46. Jimenez H, Wang M, Zimmerman JW, Pennison MJ, Sharma S, et al. Tumour-specific amplitude-modulated radiofrequency electromagnetic fields induce differentiation of hepatocellular carcinoma via targeting Cav3.2 T-type voltage-gated calcium channels and Ca^{2+} influx. *EBiomedicine* 2019. 44:209-24.
47. Arzumanyan A, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer* 2013;13:123-35.
48. Kew MC. Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011;26:144-52.
49. Geng M, Xin X, Bi LQ, Zhou LT, Liu XH. Molecular mechanism of hepatitis B virus X protein function in hepatocarcinogenesis. *World J Gastroenterol* 2015;21:10732-8.
50. Tarocchi M, Polvani S, Marroncini G, Galli A. Molecular mechanism of hepatitis B virus-induced hepatocarcinogenesis. *World J Gastroenterol* 2014;20:11630-40.
51. Casciano JC, Bouchard MJ. Hepatitis B virus X protein modulates cytosolic Ca^{2+} signaling in primary human hepatocytes. *Virus Res* 2018;246:23-7.
52. Bouchard MJ, Wang LH, Schneider RJ. Calcium signaling by HBx protein in hepatitis B virus DNA replication. *Science* 2001;294:2376-8.
53. Yao JH, Liu ZJ, Yi JH, Wang J, Liu YN. Hepatitis B Virus X protein upregulates intracellular calcium signaling by binding C-terminal of orail protein. *Curr Med Sci* 2018;38:26-34.
54. Yen TT, Yang A, Chiu WT, Li TN, Wang LH, et al. Hepatitis B virus PreS2-mutant large surface antigen activates store-operated calcium entry and promotes chromosome instability. *Oncotarget* 2016;7:23346-60.
55. Zhang Z, Chen J, He Y, Zhan X, Zhao R, et al. miR-125b inhibits hepatitis B virus expression in vitro through targeting of the SCNN1A gene. *Arch Virol* 2014;159:3335-43.
56. Taylor JM, Han Z. Purinergic receptor functionality is necessary for infection of human hepatocytes by hepatitis delta virus and hepatitis B virus. *PloS One* 2010;5:e15784.
57. Wirth TC, Manns MP. The impact of the revolution in hepatitis C treatment on hepatocellular carcinoma. *Ann Oncol* 2016;27:1467-74.
58. Jahan S, Ashfaq UA, Qasim M, Khaliq S, Saleem MJ, et al. Hepatitis C virus to hepatocellular carcinoma. *Infect Agent Cancer* 2012;7:2.
59. Griffin SD, Beales LP, Clarke DS, Worsfold O, Evans SD, et al. The p7 protein of hepatitis C virus forms an ion channel that is blocked by the antiviral drug, Amantadine. *FEBS Lett* 2003;535:34-8.

60. Chandler DE, Penin F, Schulten K, Chipot C. The p7 protein of hepatitis C virus forms structurally plastic, minimalist ion channels. *PLoS Comput Biol* 2012;8:e1002702.
61. Foster TL, Verow M, Wozniak AL, Bentham MJ, Thompson J, et al. Resistance mutations define specific antiviral effects for inhibitors of the hepatitis C virus p7 ion channel. *Hepatology* 2011;54:79-90.
62. Jones CT, Murray CL, Eastman DK, Tassello J, Rice CM. Hepatitis C virus p7 and NS2 proteins are essential for production of infectious virus. *J Virol* 2007;81:8374-83.
63. Steinmann E, Penin F, Kallis S, Patel AH, Bartenschlager R, et al. Hepatitis C virus p7 protein is crucial for assembly and release of infectious virions. *PLoS Pathog* 2007;3:e103.
64. Wozniak AL, Griffin S, Rowlands D, Harris M, Yi M, et al. Intracellular proton conductance of the hepatitis C virus p7 protein and its contribution to infectious virus production. *PLoS Pathog* 2010;6:e1001087.
65. Griffin S, Stgelais C, Owsianka AM, Patel AH, Rowlands D, et al. Genotype-dependent sensitivity of hepatitis C virus to inhibitors of the p7 ion channel. *Hepatology* 2008;48:1779-90.
66. Steinmann E, Whitfield T, Kallis S, Dwek RA, Zitzmann N, et al. Antiviral effects of amantadine and iminosugar derivatives against hepatitis C virus. *Hepatology* 2007;46:330-8.
67. Shiryayev VA, Radchenko EV, Palyulin VA, Zefirov NS, Bormotov NI, et al. Molecular design, synthesis and biological evaluation of cage compound-based inhibitors of hepatitis C virus p7 ion channels. *Eur J Med Chem* 2018;158:214-35.
68. Pietschmann T. Clinically approved ion channel inhibitors close gates for hepatitis C virus and open doors for drug repurposing in infectious viral diseases. *J Virol* 2017;91.
69. Breiting U, Farag NS, Ali NKM, Breiting HA. Patch-clamp study of hepatitis C p7 channels reveals genotype-specific sensitivity to inhibitors. *Biophys J* 2016;110:2419-29.
70. Hong W, Lang Y, Li T, Zeng Z, Song Y, et al. A p7 ion channel-derived peptide inhibits hepatitis C virus infection in vitro. *J Biol Chem* 2015;290:23254-63.
71. Pavlovic D, Neville DC, Argaud O, Blumberg B, Dwek RA, et al. The hepatitis C virus p7 protein forms an ion channel that is inhibited by long-alkyl-chain iminosugar derivatives. *Proc Natl Acad Sci U S A* 2003;100:6104-8.
72. Manzoor S, Idrees M, Ashraf J, Mehmood A, Butt S, et al. Identification of ionotropic purinergic receptors in Huh-7 cells and their response towards structural proteins of HCV genotype 3a. *Virol J* 2011;8:431.
73. Manzoor S, Akhtar A, Naseem S, Khalid M, Mazhar M, et al. Ionotropic purinergic receptors P2X4 and P2X7: proviral or antiviral? An insight into P2X receptor signaling and hepatitis C virus infection. *Viral Immunol* 2016;29:401-8.
74. Sidorkiewicz M, Brocka M, Bronis M, Grek M, Jozwiak B, et al. The altered expression of alpha1 and beta3 subunits of the gamma-aminobutyric acid A receptor is related to the hepatitis C virus infection. *Eur J Clin Microbiol Infect Dis* 2012;31:1537-42.
75. Streba LA, Vere CC, Rogoveanu I, Streba CT. Nonalcoholic fatty liver disease, metabolic risk factors, and hepatocellular carcinoma: an open question. *World J Gastroenterol* 2015;21:4103-10.
76. Kikuchi L, Oliveira CP, Carrilho FJ. Nonalcoholic fatty liver disease and hepatocellular carcinoma. *Biomed Res Int* 2014;2014:106247.
77. Dhamija E, Paul SB, Kedia S. Non-alcoholic fatty liver disease associated with hepatocellular carcinoma: an increasing concern. *Indian J Med Res* 2019;149:9-17.
78. Wulff H, Castle NA. Therapeutic potential of KCa3.1 blockers: recent advances and promising trends. *Expert Rev Clin Pharmacol* 2010;3:385-96.
79. Freise C, Heldwein S, Erben U, Hoyer J, Kohler R, et al. K⁺-channel inhibition reduces portal perfusion pressure in fibrotic rats and fibrosis associated characteristics of hepatic stellate cells. *Liver Int* 2015;35:1244-52.
80. Paka L, Smith DE, Jung D, McCormack S, Zhou P, et al. Anti-steatotic and anti-fibrotic effects of the KCa3.1 channel inhibitor, Senicapoc, in non-alcoholic liver disease. *World J Gastroenterol* 2017;23:4181-90.
81. Basseti Fantauzzi C, Menini S, Iacobini C, Rossi C, Santini E, et al. Deficiency of the purinergic receptor 2X7 attenuates nonalcoholic steatohepatitis induced by high-fat diet: possible role of the NLRP3 inflammasome. *Oxid Med Cell Longev* 2017;2017:8962458.
82. Shang Y, Li XF, Jin MJ, Li Y, Wu YL, et al. Leucodin attenuates inflammatory response in macrophages and lipid accumulation in steatotic hepatocytes via P2x7 receptor pathway: a potential role in alcoholic liver disease. *Biomed Pharmacother* 2018;107:374-81.
83. Huang C, Yu W, Cui H, Wang Y, Zhang L, et al. P2X7 blockade attenuates mouse liver fibrosis. *Mol Med Rep* 2014;9:57-62.
84. Wilson CH, Ali ES, Scrimgeour N, Martin AM, Hua J, et al. Steatosis inhibits liver cell store-operated Ca²⁺ entry and reduces ER Ca²⁺ through a protein kinase C-dependent mechanism. *Biochem J* 2015;466:379-90.
85. Grimm C, Holdt LM, Chen CC, Hassan S, Muller C, et al. High susceptibility to fatty liver disease in two-pore channel 2-deficient mice. *Nat Commun* 2014;5:4699.
86. Li Q, Li L, Wang F, Chen J, Zhao Y, et al. Dietary capsaicin prevents nonalcoholic fatty liver disease through transient receptor potential vanilloid 1-mediated peroxisome proliferator-activated receptor delta activation. *Pflugers Arch* 2013;465:1303-16.
87. Li L, Chen J, Ni Y, Feng X, Zhao Z, et al. TRPV1 activation prevents nonalcoholic fatty liver through UCP2 upregulation in mice. *Pflugers Arch* 2012;463:727-32.
88. Zhan L, Li J. The role of TRPV4 in fibrosis. *Gene* 2018;642:1-8.
89. Iyer SC, Kannan A, Gopal A, Devaraj N, Halagowder D. Receptor channel TRPC6 orchestrate the activation of human hepatic stellate cell under hypoxia condition. *Exp Cell Res* 2015;336:66-75.
90. Fang L, Zhan S, Huang C, Cheng X, Lv X, et al. TRPM7 channel regulates PDGF-BB-induced proliferation of hepatic stellate cells via PI3K and ERK pathways. *Toxicol Appl Pharmacol* 2013;272:713-25.
91. Pan CX, Wu FR, Wang XY, Tang J, Gao WF, et al. Inhibition of ASICs reduces rat hepatic stellate cells activity and liver fibrosis: an in

- vitro and in vivo study. *Cell Biol Int* 2014;38:1003-12.
92. Starr SP, Raines D. Cirrhosis: diagnosis, management, and prevention. *Am Fam Physician* 2011;84:1353-9.
 93. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet* 2014;383:1749-61.
 94. Romanelli RG, Stasi C. Recent advancements in diagnosis and therapy of liver cirrhosis. *Curr Drug Targets* 2016;17:1804-17.
 95. Liu G, Xie C, Sun F, Xu X, Yang Y, et al. Clinical significance of transient receptor potential vanilloid 2 expression in human hepatocellular carcinoma. *Cancer Genet Cytogenet* 2010;197:54-9.
 96. Zúñiga-García V, Chávez-López Mde G, Quintanar-Jurado V, Gabiño-López NB, Hernández-Gallegos E, et al. Differential expression of ion channels and transporters during hepatocellular carcinoma development. *Dig Dis Sci* 2015;60:2373-83.
 97. Dufour JF, Luthi M, Forestier M, Magnino F. Expression of inositol 1,4,5-trisphosphate receptor isoforms in rat cirrhosis. *Hepatology* 1999;30:1018-26.
 98. Xian ZH, Cong WM, Wang YH, Wang B, Wu MC. Expression and localization of aquaporin-1 in human cirrhotic liver. *Pathol Res Pract* 2009;205:774-80.
 99. Yokomori H, Oda M, Yoshimura K, Kaneko F, Hibi T. Aquaporin-1 associated with hepatic arterial capillary proliferation on hepatic sinusoid in human cirrhotic liver. *Liver Int* 2011;31:1554-64.
 100. Fabrega E, Berja A, García-Unzueta MT, Guerra-Ruiz A, Cobo M, et al. Influence of aquaporin-1 gene polymorphism on water retention in liver cirrhosis. *Scand J Gastroenterol* 2011;46:1267-74.
 101. Rodríguez-Vilarrupla A, Graupera M, Matei V, Bataller R, Abalde JG, et al. Large-conductance calcium-activated potassium channels modulate vascular tone in experimental cirrhosis. *Liver Int* 2008;28:566-73.
 102. Yu Z, Serra A, Sauter D, Loffing J, Ackermann D, et al. Sodium retention in rats with liver cirrhosis is associated with increased renal abundance of NaCl cotransporter (NCC). *Nephrol Dial Transplant* 2005;20:1833-41.
 103. Yang XW, Liu JW, Zhang RC, Yin Q, Shen WZ, et al. Inhibitory effects of blockage of intermediate conductance Ca^{2+} -activated K^{+} channels on proliferation of hepatocellular carcinoma cells. *J Huazhong Univ Sci Technolog Med Sci* 2013;33:86-89.
 104. Freise C, Ruehl M, Seehofer D, Hoyer J, Somasundaram R. The inhibitor of Ca^{2+} -dependent K^{+} channels TRAM-34 blocks growth of hepatocellular carcinoma cells via downregulation of estrogen receptor alpha mRNA and nuclear factor-kappaB. *Invest New Drugs* 2013;31:452-7.
 105. Fan H, Zhang M, Liu W. Hypermethylated KCNQ1 acts as a tumor suppressor in hepatocellular carcinoma. *Biochem Biophys Res Commun* 2018;503:3100-7.
 106. Zhang K, Mu L, Ding MC, Xu R, Ding ZJ, et al. NFkappaB mediated elevation of KCNJ11 promotes tumor progression of hepatocellular carcinoma through interaction of lactate dehydrogenase A. *Biochem Biophys Res Commun* 2018;495:246-53.
 107. Pardo LA, Stuhmer W. EagI: an emerging oncological target. *Cancer Res* 2008;68:1611-3.
 108. Wulff H, Castle NA, Pardo LA. Voltage-gated potassium channels as therapeutic targets. *Nat Rev Drug Discov* 2009;8:982-1001.
 109. Pardo LA, del Camino D, Sanchez A, Alves F, Bruggemann A, et al. Oncogenic potential of EAG K^{+} channels. *EMBO J* 1999;18:5540-7.
 110. Rodríguez-Rasgado JA, Acuña-Macías I, Camacho J. EagI channels as potential cancer biomarkers. *Sensors (Basel)* 2012;12:5986-95.
 111. Farias LM, Ocana DB, Diaz L, Larrea F, Avila-Chavez E, et al. Ether a go-go potassium channels as human cervical cancer markers. *Cancer Res* 2004;64:6996-7001.
 112. Gómez-Varela D, Zwick-Wallasch E, Knötgen H, Sánchez A, Hettmann T, et al. Monoclonal antibody blockade of the human EagI potassium channel function exerts antitumor activity. *Cancer Res* 2007;67:7343-9.
 113. García-Becerra R, Diaz L, Camacho J, Barrera D, Ordaz-Rosado D, et al. Calcitriol inhibits Ether-a go-go potassium channel expression and cell proliferation in human breast cancer cells. *Exp Cell Res* 2010;316:433-42.
 114. Weber C, Mello de Queiroz F, Downie BR, Suckow A, Stuhmer W, et al. Silencing the activity and proliferative properties of the human EagI Potassium Channel by RNA Interference. *J Biol Chem* 2006;281:13030-7.
 115. de Guadalupe Chávez-López M, Pérez-Carreón JI, Zúñiga-García V, Díaz-Chávez J, Herrera LA, et al. Astemizole-based anticancer therapy for hepatocellular carcinoma (HCC), and EagI channels as potential early-stage markers of HCC. *Tumour Biol* 2015;36:6149-58.
 116. Dziegielewska B, Gray LS, Dziegielewski J. T-type calcium channels blockers as new tools in cancer therapies. *Pflugers Arch* 2014;466:801-10.
 117. Li Y, Liu S, Lu F, Zhang T, Chen H, et al. A role of functional T-type Ca^{2+} channel in hepatocellular carcinoma cell proliferation. *Oncol Rep* 2009;22:1229-35.
 118. Xie R, Xu J, Wen G, Jin H, Liu X, et al. The P2Y2 nucleotide receptor mediates the proliferation and migration of human hepatocellular carcinoma cells induced by ATP. *J Biol Chem* 2014;289:19137-49.
 119. Maynard JP, Lee JS, Sohn BH, Yu X, Lopez-Terrada D, et al. P2X3 purinergic receptor overexpression is associated with poor recurrence-free survival in hepatocellular carcinoma patients. *Oncotarget* 2015;6:41162-79.
 120. Yang N, Tang Y, Wang F, Zhang H, Xu D, et al. Blockade of store-operated Ca^{2+} entry inhibits hepatocarcinoma cell migration and invasion by regulating focal adhesion turnover. *Cancer Lett* 2013;330:163-9.
 121. Tang BD, Xia X, Lv XF, Yu BX, Yuan JN, et al. Inhibition of Orai1-mediated Ca^{2+} entry enhances chemosensitivity of HepG2 hepatocarcinoma cells to 5-fluorouracil. *J Cell Mol Med* 2017;21:904-15.
 122. Wang R, Kang B, Hu R, Huang Y, Qin Z, et al. CIC-3 chloride channel protein induces G1 arrest in hepatocellular carcinoma Hep3B cells. *Oncol Rep* 2018;40:472-8.
 123. Wei X, Li J, Xie H, Wang H, Wang J, et al. Chloride intracellular channel 1 participates in migration and invasion of hepatocellular carcinoma by targeting maspin. *J Gastroenterol Hepatol* 2015;30:208-16.
 124. Guo G, Cui Y, Chen H, Zhang L, Zhao M, et al. Analgesic-antitumor peptide inhibits the migration and invasion of HepG2 cells by an

- upregulated VGSC beta1 subunit. *Tumour Biol* 2016;37:3033-41.
125. He Z, Dong W, Hu J, Ren X. AQP5 promotes hepatocellular carcinoma metastasis via NF-kappaB-regulated epithelial-mesenchymal transition. *Biochem Biophys Res Commun* 2017;490:343-8.
 126. Zhang Z, Han Y, Sun G, Liu X, Jia X, et al. MicroRNA-325-3p inhibits cell proliferation and induces apoptosis in hepatitis B virus-related hepatocellular carcinoma by down-regulation of aquaporin 5. *Cell Mol Biol Lett* 2019;24:13.
 127. Li CF, Zhang WG, Liu M, Qiu LW, Chen XF, et al. Aquaporin 9 inhibits hepatocellular carcinoma through up-regulating FOXO1 expression. *Oncotarget* 2016;7:44161-70.
 128. Zhang WG, Li CF, Liu M, Chen XF, Shuai K, et al. Aquaporin 9 is down-regulated in hepatocellular carcinoma and its over-expression suppresses hepatoma cell invasion through inhibiting epithelial-to-mesenchymal transition. *Cancer Lett* 2016;378:111-9.
 129. Xu J, Yang Y, Xie R, Liu J, Nie X, et al. The NCX1/TRPC6 complex mediates TGFbeta-driven migration and invasion of human hepatocellular carcinoma cells. *Cancer Res* 2018;78:2564-76.
 130. Tian Y, Zhu MX. A novel TRPC6-dependent mechanism of TGF-beta-induced migration and invasion of human hepatocellular carcinoma cells. *Sci China Life Sci* 2018;61:1120-2.
 131. Wen L, Liang C, Chen E, Chen W, Liang F, et al. Regulation of multi-drug resistance in hepatocellular carcinoma cells is TRPC6/calcium dependent. *Sci Rep* 2016;6:23269.
 132. Selli C, Erac Y, Kosova B, Erdal ES, Tosun M. Silencing of TRPC1 regulates store-operated calcium entry and proliferation in Huh7 hepatocellular carcinoma cells. *Biomed Pharmacother* 2015;71:194-200.
 133. Selli C, Pearce DA, Sims AH, Tosun M. Differential expression of store-operated calcium- and proliferation-related genes in hepatocellular carcinoma cells following TRPC1 ion channel silencing. *Mol Cell Biochem* 2016;420:129-40.
 134. Miao X, Liu G, Xu X, Xie C, Sun F, et al. High expression of vanilloid receptor-1 is associated with better prognosis of patients with hepatocellular carcinoma. *Cancer Genet Cytogenet* 2008;186:25-32.
 135. Chen WT, Lin GB, Lin SH, Lu CH, Hsieh CH, et al. Static magnetic field enhances the anticancer efficacy of capsaicin on HepG2 cells via capsaicin receptor TRPV1. *PLoS One* 2018;13:e0191078.
 136. Hu Z, Cao X, Fang Y, Liu G, Xie C, et al. Transient receptor potential vanilloid-type 2 targeting on stemness in liver cancer. *Biomed Pharmacother* 2018;105:697-706.
 137. Fang Y, Liu G, Xie C, Qian K, Lei X, et al. Pharmacological inhibition of TRPV4 channel suppresses malignant biological behavior of hepatocellular carcinoma via modulation of ERK signaling pathway. *Biomed Pharmacother* 2018;101:910-9.
 138. Chen Y, Yu Y, Sun S, Wang Z, Liu P, et al. Bradykinin promotes migration and invasion of hepatocellular carcinoma cells through TRPM7 and MMP2. *Exp Cell Res* 2016;349:68-76.
 139. Jin C, Ye QH, Yuan FL, Gu YL, Li JP, et al. Involvement of acid-sensing ion channel 1alpha in hepatic carcinoma cell migration and invasion. *Tumour Biol* 2015;36:4309-17.
 140. Pittala S, Krelin Y, Shoshan-Barmatz V. Targeting liver cancer and associated pathologies in mice with a mitochondrial VDAC1-based peptide. *Neoplasia* 2018;20:594-609.
 141. Guerra MT, Florentino RM, Franca A, Lima Filho AC, Dos Santos ML, et al. Expression of the type 3 InsP3 receptor is a final common event in the development of hepatocellular carcinoma. *Gut* 2019;68:1676-87.
 142. Hartke J, Johnson M, Ghabril M. The diagnosis and treatment of hepatocellular carcinoma. *Semin Diagn Pathol* 2017;34:153-9.
 143. Stühmer W, Alves F, Hartung F, Zientkowska M, Pardo LA. Potassium channels as tumour markers. *FEBS Lett* 2006;580:2850-2.
 144. Black JA, Waxman SG. Noncanonical roles of voltage-gated sodium channels. *Neuron* 2013;80:280-91.
 145. Downie BR, Sánchez A, Knötgen H, Contreras-Jurado C, Gymnopoulos M, et al. Eag1 expression interferes with hypoxia homeostasis and induces angiogenesis in tumors. *J Biol Chem* 2008;283:36234-40.
 146. Buchanan PJ, McCloskey KD. Ca_v channels and cancer: canonical functions indicate benefits of repurposed drugs as cancer therapeutics. *Eur Biophys J* 2016;45:621-33.
 147. Lee H, Kang S, Kim W. Drug repositioning for cancer therapy based on large-scale drug-induced transcriptional signatures. *PLoS One* 2016;11:e0150460.
 148. Ellegaard AM, Dehlendorff C, Vind AC, Anand A, Cedervik L, et al. Repurposing cationic amphiphilic antihistamines for cancer treatment. *EBioMedicine* 2016;9:130-9.

Review

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Cytotoxic immune cell-based immunotherapy for hepatocellular carcinoma

Jing Li¹, Longxiang Tao², Xuefu Wang³

¹School of Life Sciences, Anhui Medical University, Hefei 230032, Anhui, China.

²Department of Radiology, The First Affiliated Hospital of Anhui Medical University, Hefei 230022, Anhui, China.

³School of Pharmacy, Anhui Medical University, Hefei 230032, Anhui, China.

Correspondence to: Xuefu Wang, PhD, School of Pharmacy, Anhui Medical University, #81, Meishan Road, Hefei 230022, Anhui, China.
E-mail: wangxuefu@ahmu.edu.cn

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common solid tumors with poor clinical prognosis. Novel therapeutic regimens are urgently required for patients with advanced HCC. Both pre-clinical and clinical studies suggest immunotherapy as an attractive alternative for advanced HCC treatment. Natural killer (NK) cells and CD8⁺ T cells are the most important cytotoxic immune cells involved in cancer treatment and elimination. Reinvigorating the anticancer activity of NK and CD8⁺ T cells is the fundamental guarantee for the success of immunotherapy in advanced HCC treatment. Therefore, in this review, we aim to summarize the characteristics and roles of NK and CD8⁺ T cells in HCC development, describe the frontiers of immunotherapy for advanced HCC based on immune checkpoint inhibitors and adoptive cell transfer, and discuss their limitations and scope for future improvement.

Keywords: Hepatocellular carcinoma, immunotherapy, natural killer cells, CD8⁺ T cells

INTRODUCTION

Although hepatocellular carcinoma (HCC) is only the fifth-most common cancer worldwide, it ranks second in cancer-related mortality^[1]. Local regional therapies such as surgical resection, cryoablation, radiofrequency ablation, transarterial chemoembolization, and liver transplantation are effective only for patients with early-stage HCC^[2]. Multi-targeted tyrosine kinase inhibitors (TKIs) provide options for systemic treatment of



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patients with advanced HCC. The first-line agent sorafenib modestly extends the survival in advanced HCC by about 3 months^[3]. Lenvatinib has been approved for HCC treatment based on the results of a randomized phase III trial, in that lenvatinib is non-inferior to sorafenib in overall survival assessment in patients with advanced HCC, with similar safety and tolerability profiles as sorafenib^[4]. Regorafenib and cabozantinib have been approved as second-line options for patients with progressive HCC despite on-going sorafenib treatment, in that they improve overall survival in such patients^[5-7]. Nonetheless, the survival benefit from the TKIs is limited and unexpected. Therefore, novel clinical therapies are urgently required for treatment of early-stage and advanced HCC. Recently, immunotherapy with immune checkpoint blockade and adoptive immune cell transfer has been clinically tested in various types of cancers, which provides a novel therapeutic strategy for difficult-to-treat HCC cases. The present review aims to summarize the characteristics and roles of natural killer (NK) cells and CD8⁺ T cells during HCC development, describe the frontiers of immunotherapy for advanced HCC based on immune checkpoint inhibitors (ICIs) and adoptive cell transfer (ACT), and discuss their limitations and scope for future improvement.

NK CELLS AND CD8⁺ T CELLS IN HCC

The liver contains diverse types of immune cells such as T cells, NK cells, B cells, NKT, and Kupffer cells^[8,9]. However, the liver is a tolerogenic immune organ in the physiological state, in that the liver remains tolerant to stimuli from the hepatic artery and portal vein, such as bacterial products, environmental toxins, and food antigens, to avoid tissue damage^[10,11]. This immune-tolerant microenvironment of the liver contributes to the immunoescape of HCC^[12]. Studies have extensively discussed the properties and contribution of immunosuppressive cells during HCC progression^[13]. However, to our best knowledge, the characteristics and contribution of the two most important anticancer immune cells in the liver - NK cells and CD8⁺ T cells - have not been well-documented.

NK cells in HCC

Innate lymphoid cells (ILCs) function as the first line of immune defense against infections and cancers. Paralleling with T cell subsets, ILCs comprise NK cells, ILC1, ILC2, and ILC3, amongst which the NK and ILC1 cells are abundant in the liver^[14,15]. Unlike helper ILC subsets, NK cells are classified as a cytotoxic ILC subset because of their direct killing of cancer cells and infected cells via cytotoxicity and cytokine secretion^[16]. NK cells express activating receptors such as CD16, NKp30, NKp44, NKp46, NKp80, NKG2D, CD244, CD226; cytokine receptors such as IL-2R, IL-28R, IL-12R, IFNR, IL-15R, IL-18R, IL-1R8, IL-10R, and TGF-βR; and inhibitory receptors such as NKG2A, KLRG1, KIRs, TIGIT, TIM3, Siglecs, PD-1, LAG3, A2AR, LAIRs, and ILT^[17,18]. The activation of NK cells is determined by the net value of activating signal strength determined by the competition between activating and inhibitory receptors^[17,19]. The abundance and activity of NK cells are modulated by multiple signals within the tumor microenvironment, which significantly influence cancer development. Compared with healthy controls, HCC patients have a dramatic reduction of tumor-infiltrating NK cells; their abundance in HCC tissues is positively correlated with patient survival^[20]. NK cells in the HCC tissues of patients with advanced HCC show dysfunctional or exhausted state^[20], suggesting that NK-cell exhaustion contributes to HCC progression. The regulators for NK cell exhaustion have been extensively investigated. For example, the up-regulation of inhibitory receptors on NK cells leads to NK-cell exhaustion and predicts poor prognosis in HCC patients^[21]. TGF-β and IL-10 promoted NK-cell exhaustion in HCC^[22,23]. Hypoxia-induced mitochondrial fragmentation limits NK-cell anticancer activity^[24]. Additionally, immunosuppressive cells such as myeloid-derived suppressor cells, monocyte/macrophage, and HCC-associated fibroblasts in intratumor tissues of HCC contribute to NK-cell exhaustion^[25-27]. The plasticity of NK cell activity provides the foundation of NK-cell-based cancer immunotherapy. Immunotherapeutic drugs triggering NK-cell activation are being developed and assessed in pre-clinical and clinical trials. Nevertheless, it is still crucial to decipher the mechanisms by which NK cells undergo exhaustion in patients with advanced HCC, in order to offer more patient benefits in terms of effective reinvigoration of NK-cell anticancer activity and precision therapy in HCC.

NK cells have been reported to account for 20%-40% of human hepatic lymphocytes and 10%-20% of murine hepatic lymphocytes, more than half of which *bona fide* comprise ILC1 or liver-resident NK cells^[28]. The observations from the parabiosis model show that the liver contains conventional NK cells and liver-resident NK cells^[29], further supported by the findings that hepatic irradiation could persistently eliminate liver-resident NK cells^[30]. Early evidence has shown that liver-resident NK cells expressed higher levels of CD160, CD69, CD44, CXCR3, CXCR6, TRAIL, FasL, GM-CSF, and TNF- α ^[29]. CXCR6 is required for the retention of liver-resident NK cells within the liver^[31]. CD8⁺ T cells promote liver-resident NK cell maturation through the CD70-CD27 axis^[32]. Liver-derived TGF- β maintains the property of liver-resident NK cells^[33]. Functionally, liver-resident NK cells were originally found to mediate skin-contact inflammation^[29]. Zhou *et al.*^[34] reported that liver-resident NK cells inhibited T cell antiviral activity via PD-L1 during viral infection. Additionally, liver-resident NK cells can suppress autoimmune cholangitis by limiting the expansion of CD4⁺ T cells^[35]. These findings suggest that liver-resident NK cells play versatile roles in liver diseases. Human liver-resident NK cells are CD56^{bright} Eomes^{hi} Tbet^{lo} Hobit⁺ TIGIT⁺ CD69⁺ CXCR6⁺ CD49e⁻; express higher levels of NKG2D, NKP46, TRAIL, and FasL; and possess cytotoxicity against HCC cells^[36]. However, liver-resident NK cells within the HCC tissue down-regulate NKG2D^[37]. Moreover, liver-resident NK cells express more types of inhibitory receptors such as PD-1, CD96, and TIGIT^[38]. Therefore, liver-resident NK cells undergo exhaustion during HCC progression. Fortunately, IL-15 could recover HCC-induced liver-resident NK-cell dysfunction^[37]. In addition, the mTOR inhibitor - everolimus - enhances their anticancer activity through upregulation of TRAIL^[39]. Thus, liver-resident NK cells have the potential for application in HCC therapy, although the complete underlying mechanism of liver-resident NK cells' exhaustion remains unclear.

CD8⁺ T cells in HCC

The abundance of tumor-infiltrating CD8⁺ T cells and the frequency of IFN- γ ⁺ CD8⁺ T cells were associated with improved survival of HCC patients^[40,41]. CD8⁺ T cells were enriched in early-stage HCC, but progressively reduced with tumor progression, accompanied with increased expression of checkpoints on tumor-infiltrating CD8⁺ T cells^[42]. Therefore, CD8⁺ T cells in HCC tissues progressively underwent functional compromise during cancer progression, characterized by high levels of immune checkpoints, low effector cytokines, and impaired cytotoxicity and proliferation. In detail, however, CD8⁺ T cells in HCC tissues expressed different PD-1 levels and displayed different anticancer capacity^[43]. Among PD-1^{high} CD8⁺ T cells, 4-1BB⁺ PD-1^{high} CD8⁺ T cells displayed stronger anticancer activity and proliferative potential^[44]. In recent times, several studies have reported that TCF-1⁺ PD-1⁺ T cells sustained the stemness and response to immune checkpoint blockade in certain types of cancers^[45,46]. Therefore, the identification of functional tumor-infiltrating CD8⁺ T cells for immunotherapy will likely benefit clinical outcomes and promote precision medicine for HCC patients.

The systemic, local, cellular, and molecular mechanisms of T-cell exhaustion in HCC have been extensively investigated. The hepatic inflammatory microenvironment had been confirmed to be critical for HCC development^[47]. Lim *et al.*^[48] found that HBV-related HCC microenvironment displayed more immunosuppression than non-viral-related HCC microenvironment, indicating increased difficulty in the immunotherapy of HBV-related HCC. Hepatoma cells, LSECs, suppressive immune cells, inhibitory receptors, and cytokines have been found to trigger tumor-infiltrating CD8⁺ T-cell exhaustion^[49]. For instance, myeloid-derived suppressor cells and T regulatory cells in HCC tissues had been found to impair T-cell functionality^[50]. The inhibitory cytokine - IL-35 - dampened CD8⁺ T cells activity in HCC patients^[51]. 14-3-3 ζ , a suppressor of apoptosis, is highly expressed in HCC and promotes epithelial-mesenchymal transition of HCC cells^[52]. Wang *et al.*^[53] reported that 14-3-3 ζ delivered by HCC-derived exosomes contributed to impaired anticancer activity of CD8⁺ T cells. The thymocyte selection-associated high mobility group box (TOX) transcription factor belongs to an evolutionarily conserved DNA-binding protein family and regulates the development of T cells^[54]. Recently, several studies have confirmed that TOX was critical for CD8⁺ T cell exhaustion^[55,56]. Moreover, it was found that TOX could promote CD8⁺ T-cell exhaustion in

HCC tissues by restraining PD-1 degradation^[57]. The complicated immunosuppressive microenvironment in HCC tissues severely impairs the efficacy of immunotherapy. Therefore, the mechanisms of CD8⁺ T-cell exhaustion in HCC needs to be further elucidated.

CYTOTOXIC IMMUNE CELL-BASED IMMUNOTHERAPY OF HCC

The increased understanding of CD8⁺ T cells and NK cells promotes the development of effective immunotherapy. These two immune cell populations follow many similar patterns and/or complementary patterns to eliminate cancer cells. Moreover, their activities are regulated by common immune checkpoints. Here, we discuss the application and outcomes of cytotoxic cell-based ICIs and ACT in HCC treatment.

Immune checkpoint inhibitors

ICIs have displayed impressive efficacy in treating a variety of cancers. An increasing number of studies are being conducted on novel immune checkpoints and their inhibitors. Additionally, studies on ICI-based immunotherapy for advanced HCC treatment are also increasing, with some showing encouraging therapeutic effects.

Anti-PD-1 antibody and anti-PDL1 antibodies

PD-1 is mainly expressed on CD8⁺ T cells, whose binding with PD-L1/PD-L2 induces CD8⁺ T-cell exhaustion^[58]. T cells from HCC tissues express high levels of PD-1^[59,60]. Interestingly, PD-1^{high} B cells in HCC tissues suppressed CD8⁺ T-cell anticancer immunity by secreting IL-10^[61]. Moreover, PD-1⁺ dendritic cells (DCs) in HCC tissues also suppressed CD8⁺ T-cell anticancer immunity^[62]. In addition, PD-1 ligands are associated with aggressiveness and recurrence of HCC^[63,64]. Wu *et al.*^[65] reported that PD-L1 on Kupffer cell blocks CD8⁺ T-cell anti-HCC activity. Besides, hepatoma cell-expressed PD-L1 induces apoptosis of CD8⁺ T cells and promotes HCC recurrence^[66]. Additionally, PD-L1-expressing monocytes induce polarization of Th22 cells through PD-1 in HCC tissues^[67]. PD-L1 on intratumoral hepatic stellate cells or peritumoral neutrophils also contributes to the impairment of T cell-mediated anti-HCC immunity^[68,69]. These findings indicate that blockade of PD-1/PD-L might be promising immunotherapy for HCC. Nivolumab and pembrolizumab gained approval for treatment of advanced HCC based on encouraging results from phase I/II studies in advanced HCC patients with objective response rates of 17%-20%^[70,71]. However, results from two phase III clinical studies did not reveal statistically significant improvement in survival benefit^[72,73]. There are several ongoing trials with monoclonal antibodies against PD-1, such as nivolumab, pembrolizumab, tislelizumab, and camrelizumab, in HCC patients, either as monotherapy or in combination with other treatments. A phase-I/II study reported that the combination of atezolizumab and bevacizumab resulted in a 62% response rate in advanced HCC patients^[74]. The breakthrough therapy of atezolizumab in combination with bevacizumab is recently approved as a first-line treatment for patients with advanced or metastatic HCC. Moreover, a randomized phase III study demonstrated superior overall survival and progression-free survival compared to sorafenib in the first-line treatment of advanced HCC^[75]. In addition, another antiangiogenic drug ramucirumab has been approved as a second-line therapy for advanced HCC following first-line therapy with sorafenib^[76]. The clinical efficacy from the combination of anti-PD-L1 with antiangiogenic agents encourages researchers to extensively develop novel combination strategies to improve the clinical efficacy of HCC treatment.

Anti-CTLA-4 antibody

CTLA-4 is expressed on Treg cells and activated T cells and inhibits T-cell activation by competing for CD80/CD86 with CD28^[77]. Liu *et al.*^[78] found that CTLA-4 polymorphism may have negative effects on HCC. CTLA-4⁺ Treg cells impair T cell-mediated anti-HCC immunity^[50]. HCC-derived Treg cells limit DCs function by CTLA-4^[79]. Furthermore, CTLA-4⁺ DCs suppress T cell-mediated anti-HCC immunity by IL-10 and IDO^[80]. Fortunately, CTLA-4 blockade with glucocorticoid-induced tumor necrosis factor receptor family-related protein (GITR) engagement completely abrogates Treg-mediated immunosuppression in

HCC^[81]. These findings indicate that CTLA-4 is a promising target for HCC treatment. Recently, the CTLA-4 blockade agent, ipilimumab, has been tested in clinical trials of HCC treatment, with a partial response rate of 18% and a disease control rate of 76%^[82]. Duffy *et al.*'s^[83] study demonstrated that tremelimumab could achieve a partial response rate of 26% and a disease control rate of 84%. Furthermore, the combination therapy of ipilimumab and nivolumab could achieve objective response rates of 31% and a median duration of 17.48 months in advanced HCC patients^[84]. As with other cancers, the combination regimens of nivolumab and ipilimumab led to grade 3-4 treatment-related adverse events occurring in 37.7% patients, which were more frequently observed with combination regimens than with single-agents, especially in patients who received higher dosages of ipilimumab. However, most of the adverse events were manageable with timely recognition, steroid treatment, and discontinuation of immunotherapy, with a very low rate of liver failure^[73,85,86].

Other immune checkpoint inhibitors

The binding of inhibitory killer-immunoglobulin-like receptors (KIRs) expressed on NK cells with HLA class I molecules inhibit the activation of NK cells^[87]. Antibodies against inhibitory KIRs enhance NK cell cytotoxicity. It was reported that KIR/HLA immunogenetic background influenced the evolution of HCC^[88,89]. Anti-KIR antibodies - IPH2101 and IPH2102 - were well tolerated in patients with relapsed multiple myeloma^[90,91]. However, little evidence emerged to confirm the efficacy of these anti-KIR antibodies against HCC. NKG2A is an inhibitor receptor expressed on both CD8⁺ T cells and NK cells^[92,93]. Therefore, anti-NKG2A mAb promoted anticancer immunity of both CD8⁺ T and NK cells^[94,95]. It has been shown that NKG2A mediated NK-cell exhaustion in patients with HCC^[22]. However, the effect of anti-NKG2A mAb on HCC needs to be confirmed in clinical trials. Increasing inhibitory receptors such as IL-1R8, TIM3, TIGIT, and CD96 have been found to be important for regulating NK cell activity against tumors^[96-100]. Clinical trials have been performed to evaluate the efficiency of antibodies against these checkpoints for cancer therapy. The efficacy of NK cell-based checkpoint inhibitors in HCC needs further preclinical and clinical studies.

Combination therapy with immune checkpoint inhibitors

Immune checkpoint blockade leads to recovery of immune response against HCC cells and suppression of tumor growth in HCC. However, most HCC patients still do not achieve clinical benefit from ICI immunotherapy, highlighting the need for creative strategies to improve therapeutic efficacy. First, novel checkpoints need to be identified in HCC. For example, B and T cell lymphocyte attenuator has been found to participate in suppressing CD4⁺ T cell function in HCC^[101]. Siglec-15 has been confirmed to be an immune suppressor and displays promising efficacy in cancer immunotherapy^[102]. The roles of novel checkpoints in HCC have not been addressed. Second, novel combinations of checkpoints need to be designed. Zhou *et al.*^[42] found that T cells isolated from HCC tissue expressed high levels of PD-1, CTLA4, TIM3, and LAG3, suggesting the involvement of multiple checkpoints in T-cell exhaustion in HCC. The efficacy achieved by combining blockade of checkpoints was better than that by single checkpoint alone^[42]. Therefore, a combination of ICIs might achieve better results than just monotherapy for HCC treatment. Moreover, the individualized combination for HCC patients can be designed based on omics-data to achieve precision medicine. Third, novel comprehensive combination needs to be tested. Wehrenberg-Klee *et al.*^[103] reported that combining radioembolization with nivolumab could enhance the ICI-induced anticancer immune response. Shigeta *et al.*^[104] found that dual anti-PD-1/VEGFR-2 therapy enhanced CD8⁺ cytotoxic T cell anticancer immune response in HCC. PD-1/PD-L1 double blockade increased anticancer immune response of vaccine-induced CD8⁺ T cells in advanced HCC patients^[105]. To improve the benefits of ICI, it is necessary to integrate ICI therapy with targeted agents, locoregional therapy, vaccines, or other forms of therapy. Of note, the clinical outcomes of such integration require further investigation in future studies.

Adoptive cell transfer

NK cells and CD8⁺ T cells eliminate cancer cells by direct cytotoxicity. In advanced HCC, the scarcity of NK cells and CD8⁺ T cells in HCC tissues eliminates the ICI-induced anticancer efficacy. In this setting, it is absolutely necessary to adaptively transfer cytotoxic immune cells into patients with advanced HCC.

NK cell therapy

NK cells have potent anticancer capacity. HLA class I molecule-independent activation endows NK cells with more potential for extensive applications. HLA class I molecules block the NK cell killing through interaction with KIRs or CD94/NKG2A/B on NK cells^[106]. Meanwhile, stress-induced ligands on cancer cells can activate NK cells by interacting with activation receptors on them^[107]. However, NK cell function is impaired and hardly restored in advanced cancers. Hence, adoptive transfer of NK cells is a valuable option for cancer therapy. However, adoptive transfer of autologous lymphokine-activated NK cells with IL-2 into patients with metastatic cancer led to a poor clinical outcome^[108], which might be attributed to high levels of HLA class I molecules on cancer cells and the exhausted function of patients' NK cells. To overcome these defects, allogeneic NK cells - especially allogeneic haploidentical NK cells - are harnessed to treat various malignancies^[109]. Encouraging clinical efficacy has been observed in trials of acute myeloid leukemia^[110]. Moreover, cryoablation combined with allogeneic NK cell therapy markedly improved the progression-free survival of patients with advanced HCC^[111]. Besides autologous and allogeneic NK cells, NK-92 cells, an NK cell line, is also used in clinical trials of cancer therapy, with encouraging results observed in patients with advanced lung cancer^[112]. To enhance the targetability of NK cells, Chimeric antigen receptor (CAR)-NK cells have also been developed and pre-clinically evaluated. NK-92 cells with CD19-CAR display potent ability to kill CD19⁺ leukemia cell lines and lymphoblasts from patients with leukemia^[113]. NK-92 cells with GPC3-CAR show significant *in vitro* cytotoxicity to GPC3⁺ HCC cells and potent anticancer activity in HCC xenografts^[114]. Accumulating evidence indicates that NK cell therapy is a potential approach for HCC treatment with technical improvements in the activation and expansion of NK cells.

Cytokine-induced killer cell adjuvant therapy

Cytokine-induced killer (CIK) cells generated from blood mononuclear cells cultured with IFN- γ , anti-CD3, and IL-2 show potent anticancer activity^[115]. Jia *et al.*^[116] reported that CIK cells improved overall survival in HCC. CIK cell adjuvant therapy also reduces the recurrence in HCC patients undergoing curative treatment^[117]. Lee *et al.*^[118] found that the efficacy of CIK cells in patients with HCC lasted over 5 years. Chang *et al.*^[119] reported that the high number of PD-1⁺ tumor infiltrating lymphocytes could predict the response and clinical benefits of CIK cell adjuvant immunotherapy in HCC patients. Pan *et al.*^[120] reported that CIK cell cytotoxicity is a predictive biomarker for adjuvant CIK cell immunotherapy of HCC patients after surgery. Collectively, increasing evidence suggests that CIK cell-based adjuvant immunotherapy shows modest efficacy in early-stage HCC. Although Wang *et al.*^[121] showed that intraperitoneal perfusion of CIK cells with local hyperthermia was safe for patients with advanced HCC, more clinical data on the efficacy of CIK cell therapy in advanced HCC is currently lacking. Further detailed studies on the characteristics of CIK cells and their recognition and effector function are required to improve the clinical outcomes of CIK cell adjuvant immunotherapy in HCC.

CAR-redirected T cell therapy

CAR-T cells have shown tremendous clinical efficacy in the therapy of hematological malignancies^[122]. Moreover, CAR-T cell therapy is expected to convert cold tumors into hot tumors, which represents a promising immunotherapeutic option for HCC treatment. Glypican-3 (GPC3) is a membrane heparan sulfate and is highly expressed in HCC tissues^[123,124]. Unfortunately, the GPC3-targeted antibody - GC33 - was unsuccessful in bringing about clinical benefit to patients with HCC^[125]. However, the anti-GPC3/anti-CD3 bispecific antibody - ERY974 - could activate T cells and convert the microenvironment of a cold tumor to that of a hot one^[126]. Therefore, GPC3 is a promising target of CAR-T cells in HCC. Indeed, GPC3-CAR-T cells could eliminate GPC3⁺ HCC cells and tumors in a patient-derived xenograft model^[127]. GPC3-CAR-T therapies have been registered for clinical trials. To overcome T-cell exhaustion induced by checkpoints, an enhanced version of CAR-T cells is being currently designed. For instance, PD-1 is disrupted via CRISPR/Cas9 to enhance the activity of GPC3-CAR T cells against HCC^[128]. A soluble PD-1-CH3 fusion protein is expressed to increase anticancer activities of GPC3-CAR-T cells^[129]. Co-expressing GPC3-CAR and co-

stimulatory molecule ICOSL-41BB promotes CAR-T cell proliferation and tumor rejection^[130]. Besides GPC3, MUC-1, EpCAM, AFP, and CEA might be potential targets of CAR T cells for HCC treatment, which have been registered for clinical trials on the applicability of CAR-T therapy as a treatment strategy for HCC^[131]. Moreover, these classical tumor-associated antigens and ligands for receptors expressed on T cells also act as the targets for HCC recognition. For instance, NKG2D-based CAR-T cells could potentially eliminate NKG2DL⁺ HCC cells^[132]. A CD147-targeted inducible CAR-T cell system has been developed for HCC treatment^[133]. Although clinical trials of CAR-T therapy against HCC have not been completed, CAR-T therapy might provide effective therapeutic modalities for HCC treatment. Nonetheless, to date, the therapeutic efficacy of CAR-T cells remains limited owing to the lack of cancer-specific targets, weak expansion, poor infiltration, and induced exhaustion of CAR-T cells. Hence, smarter optimization strategies and more clinical trials are required for the confirmation and improvement of clinical outcomes of CAR-T cells in HCC treatment.

T cell receptor-genetically engineered T cell therapy

The success of T cell receptor-genetically engineered T (TCR-T) cells in melanoma treatment has encouraged the use of TCR-T cells in HCC treatment. Autologous T cells forced to express an HBV-specific TCR recognized HBsAg⁺ HCC cells and decreased HBsAg levels in a patient who underwent liver transplant^[134]. T cells genetically engineered with HCV NS3:1406-1415-reactive TCR recognized the naturally processed antigen and led to suppression of HCV⁺ HCC *in vivo*^[135]. T cells genetically engineered with AFP-specific TCR specifically recognized and killed AFP⁺ HepG2 cells, both *in vitro* and *in vivo*^[136]. Although there remain many challenges such as off-target cross-reactivity and low TCR affinity that need to be overcome before successful translation into clinical practice^[137,138], increasing findings suggest that TCR-T therapy might be an attractive alternative immunotherapeutic modality for HCC treatment.

DC-vaccines adjuvant immunotherapy

Briefly, DCs are professional antigen-presenting cells with the capacity to prime antigen-specific T-cell immunity. DC vaccines are recognized as promising agents for activating T cells to eliminate cancer cells; their role and functions have been evaluated in some malignancies in clinical trials, including HCC. A phase II study using intravenous vaccination with DCs pulsed with HepG2 lysate was found to be safe and showed evidence of anticancer efficacy in some patients with advanced HCC^[139]. Another phase I/II study reported that vaccination with DCs pulsed with AFP peptides induced strong T-cell immunity against AFP but no clinical responses in HCC patients^[140]. Other phase I/IIa studies also reported that subcutaneous vaccination with DC pulsed with multiple antigens such as AFP, glypican-3 (GPC-3), and melanoma-associated antigen 1 (MAGE-1) enhanced anticancer immunity and prolonged time-to-recurrence and recurrence-free survival in HCC patients^[141-143]. Interestingly, Lu *et al.*^[144] showed that exosomes derived from AFP-expressing DCs elicited potent anticancer immune responses and cancer regression in HCC mice, thus providing a novel option for vaccine-based immunotherapy of HCC. Pang *et al.*^[145] reported that DCs fused with cancer stem cells could efficiently stimulate T lymphocytes to generate specific CD8 T cells against cancer stem cells. Collectively, several studies indicate that DC vaccine-based adjuvant therapy enhances anticancer immunity and improves the survival of patients with HCC. Nonetheless, further improvements such as specific immunogenic neoantigens for HCC, safe and feasible DC source, potent adjuvant, and access to vaccination are required for future success of DC-based HCC immunotherapy.

Combination therapy of immune checkpoint blockade and adoptive cell transfer

The existence of cancer immunosuppressive microenvironment limits the effector function of adoptive immune cells. Therefore, it is reasonable to improve the cancer immunosuppressive microenvironment to enhance the curative efficacy of adoptive immune cells on HCC. Kodumudi *et al.*^[146] reported that adoptive transfer of tumor infiltrating lymphocytes from tumors with anti-PD-L1 antibody treatment led to a significant delay in tumor growth, suggesting that pretreatment with immune checkpoint blockade could be

an effective strategy to improve tumor infiltrating lymphocyte infiltration and function. Moreover, CAR-T cell therapy in combination with PD-1 blockade overcomes PD-L1-mediated cancer immunosuppression and leads to enhanced therapeutic efficacy^[147]. The combination of CTLA-4 blockade with ACT also generates greater therapeutic efficacy^[148]. Besides systemic delivery of checkpoint blockade, the knockout of immune checkpoint in CAR-T cells by gene-editing technologies improves anti-tumor efficacy of CAR-T cells in various cancer models by enhancing effector function and survival of T-cells^[149]. It is understood that the immunosuppressive trait of the HCC microenvironment requires combinational therapeutic modalities for effective outcome^[150]. These findings support combination immunotherapy with immune checkpoint blockade and ACT for cancers, which is expected to result in greater anticancer immune response than with either intervention alone.

CONCLUSION

Following significant therapeutic progress made on the basis of basic research, immunological studies offer a new era of clinical application. Immunotherapy brings a new hope to depressed patients with chronic infection, autoimmune disease, or cancers. More importantly, cytotoxic immune cell-based immunotherapy has markedly improved survival in patients with advanced cancers. The high mortality of patients with advanced HCC owing to resistance to chemotherapy highlights the importance and value of immunotherapy in HCC treatment, although the clinical efficiency has not been as promising as expected. The development of novel ICIs, cytokines, tumor-specific antigens, gene-modified/CAR NK cells, and TCR/CAR CD8⁺ T cells is expected to improve the curative effect. Furthermore, the flexible combination of immunotherapy and other therapies might offer the much required breakthrough in clinical efficacy of HCC treatment.

DECLARATIONS

Authors' contributions

Contributed to conception and design of the study and manuscript writing: Li J, Tao L, Wang X
Final approval of manuscripts: Li J, Tao L, Wang X

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable

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REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359-86.
2. Gosalia AJ, Martin P, Jones PD. Advances and future directions in the treatment of hepatocellular carcinoma. *Gastroenterol Hepatol (N Y)* 2017;13:398-410.
3. Raoul JL, Kudo M, Finn RS, Edeline J, Reig M, et al. Systemic therapy for intermediate and advanced hepatocellular carcinoma: sorafenib and beyond. *Cancer Treat Rev* 2018;68:16-24.
4. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018;391:1163-73.
5. Bruix J, Qin S, Merle P, Granito A, Huang YH, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;389:56-66.
6. Finn RS, Merle P, Granito A, Huang YH, Bodoky G, et al. Outcomes of sequential treatment with sorafenib followed by regorafenib for HCC: additional analyses from the phase III RESORCE trial. *J Hepatol* 2018;69:353-8.
7. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N Engl J Med* 2018;379:54-63.
8. Racanelli V, Rehermann B. The liver as an immunological organ. *Hepatology* 2006;43:S54-62.
9. Crispe IN. The liver as a lymphoid organ. *Ann Rev Immunol* 2009;27:147-63.
10. Heymann F, Tacke F. Immunology in the liver--from homeostasis to disease. *Nat Rev Gastroenterol Hepatol* 2016;13:88-110.
11. Kubes P, Jenne C. Immune Responses in the Liver. *Ann Rev Immunol* 2018;36:247-77.
12. Makarova-Rusher OV, Medina-Echeverez J, Duffy AG, Greten TF. The yin and yang of evasion and immune activation in HCC. *J Hepatol* 2015;62:1420-9.
13. Lu C, Rong D, Zhang B, Zheng W, Wang X, et al. Current perspectives on the immunosuppressive tumor microenvironment in hepatocellular carcinoma: challenges and opportunities. *Mol Cancer* 2019;18:130.
14. Cherrier DE, Serafini N, Di Santo JP. Innate lymphoid cell development: a T cell perspective. *Immunity* 2018;48:1091-103.
15. Peng H, Tian Z. Re-examining the origin and function of liver-resident NK cells. *Trends Immunol* 2015;36:293-9.
16. Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. *Nat Rev Immunol* 2018;18:671-88.
17. Martinet L, Smyth MJ. Balancing natural killer cell activation through paired receptors. *Nat Rev Immunol* 2015;15:243-54.
18. Pegram HJ, Andrews DM, Smyth MJ, Darcy PK, Kershaw MH. Activating and inhibitory receptors of natural killer cells. *Immunol Cell Biol* 2011;89:216-24.
19. Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: integration of signals for activation and inhibition. *Ann Rev Immunol* 2013;31:227-58.
20. Cai L, Zhang Z, Zhou L, Wang H, Fu J, et al. Functional impairment in circulating and intrahepatic NK cells and relative mechanism in hepatocellular carcinoma patients. *Clin Immunol* 2008;129:428-37.
21. Sun C, Sun H, Zhang C, Tian Z. NK cell receptor imbalance and NK cell dysfunction in HBV infection and hepatocellular carcinoma. *Cell Mol Immunol* 2015;12:292-302.
22. Sun C, Xu J, Huang Q, Huang M, Wen H, et al. High NKG2A expression contributes to NK cell exhaustion and predicts a poor prognosis of patients with liver cancer. *Oncoimmunology* 2017;6:e1264562.
23. Sun H, Huang Q, Huang M, Wen H, Lin R, et al. Human CD96 correlates to natural killer cell exhaustion and predicts the prognosis of human hepatocellular carcinoma. *Hepatology* 2019;70:168-83.
24. Zheng X, Qian Y, Fu B, Jiao D, Jiang Y, et al. Mitochondrial fragmentation limits NK cell-based tumor immunosurveillance. *Nat Immunol* 2019;20:1656-67.
25. Hoechst B, Voigtlaender T, Ormandy L, Gamrekashvili J, Zhao F, et al. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the Nkp30 receptor. *Hepatology* 2009;50:799-807.
26. Wu Y, Kuang DM, Pan WD, Wan YL, Lao XM, et al. Monocyte/macrophage-elicited natural killer cell dysfunction in hepatocellular carcinoma is mediated by CD48/2B4 interactions. *Hepatology* 2013;57:1107-16.
27. Li T, Yang Y, Hua X, Wang G, Liu W, et al. Hepatocellular carcinoma-associated fibroblasts trigger NK cell dysfunction via PGE2 and IDO. *Cancer Lett* 2012;318:154-61.
28. Tian Z, Chen Y, Gao B. Natural killer cells in liver disease. *Hepatology* 2013;57:1654-62.
29. Peng H, Jiang X, Chen Y, Sojka DK, Wei H, et al. Liver-resident NK cells confer adaptive immunity in skin-contact inflammation. *J Clin Invest* 2013;123:1444-56.
30. Nakano R, Ohira M, Yano T, Imaoka Y, Tanaka Y, et al. Hepatic irradiation persistently eliminates liver resident NK cells. *PloS One* 2018;13:e0198904.
31. Stegmann KA, Robertson F, Hansi N, Gill U, Pallant C, et al. CXCR6 marks a novel subset of T-bet(lo)Eomes(hi) natural killer cells residing in human liver. *Sci Rep* 2016;6:26157.
32. Bai L, Peng H, Hao X, Tang L, Sun C, et al. CD8⁺ T cells promote maturation of liver-resident NK cells through the CD70-CD27 axis. *Hepatology* 2019;70:1804-15.
33. Harmon C, Jameson G, Almuaili D, Houlihan DD, Hoti E, et al. Liver-derived TGF-beta maintains the Eomes(hi)Tbet(lo) phenotype of liver resident natural killer cells. *Front Immunol* 2019;10:1502.
34. Zhou J, Peng H, Li K, Qu K, Wang B, et al. Liver-resident NK cells control antiviral activity of hepatic T cells via the PD-1-PD-L1 axis. *Immunity* 2019;50:403-17.e4.
35. Zhao ZB, Lu FT, Ma HD, Wang YH, Yang W, et al. Liver-resident NK cells suppress autoimmune cholangitis and limit the

- proliferation of CD4⁺ T cells. *Cell Mol Immunol* 2019;17:178-89.
36. Hwang S, Han J, Baek JS, Tak E, Song GW, et al. Cytotoxicity of human hepatic intrasinusoidal CD56^{bright} natural killer cells against hepatocellular carcinoma cells. *Int J Mol Sci* 2019;20:1564.
 37. Easom NJW, Stegmann KA, Swadling L, Burton AR, et al. IL-15 overcomes hepatocellular carcinoma-induced NK cell dysfunction. *Front Immunol* 2018;9:1009.
 38. Sun H, Liu L, Huang Q, Liu H, Huang M, et al. Accumulation of tumor-infiltrating CD49a⁺ NK cells correlates with poor prognosis for human hepatocellular carcinoma. *Cancer Immunol Res* 2019;7:1535-46.
 39. Saparbay J, Tanaka Y, Tanimine N, Ohira M, Ohdan H. Everolimus enhances TRAIL-mediated anti-tumor activity of liver resident natural killer cells in mice. *Transpl Int* 2020;33:229-43.
 40. Gabrielson A, Wu Y, Wang H, Jiang J, Kallakury B, et al. Intratumoral CD3 and CD8 T-cell densities associated with relapse-free survival in HCC. *Cancer Immunol Res* 2016;4:419-30.
 41. Kalathil SG, Hutson A, Barbi J, Iyer R, Thanavala Y. Augmentation of IFN-gamma+ CD8+ T cell responses correlates with survival of HCC patients on sorafenib therapy. *JCI Insight* 2019;4.
 42. Zhou G, Sprengers D, Boor PPC, Doukas M, Schutz H, et al. Antibodies against immune checkpoint molecules restore functions of tumor-infiltrating T cells in hepatocellular carcinomas. *Gastroenterology* 2017;153:1107-19.e10.
 43. Wang X, He Q, Shen H, Lu XJ, Sun B. Genetic and phenotypic difference in CD8⁺ T cell exhaustion between chronic hepatitis B infection and hepatocellular carcinoma. *J Med Genet* 2019;56:18-21.
 44. Kim HD, Park S, Jeong S, Lee YJ, Lee H, et al. 4-1BB delineates distinct activation status of exhausted tumor-infiltrating CD8⁺ T cells in hepatocellular carcinoma. *Hepatology* 2020;71:955-71.
 45. Miller BC, Sen DR, Al Abosy R, Bi K, Virkud YV, et al. Subsets of exhausted CD8⁺ T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat Immunol* 2019;20:326-36.
 46. Siddiqui I, Schaeuble K, Chennupati V, Fuentes Marraco SA, Calderon-Copete S, et al. Intratumoral Tcf1⁺ PD-1⁺ CD8⁺ T cells with stem-like properties promote tumor control in response to vaccination and checkpoint blockade immunotherapy. *Immunity* 2019;50:195-211.e10.
 47. Yang YM, Kim SY, Seki E. Inflammation and liver cancer: molecular mechanisms and therapeutic targets. *Semin Liver Dis* 2019;39:26-42.
 48. Lim CJ, Lee YH, Pan L, Lai L, Chua C, et al. Multidimensional analyses reveal distinct immune microenvironment in hepatitis B virus-related hepatocellular carcinoma. *Gut* 2019;68:916-27.
 49. Yarchoan M, Xing D, Luan L, Xu H, Sharma RB, et al. Characterization of the immune microenvironment in hepatocellular carcinoma. *Clin Cancer Res* 2017;23:7333-9.
 50. Kalathil S, Lugade AA, Miller A, Iyer R, Thanavala Y. Higher frequencies of GARP⁺ CTLA-4⁺ Foxp3⁺ T regulatory cells and myeloid-derived suppressor cells in hepatocellular carcinoma patients are associated with impaired T-cell functionality. *Cancer Res* 2013;73:2435-44.
 51. Yang L, Shao X, Jia S, Zhang Q, Jin Z. Interleukin-35 dampens CD8⁺ T cells activity in patients with non-viral hepatitis-related hepatocellular carcinoma. *Front Immunol* 2019;10:1032.
 52. Huang XY, Ke AW, Shi GM, Zhang X, Zhang C, et al. alphaB-crystallin complexes with 14-3-3zeta to induce epithelial-mesenchymal transition and resistance to sorafenib in hepatocellular carcinoma. *Hepatology* 2013;57:2235-47.
 53. Wang X, Shen H, Zhangyuan G, Huang R, Zhang W, et al. 14-3-3zeta delivered by hepatocellular carcinoma-derived exosomes impaired anti-tumor function of tumor-infiltrating T lymphocytes. *Cell Death Dis* 2018;9:159.
 54. Aliahmad P, Seksenyan A, Kaye J. The many roles of TOX in the immune system. *Curr Opin Immunol* 2012;24:173-7.
 55. Seo H, Chen J, González-Avalos E, Samaniego-Castruita D, Das A, et al. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8⁺ T cell exhaustion. *Proc Natl Acad Sci U S A* 2019;116:12410-5.
 56. Scott AC, Dündar F, Zumbo P, Chandran SS, Klebanoff CA, et al. TOX is a critical regulator of tumour-specific T cell differentiation. *Nature* 2019;571:270-4.
 57. Wang X, He Q, Shen H, Xia A, Tian W, et al. TOX promotes the exhaustion of antitumor CD8⁺ T cells by preventing PD1 degradation in hepatocellular carcinoma. *J Hepatol* 2019;71:731-41.
 58. Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, et al. Defining CD8⁺ T cells that provide the proliferative burst after PD-1 therapy. *Nature* 2016;537:417-21.
 59. Gehring AJ, Ho ZZ, Tan AT, Aung MO, Lee KH, et al. Profile of tumor antigen-specific CD8 T cells in patients with hepatitis B virus-related hepatocellular carcinoma. *Gastroenterology* 2009;137:682-90.
 60. Hsu PN, Yang TC, Kao JT, Cheng KS, Lee YJ, et al. Increased PD-1 and decreased CD28 expression in chronic hepatitis B patients with advanced hepatocellular carcinoma. *Liver Int* 2010;30:1379-86.
 61. Xiao X, Lao XM, Chen MM, Liu RX, Wei Y, et al. PD-1hi Identifies a novel regulatory B-cell population in human hepatoma that promotes disease progression. *Cancer Discov* 2016;6:546-59.
 62. Lim TS, Chew V, Sieow JL, Goh S, Yeong JP, et al. PD-1 expression on dendritic cells suppresses CD8⁺ T cell function and antitumor immunity. *Oncoimmunology* 2016;5:e1085146.
 63. Gao Q, Wang XY, Qiu SJ, Yamato I, Sho M, et al. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. *Clin Cancer Res* 2009;15:971-9.
 64. Jung HI, Jeong D, Ji S, Ahn TS, Bae SH, et al. Overexpression of PD-L1 and PD-L2 is associated with poor prognosis in patients with hepatocellular carcinoma. *Cancer Res Treat* 2017;49:246-54.
 65. Wu K, Kryczek I, Chen L, Zou W, Welling TH. Kupffer cell suppression of CD8⁺ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. *Cancer Res* 2009;69:8067-75.
 66. Shi F, Shi M, Zeng Z, Qi RZ, Liu ZW, et al. PD-1 and PD-L1 upregulation promotes CD8(+) T-cell apoptosis and postoperative

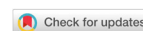
- recurrence in hepatocellular carcinoma patients. *Int J Cancer* 2011;128:887-96.
67. Kuang DM, Xiao X, Zhao Q, Chen MM, Li XF, et al. B7-H1-expressing antigen-presenting cells mediate polarization of protumorigenic Th22 subsets. *J Clin Invest* 2014;124:4657-67.
68. Xia Y, Chen R, Ye SL, Sun R, Chen J, et al. Inhibition of T-cell responses by intratumoral hepatic stellate cells contribute to migration and invasion of hepatocellular carcinoma. *Clin Exp Metastasis* 2011;28:661-74.
69. He G, Zhang H, Zhou J, Wang B, Chen Y, et al. Peritumoral neutrophils negatively regulate adaptive immunity via the PD-L1/PD-1 signalling pathway in hepatocellular carcinoma. *J Exp Clin Cancer Res* 2015;34:141.
70. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
71. Zhu AX, Finn RS, Edeline J, Cattani S, Ogasawara S, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018;19:940-52.
72. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, et al. Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: a randomized, double-blind, Phase III trial. *J Clin Oncol* 2020;38:193-202.
73. Cheng AL, Hsu C, Chan SL, Choo SP, Kudo M. Challenges of combination therapy with immune checkpoint inhibitors for hepatocellular carcinoma. *J Hepatol* 2020;72:307-19.
74. Floudas CS, Brar G, Greten TF. Immunotherapy: current status and future perspectives. *Dig Dis Sci* 2019;64:1030-40.
75. Reck M, Mok TSK, Nishio M, Jotte RM, Cappuzzo F, et al. Atezolizumab plus bevacizumab and chemotherapy in non-small-cell lung cancer (IMpower150): key subgroup analyses of patients with EGFR mutations or baseline liver metastases in a randomised, open-label phase 3 trial. *Lancet Respir Med* 2019;7:387-401.
76. Chau I, Peck-Radosavljevic M, Borg C, Malfertheiner P, Seitz JF, et al. Ramucirumab as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib: patient-focused outcome results from the randomised phase III REACH study. *Eur J Cancer* 2017;81:17-25.
77. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med* 1995;182:459-65.
78. Liu Z, Song Z, Sun J, Sun F, Li C, et al. Association between CTLA-4 rs231775 polymorphism and hepatocellular carcinoma susceptibility. *Int J Clin Exp Pathol* 2015;8:15118-22.
79. Chen X, Du Y, Hu Q, Huang Z. Tumor-derived CD4⁺CD25⁺regulatory T cells inhibit dendritic cells function by CTLA-4. *Pathol Res Pract* 2017;213:245-9.
80. Han Y, Chen Z, Yang Y, Jiang Z, Gu Y, et al. Human CD14⁺ CTLA-4⁺ regulatory dendritic cells suppress T-cell response by cytotoxic T-lymphocyte antigen-4-dependent IL-10 and indoleamine-2,3-dioxygenase production in hepatocellular carcinoma. *Hepatology* 2014;59:567-79.
81. Pedroza-Gonzalez A, Zhou G, Singh SP, Boor PP, Pan Q, et al. GITR engagement in combination with CTLA-4 blockade completely abrogates immunosuppression mediated by human liver tumor-derived regulatory T cells ex vivo. *Oncoimmunology* 2015;4:e1051297.
82. Sangro B, Gomez-Martin C, de la Mata M, Iñarrairaegui M, Garralda E, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol* 2013;59:81-8.
83. Duffy AG, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J Hepatol* 2017;66:545-51.
84. Yau T, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, et al. Nivolumab (NIVO) + ipilimumab (IPI) combination therapy in patients (pts) with advanced hepatocellular carcinoma (aHCC): results from CheckMate 040. *J Clin Oncol* 2019;37:4012.
85. Kaseb A, Vence L, Blando J, Yadav S, Ikoma N, et al. Randomized, open-label, perioperative phase II study evaluating nivolumab alone versus nivolumab plus ipilimumab in patients with resectable HCC. *Ann Oncol* 2019;30:iv112.
86. Hellmann MD, Paz-Ares L, Bernabe Caro R, Zurawski B, Kim SW, et al. Nivolumab plus ipilimumab in advanced non-small-cell lung cancer. *N Engl J Med* 2019;381:2020-31.
87. Purdy AK, Campbell KS. Natural killer cells and cancer: regulation by the killer cell Ig-like receptors (KIR). *Cancer Biol Ther* 2009;8:2211-20.
88. Cariani E, Missale G. KIR/HLA immunogenetic background influences the evolution of hepatocellular carcinoma. *Oncoimmunology* 2013;2:e26622.
89. Pan N, Qiu J, Sun H, Miao F, Shi Q, et al. Combination of human leukocyte antigen and killer cell immunoglobulin-like receptor genetic background influences the onset age of hepatocellular carcinoma in male patients with hepatitis B virus infection. *Clin Dev Immunol* 2013;2013:874514.
90. Vey N, Karlin L, Sadot-Lebouvier S, Broussais F, Berton-Rigaud D, et al. A phase 1 study of lirilumab (antibody against killer immunoglobulin-like receptor antibody KIR2D; IPH2102) in patients with solid tumors and hematologic malignancies. *Oncotarget* 2018;9:17675-88.
91. Benson DM Jr, Hofmeister CC, Padmanabhan S, Suvannasankha A, Jagannath S, et al. A phase 1 trial of the anti-KIR antibody IPH2101 in patients with relapsed/refractory multiple myeloma. *Blood* 2012;120:4324-33.
92. van Montfoort N, Borst L, Korner MJ, Sluijter M, Marijt KA, et al. NKG2A blockade potentiates CD8 T cell immunity induced by cancer vaccines. *Cell* 2018;175:1744-55.e15.
93. Xu HC, Huang J, Pandya AA, Lang E, Zhuang Y, et al. Lymphocytes negatively regulate NK cell activity via Qa-1b following viral infection. *Cell Rep* 2017;21:2528-40.
94. André P, Denis C, Soulas C, Bourbon-Caillet C, Lopez J, et al. Anti-NKG2A mAb is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both T and NK cells. *Cell* 2018;175:1731-43.e13.
95. Kamiya T, Seow SV, Wong D, Robinson M, Campana D. Blocking expression of inhibitory receptor NKG2A overcomes tumor resistance to NK cells. *J Clin Invest* 2019;129:2094-106.

96. Zhang Q, Bi J, Zheng X, Chen Y, Wang H, et al. Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. *Nat Immunol* 2018;19:723-32.
97. Stanitsky N, Simic H, Arapovic J, Toporik A, Levy O, et al. The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc Natl Acad Sci U S A* 2009;106:17858-63.
98. Molgora M, Bonavita E, Ponzetta A, Riva F, Barbagallo M, et al. IL-1R8 is a checkpoint in NK cells regulating anti-tumour and anti-viral activity. *Nature* 2017;551:110-4.
99. Ndhlovu LC, Lopez-Vergès S, Barbour JD, Jones RB, Jha AR, et al. Tim-3 marks human natural killer cell maturation and suppresses cell-mediated cytotoxicity. *Blood* 2012;119:3734-43.
100. Chan CJ, Martinet L, Gilfillan S, Souza-Fonseca-Guimaraes F, Chow MT, et al. The receptors CD96 and CD226 oppose each other in the regulation of natural killer cell functions. *Nat Immunol* 2014;15:431-8.
101. Zhao Q, Huang ZL, He M, Gao Z, Kuang DM. BTLA identifies dysfunctional PD-1-expressing CD4⁺ T cells in human hepatocellular carcinoma. *Oncoimmunology* 2016;5:e1254855.
102. Wang J, Sun J, Liu LN, Flies DB, Nie X, et al. Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy. *Nat Med* 2019;25:656-66.
103. Wehrenberg-Klee E, Goyal L, Dugan M, Zhu AX, Ganguli S. Y-90 radioembolization combined with a PD-1 inhibitor for advanced hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 2018;41:1799-802.
104. Shigeta K, Datta M, Hato T, Kitahara S, Chen IX, et al. Dual programmed death receptor-1 and vascular endothelial growth factor receptor-2 blockade promotes vascular normalization and enhances antitumor immune responses in hepatocellular carcinoma. *Hepatology* 2019.
105. Sawada Y, Yoshikawa T, Shimomura M, Iwama T, Endo I, et al. Programmed death-1 blockade enhances the antitumor effects of peptide vaccine-induced peptide-specific cytotoxic T lymphocytes. *Int J Oncol* 2015;46:28-36.
106. Ljunggren HG, Kärre K. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today* 1990;11:237-44.
107. Shiffrin N, Raulet DH, Ardolino M. NK cell self tolerance, responsiveness and missing self recognition. *Semin Immunol* 2014;26:138-44.
108. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N Engl J Med* 1985;313:1485-92.
109. Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* 2005;105:3051-7.
110. Romee R, Rosario M, Berrien-Elliott MM, Wagner JA, Jewell BA, et al. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Sci Transl Med* 2016;8:357ra123.
111. Lin M, Liang S, Wang X, Liang Y, Zhang M, et al. Cryoablation combined with allogeneic natural killer cell immunotherapy improves the curative effect in patients with advanced hepatocellular cancer. *Oncotarget* 2017;8:81967-77.
112. Tonn T, Schwabe D, Klingemann HG, Becker S, Esser R, et al. Treatment of patients with advanced cancer with the natural killer cell line NK-92. *Cytotherapy* 2013;15:1563-70.
113. Romanski A, Uherek C, Bug G, Seifried E, Klingemann H, et al. CD19-CAR engineered NK-92 cells are sufficient to overcome NK cell resistance in B-cell malignancies. *J Cell Mol Med* 2016;20:1287-94.
114. Yu M, Luo H, Fan M, Wu X, Shi B, et al. Development of GPC3-specific chimeric antigen receptor-engineered natural killer cells for the treatment of hepatocellular carcinoma. *Mol Ther* 2018;26:366-78.
115. Xiao Z, Wang CQ, Feng JH, Zhou MH, Wang YZ, et al. Effectiveness and safety of chemotherapy with cytokine-induced killer cells in non-small cell lung cancer: a systematic review and meta-analysis of 32 randomized controlled trials. *Cytotherapy* 2019;21:125-47.
116. Jia CC, Chen YH, Cai XR, Li Y, Zheng XF, et al. Efficacy of cytokine-induced killer cell-based immunotherapy for hepatocellular carcinoma. *Am J Cancer Res* 2019;9:1254-65.
117. Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, et al. Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. *Gastroenterology* 2015;148:1383-91.e6.
118. Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, et al. Sustained efficacy of adjuvant immunotherapy with cytokine-induced killer cells for hepatocellular carcinoma: an extended 5-year follow-up. *Cancer Immunol Immunother* 2019;68:23-32.
119. Chang B, Shen L, Wang K, Jin J, Huang T, et al. High number of PD-1 positive intratumoural lymphocytes predicts survival benefit of cytokine-induced killer cells for hepatocellular carcinoma patients. *Liver Int* 2018;38:1449-58.
120. Pan QZ, Liu Q, Zhou YQ, Zhao JJ, Wang QJ, et al. CIK cell cytotoxicity is a predictive biomarker for CIK cell immunotherapy in postoperative patients with hepatocellular carcinoma. *Cancer Immunol Immunother* 2020; Epub ahead of print. doi:10.1007/s00262-020-02486-y
121. Wang XP, Xu M, Gao HF, Zhao JF, Xu KC. Intraperitoneal perfusion of cytokine-induced killer cells with local hyperthermia for advanced hepatocellular carcinoma. *World J Gastroenterol* 2013;19:2956-62.
122. Liu Y, Chen X, Han W, Zhang Y. Tisagenlecleucel, an approved anti-CD19 chimeric antigen receptor T-cell therapy for the treatment of leukemia. *Drugs Today (Barc)* 2017;53:597-608.
123. Zhu ZW, Friess H, Wang L, Abou-Shady M, Zimmermann A, et al. Enhanced glypican-3 expression differentiates the majority of hepatocellular carcinomas from benign hepatic disorders. *Gut* 2001;48:558-64.
124. Filmus J, Capurro M. Glypican-3: a marker and a therapeutic target in hepatocellular carcinoma. *FEBS J* 2013;280:2471-6.
125. Abou-Alfa GK, Puig O, Daniele B, Kudo M, Merle P, et al. Randomized phase II placebo controlled study of codrituzumab in previously treated patients with advanced hepatocellular carcinoma. *J Hepatol* 2016;65:289-95.
126. Ishiguro T, Sano Y, Komatsu SI, Kamata-Sakurai M, Kaneko A, et al. An anti-glypican 3/CD3 bispecific T cell-redirecting antibody for treatment of solid tumors. *Sci Transl Med* 2017;9:eal4291.
127. Gao H, Li K, Tu H, Pan X, Jiang H, et al. Development of T cells redirected to glypican-3 for the treatment of hepatocellular carcinoma. *Clin Cancer Res* 2014;20:6418-28.

128. Guo X, Jiang H, Shi B, Zhou M, Zhang H, et al. Disruption of PD-1 enhanced the anti-tumor activity of chimeric antigen receptor T cells against hepatocellular carcinoma. *Front Pharmacol* 2018;9:1118.
129. Pan Z, Di S, Shi B, Jiang H, Shi Z, et al. Increased antitumor activities of glypican-3-specific chimeric antigen receptor-modified T cells by coexpression of a soluble PD1-CH3 fusion protein. *Cancer Immunol Immunother* 2018;67:1621-34.
130. Hu W, Huang X, Huang X, Chen W, Hao L, et al. Chimeric antigen receptor modified T cell (CAR-T) co-expressed with ICOSL-41BB promote CAR-T proliferation and tumor rejection. *Biomed Pharmacother* 2019;118:109333.
131. Chen Y, E CY, Gong ZW, Liu S, Wang ZX, et al. Chimeric antigen receptor-engineered T-cell therapy for liver cancer. *Hepatobiliary Pancreat Dis Int* 2018;17:301-9.
132. Sun B, Yang D, Dai H, Liu X, Jia R, et al. Eradication of hepatocellular carcinoma by NKG2D-based CAR-T cells. *Cancer Immunol Res* 2019;7:1813-23.
133. Zhang RY, Wei D, Liu ZK, Yong YL, Wei W, et al. Doxycycline inducible chimeric antigen receptor T cells targeting CD147 for hepatocellular carcinoma therapy. *Front Cell Dev Biol* 2019;7:233.
134. Qasim W, Brunetto M, Gehring AJ, Xue SA, Schurich A, et al. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient. *J Hepatol* 2015;62:486-91.
135. Spear TT, Callender GG, Roszkowski JJ, Moxley KM, Simms PE, et al. TCR gene-modified T cells can efficiently treat established hepatitis C-associated hepatocellular carcinoma tumors. *Cancer Immunol Immunother* 2016;65:293-304.
136. Zhu W, Peng Y, Wang L, Hong Y, Jiang X, et al. Identification of alpha-fetoprotein-specific T-cell receptors for hepatocellular carcinoma immunotherapy. *Hepatology* 2018;68:574-89.
137. Spear TT, Evavold BD, Baker BM, Nishimura MI. Understanding TCR affinity, antigen specificity, and cross-reactivity to improve TCR gene-modified T cells for cancer immunotherapy. *Cancer Immunol Immunother* 2019;68:1881-9.
138. Tendeiro Rego R, Morris EC, Lowdell MW. T-cell receptor gene-modified cells: past promises, present methodologies and future challenges. *Cytotherapy* 2019;21:341-57.
139. Palmer DH, Midgley RS, Mirza N, Torr EE, Ahmed F, et al. A phase II study of adoptive immunotherapy using dendritic cells pulsed with tumor lysate in patients with hepatocellular carcinoma. *Hepatology* 2009;49:124-32.
140. Butterfield LH, Ribas A, Disette VB, Lee Y, Yang JQ, et al. A phase I/II trial testing immunization of hepatocellular carcinoma patients with dendritic cells pulsed with four alpha-fetoprotein peptides. *Clin Cancer Res* 2006;12:2817-25.
141. Tada F, Abe M, Hirooka M, Ikeda Y, Hiasa Y, et al. Phase I/II study of immunotherapy using tumor antigen-pulsed dendritic cells in patients with hepatocellular carcinoma. *Int J Oncol* 2012;41:1601-9.
142. Lee JH, Lee Y, Lee M, Heo MK, Song JS, et al. A phase I/IIa study of adjuvant immunotherapy with tumour antigen-pulsed dendritic cells in patients with hepatocellular carcinoma. *Br J Cancer* 2015;113:1666-76.
143. Lee JH, Tak WY, Lee Y, Heo MK, Song JS, et al. Adjuvant immunotherapy with autologous dendritic cells for hepatocellular carcinoma, randomized phase II study. *Oncoimmunology* 2017;6:e1328335.
144. Lu Z, Zuo B, Jing R, Gao X, Rao Q, et al. Dendritic cell-derived exosomes elicit tumor regression in autochthonous hepatocellular carcinoma mouse models. *J Hepatol* 2017;67:739-48.
145. Pang YB, He J, Cui BY, Xu S, Li XL, et al. A potential antitumor effect of dendritic cells fused with cancer stem cells in hepatocellular carcinoma. *Stem Cells Int* 2019;2019:5680327.
146. Kodumudi KN, Siegel J, Weber AM, Scott E, Sarnaik AA, et al. Immune checkpoint blockade to improve tumor infiltrating lymphocytes for adoptive cell therapy. *PloS One* 2016;11:e0153053.
147. John LB, Devaud C, Duong CP, Yong CS, Beavis PA, et al. Anti-PD-1 antibody therapy potentially enhances the eradication of established tumors by gene-modified T cells. *Clin Cancer Res* 2013;19:5636-46.
148. Mahvi DA, Meyers JV, Tatar AJ, Contreras A, Suresh M, et al. Ctl4-4 blockade plus adoptive T-cell transfer promotes optimal melanoma immunity in mice. *J Immunother* 2015;38:54-61.
149. Jung IY, Lee J. Unleashing the therapeutic potential of CAR-T cell therapy using gene-editing technologies. *Mol Cells* 2018;41:717-23.
150. Zhang R, Zhang Z, Liu Z, Wei D, Wu X, et al. Adoptive cell transfer therapy for hepatocellular carcinoma. *Front Med* 2019;13:3-11.

Original Article

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Treatment efficacy for patients with chronic hepatitis C and preexisting hepatocellular carcinoma by directly acting antivirals

Chia-Yen Dai^{1,4}, Chung-Feng Huang^{1,3,4}, Meng-Hsuan Hsieh^{1,2}, Ching-I Huang¹, Ming-Lun Yeh^{1,3}, Pei-Chien Tsai¹, Ching-Chih Lin¹, Meng-Szu Lee², Jeng-Fu Yang^{1,2}, Po-Yao Hsu¹, Yu-Ju Wei¹, Cheng-Ting Hsu¹, Po-Cheng Liang¹, Yi-Hung Lin¹, Jee-Fu Huang^{3,4}, Wan-Long Chuang^{3,4}, Ming-Lung Yu^{1,3,4}

¹Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan.

²Health Management Center & Department of Community Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan.

³Faculty of Internal Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 807, Taiwan.

⁴Department of Biological Science and Technology, College of Biological Science and Technology, National Chiao Tung University, Hsin-Chu, Kaohsiung 807, Taiwan.

Correspondence to: Dr. Ming-Lung Yu, Department of Internal Medicine, Kaohsiung Medical University Hospital, 100, Shi-Tzyou 1st Road, Kaohsiung 807, Taiwan. E-mail: fish6069@gmail.com

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Abstract

Aim: Despite the high cure rate of interferon-free directly acting antivirals (DAAs) for chronic hepatitis C (CHC) patients, the treatment efficacy for patients with preexisting hepatocellular carcinoma (HCC) remains undefined. We aimed in the present study to address the issue by using novel DAAs in treating CHC patients who were adherent to treatment in Taiwan.

Methods: CHC patients with or without HCC were consecutively enrolled. The primary objective was sustained virological response (SVR) defined as undetectable HCV RNA throughout 12 weeks of a post-treatment follow-up period (SVR12). Only patients with available SVR12 were enrolled for final analysis.

Results: A total of 1237 patients (1113 non-HCC, 101 inactive HCC and 23 active HCC) were enrolled. The overall SVR12 rate was 98.9%, and was similar between HCV patients with and without pre-existing HCC (98.4% vs. 98.9%, $P = 0.64$). While HCC patients were classified as those who had active or inactive HCC, the SVR12 was also similar between patients with and without active HCC (95.7% vs. 99.0%, $P = 0.34$). Among the 101 patients without viable HCC at the time of DAA initiation, eighty-four patients exhibited curative therapy and the other 17



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patients experienced HCC recurrence before DAAs. Among the 23 patients with viable HCC at the time of DAA treatment, 10 patients had received curative therapy for HCC whereas the remaining 13 patients had HCC that was never cured. The SVR12 rates were also similar among the four subpopulations, being 98.8% (83/84), 100% (17/17), 90% (9/10) and 100% (13/13) respectively.

Conclusion: CHC patients with HCC who were adherent to potent DAAs achieved similar SVR12 rate compared to those without HCC and could be effectively treated.

Keywords: Directly acting antiviral, chronic hepatitis C, hepatitis C virus, hepatocellular carcinoma, sustained virological response

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies^[1], which attributes to the second cause of cancer-related death worldwide^[2]. Hepatitis C virus (HCV) infection is one of the leading etiologies of HCC, which may account for one third of HCC patients^[3]. On the other hand, the risk of HCC decreases drastically after successful antiviral therapy^[4]. Interferon-free all oral directly acting antivirals (DAAs) have become a standard of care since 2014, providing ultimately high HCV cure rate and satisfactory safety profiles^[5,6]. Emerging evidence has also shown the benefit of DAAs in reducing the development of HCV-related HCC^[7].

Recently, the issue concerning whether pre-existing HCC would compromise the sustained virological response (SVR) rate in chronic hepatitis C (CHC) patients with DAAs has been raised, although discordant results might in part be attributed to different treatment regimens and patient characteristics^[8]. In patients with pre-existing HCC, a recent meta-analysis has shown that different treatment responses might exist between patients with or without viable HCC at the time of initiating DAAs^[9]. Some of the earlier studies used suboptimal treatment regimens that could not truly reflect the real world situation nowadays^[10]. By using current DAAs, an SVR rate of > 95% could be accomplished across populations^[11,12]. It is therefore crucial to revisit the issue by using potent DAAs in daily practice. In addition, HCC patients might have more safety concerns and are more likely to have experienced treatment discontinuation^[5]. Unequal tolerability might further compromise efficacy evaluation in these patients. Herein, we aimed to explore the issue by recruiting a well-characterized patient group in terms of HCC status who were adherent to novel DAA regimens.

METHODS

Patients receiving DAAs were consecutively enrolled from Aug 2015 to Mar 2019. The treatment strategies were based on regional guidelines^[13] and regulations of the Ministry of Health and Welfare of Taiwan. Daclatasvir (DCV)/Asunaprevir (ASV) for HCV genotype 1 (HCV-1) and Sofosbuvir (SOF)/ribavirin for HCV-2 have been reimbursed in Taiwan since 2017. Due to the previous relatively suboptimal treatment responses, patients who used these regimens were excluded in the current study.

The diagnosis of HCC was ascertained by pathology or clinical judgments based on the guidelines of the Asian Pacific Association for the Study of the Liver^[14] and the American Association for the Study of Liver Diseases^[2]. HCC was defined as curative if the initial presentation could be managed by surgical resection, local ablation or liver transplantation. The inactive HCC indicated that patients who had non-viable HCC were defined as if there were no image evidence of recurrence within 6 months before initiating DAA treatment. Other patients were defined with active HCC. The Review Board of the Kaohsiung Medical University Hospital approved the protocols that followed the guidelines of the International Conference on Harmonization for Good Clinical Practice. All patients provided written informed consent.

Table 1. Basic characteristics and treatment regimens of the patients with or without HCC

	All patients (n = 1237)	Non-HCC (n = 1113)	HCC (n = 124)	P value
Male gender, n (%)	542 (43.8)	477 (42.9)	65 (52.4)	0.04
Age, years (mean ± SD)	61.8 ± 11.8	61.1 ± 11.8	68.5 ± 9.4	< 0.001
Body weight, kg (mean ± SD)	63.7 ± 12.4	64.0 ± 12.5	61.0 ± 10.7	0.005
Diabetes, n (%)	273 (22.1)	244 (21.9)	29 (23.4)	0.71
Hypertension, n (%)	502 (40.6)	446 (40.1)	56 (45.2)	0.27
Platelet count, × 1000/mm ³ (mean ± SD)	170 ± 70	173 ± 68	144 ± 84	< 0.001
AST, IU/L (mean ± SD)	68.4 ± 48.0	67.1 ± 45.6	79.6 ± 49.9	0.009
ALT, IU/L (mean ± SD)	77.6 ± 64.4	77.7 ± 65.3	76.8 ± 56.4	0.88
Serum albumin, g/dL (mean ± SD)	4.3 ± 0.4	4.3 ± 0.4	4.0 ± 0.5	< 0.001
Serum bilirubin, mg/dL (mean ± SD)	1.0 ± 0.5	1.0 ± 0.5	1.1 ± 0.6	0.009
FIB-4 (mean ± SD)	3.80 ± 3.47	3.60 ± 3.39	5.62 ± 3.71	< 0.001
HCV RNA, log IU/mL	5.65 ± 1.01	5.67 ± 1.01	5.49 ± 0.99	0.06
HCV genotype, n (%)				
1	923 (74.6)	824 (74.0)	99 (79.8)	0.16
Non-1	314 (25.4)	289 (26.0)	25 (20.0)	
Liver cirrhosis, n (%)	597 (48.3)	516 (46.4)	81 (65.3)	< 0.001
Decompensation, n (%)	28 (4.7)	20 (3.9)	8 (9.9)	0.04
Prior treatment experienced*, n (%)	335 (27.1)	286 (25.7)	49 (39.5)	0.001
HBsAg (+), n (%)	83 (6.7)	74 (6.6)	9 (7.3)	0.8
HIV (+), n (%)	19 (1.5)			
PWID				0.64
Past usage	31 (2.5)	29 (2.6)	2 (1.6)	
Current usage	4 (0.3)	4 (0.4)	0 (0)	
Regimen, n (%)				0.03
PrOD ± RBV	423 (34.2)	370 (33.2)	53 (42.7)	
SOF/LDV ± RBV	338 (27.3)	309 (27.8)	29 (23.4)	
SOF/DCV ± RBV	122 (9.9)	105 (9.4)	17 (13.7)	
ELB/GRZ	157 (12.7)	141 (12.7)	16 (12.9)	
GLE/PIB	193 (15.6)	184 (16.5)	9 (7.3)	
SOF/VEL	4 (0.3)	4 (0.4)	0 (0)	
Sustained virological response, n (%)	1223 (98.9)	1101 (98.9)	122 (98.4)	0.64

AST: aspartate aminotransferase; ALT: alanine aminotransferase; FIB-4: fibrosis-4 index; HBsAg: hepatitis B surface antigen; PWID: patients who inject drugs; HIV: human immunodeficiency virus; PrOD: Paritaprevir/ritonavir/Ombitasvir/Dasabuvir; DCV: Daclatasvir; SOF: Sofosbuvir; LDV: Ledipasvir; ELB: Elbasvir; GRZ: Grazoprevir; VEL: Velpatasvir; GLE: Glecaprevir; PIB: Pibrentasvir; RBV: ribavirin; HCC: hepatocellular carcinoma. *All interferon-based therapy

The primary outcome was treatment efficacy defined as undetectable HCV RNA at the 12-week follow-up period after completing the anti-HCV therapy (SVR12). Only patients with available SVR12 were enrolled for final analysis.

The HCV RNA and HCV genotypes were tested by using real-time PCR assay (RealTime HCV; Abbott Molecular, Des Plaines IL, USA; with the detection limit: 12 IU/mL)^[15] defined by any of the following: liver histology^[16], transient elastography (FibroScan®; Echosens, Paris, France) > 12 kPa^[17], acoustic radiation force impulse (> 1.98 m/s)^[18], fibrosis-4 index (FIB-4, > 6.5)^[19] and/or the presence of clinical, radiological, endoscopic, or laboratory evidence of cirrhosis and/or portal hypertension.

Statistical analyses

Frequency was compared between groups using the χ^2 test with the Yates correction or Fisher's exact test. Group means (presented as the mean standard deviation) were compared using analysis of variance and Student's *t*-test or the nonparametric Mann-Whitney test when appropriate. The fibrosis-4 score (FIB-4) was calculated as age (years) × AST (U/L)/{platelets (10⁹/L) × [alanine transaminase (ALT) (U/L)]}^{1/2}. The statistical analyses were performed by using the SPSS 12.0 statistical package (SPSS, Chicago, IL, USA). All the statistical analyses were based on two-sided hypothesis tests with a significance level of *P* < 0.05.

RESULTS

Patient characteristics

As shown in [Table 1](#), 1237 patients were enrolled in the current study, with their patient and viral characteristics and treatment regimens also shown. The mean age was 61.8 years with 43.8% being males; the most common viral genotype was HCV genotype-1 (HCV-1, 74.6%); and the proportion of patients with liver cirrhosis was 48.3% ($n = 597$), whereas 28 patients (4.7%) had decompensated liver cirrhosis. Three hundred and thirty-five patients (27.1%) failed previous interferon-based regimens, and 83 patients (6.7%) were dually infected with the hepatitis B virus. The most commonly used DAA regimen was Paritaprevir/ritonavir plus Ombitasvir and Dasabuvir (PrOD) (34.2%), followed by SOF plus Ledipasvir (LDV).

One hundred and twenty-four patients (10.0%) had previous history of HCC before treatment. Of them, 101 patients (8.2%) had inactive HCC whereas the remaining 23 patients (1.9%) had active HCC at the time of DAA initiation.

Compared to patients without HCC, those with pre-existing HCC were older, had higher pretreatment aspartate aminotransferase, FIB-4 and bilirubin levels, lower body weight, albumin and platelet counts, and a higher proportion were males, had liver cirrhosis, and interferon-experienced history. Treatment regimens differed among patients with or without HCC; HCC patients had a higher proportion of PrOD usage than those without.

Treatment responses

The SVR12 rate was 98.9%, and was 99.3%, 98.7%, 97.9%, 100%, 99% and 100% in patients who received PrOD, Elbasvir (EBR)/Grazoprevir (GZR), SOF/LDV, SOF/DCV, Glecaprevir (GLE)/Pibrentasvir (PIB) and SOF/Velpatasvir (VEL) respectively. The SVR12 was similar between patients with and without pre-existing HCC (98.4% vs. 98.9%, $P = 0.64$) [[Table 1](#)]. While HCC patients were classified as those with active or inactive HCC, the SVR12 was also similar between patients with and without active HCC (95.7% vs. 99.0%, $P = 0.34$).

Among the 101 patients without viable HCC at the time of DAA initiation, eighty-four patients were with curative therapy and the other 17 patients experienced HCC recurrence before DAAs. Among the 23 patients with viable HCC at the time of DAA treatment, ten patients with HCC had ever received curative therapy whereas the remaining 13 patients with HCC had never received this. Patient and viral characteristics as well as treatment regimens are shown in [Table 2](#). The SVR12 rates were similar among the four populations, being 98.8% (83/84), 100% (17/17), 90% (9/10) and 100% (13/13) respectively. None of the clinical factor including the HCC status was associated with SVR to the DAA treatment [[Table 3](#)].

DISCUSSION

In the present study in Taiwanese patients with HCC, in addition to the similar effectiveness compared to patients without HCC by high potency DAAs, we demonstrate equivalent effectiveness also in both patients with and without HCC. With a relatively high SVR rate by interferon-based therapy in Taiwan compared to Western countries^[20,21], the Taiwanese National Health Insurance Scheme has reimbursed the cost of the PegIFN/RBV therapy since 2013^[22]. Due to the adverse effects of the IFN-based regimen, the treatment of patients with HCC is quite limited, even though the SVR rate is equivalent in patients with and without HCC when patients achieved good adherence, particularly in CHC patients after successful eradication of HCC^[23]. Since 2017, DAAs have been reimbursed by TNHI (free of charge for DAA medication) in patients with limited to advanced fibrosis and cirrhosis. The DAA treatment then became the standard treatment of CHC in Taiwan for patients fulfilling the reimbursement criteria, instead of interferon-based therapy. The high SVR rate has been reported as more than 97% in patients who completed the duration of therapy by ASV plus DCV if the patients had no NS5A mutants, PrOD with/without RBV and GZR/EBR with/without

Table 2. Characteristics of patient with HCC history and cancer status at the time of DAA treatment

	Inactive HCC		Active HCC	
	Curative & non-viable (<i>n</i> = 84)	Recurrent & non-viable (<i>n</i> = 17)	Once curative & viable (<i>n</i> = 10)	Never curative & viable (<i>n</i> = 13)
Male gender, <i>n</i> (%)	40 (47.6)	11 (64.7)	6 (60)	8 (61.5)
Age, years (mean ± SD)	68.8 ± 9.9	67.8 ± 7.3	70.2 ± 8.3	66.6 ± 9.7
Body weight, kg (mean ± SD)	60.7 ± 10.5	59.9 ± 10.9	61.9 ± 12.8	63.8 ± 11.1
Diabetes, <i>n</i> (%)	15 (17.9)	8 (47.1)	2 (20)	4 (30.8)
Hypertension, <i>n</i> (%)	39 (46.4)	8 (47.1)	5 (50.0)	4 (30.8)
Platelet count, × 1000/mm ³ (mean ± SD)	146 ± 94	140 ± 53	152 ± 55	135 ± 65
AST, IU/L (mean ± SD)	82.3 ± 56.1	72.9 ± 36.7	71.5 ± 34.7	77.2 ± 29.8
ALT, IU/L (mean ± SD)	77.3 ± 59.7	71.6 ± 48.8	77.6 ± 59.8	80.3 ± 45.8
Serum albumin, g/dL (mean ± SD)	4.0 ± 0.5	3.9 ± 0.5	4.1 ± 0.3	3.9 ± 0.6
Serum bilirubin, mg/dL (mean ± SD)	1.1 ± 0.5	1.0 ± 0.4	1.0 ± 0.5	1.3 ± 0.9
FIB-4 (mean ± SD)	5.77 ± 3.73	5.31 ± 3.84	4.49 ± 2.47	5.93 ± 4.41
HCV RNA, log IU/mL	5.50 ± 1.00	5.51 ± 0.93	5.48 ± 0.97	5.40 ± 1.07
HCV genotype 1, <i>n</i> (%)	70 (83.3)	13 (76.5)	6 (60.0)	10 (76.9)
Liver cirrhosis, <i>n</i> (%)	54 (64.3)	10 (58.8)	6 (60.0)	11 (84.6)
Decompensation, <i>n</i> (%)	5 (6.0)	1 (5.9)	0 (0)	2 (15.4)
Prior treatment experienced*, <i>n</i> (%)	36 (42.9)	4 (23.5)	5 (50.0)	4 (30.8)
HBsAg (+)	3 (3.6)	5 (29.4)	1 (10.0)	0 (0)
PrOD ± RBV	42 (50.0)	3 (17.6)	5 (50.0)	3 (23.3)
SOF/LDV ± RBV	22 (26.2)	3 (17.6)	1 (10.0)	3 (23.3)
SOF/DCV ± RBV	8 (9.5)	4 (23.5)	3 (30.0)	2 (15.4)
ELB/GRZ	6 (7.1)	6 (35.3)	1 (10.0)	3 (23.3)
GLE/PIB	6 (7.1)	1 (5.9)	0 (0)	2 (15.4)
Sustained virological response, <i>n</i> (%)	83 (98.8)	17 (100)	9 (90)	13 (100)

HCC: hepatocellular carcinoma; DAA: directly acting antivirals; AST: aspartate aminotransferase; ALT: alanine aminotransferase; FIB-4: fibrosis-4 index; HBsAg: hepatitis B surface antigen; PrOD: Paritaprevir/ritonavir/Ombitasvir/Dasabuvir; DCV: Daclatasvir; SOF: Sofosbuvir; LDV: Ledipasvir; ELB: Elbasvir; GRZ: Grazoprevir; VEL: Velpatasvir; GLE: Glecaprevir; PIB: Pibrentasvir; RBV: ribavirin. *All interferon-based therapy

RBV^[24]. Since 2019, patients with all stages of fibrosis have been reimbursed for DAA therapy, and all high potency first-line DAAs are available. The treatment of CHC has come to a new era including all subgroups of patients including patients with HCC particularly in patients with liver function impairment with the administration of new agents with SOF/LDV or SOF/VEL but not protease inhibitors.

The cure for HCV in HCC patients is encouraged by association with increased overall survival in these patients by interferon-based therapy, as per reports by Singal *et al.*^[25] and Morgan *et al.*^[26] Recently the benefits of the eradication of HCV infection by DAAs have also been elucidated by Kamp *et al.*^[27] and Dang *et al.*^[28] Nevertheless, the potentially suboptimal antiviral treatment efficacy by DAAs has been reported by some studies indicating treatment inferiority for HCC patients. Beste *et al.*^[29] reported the presence of HCC being associated with lower likelihood of SVR with SOF, SOF/LDV, and PrOD with or without ribavirin. Saberi *et al.*^[10] have observed a high rate of viral relapse after DAA treatment in patients with a concurrent HCC diagnosis in their case series. Prenner *et al.*^[30] have reported that when considering the treatment efficacy, presence of active HCC at the initiation of HCV therapy is significantly associated with DAA treatment failure. Radhakrishnan *et al.*^[31] reported the presence of HCC was associated with significantly lower odds of achieving SVR compared to those who had no HCC. However, HCC treatment status was not associated with SVR among those with HCC.

A recent meta-analysis including 49 studies from 15 countries concluded that compared to those without HCC, SVR rates were lower in patients with HCC, especially with active HCC^[9]. Patients with HCC treated with SOF/LDV had lower SVR rates than patients without HCC (92.6%, *n* = 884 vs. 97.8%, *P* = 0.026) and active/residual HCC than patients with inactive/ablated HCC (SVR 73.1% vs. 92.6%, *P* = 0.002). The role of

Table 3. Factors associated with SVR

	Non-SVR (<i>n</i> = 14)	SVR (<i>n</i> = 1223)	<i>P</i> value
Male gender, <i>n</i> (%)	8 (57.1)	534 (43.7)	0.31
Age, years (mean ± SD)	58.8 ± 11.3	61.9 ± 11.8	0.33
Body weight, kg (mean ± SD)	70.9 ± 16.8	63.3 ± 12.3	0.13
Diabetes, <i>n</i> (%)	3 (21.4)	270 (22.1)	1.00
Hypertension, <i>n</i> (%)	3 (21.4)	499 (40.8)	0.14
Platelet count, × 1000/mm ³ (mean ± SD)	176 ± 79	170 ± 70	0.77
AST, IU/L (mean ± SD)	79.8 ± 58.0	68.2 ± 47.8	0.47
ALT, IU/L (mean ± SD)	86.8 ± 52.9	77.5 ± 64.6	0.53
Serum albumin, g/dL (mean ± SD)	4.3 ± 0.5	4.3 ± 0.4	0.48
Serum bilirubin, mg/dL (mean ± SD)	1.1 ± 0.5	1.0 ± 0.5	0.34
FIB-4 (mean ± SD)	5.08 ± 7.21	3.79 ± 3.41	0.17
HCV RNA, log IU/mL	5.88 ± 0.97	5.65 ± 1.01	0.40
HCV genotype 1, <i>n</i> (%)	10 (71.4)	913 (74.7)	0.76
Liver cirrhosis, <i>n</i> (%)	5 (35.7)	592 (48.4)	0.43
Decompensation, <i>n</i> (%)	0 (0)	28 (0.2)	1.00
Prior treatment experienced*, <i>n</i> (%)	4 (28.6)	331 (27.1)	1.00
HBsAg (+)	0 (0)	83 (6.8)	0.62
Regimen, <i>n</i> (%)			0.44
PrOD ± RBV	3 (21.4)	420 (34.3)	
SOF/LDV ± RBV	7 (50.0)	331 (27.1)	
SOF/DCV ± RBV	0 (0)	122 (10.0)	
ELB/GRZ	2 (14.3)	155 (12.7)	
GLE/PIB	2 (14.3)	191 (15.6)	
SOF/VEL	0 (0)	4 (0.3)	
HCC history, <i>n</i> (%)			0.34
No	12 (85.7)	1101 (90.0)	
Yes, non-viable	1 (7.1)	100 (8.2)	
Yes, viable	1 (7.1)	22 (1.8)	

AST: aspartate aminotransferase; ALT: alanine aminotransferase; FIB-4: fibrosis-4 index; HBsAg: hepatitis B surface antigen; PrOD: Paritaprevir/ritonavir/Ombitasvir/Dasabuvir; DCV: Daclatasvir; SOF: Sofosbuvir; LDV: Ledipasvir; ELB: Elbasvir; GRZ: Grazoprevir; VEL: Velpatasvir; GLE: Glecaprevir; PIB: Pibrentasvir; RBV: ribavirin; HCC: hepatocellular carcinoma; SVR: sustained virological response. *All interferon-based therapy

the different DAA regimens on the impact of SVR in patients with HCC remains unclear^[32]. It is noteworthy that in some studies, patients may receive possibly inadequate or low-potency treatments such as simeprevir/SOF 12 weeks, SOF/LDV 12 weeks in treatment-experienced patients or SOF/ribavirin regimens. It is interesting as to whether the regimens influence the SVR rate. In the present study, we observed similar SVR rates between patients with and without HCC, or HCC patients with and without active diseases in patients with adherence to potent DAAs. The very high SVR rates (> 98%) might prevent negative impact on the responses. Our study has emphasized the importance of potent DAAs in addition to good compliance in patients with HCC.

It is not clear why the presence or history of HCC might influence the likelihood of achieving SVR. We propose some mechanisms for the suboptimal effects of DAAs in patients with HCC. Firstly, tumor cells serve as a sanctuary site for HCV where the replication of the HCV is preserved^[33]. Secondly, HCV within tumor cells might evade the antiviral effects of DAA therapy due to ineffective blood delivery to the target site^[34]. Thirdly, poor cancer immunity and altered tumor microenvironment has also been linked to altered antiviral efficacy of DAA therapy^[35]. Since the SVR is highly related to the effective distribution of the drug, different vascularity changes in HCC or the treated HCC might also be causative. In our patients, good adherence and sufficient duration and dosage of the DAAs might have possibly overcome the barriers for efficacy by HCC.

There are some limitations in the present study. Firstly, a retrospective observational study design may possess some selection bias in obtaining the real efficacy of the therapy. Secondly, we did not record the real-world withdrawal rate of the HCC patients receiving the DAAs although the treatment duration was shortened and the potency increased by the currently widely used DAAs, which may have improved the rate of complete treatment and discontinuation significantly. Thirdly, with a high cure rate for HCV, we did not observe any impact of the DAA therapy on the natural course of the HCC. The controversies of the influence of HCV therapy by DAAs have been discussed with more evidence from the recent systematic reviews and meta-analyses disputing the unfavorable effects on the development of more advanced recurrence or early recurrence in patients with HCC^[35]. Lastly, because of the relatively small number of HCC patients treated by DAAs in our study, the statistical non-significance in HCC and non-HCC patients possibly needs further large-scale studies for validation.

In conclusion, we have demonstrated similar SVR rates in patients with HCC, either active or inactive, receiving a complete course of potent DAAs in Taiwan. With high potent DAAs available and easier and more convenient care including shorter duration and less adverse effects during treatment, our results suggest the importance of adherence to DAA therapy and the preference of treating HCV aggressively for HCC patients in clinical settings in Taiwan.

DECLARATIONS

Authors' contributions

Conceived and planned the experiments: Dai CY, Huang CF, Chuang WL, Yu ML

Performed the analytic calculations: Dai CY, Huang CF, Tsai PC, Lin CC, Yu ML

Contributed to patient and sample preparation: Dai CY, Huang CF, Hsieh MH, Huang CI, Yeh ML, Yang JF, Wei YJ, Hsu CT, Liang PC, Lin YH, Huang JF, Chuang WL, Yu ML

Contributed to the interpretation of the results: Dai CY, Huang CF, Tsai PC, Lin CC, Lee MS, Chuang WL, Yu ML

Took the lead in writing the manuscript: Dai CY, Huang CF, Yu ML

Supervised the project: Yu ML

Availability of data and materials

The data source came from Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital. Data could be released to the public only when approval of the owner.

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None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The institutional review board of the Kaohsiung Medical University Hospital approved the protocols, which followed the guidelines of the International Conference on Harmonization for Good Clinical Practice. All patients provided written informed consent. There was no specific ethic consideration during the study.

Consent for publication

Not applicable.

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REFERENCES

1. WHO. Global Cancer Observatory. Available from: <http://globocan.iarc.fr/old/FactSheets/cancers/liver-new>. [Last accessed on 30 Mar 2020]
2. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358-80.
3. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al.; Global Burden of Disease Liver Cancer Collaboration. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. *JAMA Oncol* 2017;3:1683-91.
4. Yu ML, Huang CF, Yeh ML, Tsai PC, Huang CI, et al. Time-degenerative factors and the risk of hepatocellular carcinoma after antiviral therapy among hepatitis c virus patients: a model for prioritization of treatment. *Clin Cancer Res* 2017;23:1690-7.
5. Huang CF, Yeh ML, Huang CI, Liang PC, Lin YH, et al. Equal treatment efficacy of direct-acting antivirals in patients with chronic hepatitis C and hepatocellular carcinoma? A prospective cohort study. *BMJ Open* 2019;9:e026703.
6. Huang CF, Iio E, Jun DW, Ogawa E, Toyoda H, et al. Direct-acting antivirals in East Asian hepatitis C patients: real-world experience from the REAL-C Consortium. *Hepatol Int* 2019;13:587-98.
7. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol* 2017;S0168-8278(17)32273-0.
8. Huang CF, Yu ML. Direct-acting antivirals response in hepatocellular carcinoma: does the presence of hepatocellular carcinoma matter? *Clin Mol Hepatol* 2019;25:168-71.
9. Ji F, Yeo YH, Wei MT, Ogawa E, Enomoto M, et al. Sustained virologic response to direct-acting antiviral therapy in patients with chronic hepatitis C and hepatocellular carcinoma: A systematic review and meta-analysis. *J Hepatol* 2019;71:473-85.
10. Saberi B, Dadabhai AS, Durand CM, Philosophie B, Cameron AM, et al. Challenges in treatment of hepatitis C among patients with hepatocellular carcinoma. *Hepatology* 2017;66:661-3.
11. AASLD-IDSA HCV Guidance Panel. Hepatitis C guidance 2018 update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. *Clin Infect Dis* 2018;67:1477-92.
12. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2018. *J Hepatol* 2018;69:461-511.
13. Omata M, Kanda T, Wei L, Yu ML, Chuang WL, et al. APASL consensus statements and recommendations for hepatitis C prevention, epidemiology, and laboratory testing. *Hepatol Int* 2016;10:681-701.
14. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017;11:317-70.
15. Vermehren J, Yu ML, Monto A, Yao JD, Anderson C, et al. Multi-center evaluation of the Abbott RealTime HCV assay for monitoring patients undergoing antiviral therapy for chronic hepatitis C. *J Clin Virol* 2011;52:133-7.
16. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol* 1991;13:372-4.
17. Castéra L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005;128:343-50.
18. Lin YH, Yeh ML, Huang CI, Yang JF, Liang PC, et al. The performance of acoustic radiation force impulse imaging in predicting liver fibrosis in chronic liver diseases. *Kaohsiung J Med Sci* 2016;32:362-6.
19. Wang CC, Liu CH, Lin CL, Wang PC, Tseng TC, et al. Fibrosis index based on four factors better predicts advanced fibrosis or cirrhosis than aspartate aminotransferase/platelet ratio index in chronic hepatitis C patients. *J Formos Med Assoc* 2015;114:923-8.
20. Yu ML, Dai CY, Huang JF, Hou NJ, Lee LP, et al. A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. *Gut* 2007;56:553-9.
21. Yu ML, Dai CY, Huang JF, Chiu CF, Yang YH, et al. Rapid virological response and treatment duration for chronic hepatitis C genotype 1 patients: a randomized trial. *Hepatology* 2008;47:1884-93.
22. Dai CY, Yeh ML, Huang JF, Chuang WL, Yu ML. Insurance status and treatment candidacy of patients with hepatitis C: Taiwanese patients are luckier. *Hepatology* 2011;53:1399.
23. Huang JF, Yu ML, Huang CF, Chiu CF, Dai CY, et al. The efficacy and safety of pegylated interferon plus ribavirin combination therapy in chronic hepatitis c patients with hepatocellular carcinoma post curative therapies - a multicenter prospective trial. *J Hepatol* 2011;54:219-26.
24. Dai CY, Chen DS, Chen PJ, Kao JH, Liu CJ, et al. National registration database for the first year (2017) national health insurance reimbursed direct antiviral agents therapy for chronic hepatitis C in Taiwan. *Hepatology* 2018;68:403A.
25. Singal AK, Singh A, Jaganmohan S, Guturu P, Mummadi R, et al. Antiviral therapy reduces risk of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis. *Clin Gastroenterol Hepatol* 2010;8:192-9.
26. Morgan RL, Baack B, Smith BD, Yartel A, Pitasi M, et al. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann Int Med* 2013;158:329-37.
27. Kamp WM, Sellers CM, Stein S, Lim JK, Kim HS. Impact of direct acting antivirals on survival in patients with chronic hepatitis C and hepatocellular carcinoma. *Sci Rep* 2019;9:17081.
28. Dang H, Yeo YH, Yasuda S, Huang CF, Iio E, et al. Cure with interferon free DAA is associated with increased survival in patients with HCV related HCC from both East and West. 2019; Epub ahead of print. doi: 10.1002/hep.30988.
29. Beste LA, Green PK, Berry K, Kogut MJ, Allison SK, et al. Effectiveness of hepatitis C antiviral treatment in a USA cohort of veteran patients with hepatocellular carcinoma. *J Hepatol* 2017;67:32-9.
30. Prenner SB, VanWagner LB, Flamm SL, Salem R, Lewandowski RJ, et al. Hepatocellular carcinoma decreases the chance of successful hepatitis C virus therapy with direct-acting antivirals. *J Hepatol* 2017;66:1173-81.
31. Radhakrishnan K, Di Bisceglie AM, Reddy KR. Treatment status of hepatocellular carcinoma does not influence rates of sustained

- virologic response: an HCV-TARGET analysis. *Hepatol Commun* 2019;3:1388-99.
32. Ji F, Yeo YH, Wei MT, Wei B, Dang S, et al. Hepatocellular carcinoma decreases the effectiveness of hepatitis C antiviral treatment: Do direct-acting antiviral regimens matter? *Hepatology* 2018;67:1180-2.
 33. Niu J, Kumar U, Monjardino J, Goldin R, Rosin D, et al. Hepatitis C virus replication in hepatocellular carcinoma. *J Clin Pathol* 1995;48:880-2.
 34. Konjeti VR, John BV. Interaction between hepatocellular carcinoma and hepatitis C eradication with direct-acting antiviral therapy. *Curr Treat Options Gastroenterol* 2018;16:203-14.
 35. Sachdeva M, Chawla YK, Arora SK. Immunology of hepatocellular carcinoma. *World J Hepatol* 2015;7:2080-90.

Review

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Direct acting antivirals therapy and hepatocellular carcinoma risk in patients with hepatitis C virus

Monika Pazgan-Simon

Department of Infectious Diseases and Hepatology, Wroclaw Medical University, Wroclaw 51-149, Poland.

Correspondence to: Monika Pazgan-Simon, MD, PhD, Department of Infectious Diseases and Hepatology, Wroclaw Medical University, Poland. E-mail: monikapazgansimon@gmail.com

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Abstract

The estimated number of people with active hepatitis C virus infection worldwide is about 70 million. The estimated number of people with active hepatitis C virus infection worldwide is about 70 million. Approximately 30% of infected individuals develop cirrhosis, whilst some develop liver cancer, the fifth most common cancer worldwide. Currently available treatments, high-efficacy antiviral agents mostly short-term (8-12 weeks) and pangenotypic, have efficacy rates of over 96%. Some patients, especially those with cirrhosis, develop primary liver cancer even after effective hepatitis C virus treatment. In order to diagnose hepatocellular carcinoma early, patients at risk should be enrolled in a surveillance program.

Keywords: Hepatitis C virus, direct acting antivirals treatment, oncogenesis

INTRODUCTION

The main causal agents in viral hepatitis are primary hepatotropic viruses: A (HAV), B (HBV), C (HCV), D (HDV), E (HEV). There are other secondary hepatotropic viruses that can also cause viral hepatitis.

HCV is caused by the RNA virus of the Flaviviridae family, which has a single strand of 3011 bases and a lipid membrane envelope sized 60-70 nm. The virus structure encompasses a genome, core envelope proteins (E1, E2) and 7 non-structural proteins (NS1, NS2, NS3, NS4A, NS4B, NS5A and NS5B)^[1,2].

Hepatitis C is a blood-borne infection, transmitted as a result of skin impairment with non-sterile medical equipment, in particular intravenous drug injections and tattooing, during sex. This is especially the case for passive anal intercourse, vertically (from mother onto the child) during childbirth, and less often during



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organ transplantation. An acute viral infection develops within 14 to 180 days following the exposure. Only humans and chimpanzees are affected.

To date, seven HCV genotypes with variable prevalence have been identified worldwide. Genotype 1 HCV has 2 subtypes: 1a predominant in North America and 1b predominant in Europe. Genotype 2 HCV is fairly rare, occurring in North America. Genotype 3 HCV is the most common among intravenous drug users, whilst genotype 4 HCV is the most prevalent in North Africa^[3]. The remaining genotypes are very uncommon. Individuals infected with genotypes 1a and 1b are more likely to develop chronic hepatitis, whereas those with genotype 3 are more likely to develop fatty liver disease. The highest prevalence of HCV is noted in Asia, south frontiers of North America and Brazil. The estimated number of people with active HCV infection worldwide is about 70 million, with over 100 million testing positive for anti-HCV antibodies (serological evidence of past HCV infection), and another 3-4 million people get infected every year. Unfortunately, despite ongoing research the HCV vaccine has not been developed to date.

Intravenous drug users, homosexual men, rough sleepers, individuals with HIV co-infection, migrants, blood and blood product recipients and prisoners are at particular risk of HCV infection.

Whereas HCV infection can be chronic, the virus does not embed in human genome (just as the HBV does), despite long-term persistence in hepatocytes or liver-resident lymphocytes.

NATURAL COURSE OF HCV INFECTION

After an acute infection, spontaneous virus clearance occurs in up to 30% of infected individuals within 6 months. However, 70% of affected patients develop chronic condition. Up to 80% of infected individuals are asymptomatic, so the infection may remain undiagnosed in many cases. About 30% of infected individuals develop cirrhosis. Some of them may present with hepatocellular carcinoma (HCC) in a 30-year follow-up study^[4].

Patients with chronic infection often present with extrahepatic manifestations. Their symptoms are usually caused by cryoglobulins, leading to vasculitis and kidney damage. Osteoarticular, cardiovascular, endocrine (especially pancreatic and thyroid), central nervous system or skin involvement are also possible. They may develop lymphomas. Some patients with extrahepatic manifestations may have no identifiable liver disease.

PRIMARY LIVER CANCER AND HCV INFECTION

In patients with HCV-associated cirrhosis, the annual risk of HCC is 2%-8%, whereas the overall risk of cancer in all HCV-infected patients is 14.4%. HCC recurrence within 5 years following effective treatment (different therapies) affects 70% of patients. Most HCC cases are found in Japan, Pakistan, Asia, Europe and the United States.

In HCV-infected patients, HCC development is associated with the progression of fibrosis and cirrhosis. Structural and non-structural viral proteins virus play the key role in malignant transformation inducing oxidative stress, aberrant proliferation and apoptosis, chronic inflammation, dysregulated lipid synthesis as well as excessive and abnormal angiogenesis. Through methylation, HCV core proteins reduce CDKN2A activity, causing telomerase dysfunction (TERT promoter mutations), the most common genetic aberrations seen in HCC^[5].

Furthermore, NS3 and NS5A proteins may disrupt the p53 synthesis pathway, blocking apoptosis and nucleocytoplasmic shuttling of p53^[6].

Table 1. Risk factors of HCC development in patients treated with interferon depending on the time since the end of treatment

Up to 2 years following the end of treatment	Up to 4 years following the end of treatment	Over 4 years following the end of treatment
Older age	Older age	Diabetes
Male sex	Diabetes	
Advanced fibrosis	Advanced fibrosis	
Fatty liver disease	Elevated AFP levels	
Elevated AFP level at the end of treatment		

HCC: hepatocellular carcinoma

INTERFERON TREATMENTS

In the 1980s, natural interferon was approved for the treatment of HCV infection, followed by alpha-2a and 2b interferon, and pegylated alpha-2a and alpha-2b interferon approved in 2002. These were administered subcutaneously, offering the efficacy below 50%, even when administered in combination with ribavirin, with peginterferons being the most effective. The treatment was associated with numerous adverse effects, frequently causing premature drug discontinuation. However, apart from their antiviral effect, interferons also exerted antifibrotic and antineoplastic effect. Interferon therapy could not be used in some patients and was only limited to those with non-advanced liver disease, without numerous contraindications for the use of interferon alpha.

The natural course of HCV infection may lead to the development of HCC in some cases [Table 1]. The risk of HCC development in patients with cirrhosis (CTP A) treated with interferon was lower in responders (SVR achieved) than in non-responders.

INTERFERON-FREE TREATMENTS

The first interferon-free treatment of HCV infection, sofosbuvir, was approved in 2014. It quickly became clear that due to virus variation, monotherapy was ineffective. When combined therapy with direct acting antivirals (DAA), different targets had to be used. The currently available interferon-free treatments are effective in the majority (over 95%) of patients, regardless of their age, sex, ethnicity, body weight, liver disease stage or co-infection with HIV.

DAA medications act on known HCV replication targets, effectively leading to a complete virion elimination. The agents currently used include a pangenotypic combination of (1) sofosbuvir (NS5B polymerase inhibitor)/velpatasvir (NS5A inhibitor) with or without voxilaprevir (NS3/4A inhibitor); (2) ombitasvir (NS5A inhibitor)/paritaprevir (NS3/4A inhibitor) - ritonavir with or without dasabuvir (non-nucleotide NS5A inhibitor), glecaprevir NS3/4A inhibitor/pibrentasvir (NS5A inhibitor); and (3) grazoprevir (NS3/4A protease inhibitor)/elbasvir (NS5A inhibitor), which is effective in genotype 1 and 4 HCV infection^[7,8].

EASL AND AASLD RECOMMENDATIONS FOR THE MANAGEMENT OF PATIENTS WITH HCV AND HCC

EASL guidelines advise against declining antiviral therapy in patients with cirrhosis, including advanced cirrhosis. However, continued surveillance is still required following successful antiviral therapy. The risk of HCC for patients with HCV-related cirrhosis who develop SVR after DAA treatment is lowered, but not eliminated.

Whilst direct-acting antiviral therapies definitely improve survival in patients with cirrhosis previously treated for HCC, the impact of HCV eradication by DAAs on the future risk of HCC is uncertain. Therefore, patients with HCV-related cirrhosis and history of HCC should be considered for treatment with DAAs but should also continue to undergo surveillance.

AASLD pointed out that patients with cirrhosis and HCC have lower SVR rates than those with cirrhosis without HCC. Longer regimens may improve treatment response. Patients with compensated cirrhosis should be treated with sofosbuvir/ledipasvir or sofosbuvir/velpatasvir (with ribavirin) for 12 weeks. In those with decompensated cirrhosis and HCC, the above treatment regimen should be extended to 24 weeks^[8,9].

GLOBAL HEALTH SECTOR STRATEGY ON VIRAL HEPATITIS

In 2016, the WHO published the global health sector strategy on viral hepatitis which aims at reducing viral hepatitis-related mortality. The strategy aims to identify 80% of HCV and HBV infected patients and start treatment in 80% of them, to minimise the risk of infection during blood transfusion and phlebotomy, as well as to ensure access to sterile injecting equipment for people who inject drugs by 2030. Available, easy-to-administer treatments increase the chances to effectively implement this strategy in relation to HCV infection. Many countries, such as Georgia and Australia, have already approached the targets set by the WHO for 2030.

CARCINOGENESIS IN PATIENTS AFTER DAA TREATMENT - MOLECULAR ASPECTS

As the HCV does not embed in human genome, it does not exert a direct carcinogenic effect.

There is a number of hypotheses to explain the development of primary liver cancer after DAA treatment. The first of them assumes that DAA causes rapid virus clearance, which results in immune dysfunction, cytokine activation, dysregulation of apoptosis and reactive oxygen species which, in turn, stimulate angiogenesis directly involved in tumour growth^[9].

DAA treatment is associated with rapid viral clearance and modulating antiviral immunity in favour of promoting oncogenesis. DAAs change the risk of HCC by facilitating restoration of innate immunity, downregulation of interferon II and III genes and their receptors, as well as reducing the mi-R-122 levels, which promotes proliferation^[10].

The evidence seems to indicate that carcinogenesis after DAA therapy is associated with HCV-induced imbalance between life- and apoptosis-promoting factors.

Viral clearance is associated with expression of a number of apoptosis-regulating genes, such as *TP53*, *FAS*, *TGF*, *VEGF*, which trigger tumour development and spread. Furthermore, other genes, such as *HGF*, *ROS* and *FGF* stimulate and upregulate angiogenesis.

In the case of antiviral drug resistance, natural killer (NK) cell dysfunction leads to virus proliferation and reduced expression of interferon genes, disrupting natural defence mechanisms. Decreased interferon gamma levels stimulates carcinogenesis, while impaired interferon gene expression results in HCC development.

Another hypothesis postulates that carcinogenesis occurs in response to cytotoxic cell silencing caused by transient immunosuppression during viral clearance. As NKG2D expression on NK cells plummets in response to DAA treatment, HCC develops.

The immune markers present at baseline, which are associated with a higher risk of HCC, include the TNF alpha, which is constantly secreted in patients with HCC, unlike cancer-free patients, in whom TNF alpha suppression has been shown [Table 2].

HCC OCCURRENCE IN PATIENTS TREATED WITH DAA

In 2016 and 2017, shortly after the first DAA approval, first reports of post-SVR development of HCC in patients with cirrhosis treated with DAA were published [Table 3]. However, the study groups in these

Tabel 2. Genes implicated in viral clearance and HCC development**Genes implicated in viral clearance and HCC development**

Toll-like receptor-4 (<i>TLR-4</i>)
Toll-like receptor-2 (<i>TLR-2</i>)
MHC class 1 polipeptide-related sequence A (<i>MIC-A</i>)
Cellular tumor antigen p53
SET domain containing-5 (<i>SETD5</i>)
Retinoblastoma-associated protein RB
Secreted apoptosis-related protein-2 (<i>SFRP2</i>)
Signal transducer and activator of transcription (<i>STAT</i>)-3
Glutathione S-transferase Mu 1 (<i>GSTM1</i>)
Interferone lambda (<i>IFNL3</i>)
Interferone lambda (<i>IFNL4</i>)
Hepatocyte growth factor (<i>HGF</i>)
Interleukins 6 and 17 (<i>IL-6, IL-17</i>)
Matrix metalloproteinases 2,9

HCC: hepatocellular carcinoma; MHC: major histocompatibility complex

reports were small and there was no control group to provide a comparison. It should be noted, however, that safe DAA therapy was first administered to patients with the most advanced liver disease, compensated and decompensated cirrhosis, which alone is a risk factor for HCC.

In 2017, a study in 22,500 American veterans (96.7% of men) with hepatitis C was published. The calculated risk of HCC development in 1-year follow up was lower by 72% in patients who achieved SVR than in those who were not cured^[11].

In 2018, a meta-analysis of an over 2-year follow up of 9895 French patients with hepatitis C, treated with interferon or DAAs demonstrated that DAA exposure did not increase the risk of HCC, when the results were adjusted for patient age, liver disease stage, diabetes, hypertension, as well as biological variables at screening.

In 2019, the results of almost 3-year follow-up 7344 patients with hepatitis C treated with DAA and 2551 untreated patients with hepatitis C were published. DAA exposure was associated with a decrease in all-cause mortality (HR = 0.48) and *de novo* HCC occurrence (HR = 0.66) and was not associated with decompensated cirrhosis.

MANAGEMENT OF PATIENTS WITH OR AT RISK OF HCC CURRENTLY OR PREVIOUSLY TREATED WITH DAA - RECOMMENDATIONS

Patients with advanced fibrosis or cirrhosis should be screened for HCC before the commencement of treatment with DAA.

After the end of DAA, patients with SVR should be regularly monitored every 6 months with laboratory blood tests, including AFP level, and an abdominal ultrasound.

Antiviral treatment in patients with active malignancy is associated with a worse treatment response, hence, radical treatment, if possible, is recommended first, followed by a course of DAAs.

However, in patients with advanced HCC (BCLC stage C and D) who cannot undergo radical treatment, the decision to start DAA therapy should be made on a case-by-case basis, considering patient's clinical status, their potential survival, or individual preferences.

Table 3. Molecular risk factors of carcinogenesis in patients treated with DAA

Molecular risk factors of carcinogenesis in patients treated with DAA
Cirrhosis
Reduced interferon production
Decreased micro-RNA-122 levels
T-cell dysfunction
Hyporesponsive NK cells
Rapid decrease of chronic inflammation

DAA: direct acting antiviral; NK: natural killer

In patients waiting for a liver transplant because due to HCV-associated cirrhosis and HCC, the moment of DAA treatment commencement should be decided depending on the patient's position on the list, expected waiting time, and liver disease stage.

In patients with HCC treated with radical methods: transplantation, resection or embolization, treatment with DAAs should be considered after they have remained clinically stable and relapse-free for 6 months.

Patients with cirrhosis and history of HCC, who completed treatment with DAA, regardless of their SVR status, should be carefully monitored every 3-6 months, with an abdominal ultrasound and, in some cases, also abdominal CT and MRI^[12].

HCC RECURRENCE IN PATIENTS TREATED WITH DAA

Recurrence is seen in some patients treated for HCC subsequently treated effectively with DAA [Table 4]. At times, it may be aggressive and rapidly lead to death. In 2017 and 2018, a number of often contradictory reports from different centres were published.

The main cause of recurrence is a simultaneous rapid clearance of HCV and liver tissue-resident memory T-cells, which reduces local immunosuppression and promotes recurrent carcinogenesis^[13]. HCC recurrence after effective treatment HCV proteins are known modulators of intracellular signalling pathways, which may induce carcinogenesis in infected individuals. The expression of treatment-induced, mutated viral proteins also plays a role in HCC recurrence. Despite viral clearance, the ongoing oncogenesis does not cease, and the tumour develops further spreading to other sites as metastases.

HCC RECURRENCE IN PATIENTS TREATED WITH DAA - SIGNIFICANT PUBLICATIONS

In a retrospective study by Nagata *et al.*^[14], patients with HCV and a history of HCC were treated either with INF ($n = 60$) or with DAA ($n = 83$), with the mean follow up of 7.5 years. The recurrence rates were comparable in patients treated with IFN and DAA (47% at 5 years post-SVR vs. 22.9% at 3 years post-SVR, respectively).

In a prospective study in Italy, Cabibbo *et al.*^[15] studied patients with history of HCC treated with DAA, demonstrating HCC recurrence in 20.38% of patients treated with DAA. Previous HCC recurrence prior to treatment with DAA and tumour size above 2.5 cm at baseline were associated with higher risk of recurrence.

In 2018, Singal published a large meta-analysis of 793 patients who completed HCC treatment in the USA and Canada and were treated for HCV with DAA ($n = 304$) or untreated ($n = 489$). There were 128 (42%) cases of relapse in patients treated with DAA and 228 (58.9%) cases of relapse in those untreated for HCV infection, which clearly indicates the beneficial role of DAAs in preventing HCC relapse in individuals with chronic hepatitis C. The only factor significantly correlated with relapse was the baseline HCC stage (BCLC)^[16].

Table 4. Clinical risk factors of carcinogenesis in patients treated with DAA

Clinical risk factors of carcinogenesis in patients treated with DAA
Previous history of HCC
Cirrhosis
Male sex
Hypoalbuminaemia
Thrombocytopaenia
Elevated AFP levels

DAA: direct acting antiviral; HCC: hepatocellular carcinoma

CONCLUSION

The natural course of HCV infection may lead to the development of HCC in some cases. DAA therapy reduced risk of HCV related HCC and death. But in some cases HCV therapy is related with liver cancer development. Patients after DAA treatment, mainly with liver cirrhosis, require HCC surveillance.

DECLARATIONS

Authors' contributions

The author contributed solely to the article.

Availability of data and materials

Not applicable.

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Conflicts of interest

The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

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REFERENCES

1. The World Organization. Hepatitis C fact sheet. Available from: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-c> 07.04.202. [Last accessed on 7 Apr 2020]
2. Bartenschlager R, Lohmann V, Penin F. The molecular and structural basis of advanced antiretroviral therapy for hepatitis virus C infection. *Nat Rev Microbiol* 2013;11:482-96.
3. Patin E, Kotalik Z, Guernon J, Bibert S, Nalps B, et al. Genome-wide association study identifies variants associated with progression of liver fibrosis from HCV infection. *Gastroenterology* 2012;143:1244-52.e12.
4. Westbrook RH, Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014;61:58-68.
5. Nault JC, Mallet M, Pilati C, Calderano J, Bioulac-Sage P, et al. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat Commun* 2013;4:2281.
6. Kao CF, Chen SY, Chen JY, Wu Lee YH. Modulation of 53 transcription regulatory activity and post-translational modification by hepatitis C virus core protein. *Oncogene* 2004;23:2472-83.
7. European Association for the Study of the Liver. EASL recommendation on treatment of hepatitis C 2018. *J Hepatol* 2018;69:461-511.
8. Ghany MG, Morgan TR; AASLD-IDS A Hepatitis C Guidance Panel. Hepatitis C guidance 2019 update: American association for the study of liver diseases-infectious diseases society of america recommendations for testing, managing, and treating hepatitis C virus

- infection. *Hepatology* 2020;71:686-721.
9. Butt AS, Sharif F, Abid S, Impact of direct acting antivirals on occurrence and recurrence of hepatocellular carcinoma: Biologically plausible or an epiphenomenon? *World J Hepatol* 2018;10:267-76.
 10. Kobzial K, Moser S, Schwarzer R, Laferl H, Al-Zoairy R, et al. Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. *J Hepatol* 2016;65:856-8.
 11. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, et al. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. *Gastroenterology* 2017;153:996-1005.e1.
 12. Singal AG, Lim JK, Kanwal F. AGA clinical practice update on interaction between oral direct-acting antivirals for chronic hepatitis C and hepatocellular carcinoma: expert review. *Gastroenterology* 2019;145:2149-57.
 13. Ioannou GN, Feld JJ. What are the benefits of a sustained virologic response to direct-acting antiviral therapy for hepatitis C virus infection? *Gastroenterology* 2019;156:446-60.e2.
 14. Nagata H, Nakagawa M, Asahina Y, Sato A, Asano Y, et al. Effect of interferon-based and -free therapy on early occurrence and recurrence of hepatocellular carcinoma in chronic hepatitis C. *J Hepatol* 2017;67:933-9.
 15. Cabibbo G, Petta S, Calvaruso V, Cacciola I, Cannavo MR, et al. Is early recurrence of hepatocellular carcinoma in HCV cirrhotic patients affected by treatment with direct-acting antivirals? A prospective multicentre study. *Aliment Pharmacol Ther* 2017;46:688-95.
 16. Singal AG, Rich NE, Metha N, Branch A, Pillai A, et al. Direct-acting antiviral therapy not associated with recurrence of hepatocellular carcinoma in a multicenter North American cohort study. *Gastroenterology* 2019;156:1683-92.e1.

Review

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Epidemiology of non-alcoholic fatty liver disease-related hepatocellular carcinoma: a western perspective

Ann Farrell^{1,2}, Marno Ryan^{1,2}, Jessica Howell^{1,2,3}

¹Department of Gastroenterology, St. Vincent's Hospital Melbourne, Melbourne 3065, Australia.

²Department of Medicine, The University of Melbourne, Melbourne 3010, Australia.

³Disease elimination program, Burnet Institute, Melbourne 3004, Australia.

Correspondence to: Dr. Ann Farrell, Department of Gastroenterology, St Vincent's Hospital Melbourne, Fitzroy 3065, Australia.
E-mail: ann.farrell@svha.org.au

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is now the most common cause of liver disease worldwide, and represents an increasingly important cause of hepatocellular carcinoma (HCC). As the prevalence of NAFLD has increased, the burden of NAFLD-related HCC has been rising in parallel. This is particularly evident in Western countries, where NAFLD is estimated to account for 10%-59% of all HCC. NAFLD-related HCC can occur in the presence or absence of cirrhosis, and, while those with cirrhosis remain at the greatest risk, factors such as steatohepatitis, age, genetic polymorphisms, type 2 diabetes mellitus and obesity also appear have an impact on the risk of developing HCC in NAFLD. In this review, we present the epidemiology of NAFLD-related HCC from a Western perspective, highlighting gaps in current knowledge and future directions for research in this field.

Keywords: Non-alcoholic fatty liver disease, steatohepatitis, cirrhosis, hepatocellular carcinoma, epidemiology

INTRODUCTION

Over the past two decades, non-alcoholic fatty liver disease (NAFLD) has rapidly become the most common cause of liver disease worldwide, affecting approximately a quarter of the global population^[1]. It is considered to be a hepatic end organ effect of the metabolic syndrome and its rise to prominence has



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coincided with the onset of the obesity and type 2 diabetes mellitus (T2DM) epidemics^[2]. Previously, due to the strong association between NAFLD and the metabolic syndrome, NAFLD was considered a disease of affluent nations; however, with the epidemiological transition and increasing urbanisation in low and middle income countries, prevalence of the metabolic syndrome and NAFLD is now increasing rapidly across the globe^[3-5]. This high prevalence and subsequent burden of progressive liver disease has resulted in NAFLD becoming an increasingly important cause of hepatocellular carcinoma (HCC)^[6-8]. This review outlines the epidemiology of NAFLD-related HCC, with a focus on Western countries.

METHODS

We conducted a search of the Medline, PubMed and Cochrane databases to identify English language, original research articles conducted in adults over the age of 18, and published between 1 January 1990 and 1 September 2019 using the Medical Subject Headings “Nonalcoholic Fatty Liver Disease” and “Hepatocellular carcinoma”. Duplicate results were removed using Endnote X9, before screening by title and abstract was performed to find research relating to HCC in patients with NAFLD (single reviewer - AF). Original research articles were eligible for selection, including meta-analyses, controlled trials, cohort studies and case-series, which clearly described their study population including their diagnostic criteria for NAFLD, and described the incidence or prevalence of NAFLD-related HCC.

NAFLD, nonalcoholic steatohepatitis and the natural history of disease

NAFLD is a broad term used to describe a spectrum of liver diseases characterised by excessive hepatic fat accumulation with associated insulin resistance, in the absence of a secondary cause or significant alcohol consumption^[9,10]. More precisely, NAFLD is defined as the presence of steatosis in > 5% of hepatocytes histologically or by proton density fat fraction on magnetic resonance imaging (MRI-PDFF), and it can be subclassified into nonalcoholic fatty liver (NAFL) or nonalcoholic steatohepatitis (NASH)^[9,10]. While NAFL refers to simple steatosis, NASH is characterised by hepatic lobular inflammation and is associated with an increased risk of progression to fibrosis, cirrhosis and hepatic decompensation^[9]. NASH can only reliably be differentiated from NAFL histologically, and the prevalence of NASH in liver biopsies from NAFLD patients is estimated to be 59.10% (95%CI: 47.55%-69.73%) from pooled NASH prevalence data^[1]. This estimate is higher than previous estimates, suggesting NASH occurred in approximately one third of NAFLD patients; however, retrospective biopsy studies are inherently affected by selection bias of patients in whom there is high clinical suspicion for steatohepatitis^[11,12].

Fibrosis is an important prognostic factor in NAFLD. Fibrosis stage is independently associated with mortality, liver transplantation and liver-related events, with fibrosis occurring at twice the rate in patients with NASH compared with NAFL^[13,14]. Furthermore, liver-related mortality increases significantly with increasing fibrosis stage^[15]. In a meta-analysis of 411 patients from 11 cohort studies with biopsy proven NAFLD (2145.5 person-years of follow-up), the annual fibrosis progression rate in patients with NAFL and stage 0 fibrosis at baseline was 0.07 stages per year (95%CI: 0.02-0.11 stages/year), and 0.14 stages per year in NASH (95%CI: 0.07-0.21 stages per year)^[16]. This is the equivalent of one stage of progression over 14.3 years (95%CI: 9.1-50 years) in NAFL vs. 7.1 years (95%CI: 4.8-14.3 years) for NASH. In patients with NASH, approximately 20% of those with advanced (F3) fibrosis will progress to cirrhosis, and 20% of compensated NASH cirrhotics will develop hepatic decompensation over a two-year period^[17,18].

Current global trends in HCC

Liver cancer, of which HCC is the main subtype, has the sixth highest cancer incidence, and is the fourth leading cause of cancer-related death worldwide^[19]. There are significant geographic variations in the incidence of HCC, reflecting the differences in aetiology of liver diseases in each region^[20]. In Asian countries where the highest incidence rates of HCC are reported (20 per 100,000 in men and 6.9 in

women), viral hepatitis, particularly hepatitis B, accounts for the majority of cases with the population attributable fraction due to hepatitis B approximately 51%-57%^[21,22]. Comparatively, in high income regions of the world such as Western Europe, North America and Australasia, where the predominant aetiologies are hepatitis C and alcohol, HCC incidence rates are much lower^[8]. In Europe, the age-standardised incidence rate is estimated at 6.8 per 100,000 population in men and 2.2 in women, while, in the US, the overall incidence is 11.6 per 100,000 population in men and 4.3 per 100,000 in women^[21,23]. This is on the background of an almost three-fold increase in HCC cases in the United States over the past two decades^[24,25]. Australia and New Zealand have also demonstrated a significant increase in HCC with a 7.5-fold increase between 1982 and 2014^[26]. In 2015, the Australian incidence of HCC was 7.6 per 100,000 persons, again with higher rates in men (12 per 100,000) than women (3.9 per 100,000), which is expected to have increased to 8.6 cases per 100,000 persons in 2019^[27]. This increase is likely due in part to an aging population, with a high prevalence of hepatitis C; however, the rise of the obesity epidemic, and an associated increase in the prevalence of NAFLD-related HCC, may also be contributing^[28,29]. Furthermore, within these Western countries, particularly in the US, NAFLD, fibrosis and HCC are more prevalent in some ethnic sub-populations. This has been seen in Hispanic, Pacific Islander and subcontinental Indian groups, whereas other sub-populations, such as African Americans, appear to have lower rates despite similar risk factors^[30-32]. Whether this difference is due to disparities in socioeconomic status or diet remains to be seen; however, there may also be inherent biological differences with a higher prevalence of the PNPLA3 genetic polymorphism noted in the Hispanic population^[33].

Prevalence of NAFLD-related HCC

NAFLD has become the most rapidly increasing cause of HCC in Western countries, with the proportion of HCC attributable to NAFLD increasing significantly over the past two decades^[34,35]. This shift has mirrored the rise of obesity and the metabolic syndrome, while also coinciding with the development of effective treatments for viral hepatitis and greater coverage of hepatitis B vaccination programmes^[6]. This increase in NAFLD-related HCC is most pronounced in Western nations: in the BRIDGE study, a multiregional, large-scale longitudinal cohort study of consecutive newly diagnosed HCC cases, NAFLD accounted for 10%-12% of HCC cases in North America and Europe, but only 1%-6% in Asian countries^[7]. In North America, NAFLD is a common cause of HCC. In a large early cohort study of HCC patients identified from a healthcare claims database in the USA, NAFLD was found to be the most common aetiology, accounting for 59% of their 4406 cases^[36]. However, subsequent similar US-based longitudinal cohort studies have reported a much lower proportion of NAFLD-related HCC. The Surveillance, Epidemiology, and End Results (SEER) cancer registry database found that NAFLD was the predominant aetiology in 14.1% of their 4929 HCC cases; this was even lower in the Veteran Affairs (VA) Hospitals cohort, where 8% of 1500 HCCs were attributable to NAFLD^[8,37]. This may reflect differences in the diversity of the groups studied here, with the VA group enriched with older males with hepatitis C. NAFLD is now the second leading cause of liver transplantation for HCC in the United States after Hepatitis C virus (HCV), and is the most rapidly growing indication for HCC-related liver transplantation, having increased from 8.3% in 2002 to 13.5% in 2012^[38]. Moreover, the number of people with NAFLD-related HCC on liver transplant waiting lists in the United States has also risen from 2.1% in 2002 to 17.9% in 2017^[38,39].

European and Australasian studies have also described an increase in the prevalence of NAFLD-related HCC. In the UK, between 2000 and 2010 there was a 10-fold increase in NAFLD-related HCC, with histologically or radiologically proven NAFLD accounting for 34.8% of all HCC cases in 2010 in one cohort of 633 patients^[34]. Data from the European Transplant Registry also show an increase in transplantation for NAFLD-related HCC from 0.2% in 2007 to 1.2% in 2017^[40]. In Australia and New Zealand, NAFLD is the third leading cause of HCC in those who underwent transplantation, and an Australian cohort of 272 HCC patients found NAFLD to be the underlying aetiology with 14% of cases^[41,42]. A summary of these cohorts is outlined in Table 1.

Table 1. Overview of trials

Author	Country	Design/Method	Number of participants	Definition of NAFLD	Results	Proportion of NAFLD with cirrhosis (%)	Limitations/Bias
Marrero <i>et al.</i> ^[69]	USA	Single centre retrospective cohort of consecutive HCC cases	105	Histology	Cryptogenic cirrhosis 2nd leading cause of HCC ($n = 30$, 29%) and 6 of those cases had histological features of NASH, while 8 had clinical features of NAFLD	N/A	Retrospective study design
Sanyal <i>et al.</i> ^[47]	USA	Single centre prospective cohort of patients with NASH cirrhosis	152	Histologic features of steatohepatitis and cirrhosis	NASH had a lower risk of developing hepatocellular carcinoma compared to those with HCV cirrhosis (10/149 patients <i>vs.</i> 25/147 patients at risk; $P < 0.01$)	100%	Small cohort, with low numbers of HCC and comparator group was those with treatment naïve HCV or treatment non-responders in pre DAA therapy era
Paradis <i>et al.</i> ^[70]	France	Retrospective review of HCC liver resection specimens between 1995 and 2007	128	N/A	65.5% of cases with Met Sy associated HCC had F0-2 fibrosis	N/A	Surgical resection bias could account for high proportion of patients without significant fibrosis
Yatsuji <i>et al.</i> ^[46]	Japan	Prospective cohort study of patients with NASH and HCV cirrhosis	68	Histologic features of steatohepatitis and cirrhosis	5-year HCC rate was 11.3% for NASH and 30.5% for HCV	100%	Small cohort, with low numbers of HCC and comparator group was those with treatment naïve HCV or treatment non-responders in pre DAA therapy era
Ascha <i>et al.</i> ^[44]	USA	Prospective analysis of patients with NASH and HCV cirrhosis between 2003 and 2007	195	Histologic features of NASH or cryptogenic cirrhosis in the presence of metabolic syndrome and without a history of significant alcohol intake	NASH Cirrhotics had a yearly cumulative incidence of HCC 2.6% <i>c/</i> w 4% in HCV cirrhosis NASH patients were older at diagnosis of cirrhosis (56.6 years <i>vs.</i> 48.2 years)	100%	Small cohort, with low numbers of HCC and comparator group was those with treatment naïve HCV or treatment non-responders in pre DAA therapy era
Sanyal <i>et al.</i> ^[36]	USA	Retrospective review of HCC cases identified from a large healthcare claims database between 2002 and 2008	4406	Documentation of diagnosis based on ICD-9 coding	NAFLD (59%) most common aetiology of HCC followed by diabetes (36%) Of 729,018 patients with NAFLD/ NASH codes, 19,217 (2.7%) had HCC at one time (incident or prevalent case)	46%	Use of coding criteria likely underestimates NAFLD cases when estimating incidence or prevalence
Bhala <i>et al.</i> ^[45]	Australia, USA, UK, Italy	Prospective multicentre natural history study of NAFLD patients with advanced fibrosis or Child's Pugh A cirrhosis	247	Histologic features of advanced fibrotic NAFLD as well as elevated aminotransferases for at least 6 months	HCC occurred more commonly in patients with HCV cirrhosis than in NAFLD [$n = 18$ (6.8%) <i>vs.</i> 6 (2.4%); $P = 0.03$]	52.2%	Small cohort, with low numbers of HCC and comparator group was those with treatment naïve HCV or treatment non-responders in pre DAA therapy era
Ertle <i>et al.</i> ^[50]	Germany	Single centre review of new HCC cases within a 12-month period between 2007 and 2008	162	NAFLD/NASH was defined according to the histological features of NASH, when available or cryptogenic cirrhosis in the presence of MetSy and without a history of significant alcohol intake	NAFLD and NASH most common aetiology 24% followed by HCV 23.3% but cryptogenic made up 15.3%	58.3%	Small cohort. All NASH patients were cirrhotic with no non-cirrhotic NASH controls

Wong <i>et al.</i> ^[38]	USA	Retrospective cohort study of OLT recipients on organ registry for HCC in US from 2002 to 2012.	10,061	Documented diagnosis of NASH or obese patients with cryptogenic/unknown aetiology of liver disease	NASH was 2nd leading cause of OLT for HCC (8.3% in 2002 vs. 10.3% in 2007 vs. 13.5% in 2012) behind HCV but the most rapidly growing indication (4x increase in NASH, 2x increase in HCV) Non-NASH-related HCC patients were more likely to be men (79.2% vs. 70.6%) and were younger at time of OLT (57.2 ± 7.6 vs. 59.3 ± 7.3) NASH-related patients had higher BMI (33.6 ± 4.3 vs. 27.3 ± 4.9) and higher rates of diabetes (42.8% vs. 20.8%)	N/A	Used a modified diagnostic criterion for NASH. Did not present proportion of cirrhotic patients. Transplant patients tend to have high adherence and engagement with medical care, which may account for the older and heavier patients undergoing transplant with NASH
Dyson <i>et al.</i> ^[34]	UK	Retrospective longitudinal cohort of consecutive HCC cases between 2000 and 2010	632	Evidence of a fatty liver on biopsy or imaging, with an otherwise negative liver screen, drinking < 21 or < 14 units of alcohol per week respectively for at least 5 years prior to their first presentation with liver disease	NAFLD accounted for 34.8% of HCC cases NAFLD patients were older (71.3 vs. 67) Survival similar to other aetiologies but higher incidental presentation (38.2%) and lower prevalence of cirrhosis (77.2%)	77.2%	Retrospective single centre study in a region which high rates of obesity and diabetes
Park <i>et al.</i> ^[7]	Multi-regional	The BRIDGE study: Observational longitudinal cohort in 42 countries between 2005 and 2012	18,301 (Asia 67% of patients, Europe 20% and North America 13%)	Medical chart review	Proportion of HCC attributable to NAFLD: North America 12%, Europe 10%, China 1%, Taiwan 5%, South Korea 6%	N/A	Multiple centres in different regions without a clear definition of NAFLD used
Younossi <i>et al.</i> ^[8]	USA	Longitudinal cohort study using SEER registries to identify patients with HCC between 2004 and 2009	4929	ICD codes NAFLD also defined by clinical diagnosis (cryptogenic cirrhosis, obese-diabetics with cryptogenic liver disease)	14.1% of HCC linked to NAFLD and a 9% annual increase in NAFLD-related HCC NAFLD HCC were older, had shorter survival time, more heart disease and more likely to die from primary liver cancer Only 5% who received transplant post HCC were for NAFLD	N/A	Coding information can underestimate NAFLD and obesity
Mittal <i>et al.</i> ^[37]	USA	Retrospective cohort analysis of patients with HCC in US Veterans Affairs hospitals between 2005 and 2010	1500	Histology or presence of the metabolic syndrome in the absence of other causes of liver disease	NAFLD HCC accounted for 8% of HCC 1-year survival comparable between aetiologies	58.3%	The vast majority of the cohort were male, which does not represent the general population
Perumpail <i>et al.</i> ^[71]	USA	Case series of HCC patients	44	Radiological or histological evidence of hepatic steatosis, with no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders	9 cases of HCC in the absence of cirrhosis, and 6 were attributable to NAFLD/NASH	N/A	Very small case series

Piscaglia <i>et al.</i> ^[49]	Italy	Multicentre observational prospective cohort of patients with NAFLD-related HCC	145	NAFLD if all other known aetiologies of liver disease could be ruled out and if consistent present or past histological or ultrasonographic features of fatty liver and alcohol intake < 30 g/day	46.2% of NAFLD associated HCC were non-cirrhotic compared to 2.8% in those with HCV 73.1% of NAFLD-related HCC patients have diabetes mellitus compared with only 24.9% of HCV-related HCC patients NAFLD HCC detected at later stage, had larger volume and more infiltrative pattern compared to HCV-related HCC Non-significant survival difference NAFLD 28.5 months vs. 35 months in HCV	53.8%	Median follow up of 13 months so longer term survival results may not be truly reflective of the general population
Mohamad <i>et al.</i> ^[48]	USA	Retrospective analysis of patients diagnosed with HCC and NAFLD at the Cleveland Clinic between 2003 and 2012	83	Histologically	43.4% of NAFLD-related HCC occurred with a non-cirrhotic liver NAFLD HCC were older and more likely to present with a single nodule, larger tumour and more likely to undergo resection, but did have higher rates of recurrence	56.6%	Retrospective case series with selection bias for patients who underwent liver biopsy
Kanwal <i>et al.</i> ^[32]	USA	Prospective longitudinal cohort of NAFLD patients with matched controls	296,707	2 or more elevated ALT values (> 40 IU/mL for men and > 31 IU/mL for women) in the outpatient setting in the absence of other causes of liver disease or any ICD codes for alcohol use	Risk of HCC 0.21/1000 PYs in those with NAFLD HR: 7.62; 95%CI: 5.76-10.09 NAFLD with cirrhosis a/w higher risk 10.6/1000 PYs 20% of NAFLD associated HCC were not cirrhotic	80%	ALT is not always an accurate diagnostic marker for NAFLD and this method may be underestimate prevalence in both NAFLD and control group
Hong <i>et al.</i> ^[42]	Australia	Multicentre prospective cohort of newly diagnosed HCC between 2012 and 2013	272	Medical chart review	NAFLD associated HCC accounted for 14% of cases	N/A	No clear definition of NAFLD used
Bengtsson <i>et al.</i> ^[52]	Sweden	Single centre retrospective cohort study of all HCC patients at Karolinska University Hospital Stockholm from 2004 to 2017	225	NAFLD defined by no other cause of liver disease and one of: (1) Liver biopsy supporting NAFLD; (2) Radiological features supporting steatosis; (3) BMI ≥ 25 and T2DM or BMI ≥ 30	Non-cirrhotic NAFLD-HCC were older (74 years vs. 70 years), had a lower prevalence of T2DM (66% vs. 80%), larger tumours, less frequently underwent liver transplantation (0% vs. 11%) and more frequently underwent resection (35% vs. 8%) compared to those with cirrhosis Mortality was similar (aHR for non-cirrhotic NAFLD-HCC vs. cirrhotic NAFLD-HCC 0.93; 95%CI: 0.58-1.51)	63%	Retrospective design and cohort derived from a tertiary referral centre which may be affected by referral bias, with those not suitable for curative intervention not referred for review

NASH: nonalcoholic steatohepatitis; HCC: hepatocellular carcinoma; NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; HCV: hepatitis C virus; SEER: surveillance, epidemiology, and end results; HR: hazard ratio; aHR: adjusted hazard ratio; OLT: orthotopic liver transplantation; T2DM: type 2 diabetes mellitus; ALT: alanine aminotransferase; LT: in Wong USA 2012 please change the 'LT' in the methods column to OLT; DAA: direct-acting antiviral agents; ICD: international classification of diseases; N/A: not applicable; PYs: person years

Incidence of HCC in NAFLD

Although NAFLD represents an increasing proportion of HCC cases, the true risk of HCC in NAFLD patients with and without cirrhosis is not clear. As HCC is an infrequent outcome of chronic liver disease, there are limited studies with the adequate cohort size and duration of follow up required to accurately assess incidence rates. As a result, several meta-analyses have been performed to pool data and increase the sample size. A systematic review by White *et al.*^[43] found the risk of HCC in NAFLD patients to be in the range of 0%-38% over a median of 5-10 years of follow up. More recently, Younossi *et al.*^[1] performed a meta-analysis to evaluate the global prevalence and outcomes of NAFLD, and calculated an annual HCC incidence of 0.44 per 1000 person years (95%CI: 0.29-0.66) in patients with NAFLD, with much higher rates in those with NASH, 5.29 per 1000 person years (95%CI: 0.75-37.56). These meta-analyses are somewhat limited by the heterogeneity of the included studies, with small retrospective cohorts and inconsistent methods of defining NAFLD. As a result, a large United States longitudinal study led by Kanwal *et al.*^[32] compared 296,707 patients with NAFLD to 296,707 matched healthy controls to determine the incidence of HCC in these cohorts. NAFLD was defined by persistently elevated transaminases (> 40 IU/mL for men and > 31 IU/mL for women, with a minimum of two results at least six months apart), in the absence of any significant alcohol intake or another cause of liver disease. The healthy controls had normal liver function tests, no other history of liver disease and minimal alcohol intake. The overall incidence of HCC in the NAFLD patients was low, 0.21 per 1000 patient years; however, HCC incidence was much higher in patients with NAFLD cirrhosis, 10.6 per 1000 patient years. Overall, cirrhosis in NAFLD appears to be the most significant risk factor for HCC [Figure 1], with approximately 6%-13% of patients with NAFLD cirrhosis developing a HCC during 3-10 years of follow up in a number of prospective observational cohorts^[44-47].

NAFLD-related HCC in the absence of cirrhosis

NAFLD patients without liver cirrhosis are also at increased risk of HCC compared to those without liver disease^[32,43]. This is particularly concerning given the high population prevalence of NAFLD, and the implications for HCC screening and increasing disease burden^[1]. In the US, Sanyal *et al.*^[36] found that, among patients with NAFLD-related HCC, as many as 54% were not known to be cirrhotic based on health care coding information. The proportion of non-cirrhotic patients was similarly high in the smaller Cleveland clinic cohort, which found that 43.4% of cases occurred in the absence of cirrhosis^[48]. Those patients tended to be older (67.5 ± 12.3 years vs. 62.7 ± 8.1 years), and less likely to be obese (52% vs. 83%) or have type 2 diabetes (38% vs. 83%), than their cirrhotic counterparts. Similar findings have also been reported from European cohort studies, with a high proportion of NAFLD-related HCC occurring in non-cirrhotic patients: 46.2% in a multicentre Italian study, 41.7% in a single centre German study and 22.7% in a UK-based cohort^[34,49,50]. In the cohort study by Kanwal *et al.*^[32], the risk of HCC in non-cirrhotic NAFLD was higher than in the healthy controls, but the overall incidence rates were low (0.08 vs. 0.02 per 1000 person years). Despite this, due to the high global prevalence of NAFLD, the absolute burden of NAFLD-related HCC, including those with and without cirrhosis, remains significant.

Clinical outcomes in NAFLD-related HCC

Patients with NAFLD-related HCC tend to present at an older age compared to patients with HCC due to other aetiologies^[8,28,31]. In the SEER cohort, NAFLD-related HCC patients were older (73 ± 8 years vs. 66 ± 11 years), had more heart disease (35.1% vs. 7%-27%) and had a shorter mean survival time (14.17 ± 17.14 months vs. 17.85 ± 21.47 months), compared to other aetiologies; and 84.3% died of their primary liver cancer^[8]. This group found higher odds of one-year mortality (OR: 1.21, 95%CI: 1.01-1.45) in those with NAFLD-related HCC, although other large HCC cohorts have found no difference in overall survival^[34,37,49]. Furthermore, a Canadian study of 929 patients who underwent a transplant for HCC, including 60 with NAFLD-related HCC, also found that these patients had similar one-, three- and five-year survival outcomes (98%, 96% and 80%, respectively) compared to non-NAFLD HCC (95%, 84% and 78%, respectively), with no difference in tumour recurrence (13.3% vs. 14%)^[51]. As patients with NAFLD-related

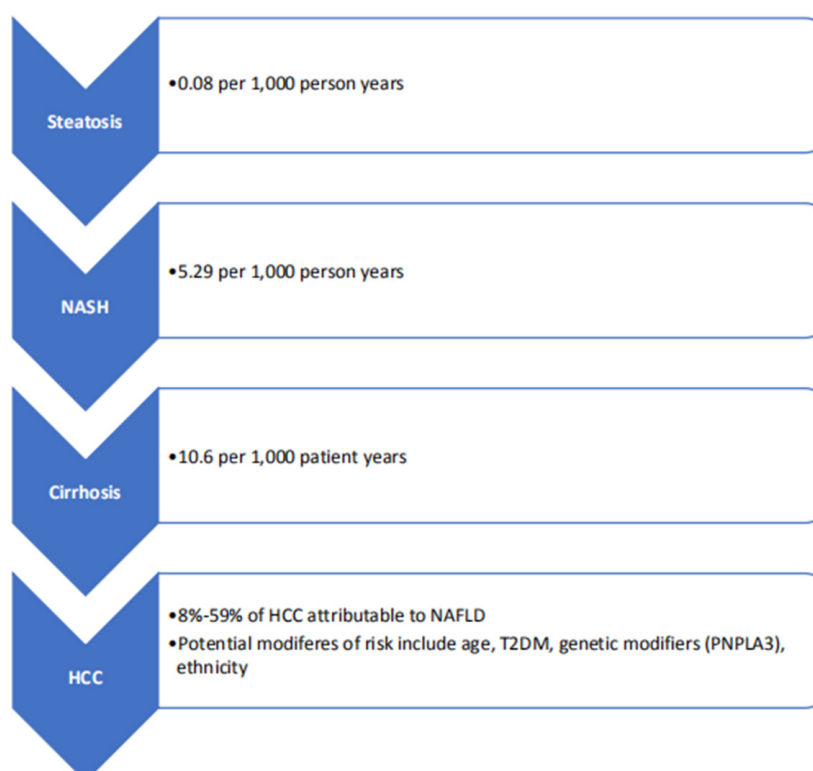


Figure 1. Increasing risk of HCC with stage of disease^[1,7,32,36-38]. NASH: nonalcoholic steatohepatitis; HCC: hepatocellular carcinoma; NAFLD: non-alcoholic fatty liver disease; T2DM: type 2 diabetes mellitus

HCC tend to be older, with greater metabolic and vascular comorbidities than those presenting with HCC from other causes, the relative equivalence in survival may be due to the proportion of patients without cirrhosis in some of these cohorts (22.8% and 41.7% in the studies by Dyson and Mittal, respectively)^[8,34,37].

Although non-cirrhotic and cirrhotic NAFLD-related HCC may present differently, overall survival rates between these two groups appear to be the same. A review of NAFLD-related HCC cases at the Cleveland clinic found that HCC in non-cirrhotic patients was more likely to present as a single nodule (80.6% vs. 52.2%) and with a larger nodule size (> 5 cm) (77.8% vs. 10.6%)^[48]. From a treatment perspective, they were more likely to receive hepatic resection (66.7% vs. 17%) but less likely to receive loco-regional therapy (22.3% vs. 61.7%) or orthotopic liver transplantation (OLT) (0% vs. 72.3%). They also found that HCC in non-cirrhotic NAFLD had a higher rate of recurrence than in those with cirrhosis (86% vs. 14%); however, after adjusting for age or OLT, there was no difference in overall survival between the two groups. This is consistent with data from a large European cohort of NAFLD-related HCC patients in Sweden, which found non-cirrhotic patients to be older (median 74 years vs. 70 years), with larger tumours^[52]. Similarly, in this group, those without cirrhosis were more likely to have a resection (35% vs. 8%), and less frequently underwent OLT (0% vs. 11%), but no significant difference was found in the overall mortality rates [adjusted hazard ratio (aHR): 0.93, 95%CI: 0.58-1.51, $P = 0.78$]. On further analysis, parameters independently associated with a higher risk for overall mortality in non-cirrhotic patients included; Barcelona Clinic Liver Cancer class C or D (aHR compared with class 0: 7.03, 95%CI: 2.89-17.11, $P < 0.001$), the presence of four or more tumours (aHR compared with one tumour: 9.26, 95%CI: 3.20-26.81, $P < 0.001$), higher serum albumin (aHR 0.29 per increased g/dL, 95%CI: 0.15-0.56, $P < 0.001$) and the presence of T2DM (aHR: 3.65, 95%CI: 1.69-7.89, $P = 0.001$).

Obesity and type 2 diabetes and HCC risk

Obesity and T2DM are metabolic comorbidities closely associated with NAFLD, which have also been independently linked to the development of HCC^[53,54]. T2DM has been found to be associated with increased HCC risk, particularly in patients with non-viral liver disease. A 2012 meta-analysis including 17 case-control studies and 32 cohort studies showed a statistically significant increased risk of HCC prevalence among diabetic individuals (RR: 2.31, 95%CI: 1.87-2.84)^[53]. The pooled risk estimate from 17 case-control studies (OR: 2.40, 95%CI: 1.85-3.11) was slightly higher than that from the 25 cohort studies included (RR: 2.23, 95%CI: 1.68-2.96). This analysis was limited by lower patient numbers, which prevented adequate subgroup analysis, and, while there was some adjustment for the presence of viral hepatitis and cirrhosis, the possible presence of underlying NAFLD was not accounted for. In a study from the Mayo Clinic, the hazard ratio of HCC in cirrhotic patients without HCV infection was found to be two times higher in patients with T2DM compared to those without [Hazard Ratio (HR): 2.1, 95%CI: 1.1-4.1]^[55]. Additionally, T2DM has also been found to be an independent risk factor for HCC in patients with cryptogenic cirrhosis; however, these patients also demonstrated features of NAFLD based on markers of insulin resistance and lipid profiles^[56]. Overall, while T2DM does appear to be associated with an increased risk of HCC in those with or without known liver disease, current studies are limited by confounding and the difficulty of excluding underlying NAFLD as a contributing factor.

Obesity is associated with an increased risk of many types of malignancy, and several epidemiological studies suggest a modest increase in the relative risk of HCC in obese populations^[57]. A 2012 meta-analysis of 26 prospective cohort studies, including 25,337 individuals, found excess body weight [body mass index (BMI) ≥ 25 kg/m²] and obesity (BMI ≥ 30 kg/m²) to be associated with an increased risk of primary liver cancer [Summary Relative Risks (SRR) 1.48, 95%CI: 1.31-1.67; and SRR 1.83, 95%CI: 1.59-2.11, respectively]^[54]. While this evidence is somewhat limited by heterogeneity, on subgroup analysis, there was an increased risk in males, those with HCV and cirrhosis from any aetiology compared with the general population, suggesting that obesity may be an important cofactor in the development of HCC.

Genetic markers in NAFLD-related HCC

Several host genetic polymorphisms have been linked to the presence of NAFLD, risk of fibrosis progression and the development of NAFLD-related HCC^[58]. These are particularly relevant as they may present novel biomarkers to help triage those at most risk of HCC and therefore in need of screening, particularly among those with NAFLD and no cirrhosis. The most studied of these is a single-nucleotide polymorphism (SNP) in PNPLA3. The association between PNPLA3 (rs738409) and hepatic steatosis was first identified in the genome-wide survey performed on the > 2000 participants of the Dallas Heart Study who underwent Magnetic Resonance Spectroscopy of the liver for quantification of liver fat^[59]. The presence of this SNP has since been found to be associated with an increased risk of advanced fibrosis in all patients with liver disease, and a 2014 meta-analysis confirmed that it was also associated with an increased risk of HCC in patients with NASH and alcohol-related cirrhosis (OR: 1.67, 95%CI: 1.27-2.21), but not in other aetiologies (OR: 1.33, 95%CI: 0.96-1.82)^[60]. Moreover, in a large well-characterised Northern European cohort of patients with histologically proven NAFLD patients, 100 of whom had NAFLD-related HCC, it was shown that PNPLA3 rs738409, especially the C > G polymorphism, was associated with an increased risk of HCC independent of other traditional risk factors including age, gender, BMI, presence of T2DM or the presence of advanced fibrosis or cirrhosis^[61]. Despite this evidence, the potential use of this SNP as a predictive biomarker to identify high-risk individuals for HCC screening has not yet been validated in larger cohorts, and it is not widely used.

Other potential genetic risk modifiers of interest include a variant in the *MBOAT7* gene, which was associated with an increased risk of NAFLD-related HCC in an Italian cohort of NAFLD patients (OR: 1.65, 95%CI: 1.08-2.55; $n = 765$), particularly in those without advanced fibrosis ($P < 0.001$), and TERT

promoter mutations that can be found in low and high grade dysplastic lesions, and in increased frequency in early HCC^[62-64]. The *TM6SF2* genetic variant on chromosome 19 has also been shown to be associated with an increased risk of NAFLD and NAFLD-related hepatic fibrosis and cirrhosis, independent of other known traditional risk factors including PNPLA3 status^[65]. In one cohort of 99 biopsy-confirmed HCC patients, homozygous carriage of the *TM6SF2* rs58542926 minor allele was associated with an increased risk of NAFLD-related HCC. However, this was not shown to be significant on multivariate analysis when adjusting for traditional risk factors such as age, gender or cirrhosis^[66]. Further investigation is required to determine the clinical significance of these associations and establish whether they may be of prognostic or diagnostic significance in the future. Consideration of cost, access to and acceptability of genetic sequencing as a screening or prognostic tool for patients with NAFLD must also be explored.

Should patients with NAFLD undergo HCC surveillance?

Routine HCC surveillance is recommended for all patients with cirrhosis regardless of the aetiology of their liver disease^[9,10], and as such all patients with NAFLD cirrhosis should be enrolled in a screening program. While HCCs that are detected by surveillance programs are found at an earlier stage than those found incidentally, patients with NAFLD-related HCC are less likely to have recognised liver disease, and as such have a lower proportion of HCCs detected through screening compared with other causes of liver disease^[67]. In the UK Newcastle cohort and the HCC NAFLD Italian groups, only 22.8%-47.6% of NAFLD-related HCC cases were detected during surveillance compared with 46.2%-63.3% in HCV^[34,49]. Additionally, even in high risk patients who have NAFLD cirrhosis, screening is less likely to detect early stage HCCs compared with other aetiologies^[34,37,49]. This is likely attributable to the current recommended screening modality of ultrasound, which can be technically challenging in obese patients with excess visceral adiposity, and is highly operator dependant^[68]. Cross-sectional imaging such as MRI may be used as an alternative in patients in whom adequate views of the liver are unable to be obtained via ultrasound, however the suitability and cost-effectiveness of this approach has yet to be determined. Concern remains regarding whether NAFLD patients without cirrhosis should also be screened, considering that 23%-54% of NAFLD-related HCC occurs in non-cirrhotic patients. However, due to the large volume of NAFLD patients and low annual HCC incidence of less than 2% in this cohort, routine screening is not currently recommended^[34,36,37,49]. The development of risk stratification tools may be of use in identifying high-risk patients who would benefit from screening, and may include the use of genetic markers.

Limitations in current estimates and Future directions

While these observational studies are informative in reviewing trends, there are several limitations to this method of determining the true burden of disease. The largest cohorts have typically identified NAFLD using coded registry data, or blood tests, which can be inconsistent and will not always reflect whether there is underlying fibrosis or steatohepatitis. Additionally, HCC registry information tends to be more representative of tertiary care and patients who have been referred for active management. As NAFLD-related HCC patients tend to be older and more comorbid, it is possible that not all cases are referred to these specialist centres, and they may have remained in community care settings. Similarly, as the SEER cohort demonstrated, patients with NAFLD-related HCC are less likely to be transplanted compared to HCC due to other causes, thus transplant registry information is also likely to underestimate the true burden of disease. Another limitation of cross-sectional retrospective studies is the evolving nature of NAFLD and NASH. These are not static disease states, but can progress or regress over time with changes in weight, which is often difficult to assess. While these studies have examined the risk associated with NAFLD alone, the increasing prevalence of obesity and diabetes in HCC of all aetiologies suggests that these metabolic factors including NAFLD could be an important co-factor in hepatic carcinogenesis^[34]. In the future, the development of a simple, cost-effective population-based diagnostic test would be of benefit to improve the detection rate of NAFLD and trigger screening for underlying fibrosis and cirrhosis. Additionally, the development of prognostic markers or algorithms to identify high-risk patients for HCC surveillance is also an area of ongoing research. Further, as treatment for NAFLD progresses, and weight

management becomes a greater focus of therapy, the ongoing risk of HCC in individuals with NAFLD who have subsequently lost weight or undergone therapy will need to be evaluated.

CONCLUSION

NAFLD is an important cause of liver disease worldwide and is associated with an increased risk of developing HCC, particularly in the presence of liver cirrhosis. While the absolute risk appears to be low, prevalence of NAFLD-related HCC is increasing in parallel with the prevalence of NAFLD worldwide, particularly in the West. The future global burden of NAFLD-related HCC represents a major public health threat and further research to identify cost-effective prevention and treatment strategies are urgently required.

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Authors' contributions

Performed the literature review and generated the manuscript: Farrell A
Assisted in developing the outline for the manuscript: Howell J
Responsible for review of the manuscript: Ryan M, Howell J

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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REFERENCES

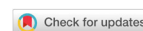
1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73-84.
2. Kim D, Touro A, Kim WR. Nonalcoholic fatty liver disease and metabolic syndrome. *Clin Liver Dis* 2018;22:133-40.
3. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. *J Hepatol* 2018;69:896-904.
4. Ford ES, Giles WH, Mokdad AH. Increasing prevalence of the metabolic syndrome among u.s. Adults. *Diabetes Care* 2004;27:2444-9.
5. Saklayen MG. The global epidemic of the metabolic syndrome. *Curr Hypertens Rep* 2018;20:12.
6. Bertuccio P, Turati F, Carioli G, Rodriguez T, La Vecchia C, et al. Global trends and predictions in hepatocellular carcinoma mortality. *J Hepatol* 2017;67:302-9.
7. Park JW, Chen M, Colombo M, Roberts LR, Schwartz M, et al. Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. *Liver Int* 2015;35:2155-66.
8. Younossi ZM, Otgonsuren M, Henry L, Venkatesan C, Mishra A, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015;62:1723-30.
9. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association

- for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1388-402.
10. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018;67:328-57.
 11. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011;140:124-31.
 12. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006;43:S99-112.
 13. Ekstedt M, Hagström H, Nasr P, Fredrikson M, Stål P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015;61:1547-54.
 14. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Björnsson ES, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2015;149:389-97.e10.
 15. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. *Hepatology* 2017;65:1557-65.
 16. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, et al. Fibrosis progression in nonalcoholic fatty liver vs. nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015;13:643-54.e1-9.
 17. Loomba R, Adams LA. The 20% rule of NASH progression: the natural history of advanced fibrosis and cirrhosis caused by NASH. *Hepatology* 2019;70:1885-88.
 18. Sanyal AJ, Harrison SA, Ratzliff V, Abdelmalek MF, Diehl AM, et al. The natural history of advanced fibrosis due to nonalcoholic steatohepatitis: data from the simtuzumab Trials. *Hepatology* 2019;70:1913-27.
 19. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, et al.; Global Burden of Disease Collaboration. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. *JAMA Oncol* 2017;3:524-48.
 20. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
 21. Wong MC, Jiang JY, Goggins WB, Liang M, Fang Y, et al. International incidence and mortality trends of liver cancer: a global profile. *Sci Rep* 2017;7:45846.
 22. Baecker A, Liu X, La Vecchia C, Zhang ZF. Worldwide incidence of hepatocellular carcinoma cases attributable to major risk factors. *Eur J Cancer Prev* 2018;27:205-12.
 23. Kim D, Li AA, Perumpail BJ, Gadiparthi C, Kim W, et al. Changing trends in etiology-based and ethnicity-based annual mortality rates of cirrhosis and hepatocellular carcinoma in the United States. *Hepatology* 2019;69:1064-74.
 24. El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: where are we? Where do we go? *Hepatology* 2014;60:1767-75.
 25. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol* 2009;27:1485-91.
 26. Wallace MC, Preen DB, Short MW, Adams LA, Jeffrey GP. Hepatocellular carcinoma in Australia 1982-2014: increasing incidence and improving survival. *Liver Int* 2019;39:522-30.
 27. Australian Institute of Health and Welfare. Cancer in Australia 2019. Available from: <https://www.aihw.gov.au/reports/cancer/cancer-in-australia-2019/formats> [Last accessed on 12 Mar 2020]
 28. Hajarizadeh B, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol* 2013;10:553-62.
 29. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013;10:686-90.
 30. Yang B, Liu JB, So SK, Han SS, Wang SS, et al. Disparities in hepatocellular carcinoma incidence by race/ethnicity and geographic area in California: implications for prevention. *Cancer* 2018;124:3551-59.
 31. Pham C, Fong TL, Zhang J, Liu L. Striking racial/ethnic disparities in liver cancer incidence rates and temporal trends in California, 1988-2012. *J Natl Cancer Inst* 2018;110:1259-69.
 32. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, et al. Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease. *Gastroenterology* 2018;155:1828-37.e2.
 33. Danford CJ, Yao ZM, Jiang ZG. Non-alcoholic fatty liver disease: a narrative review of genetics. *J Biomed Res* 2018;32:389-400.
 34. Dyson J, Jaques B, Chattopadhyay D, Lochan R, Graham J, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014;60:110-7.
 35. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011;34:274-85.
 36. Sanyal A, Poklepovic A, Moyneur E, Barghout V. Population-based risk factors and resource utilization for HCC: US perspective. *Curr Med Res Opin* 2010;26:2183-91.
 37. Mittal S, Sada YH, El-Serag HB, Kanwal F, Duan Z, et al. Temporal trends of nonalcoholic fatty liver disease-related hepatocellular carcinoma in the veteran affairs population. *Clin Gastroenterol Hepatol* 2015;13:594-601.e1.
 38. Wong RJ, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014;59:2188-95.
 39. Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, et al. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology* 2019;69:2672-82.
 40. Belli LS, Perricone G, Adam R, Cortesi PA, Strazzabosco M, et al. Impact of DAAs on liver transplantation: Major effects on the evolution of indications and results. An ELITA study based on the ELTR registry. *J Hepatol* 2018;69:810-17.

41. Calzadilla-Bertot L, Jeffrey GP, Jacques B, McCaughan G, Crawford M, et al. Increasing incidence of nonalcoholic steatohepatitis as an indication for liver transplantation in Australia and New Zealand. *Liver Transpl* 2019;25:25-34.
42. Hong TP, Gow PJ, Fink M, Dev A, Roberts SK, et al. Surveillance improves survival of patients with hepatocellular carcinoma: a prospective population-based study. *Med J Aust* 2018;209:348-54.
43. White DL, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clin Gastroenterol Hepatol* 2012;10:1342-59.e2.
44. Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, et al. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010;51:1972-8.
45. Bhala N, Angulo P, van der Poorten D, Lee E, Hui JM, et al. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. *Hepatology* 2011;54:1208-16.
46. Yatsuji S, Hashimoto E, Tobari M, Tani M, Tokushige K, et al. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. *J Gastroenterol Hepatol* 2009;24:248-54.
47. Sanyal AJ, Banas C, Sargeant C, Luketic VA, Sterling RK, et al. Similarities and differences in outcomes of cirrhosis due to nonalcoholic steatohepatitis and hepatitis C. *Hepatology* 2006;43:682-9.
48. Mohamad B, Shah V, Onyshchenko M, Elshamy M, Aucejo F, et al. Characterization of hepatocellular carcinoma (HCC) in non-alcoholic fatty liver disease (NAFLD) patients without cirrhosis. *Hepatol Int* 2016;10:632-9.
49. Piscaglia F, Svegliati-Baroni G, Barchetti A, Pecorelli A, Marinelli S, et al. Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: a multicenter prospective study. *Hepatology* 2016;63:827-38.
50. Ertle J, Dechène A, Sowa JP, Penndorf V, Herzer K, et al. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *Int J Cancer* 2011;128:2436-43.
51. Sadler EM, Mehta N, Bhat M, Ghanekar A, Greig PD, et al. Liver transplantation for NASH-related hepatocellular carcinoma versus non-NASH etiologies of hepatocellular carcinoma. *Transplantation* 2018;102:640-47.
52. Bengtsson B, Stål P, Wahlin S, Björkström NK, Hagström H. Characteristics and outcome of hepatocellular carcinoma in patients with NAFLD without cirrhosis. *Liver Int* 2019;39:1098-108.
53. Wang P, Kang D, Cao W, Wang Y, Liu Z. Diabetes mellitus and risk of hepatocellular carcinoma: a systematic review and meta-analysis. *Diabetes Metab Res Rev* 2012;28:109-22.
54. Chen Y, Wang X, Wang J, Yan Z, Luo J. Excess body weight and the risk of primary liver cancer: an updated meta-analysis of prospective studies. *Eur J Cancer* 2012;48:2137-45.
55. Yang JD, Mohamed HA, Cvinar JL, Gores GJ, Roberts LR, et al. Diabetes Mellitus Heightens the Risk of Hepatocellular Carcinoma Except in Patients With Hepatitis C Cirrhosis. *Am J Gastroenterol* 2016;111:1573-80.
56. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002;123:134-40.
57. Pischon T, Nimpf K. Obesity and risk of cancer: an introductory overview. *Recent Results Cancer Res* 2016;208:1-15.
58. Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 2019;16:411-28.
59. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40:1461-5.
60. Singal AG, Manjunath H, Yopp AC, Beg MS, Marrero JA, et al. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014;109:325-34.
61. Liu YL, Patman GL, Leathart JB, Piguet AC, Burt AD, et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014;61:75-81.
62. Donati B, Dongiovanni P, Romeo S, Meroni M, McCain M, et al. MBOAT7 rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals. *Sci Rep* 2017;7:4492.
63. Donati B, Pietrelli A, Pingitore P, Dongiovanni P, Caddeo A, et al. Telomerase reverse transcriptase germline mutations and hepatocellular carcinoma in patients with nonalcoholic fatty liver disease. *Cancer Med* 2017;6:1930-40.
64. Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM. Genetic Landscape and Biomarkers of Hepatocellular Carcinoma. *Gastroenterology* 2015;149:1226-39.e4.
65. Zhang X, Liu S, Dong Q, Xin Y, Xuan S. The genetics of clinical liver diseases: insight into the TM6SF2 E167K variant. *J Clin Transl Hepatol* 2018;6:326-31.
66. Liu YL, Reeves HL, Burt AD, Tiniakos D, McPherson S, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014;5:4309.
67. Taylor EJ, Jones RL, Guthrie JA, Rowe IA. Modeling the benefits and harms of surveillance for hepatocellular carcinoma: information to support informed choices. *Hepatology* 2017;66:1546-55.
68. Cengiz M, Sentürk S, Cetin B, Bayrak AH, Bilek SU. Sonographic assessment of fatty liver: intraobserver and interobserver variability. *Int J Clin Exp Med* 2014;7:5453-60.
69. Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, et al. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology* 2002;36:1349-54.
70. Paradis V, Zalinski S, Chelbi E, Guedj N, Degos F, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. *Hepatology* 2009;49:851-9.
71. Perumpail RB, Wong RJ, Ahmed A, Harrison SA. Hepatocellular carcinoma in the setting of non-cirrhotic nonalcoholic fatty liver disease and the metabolic syndrome: US experience. *Dig Dis Sci* 2015;60:3142-8.

Review

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Diagnosing non-hepatocellular carcinoma malignancies on CT/MRI and contrast enhanced ultrasound: the Liver Imaging Reporting and Data System approach

Kathryn J. Fowler¹, Guilherme Moura Cunha¹, Tae Kyoung Kim²

¹Department of Diagnostic Radiology, University of California at San Diego, San Diego, CA 92130, USA.

²University of Toronto, Joint Department of Medical Imaging, University Health Network/Mount Sinai Hospital/Women's College Hospital, Toronto M5G 2N2, Canada.

Correspondence to: Dr. Kathryn J. Fowler, Department of Diagnostic Radiology, University of California at San Diego, 200 West Arbor Drive #8756, San Diego, CA 92130, USA. E-mail: klfowler@health.ucsd.edu

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Abstract

The Liver Imaging Reporting and Data System (LI-RADS) provides a stepwise algorithmic approach that is proven to be highly accurate in diagnosing hepatocellular carcinoma (HCC) in patients at risk. An essential and early step in the algorithm is the diagnosis of malignancies other than HCC, such as cholangiocarcinoma and combined tumors, by application of LR-M features and criteria. The LR-M category captures most non-HCC malignancies and some atypical HCCs. The exclusion of non-HCC malignancies is important for maintaining the high specificity of the LR-5, definite HCC category. This review provides an overview of the approach to diagnosing non-HCC malignancies using LI-RADS CT/MRI and contrast enhanced ultrasound algorithms.

Keywords: Magnetic resonance imaging, computer tomography, contrast-enhanced ultrasound

INTRODUCTION

According to most major liver societies, screening/surveillance imaging is recommended for patients with cirrhosis to detect early stage hepatocellular carcinoma (HCC). While HCC is the most common primary liver malignancy, cirrhosis and chronic viral hepatitis also place patients at risk for non-HCC malignancies such as intrahepatic cholangiocarcinoma (iCCA) and combined hepatocellular-cholangiocarcinomas (cHCC-CCA)^[1,2]. As a result, these non-HCC malignancies are occasionally found on surveillance imaging



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in high-risk patients. There are substantial differences in the treatment strategy and prognosis between HCC and non-HCC malignancies and it is critical to differentiate between the two.

The Liver Imaging Reporting and Data System (LI-RADS) is endorsed by the American College of Radiology and the American Association for the Study of Liver Diseases^[3]. LI-RADS provides the most comprehensive guidance for imaging in at risk patients, with imaging algorithms covering ultrasound screening/surveillance, diagnosis on CT/MRI, diagnosis on contrast enhanced ultrasound (CEUS), and treatment response on CT/MRI. This manuscript will provide an overview of the imaging appearances and LI-RADS approach to diagnosing iCCA and cHCC-CCA in at risk patients, both on CT/MRI and on CEUS.

CT/MRI

Imaging appearance of iCCA and cHCC-CCA

On CT and MRI, both iCCA and HCC may show arterial phase hyperenhancement (APHE) and washout. It is the morphology of the enhancement during the dynamic postcontrast phases that helps differentiate between them. These differences in morphology are likely attributable to different histological composition. Intrahepatic mass forming cholangiocarcinomas frequently show rim APHE, peripheral washout appearance and often delayed central enhancement^[4]. It is hypothesized that this targetoid pattern on dynamic imaging may reflect the peripheral cellularity, central necrosis and dense fibrous stroma seen in iCCA^[5]. This same histological constitution likely also explains the targetoid appearance seen on MRI diffusion weighted and hepatobiliary phase imaging that has been described in iCCA^[6-10]. Other ancillary features reported in iCCA include capsular retraction, peripheral biliary duct dilation, and central T2 hypointensity.

On the contrary, HCC tends to have nonrim APHE, nonperipheral washout appearance and delayed enhancing capsule (i.e., major features). These features are likely attributable to the changes during hepatocarcinogenesis that result in the development of unpaired arteries and loss of portal tracts^[5]. Unlike iCCA, HCC may be encapsulated by a true fibrous capsule or compressed liver parenchyma, which may explain the delayed enhancing “capsule” described on imaging.

Small iCCAs can have overlaps in imaging appearance with HCC^[11,12]. Small iCCAs have a greater propensity for showing nonrim APHE and nonperipheral washout appearances, potentially due to a preservation of portal tract architecture and the lack of significant central necrosis in smaller, early stage lesions^[5,13]. Atypical appearances and overlap with HCC may be more commonly observed in patients with risk factors for HCC such as background liver cirrhosis^[14]. The reason for this is not entirely understood but could relate to alterations in background liver blood supply (e.g., in the setting of cirrhosis, the liver receives more arterial supply and relatively less portal venous supply).

LI-RADS approach to diagnosis on CT/MRI

The LI-RADS CT/MRI diagnostic algorithm provides a step-wise approach for arriving at a highly specific diagnosis of HCC. The intention of the algorithm is to achieve a greater than 95 percent positive predictive value of the LR-5 (definite HCC) category for the diagnosis of HCC. The philosophy behind this approach rests in the fact that liver transplantation is considered the optimal cure for patients in the United States and Canada with cirrhosis and early stage HCC. In this context, definitive imaging diagnosis of HCC is sufficient for assigning priority on the transplantation waitlist and as such, demands a near zero false positive rate.

The algorithm begins with assessment of imaging adequacy and assignment of LR-NC (not categorizable) if imaging is not adequate to narrow in on a final diagnostic category. Second, the radiologist should determine if there is advanced disease, such as tumor in vein (LR-TIV). The next step involves identifying definitely or probably benign entities (e.g., perfusional shunts or hemangiomas) and assigning them an LR-1 or LR-2 categorization. Next, the radiologist is tasked with identifying possible or probable non-HCC malignancy

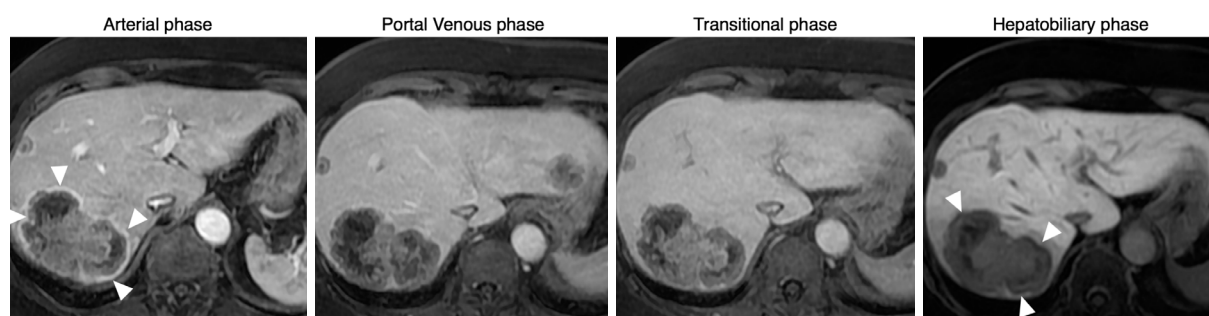


Figure 1. Gadoxetate enhanced MRI: 55 year-old male with chronic hepatitis B infection. A targetoid observation (65 mm) exhibits features of LR-M: rim arterial phase hyperenhancement and peripheral washout (arrowheads). After biopsy, the lesion was diagnosed as liver metastasis from colorectal cancer

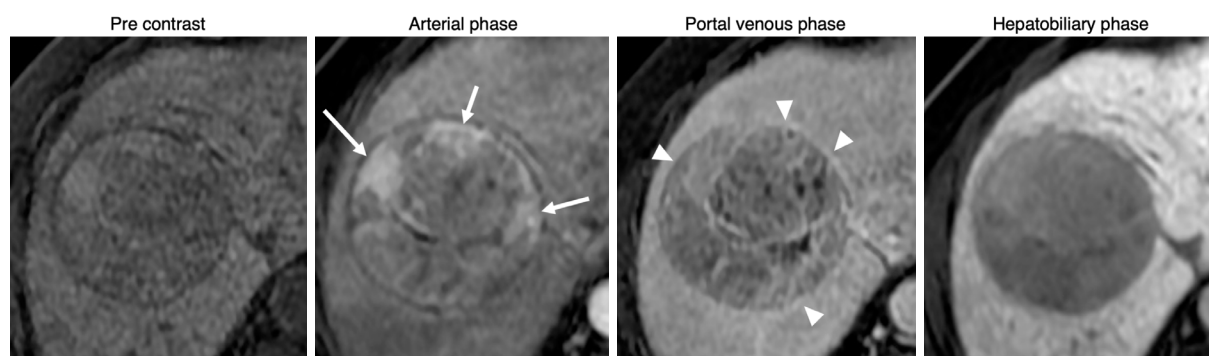


Figure 2. Gadoxetate enhanced MRI: 65 year-old male with chronic hepatitis C cirrhosis. A 60-mm observation exhibits features of LR-5: non rim arterial phase hyperenhancement (arrows), washout and capsule (arrowheads) indicating definite hepatocellular carcinoma. Ancillary features seen are a mosaic architecture and hepatobiliary phase hypointensity

(LR-M). This task will be expanded on below. Once TIV, benign entities, and other malignancies have been excluded in a step-wise fashion, the radiologist should be left with solid hepatocellular nodules that are further classified as intermediate (LR-3), probable (LR-4) or definite HCC (LR-5), according to the presence and number of major features. In LI-RADS, the major features include size, APHE, washout appearance, delayed enhancing capsule, and threshold growth.

The LR-M category is a very important step in the algorithm, as non-HCC malignancies must be considered to avoid false positives in diagnosing LR-5. The features of LI-RADS LR-M primarily reflect the features of iCCA as described above: targetoid patterns in dynamic enhancement, diffusion weighted imaging, and hepatobiliary phase appearances^[15]. Figure 1 demonstrates a typical non-HCC malignancy with LR-M targetoid features. For comparison, Figure 2 shows a typical LR-5, definite HCC, on MRI. The presence of any targetoid feature is sufficient for LR-M categorization, even if the observation has some features of HCC. Additional features may be applied in malignant observations that do not meet LR-5 or TIV criteria: marked diffusion restriction, necrosis, and infiltrative appearances. Beyond the LR-M features described here, there are ancillary features that favor malignancy in general (e.g., subthreshold growth, corona appearance, hepatobiliary phase hypointensity); however, these ancillary features are not, by themselves, sufficient to categorize an observation as LR-M.

LI-RADS recognizes that imaging features and morphologies are subjective in nature. To help radiologists, there are tie breaking rules both for features and final diagnostic categories. When in doubt, the radiologist should refer to the category or feature that is less specific for HCC. For example, if there is a question of whether APHE is rim or nonrim, the radiologist should assign rim APHE. Likewise, if there is doubt between LR-5 vs. LR-M, the radiologist should assign LR-M.

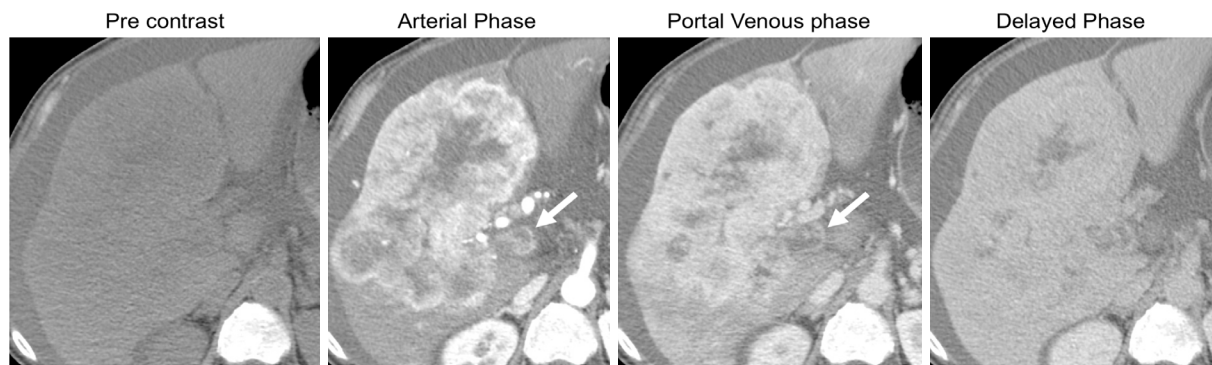


Figure 3. Contrast enhanced CT: 70 year-old male with chronic hepatitis B. A 120-mm mass in the right liver lobe exhibits a targetoid appearance in the arterial phase with central progressive enhancement in the portal venous and delayed phases, indicating a LR-M observation. There are also features of tumor in vein (arrows), shown as portal vein expansion with heterogeneous contrast enhancement. Biopsy results indicated a cholangiocarcinoma

How accurate is CT/MRI LI-RADS in differentiating iCCA from HCC?

The LI-RADS approach accurately assigns most non-HCC malignancies, including iCCA, to the LR-M category^[16-19]. It is important to note that unlike LR-5, the LR-M category is aimed at high sensitivity rather than high specificity for non-HCC malignancies. Hence, it is not unexpected that some atypical HCCs will be categorized as LR-M. In a systematic review and meta-analysis of 17 journal articles published by van der Pol *et al.*^[20], the vast majority (93%CI: 87%-97%) of lesions classified as LR-M were malignant and about two thirds were non-HCC malignancies. In other words, about 1/3 of LR-M lesions were in fact atypical HCC. Atypical HCC may represent cirrhotomimetic, sarcomatoid, macrotrabeular massive, schirrhous or other variants that may show LR-M features^[21-24]. Emerging data suggest that LR-M categorization may provide prognostic information for HCC and cHCC-CCA tumors, with shorter disease free progression and overall survival compared to tumors that are categorized as LR-5^[25]. Figure 3 shows an aggressive LR-M observation with signs of TIV.

CEUS

Imaging appearance

On CEUS, both iCCA and HCC often show APHE and washout. CEUS can detect APHE of liver observations more sensitively than CT/MRI due to real-time assessment of arterial phase enhancement. Therefore, earlier studies raised a concern for misdiagnosis of iCCA as HCC as both often show APHE and washout^[26]. However, more recent studies have consistently demonstrated that CEUS reliably differentiates iCCA from HCC if the pattern of APHE and the degree and timing of washout are considered^[27-30]. On CEUS, iCCAs commonly show rim APHE which is uncommon in HCC. Early washout within 60 s after contrast injection and marked washout are consistently demonstrated in iCCA^[31,32]. Very early washout within the arterial phase time frame, which is frequently seen in iCCA on CEUS, may explain their frequent arterial-phase hypoenhancing appearance on CT or MRI [Figure 4]^[33]. On the other hand, HCCs typically show late (> 60 s after contrast injection) and mild washout^[31,32].

The LI-RADS approach on CEUS

The LI-RADS step-wise algorithmic approach on CEUS mirrors that of CT/MRI. Similar to CT/MRI, the LR-M categorization is an early step in the process intended to identify malignant nodules that may not be HCC. CEUS LR-M criteria include: rim-APHE, early (< 60 s) washout, or marked washout visible within the first 120 s [Figures 4 and 5]^[34,35]. The CEUS LI-RADS M criteria, like CT/MRI LR-M, reflect the imaging appearance of iCCA on CEUS.

CEUS LR-M criteria differ from CT/MRI LR-M criteria mostly due to the different properties of the contrast agents^[33,36]. Figure 6 compares the criteria for LR-M and LR-5 between CT/MRI and CEUS. Microbubble

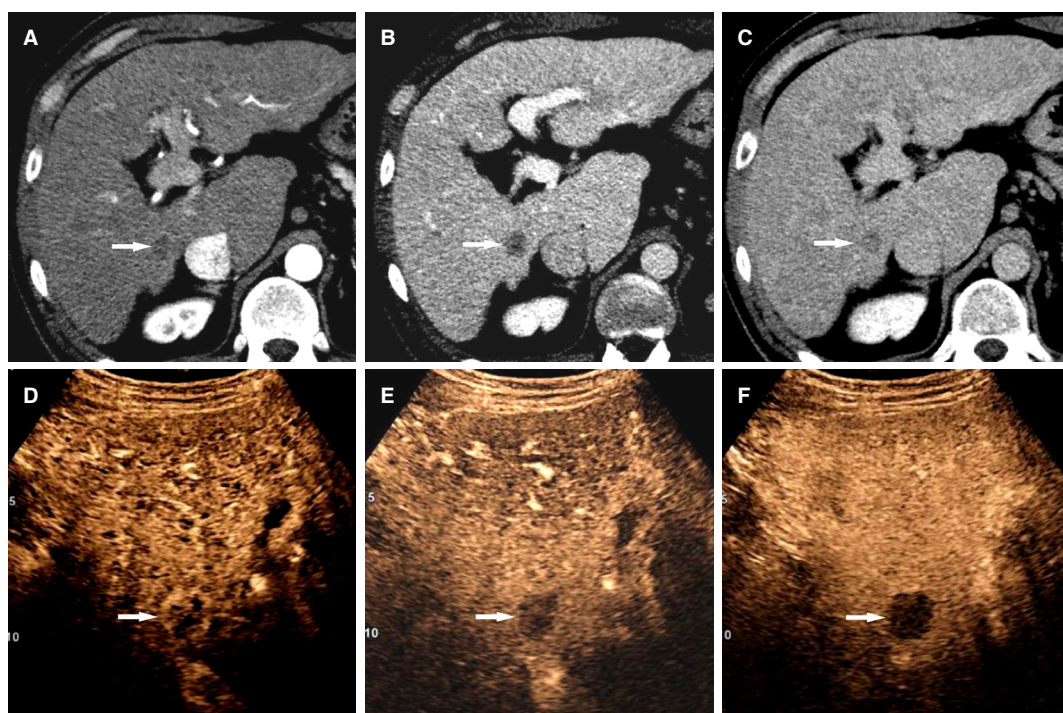


Figure 4. Intrahepatic cholangiocarcinoma in a 51-year-old man with hepatitis-C related liver cirrhosis. CT scans in the arterial (A), portal venous (B), and delayed (C) phases demonstrate a hypoattenuating hepatic nodule (arrow); C: the nodule shows a mild central enhancement in the delayed phase; D: CEUS in the arterial phase shows a rim arterial-phase hyperenhancement in the nodule (arrow); E: CEUS at 42 s after contrast injection demonstrates early washout (arrow); F: CEUS at 104 s after contrast injection shows marked washout (arrow). CEUS: contrast enhanced ultrasound

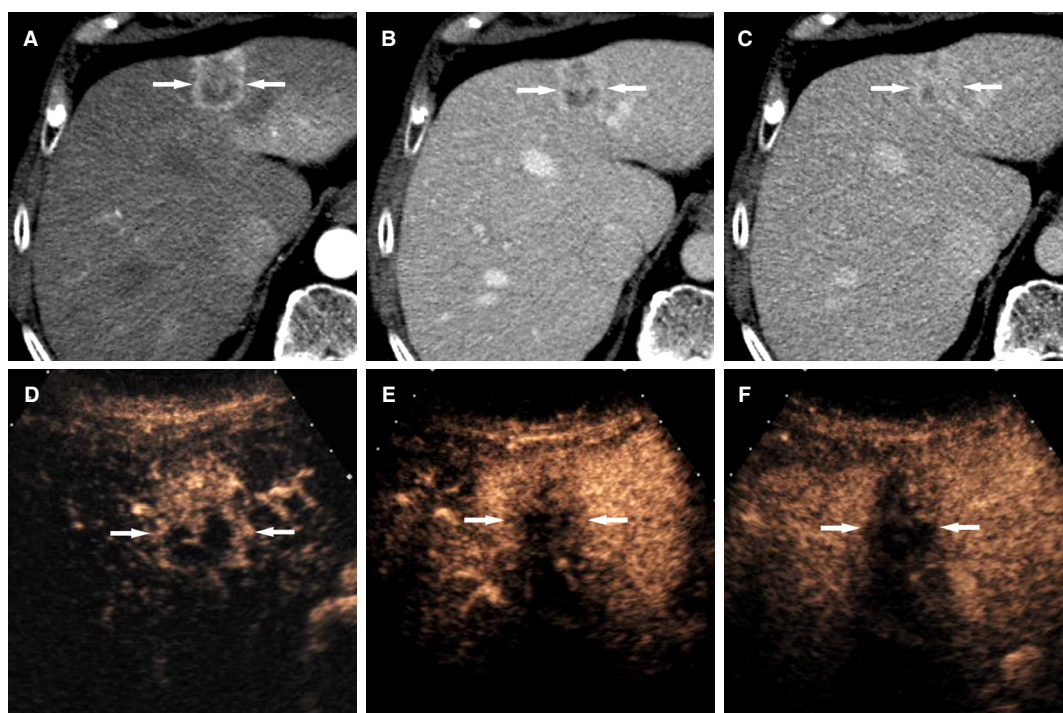


Figure 5. Intrahepatic cholangiocarcinoma in a 78-year-old man with hepatitis-C related liver cirrhosis. A: CT scans in the arterial phase shows a mass with rim arterial-phase hyperenhancement (arrows); CT scans in the portal venous (B), and delayed (C) phases demonstrates gradual enhancement of the mass (arrows); C: there is also heterogeneous hyperenhancement in the delayed phase; D: CEUS in the arterial phase shows irregular rim arterial-phase hyperenhancement in the mass (arrows); E: CEUS at 48 s after contrast injection demonstrates early washout (arrows); F: CEUS at 115 s shows marked washout (arrows). CEUS: contrast enhanced ultrasound

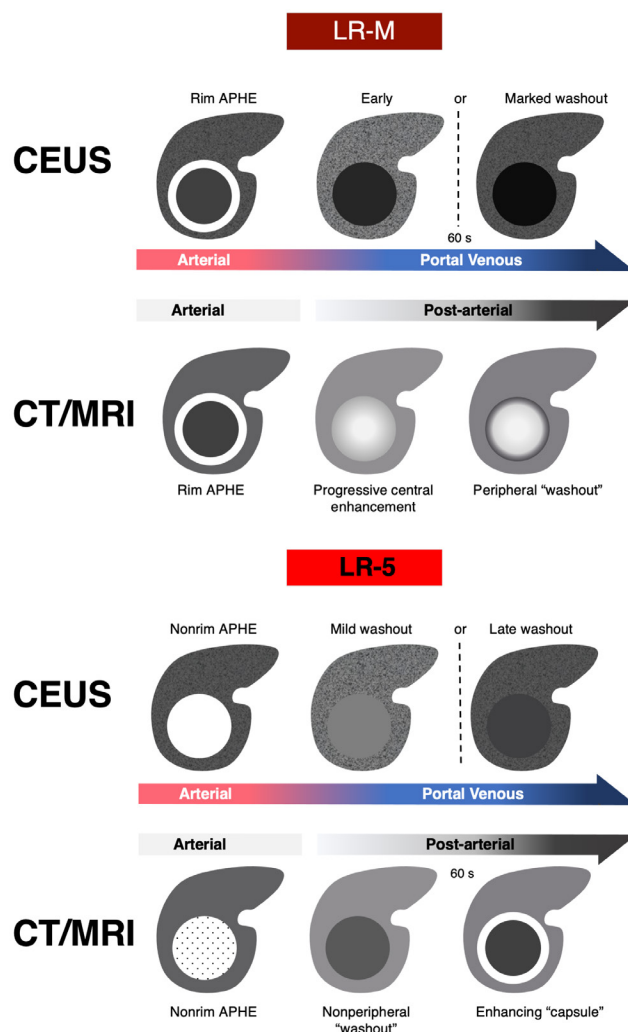


Figure 6. Schematics illustrating imaging features of LR-M and major imaging features of LR-5 according to CEUS LI-RADS and CT/MRI LI-RADS. CEUS: contrast enhanced ultrasound; LI-RADS: Liver Imaging Reporting and Data System; APHE: arterial phase hyperenhancement

contrast agents in CEUS are strictly intravascular because the large size of microbubbles does not permit them to pass through the vascular endothelium into the interstitial space, whereas the small molecules of CT/MRI contrast agents can pass through vascular endothelium. This difference in distribution of contrast translates to differences in imaging appearance. On CT/MRI, iCCAs typically demonstrate a gradual enhancement over time and often show delayed central enhancement due to hyperpermeability of the vascular endothelium and tumor interstitial constituents like necrosis or fibrosis that expand the extracellular space [Figure 5]. In contradistinction, the concentration of the microbubbles is uniformly distributed in the blood pool in the late phase and the degree of enhancement in the late phase mainly reflects the vascular volume on CEUS. The vascular volume in iCCA with fibrosis and absence of sinusoid-like structures is particularly low. Therefore, iCCAs invariably show marked washout in the late phase^[37].

How accurate is CEUS LR-M for differentiating iCCA from HCC?

The main role of the LR-M category is to prevent misdiagnosis of non-HCC malignancy as HCC. Therefore, the LR-M category should have a high sensitivity to diagnose non-HCC malignancy. In a large retrospective study including 1006 hepatic nodules by Terzi *et al.*^[38], 31/40 iCCAs were categorized as LR-M. The remaining 9/40 iCCAs were categorized as LR-3 or LR-4. No iCCAs were categorized as LR-5. However, it

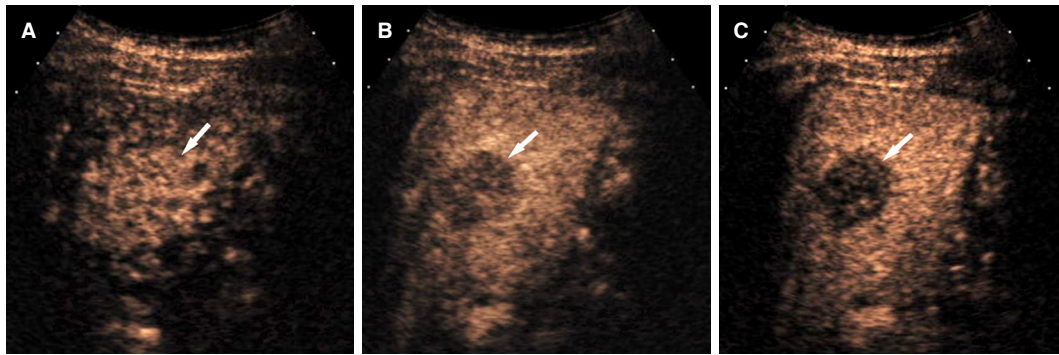


Figure 7. Combined hepatocellular-cholangiocarcinoma in a 77-year-old man with hepatitis-B related liver cirrhosis. A: contrast-enhanced CT or MRI could not be performed due to renal failure. CEUS in the arterial phase shows a mass with homogeneous arterial phase hyperenhancement (arrow); B: CEUS at 31 s after contrast injection shows early washout (arrow); C: CEUS at 60 s after contrast injection shows marked washout (arrow). CEUS: contrast enhanced ultrasound

is important to note that 48% (39/82) of LR-M observations were HCCs^[38]. Later studies also confirmed the high sensitivity of CEUS LR-M criteria for diagnosing non-HCC malignancies^[39-41].

MANAGEMENT CONSIDERATIONS

Histologic diagnosis by biopsy is usually recommended for LR-M lesions. This is both to direct care for non-HCC malignancies and to identify the significant minority of atypical HCCs that are categorized as LR-M observations^[17,18]. While retrospective data suggest that LR-M carries prognostic significance in the context of HCC, there is no prospective data to guide management decisions for these radiology-pathology discordant lesions. As of now, in the United States and Canada, if a lesion is biopsy proven to be HCC, it may be considered for standard HCC therapies including transplantation, despite its LR-M categorization. With additional data to support the prognostic significance of LR-M categorization, radiological appearances may be incorporated into future treatment algorithms for atypical HCC.

Challenges and considerations-combined tumors

While LI-RADS accurately differentiates HCC from iCCA, combined tumors (cHCC-CCA) still present a challenge. Combined hepatocellular cholangiocarcinomas are relatively rare primary liver carcinomas and as a result, the true epidemiology and risk factors are less well known. Several studies have shown some overlap in the risk factor profile for cHCC-CCA and HCC^[42]. In the latest World Health Organization classification of tumors 5th edition, cHCC-CCA are a distinct entity defined by the unequivocal presence of both hepatocytic and cholangiocytic differentiation within the same tumor^[43]. In a retrospective review of 3103 adult liver transplantation from database entries by Jung *et al.*^[44], cHCC-CCA constituted 2.7% (32/1173) of patients with primary liver malignancies.

Imaging diagnosis is challenging because cHCC-CCA is not a homogeneous tumor. On CT/MRI, cHCC-CCA appear to have more similarity with iCCA than HCC, often showing targetoid features^[4,45,46]. However, there may be overlap in appearance with HCC in a minority of cases^[4,45]. CEUS findings inevitably overlap with HCC and iCCA, depending on the amount of each component^[47]. In a recent study by Zheng *et al.*^[48], 20/24 (83%) of cHCC-CCA were categorized as LR-M on CEUS [Figure 7] and the remaining 4/24 (17%) as LR-5.

Challenges and considerations-reader agreement

The main philosophy of LI-RADS is to preserve high specificity/PPV for the diagnosis of HCC. LI-RADS suggests that the major features of HCC be applied only if unequivocally present, and that when considering whether a feature represents a major vs. LR-M feature, it should be classified as LR-M. For instance, if in

doubt about whether CEUS washout is marked or mild, it should be classified as marked. Likewise, if in doubt about rim APHE *vs.* nonrim APHE, we should call it rim APHE. These rules help direct individuals in the application of the LI-RADS algorithm, but do not necessarily address inter-reader variability, which is invariably encountered in clinical practice. While the literature suggests that agreement is good to excellent for LR-M *vs.* LR-5 categorization^[4,49], its reliability in routine practice is not well known and this remains an area of continued research and improvement for LI-RADS.

FUTURE DIRECTIONS

The prognostic value of LR features and categories is an area of active research with opportunities to investigate relationships of features with molecular profiles, immune landscapes, and histological subtypes of HCC that may inform individualized therapy.

As radiology continues to evolve toward big data and natural language processing applications, the LI-RADS categorical approach may be replaced or augmented by radiomics or deep learning models that have the potential to provide a more granular probability of diagnosing iCCA, cHCC-CCA, and HCC.

CEUS LI-RADS currently only addresses contrast agents that are FDA approved in the United States. Future versions may incorporate hepatocyte specific agents.

CONCLUSION

LI-RADS LR-M category is intended to capture all non-HCC malignancies. The criteria differs depending on modality. On CT/MRI, targetoid dynamic enhancement, diffusion weight, and hepatobiliary phase imaging are the primary features of LR-M. On CEUS, the presence of rim APHE and marked or early washout are the primary features of LR-M. When applied, LR-M criteria accurately capture almost all non-HCC malignancies and some atypical HCCs.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and design of the study and performed data analysis and interpretation: Fowler KJ, Cunha GM, Kim TK

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Dr. Fowler KJ receives research support from Bayer and General Electric (GE healthcare) and from Innovis, Medscape and Bayer for consulting. Dr. Cunha GM and Dr. Kim TK declared that there are no conflict of interest with regard to this manuscript.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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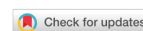
REFERENCES

1. El-Serag HB, Engels EA, Landgren O, Chiao E, Henderson L, et al. Risk of hepatobiliary and pancreatic cancers after hepatitis C virus infection: a population-based study of U.S. veterans. *Hepatology* 2009;49:116-23.
2. Yamamoto S, Kubo S, Hai S, Uenishi T, Yamamoto T, et al. Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. *Cancer Sci* 2004;95:592-5.
3. Chernyak V, Fowler KJ, Kamaya A, Kielar AZ, Elsayes KM, et al. Liver Imaging Reporting and Data System (LI-RADS) Version 2018: imaging of hepatocellular carcinoma in at-risk patients. *Radiology* 2018;289:816-30.
4. Fraum TJ, Tsai R, Rohe E, Ludwig DR, Salter A, et al. Differentiation of hepatocellular carcinoma from other hepatic malignancies in patients at risk: diagnostic performance of the liver imaging reporting and data system version 2014. *Radiology* 2018;286:158-72.
5. Tang A, Bashir MR, Corwin MT, Cruite I, Dietrich CF, et al. Evidence supporting LI-RADS major features for CT- and MR imaging-based diagnosis of hepatocellular carcinoma: a systematic review. *Radiology* 2018;286:29-48.
6. Kang Y, Lee JM, Kim SH, Han JK, Choi BI. Intrahepatic mass-forming cholangiocarcinoma: enhancement patterns on gadoxetic acid-enhanced MR images. *Radiology* 2012;264:751-60.
7. Peporte AR, Sommer WH, Nikolaou K, Reiser MF, Zech CJ. Imaging features of intrahepatic cholangiocarcinoma in Gd-EOB-DTPA-enhanced MRI. *Eur J Radiol* 2013;82:e101-6.
8. Hwang J, Kim YK, Park MJ, Lee MH, Kim SH, et al. Differentiating combined hepatocellular and cholangiocarcinoma from mass-forming intrahepatic cholangiocarcinoma using gadoxetic acid-enhanced MRI. *J Magn Reson Imaging* 2012;36:881-9.
9. Chong YS, Kim YK, Lee MW, Kim SH, Lee WJ, et al. Differentiating mass-forming intrahepatic cholangiocarcinoma from atypical hepatocellular carcinoma using gadoxetic acid-enhanced MRI. *Clin Radiol* 2012;67:766-73.
10. Park HJ, Kim YK, Park MJ, Lee WJ. Small intrahepatic mass-forming cholangiocarcinoma: target sign on diffusion-weighted imaging for differentiation from hepatocellular carcinoma. *Abdom Imaging* 2013;38:793-801.
11. Rimola J, Forner A, Reig M, Vilana R, de Lope CR, et al. Cholangiocarcinoma in cirrhosis: absence of contrast washout in delayed phases by magnetic resonance imaging avoids misdiagnosis of hepatocellular carcinoma. *Hepatology* 2009;50:791-8.
12. Kim SJ, Lee JM, Han JK, Kim KH, Lee JY, et al. Peripheral mass-forming cholangiocarcinoma in cirrhotic liver. *AJR Am J Roentgenol* 2007;189:1428-34.
13. Nakanuma Y, Komuta M, Zen Y. WHO Classification of tumors. Digestive System Tumors. 5th ed. Lyon (France): International Agency for Research on Cancer; 2019.
14. Fraum TJ, Cannella R, Ludwig DR, Tsai R, Naeem M, et al. Assessment of primary liver carcinomas other than hepatocellular carcinoma (HCC) with LI-RADS v2018: comparison of the LI-RADS target population to patients without LI-RADS-defined HCC risk factors. *Eur Radiol* 2020;30:996-1007.
15. Fowler KJ, Potretzke TA, Hope TA, Costa EA, Wilson SR. LI-RADS M (LR-M): definite or probable malignancy, not specific for hepatocellular carcinoma. *Abdom Radiol (NY)* 2018;43:149-57.
16. Horvat N, Nikolovski I, Long N, Gerst S, Zheng J, et al. Imaging features of hepatocellular carcinoma compared to intrahepatic cholangiocarcinoma and combined tumor on MRI using liver imaging and data system (LI-RADS) version 2014. *Abdom Radiol (NY)* 2018;43:169-78.
17. Ludwig DR, Fraum TJ, Cannella R, Ballard DH, Tsai R, et al. Hepatocellular carcinoma (HCC) versus non-HCC: accuracy and reliability of Liver Imaging Reporting and Data System v2018. *Abdom Radiol (NY)* 2019;44:2116-32.
18. Shao S, Liang Y, Kuang S, Chen J, Shan Q, et al. Diagnostic performance of LI-RADS version 2018 in differentiating hepatocellular carcinoma from other hepatic malignancies in patients with hepatitis B virus infection. *Bosn J Basic Med Sci* 2020; Epub ahead of print. doi: 10.17305/bjbm.2019.4576.
19. Lee HS, Kim MJ, An C. How to utilize LR-M features of the LI-RADS to improve the diagnosis of combined hepatocellular-cholangiocarcinoma on gadoxetate-enhanced MRI? *Eur Radiol* 2019;29:2408-16.
20. van der Pol CB, Lim CS, Sirlin CB, McGrath TA, Salameh JP, et al. Accuracy of the liver imaging reporting and data system in computed tomography and magnetic resonance image analysis of hepatocellular carcinoma or overall malignancy-a systematic review. *Gastroenterology* 2019;156:976-86.
21. Park YS, Lee CH, Kim BH, Lee J, Choi JW, et al. Using Gd-EOB-DTPA-enhanced 3-T MRI for the differentiation of infiltrative hepatocellular carcinoma and focal confluent fibrosis in liver cirrhosis. *Magn Reson Imaging* 2013;31:1137-42.
22. Lim S, Kim YK, Park HJ, Lee WJ, Choi D, et al. Infiltrative hepatocellular carcinoma on gadoxetic acid-enhanced and diffusion-weighted MRI at 3.0T. *J Magn Reson Imaging* 2014;39:1238-45.
23. Choi SY, Kim YK, Min JH, Kang TW, Jeong WK, et al. Added value of ancillary imaging features for differentiating scirrhous hepatocellular carcinoma from intrahepatic cholangiocarcinoma on gadoxetic acid-enhanced MR imaging. *Eur Radiol* 2018;28:2549-60.
24. Seo N, Kim MJ, Rhee H. Hepatic sarcomatoid carcinoma: magnetic resonance imaging evaluation by using the liver imaging reporting and data system. *Eur Radiol* 2019;29:3761-71.
25. Choi SH, Lee SS, Park SH, Kim KM, Yu E, et al. LI-RADS Classification and prognosis of primary liver cancers at gadoxetic acid-enhanced MRI. *Radiology* 2019;290:388-97.
26. Vilana R, Forner A, Bianchi L, Garcia-Criado A, Rimola J, et al. Intrahepatic peripheral cholangiocarcinoma in cirrhosis patients may

- display a vascular pattern similar to hepatocellular carcinoma on contrast-enhanced ultrasound. *Hepatology* 2010;51:2020-2029.
27. Wildner D, Bernatik T, Greis C, Seitz K, Neurath MF, et al. CEUS in hepatocellular carcinoma and intrahepatic cholangiocellular carcinoma in 320 patients - early or late washout matters: a subanalysis of the DEGUM multicenter trial. *Ultraschall Med* 2015;36:132-9.
 28. Bhayana D, Kim TK, Jang HJ, Burns PN, Wilson SR. Hypervascular liver masses on contrast-enhanced ultrasound: the importance of washout. *AJR Am J Roentgenol* 2010;194:977-83.
 29. Han J, Liu Y, Han F, Li Q, Yan C, et al. The degree of contrast washout on contrast-enhanced ultrasound in distinguishing intrahepatic cholangiocarcinoma from hepatocellular carcinoma. *Ultrasound Med Biol* 2015;41:3088-95.
 30. Li R, Yuan MX, Ma KS, Li XW, Tang CL, et al. Detailed analysis of temporal features on contrast enhanced ultrasound may help differentiate intrahepatic cholangiocarcinoma from hepatocellular carcinoma in cirrhosis. *PLoS One* 2014;9:e98612.
 31. Jang HJ, Kim TK, Burns PN, Wilson SR. Enhancement patterns of hepatocellular carcinoma at contrast-enhanced US: comparison with histologic differentiation. *Radiology* 2007;244:898-906.
 32. Jang HJ, Kim TK, Burns PN, Wilson SR. CEUS: an essential component in a multimodality approach to small nodules in patients at high-risk for hepatocellular carcinoma. *Eur J Radiol* 2015;84:1623-35.
 33. Wilson SR, Kim TK, Jang HJ, Burns PN. Enhancement patterns of focal liver masses: discordance between contrast-enhanced sonography and contrast-enhanced CT and MRI. *AJR Am J Roentgenol* 2007;189:W7-12.
 34. Wilson SR, Lyshchik A, Piscaglia F, Cosgrove D, Jang HJ, et al. CEUS LI-RADS: algorithm, implementation, and key differences from CT/MRI. *Abdom Radiol (NY)* 2018;43:127-42.
 35. Lyshchik A, Kono Y, Dietrich CF, Jang HJ, Kim TK, et al. Contrast-enhanced ultrasound of the liver: technical and lexicon recommendations from the ACR CEUS LI-RADS working group. *Abdom Radiol (NY)* 2018;43:861-79.
 36. Kim TK, Noh SY, Wilson SR, Kono Y, Piscaglia F, et al. Contrast-enhanced ultrasound (CEUS) liver imaging reporting and data system (LI-RADS) 2017 - a review of important differences compared to the CT/MRI system. *Clin Mol Hepatol* 2017;23:280-9.
 37. Yang HK, Burns PN, Jang HJ, Kono Y, Khalili K, et al. Contrast-enhanced ultrasound approach to the diagnosis of focal liver lesions: the importance of washout. *Ultrasonography* 2019;38:289-301.
 38. Terzi E, Iavarone M, Pompili M, Veronese L, Cabibbo G, et al. Contrast ultrasound LI-RADS LR-5 identifies hepatocellular carcinoma in cirrhosis in a multicenter retrospective study of 1,006 nodules. *J Hepatol* 2018;68:485-92.
 39. Li F, Li Q, Liu Y, Han J, Zheng W, et al. Distinguishing intrahepatic cholangiocarcinoma from hepatocellular carcinoma in patients with and without risks: the evaluation of the LR-M criteria of contrast-enhanced ultrasound liver imaging reporting and data system version 2017. *Eur Radiol* 2020;30:461-70.
 40. Chen LD, Ruan SM, Liang JY, Yang Z, Shen SL, et al. Differentiation of intrahepatic cholangiocarcinoma from hepatocellular carcinoma in high-risk patients: A predictive model using contrast-enhanced ultrasound. *World J Gastroenterol* 2018;24:3786-98.
 41. Chen LD, Ruan SM, Lin Y, Liang JY, Shen SL, et al. Comparison between M-score and LR-M in the reporting system of contrast-enhanced ultrasound LI-RADS. *Eur Radiol* 2019;29:4249-57.
 42. Brunt E, Aishima S, Clavien PA, Fowler K, Goodman Z, Gores G, Gouw A, et al. cHCC-CCA: Consensus terminology for primary liver carcinomas with both hepatocytic and cholangiocytic differentiation. *Hepatology* 2018;68:113-126.
 43. Sciarra A, Park YN, Sempoux C. Updates in the diagnosis of combined hepatocellular-cholangiocarcinoma. *Hum Pathol* 2020;96:48-55.
 44. Jung DH, Hwang S, Song GW, Ahn CS, Moon DB, et al. Longterm prognosis of combined hepatocellular carcinoma-cholangiocarcinoma following liver transplantation and resection. *Liver Transpl* 2017;23:330-41.
 45. Potretzke TA, Tan BR, Doyle MB, Brunt EM, Heiken JP, et al. Imaging features of biphenotypic primary liver carcinoma (Hepatocholangiocarcinoma) and the potential to mimic hepatocellular carcinoma: LI-RADS analysis of CT and MRI features in 61 Cases. *AJR Am J Roentgenol* 2016;207:25-31.
 46. Wells ML, Venkatesh SK, Chandan VS, Fidler JL, Fletcher JG, et al. Biphenotypic hepatic tumors: imaging findings and review of literature. *Abdom Imaging* 2015;40:2293-305.
 47. Li F, Han J, Han F, Wang JW, Luo RZ, et al. Combined hepatocellular cholangiocarcinoma (Biphenotypic) tumors: potential role of contrast-enhanced ultrasound in diagnosis. *AJR Am J Roentgenol* 2017;209:767-74.
 48. Zheng W, Li Q, Zou XB, Wang JW, Han F, et al. Evaluation of Contrast-enhanced US LI-RADS version 2017: application on 2020 liver nodules in patients with hepatitis B infection. *Radiology* 2020;294:299-307.
 49. Abdel Razek AAK, El-Serougy LG, Saleh GA, Abd El-Wahab R, Shabana W. Interobserver agreement of magnetic resonance imaging of liver imaging reporting and data system version 2018. *J Comput Assist Tomogr* 2020;44:118-23.

Review

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Hepatocellular carcinoma and hepatitis C virus infection in Latin America: epidemiology, diagnosis and treatment

Marina Galicia-Moreno¹, Hugo Christian Monroy-Ramirez¹, Marina Campos-Valdez¹, Jaime Sanchez-Meza¹, Laura Sanchez-Orozco¹, Juan Armendariz-Borunda^{1,2}

¹University of Guadalajara, Institute of Molecular Biology in Medicine and Gene Therapy, Department of Molecular Biology and Genomics, Health Science University Center (CUCS), Jalisco 44340, Mexico.

²Tecnologico de Monterrey, Campus Guadalajara, Jalisco 45138, Mexico.

Correspondence to: Dr. Juan Armendariz-Borunda, Institute of Molecular Biology in Medicine and Gene Therapy, University Center for Health Science (CUCS), Department of Molecular Biology and Genomics, University of Guadalajara, Sierra Mojada 950, Independencia Oriente, Apdo, Postal 2-123, Guadalajara, Jalisco 44340, Mexico. E-mail: armdbo@gmail.com

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Abstract

Hepatocellular carcinoma (HCC) is the most common cancer associated with chronic liver disease and cirrhosis. The most common cause of HCC is chronic hepatitis C virus infection and many studies in Europe, Asia and North America have focused on its etiology, epidemiology, diagnostic tools, and therapeutic options. However, little is known about these issues in Latin America. The aim of this review is to address these aspects of HCC in Latin America. The main risk factors associated with developing HCC in this region are: age, concomitant cirrhosis, hepatitis C infection, obesity and hereditary disease such as hemochromatosis. On the other hand, screening tests and diagnostic methods of HCC are mostly serum alpha fetoprotein quantification, liver ultrasound, computed tomography, magnetic resonance, and histopathology. Novel diagnostic methods include gut microbiota analysis and the use of nanotechnology and they continue to be tested. Finally, according to the Barcelona Clinic Liver Cancer, curative treatments used in HCC patients are mainly liver resection, liver transplantation, and local ablation, each with advantages and disadvantages. In conclusion, clear strategies are urgently needed to understand the extent of HCC and related problems in this part of the world. This review provides greater knowledge of HCC for the proper design of preventive programs by taking into consideration specific characteristics of our population. Also, this review allows for an understanding of individualizing treatments according to the patient's needs.

Keywords: Liver, hepatitis C, epidemiology, diagnosis, treatment, hepatocellular carcinoma, Latin America



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INTRODUCTION

Latin America is one of the most urbanized regions in the world, made up of 20 countries and 13 departments with an estimated population of 626 million^[1].

In this and other regions with large populations, access to health care is the main impediment for early diagnosis and correct treatment of HCC; and therefore, for the implementation of surveillance programs^[2]. HCC is the most common cancer associated with chronic liver disease and cirrhosis, and is the second leading cause of cancer-related deaths worldwide^[3]. Etiology factors for HCC varies according to its geographical area^[4], being the most reported causes of HCC around the world chronic hepatitis C and B viruses (HCV, HBV) infections, and alcohol consumption^[5]. Recently, it has been considered that non-alcoholic fatty liver disease could also be an important risk factor for HCC development^[6].

Globally, liver cancer is the sixth cause of incidence and fourth in cancer-related mortality. New cases of liver cancer in 2018 were 841,080 that correspond to 4.7% of all registered cancer cases. Worldwide in that same year, there were 781,631 deaths caused by liver cancer, number that represent 8.2% of deaths in that year^[7].

In 2018, Latin America registered an incidence of 38,400 HCC cases; 11,229 of which corresponded to Central America, 24,248 to South America and 2,923 to the Caribbean, being more affected the masculine gender. Brazil was the country with most patients with HCC, followed by Mexico, Argentina and Peru^[7]. On the other hand, regarding mortality data, HCC was the main cause of death in Latin America and Caribbean countries during 2018, affected mainly masculine gender, Brazil and Mexico were the countries in the LAC region with the highest mortality^[7]. The incidence, mortality, cumulative risk data and prevalence for the countries belonging to Latin America are shown in Table 1.

At present, many studies have been focused on studying the etiology, epidemiology, diagnosis tools, and therapeutic options in Europe, Asia and North America. However, little is known about these issues in Latin America. The aim of this work is to provide a comprehensive review about the current situation of HCC of viral origin (HCV) in Latin American, showing information about diagnosis and therapeutic strategies employed in this region.

EPIDEMIOLOGY OF CHRONIC HCV INFECTION IN RELATION TO HCC: PREVALENCE AND MORTALITY

The main causes of HCC in different geographical areas around the world have been related to infective agents such as HCV^[8]. Of the total number of cancer cases, HCV caused 160,000 cases of HCC in 2018, which is equivalent to 7.1% of cases worldwide caused by infectious agents. Of these, 3900 were reported in Central America, 7400 in South America and 782 cases in the Caribbean^[7].

Other data show that in 2016, of 7.2 million people worldwide who were living with chronic hepatitis C, 57% corresponded to Latin America and 3% were from the Caribbean [Figure 1 and Table 2]^[9,10]. The number of people diagnosed and treated for chronic HCV infection in the Americas is very low - only around 25% of all cases are diagnosed but just 14% are from Latin America and the Caribbean. Approximately 301,000 were treated in 2016, which is equivalent to only 16% of the diagnosed population in America, whereas in Latin America and the Caribbean, just 5% were under treatment. Determining the precise number of patients with known HCV status and receiving care has been difficult^[9].

In 2013 in America, of 125,700 deaths due to HCV and HBV, 80% were attributable to HCV of which 39% occurred in the Americas. Compared to 1990, the number of deaths has increased by 134%, and 8% since 2010^[9].

Table 1. Incidence, mortality and prevalence of HCC in Latin America and the Caribbean in 2018

Latin American Country	New cases			Deaths			5-year prevalence
	Number	Rank (of all types of cancer)	Cum. risk	Number	Rank	Cum. risk	
Argentina	2343	16	0.43	2113	9	0.38	1599
Bolivia	678	7	0.64	667	5	0.62	505
Brazil	12,463	12	0.54	11,797	7	0.51	8873
Chile	1582	10	0.64	1448	8	0.58	1060
Colombia	2279	13	0.43	2216	6	0.42	1552
Costa Rica	427	8	0.71	395	5	0.61	303
Cuba	837	17	0.44	773	9	0.39	588
Dominican Republic	718	7	0.77	650	5	0.68	560
Ecuador	979	10	0.54	953	4	0.52	686
El Salvador	514	5	0.71	500	3	0.69	358
Guatemala	1787	4	1.72	1741	1	1.69	1359
Mexico	7265	9	0.63	6868	3	0.60	5434
Peru	2317	10	0.73	2239	4	0.70	1709
Puerto Rico	351	11	0.62	375	5	0.64	245
Venezuela	1193	14	0.40	1152	8	0.38	920

The incidence, mortality and prevalence of HCC in countries belonging to Latin America and the Caribbean are shown. In addition to the above, the rank that the HCC occupies among all the types of cancer reported for each country is shown. Data obtained from GLOBOCAN 2019. HCC: hepatocellular carcinoma

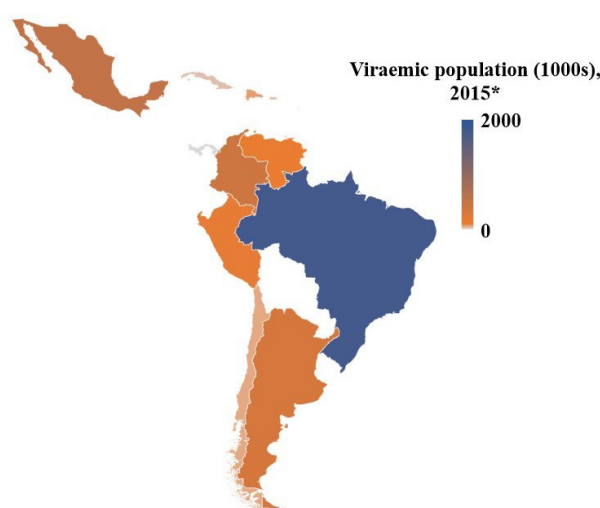


Figure 1. Map of the estimated hepatitis C virus viremic population in countries from Latin America and the Caribbean. Countries that are not shown or are in white color do not have available data

From the available data, ranking of the prevalence of HCV (% of total population) in the LAC countries in 2015 were: Puerto Rico (1.0%), Brazil (0.9%), Argentina and Colombia (0.8%), Dominican Republic (0.5%), Peru (0.5%), México and Venezuela (0.4%), and Guadeloupe, Panama, Chile and Cuba (0.3%)^[10].

RELATIONSHIP BETWEEN HCV CHRONIC INFECTION AND HCC

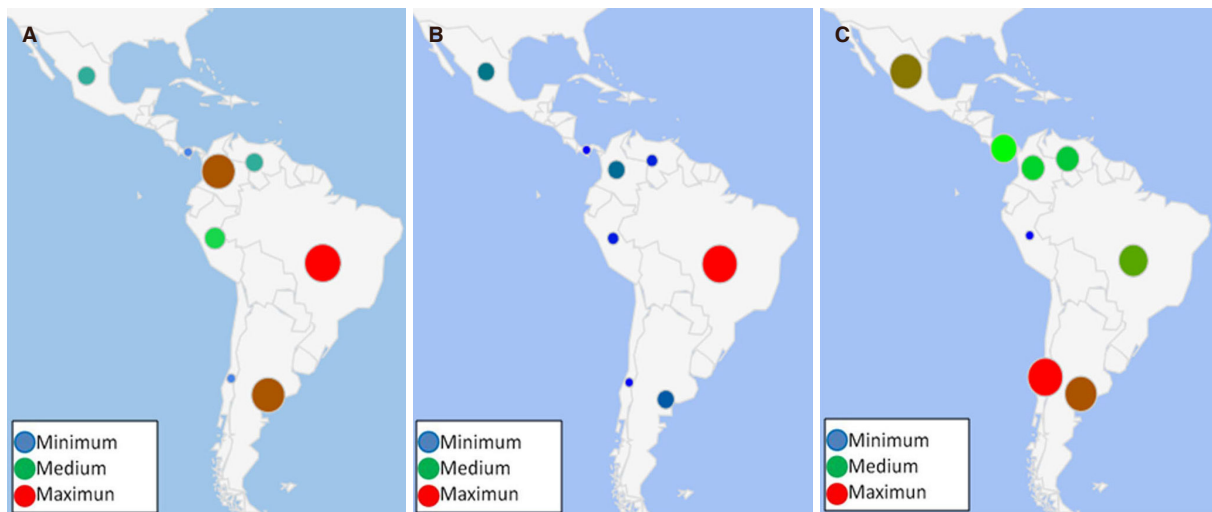
In 2015, the WHO estimated that viral hepatitis accounted for 1.34 million deaths. These deaths resulted from chronic liver disease (720,000 due to cirrhosis) and primary liver cancer (470,000 cases). Each year, the number of deaths related to viral hepatitis has been growing and is represented by the increase in mortality related to viral hepatitis by 22% since 2000^[11].

Around 75% of people exposed to HCV infection will not be able to eradicate the virus - 60%-70% of them will develop chronic liver diseases and of the remainder, 5%-20% will develop cirrhosis over a period of 20-30 years and 1%-5% will die from cirrhosis or HCC^[12].

Table 2. Estimated number of viremic people in Latin America and the Caribbean in 2016

Region	Estimated number of viremic (HCV-RNA) people, 2016
Latin America	3.8 million (2.6-4.2 million)
Caribbean	240,000 (180,000-350,000)
Latin America and the Caribbean	4.1 million (2.8-4.6 million)

HCV: hepatitis C virus

**Figure 2.** Choropleth maps related to HCV related to disease and death in Latin America. A: HCV prevalence in 2015; B: estimated population with cirrhosis related to HCV in 2015; C: HCC mortality rate caused by HCV by 100,000 inhabitants, 2013. HCV: hepatitis C virus; HCC: hepatocellular carcinoma

Globally, the estimated annual percentage change in liver cancer due to age-standardized HCV incidence rate has increased 0.57 (95%CI: 0.48-0.66) between 1990 and 2016. This pattern is heterogeneous across regions and countries. This rate was also higher in low- and middle-income countries than in high-income countries^[13].

In 2016, the percentage contribution of HCV to absolute liver cancer incidence (in both sexes) in Tropical, Southern, Central and Andean Latin America were 37.4%, 47.9%, 34.2% and 13.6%, respectively^[13]. Deaths due to HCC related to HCV infection (in 2013) had the highest incidence in the Dominican Republic at 3.27 per 100,000 people, followed by Chile with 3.22, Cuba with 2.96, Argentina with 2.84, and Mexico with 2.68^[9,10] [Figure 2 and Table 3].

DISTRIBUTION OF HCV GENOTYPES IN LATIN AMERICA

Studies on HCV genotypes related to different stages of liver disease have been reported even though the results are controversial. Nevertheless, cofactors such as age, sex, obesity, diabetes and alcohol consumption must be taken into consideration since they have an impact on the progress of chronic liver disease^[14]. Worldwide, genotype 1 is the most prevalent, causing 44% of all infections, followed by genotype 3 with 25% and then genotype 4 with 15%. HCV genotype 1 is also the most prevalent in Latin America [Figure 3] and genotype 1b is predominant in the LAC^[15].

According to Maucourt-Boulch *et al.*^[16], HCV was the cause of HCC in 58.7% of cases in Mexico, 50.0% in Brazil and 35%-38.8% in the rest of the LAC countries that were analyzed^[3,10,16] [Figure 3].

Table 3. HCV infections, genotypes and mortality rates in Latin America and the Caribbean

Country	Viremic prevalence, 2015*	Most prevalent genotype, 2015	Population living with HCV-related liver cirrhosis (1000s), 2015	Mortality rates from acute and chronic hepatitis C, 2013	Mortality rates from cirrhosis due to hepatitis C, 2013	Mortality rates from liver cancer due to hepatitis C, 2013
Dominican Republic	0.6%	1a (58.9%)	6.80	0.08	6.60	3.27
Chile	0.3%	1b (72.7%)	6.80	0.00	11.38	3.22
Cuba	0.3%	1b (81%)	4.30	0.00	4.19	2.96
Argentina	0.8%	1b (38.1%)	48.20	0.02	6.62	2.84
Mexico	0.4 %	1a (45.4%)	62.70	0.05	12.55	2.68
Brazil	0.9%	1b (33.4%)	267.00	0.04	5.93	2.46
Colombia	0.8%	1b (82.8%)	57.40	0.03	3.78	1.86
Venezuela	0.4%	1a (37%)	0.21	0.05	3.94	1.80
Peru	0.5%	1a (74%)	16.90	0.02	6.68	0.90

*2015 year-end estimate is a model output projection based on historic data. HCV: hepatitis C virus

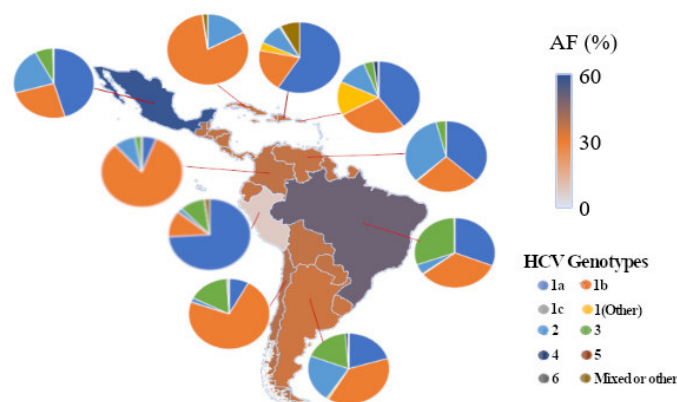


Figure 3. Choropleth map of the fraction of liver cancer attributable to HCV in some Latin America and the Caribbean countries, 2012. Pie charts represent the genotypes present in some of those countries and the proportion of cases that are related to each genotype. Genotype distribution data are either taken from the literature or based on regional averages in the absence of country-specific data. HCV: hepatitis C virus; AF: attributable fraction

ETIOLOGY AND RISK FACTORS IN HCC DEVELOPMENT

The etiology of HCC depends on the geographic location. For example, in countries where HCC is endemic such as Africa, Asia and Alaska, the most common cause is HBV infection. In countries where the risk of HCC is low, cirrhosis is the main cause of HCC in spite of the etiology^[17].

The main risk factors associated with developing HCC are as follows.

Age

The risk of developing HCC is higher during the seventh decade of life. Nevertheless, HCC tends to affect people in their sixties in Mexico^[18].

Cirrhosis

This is the main risk factor for HCC^[18]. Several etiologic agents are implicated with cirrhosis development including chronic viral hepatitis, alcohol consumption, hemochromatosis, metabolic diseases such as nonalcoholic fatty liver disease, *etc.* In Mexico, the main risk factor for cirrhosis is alcohol consumption^[19]. Long term studies have reported that 1%-8% of cirrhotic patients will develop HCC (3%-8% in patients infected with HCV)^[2].

Hepatitis C

HCV infection is another important risk factor for developing HCC. New cases of HCC develop in 3%-5% of patients with cirrhosis due to HCV per year^[20]. HCV Genotype 1b has been identified as a high risk factor for HCC development. Studies conducted in Latin America and the Caribbean have reported several other risk factors for HCV infection and eventual HCC in specific social minority groups such as drug users^[21], prison inmates^[22], and sex workers^[23].

Aflatoxin

This is an important risk factor related to HCC development, mainly in Africa and Asia. Aflatoxin is produced by *Aspergillus flavus* and is found in maize and peanuts, which causes modifications in the DNA of hepatocytes^[17].

Nonalcoholic fatty liver disease/nonalcoholic steatohepatitis

Due to the growing obesity epidemic, nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease which includes a clinic-pathologic spectrum of disease ranging from isolated hepatic steatosis to nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis or HCC. Once cirrhosis has developed, NASH pathology may be difficult to evaluate because fatty deposition and inflammation often disappear. Between 40% to 60% of patients with NASH-induced cirrhosis may develop a complication such as HCC after a period of 5 to 7 years. A meta-analysis conducted by Singal *et al.*^[24], showed an association between the PNPLA3 variant and an increased risk for HCC, especially in patients with NAFLD-related cirrhosis. According to the Centers for Disease Control, the incidence of HCC was higher in Latinos than in non-Latinos. Latinos with HCC also have shorter survival rates than non-Latinos^[25].

Obesity

This is yet another important risk factor that leads to an increase in the incidence of HCC. In subjects with a body mass index of 35 or above, tumor development is more frequent^[26].

In Latin America and the Caribbean, a heterogeneous obesity pattern across the countries has been found. However, the prevalence of obesity has been increasing, not only among rural populations, the poor and least educated, but also the urban populations, the rich and highly educated^[27]. Chile and Mexico have the highest prevalence of overweight and obese boys at 11.9% (9.6-14.3) and 10.5% (8.8-12.4) respectively, while for girls, the prevalence in Uruguay and Costa Rica are 18.1% (14.9-21.9) and 12.4% (10.0-15.1) respectively^[28]. In Mexico, mortality data regarding obesity has been calculated and consequently, NAFLD will be the second most important cause of liver disease in the future, around 2050^[29]. These data and evidence for the association between obesity and NAFLD as the third cause of HCC in Latin America, predict an increase in the incidence of HCC incidence in the near future. Thus, the implementation of measures to incorporate healthy diets and physical activity in the general population is urgent, in order to achieve healthy body weights to reduce the incidence of cancer among other chronic diseases such as diabetes mellitus.

Hereditary hemochromatosis

This condition is related with a 200-fold increase in risk for HCC. The formation of free radicals and lipoperoxidation products produce iron toxicity in the liver that eventually, can cause cirrhosis and HCC^[30].

Wilson's disease

This is a heritable disease that is the result of a mutation in the *ATP7B* gene and causes alterations in plasma copper circulation. A high content of free copper in circulation induces cytoplasmic cell damage, cirrhosis, and eventually HCC^[30].

SCREENING AND DIAGNOSTIC METHODS FOR HCC

The diagnosis of HCC includes screening tests, histopathological and imaging methods. The most widely used tests for screening are as follows.

Serum alpha-fetoprotein

Produced by the fetal liver and yolk cells as well as regenerating hepatocytes. By itself, serum alpha-fetoprotein (AFP) quantification is more sensitive than other markers. AFP has a cut off point of 10.9 ng/mL^[31] but its sensitivity is low (25%-48%) since its concentration depends on tumor size. It is important to mention that some patients with HCC (30% to 50%) do not have high levels of AFP even in advanced stages. Due to this, it is not recommended as the only screening test^[32].

Liver ultrasound

Liver ultrasound (LU) is considered the first choice screening test for HCC detection because it has a sensitivity between 60%-80%, and its specificity is above 90%^[33]. According to international guidelines (EASL and AASLD), a LU every six months is suggested for early detection of HCC in cirrhosis patients^[34,35]. This test is able to detect lesions larger than 1 cm in diameter, is safe, low-cost and does not have secondary effects.

LU + alpha-fetoprotein

Together, both strategies add only 6 to 8% of cases of previously undetected HCC. Combining both markers actually increases the number of false positive results. These diagnostic tests are suggested in subjects with a high risk of HCC at a frequency of 6 to 12 months.

Others methods used in diagnosing HCC are as follows.

Computed tomography and magnetic resonance imaging

The diagnosis and prognosis of HCC depends on the stage at which the tumor is detected. If detected at an early stage of HCC, long-term patient survival is more probable. Noninvasive imaging tests, including computed tomography (CT) and magnetic resonance imaging (MR) have been recommended by several clinical practice guidelines as the first-line diagnostic tools for the screening, diagnosis, staging and surveillance of HCC^[35-37]. One characteristic about a liver nodule that suggests dysplasia is decreased hepatic artery flow, and the maintenance of portal venous flow. The presence of new unpaired arteries not accompanied by bile ducts is also a classic characteristic for differentiated neoplastic nodules from typical, regeneratiing nodules^[38,39]. This, and other changes can be evaluated with current imaging techniques. The hemodynamic alterations that occur in HCC represent pathological markers for current, noninvasive diagnosis of HCC through CT and MR. Through the evaluation of dynamic images, the diagnosis of HCC is established by the detection of contrast-hyperenhancement in the arterial phase, and hypoenhancement in the portal venous phase. This response is defined as the "HCC radiological hallmark". This vascular pattern allows HCC diagnosis with almost 100% specificity and positive predictive value for nodules with diameters of at least 1 cm^[40-42]. According to recent meta-analyses, the sensitivity of CT and MR were 63%-73% and 77%-90% respectively, with a specificity of 87%-98% and 84%-97%, respectively^[43-46]. Today, new imaging techniques have been developed to improve the non-invasive evaluation of HCC. The most significant techniques are diffusion weighted imaging (DWI) and hepatobiliary contrast agents.

DWI is a functional magnetic resonance technique that consists of quantifying proton diffusion in tissues^[44]. There is cellular increase in HCC and this cellular proliferation restricts water proton diffusion^[47]. It is important to mention that DWI quantification demonstrates restricted specificity for HCC because some lesions can show restricted diffusion on DWI^[48,49].

On the other hand, hepatobiliary contrast agents such as gadobenate dimeglumine (Gd-BOPTA) and gadoxetate disodium (Gd-EOB-DTPA) can provide information about tumor vasculature and hepatocyte function in a single examination^[50].

Histopathology

This diagnostic method can only be considered when evaluating nodules greater than 2 cm, or if radiological findings are not compatible with HCC. However, liver biopsies can yield false negative results^[51]. Histological evaluation is considered positive if the sample is positive for at least two of glypican 3, heat shock protein 70 and glutamine synthase, which represents a sensitivity of 72% and a specificity of 100%^[52].

Finally, we will review two novel, unconventional, diagnostic methods of HCC.

Gut microbiota analysis

The gut microbiota has an important role in the maintenance of homeostasis in humans. Evidence demonstrates connections between gut microbiota and HCC development. Ponziani *et al.*^[53] demonstrated that translocated bacterial elements from the gut to the liver can start an inflammatory process through toll-like receptors (TLRs). Lipopolysaccharides from Gram (-) bacteria can bind TLR-4; TLR-2 recognizes the bacterial triacylated lipopeptide, and TLR-5 can recognize flagellin, a protein component of bacterial flagella^[54,55]. In all cases, the final effect is the production of inflammatory cytokines such as TNF- α , IL-1 β and IL-6 from the NF- κ B pathway^[56]. The gut microbiota and the development of HCC is also linked directly via the JAK or STAT3 pathway, which are mainly activated by IL-6^[57]. Due to this, gut microbiota evaluation could improve diagnostic reliability. Pre-clinical and clinical trials have shown a direct correlation between Gram (-) bacteria and inflammatory changes related to the development of HCC. All these observations suggest that evaluation of the proportion of harmful and beneficial bacteria could be considered as a promising tool for the early diagnosis of HCC. Some limitations have been considered for these purposes as sometimes, HCC patients are subjected to antibiotic treatment, which may alter the composition and function of their microbiota, limiting diagnostic use.

Nanotechnology for HCV diagnosis

HCV infection is the main etiologic agent of hepatic cirrhosis and HCC. There are 8 main genotypes and 86 subtypes described in the last few years^[58]. These antiviral agents are effective in reducing the probability of developing cirrhosis and HCC. However, these are highly expensive drugs and inaccessible to most patients, especially in low-income countries. Another important aspect to consider is that only a few highly-specialized laboratories perform molecular diagnosis for HCV, which is essential for the best diagnosis and treatment^[59].

Nanotechnology is a new, interesting and promising diagnosis tool for infectious diseases that uses nanoparticles and specific nanoscale tests directed at the pathogen genome^[59]. There are different types of nanoparticles and gold is suitable for efficient diagnosis. It has been used to identify pathogens such as *Mycobacterium tuberculosis*^[60], *Helicobacter pylori*^[61], the dengue virus^[62], and influenza A^[63]. On the other hand, RNA aptamers are single-stranded RNA oligonucleotides that specifically bind to a target molecule, which makes them good alternatives for the development of HCV serological tests^[59]. Nanoparticles and aptamers, can be used as biosensors for diagnosis as the fusion of both can be bound to the HCV core protein, enabling easy detection of this protein^[64].

There are several reasons that can lead to diagnostic failure in early HCC and as a consequence, failure in the correct selection of therapies. First, the absence of early identification of the at-risk population; second, no application of routine surveillance (e.g., performance of ultrasound twice a year); and finally, mistakes in the interpretation of screening tests^[1]. There is no evidence to suggest that HCC screening improves survival in high-risk groups, although many medical professionals do use several diagnostic strategies such as AFP and liver ultrasonography for HCC screening^[65]. Nevertheless, screening tests for HCC are very important to increase survival and quality of life. For example, when detected early, 5-year survival is greater than 50% but when diagnosed late and patients are symptomatic or the cancer is late stage, 5-years survival is less than 10%^[18].

Table 4. Liver transplants in Latin America and the Caribbean in 2016

Country	Number of transplants	PMP	Waiting list*
Argentina	368	8.4	2008
Bolivia	1	0.1	4
Brazil	1880	9.0	4673
Chile	93	5.1	256
Colombia	240	4.9	74
Costa Rica	13	2.7	60
Cuba	20	1.8	32
Dominican Republic	3	0.3	20
Ecuador	31	1.9	34
El Salvador	-	-	-
Guatemala	0	0.0	-
Mexico	178	1.4	681
Peru	23	0.7	40
Puerto Rico	42	12.0	32
Venezuela	2	< 0.1	19

*Total number of patients who were active on the waiting list in 2016; -data not found. PMP: per million population

THERAPY

The Latin America Association for the Study of the Liver (ALEH) published the clinical guidelines for the management of HCC in the region. The ALEH indicates the staging procedures that should be carried out. Also, one of its main objectives is to define the best therapeutic strategy for each patient. The most widely used staging system is the Barcelona Clinic Liver Cancer (BCLC) system since it relates each stage of HCC with the most appropriate treatment according to scientific evidence^[5,66]. Generally, HCC can be approached by curative or systemic treatments. Curative treatment is possible if HCC is diagnosed at an early stage.

Curative treatment

According to the BCLC classification, three curative treatments are available: liver resection, liver transplantation, and local ablation^[67].

Liver resection is the best therapeutic option for HCC patients with or without cirrhosis, when the liver is still functional^[18]. The aim of this surgical procedure is to obtain at least 2 cm margins through anatomic resection, except when the cirrhotic patient's healthy residual liver is compromised^[70-73]. Liver resection and liver transplantation are the only curative treatments for HCC patients, but unfortunately only 5% to 10% of patients are candidates because most have advanced disease and poor liver function^[68]. This option has shown good results with up to 60% 5-year survival and low perioperative mortality (0.8%-3%)^[69]. If liver transplantation is contraindicated, the alternative is locoregional therapy. To select the ideal candidate, CT or MR evaluation of tumor size, presence of satellite lesions and vascular involvement are very important^[5]. In some Latin American regions, HCC resection is recommended in patients classified as intermediate stage, when the liver has not completely lost its function, and survival of 5 years can still be achieved (patients with Child-Pugh A)^[66,74].

Liver transplantation is the best option for treatment taking into account the tumor and the concomitant disease. In Latin America, the main problem is the absence of an organ donation culture^[18]. Liver resection and transplantation are curative surgical treatments for HCC by removing both the tumor and cirrhosis. It is important then, to consider: (1) the candidate according to their tumor stage, liver function, physiological status, (2) the experience of the medical staff performing the surgery^[75]. BCLC guidelines are the most widely accepted for assessing a HCC patient's prognosis^[76]. According to Mazzaferro *et al.*^[77], a patient could be eligible for liver transplantation when its expected survival is at least 70% at 5 years; this survival also depends of lesions size. In Mexico, liver transplantation is the first choice treatment for patients with Child-

Pugh C score and HCV co-infection. Currently, Latin America has transplant programs and more than 2500 liver transplants are performed in the region every year^[8]. Worldwide, in 2017, an estimated total of 32,348 liver transplants were performed with 2894 in Latin America. Table 4 shows the number of liver transplants performed as well as the number of patients on the waiting list in the LAC countries^[78,79].

Loco-regional therapy

Several minimally invasive treatment options for patients with unresectable HCC have been developed including (1) curative therapies such as radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), microwave ablation (MWA), cryoablation (CA), irreversible electroporation (IRE), and (2) palliative therapies such as chemoembolization transcatheter-arterial chemoembolization (TACE) or trans-arterial radioembolization (TARE).

Curative options: liver ablation

These are ablative techniques that use chemical or thermal energy. EASL clinical practice guidelines recommend the use of this ablative therapy in very early (single lesion < 2 cm) and early stage (2-3 nodules < 3 cm) HCC. Ablation is recommended when resection or transplantation are not an option for HCC patients^[80]. Tumor cell destruction can be produced through chemical substance injection (PEI) or through temperature alteration (radiofrequency, microwave, laser or cryotherapy). Although there are several options, RFA is the first choice^[5].

RFA is uses an alternating electric current between 460-500 kHz which is applied to the lesion via a radiofrequency electrode. It induces the electromagnetic field to produce an oscillation of tissue ions and frictional heating, leading to coagulative necrosis and cell death a temperatures of 60-100 °C^[80]. RFA is more beneficial than PEI in patients with early stage HCC. It offers 5-year-survival rates up to 76% when used as the main therapy in patients with resectable HCC. Based on BCLC criteria^[81], it is important to note that results of RFA are optimal in patients with tumor > 3 cm^[82]. In Mexico, PEI and RFA are available treatment options with successful results^[18].

On the other hand, PEI is a good option for nodular HCC. This is the most widely used method for chemical ablation but has the disadvantages of non-uniform diffusion and uncontrollability of injected alcohol in large tumors. Hence, PEI is applied for the treatment of small HCC. It produces complete necrosis in 90% of tumors < 2 cm, and in 50% of tumors measuring 3-5 cm^[83,84]. The main drawback of PEI is the high local recurrence rate, which is 43% in lesions > 3 cm^[85]. In Brazil, percutaneous ablation of early HCC is recommended more frequently than in other LAC countries^[74].

Regarding MWA, it was initially developed to work around the heat sink and tissue impedance limitations of RFA in the liver. RFA and MWA have similar mechanism of inducing cell death through increasing tissue temperature by the continuous realignment of polar molecules within a microwave field at frequencies of 915/2450 MHz^[86]. Microwaves radiate throughout all tissue without impedance, allowing a larger tissue to be heated with each application. This technique is less invasive than hepatic resection and may be considered for patients for whom resection may be contraindicated because of age or comorbidities such as portal hypertension^[87].

Other modalities of ablation currently employed in clinical practice are CA and IRE. CA destroys tissue, causing necrosis of the tumor by freezing at temperatures between -35 °C to -20 °C using the Joule-Thomson theory in the thawing process. This theory describes the temperature change of a gas when it is forced through a valve while it is kept insulated so that no heat is exchanged with the environment^[80]. Tissue destruction is carried out through two mechanisms, a direct cellular injury and a vascular related injury. With respect to patient selection, it is necessary to consider: (1) patients with a single HCC < 5 cm in

diameter, or up to three HCCs with each < 3 cm in diameter; (2) absence of portal venous thrombosis; (3) Child-Pugh A or B, and (4) no significant coagulopathy^[88].

Finally, IRE is a novel, non-thermal form of tumor ablation that produces less collateral damage. IRE relies on short pulses of high frequency energy to induce pores in the lipid bilayer of cells, leading to cell death via apoptosis^[89]. Patients who are candidates for RFA or MWA, but have tumors adjacent to structures that would cause either heat sink or collateral damage, can therefore be treated with IRE.

Palliative options, chemoembolization

This treatment consists of the administration of a chemotherapeutic agent into the tumor followed by an embolizing agent. This process increases the survival rate to 41% in 2 years^[90]. TACE is the gold standard in patients unsuitable for surgery and for percutaneous ablation techniques with multinodular HCC, but have preserved liver function without vascular invasion or extrahepatic spread. This technique is based on occlusion of the arterial blood supply of the target neoplastic lesion by embolizing microparticles, in combination with an injection of chemotherapeutic drugs^[80]. A variety of chemotherapeutic agents have been used, including monotherapy with doxorubicin or cisplatin, or a mixture with cisplatin, doxorubicin and mitomycin C. Contraindications to TACE include decompensated cirrhosis (Child-Pugh C), encephalopathy, active infection and uncorrectable bleeding diathesis. Other relative contraindications include serum bilirubin of 3-7 mg/dL, advanced cancer stage, portal vein thrombosis, iodinated contrast allergy, biliary obstruction and renal insufficiency^[90].

TARE is an important loco-regional treatment, used in patients with intermediate or advanced stages of HCC who are not candidates for TACE or Sorafenib. TARE, also known as selective internal radiation therapy, consists of intra-arterial delivery of a radioactive material to the tumor to limit systemic irradiation and preserve healthy liver. Yttrium-90 (⁹⁰Y) has suitable characteristics for the treatment of tumors. It is a pure β emitter characterized by a short half-life (64.2 h) and has limited tissue penetration. Two types of microspheres are available, TheraSphere which is made of glass, and SIR-Spheres made of resin, and both have been demonstrated effective and safe in treating primary and secondary liver cancers^[91]. Emission of a β particle with decay ⁹⁰Y to ⁹⁰Zr (zirconium) enables delivery of targeted radiation to the lesion, limiting radiation exposure to normal parenchyma while reducing the risk of radiation exposure induced liver disease^[92].

According to the clinical practice guidelines for the management of HCC by the Latin American Association for the Study of the Liver (LAASL), chemoembolization is an option for patients, particularly those who are not candidates for resection, liver transplantation, or percutaneous ablation^[5].

Systemic therapies

These are options for patients with the diagnosis of advanced stage HCC or in patients with tumor progression after loco-regional therapy^[93].

Sorafenib

This is considered and approved as first-line treatment for patients with advanced HCC^[93]. It is a multi-kinase inhibitor that targets Raf-1, B-Raf, vascular endothelial growth factor receptor, platelet derived growth factor receptor, and c-kit receptors. It inhibits tyrosine kinase activity and serine-threonine kinase receptor, acting as an antiproliferative and anti-angiogenic agent. Its efficacy has been demonstrated in phase II and III clinical trials^[93].

HCC patients with Child-Pugh category A and advanced disease and with an Eastern Cooperative Oncology Group score of 0-2 are eligible to receive Sorafenib^[35]. However, the main disadvantage is its high cost that makes it non-affordable to most patients. A retrospective analysis of 127 HCC cases treated with sorafenib in

8 medical centers in 5 South American countries between January 2010 and June 2017, showed that 38% of cases was due to HCV and the median survival after initiation of treatment was 7.5 months (IQR 2-17) in all subjects^[94].

According to current guidelines established by ALEH, Sorafenib is standard systemic therapy for HCC in patients categorized as Child-Pugh C, or with underlying cirrhosis and advanced tumor (stage C according to BCLC guidelines), or with tumor progression even after loco-regional therapy. However, there are no other therapeutic alternatives in the event of treatment failure with Sorafenib^[5].

Levatinib

This is a tyrosine kinase inhibitor that blocks VEGFR1-3, fibroblast growth factor receptors (FGFRs) 1-4, PEGFR, RET, and KIT. The overall mean survival time after treatment is 13.6 months and the rate of objective response (according to response evaluation criteria in solid tumors) is 18.8% for patients receiving levatinib (< 1% complete response and 18% partial response). The most common adverse events for this treatment are hypertension, diarrhea, decreased appetite, and weight loss^[95].

Sorafenib and levatinib are approved as first-line therapy while regorafenib and cabozantinib are indicated in patients who have progressed or are intolerant to sorafenib^[96].

Regorafenib

This is a multi-target inhibitor of VEGFR1, TIE-2, RETRA-1, BRAF, PDGFR, FGFR, and CSF1R. It improves overall survival with a hazard ratio of 0.63, and the median duration of overall survival of patients who received it is 10.6 months. Adverse events include hypertension, hand and foot skin reactions, and diarrhea^[97].

Cabozantinib

This targets the mesenchymal-epithelial transition factor (c-Met) pathway, as well as the VEGF and RET receptors. Compared to placebo, cabozantinib increased the median overall survival (10.2 months *vs.* 8.0 months). The hazard ratio for death by treatment is 0.76; (95%CI: 0.63-0.92). However, grade 3 or 4 adverse events occurred in 68% and 36% of patients in the cabozantinib and placebo groups respectively. The most common high-grade adverse events include palmar-plantar erythrodysesthesia, hypertension, increased aspartate aminotransferase level, fatigue and diarrhea^[98].

Ramucirumab

This is a fully humanized IgG1 monoclonal antibody directed against the extracellular domain of VEGFR-2. It has shown clinical efficacy either alone or in combination with chemotherapy in the treatment of a number of malignancies. It can be given on a twice or thrice weekly schedule and binds with much higher affinity to VEGFR-2 than its natural ligands, thus preventing the VEGF-VEGFR-2 interaction. Ramucirumab has also shown an advantage in delaying the worsening of disease-related symptoms and prolonging overall survival compared with placebo^[99].

Nivolumab

This is a fully human immunoglobulin G4, anti-PD-1 monoclonal antibody that has been approved for the treatment of multiple advanced malignancies. In the phase I/II trial Checkmate-040, nivolumab showed response across all cohorts in 14%-20% of patients. The most common adverse events were fatigue, pruritus and rash, but manageable. Grade 3/4 serious adverse events occurred in 4%^[100].

Doxorubicin

This is the most studied and widely used chemotherapeutic agent for HCC treatment and is chosen when the patient's disease is critical. It is a DNA intercalating agent and its mechanism of action is related to inhibition

of topoisomerase II^[101]. Its use though is not recommended by many clinical guidelines such as AASLD or EASLD. In Latin America, patients receiving doxorubicin with arterial embolization have a 58% survival rate at 2 years^[5]. In patients with advanced HCC however, doxorubicin administration failed to improve survival^[5]. The combined effects of doxorubicin and sorafenib have been studied but favorable results were not obtained because of higher toxicity, mainly cardiotoxicity and neutropenia^[102].

Interferon

These are agents with an immunomodulatory and antiproliferative effect on tumor cells in HCC. Adjuvant interferon (IFN) therapy has been demonstrated to reduce the recurrence of HCC, but does not improve the survival of HCV-related HCC patients. IFN is effective in intermediate and advanced HCC patients^[103]. In terms of survival, tumor response and toxicity, IFN administration is superior to doxorubicin in patients with HCC.

IFN has several properties including antiviral, anti-tumor, and immunomodulatory effects^[104]. Three IFN subtypes have been identified: type 1 (IFN α and β), 2 (IFN- γ), and 3 (IFN- λ), but only IFN-type 1 is used in the treatment of chronic viral hepatitis to reduce the risk of HCC in patients infected with HCV^[103]. Evidence was first obtained from a randomized controlled trial with 90 patients where IFN treatment was effective in decreasing the incidence of HCC^[105]. In patients with HCV-related cirrhosis treated with IFN, there was a sustained virological response (SVR) after treatment that in turn, resulted in improved clinical outcomes including a lower risk of decompensation and HCC development^[106].

In Latin America and according to the LAASL, the preventive effect of antiviral therapy in patients with HCV is more effective when using pegylated IFN plus ribavirin, which achieves higher SVRs. It is important to note however, that chronic administration of low dose pegylated IFN, without achieving SVR, fails to reduce the risk of HCC^[5].

Direct acting antivirals

As previously mentioned, chronic HCV is the leading cause of HCC worldwide. The implementation of *in vitro* replication models using sub-genomic replicons and the cell culture system of HCV enabled the discovery and development of direct-acting antivirals (DAAs) for the treatment of chronic HCV. These antivirals have considerably improved the sustained viral response in the treatment of all HCV genotypes, with cure rates of more than 95%^[107]. Treatment with DAAs in Latin America lagged behind Europe, Asia and North America but results have been really good in terms of viral eradication. Nevertheless, this must still be interpreted with caution since the number of studies in Latin America are still scarce.

In a multicenter study performed in Latin America to evaluate the association between DAAs and HCC waitlist progression or its recurrence following liver transplantation (LT) between 2012 and 2018, 503 patients without chronic HCV and 481 patients with chronic HCV were recruited (197, 19, 24, 180, 18, 5, 45 and 3 patients each from Argentina, Uruguay, Chile, Brazil, Mexico, Peru, Colombia and Ecuador respectively). From these HCV⁺ patients, 327 were not treated with DAAs while 164 were. The most commonly used DAA regimen was sofosbuvir/daclatasvir in 68.3% (112) of patients, followed by peritaprevir/ritonavir/ombitasvir/dasabuvir in 12.2% ($n = 20$), and sofosbuvir/ledipasvir in 6.7% ($n = 11$). The overall SVR was 89.8% 95%CI (81.0-97.1), which was not statistically different between patients treated before 90.6%CI (83.9-94.1) or after transplantation 89.2%CI (80.4-94.9). While the patients were on the waiting list period, 13.4% ($n = 66$) of HCV patients received DAA treatment and 86.6% ($n = 425$) did not. The cumulative incidence of HCC progression was 24.2% ($n = 222$). Patients treated with DAAs before LT presented a similar cumulative incidence of tumor waitlist progression when compared with the HCV⁺ untreated DAA group (26.2% vs. 26.9% $P = 0.47$); both had a similar HCC drop-out rate [12.1%CI (0.4-8.1) vs. 12.9 %CI (3.8-27.2)]. A non-significant but numerically higher proportion of patients with pre-LT DAAs presented with extrahepatic progression

or vascular invasion when compared to those without DAAs (4/66, 6.1% *vs.* 17/425, 4%; $P = 0.12$). A lower incidence of post-transplant HCC recurrence among HCV⁺ patients treated with pre- or post-LT DAAs was observed [0.7%CI (0.2-4.9)]. Although some patients with DAA treatment developed HCC, DAA treatment was neither associated with increased HCC recurrence after LT nor with waitlist tumor progression^[108].

A prospective, multicenter cohort study was performed in 23 hospitals (from Argentina, Brazil, Chile, Colombia and Uruguay) from Latin America with 1760 patients treated with DAAs, in order to evaluate disease progression during a median follow-up of 26.2 months. Results showed an overall, cumulative incidence of disease progression of 8.3 in non SVR *vs.* 3.9 after SVR achievement. Disease progression was seen with the development of liver fibrosis (HR = 3.4; 95%CI: 1.2-9.6), clinically significant portal hypertension (HR = 2.1; 95%CI: 1.2-3.8) and *de novo* HCC (HR = 0.2; 95%CI: 0.1-0.8) in the overall cohort. SVR was associated with an 80% reduction in disease progression when compared with DAA failure, which supports significant reduction in the risk of new liver-related complications^[109] after treatment of HCV infection with DAAs.

CONCLUSION

HCC is the second leading cause of cancer related-deaths worldwide according to the WHO in 2015. This global health problem has caused the death of about 1.34 million people. Governments worldwide have implemented strategies to reduce this mortality but critical issues such as early diagnosis and appropriate treatment in at risk populations remain to be addressed. In Latin America, it is imperative that clearer strategies to understand the extent of the problem in this region be implemented. One possible strategy could be conducting annual epidemiological studies to identify high-risk populations and the main etiological causes. The updated data might then provide health authorities with more effective preventive approaches and enable implementation of the most effective treatments on time.

DECLARATIONS

Authors' contributions

Contributed to the planning, bibliographic revision and writing of the manuscript: Galicia-Moreno M

Contributed to bibliographic revision, figure design, and manuscript writing: Campos-Valdez M, Sanchez-Meza J

Responsible for the planning of, conducting the study and figure design: Monroy-Ramirez HC

Responsible for manuscript revision: Sanchez-Orozco L

Responsible for manuscript planning and revision: Armendariz-Borunda J

Approved the final manuscript: Galicia-Moreno M, Monroy-Ramirez HC, Campos-Valdez M, Sanchez-Meza J, Sanchez-Orozco L, Armendariz-Borunda J

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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REFERENCES

1. Piñero F, Poniachik J, Ridruejo E, Silva M. Hepatocellular carcinoma in Latin America: diagnosis and treatment challenges. *World J Gastroenterol* 2018;24:4224-9.
2. Fassio E, Díaz S, Santa C, Reig ME, Martínez Artola Y, et al. Etiology of hepatocellular carcinoma in Latin America: a prospective, multicenter, international study. *Ann Hepatol* 2010;9:63-9.
3. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mather C, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *In J Cancer* 2015;136:E359-86.
4. McGlynn KA, Petrick JL, London WT. Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability. *Clin Liver Dis* 2015;19:223-38.
5. Méndez-Sánchez N, Ridruejo E, Alves de Mattos A, Chávez-Tapia NC, Zapata R, et al. Latin American Association for the Study of the Liver (LAASL) clinical practice guidelines: management of hepatocellular carcinoma. *Ann Hepatol* 2014;13:S4-40.
6. Piñero F, Pages J, Marciano S, Fernández N, Silva J, et al. Fatty liver disease, an emerging etiology of hepatocellular carcinoma in Argentina. *World J Hepatol* 2018;10:41-50.
7. IARC/WHO. GLOBOCAN 2019: Estimated cancer incidence, mortality and prevalence worldwide in 2019. Cancer today data base, Population Fact Sheets. Available from: <https://gco.iarc.fr/today/fact-sheets-populations> [Last accessed on 16 Apr 2020]
8. Piñero F, Costa P, Boteon YL, Duque SH, Marciano S, et al. A changing etiologic scenario in liver transplantation for hepatocellular carcinoma in a multicenter cohort study from Latin America. *Clin Res Hepatol Gastroenterol* 2018;42:443-52.
9. World Health Organization. Hepatitis B and C in the spotlight, a public health response in the Americas. 2016. Available from: <http://iris.paho.org/xmlui/bitstream/handle/123456789/31449/9789275119297eng.pdf?sequence=5&isAllowed=y> [Last accessed on 16 Apr 2020]
10. Blach S, Zeuzem S, Manns M, Altraif I, Duberg AS, et al. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol Hepatol* 2017;2:161-76.
11. World Health Organization. Global Hepatitis Report, World Health Organization. 2017. Available from: <http://www.who.int/hepatitis> [Last accessed on 16 Apr 2020]
12. Centers for Disease Control and Prevention. The ABCs of Hepatitis Fact Sheet. 2016. Available from: www.cdc.gov/hepatitis [Last accessed on 16 Apr 2020]
13. Liu Z, Jiang Y, Yuan H, Fang Q, Cai N, et al. The trends in incidence of primary liver cancer caused by specific etiologies: results from the Global Burden of Disease Study 2016 and implications for liver cancer prevention. *J Hepatol* 2019;70:674-83.
14. Zein NN. Clinical significance of hepatitis C virus genotypes. *Clin Microbiol Rev* 2000;13:223-35.
15. Kershenovich D, Razavi HA, Sánchez-Avila JF, Bessone F, Coelho HS, et al. Trends and projections of hepatitis C virus epidemiology in Latin America. *Liver Int* 2011;31:18-29.
16. Maucort-Boulch D, de Martel C, Franceschi S, Plummer M. Fraction and incidence of liver cancer attributable to hepatitis B and C viruses worldwide. *Int J Cancer* 2018;142:2471-7.
17. Motola-Kuba D, Zamora-Valdes D, Uribe M, Méndez-Sánchez N. Hepatocellular carcinoma. An overview. *Ann Hepatol* 2006;5:16-24.
18. González Huezo MS, Sánchez Ávila JF; Asociación Mexicana de Hepatología; Asociación Mexicana de Gastroenterología; Sociedad Mexicana de Radiología e Imagen, et al. Mexican consensus on the diagnosis and management of hepatocellular carcinoma. *Rev Gastroenterol Mex* 2014;79:250-62.
19. Méndez-Sánchez N, Aguilar-Ramírez JR, Reyes A, Dehesa M, Juárez A, et al. Etiology of liver cirrhosis in Mexico. *Ann Hepatol* 2004;3:30-3.
20. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004;127:S35-50.
21. Weissenbacher M, Rossi D, Radulich G, Sosa-Estáñi S, Vila M, et al. High seroprevalence of bloodborne viruses among street-recruited injection drug users from Buenos Aires, Argentina. *Clin Infect Dis* 2003;15:S348-52.
22. Guimarães T, Granato CF, Varella D, Ferraz ML, Castelo A, et al. High prevalence of hepatitis C infection in a Brazilian prison: identification of risk factors for infection. *Braz J Infect Dis* 2001;5:111-28.
23. Pando MA, Bautista CT, Maulen S, Duranti R, Marone R, et al. Epidemiology of human immunodeficiency virus, viral hepatitis (B and C), *treponema pallidum*, and human T-cell, and human T-cell lymphotropic I/II virus among men who have sex with men in Buenos Aires Argentina. *Sex Transm Dis* 2006;33:307-13.
24. Singal AG, Manjunath H, Yopp AC, Beg MS, Marrero JA, et al. The effect of PNPLA3 on fibrosis progression and development of the hepatocellular carcinoma a meta-analysis. *Am J Gastroenterol* 2014;109:325-24.
25. Saab S, Manne V, Nieto J, Schwimmer JB, Chalasani NP. Nonalcoholic fatty liver disease in Latinos. *Clin Gastroenterol Hepatol* 2016;14:5-12.
26. Nordenstedt H, White D, El-Serag HB. The changing pattern of epidemiology in hepatocellular carcinoma. *Dig Liver Dis* 2010;42:s206-14.
27. Jiwani SS, Carrillo Larco RM, Hernández-Vásquez A, Barrientos-Gutiérrez T, Basto-Abreu A, et al. The shift of obesity burden by socioeconomic status between 1998 and 2017 in Latin America and the Caribbean: a cross-sectional series study. *Lancet Glob Health* 2019;7:e1644-54.
28. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet*

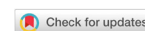
- 2017;390:2627-42.
29. Méndez-Sánchez N, Villa AR, Chávez-Tapia NC, Ponciano-Rodríguez G, Almeda-Valdés P, et al. Trends in liver disease prevalence in Mexico from 2005 to 2050 through mortality data. *Ann Hepatol* 2005;4:52-5.
30. Bailey MA, Brunt EM. Hepatocellular carcinoma: predisposing conditions and precursor lesions. *Gastroenterol Clin North Am* 2002;31:641-62.
31. Marrero JA, Feng Z, Wang Y, Nguyen MH, Befeler AS, et al. Alpha-protein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009;137:110-28.
32. El-Serag HB, Kramer JR, Chen GJ, Duan Z, Richardson PA, et al. Effectiveness of AFP and ultrasound tests on hepatocellular carcinoma mortality in HCV-infected patients in the USA. *GUT* 2011;60:992-7.
33. Marrero JA. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009;30:37-47.
34. European Association for the study of the liver, European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012;56:908-43.
35. Heimbach J, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358-80.
36. Marrero JA, Ahn J, Rajender Reddy K; American College of Gastroenterology. ACG clinical guideline: the diagnosis and management of focal liver lesions. *Am J Gastroenterol* 2014;109:1328-47.
37. Kudo M, Matsui O, Izumi N, Iijima H, Kadoya M, et al. JSH-consensus-based clinical practice guideline for the management of hepatocellular carcinoma: 2014 update by the liver cancer study group of Japan. *Liver Cancer* 2014;3:458-68.
38. Matsui O. Analysis of intranodular hemodynamics of dysplastic nodules in cirrhotic livers. *Radiology* 2001;218:603-5.
39. Ueda K, Terada T, Nakanuma Y, Matsui O. Vascular supply in adenomatous hyperplasia of the liver and hepatocellular carcinoma: a morphometric study. *Hum Pathol* 1992;23:619-26.
40. Forner A, Vilana R, Ayuso C, Bianchi L, Solé M, et al. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: prospective validation of the noninvasive diagnosis criteria for hepatocellular carcinoma. *Hepatology* 2008;47:97-104.
41. Sangiovanni A, Manini MA, Iavarone M, Romeo R, Forzenigo LV, et al. The diagnosis and economic impact of contrast imaging techniques in the diagnosis of small hepatocellular carcinoma in cirrhosis. *Gut* 2010;59:638-44.
42. Bolondi L, Gaiani S, Celli N, Golfieri R, Grigioni WR, et al. Characterization of small nodules in cirrhosis by assessment of vascularity: the problem of hypovascular hepatocellular carcinoma. *Hepatology* 2005;42:27-34.
43. Hanna RF, Milousshev VZ, Tang A, Finklestone LA, Brejt SZ, et al. Comparative 13-year meta-analysis of the sensitivity and positive predictive value of ultrasound, CT, and MRI for detecting hepatocellular carcinoma. *Abdom Radiol* 2016;41:71-90.
44. Lee YJ, Lee JM, Lee JS, Lee HY, Park BH, et al. Hepatocellular carcinoma: diagnosis performance of multidetector CT and MR imaging a system review and meta-analysis. *Radiology* 2015;275:97-109.
45. Roberts LR, Sirlin CB, Zaiem F, Almasri J, Prokop LJ, et al. Imaging for the diagnosis of hepatocellular carcinoma: a systematic review and meta-analysis. *Hepatology* 2018;67:401-21.
46. Kierans AS, Kang SK, Rosenkrantz AB. The diagnosis performance of dynamic contrast-enhanced MR imaging for detection of small hepatocellular carcinoma measuring up to 2 cm: a meta-analysis. *Radiology* 2016;278:82-94.
47. Kele PG, Van der Jagt EJ. Diffusion weighted imaging in the liver. *World J Gastroenterol* 2010;16:1567-76.
48. Miller FH, Hammond N, Siddiqi AJ, Shroff S, Khatri G, et al. Utility of diffusion-weighted MRI in distinguishing benign and malignant hepatic lesion. *J Magn Reson Imaging* 2010;32:138-47.
49. Galea N, Cantisani V, Taouli B. Liver lesion detection and characterization: role of diffusion-weighted imaging. *J Magn Reson Imaging* 2013;37:1260-76.
50. Van Beers BE, Pastor CM, Hussain HK. Primovist, Eovist: what to expect? *J Hepatol* 2012;57:421-9.
51. Caturelli E, Solmi L, Anti M, Fusilli S, Roselli P, et al. Ultrasound guided fine needle biopsy of early hepatocellular carcinoma complicating liver cirrhosis: a multicentre study. *Gut* 2004;53:1356-62.
52. International Consensus Group for Hepatocellular Neoplasia, The International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 2009;49:658-64.
53. Ponziani FR, Nicoletti A, Gasbarrini A, Pompili M. Diagnostic and therapeutic potential of the gut microbiota in patients with early hepatocellular carcinoma. *Ther Adv Med Oncol* 2019;11:1758835919848184.
54. Kawai T, Akira S. The roles of TLRs, RLSs and NLRs in pathogen recognition. *Int Immunol* 2009;21:317-37.
55. Miao EA, Andersen-Nissen E, Warren SE, Aderem A. TLR5 and Ipaf: dual sensors of bacterial flagellin in the innate immune system. *Semin Immunopathol* 2007;29:275-88.
56. Luedde T, Schwabe RF. NF- κ B in the liver-linking injury, fibrosis and hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2011;8:108-28.
57. Jung IH, Choi JH, Chung YY, Lim GL, Park YN, et al. Predominant activation of JAK/STAT3 pathway by interleukin-6 is implicated in hepatocarcinogenesis. *Neoplasia* 2015;17:586-697.
58. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: update criteria and genotype assignment web resource. *Hepatology* 2013;59:318-27.
59. Arca-Lafuente S, Martínez-Román P, Mateo-Cano I, Madrid R, Briz V. Nanotechnology: a reality for diagnosis of HCV infectious disease. *J Infect* 2019;30:30281-6.
60. Hussain MM, Samir TM, Azzazy HME. Unmodified gold nanoparticles for direct and rapid detection of mycobacterium tuberculosis complex. *Clin Biochem* 2013;46:633-7.
61. Gill P, Alvandi A, Abdul-tehrani H, Sadeghizadeh M. Colorimetric detection of helicobacter pylori DNA using isothermal helicase-

- dependent amplification and gold nanoparticle probes. *Diagn Microbiol Infect Dis* 2008;62:119-24.
62. Carter JR, Balaram V, Kucharski CA, Fraser TS, Fraser MJ. A novel dengue virus detection method that couples DNazyme and gold nanoparticle approaches. *Virol J* 2013;10:201.
63. Liu Y, Zhang L, Wei W, Zhao H, Zhou Z, et al. Colorimetric detection in influenza A virus using antibody-functionalized gold nanoparticles. *Analyst* 2015;140:3989-95.
64. Wang C, Zhang L, Shen X. Development of a nucleic acid lateral flow strip for detection of hepatitis C virus (HCV) core antigen. *Nucleosides Nucleotides Nucl Acids* 2013;32:59-68.
65. Daniele B, Bencivenga A, Megna AS, Tinessa V. α -fetoprotein and ultrasonography screening for hepatocellular carcinoma. *Gastroenterology* 2004;127:S108-12.
66. Fassio E, Mazzolini G, AAEH. Consenso y guías Argentinas para la vigilancia, Diagnóstico y Tratamiento del Hepatocarcinoma. *Acta Gastroenterol Latinoam* 2016;46:350-74. (in Spanish)
67. Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999;19:329-38.
68. Meza-Junco J, Montañón-Loza A, Myrna C. Treatment modalities in patients with hepatocellular carcinoma: a retrospective series in a single institution in Mexico. *Gastroenterol Hepatol* 2004;27:11-7.
69. Grazi GL, Ercolani G, Pierangeli F, Del Gaudio M, Cescon M, et al. Improved results of liver resection for hepatocellular carcinoma on cirrhosis give the procedure added value. *Ann Surg* 2001;234:71-8.
70. Poon RT, Fan ST, Ng IO, Wong J. Significance of resection margin in hepatectomy for hepatocellular carcinoma: a critical reappraisal. *Ann Surg* 2000;23:544-51.
71. Imamura H, Matsuyama Y, Miyagawa Y, Ishida K, Shimada R, et al. Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. *Br J Surg* 1999;86:1032-38.
72. Hasegawa K, Kokudo N, Imamura H, Matsuyama Y, Aoki T, et al. Prognostic impact of anatomic resection for hepatocellular carcinoma. *Ann Surg* 2005;242:252-9.
73. Kosuge T, Makuuchi M, Takayama T, Yamamoto J, Shimada K, et al. Long-term results after resection of hepatocellular carcinoma: experience of 480 cases. *Hepatogastroenterology* 1993;40:328-32.
74. Carrilho FJ, Kikuchi L, Branco F, Sandoval Goncalves C, Aves de Mattos A, et al. Clinical and epidemiological aspects of hepatocellular carcinoma in Brazil. *Clinics* 2010;65:1285-90.
75. Fan ST. Hepatocellular carcinoma- resection or transplant? *Nat Rev Gastroenterol Hepatol* 2012;9:732-37.
76. Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, et al. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012;13:e11-22.
77. Mazzaferro V, Bhoori S, Sposito C, Bongini M, Langer M, et al. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011;17:S44-57.
78. Sociedad de Trasplantes de América Latina y el Caribe (Stalyc). Newsletter transplant international figures on donation and transplantation 2016. Available from: <https://stalyc.net/utilidades/biblioteca/publicaciones/file/94-newsletter-transplant-22.html> [Last accessed on 16 Apr 2020]
79. Sociedad de Trasplantes de América Latina y el Caribe (Stalyc). Latin America Transplantation Registry Report 2015-2016. Available from: <https://www.stalyc.net/registros/file/74-latin-america-transplantation-registry-report-2015-2016.html> [Last accessed on 16 Apr 2020]
80. Inchingolo R, Posa A, Mariappan M, Spiliopoulos S. Locoregional treatments for hepatocellular carcinoma: current evidence and future directions. *World J Gastroenterol* 2019;28:4614-28.
81. N'Kontchou G, Mahamoudi A, Aout M, Ganne-Carrié N, Grando V, et al. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 Western patients with cirrhosis. *Hepatology* 2009;50:1475-83.
82. Lu DS, Yu NC, Raman SS, Limanond P, Lassman C, et al. Radiofrequency ablation of hepatocellular carcinoma: treatment success as defined by histologic examination of the explanted liver. *Radiology* 2005;234:954-60.
83. Sato S, Shiratori Y, Imamura M, Teratani T, Obi S, et al. Power Doppler signals after percutaneous ethanol injection therapy for hepatocellular carcinoma predict local recurrence of tumors: a prospective study using 199 consecutive patients. *J Hepatol* 2001;35:225-34.
84. Lencioni R, Crocetti L. Local-regional treatment of hepatocellular carcinoma. *Radiology* 2012;262:43-58.
85. Khan KN, Yatsushashi H, Yamasaki K, Yamasaki M, Inoue O, et al. Prospective analysis of risk factors for early intrahepatic recurrence of hepatocellular carcinoma following ethanol injection. *J Hepatol* 2000;32:269-78.
86. Dou JP, Liang P, Yu J. Microwave ablation for liver tumors. *Abdom Radiol* 2016;41:650-8.
87. Glassberg MB, Ghosh S, Clymer JW, Wright GWJ, Ferko N, et al. Microwave ablation compared with hepatic resection for the treatment of hepatocellular carcinoma and liver metastases: a systematic review and meta-analysis. *World J Surg Oncol* 2019;17:98.
88. Doo Song K. Percutaneous cryoablation for hepatocellular carcinoma. *Clin Mol Hepatol* 2016;22:509-15.
89. Zimmerman A, Grand D, Charpentier KP. Irreversible electroporation of hepatocellular carcinoma: patient selection and perspectives. *J Hepatocell Carcinoma* 2017;13:49-58.
90. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology* 2003;37:429-42.
91. Bouvry C, Palard X, Edeline J, Ardisson V, Loyer P, et al. Transarterial radioembolization (TARE) agents beyond 90Y microspheres. *Biomed Res International* 2018;2018:1-14.
92. Hsieh TC, Wu YC, Sun SS, Yen KY, Kao CH. Treating hepatocellular carcinoma with 90Y-bearing microspheres: a review. *Biomedicine* 2016;6:19.
93. Bruix J, Sherman M; American Association for the Study of the Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53:1020-2.

94. Leathers JS, Balderramo D, Prieto J, Diehl F, Gonzalez-Ballerga E, et al. Sorafenib for treatment of hepatocellular carcinoma: a survival analysis from the south american liver research network. *J Clin Gastroenterol* 2019;53:464-9.
95. Greten TF, Lai CW, Li G, Staveley-O'Carroll KF. Targeted and immune-based therapies for hepatocellular carcinoma. *Gastroenterol* 2019;156:510-24.
96. De Mattia E, Cecchin E, Guardascione M, Foltran L, Raimo TD, et al. Pharmacogenetics of the systemic treatment in advanced hepatocellular carcinoma. *World J Gastroenterol* 2019;25:3870-96.
97. Bruix J, Qin S, Merle P, Granito A, Huang YH, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;389:56-66.
98. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N Engl J Med* 2018;379:54-63.
99. Turkes F, Chau I. Ramucirumab and its use in the treatment of hepatocellular carcinoma. *Future Oncol* 2019;15:979-88.
100. Finkelmeier F, Waidmann O, Trojan J. Nivolumab for the treatment of hepatocellular carcinoma. *Expert Rev Anticancer Ther* 2018;18:1169-75.
101. Wang J, Ma L, Tang X, Zhang X, Qiao Y, et al. Doxorubicin induces apoptosis by targeting Madcam 1 and AKT and inhibiting protein translation initiation in hepatocellular carcinoma cells. *Oncotarget* 2015;6:24075-91.
102. Zhou Y, Li K, Li F, Han S, Wang Y, et al. Doxorubicin and ABT-199 coencapsulated nanocarriers for targeted delivery and synergistic treatment against hepatocellular carcinoma. *J Nanomat* 2019;2019:13.
103. Zhuang L, Zeng X, Yang Z, Meng Z. Effect and safety of interferon for hepatocellular carcinoma: a systematic review and meta-analysis. *PloS One* 2013;8:e61361.
104. George PM, Badiger R, Alazawi W, Foster GR, Mitchell JA. Pharmacology and therapeutic potential of interferons. *Pharmacol Ther* 2012;135:44-53.
105. Nishiguchi S, Kuroti T, Nakatani S, Morimoto H, Takeda T, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995;346:1051-5.
106. Bruno S, Stroffolini T, Colombo M, Bollani S, Benvegnù L, et al. Sustained virological response to interferon-alpha is associated with improved outcome in HVC-related cirrhosis: a retrospective study. *Hepatology* 2007;45:579-87.
107. Baumert TF, Berg T, Lim JK, Nelson DR. Status of direct-acting antiviral therapy for hepatitis C virus infection and remaining challenges. *Gastroenterology* 2019;156:431-45.
108. Piñero F, Boin I, Chagas A, Quiñonez E, Marciano S, et al. Direct-acting antivirals and hepatocellular carcinoma: no evidence of higher waitlist progression or post-transplant recurrence. *Liver Transpl* 2020; epub ahead of print. doi: 10.1002/lt.25744
109. Mendizabal M, Piñero F, Ridruejo E, Wolff FH, Anders M, et al. Disease progression in patients with hepatitis C virus infection treated with direct-acting antiviral agents. *Clin Gastroenterol Hepatol* 2020;28:30263-9.

Review

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The impact of direct-acting antivirals on hepatitis C associated hepatocellular carcinoma

Tai-Ping Lee, David Bernstein

Division of Hepatology and Sandra A. Bass Center for Liver Diseases at Northwell Health, Manhasset, NY 11030, USA.

Correspondence to: Dr. Tai-Ping Lee, Division of Hepatology and Sandra A. Bass Center for Liver Diseases at Northwell Health, Manhasset, NY 11030, USA. E-mail: taiplee@gmail.com

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Abstract

The increased incidence of hepatocellular carcinoma (HCC) in the last several decades in the United States and worldwide has partly resulted from an increase in hepatitis C virus (HCV) infection. HCV carcinogenesis is speculated to be indirectly related to multiple steps from inflammation to fibrosis and advanced fibrosis/cirrhosis over 20 or more years. However, the direct carcinogenic potential from HCV may explain HCC occurring in non-cirrhotic HCV patients. Highly potent direct-acting antivirals (DAAs) in recent years have changed hepatitis C treatment significantly and have resulted in the sustained virologic response (SVR) rate exceeding 90%. Although initial reports concerned the increase in de novo and recurrent HCC associated with DAAs, more recent studies showed that DAA-induced SVR on the contrary reduced risk of HCV-associated HCC without increasing its recurrence. The International Consortium of Hepatitis C Therapeutic Registry and Research Network (HCV-TARGET) database and other resources of HCV patients treated with DAA collectively in the near future most likely will be able to show definitive evidence on the risk of HCC occurrence and recurrence after DAA with SVR. The long-term risk of HCC in chronic hepatitis C patients with advanced fibrosis or cirrhosis remains high after DAAs with SVR. Thus, HCC surveillance on this sub-group of patients is important for early detection and intervention of HCC.

Keywords: Direct-acting antivirals, hepatitis C virus infection, risk of hepatocellular carcinoma

INTRODUCTION

Liver cancer was predicted to be the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide in 2018. Statistically, hepatocellular carcinoma (HCC) comprises 75%-85% cases of liver cancer^[1].



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The sharply increased incidence of HCC in the last several decades in the United States and worldwide has partly resulted from an increase in hepatitis C virus (HCV) infection^[2]. In the United States, chronic hepatitis C accounts for approximately 20%-31% of HCC deaths^[2,3].

HCV AND HCC

HCV carcinogenesis is speculated to be indirectly related to multiple steps from chronic inflammation to fibrosis, advanced fibrosis and cirrhosis with somatic genetic/epigenetic alterations, and malignant transformation of hepatocytes over 20 or more years^[4,5]. Patients with HCV cirrhosis had a three-fold higher adjusted risk of HCC than those with cirrhosis from other etiologies, implying direct carcinogenic effects of the virus^[6-9]. HCC may develop in non-cirrhotic HCV patients, suggesting a direct HCV oncogenic effect^[10]. Additionally, HCV core protein was shown to have oncogenic potential by using transgenic mouse models, indicating its direct involvement in carcinogenesis^[11].

HCV-infected patients with advanced fibrosis or cirrhosis and older age are well-established risk for HCC development^[4,12-14]. The prevalence of HCC is especially high in cirrhotic HCV patients with an estimated annual risk of 2%-4%^[14].

HCV ERADICATION BY INTERFERON-BASED THERAPY AND HCC RISKS

Long-term eradication of HCV reduced HCC risk, which was initially demonstrated in patients who achieved SVR by interferon (IFN)-based therapies^[14-18]. An analysis from 12 observational studies demonstrated that IFN-induced SVR led to nearly four-fold HCC risk reduction irrespective of liver disease stage^[19].

Van der Meer *et al.*^[20] found that the 10-year cumulative HCC incidence with SVR was 5.1%, vs. 21.8% in those without SVR ($P < 0.001$).

Although IFN has potential anti-inflammatory and/or immunomodulatory effects for the prevention of HCC, HCV eradication does not eliminate the risk of HCC^[21,22]. El-Serag *et al.*^[22] reported an overall incidence rate of 0.33% in new HCC development, which could occur more than 10 years after HCV eradication by IFN-based therapy.

HCV ERADICATION BY DAAS AND HCC RISKS

DAAs for HCV infection directly targeting viral protease, polymerase, or non-structural proteins have replaced IFN-based therapy over the past few years. They have changed the management of hepatitis C virus infection significantly, as the treatment is easy to administer, well-tolerated, safe, and highly effective with an SVR rate exceeding 90%^[23-25].

DAAs can be used in HCV infection with advanced and complicated liver disease^[25-31]. Multiple large cohort studies have shown that DAA-induced SVR is associated with a reduced risk of HCC^[14,32-35]. Kanwal *et al.*^[33] reported a significantly reduced risk of HCC (0.9 vs. 3.45 HCC/100 person-years) in 22,500 hepatitis C patients treated by DAAs with SVR compared to those without.

Piñero *et al.*^[35] showed an overall 73% relative risk reduction for *de novo* HCC in DAAs-treated HCV patients with SVR, but the risk remained high in patients with advanced fibrosis and cirrhosis. Furthermore, reduced HCC risk by DAAs with SVR was demonstrated in patients with or without cirrhosis by Ioannou *et al.*^[34].

HCC RISK IN CHRONIC HEPATITIS C TREATED BY DAA COMPARED TO THAT OF IFN THERAPY

IFN, an immune modulator, inhibits proliferation and may prevent the development of HCC. IFN-based HCV antiviral therapy due to its potential side effects was used mostly on patients without cirrhosis.

On the contrary, DAAs have been used on HCV patients with advanced fibrosis and cirrhosis who are at high risk of HCC. It was speculated that there would be more HCV-associated HCCs after DAA with SVR than those post-IFN with SVR ones in the United States, given that the largest cohort of chronic hepatitis C patients in the United States are baby boomers with advanced age, cirrhosis^[36], and rising prevalence of metabolic syndrome-associated co-morbidities^[37].

Waziry *et al.*^[38] reported a random-effects meta-analysis comparing HCC occurrence and recurrence in patients treated by DAA and IFN therapy and showed no evidence of difference in HCC risk between the two groups after meta-regression adjustment of age and study follow up duration. Ioannou *et al.*^[34] published a large VA cohort study of 21,498 chronic hepatitis C (CHC) patients with DAA-induced SVR, showing that it is associated with reduced risk of *de novo* HCC compared to treatment failure and that the risk for HCC after DAA therapy is similar to the risk after IFN therapy.

Singer *et al.*^[39] using administrative claims data demonstrated that the risk of HCC was lower in DAA-treated patients (adjusted HR = 0.69; 95%CI: 0.59-0.81).

DAAS AND DE NOVO HCC

Earlier studies of first-generation DAAs showed increased risk for *de novo* and recurrent HCC, which brought concerns that DAAs might have carcinogenic effects^[40-43]. A retrospective multicenter study from Spain reported a short-term HCC incidence of 3.73 HCC/100 patient-years (95%CI: 2.96-4.70), within a median 10.3 months after starting DAA therapy on 1123 HCV patients with cirrhosis^[44].

HCC risk with DAAs is related to the severity of liver histology^[33,45,46]. The annual incidence of HCC after SVR was higher in those with cirrhosis than those without cirrhosis (1.82 vs. 0.34/100 person-years)^[14,33].

Ioannou *et al.*^[47] reported that an increased risk for HCC in hepatitis C patients with baseline cirrhosis or high FIB-4 treated with either IFN-based therapy or DAAs could persist up to 10 years after SVR. Kanwal *et al.*^[48] also showed that an increased risk for HCC after DAAs with SVR remained for up to 3.6 years of follow up, and it was particularly high in patients with cirrhosis.

DAAS AND RECURRENT HCC

Hepatitis C virus stimulates immune response. HCV-specific T cells produce cytokines including IFN with anti-HCC effects^[49-52]. The recurrence of HCC was speculated to be due to reduced immune surveillance, cytokine imbalance, and angiogenesis^[50-53].

A meta-analysis by Singal *et al.*^[54] demonstrated that IFN-based treatment for HCV patients after curative HCC therapy reduced HCC recurrence and improved the outcomes. Nishibatake Kinoshita *et al.*^[55] reported no significant difference of HCV-related early HCC recurrence after HCC treatment between 156 patients in the IFN-based group and 147 patients in the DAA group.

Several earlier studies showed different results regarding DAAs and the risk of HCC recurrence^[56-58]. Some studies that reported an increased risk for HCC recurrence with use of DAAs correlated earlier HCC recurrence with a shorter interval between complete response to HCC treatment and the DAA agent^[40,59].

A meta-analysis of HCC recurrence after DAAs by Saraiya pointed out that some studies lacked a comparison cohort or had different patient selection criteria, timing of DAA therapy, and follow up schedules. Nevertheless, they found no significant difference in HCC recurrence among the study groups^[60].

The benefits of DAA therapy including regression of fibrosis, decrease in portal hypertension, and hepatic failure are weighed against potential risk for HCC recurrence. A large retrospective study of 793 patients in

North America (304 received DAA therapy vs. 489 received no HCV therapy) published by Singal *et al.*^[61] showed no association between DAA therapy and HCC recurrence (HR = 0.90; 95%CI: 0.70-1.16).

Dang *et al.*^[62] reported a 60%-70% improvement in 5-year all-cause and liver-related mortality in HCV-related HCC patients after DAAs with SVR, compared to patients untreated for HCV. Singal *et al.*^[14] hypothesized that, by decreasing HCV viral load and slowing or preventing liver decompensation, DAA therapy could reduce the risk for late HCC recurrence.

ACTIVE HCC EFFECT ON SVR, AND TIMING OF DIRECT-ACTING ANTIVIRAL THERAPY

Lower HCV SVR rates were reported in the presence of HCC^[63-69]. It is speculated that the low HCV SVR rates were due to altered inflammatory state in the tumor microenvironment, DAA uptake into hepatocyte, resistant profiles in the context of HCC, immune escape mechanism, HCC reservoir, and penetration of DAAs to HCV-infected HCC tissue^[63,69].

Although Ahmed *et al.*^[70] showed that pre-liver transplant HCV treatment with DAAs provided great outcomes and the most cost-effective management for CHC patients with HCC or decompensated cirrhosis while waiting for liver transplant in the US, a study on US veterans with HCV observed an SVR rate of 74.4% in patients who received DAAs during active HCC compared to 91.1% in patients without HCC^[64]. Deferring DAA therapy until six months after completion of either liver resection or ablation is recommended in HCC patients who are eligible for curative HCC treatment^[14].

Radhakrishnan *et al.*^[71] using HCV-TARGET database demonstrated a 50% reduced SVR in HCV patients with HCC, but SVR was not different among patients who received complete, partial, or no treatment at all.

Median wait time, availability of hepatitis C-positive organ, and severity of liver decompensation are the determinants of timing of DAA therapy in HCV-associated HCC patients who are on the liver transplantation (LT) list. DAA therapy for patients awaiting LT is usually deferred until after transplant so patients will be eligible to receive an HCV positive donor^[14].

Reduced liver-related deaths on LT waiting list and decreased progression of liver disease from post-transplant HCV reinfection by DAA have been observed^[72,73]. Some patients treated by DAAs with SVR while awaiting LT had sufficient improvement in liver function to receive other curative therapies or forego transplant^[73-75]. Although Yang *et al.*^[76] suggested DAAs might be associated with a higher rate of HCC recurrence post-LT in a small group of patients, Emamaullee and colleagues demonstrated that HCV eradication pre-LT did not impact rates of delisting for HCC progression or rates of HCC recurrence post-LT in a larger retrospective study^[77].

DAA THERAPY IN PATIENTS WITH UNTREATED ADVANCED HCC

Limited data are available regarding the use of DAAs in hepatitis C patients with untreated advanced HCC. A theoretical benefit from DAAs in this setting is that it may improve liver decompensation and allow continued HCC therapy. Tumor burden, life expectancy, and patient preference need to be considered for DAA therapy since it is palliative^[14].

SUMMARY

Highly potent DAAs in recent years have revolutionized hepatitis C treatment and have high SVR rate exceeding 90%. Numerous studies have shown that DAA-induced SVR reduces risk of hepatitis C-associated HCC. Although recent research demonstrated no increased risk of HCC in HCV patients after DAA with SVR, the HCV-TARGET database and other resources, such as DAA manufacturers' database and

Surveillance, Epidemiology and End Results Program in the United States, collectively are most likely to show definitive evidence on the risk of HCC occurrence and recurrence after DAA with SVR.

Nevertheless, the risk of HCC in chronic hepatitis C patients with advanced fibrosis or cirrhosis remains after DAAs with SVR. The concerns of DAA-associated *de novo* HCC and its recurrence in HCV patients warrant further investigation. Clinical parameters and/or potential molecular biomarkers in the near future may enable better identification of HCC in high-risk HCV patients treated by DAAs with SVR. Thus, this subset of patients will benefit from proper surveillance, early detection, and intervention of HCC.

DECLARATIONS

Authors' contributions

Study concept and design, literature search, drafting of the manuscript: Lee TP
Administrative support: Bernstein D

Availability of data and materials

Not applicable.

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None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
2. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al.; Global Burden of Disease Liver Cancer Collaboration. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the Global Burden of Disease Study 2015. *JAMA Oncol* 2017;3:1683-91.
3. Islami F, Goding Sauer A, Miller KD, Siegel RL, Fedewa SA, et al. Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States. *CA Cancer J Clin* 2018;68:31-54.
4. Goossens N, Hoshida Y. Hepatitis C virus-induced hepatocellular carcinoma. *Clin Mol Hepatol* 2015;21:105-14.
5. Tan A, Yeh SH, Liu CJ, Cheung C, Chen PJ. Viral hepatocarcinogenesis: from infection to cancer. *Liver Int* 2008;28:175-88.
6. Kamegaya Y, Hiasa Y, Zukerberg L, Fowler N, Blackard JT, et al. Hepatitis C virus acts as a tumor accelerator by blocking apoptosis in a mouse model of hepatocarcinogenesis. *Hepatology* 2005;41:660-7.
7. Lemon SM, McGivern DR. Is hepatitis C carcinogenic? *Gastroenterology* 2012;142:1274-8.
8. Chen CL, Tsukamoto H, Machida K. Oncogenic signaling pathways and origins of tumor-initiating stem-like cells of hepatocellular carcinomas induced by hepatitis C virus, alcohol and/or obesity. *Hepatol Int* 2014;8:330-8.
9. Zhang Y. Detection of epigenetic aberrations in the development of hepatocellular carcinoma. *Cancer Epigenetics* 2014;1238:709-31.
10. Yeh MM, Daniel HDJ, Torbenson M. Hepatitis C associated hepatocellular carcinoma in non-cirrhotic livers. *Mod Pathol* 2010;23:276-83.
11. Koike K. Hepatitis C virus contributes to hepatocarcinogenesis by modulating metabolic and intracellular signaling pathways. *J Gastroenterol Hepatol* 2007;22:S108-11.
12. Hoshida Y, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular

- carcinoma. *J Hepatol* 2014;61:S79-90.
13. Axley P, Ahmed Z, Ravi S, Singal AK. Hepatitis C virus and hepatocellular carcinoma: a narrative review. *J Clin Transl Hepatol* 2018;6:79-84.
 14. Singal AG, Lim JK, Kanwal F. AGA clinical practice update on interaction between oral direct-acting antivirals for chronic hepatitis C infection and hepatocellular carcinoma: expert review. *Gastroenterology* 2019;156:2149-57.
 15. Yu ML, Lin SM, Chuang WL, Dai CY, Wang JH, et al. A sustained virological response to interferon or interferon/ribavirin reduces hepatocellular carcinoma and improves survival in chronic hepatitis C: a nationwide, multicenter study in Taiwan. *Antivir Ther* 2006;11:985-94.
 16. Singal AG, Volk ML, Jensen D, Di Bisceglie AM, Schoenfeld PS. A sustained viral response is associated with reduced liver-related morbidity and mortality in patients with hepatitis C virus. *Clin Gastroenterol Hepatol* 2010;8:280-8,288.e1.
 17. Ogawa E, Furusyo N, Kajiura E, Takahashi K, Nomura H, et al. Efficacy of pegylated interferon alpha-2b and ribavirin treatment on the risk of hepatocellular carcinoma in patients with chronic hepatitis C: a prospective, multicenter study. *J Hepatol* 2013;58:495-501.
 18. Moon C, Jung KS, Kim DY, Baatarkhuu O, Park JY, et al. Lower incidence of hepatocellular carcinoma and cirrhosis in hepatitis C patients with sustained virological response by pegylated interferon and ribavirin. *Dig Dis Sci* 2015;60:573-81.
 19. Morgan RL, Baack B, Smith BD, Yartel A, Pitasi M, et al. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann Intern Med* 2013;158:329-37.
 20. van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012;308:2584-93.
 21. Janjua NZ, Chong M, Kuo M, Woods R, Wong J, et al. Long-term effect of sustained virological response on hepatocellular carcinoma in patients with hepatitis C in Canada. *J Hepatol* 2017;66:504-13.
 22. El-Serag HB, Kanwal F, Richardson P, Kramer J. Risk of hepatocellular carcinoma after sustained virological response in Veterans with hepatitis C virus infection. *Hepatology* 2016;64:130-7.
 23. Chung RT, Baumert TF. Curing chronic hepatitis C-the arc of a medical triumph. *N Engl J Med* 2014;370:1576-8.
 24. McGlynn EA, Adams JL, Kramer J, Sahota AK, Silverberg MJ, et al. Assessing the safety of direct-acting antiviral agents for hepatitis C. *JAMA Network Open* 2019;2:e194765.
 25. American Association for the Study of Liver Diseases and Infectious Diseases Society of America. Recommendations for testing, managing, and treating hepatitis C. Available from: <http://www.hcvguidelines.org> [Last accessed on 14 Apr 2020]
 26. Kohli A, Shaffer A, Sherman A, Kottitil S. Treatment of hepatitis C: a systematic review. *JAMA* 2014;312:631-40.
 27. Wyles DL, Sulkowski MS, Dieterich D. Management of hepatitis C/HIV coinfection in the era of highly effective hepatitis C virus direct-acting antiviral therapy. *Clin Infect Dis* 2016;63 Suppl 1:S3-11.
 28. Kohli A, Alshati A, Georgie F, Manch R, Gish RG. Direct-acting antivirals for the treatment of chronic hepatitis C in patients with chronic kidney disease. *Therap Adv Gastroenterol* 2016;9:887-97.
 29. Falade-Nwulia O, Suarez-Cuervo C, Nelson DR, Fried MW, Segal JB, et al. Oral direct-acting agent therapy for hepatitis C virus infection: a systematic review. *Ann Intern Med* 2017;166:637-48.
 30. Cheung MCM, Walker AJ, Hudson BE, Verma S, McLauchlan J, et al. Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. *J Hepatol* 2016;65:741-7.
 31. Nahon P, Bourcier V, Layese R, Audureau E, Cagnot C, et al. Eradication of hepatitis C virus infection in patients with cirrhosis reduces risk of liver and non-liver complications. *Gastroenterology* 2017;152:142-6.
 32. Su F, Ioannou GN. Hepatocellular carcinoma risk after direct-acting antiviral therapy. *Clin Liver Dis (Hoboken)* 2019;13:6-12.
 33. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, et al. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. *Gastroenterology* 2017;153:996-1005.e1.
 34. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol* 2017. Epub ahead of print. doi: 10.1016/j.jhep.2017.08.030.
 35. Piñero F, Mendizabal M, Ridruejo E, Herz Wolff F, Ameigeiras B, et al. Treatment with direct-acting antivirals for HCV decreases but does not eliminate the risk of hepatocellular carcinoma. *Liver Int* 2019;39:1033-43.
 36. Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* 2010;138:513-21.
 37. Lazo M, Nwankwo C, Daya NR, Thomas DL, Mehta SH, et al. Confluence of epidemics of hepatitis C, diabetes, obesity, and chronic kidney disease in the United States Population. *Clin Gastroenterol Hepatol* 2017;15:1957-64.
 38. Waziry R, Hajarizadeh B, Grebely J, Amin J, Law M, et al. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: a systematic review, meta-analyses, and meta-regression. *J Hepatol* 2017;67:1204-12.
 39. Singer AW, Reddy KR, Telep LE, Osinusi AO, Brainard DM, et al. Direct-acting antiviral treatment for hepatitis C virus infection and risk of incident liver cancer: a retrospective cohort study. *Aliment Pharmacol Ther* 2018;47:1278-87.
 40. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016;65:727-33.
 41. Cabibbo G, Celsa C, Cammà C, Craxi A. Should we cure hepatitis C virus in patients with hepatocellular carcinoma while treating cancer? *Liver Int* 2018;38:2108-16.
 42. Kozbial K, Moser S, Schwarzer R, Laferl H, Al-Zoairy R, et al. Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. *J Hepatol* 2016;65:856-8.
 43. Nault JC, Colombo M. Hepatocellular carcinoma and direct acting antiviral treatments: controversy after the revolution. *J Hepatol* 2016;65:663-5.
 44. Mariño Z, Darnell A, Lens S, Sapena V, Díaz A, et al. Time association between HCV therapy and hepatocellular carcinoma emergence in patients with cirrhosis. The relevance of non-characterized nodules. *J Hepatol* 2019;70:874-84.

45. Nahon P, Layese R, Bourcier V, Cagnot C, Marcellin P, et al. Incidence of hepatocellular carcinoma after direct antiviral therapy for HCV in patients with cirrhosis Included in surveillance programs. *Gastroenterology* 2018;155:1436-50.
46. Ogawa E, Furusyo N, Nomura H, Dohmen K, Higashi N, et al.; Kyushu University Liver Disease Study (KULDS) Group. Short-term risk of hepatocellular carcinoma after hepatitis C virus eradication following direct-acting anti-viral treatment. *Aliment Pharmacol Ther* 2018;47:104-13.
47. Ioannou GN, Beste LA, Green PK, Singal AG, Tapper EB, et al. Increased risk for hepatocellular carcinoma persists up to 10 years after HCV eradication in patients with baseline cirrhosis or high FIB-4 Scores. *Gastroenterology* 2019;157:1264-78.
48. Kanwal F, Kramer JR, Asch SM, Cao Y, Li L, et al. Long-term risk of hepatocellular carcinoma in HCV patients treated with direct acting antiviral agents. *Hepatology* 2020;71:44-55.
49. Llovet JM, Villanueva A. Liver cancer: effect of HCV clearance with direct-acting antiviral agents on HCC. *Nat Rev Gastroenterol Hepatol* 2016;13:561-2.
50. Reig M, Boix L, Mariño Z, Torres F, Forns X, et al. Liver cancer emergence associated with antiviral treatment: an immune surveillance failure? *Semin Liver Dis* 2017;37:109-18.
51. Debes JD, van Tilborg M, Groothuismink ZMA, Hansen BE, Schulze Zur Wiesch J, et al. Levels of cytokines in serum associate with development of hepatocellular carcinoma in patients with HCV infection treated with direct-acting antivirals. *Gastroenterology* 2018;154:515-7.e3.
52. Sanduzzi-Zamparelli M, Boix L, Leal C, Reig M. Hepatocellular carcinoma recurrence in HCV patients treated with direct antiviral agents. *Viruses* 2019;11:406.
53. Villani R, Vendemiale G, Serviddio G. Molecular Mechanisms Involved in HCC Recurrence after direct-acting antiviral therapy. *Int J Mol Sci* 2019;20:49.
54. Singal AK, Freeman DH, Anand BS. Meta-analysis: interferon improves outcomes following ablation or resection of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2010;32:851-8.
55. Nishibatake Kinoshita M, Minami T, Tateishi R, Wake T, Nakagomi R, et al. Impact of direct-acting antivirals on early recurrence of HCV-related HCC: comparison with interferon-based therapy. *J Hepatol* 2019;70:78-86.
56. Colombo M, Boccaccio V. HCV therapy and risk of liver cancer recurrence: who to treat? *Nat Rev Gastroenterol Hepatol* 2018;15:392-3.
57. Reig M, Boix L, Bruix J. The impact of direct antiviral agents on the development and recurrence of hepatocellular carcinoma. *Liver Int* 2017;37:136-9.
58. Guarino M, Viganò L, Ponziani FR, Giannini EG, Lai Q, et al. Recurrence of hepatocellular carcinoma after direct acting antiviral treatment for hepatitis C virus infection: literature review and risk analysis. *Dig Liver Dis* 2018;50:1105-14.
59. Guarino M, Sessa A, Cossiga V, Morando F, Caporaso N, et al. Direct-acting antivirals and hepatocellular carcinoma in chronic hepatitis C: a few lights and many shadows. *World J Gastroenterol* 2018;24: 2582-95.
60. Saraiya N, Yopp AC, Rich NE, Odewole M, Parikh ND, et al. Systematic review with meta-analysis: recurrence of hepatocellular carcinoma following direct-acting antiviral therapy. *Aliment Pharmacol Ther* 2018;48:127-37.
61. Singal AG, Rich NE, Mehta N, Branch A, Pillai A, et al. Direct-acting antiviral therapy not associated with recurrence of hepatocellular carcinoma in a multicenter North American cohort study. *Gastroenterology* 2019;156:1683-92.
62. Dang H, Yeo YH, Yasuda S, Huang CF, Iio E, et al. Cure with interferon free DAA is associated with increased survival in patients with HCV related HCC from both East and West. *Hepatology* 2019; Epub ahead of print [PMID: 31610027 DOI: 10.1002/hep.30988]
63. Saberi B, Dadabhai AS, Durand CM, Philosophe B, Cameron AM, et al. Challenges in treatment of hepatitis C among patients with hepatocellular carcinoma. *Hepatology* 2017;66:661-3.
64. Beste LA, Green PK, Berry K, Kogut MJ, Allison SK, et al. Effectiveness of hepatitis C antiviral treatment in a USA cohort of veteran patients with hepatocellular carcinoma. *J Hepatol* 2017;67:32-9.
65. Prenner SB, VanWagner LB, Flamm SL, Salem R, Lewandowski RJ, et al. Hepatocellular carcinoma decreases the chance of successful hepatitis C virus therapy with direct-acting antivirals. *J Hepatol* 2017;66:1173-81.
66. Chang CY, Nguyen P, Le A, Zhao C, Ahmed A, et al. Real-world experience with interferon -free, direct-acting anti-hepatitis C therapies in Asian Americans with chronic hepatitis C and advanced liver disease. *Medicine (Baltimore)* 2017;96:e6128.
67. Soria A, Fabbiani M, Lapadula G, Gori A. Unexpected viral relapses in hepatitis C virus-infected patients diagnosed with hepatocellular carcinoma during treatment with direct-acting antivirals. *Hepatology* 2017;66:992-4.
68. Ji F, Yeo YH, Wei MT, Wei B, Dang S, et al. Hepatocellular carcinoma decreases the effectiveness of hepatitis C antiviral treatment: do direct-acting antiviral regimens matter? *Hepatology* 2018;67:1180-2.
69. Harrod E, Moctezuma-Velazquez C, Gurakar A, Ala A, Dieterich D, et al. Management of concomitant hepatocellular carcinoma and chronic hepatitis C: a review. *Hepatoma Res* 2019;5:28.
70. Ahmed A, Gonzalez SA, Cholaneril G, Perumpail RB, McGinnis J, et al. Treatment of patients waitlisted for liver transplant with all-oral direct-acting antivirals is a cost-effective treatment strategy in the United States. *Hepatology* 2017;66:46-56.
71. Radhakrishnan K, Di Bisceglie AM, Reddy KR, Lim JK, Levitsky J, et al. Treatment status of hepatocellular carcinoma does not influence rates of sustained virologic response: an HCV-TARGET analysis. *Hepatol Commun* 2019 ;3:1388-99.
72. Roche B, Coilly A, Duclos-Vallee JC, Samuel D. The impact of treatment of hepatitis C with DAAs on the occurrence of HCC. *Liver Int* 2018;38:139-45.
73. Belli LS, Berenguer M, Cortesi PA, Strazzabosco M, Rockenschaub SR, et al. European liver and intestine association (ELITA). Delisting of liver transplant candidates with chronic hepatitis C after viral eradication: a European study. *J Hepatol* 2016;65:524-31.
74. Gadiparthi C, Cholaneril G, Perumpail BJ, Yoo ER, Satapathy SK, et al. Use of direct-acting antiviral agents in hepatitis C virus-infected liver transplant candidates. *World J Gastroenterol* 2018;24:315-22.
75. Pascasio JM, Vinaixa C, Ferrer MT, Colmenero J, Rubin A, et al. Clinical outcomes of patients undergoing antiviral therapy while awaiting liver transplantation. *J Hepatol* 2017;67:1168-76.

76. Yang JD, Aql BA, Pungpapong S, Gores GJ, Roberts LR, et al. Direct acting antiviral therapy and tumor recurrence after liver transplantation for hepatitis C-associated hepatocellular carcinoma. *J Hepatol* 2016;65:856-68.
77. Bigam D, Shapiro AMJ, Kneteman N. HCV eradication with direct-acting antivirals does not impact HCC progression on the waiting list or HCC recurrence after liver transplantation. *Can J Gastroenterol Hepatol* 2019;2019:2509059.

Review

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Direct-acting antivirals and risk of hepatocellular carcinoma: from genetic signature to metabolic risk factors

Stefano Gitto¹, Erica Villa²

¹Department of Experimental and Clinical Medicine, University of Florence, Florence 50121, Italy.

²Department of Medical Specialties, Gastroenterology Unit, University of Modena & Reggio Emilia, Modena 41124, Italy.

Correspondence to: Prof. Erica Villa, Department of Medical Specialties, Gastroenterology Unit, University of Modena & Reggio Emilia, Via del Pozzo 71, Modena 41124, Italy. E-mail: erica.villa@unimore.it

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the second leading cause of cancer-related death. Hepatitis C virus and mainly hepatitis C virus-related cirrhosis is the chief risk factor for HCC. Many direct-acting antivirals are available for the eradication of hepatitis C virus with remarkable results in terms of virological response and with optimal safety profile. Notably, some authors have suggested that viral eradication due to these new drugs might favor both occurrence and recurrence of HCC. The exact biological mechanisms of carcinogenesis in this specific setting have not been well identified, but it has been suggested that adjustments in immune surveillance and increase in vascular endothelial growth factor expression could have a chief role. Remarkably, after publication of many large studies and meta-analyses, we can affirm that there is no increased risk on a population basis. Nonetheless, on an individual basis, sustained virological response due to direct-acting antivirals may facilitate HCC onset in some specific subgroups of patients. Among them, we could point out patients with activated neoangiogenesis but also subjects with particularly severe metabolic imbalance.

Keywords: Direct-acting antivirals, hepatocellular carcinoma, carcinogenesis

INTRODUCTION

Hepatocellular carcinoma (HCC) represents about 5.6% of cancers worldwide^[1]. It is the fifth most common malignancy and its incidence has grown in the last two decades^[2]. Notably, HCC has a strong impact on patients' outcome, being the second leading cause of cancer-related death^[3]. Hepatitis C virus (HCV) and particularly HCV-related cirrhosis, is the main risk factor for HCC^[4].



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HCV leads to chronic hepatitis C and cirrhosis in about 20% of subjects within 20 years of infection^[5] and HCC occurs in around 3.5% of cases per year^[6].

For many years, interferon (IFN)-based protocols have represented the cornerstone of antiviral therapy^[7]. Achievement of sustained virological response (SVR) with IFN is infrequent (especially in cirrhotics), and the drug leads to low compliance and high toxicity. However, viral clearance due to IFN is associated with decreased incidence of HCC and overall reduction of other liver-related events^[8].

In the last few years, new antiviral molecules with a direct action against the virus have been developed^[9]. Today, many direct-acting antivirals (DAAs) are available with amazing results in terms of SVR and safety^[10].

A few years after the introduction of DAAs, two noteworthy and innovative studies reported first that SVR due to DAA might increase the risk of both HCC occurrence and recurrence^[11,12].

Later, other authors suggested the unexpected high incidence of HCC after DAA treatment, describing the onset of extraordinarily aggressive tumors a few weeks after the end of antiviral therapy^[13].

Following the publication of these studies, many other groups reported the increased occurrence of HCC after DAA use^[14], whereas others rebutted this notion^[15-17].

The exact biological mechanisms by which oral DAAs might enhance the risk of HCC are largely unknown^[18]. Modifications in immune surveillance may potentiate the risk of HCC by accelerating the process of regeneration. Moreover, DAAs seem to increase levels of vascular endothelial growth factor (VEGF) and to modify the balance between pro- and anti-inflammatory reactions^[19].

The aim of the present review was to analyze the main evidence about the relationship between DAA and HCC onset, privileging meta-analyses and original real-life controlled trials. Moreover, we tried to identify high-risk subpopulations that can benefit from personalized post-eradication follow-up. We analyzed the state-of-the-art about this debated matter offering a critical point of view and underlining potential future perspectives.

HCC RECURRENCE AFTER SVR DUE TO DAA THERAPY

In the last years, there has been particular concern about the rates of HCC recurrence following DAA treatment^[11,12,20].

Notably, many studies about HCC recurrence are single-arm and retrospective cohort studies. Moreover, they show relevant heterogeneity in the following patterns: tumor burden, HCC treatments leading to whole response, and follow-up periods^[21]. Indeed, it is questionable whether HCC recurrence might be part of the natural history of patients with advanced liver disease or whether DAAs can have a chief role in cancer pathogenesis. Fortunately, in the last 2 years, some meta-analyses have been proposed, and these are the ideal instruments to obtain solid answers.

In 2017, Waziry *et al.*^[22] compared, using systematic review, meta-analysis, and meta-regression approaches, 15 studies on HCC recurrence after SVR. Seven studies included patients treated with IFN-based therapy and 10 comprising patients cured with DAA. The incidence of HCC recurrence was 9.21/100 per year and 12.16/100 per year in IFN and DAA subgroups, respectively. Through a meta-regression analysis and an adjustment for age and follow-up length, the authors demonstrated that patients in the DAA group did not show higher HCC recurrence in comparison with those treated with IFN.

Sometime later, Saraiya *et al.*^[21] conducted a further meta-analysis examining data from 1820 patients with SVR due to DAA treatment. A vast part of the studies considered involved less than 100 patients with consequent inexact estimates of HCC recurrence. Notably, many but not all patients were treated for HCC with radical therapy (surgical resection or ablation). In fact, a substantial percentage of subjects (25%-50%) received non-curative therapies such as transarterial chemoembolization, showing by definition a high risk of recurrence regardless of antiviral therapy.

The authors correctly underlined other methodological differences among the studies analyzed. The extent of follow-up to evaluate HCC recurrence varied among the studies (from 3 to 36 months). Furthermore, some groups defined HCC recurrence from date of cancer treatment, while others assessed recurrence from start of DAAs.

According to reported data, the HCC recurrence rate was 21.9% (with high statistical heterogeneity). Nine studies compared HCC recurrence in DAA-treated ($n = 947$), IFN-treated ($n = 210$) and/or untreated ($n = 641$) patients. The authors demonstrated that DAA-treated patients displayed lower pooled recurrence risk than did untreated patients and similar rate in comparison with subjects treated with IFN-based protocols.

In the same year (2018), another meta-analysis was published^[23]. The authors selected studies better with respect to the previous meta-analysis, since they restricted patient enrollment to those with HCC previously treated with a curative approach (surgical resection or percutaneous ablation). The authors subdivided uncontrolled and controlled studies. The pooled proportion of recurrent HCC in uncontrolled cohorts was 16.7% (with evidence of substantial statistical heterogeneity) with follow-up that highly varied (from 12 to 280 weeks). Considering controlled cohorts, the pooled proportion was 20.1% (again with substantial statistical heterogeneity) with a follow-up range that was up to 416 weeks. Remarkably, the authors themselves reported that the global quality of the evidence was low for each of the outcomes considered, suggesting the need for further studies with longer follow-up periods.

Recently, Rutledge *et al.*^[24] made strong suggestions about the complex matter of HCC recurrence after DAA therapy. The meta-analysis conducted by these authors is not only the widest among the available studies but is also particularly solid from a methodological point of view. In fact, the authors included only patients with previous curative cancer therapy and excluded studies with less than one year of follow-up. Notably, this rigorous approach allowed avoiding any rise in early HCC recurrence that could definitely be associated with the presence of small subclinical HCC at the beginning of antiviral therapy. The authors analyzed and compared 57 studies, which involved 16 IFN-treated, 33 DAA-treated and 8 untreated patients. DAA-treated patients who achieved SVR displayed a recurrence rate of 18.17/100 per year. Patients with SVR due to IFN therapy showed a similar recurrence rate (11.01/100 per year). The rate of HCC recurrence was comparable between untreated patients (25.69/100 per year) and IFN-treated patients without virological response (16.89/100 per year); subjects not responding to DAA showed a higher rate of recurrence, but the small number of patients made the value not statistically significant (44.16/100 per year).

Notably, the authors performed a subanalysis adjusting data according to HCC consolidated risk factors such as age and cirrhosis. They demonstrated that the DAA-treated group showed a lower rate of recurrent HCC (although without statistical significance).

Since overall mortality represents the most important clinical outcome, the recent findings of Singal *et al.*^[25] should be pointed out. The authors demonstrated with a multicenter cohort study ($n = 797$), that patients who achieved both complete response to any HCC treatment (resection, local ablation, transarterial chemo- or radioembolization, or radiation therapy) and SVR due to DAAs, showed a significant reduction of mortality risk in comparison with non-responders and untreated subjects.

HCC OCCURRENCE AFTER SVR ASSOCIATED WITH DAA THERAPY

Chronic viral hepatitis is the most important predisposing factor for the occurrence of HCC^[4]. Indeed, viral eradication is a main goal to decrease the incidence of HCC and, more in general, to lessen liver-related mortality^[3,8].

Considering the IFN era, Van der Meer *et al.*^[26], compared long-term outcomes of IFN monotherapy, IFN + ribavirin and PEG IFN + ribavirin, in 5 Liver Units. Recruited patients displayed advanced fibrosis and were followed up for a mean period of 8.4 years. Ten-year cumulative HCC incidence rate was 5.1% for the SVR subgroup and 21.8% for non-responders.

Ogawa *et al.*^[27] conducted a prospective national multicenter study on the effect of PEG IFN + ribavirin treatment on chronic HCV infection, focusing on HCC occurrence. The authors enrolled 1013 patients and demonstrated that 5-year cumulative incidence of HCC was 1.7% in the SVR and 7.6% in the non-SVR group with a weakening of this effect considering only cirrhotic subjects (18.9% vs. 39.4%).

Morgan *et al.*^[28] carried out a meta-analysis including 30 observational studies, with 31,528 patients enrolled. The authors confirmed that patients with SVR due to IFN-based protocol showed a lower HCC occurrence with respect to the others: 0.33 per person-year in SVR and 1.67 in non-responders.

Regarding the role of DAA therapy in favoring HCC occurrence, Conti *et al.*^[11] published in 2016 a regional multicenter study of 344 consecutive cirrhotic patients treated with DAAs, including 285 subjects who did not show previous HCC. The authors reported that after the end of DAA treatment (follow-up of 24 weeks), 9 patients (3.16%) developed HCC in a higher percentage than IFN responders. The key point of discussion should be about the main clinical patterns of enrolled patients who displayed more advanced liver disease in comparison with the classical IFN cohort. Additionally, enrolled patients were screened for HCC exclusively by ultrasound with a certain probability of small undiagnosed HCC nodules at the beginning of antiviral therapy.

Some of the previously cited meta-analyses examined the whole risk of HCC occurrence after DAAs.

Waziry *et al.*^[22], comparing IFN regimens and DAAs, demonstrated that HCC occurrence following SVR was 1.14/100 per year for patients treated with IFN and 2.96/100 per year in subjects receiving DAAs. Notably, the authors showed that occurrence rate decreased with longer follow-up and was lower in younger subjects. Finally, the authors proposed a meta-regression after adjusting data for follow-up and age, demonstrating that SVR due to DAA was not related to significantly more HCC occurrence.

Singh *et al.*^[23] analyzed a total of 39,145 patients treated with DAA (data from uncontrolled studies). Among them, 542 developed *de novo* HCC after achievement of SVR. Incident HCC showed an overall occurrence of 1.5% with substantial statistical heterogeneity among the studies included. The authors examined the HCC occurrence rate associated with DAA considering only controlled cohorts. Among five studies, the proportion of HCC occurrence was 3.3%, again with considerable statistical heterogeneity. Therefore, authors concluded that data derived from their study should be considered non-conclusive regarding the possible relationship between DAAs and increased risk of HCC occurrence.

A recently published meta-analysis^[24] examined 81 studies of HCC occurrence after DAA. In particular, the authors compared the following subgroups of studies: 31 involving 71,443 IFN-treated subjects, 44 comprising 91,249 of DAA-treated patients and 6 on 9944 untreated subjects.

The authors reported that, considering only patients with SVR due to DAA and not all the DAA population, HCC occurrence rate was 3.57 per 100 per year vs. 0.70 per 100 per year in the IFN-treated SVR group

(without statistical significance). Afterwards, the authors carried out an additional smaller meta-analysis evaluating only studies with multivariate-adjusted hazard ratios (for gender, baseline cirrhosis and age). With this approach, these authors did not find augmented hazard of HCC occurrence in subjects treated with DAA compared to the IFN-treated subgroup. Notably, the authors also sub-analyzed data according to follow-up length. Considering occurrence rates in the second year after treatment with DAAs, similar incidence rates were found (0.88 per 100 per year in the DAA group and 0.55 in the IFN group). Finally, the authors conducted a sub-analysis excluding any cases of HCC occurrence within six months after end of treatment, where they found an incidence rate of 1.12 per 100 per year for the DAA group and 3.01 for the IFN group (again without statistical significance).

HIGH RISK PATIENTS: POTENTIAL ROLE OF GENETIC SIGNATURE

The data outlined in the previous paragraphs underlined the concepts that on the whole, the risk of developing HCC after DAAs is small and DAAs represent an extraordinary option for curing hepatitis C. There is already consistent evidence that on a population basis, DAAs improve survival and decrease long-term complications such as decompensation. On an individual basis, however, DAAs might constitute a stimulus associated with higher risk of developing HCCs. In a series of patients with liver cirrhosis enrolled in a prospective study of HCC development, liver tissue of patients who subsequently developed HCC were already primed towards neoangiogenesis well before DAA treatment and ensuing HCC development^[29]. Expression level of angiopoietin-2, evaluated by immunohistochemistry, differed significantly in tumor tissue among patients with recurrent, *de novo*, and non-recurrent HCC after DAAs ($P < 0.0001$, Kruskal-Wallis test). Likewise, hepatic stiffness, spleen size, variceal size, and hepatic venous pressure gradient were related to HCC risk but only angiopoietin-2 was independently related to both risk of HCC occurrence and recurrence. Our hypothesis was that the events occurring during the progression of chronic liver disease, with occurrence of increasing portal hypertension, could be critical in activating angiopoietin-2 expression in splanchnic vascular bed hyperplasia^[29]. Of further interest, HCC developing after DAAs was often biologically aggressive, as indicated in a large proportion of them by the presence of five-gene neoangiogenic signature identified as a marker of rapidly growing and clinically aggressive HCC^[30].

The intriguing relationship between severe portal hypertension and DAA-mediated carcinogenesis could already be glimpsed in a few previously published papers. Conti *et al.*^[11] identified more severe liver fibrosis as independently associated with risk of HCC recurrence. Ravaioli *et al.*^[31] recently showed that the decrease in spleen stiffness in patients who developed HCC after DAAs was significantly less than in patients who did not develop HCC. Similar results were obtained by Ioannou *et al.*^[32] who showed that when FIB-4 remains higher than 3.25 after SVR, the risk of HCC is so consistent to merit HCC surveillance. This highlights that severe portal hypertension is a critical predisposing factor for HCC.

IMPACT OF METABOLIC RISK FACTORS ON HCC RISK

Both diabetes and obesity represent consolidated risk factors for HCC exerting a strong carcinogenic effect directly by disrupting the insulin-IGF pathway or indirectly by promoting steatosis^[33].

In a large retrospective cohort study, El-Serag *et al.*^[34] showed that SVR due to effective IFN-based treatment led to a noteworthy drop in HCC risk. Nevertheless, the authors reported that the annual risk of HCC among responding patients was not insignificant (overall 0.33%). Interestingly, residual HCC risk was associated not only with advanced stage of fibrosis and age but also with diabetes and HCV genotype 3. Relative to this point, other authors also suggested that HCV genotype 3 was correlated not only with higher rates of liver steatosis (being the more “metabolic” genotype) but also with greater HCC risk in comparison with the other genotypes^[35,36]. These data indicate that metabolic disorders might have a significant role also in determining HCC occurrence after DAA therapy.

Table 1. Available meta-analyses analyzing the possible association between DAA therapy and HCC

Ref.	Year	Country	N of studies	N of patients	Increased risk of occurrence	Increased risk of recurrence
Waziry <i>et al.</i> ^[22]	2017	Australia	41	13,875	DAA comparable to IFN	DAA comparable to IFN
Saraiya <i>et al.</i> ^[21]	2018	USA	24	1820	/	DAA comparable to IFN
Singh <i>et al.</i> ^[23]	2018	USA	44	39,145	no conclusive data	no conclusive data
Rutledge <i>et al.</i> ^[24]	2019	USA	138	177,512	DAA comparable to IFN	DAA comparable to IFN

DAA: direct-acting antiviral; HCC: hepatocellular carcinoma; IFN: interferon

Regarding the possible relationship between metabolic disorders and risk of HCC in the specific setting of DAA therapy, Nahon *et al.*^[37] reported interesting results. These authors retrospectively analyzed data from 1270 cirrhotic patients comparing the incidence of HCC in patients treated with IFN-based protocols or DAA therapy. First of all, authors demonstrated that patients treated with DAA were older and had higher rates of diabetes, suggesting that these patterns might explain the reported higher percentages of HCC occurrence in this subgroup of patients in comparison with the IFN group. However, the authors conducted a multivariate analysis, demonstrating that DAA therapy itself did not emerge as a predictor of HCC occurrence. Other factors such as increased age, past excessive alcohol consumption, virological parameters [genotype 1 (in contrast with previously reported data)], decreased platelet count and increased gamma-glutamyl transferase (GGT) levels were independently associated with post-treatment HCC onset. Concerning the metabolic issue, neither diabetes nor obesity was directly associated with HCC occurrence, but it is well known that GGT increase is a sign of altered metabolism^[38] or higher inflammatory activity^[39]. In fact, elevated GGT activity is coupled with traditional cardiovascular risk factors and particularly with metabolic syndrome^[38].

CONCLUSIONS AND FUTURE PERSPECTIVES

DAAs have constituted an extraordinary breakthrough in the therapy of hepatitis C. With an 8- to 12-week course of treatment, SVR occurs in more than 95%-99% of patients, depending on degree of liver fibrosis. These results were beyond any reasonable expectation until a few years ago. It is therefore quite conceivable that the report of an increased occurrence or recurrence of HCC during or shortly after stopping DAAs initiated a series of reactions of concern. With more data accumulating, it is now clear that there is no such risk on a population basis. In fact, as summarized in Table 1, the available high-quality meta-analyses categorically demonstrated that the risk of both HCC recurrence and occurrence after SVR is comparable between patients treated with “old” IFN-based protocols and new DAAs. As recently stated by the American Association for the Study of Liver Diseases^[40], SVR due to DAAs determine a decrease in all-cause mortality, cirrhosis, hepatic decompensation and HCC, an improvement in extra-hepatic manifestations of HCV, and an amelioration of both productivity and quality of life. Summarizing, we must treat with DAA all patients without history of HCC and all subjects with complete response to HCC treatment. Notably, as strongly demonstrated in a recently published meta-analysis^[41], patients with HCC show, for unknown reasons, lower pooled SVR rate in comparison with subjects without cancer (88.2% vs. 92.4%).

Although DAAs represent a great therapeutic option, it is possible that some subgroups of subjects may be at increased risk of HCC onset after DAA treatment. In fact, on an individual basis, administration of DAAs might be associated with facilitated HCC development in subjects who already have a predisposing hepatic condition, namely activated neoangiogenesis, as shown by increased levels of angiopoietin-2 in cirrhotic tissue^[29]. In these patients, a careful evaluation of risk-benefit of DAA treatment should be done, also in view of the aggressive features of DAA-associated HCC^[29]. Finally, in the long-term management of HCV-positive patients, it has to be considered that subjects with severe metabolic impairment seem to maintain a significant risk of HCC despite antiviral treatment and viral eradication^[37].

Concerning the possible identification of subpopulations at higher risk of HCC, some authors have analyzed the change in inflammatory balance due to DAAs as a determinant of cancer development^[19,42].

In particular, Villani *et al.*^[19] studied 103 patients treated with DAAs. Changes in levels of VEGF, epidermal growth factor, and several interleukins were evaluated. Interestingly, the authors demonstrated that VEGF increased 4-fold from baseline to week 4 of treatment. VEGF level continued to increase until the end of treatment and returned to the pre-DAA level afterwards. Of note, VEGF induces angiopoietin-2 expression and can therefore promote DAA-induced carcinogenesis as described in^[29]. On the other hand, interleukin-10 and tumor necrosis factor-alpha significantly decreased with HCV clearance. The authors of the cited study concluded that DAAs can alter the balance between inflammatory and anti-inflammatory processes, affecting antitumor surveillance of the host and favoring HCC onset after DAA therapy.

In conclusion, available data indicate that DAAs represent a great option for both patients without cancer and subjects with effectively treated HCC. Further studies may confirm whether or not some high-risk subgroups exist, which deserve a personalized surveillance approach.

DECLARATIONS

Authors' contributions

Conceived and designed the study and wrote the article together: Gitto S, Villa E

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Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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Consent for publication

Not applicable.

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REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359-86.
2. Beste LA, Leipertz SL, Green PK, Dominitz JA, Ross D, et al. Trends in burden of cirrhosis and hepatocellular carcinoma by underlying liver disease in US veterans, 2001-2013. *Gastroenterology* 2015;149:1471-82.
3. D'Ambrosio R, Della Corte C, Colombo M. Hepatocellular carcinoma in patients with a sustained response to anti-hepatitis C therapy. *Int J Mol Sci* 2015;16:19698-712.
4. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004;127:S35-50.
5. Thein HH, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. *Hepatology* 2008;48:418-31.
6. Sangiovanni A, Del Ninno E, Fasani P, De Fazio C, Ronchi G, et al. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. *Gastroenterology* 2004;126:1005-14.
7. Yano H, Iemura A, Haramaki M, Ogasawara S, Takayama A, et al. Interferon alfa receptor expression and growth inhibition by interferon alfa in human liver cancer cell lines. *Hepatology* 1999;29:1708-17.
8. Simmons B, Saleem J, Heath K, Cooke GS, Hill A. Long-term treatment outcomes of patients infected with hepatitis C virus: a systematic review and meta-analysis of the survival benefit of achieving a sustained virological response. *Clin Infect Dis* 2015;61:730-40.
9. Conteduca V, Sansonno D, Russi S, Pavone F, Dammacco F. Therapy of chronic hepatitis C virus infection in the era of direct-acting

- and host-targeting antiviral agents. *J Infect* 2014;68:1-20.
10. Sandmann L, Schulte B, Manns MP, Maasoumy B. Treatment of chronic hepatitis C: efficacy, side effects and complications. *Visc Med* 2019;35:161-70.
 11. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016;65:727-33.
 12. Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon free therapy. *J Hepatol* 2016;65:719-26.
 13. Kozbial K, Moser S, Schwarzer R, Laferl H, Al-Zoairy R, et al. Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. *J Hepatol* 2016;65:856-8.
 14. Rinaldi L, Di Francia R, Coppola N, Guerrero B, Imperato M, et al. Hepatocellular carcinoma in HCV cirrhosis after viral clearance with direct acting antiviral therapy: preliminary evidence and possible meanings. *WCRJ* 2016;3:e748.
 15. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol* 2017.
 16. ANRS collaborative study group on hepatocellular carcinoma. Lack of evidence of an effect of direct-acting antivirals on the recurrence of hepatocellular carcinoma: data from three ANRS cohorts. *J Hepatol* 2016;65:734-40.
 17. Ikeda K, Kawamura Y, Kobayashi M, Kominami Y, Fujiyama S, et al. Direct-acting antivirals decreased tumor recurrence after initial treatment of hepatitis C virus-related hepatocellular carcinoma. *Dig Dis Sci* 2017;62:2932-42.
 18. Grandhe S, Frenette CT. Occurrence and recurrence of hepatocellular carcinoma after successful direct-acting antiviral therapy for patients with chronic hepatitis C virus infection. *Gastroenterol Hepatol* 2017;13:421-5.
 19. Villani R, Facciorusso A, Bellanti F, Tamborra R, Piscazzi A, et al. DAAs rapidly reduce inflammation but increase serum VEGF Level: a rationale for tumor risk during anti-HCV treatment. *PLoS One* 2016;11:e0167934.
 20. El Kassas M, Funk AL, Salaheldin M, Shimakawa Y, Eltabbakh M, et al. Increased recurrence rates of hepatocellular carcinoma after DAA therapy in a hepatitis C infected Egyptian cohort: a comparative analysis. *J Viral Hepat* 2018;25:623-30.
 21. Saraiya N, Yopp AC, Rich NE, Odewole M, Parikh ND, et al. Systematic review with meta-analysis: recurrence of hepatocellular carcinoma following direct acting antiviral therapy. *Aliment Pharmacol Ther* 2018;48:127-37.
 22. Waziry R, Hajarizadeh B, Grebely J, Amin J, Law M, et al. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: a systematic review, metaanalyses, and meta-regression. *J Hepatol* 2017;67:1204-12.
 23. Singh S, Nautiyal A, Loke YK. Oral direct-acting antivirals and the incidence or recurrence of hepatocellular carcinoma: a systematic review and meta-analysis. *Frontline Gastroenterol* 2018;9:262-70.
 24. Rutledge SM, Zheng H, Li DK, Chung RT. No evidence for higher rates of hepatocellular carcinoma after direct-acting antiviral treatment: a meta-analysis. *Hepatoma Res* 2019;5:31.
 25. Singal AG, Rich NE, Mehta N, Branch AD, Pillai A, et al. Direct-acting antiviral therapy for hepatitis C virus infection is associated with increased survival in patients with a history of hepatocellular carcinoma. *Gastroenterology* 2019;157:1253-63.e2.
 26. Van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012;308:2584-93.
 27. Ogawa E, Furusyo N, Kajiura E, Takahashi K, Nomura H, et al. Efficacy of pegylated interferon alpha-2b and ribavirin treatment on the risk of hepatocellular carcinoma in patients with chronic hepatitis C: a prospective, multicenter study. *J Hepatol* 2013;58:495-501.
 28. Morgan RL, Baack B, Smith BD, Yartel A, Pitasi M, et al. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann Intern Med* 2013;158:329-37.
 29. Faillaci F, Marzi L, Critelli R, Milosa F, Schepis F, et al. Liver angiopoietin-2 is a key predictor of de novo or recurrent hepatocellular cancer after hepatitis C virus direct-acting antivirals. *Hepatology* 2018;68:1010-24.
 30. Villa E, Critelli R, Lei B, Marzocchi G, Cammà C, et al. Neoangiogenesis-related genes are hallmarks of fast-growing hepatocellular carcinomas and worst survival. Results from a prospective study. *Gut* 2016;65:861-9.
 31. Ravaioli F, Conti F, Brillanti S, Andreone P, Mazzella G, et al. Hepatocellular carcinoma risk assessment by the measurement of liver stiffness variations in HCV cirrhotics treated with direct acting antivirals. *Dig Liver Dis* 2018;50:573-9.
 32. Ioannou GN, Beste LA, Green PK, Singal AG, Tapper EB, et al. Increased risk for hepatocellular carcinoma persists up to 10 years after HCV eradication in patients with baseline cirrhosis or high FIB-4 scores. *Gastroenterology* 2019;157:1264-78.
 33. El-Serag HB, Kanwal F. Obesity and hepatocellular carcinoma: hype and reality. *Hepatology* 2014;60:779-81.
 34. El-Serag HB, Kanwal F, Richardson P, Kramer J. Risk of hepatocellular carcinoma after sustained virological response in Veterans with hepatitis C virus infection. *Hepatology* 2016;64:130-7.
 35. Kanwal F, Kramer JR, Ilyas J, Duan Z, El-Serag HB. HCV genotype 3 is associated with an increased risk of cirrhosis and hepatocellular cancer in a national sample of U.S. Veterans with HCV. *Hepatology* 2014;60:98-105.
 36. Kanwal F, White DL, Jiao L, Tavakoli-Tabasi S, Sangsriy S, et al. Genetic variants in interleukin-28B are associated with diabetes and diabetes-related complications in patients with chronic hepatitis C virus infection. *Dig Dis Sci* 2015;60:2030-7.
 37. Nahon P, Layese R, Bourcier V, Cagnot C, Marcellin P, et al. Incidence of hepatocellular carcinoma after direct antiviral therapy for HCV in patients with cirrhosis included in surveillance programs. *Gastroenterology* 2018;155:1436-50.
 38. Ndrepepa G, Collieran R, Kastrati A. Gamma-glutamyl transferase and the risk of atherosclerosis and coronary heart disease. *Clin Chim Acta* 2018;476:130-8.
 39. Taliani G, Badolato MC, Nigro G, Biasin M, Boddi V, et al. Serum concentration of gammaGT is a surrogate marker of hepatic TNF-alpha mRNA expression in chronic hepatitis C. *Clin Immunol* 2002;105:279-85.
 40. Ghany MG, Morgan TR; AASLD-IDS A Hepatitis C Guidance Panel. Hepatitis C guidance 2019 update: american association for the study of liver diseases-infectious diseases society of america recommendations for testing, managing, and treating hepatitis C virus infection. *Hepatology* 2020;71:686-721.

41. He S, Lockart I, Alavi M, Danta M, Hajarizadeh B, et al. Systematic review with meta-analysis: effectiveness of direct-acting antiviral treatment for hepatitis C in patients with hepatocellular carcinoma. *Aliment Pharmacol Ther* 2020;51:34-52.
42. Casadei Gardini A, Foschi FG, Conti F, Petracci E, Vukotic R, et al. Immune inflammation indicators and ALBI score to predict liver cancer in HCV-patients treated with direct-acting antivirals. *Dig Liver Dis* 2019;51:681-8.

Review

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Personalized T-cell therapy in liver transplanted patients with hepatitis B virus related hepatocellular carcinoma

Morteza Hafezi¹, Antonio Bertolotti^{1,2}, Anthony Tan¹

¹Emerging Infectious Diseases Program, Duke-NUS Medical School, Singapore 169857, Singapore.

²Singapore Immunology Network, Singapore Agency for Science, Technology & Research (A*STAR), Singapore 169857, Singapore.

Correspondence to: Prof. Antonio Bertolotti, Emerging Infectious Diseases Program, Duke-NUS Medical School, 8 College Road, Singapore 169857, Singapore. E-mail: antonio@duke-nus.edu.sg

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Abstract

Hepatocellular carcinoma (HCC) is a deadly malignancy which typically occurs in the context of chronic liver inflammation. Chronic hepatitis B virus (HBV) infection is considered a major global cause of HCC development. At the moment, liver transplantation is the only curative modality for HBV-associated HCC. However, some patients develop HBV-HCC recurrence after liver transplantation, leaving them with very limited therapeutic options. Adoptive cell therapy with HBV-specific T cell receptor (TCR) that redirects T cells against HCC relapses has shown promising results in such HBV-HCC patients. In this mini-review, we discuss the application of this personalized T cell therapy, and highlight mRNA electroporation as an efficient tool for engineering safe and efficient TCR-redirectioned T cells for the treatment of liver transplant patients with HBV-HCC metastasis.

Keywords: HBV, TCR-T cells, mRNA, HCC metastasis, adoptive cell therapy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common form of adult liver cancer and third leading cause of cancer-related mortality globally. Different etiological factors are involved in the pathogenesis of HCC and hepatotropic viruses like hepatitis B (HBV) and C (HCV) represent the major cause^[1,2]. In regions with a high incidence of HBV (i.e., South East Asia, China, and Sub-Saharan Africa), chronic HBV infection accounts for 80% of HCC^[3]. A peculiar feature of HBV-related HCC is the HBV DNA integration which



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can be detected as early as 3 days post hepatocyte infection^[3,4]. As such, HBV-HCC nodules can express either all or a portion of HBV antigens which can then be employed as a tumor-specific antigen to develop HBV-redirectioned T cells. We and other groups have recently developed immunotherapeutic approaches by engineering T cells equipped with chimeric antigen receptor (CAR)^[5] or classical T cell receptors (TCRs)^[6,7] against HBV antigens/epitopes expressed on healthy HBV-infected hepatocytes, or in HCC cells with HBV-DNA integration. Engineered TCRs or CAR redirectioned-T cells are able to lyse HCC cells expressing HBV-specific antigens *in vitro*, and showed anti-viral and anti-tumor activity in animal models^[5,7,8]. In this mini-review, we will discuss the use of such immunotherapies in the treatment of HBV-HCC relapses occurring post curative liver transplantation (LT). These patients will be under lifelong immunosuppressive regimens which in turn, create both obstacles and opportunities for the use of T cell therapy.

LIMITATIONS OF EXISTING THERAPEUTIC MODALITIES FOR HBV-HCC RELAPSES

Recurrent HCC after LT is deadly and difficult to manage. Treatment options include resection, radiofrequency ablation, percutaneous ethanol injection and transarterial chemoembolization in LT cases with solitary, intrahepatic HBV-associated HCC recurrence^[9,10]. However, most of these HBV-HCC recurrences post-LT are systemic^[11-14], which limits treatment options. In such a scenario, currently available therapies are ineffective and first-line systemic treatment with the tyrosine kinase inhibitor, Sorafenib, can only increase survival by a few months^[15]. As a new form of systemic immunotherapy, checkpoint inhibitors have shown promising outcomes in the treatment of primary HCC^[16]. Checkpoint inhibitors are designed to rejuvenate immune cell function through blocking co-inhibitory receptors (e.g., PD-1, CTLA-4) expressed on exhausted immune cells. In transplant patients, this strategy can lead to undesirable and uncontrollable immune responses against the transplanted organ^[17]. Indeed, attempts to use PD-1 inhibitors for the treatment of HCC relapses in LT patients led to graft rejection^[18]. Therefore, at present, checkpoint inhibitors are contraindicated in LT patients with HBV-HCC.

Over the past few years, new forms of immunotherapy have been developed to specifically target HCC relapses^[5,7]. In such strategies, T cells are engineered to express TCRs or CARs that are able to recognize HBV epitopes in an human leukocyte antigen (HLA)-restricted manner, or detect hepatitis B s antigen (HBsAg) respectively^[6]. We focused our efforts on engineering TCR-redirectioned T cells as a potential treatment for HBV-associated HCC patients. The unique features of HBV-HCC metastasis render TCR modified-T cells a better option than CAR engineered T cells^[19-21]. In particular, HLA matching is rarely taken into consideration for liver transplantation, hence engineered TCR-T cells could only be employed to target HBV-specific antigens associated with HLA-class I molecules expressed on HCC relapses but not on the transplanted liver^[6]. As a result, engineered TCR-T cells will not recognize HBV peptides present on the transplanted liver that has been re-infected by HBV and therefore, reduce the risk of possible graft rejection. Furthermore, HLA-restricted TCR-T cells would not bind to circulating soluble HBV antigens, which may instead, occur with HBV-specific CAR T cells^[6]. However, in animal models, which are characterized by lower quantities of serum HBsAg than in HBV infected patients, HBsAg did not suppress the ability of CAR-T cells to recognize HBV-producing hepatocytes^[5,19,20].

TREATMENT OF HBV-RELATED HCC RELAPSES WITH HBV-SPECIFIC TCR-REDIRECTED T CELLS

The feasibility of utilizing HBV-TCR-redirectioned T cells for treating HBV-associated HCC was first shown in a patient who had widespread extrahepatic HBsAg+ HCC relapse^[21]. In this scenario, HBV-specific TCR-T cells were engineered to recognize HBV-specific epitopes obtained from HBsAg+ HCC nodules. This patient was an ideal candidate for HBV-TCR T therapy due to multiple clinical features^[21]. In this unique scenario, HBsAg was secreted exclusively by the HCC metastasis while the transplanted liver was HBsAg negative. HBsAg expression in the HCC relapses was due to integration of HBV-specific envelope DNA into the

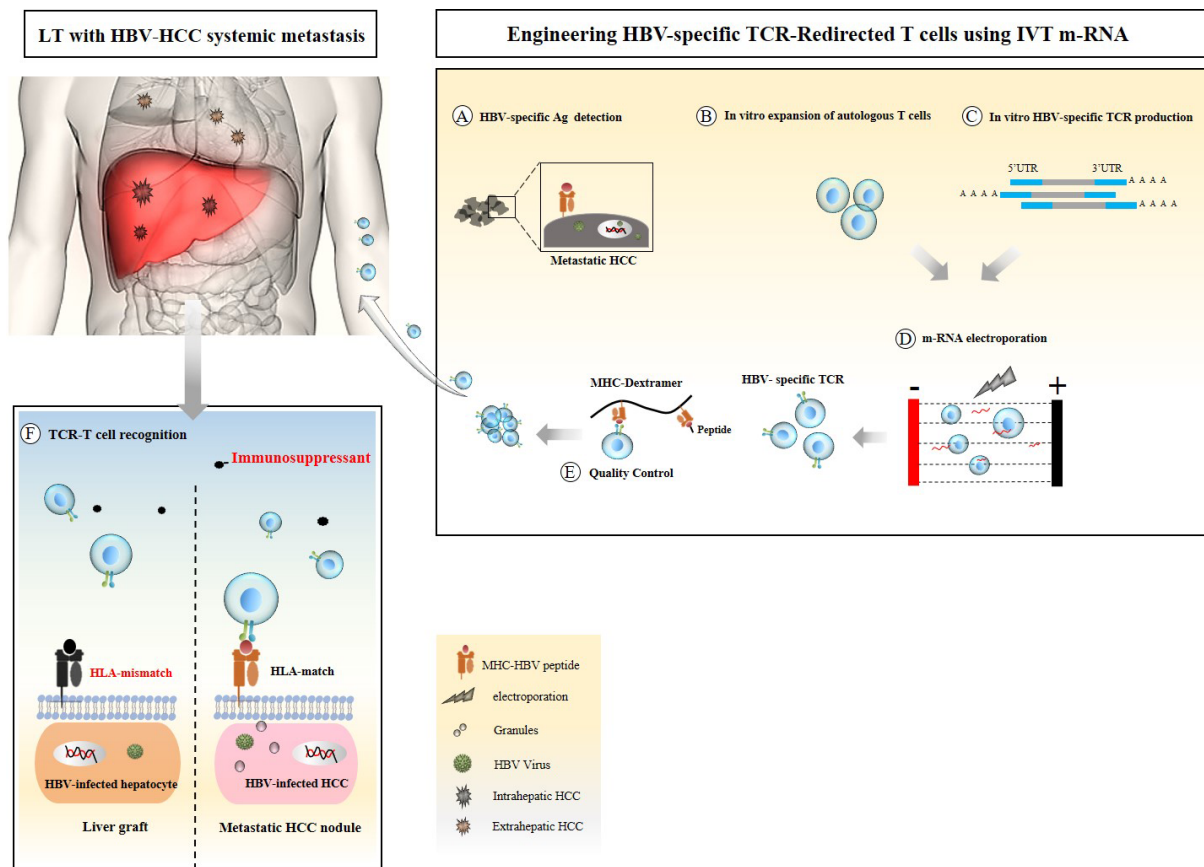


Figure 1. Schematic illustrating the development of mRNA electroporated HBV-specific TCR redirected T cells. A: identification of HBV-specific antigens expressed on metastatic tumor through molecular approaches; B: activation and expansion of autologous T cells for T cell engineering; C: *in vitro* production of mRNA encoding HBV-specific TCRs; D: developing HBV-specific TCR-redirected T cells through electroporation activated T cells with desired mRNA; E: evaluation of TCR expression and functionality of modified T cell using tetramer staining and immune assays; F: modified TCR-T cells reinfused back to LT patient to specifically target and lyse HBV-infected HCC in a HLA restricted manner with no off-tumor side effects on the HBV-infected graft. TCR: T cell receptor; HBV: hepatitis B virus; LT: liver transplantation; HCC: hepatocellular carcinoma; HLA: human leukocyte antigen; MHC: major histocompatibility complex

genome of HCC cells. Since the patient was HLA-A0201 positive, autologous T cells were engineered with a HLA-A0201-restricted TCR specific for an envelope epitope (HBs183-91). These HBV-specific TCR-redirected T cells can specifically recognize the HBsAg⁺ HCC relapses, thereby reducing the risk of affecting healthy hepatocytes during treatment [Figure 1]. Using retroviral gene editing, the coding sequences of alpha and beta TCR were introduced to the T cells *in vitro*. Following infusion of a single dose of autologous retroviral transduced TCR-T cells ($\sim 2 \times 10^4$ TCR-T cells/kg), HBV-specific T cells expanded efficiently, reaching up to $\sim 2\%$ of blood-circulating CD8 T cells and reduced serum HBsAg levels by up to 90% within 30 days post-infusion without notable side effects. Unfortunately, the patient's treatment started at a very late stage when there were already multiple metastases in the lungs, bones and neck. The patient was monitored for ~ 8 months post-T cell infusion and despite reduction of HBsAg levels, the patient's general medical condition subsequently declined and he succumbed to his medical complications. The reduction of serum HBsAg together with the expansion of the infused HBV-specific TCR-redirected T cells strongly suggest that HCC relapses expressing HBV antigens can be targeted by HBV-redirected T cells.

We therefore expanded our research efforts to better understand the treatability of HBV-related HCC relapses with this new form of immunotherapy.

ABILITY OF HBV-SPECIFIC TCR REDIRECTED T CELLS TO RECOGNIZE HCC CELLS WITH SHORT HBV-DNA INTEGRATION

As described above, the feasibility of T-cell immunotherapy was first demonstrated in the scenario wherein HBsAg was expressed and secreted only by the HCC metastases and not by the transplanted liver. However, unlike that patient, HBV-DNA integrations in HCC cells do not always involve the complete open reading frame of HBV antigens. Although HBV-DNA integration could be seen in 60%-80% of HCC cases, most HCC cells appear HBsAg negative when tested with antibodies targeting the whole HBsAg^[22,23]. This is easily explained by the detection of HBV-DNA integration that is often incomplete in normal and transformed hepatocytes and hence generate HBV-human chimeric protein^[24-26] and not whole HBV proteins.

Antibody-based techniques, which depend on the recognition of conformational epitopes, are unable to detect these chimeric proteins and therefore these HBV-HCC tumours are often negative for HBV antigens^[22,27]. This explains why utilizing HBV-related antigens as a tumor-specific antigen was highly controversial and thought to be applicable only for a minority of HCC patients presenting whole HBV antigens in their HCC relapses. However, unlike antibodies, TCRs detect short linear sequences of HBV antigens (9-10 amino acid long) that can be derived from HBV-human chimeras present in HBV serologically negative HCC cells. We have recently tested this possibility *in vitro* utilizing HCC cells negative for HBV antigens when assayed using antibody based techniques^[12]. We utilized HBV-specific CD8 T cells and antibody specific for HLA-class I/HBV epitopes and were able to demonstrate that the production and presentation of HBV-specific CD8 T cell epitopes can take place in naturally HBV serologically negative HCC cells with short HBV-DNA integration^[12]. This *in vitro* demonstration showed the possibility of utilizing short sequences of HBV-DNA integration present in the majority of HBV-related HCC as a robust tumor-associated antigen which can then be applied for HBV-specific T cell engineering. Furthermore, despite the high genetic diversity between HCC cells in the same patient^[28-30], single-cell genome sequencing of HBV-HCC cells showed remarkable homology of HBV integration across multiple single tumor cells^[31]. This suggests that TCR-redirection T cells specific for single epitopes should be able to target the majority of HCC cells present in a patient.

To show feasibility of this strategy, HBV integration profiles of HCC metastases from two LT patients with undetectable HBsAg in the serum were analyzed^[12]. HBV-specific TCR-redirection T cells targeting the epitope encoded by the detected HBV integrations were engineered. Following adoptive T cell therapy, significant volume reduction of several pulmonary metastases has been seen in one patient.

Importantly, in these patients, TCR-T cells were engineered through mRNA electroporation and not virally transduced. This approach offers inherent safety features due to the labile nature of mRNA [Figure 1]. Unlike viral DNA, mRNA transfection has no risk of causing random integration in the human genome. More importantly, controlled pharmacokinetics of the therapy allows for dose escalation by multiple repeat injections which can be personalized for each patient. Multiple infusion of mRNA TCR-T cells (~up to 600 million TCR-T cells) showed no significant increase in serum cytokine levels as well as no evidence of graft inflammation in both patients after receiving this therapy^[12]. Although this therapy was not able to completely eliminate HCC relapses in both patients, one had a dramatic reduction in size of multiple metastatic lesions in the lung without the detection of new lesions and he was clinically well for almost 2 years during treatment^[12]. Unfortunately, abdominal and retroperitoneal HCC relapses that were already present in the patient at the time of TCR therapy did not show any response, progressively expanded and ultimately led to the patient's death (Personal communication). Even though we were unable to investigate the real cause of the unresponsiveness of HCC relapses in different anatomical locations, such data suggests that a tumor's anatomical location together with its inflammatory status, could have a negative impact on TCR-T therapy outcome.

FUTURE DIRECTIONS IN HBV-HCC T CELL THERAPY

Despite encouraging results from the initial trials of TCR-redirectioned T cell therapy in LT patients with HCC relapses, there is still a clear need for improvement of such therapies. Multiple factors present in the LT sera could have an impact on the outcome of TCR-T immunotherapy^[32]. The impact of such variables also remains largely unexplored and needs to be addressed. One of the main obstacles of T cell therapy post-LT is the pharmacological immunosuppression started after transplantation to prevent graft rejection^[33]. Long-term maintenance immunosuppression typically achieved by Tacrolimus alone, or in combination with mycophenolate mofetil continues throughout the patient's life. These drugs were designed to broadly impair T effector function, therefore potentially impacting the adoptively transferred HBV-specific TCR-T cells *in vivo*. With regard to that, *in vitro* experiments in a 3D model clearly showed that the mTOR inhibitor, Rapamycin, could impair TCR-redirectioned T cell migration and cytotoxicity^[34]. Engineering T cells to be resistant to such drugs or using different subsets of T cells with inherent resistant features could allow engineered TCR-T cells to function effectively even in the face of strong immunosuppression. For instance, knock-down of FK506-binding protein 1a, tacrolimus-specific binding protein, markedly recovers T cell function in the presence of clinically-relevant concentrations of Tacrolimus, therefore the same strategy could be employed to develop drug-resistant TCR T cells for the treatment of LT with HBV-HCC relapses^[35]. Furthermore, at this moment, only $\alpha\beta$ TCR T cells are used to engineer therapeutic HBV-specific TCR T cells. Alternatively, the desired $\alpha\beta$ TCR can also be introduced into other immune cells to provide extra benefits. For example, mucosal-associated invariant T cells are non-conventional T cells representing 20%-45% of the liver T cell repertoire^[36,37]. These cells express a high level of multi-drug resistant pump called ATP-Binding cassette subfamily b member 1 (ABCB1) which allows mucosal-associated invariant T cells to retain their function in the face of chemotherapeutic intervention^[36,38]. This drug-resistant feature, together with liver specificity make these cells a highly promising candidate for developing new therapeutic T cells for the treatment of liver associated cancers. Altogether, developing drug-resistant therapeutic TCR T cells could have the potential for clinical application in the treatment of HBV-HCC relapses after curative LT, where immunotherapeutic TCR T-cell function can still be exerted in the presence of obligate immunosuppression. In our opinion, implementing these immunosuppressive drug resistant armored -TCR T cells could improve current immunotherapy outcomes in HBV-associated HCC relapses and would most likely increase survival rates by slowing down tumor progression.

DECLARATIONS

Authors' contributions

Wrote the manuscript: Hafezi M, Bertolotti A, Tan A

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al.; Global Burden of Disease Liver Cancer Collaboration. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. *JAMA Oncol* 2017;3:1683-91.
2. Ringelhan M, Pfister D, O'Connor T, Pikarsky E, Heikenwalder M. The immunology of hepatocellular carcinoma. *Nat Immunol* 2018;19:222-32.
3. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, et al. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019;16:589-604.
4. Tang KW, Alaei-Mahabadi B, Samuelsson T, Lindh M, Larsson E. The landscape of viral expression and host gene fusion and adaptation in human cancer. *Nat Commun* 2013;4:2513.
5. Krebs K, Bottinger N, Huang LR, Chmielewski M, Arzberger S, et al. T cells expressing a chimeric antigen receptor that binds hepatitis B virus envelope proteins control virus replication in mice. *Gastroenterology* 2013;145:456-65.
6. Gehring AJ, Xue SA, Ho ZZ, Teoh D, Ruedl C, et al. Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines. *J Hepatol* 2011;55:103-10.
7. Koh S, Shimasaki N, Suwanarusk R, Ho ZZ, Chia A, et al. A practical approach to immunotherapy of hepatocellular carcinoma using T cells redirected against hepatitis B virus. *Mol Ther Nucleic Acids* 2013;2:e114.
8. Kah J, Koh S, Volz T, Ceccarello E, Allweiss L, et al. Lymphocytes transiently expressing virus-specific T cell receptors reduce hepatitis B virus infection. *J Clin Invest* 2017;127:3177-88.
9. Lim HK. Radiofrequency thermal ablation of hepatocellular carcinomas. *Korean J Radiol* 2000;1:175-84.
10. Zhou B, Shan H, Zhu KS, Jiang ZB, Guan SH, et al. Chemoembolization with lobaplatin mixed with iodized oil for unresectable recurrent hepatocellular carcinoma after orthotopic liver transplantation. *J Vasc Interv Radiol* 2010;21:333-8.
11. Na GH, Hong TH, You YK, Kim DG. Clinical analysis of patients with hepatocellular carcinoma recurrence after living-donor liver transplantation. *World J Gastroenterol* 2016;22:5790-9.
12. Tan AT, Yang N, Lee Krishnamoorthy T, Oei V, Chua A, et al. Use of expression profiles of HBV-DNA integrated into genomes of hepatocellular carcinoma cells to select T cells for immunotherapy. *Gastroenterology* 2019;156:1862-76.e9.
13. Zhang HM, Shi YX, Sun LY, Zhu ZJ. Hepatocellular carcinoma recurrence in living and deceased donor liver transplantation: a systematic review and meta-analysis. *Chin Med J (Engl)* 2019;132:1599-609.
14. Sugawara Y. Living-donor liver transplantation for patients with hepatocellular carcinoma in Japan: current situations and challenge. *Hepatobiliary Pancreat Dis Int* 2020;19:1-2.
15. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-90.
16. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
17. Gassmann D, Weiler S, Mertens JC, Reiner CS, Vrugt B, et al. Liver allograft failure after nivolumab treatment-A case report with systematic literature research. *Transplant Direct* 2018;4:e376.
18. Hu B, Yang XB, Sang XT. Liver graft rejection following immune checkpoint inhibitors treatment: a review. *Med Oncol* 2019;36:94.
19. Bertolotti A, Brunetto M, Maini MK, Bonino F, Qasim W, et al. T cell receptor-therapy in HBV-related hepatocellular carcinoma. *Oncimmunology* 2015;4:e1008354.
20. Kruse RL, Shum T, Tashiro H, Barzi M, Yi Z, et al. HBsAg-redirection T cells exhibit antiviral activity in HBV-infected human liver chimeric mice. *Cytotherapy* 2018;20:697-705.
21. Qasim W, Brunetto M, Gehring AJ, Xue SA, Schurich A, et al. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient. *J Hepatol* 2015;62:486-91.
22. Wang Y, Wu MC, Sham JS, Tai LS, Fang Y, et al. Different expression of hepatitis B surface antigen between hepatocellular carcinoma and its surrounding liver tissue, studied using a tissue microarray. *J Pathol* 2002;197:610-6.
23. Wong DKH, Cheng SCY, Mak LLY, To EWP, Lo RCL, et al. Among patients with undetectable hepatitis B surface antigen and hepatocellular carcinoma, a high proportion has integration of HBV DNA into hepatocyte DNA and no cirrhosis. *Clin Gastroenterol Hepatol* 2020;18:449-56.
24. Chiu YT, Wong JK, Choi SW, Sze KM, Ho DW, et al. Novel pre-mRNA splicing of intronically integrated HBV generates oncogenic chimera in hepatocellular carcinoma. *J Hepatol* 2016;64:1256-64.
25. Furuta M, Tanaka H, Shiraishi Y, Uchida T, Imamura M, et al. Correction: characterization of HBV integration patterns and timing in liver cancer and HBV-infected livers. *Oncotarget* 2018;9:31789.
26. Furuta M, Tanaka H, Shiraishi Y, Unida T, Imamura M, et al. Characterization of HBV integration patterns and timing in liver cancer and HBV-infected livers. *Oncotarget* 2018;9:25075-88.
27. Fu S, Li N, Zhou PC, Huang Y, Zhou RR, et al. Detection of HBV DNA and antigens in HBsAg-positive patients with primary hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol* 2017;41:415-23.
28. Hou Y, Guo H, Cao C, Li X, Hu B, et al. Single-cell triple omics sequencing reveals genetic, epigenetic, and transcriptomic heterogeneity in hepatocellular carcinomas. *Cell Res* 2016;26:304-19.
29. Correction for Ling et al., extremely high genetic diversity in a single tumor points to prevalence of non-Darwinian cell evolution. *Proc Natl Acad Sci U S A* 2016;113:E663.

30. Ling S, Hu Z, Yang Z, Yang F, Li Y, et al. Extremely high genetic diversity in a single tumor points to prevalence of non-Darwinian cell evolution. *Proc Natl Acad Sci U S A* 2015;112:E6496-505.
31. Duan M, Hao J, Cui S, Worthley DL, Zhang S, et al. Diverse modes of clonal evolution in HBV-related hepatocellular carcinoma revealed by single-cell genome sequencing. *Cell Res* 2018;28:359-73.
32. Hafezi M, Bertoletti A, Tan AT. T cell immunotherapy in hepatitis B virus related hepatocellular carcinoma. *Hepatoma Res* 2018;4:16.
33. Pillai AA, Levitsky J. Overview of immunosuppression in liver transplantation. *World J Gastroenterol* 2009;15:4225-33.
34. Pavesi A, Tan AT, Koh S, Chia A, Colombo M, et al. A 3D microfluidic model for preclinical evaluation of TCR-engineered T cells against solid tumors. *JCI Insight* 2017;2.
35. De Angelis B, Dotti G, Quintarelli C, Huye LE, Zhang L, et al. Generation of Epstein-Barr virus-specific cytotoxic T lymphocytes resistant to the immunosuppressive drug tacrolimus (FK506). *Blood* 2009;114:4784-91.
36. Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood* 2011;117:1250-9.
37. Kurioka A, Walker LJ, Klenerman P, Willberg CB. MAIT cells: new guardians of the liver. *Clin Transl Immunol* 2016;5:e98.
38. Turtle CJ, Swanson HM, Fujii N, Estey EH, Riddell SR. A distinct subset of self-renewing human memory CD8+ T cells survives cytotoxic chemotherapy. *Immunity* 2009;31:834-44.

Review

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Recent advances regarding tumor microenvironment and immunotherapy in hepatocellular carcinoma

Wei Qin, Zhen-Yu Cao, Si-Yuan Liu, Xun-Di Xu

Hunan Provincial Key Laboratory of Hepatobiliary Disease Research, Division of Hepatobiliary & Pancreatic Surgery, Department of Surgery, The Second Xiangya Hospital, Central South University, Changsha 410011, Hunan, China.

Correspondence to: Dr. Xun-Di Xu, Hunan Provincial Key Laboratory of Hepatobiliary Disease Research, Division of Hepatobiliary & Pancreatic Surgery, Department of Surgery, The Second Xiangya Hospital, Central South University, Changsha 410011, Hunan, China. E-mail: xuxundi@csu.edu.cn.

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors of the liver, with poor prognosis and high mortality. Traditional treatments for patients with HCC have shown poor efficacy especially for advanced liver cancer. Compared with other organs, the liver has more natural immune cells such as Kupffer cells, natural killer cells and natural killer T cells. Immunotherapy for liver cancer has become the focus in current research. The theoretical basis of immunotherapy rests on immune tolerance and suppression in the tumor microenvironment. Common immunotherapy methods include vaccines, cytokines, adoptive cell therapies, immune checkpoint inhibitors, and oncolytic viruses. Compared with traditional treatment, immunotherapy can enhance the body's immune function, delay tumor progression, and prolong survival. This article reviews the HCC microenvironment and immunotherapy both in the clinical and basic research aspects.

Keywords: Immunotherapy, hepatocellular carcinoma, microenvironment, immune

INTRODUCTION

Epidemiological data indicate that hepatocellular carcinoma (HCC) ranks fifth among malignancies, but it is the third most common cause of cancer-related death in the world. One of the major causes of HCC is persistent infection with hepatitis B virus (HBV) or hepatitis C virus (HCV). Aflatoxin exposure is also a crucial risk factor. In addition, excessive drinking, smoking, obesity, genetic factors and dietary habits are also important factors that promote the occurrence of HCC^[1].



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With the development of surgical techniques, HCC may be cured by surgical resection or liver transplantation in the early stage. In addition, some early malignancies may be cured by prompt local treatment such as radiofrequency ablation (RFA) or percutaneous ethanol injection treatment. Unfortunately, due to the concealed and rapid progress of HCC, most patients have lost the chances of best treatment by the initial visit. Only a fraction of patients with HCC have the opportunity for these treatments. As for the patients who are not suitable for surgery or RFA, stereotactic body radiation therapy, portal vein embolization and other liver-directed therapies have become the standard treatment means^[2]. In recent years, with the great scientific and technological advances, systemic therapy has been adopted by most hepatobiliary surgeons under conditions of appropriate liver function and good physical condition.

In the past several decades, many genetic alterations in HCC have been confirmed by previous mechanism studies, such as aberrant activation of oncogenes and the inactivation of anti-oncogenes. Apart from gene mutations, changes in epigenetics such as chromatin modification, DNA methylation, gene recombination, histone modification, RNA interference and copy number variation have been proven to play a critical part in the development of primary liver cancer^[3].

The tumor microenvironment plays a vital role in tumorigenesis and cancer development and metastasis, which can be divided into an immune microenvironment characterized by immune cells and non-immune microenvironment characterized by fibroblasts. The majority of immune cells of the tumor microenvironment include T lymphocytes, B lymphocytes, macrophages, natural killer (NK) cells and antigen-presenting cells (APCs)^[4]. In the microenvironment of the liver tumor, the composition and proportion of immune cells play vital roles in the progress of tumorigenesis [Figure 1]. Compared to other organs, the liver is richer in immune cells, including natural killer T cells (NKT cells), Kupffer cells and NK cells^[5]. Moreover, inactivation of immunosuppressive cytokines (such as IL-4, -5, -8, -10, *etc.*) and immune activating cytokines (such as TNF, IL-1, *etc.*) tends to produce an immunosuppressive environment. Constant exposure to antigen at higher concentrations from the gastrointestinal tract induces the liver to develop intrinsic immune tolerance and immune evasion to ward off autoimmune injury^[6]. Since intrinsic immune tolerance and immune escape are often connected with HCC tumorigenesis, a growing number of studies point to immunotherapies (such as vaccines, adoptive cell therapies, immune checkpoint blockade, cytokines, *etc.*) targeting the microenvironment as a new strategy against hepatic cancer to bring new hope to patients with HCC. In this article, we reviewed the important role of the microenvironment in HCC tumorigenesis and the recent advances of immunotherapy in HCC.

ROLE OF IMMUNE CELLS IN LIVER TUMOR MICROENVIRONMENT

The tumor microenvironment contains a series of immune cells including NK cells, dendritic cells (DCs), macrophages, regulatory T cells (Tregs), neutrophils, T cells and eosinophils [Figure 1]. The function and number of immune cells both matter in the liver immune microenvironment, and related changes are frequently observed in the development of HCC. Multiple immune cells will promote tumor occurrence and development by activating or inhibiting various complex signaling pathways.

Tumor-associated macrophages

Previous works have established that there are monocyte-derived macrophages divided into M1 and M2 phenotypes^[7]. Tumor-associated macrophages (TAMs) are a significant component of the liver microenvironment, being very important to tumor development and thus resulting in the poor outcome of HCC patients. Preclinical studies have shown that TAMs suppress the immune system and promote tumor progression through the expression of chemokines and cytokines^[8,9]. It has been reported that CCL17, CCL18 and CCL22 can block the activation of cytotoxic T cells by attracting Tregs to tumor sites^[10-12]. TAMs interact with bone marrow-derived suppressor cells (MDSCs) to lower major histocompatibility complex II (MHCII), IL-6 and IL-12 levels and increase IL-10 production^[13]. IL-10 produced by TAMs increases the

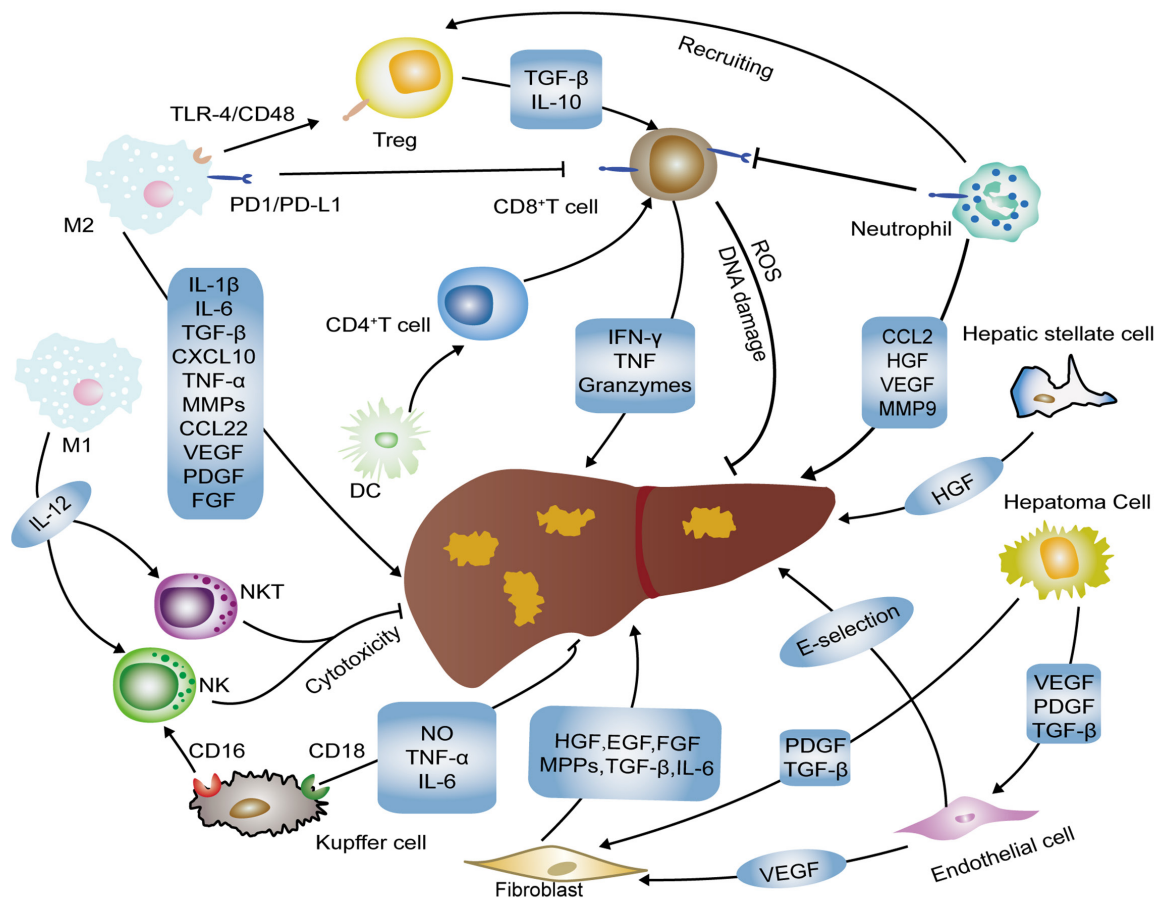


Figure 1. HCC microenvironment components and linkage. A variety of immune-related cells in the tumor microenvironment can promote or inhibit the development of hepatocellular carcinoma through various mechanisms. HCC: hepatocellular carcinoma; VEGF: vascular endothelial growth factor; NK: natural killer; DC: dendritic cell; PDGF: platelet-derived growth factor; TGF: transforming growth factor; HGF: hepatocyte growth factor; EGF: epidermal growth factor; FGF: fibroblast growth factor; MMPs: matrix metalloproteinases; NO: nitric oxide; TNF: tumor necrosis factor; NKT: natural killer T cells; ROS: reactive oxygen species

expression of FoxP3⁺ Tregs and then blocks CD4⁺ CD25⁻ T cell expression, at least promoting the progression of HCC^[14].

The chemotactic migration and selective activation functions of TAMs are achieved by osteopontin expressed by HCC cells through the CSF1-CSF1R pathway^[15]. Many studies indicate that TAMs release many important cytokines, including TNF-α, IL-6, IL-1β, and IL-23 and that they promote the expansion of Th17 cells, which protects against antitumor immunity by activating several markers^[16,17]. In addition, TAMs are able to induce angiogenesis by producing angiogenic factors and matrix metalloproteinases^[18]. Recently, more studies have determined that autophagy plays a key role in the functional control and immunosuppression of TAMs^[19].

Tumor-associated neutrophils

Similar to macrophages, tumor-associated neutrophils (TANs) have different effects on the biological behavior of tumors due to different degrees of polarization^[20]. Recent evidence suggests that TANs can be classified into N1 (suppress tumor development) and N2 (promote tumor development) phenotypes on the basis of TGF-β expression^[19,20]. Some cytokines such as type I interferons and TGF-β can modulate the activity of TANs^[21]. Studies have confirmed that the impact of neutrophils on tumor development is regulated by CD8⁺ T cells^[22].

TANs are one of the key factors for HCC progression and poor prognosis, and neutrophil-lymphocyte ratio closely correlates with tumor progression, which is a significant independent factor predicting survival after hepatectomy in patients with liver cancer^[23,24]. In peripheral blood, the highest levels of the cytokines CCL17 and CCL2 are produced by peripheral blood neutrophils activated by HCC cells and TANs. The newest discovery shows that TANs mediate the intratumoral infiltration of TAMs and regulatory T cells by overproducing CCL2 and CCL17, which then contributes to HCC progression and metastasis^[25]. Zhou *et al.*^[25] collected and analyzed HCC patients' clinical and pathological data, and the results showed that the number of CCL2⁺ or CCL17⁺ TANs was related to tumor progression and differentiation. Moreover, these authors also found that TANs triggered the secretion of microRNA 301b-3p by releasing bone morphogenic protein 2 and transforming growth factor beta 2 (TGFβ2) in HCC cells, and then increased the expression of HCC stem cell-like cells by inhibiting the expression of the limbic system-associated membrane protein and CYLD lysine 63 deubiquitinase genes. From this research, a positive feedback loop governing cancer stem-like cells and TANs in HCC were identified^[26]. TANs play a significant role in immunosuppression in HCC, unfortunately, the exact underlying mechanisms between TANs and other molecules in HCC are not quite clear.

Innate lymphocytes

The liver is a non-lymphoid organ although possessing anti-tumor capabilities, due to containing large populations of natural lymphocytes including NK cells, NKT cells, *etc.*^[27-29]. The activation of NK cells rely on a cascade of multiple activated and inactivated receptors^[30]. In the oxygen-deficient liver environment, the function of NK cells will be impaired due to hypoxia-inducible factor 1α (HIF-1α), which induces changes in MHC class I polypeptide-related sequence A (MICA)^[31]. HIF-1α is important for regulates metabolism, cell proliferation and apoptosis of in HCC microenvironment^[31,32]. In the presence of HIF-1α, proangiogenic genes including vascular endothelial growth factor (VEGF) gene are frequently overexpressed in immune cells^[33].

It is reported that α-fetoprotein (AFP) can directly influence the functions of NK cells. Short exposure of NK cells to AFP can promote the IL-2 hyperresponsive phenotype and contribute to the release of IL-6, IL-1β and TNF-α, which is related to a low recurrence rate and better overall survival of HCC patients with HBV^[34,35]. Moreover, NK cell functions can be impaired via TGF-β1, IL-8 and IL-10 released by Tregs^[36].

Tregs

Tregs belong to the subset of CD4⁺ T cells, which do not merely suppress autoimmune response, but also impair the immune response against tumors^[37,38]. Gao *et al.*^[39] demonstrated that Tregs represent an independent predictor of HCC recurrence and survival, and is related to invasiveness and intratumoral homeostasis. They also found that the combination of Treg removal and simultaneous stimulation of effector T cells was an effective immunotherapy to decrease recurrence and prolong postoperative survival. CD4⁺ CD25⁺ Tregs were used to contribute to tumor prevention in HCC patients through various contact-dependent and contact-free mechanisms. Fu *et al.*^[40] further demonstrated that CD4⁺, CD25⁺ and FoxP3⁺ Tregs impaired the function of CD8⁺ T cells effector and that the number of circulating Tregs correlated with progression in HCC patients. In addition, another study showed that that compared with normal tissues, where CD4⁺ CD25⁺ T cells were significantly increased in the area around the tumor^[41].

CD8⁺ cytotoxic T lymphocytes

CD8⁺ T cells are critical for pathogen clearance, where they contribute to the resolution of HBV and HCV infections in the liver^[42,43]. Guo *et al.*^[42] study confirmed that Fas/FasL interactions might suppress antitumor immune responses via turnover of CD8⁺ T cells. In addition, a series of immunoregulatory elements such as IL-10, IL-2, VEGF and indoleamine-2,3-dioxygenase (IDO) play an important role in inhibiting tumor-associated antigen (TAA)-specific CD8⁺ T cell responses^[42,44,45]. It has been proven that CD14⁺ DCs inhibit

the *in vitro* response of CD8⁺ T cells by affecting IL-10 and IDO^[46]. Furthermore, CD4 helper T cells are substantially important in generating functional memory CD8 T cells by inducing costimulatory molecules and promoting the expression of extracellular cytokines in DCs^[47,48].

DCs

NK cells are another participant in innate immunity, capable of initiating T cells against TAAs that are specialized APCs involved in HCC progression^[49].

In HCC, DCs can produce chemotactic cytokines, and take the initial T cells as the point of action to promote their aggregation and proliferation^[50]. Moreover, DCs can promote the maturation of B lymphocytes, enhance the immune response mediated by antitumor antibodies, and suppress immune escape^[51]. The available evidence suggests that DC maturation disorder, function decrease and reduction in peripheral blood are often observed in the HCC microenvironment, often leading to tumor development^[52,53].

In HCC, DC function is inhibited due to the production of some factors (IL-10, IL-6, *etc.*) in the tumor microenvironment^[54]. Beckebaum *et al.*^[55] confirmed that IL-10 has a considerable immunosuppressive effect on circulating DCs in HCC patients. In addition, previous studies have shown that DCs can promote immunosuppression by producing IL-10 and IDO in HCC, but that depends on the expression of CTLA4^[46].

In general, in recent years, DCs have been widely used as new vaccines in the treatment of solid tumors such as liver cancer, prostate cancer, kidney cancer, melanoma, *etc.*^[56,57].

MDSCs

Myeloid-derived suppressor cells MDSCs comprise a mixture of macrophages, monocytes, granulocytes and DCs, which are a heterogeneous population of immature myeloid cells^[58]. Some previous investigations revealed that MDSCs participate in immunosuppressive networks and are potential immunotherapy targets for HCC^[59-62]. A study by Zhou *et al.*^[63] showed that immunosuppressed CD11b⁺ CD33⁺ HLA-DR⁻ MDSCs were stimulated and amplified by hepatic CCRK by increasing IL-6 expression in human peripheral blood mononuclear cells. In addition, they also found that tumor-infiltrating CD11b⁺ CD33⁺ HLA-DR⁻ MDSCs potently inhibited autologous CD8⁺ T cell proliferation in HCC patients. MDSCs express galectin-9 and bind to TIM-3 on T cells to induce T cell apoptosis^[64].

In HCC patients, MDSC can also impair function of NK cells by inhibiting their cytotoxicity and cytokine release^[65]. Furthermore, Hu *et al.*^[66] showed that MDSCs can inhibit the production of IL-12 induced by TLR-ligand via the expression of IL-10 and can inhibit T lymphocyte activity. In summary, MDSCs play a variety of immunosuppressive roles in HCC.

Given that MDSCs play a variety of immunosuppressive roles in HCC, the immunotherapy targeted MDSC has become a research hotspot. Some previous research has shown that the combination of radiotherapy and IL-12 (RT/IL-12) may reduce accumulation of tumor-infiltrating MDSCs and reverse the intratumoral immunotolerant state and improve the immune level in HCC^[67]. In addition, targeting MDSCs and combining anti-PD-1/PD-L1 can synergistically enhance the cure of HCC^[63,68].

Hepatic stellate cells, endothelial cells and kupffer cells

In addition to that described above, hepatic stellate cells (HSC) are an important element in the microenvironment of liver tumor. Previous research has shown that HSC can secrete hepatocyte growth factor/cytokines, which lead to decreased antitumor immunity function^[69]. In addition, hepatocyte growth factor (HGF) secreted by activated HSC promotes the invasiveness and tumorigenicity of HCC^[70,71].

Compared with normal tissues, the endothelial cells of HCC are significantly different in molecular and functional aspects. Endothelial cells are involved in tumor neovasculature and play a significant role in

Table 1. Major immunotherapeutic approaches for HCC

Approach	Subject	Agent
Vaccines	Antigen peptide	AFP, GPC3, SSX-2, Htert
	Dendritic cells	Tumor antigens on DCs and tumor lysate on DCs
	HCC cells	HCC cells or lysates
	Oncolytic virus	CVV, JX-594, GLV-1h68 and G47delta
Checkpoint inhibitors	CTLA-4	Tremelimumab, tremelimumab with TACE or RFA
	PD-1	Nivolumab, pembrolizumab and pidilizumab
	PD-L1	Atezolizumab
Adoptive cells	CAR-T	GPC3, GPC3 and ASGR1
	TILs	
	NK cells	NK with K562-mb15-41BBL, sorafenib and NKG2D
	CIK	CD3 ⁺ /CD56 ⁺ T cells, CIK combination with TACE + RFA

HCC: hepatocellular carcinoma; CIK: cytokine induced killer; NK: natural killer; CAR-T: chimeric antigen receptor T cell; CTLA-4: cytotoxic T lymphocyte-associated antigen-4; CVV: cowpox virus; RFA: radiofrequency ablation; TILs: tumor-infiltrating lymphocytes; DCs: dendritic cells; TACE: transcatheter arterial chemoembolization

malignant invasion and metastasis. Previous studies have shown that various angiogenic receptors are expressed in endothelial cells, such as epidermal growth factor homology domains-2 (Tie-2), VEGF receptors, platelet-derived growth factor receptor, epidermal growth factor receptor and C-X-C chemokine receptors, which promote the formation of new blood vessels^[72,73]. Liver sinus endothelial cells are special endothelial cells that can collect sample portal venous blood and act as APCs to cross-prime T cells^[74]. In addition, the synergistic induction of tumor-derived VEGF-A and prostaglandin E2 (PGE2) can reduce antitumor immune response through excessive turnover of CD8⁺ T cells^[45].

Kupffer cells are hepatocyte macrophages that form the first line of defense against pathogens and promote local tolerance^[75]. When stimulated by inflammatory cytokines, Kupffer cells are also stimulated (TNF- α , IL-1, PDGF) to produce excess osteopontin, which plays an important part in different cellular signaling pathways, promoting inflammation, tumor invasion and metastasis^[76]. Kupffer cells produce large amounts of IL-6 in response to hepatocyte death, which contributes to compensatory proliferation of hepatocytes^[77]. In addition, IL-10 inhibits the expression of Kupffer cell-derived inflammatory TNF- α , and works in concert with NO to reduce liver inflammation^[78].

CURRENT STAGES OF IMMUNOTHERAPY

In view of the important role of the immune microenvironment in liver tumors, immunotherapy for liver cancer has become the focus in current research. According to the present study, immunotherapeutic approaches are divided into vaccines, cytokines, adoptive cell therapies, immune checkpoint inhibitors, and oncolytic viruses [Table 1].

Vaccines in HCC management

The essence of cancer vaccination is the body's own immune system activated by an antigenic substance and then attacks the tumor. With the progress of immunology and technology, tumor vaccine therapy has become the frontier in tumor treatment research. Recently, some studies showed that HCC vaccines mainly include vaccines based on antigen peptides, DCs, cancer cells and DNA.

Antigen peptide vaccines

Antigen peptide vaccines are accurate targets for HCC and are based on TAAs. Recently, the most frequently reported peptide vaccines have been AFP peptide vaccines^[79,80]. AFP is a glycoprotein belonging to the albumin family, and is normally expressed in abundance in fetal blood while aberrantly expressed on the surface of HCC cells. However, due to the immune tolerance of the liver, the immune response to AFP is limited^[81]. It is difficult for AFP to produce an effective immune response when it is synthesized in the

liver. Some clinical trials based on AFP have been initiated^[82]. Zhang *et al.*^[82] showed that cellular immune responses may be responsible for the antitumor activity against AFP-positive tumor cells in the mouse HCC model. Unfortunately, the AFP peptide vaccine also has certain limitations, where it only targets the AFP-specific immune response.

Carcinoembryonic antigen glypican-3 (GPC3) is another important antigen that targets liver cancer^[83]. In 2012, a phase I clinical trial was been initiated. Thirty-three advanced HCC patients were injected with GPC3 peptide, and nineteen HCC patients showed stable disease after 2 months treatment. The study results showed that the GPC3-derived peptide vaccination not only has good immune tolerance, but also has a clear immune response and antitumor efficacy^[84].

Studies indicate that human telomerase reverse transcriptase (hTERT) is a catalytic enzyme necessary for telomere extension^[85,86]. Mizukoshi *et al.*^[87] showed that hTERT is an important target for T cell-based immunotherapy in HCC. Another study found that 71.4% of liver cancer patients acquired TERT-specific immunity and 57.1% of patients had no HCC recurrence after vaccination with hTERT₄₆₁ peptide-specific T cells^[88]. SSX-2, a cancer-testis antigen, has been shown to be overexpressed in HCC patients. Recent evidence suggestw that a large number of SSX-2- and MAGE-A-specific CD8⁺ T cells can be found in HCC^[89].

DCs

DCs are the immune cells with the strongest antigen-presenting ability in the human immune system. Some cytokines such as recombinant human interleukin 4 (rhIL-4) and recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) can activate the function of DCs, and DCs can be sensitized via the lysis of liver cancer cells. Some research has shown that immature DCs do not cause serious immune response but suppress CD8⁺ T cell immunity^[90]. CD8⁺ T cells and polarized CD4⁺ T cells are induced by mature DCs. Therefore, mature DCs should be selected as the tumor vaccine and their maturity should be evaluated^[91]. In the body's immune system, the CD4⁺ T/CD8⁺ T ratio is used to evaluate the antitumor immunity. Encouragingly, the proportion of CD4⁺ T/CD8⁺ T has been shown to be significantly increased after DC-based immunotherapy. In addition, the latest study demonstrated that the survival rate and survival time of HCC patients were increased after DC-based immunotherapy when analyzing 1276 cases in 19 clinical trials^[92]. In addition, DC-derived exosomes are a novel class of vaccines for cancer immunotherapy. AFP-rich derived exosomes can elicit a strong antigen-specific antitumor immune response, providing a cell-free vaccine option for HCC immunotherapy^[93].

However, there are many challenges in immunotherapy with DC vaccines. To resolve the problems better, some researchers combine DC-based vaccines with checkpoint inhibitors to improve efficacy. Wilgenhof *et al.*^[94] combined DC vaccines with an immune checkpoint cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitor for advanced melanoma in phase II clinical studies. The experimental results showed that the combination of tumor vaccine and immune checkpoint inhibitor has significant advantages over single drug treatment. Furthermore, a phase I/IIa clinical study achieved encouraging results showing that the adjuvant DC vaccine for HCC was safe and well tolerated^[95].

HCC cell vaccines

HCC cells or lysates that are physically or chemically disposed to eliminate pathogenicity could be used as immunogens for tumor-specific immune responses. Nemunaitis *et al.*^[96] conducted a phase I trial of cellular immunotherapy using autologous whole-cell tumors (FANGTM). They observed that FANG manufacturing was successful in 7 of 8 attempts in this study. However, HCC vaccines are still in the early stage of clinical research and more studies are needed to prove their efficacy.

Oncolytic virus vaccines

The basic principle of antitumor oncolytic viruses is to expand them inside cancer cells and lyse them, eventually killing the cancer cells, and can selectively replicate and lyse in tumor cells without damaging

Table 2. Representative clinical trials of immune checkpoints inhibitor in hepatocellular carcinoma

Antibody features	Antibody name	Enrollment	NCT number	Status
CTLA-4	Tremelimumab	433	NCT02519348	Ongoing
		20	NCT01008358	Completed
PD1	Nivolumab	530	NCT03383458	Recruiting
		1097	NCT01658878	Completed
		1723	NCT02576509	Ongoing
		450	NCT03062358	Ongoing
	Pembrolizumab	150	NCT02702414	Ongoing
		60	NCT03163992	Recruiting
		30	NCT03419481	Recruiting
		60	NCT02595866	Recruiting
		228	NCT03419897	Ongoing
		674	NCT03412773	Ongoing
PD-L1	Durvalumab	1310	NCT03298451	Ongoing
		433	NCT02519348	Ongoing
	Atezolizumab	1732	NCT03170960	Recruiting
		740	NCT03755791	Recruiting
		480	NCT03434379	Recruiting

CTLA-4: cytotoxic T lymphocyte-associated antigen-4; PD-L1: programmed cell death-ligand 1; PD1: programmed cell death-1

normal tissue. Pexa-vec (JX-594) is an oncolytic vaccinia virus that selectively replicates in tumor cells that overexpress thymidine kinase, thereby ensuring that the virus specifically infects HCC cells, avoiding damage to normal cells. Moreover, the combined application of JX-594 and nivolumab in the treatment of liver cancer is also being evaluated (NCT03071094). Unfortunately, the phase III trial of Pexa-Vec/Nexavar combined therapy for liver cancer failed to show efficacy (NCT02562755)^[97]. Moreover, the combined application of JX-594 and nivolumab in the treatment of liver cancer is also being evaluated (NCT03071094)^[98].

The efficacy of cowpox virus (CVV) with an evolutionary tendency for cancer was studied in an animal model of metastatic HCC. The results showed that CVV may be a promising virus against metastatic HCC^[99]. In addition, GLV-1h68 and G47delta have also been used for the treatment of HCC^[100,101].

Twumasi-Boateng *et al.*^[102] commented, “As key control points in the antitumor response continue to be deciphered, OV s will provide an increasingly important platform for bioengineers to re-wire antitumor immunity”.

Immune checkpoint inhibitors in HCC management

Previous studies confirmed that immune checkpoints are frequently activated in tumor tissues compared with normal tissues and contribute to evasion of immune surveillance by tumor cells to^[103]. Tumor-associated T cells are reactivated by immune checkpoint inhibitors, and their antitumor function is increased. At present, the immune checkpoints PD-1, CTLA-4, TIM-3, VISTA and LAG-3 are most studied^[104-109]. Among them, inhibitors of PD-1 and CTLA-4 have been approved for treating melanomas by the FDA. In addition, in the treatment of HCC, great progress has been made in recent years [Table 2].

CTLA-4 inhibitors

CTLA-4 is a transmembrane receptor for T cells, and its expression is closely related to T cell activation. CTLA-4 has a higher affinity with CD80 and CD86 compared with CD28. CTLA-4 can suppress the activation of T cell by competitive antagonism of CD28 binding to CD80 and CD86^[110-112]. Blocking the binding of CTLA-4 to its ligands can stimulate the activation and proliferation of immune cells to induce or enhance the immune response.

Tremelimumab is a monoclonal antibody that targets CTLA4. In 2013, a clinical trial of tremelimumab enrolled 21 patients with chronic hepatitis C with Child-Pugh A or B cirrhosis and advanced HCC^[105]. In

this study, the HCC patients were administered tremelimumab intravenously at 15 mg/kg every 90 days until tumor development or severe toxicity. The results showed a partial response rate of 17.6% and disease control rate of 76.4%, and 45% of the patients had stable disease for more than 6 months after treatment with tremelimumab^[105]. In a phase I/II trial, tremelimumab combined with TACE or RFA were used to treat HCC. Thirty-two patients with HCC were enrolled, patients received different doses of tremelimumab (3.5 and 10 mg/kg i.v.) every 4 weeks for 6 doses and infusion for the 3 months until non-treatment criteria were reached. Subtotal RFA or chemical ablation was performed after tremelimumab. The results showed that tremelimumab combined with RFA can result in the accumulation of intratumoral CD8⁺ T cells, and dose-limiting toxicities were not observed^[113].

PD-1 and PD-L1 inhibitors

Some investigators have found that DCs, NK cells, B cells and mononuclear cells often show increased expression of PD-1^[114]. PD-1, a member of the CD28 superfamily plays a key role in delivering co-inhibitory signals to TCR receptors^[115]. Receptor binding to PD-L1 and PD-L2 were blocked by PD-1 inhibitors, resulting in T cells exerting normal efficacy against tumors^[116]. In cancer cells, PD-L1/PD-1 signals are activated by PD-L1 or PD-L2, allowing them to evade immune surveillance^[117].

Nivolumab, a monoclonal antibody targeting PD-1, has been investigated for HCC treatment. Recently, a phase 1/2 study in patients with HCC was used to assess the safety and efficacy of nivolumab in treating HCC. A total of 212 patients received nivolumab, 3 mg/kg every 2 weeks. The statistical results showed that the objective response rate was 16%, while disease control rate was 68%, and the 6-month survival rate was 82.5%^[118]. In advanced HCC patients, the safety and effectiveness of nivolumab were also assessed. In the 49 advanced HCC patients involved in a clinical trial, an objective response rate of 10% and disease control rate of 55% were seen after treatment with nivolumab for 7 months^[119]. Therefore, nivolumab shows good safety for patients with advanced HCC and has the potential to treat patients with advanced HCC. In view of those results, nivolumab was approved for the treatment of HCC patients with poor treatment after sorafenib.

Pembrolizumab is an IgG4 monoclonal antibody that targets the PD-1 receptor. In a phase II trial (KEYNOTE-224), 104 advanced HCC patients were enrolled, who were given 200 mg pembrolizumab injected every 3 weeks for about 2 years or until inappropriate. The results showed that there was objective response in 18 patients and stable disease rate of 44%, in the 104 patients studied. Statistical analyses indicated that pembrolizumab was also effective in advanced HCC patients treated with sorafenib^[120]. A randomized phase III trial was conducted in 413 patients with advanced HCC^[121]. In this study, improvement of overall survival, progression-free survival (PFS), overall response rate, and duration of response were observed in patients with pembrolizumab compared with the KEYNOTE224 study. The results showed that a good risk-benefit ratio for pembrolizumab in advanced HCC was also supported.

In 2008, 26 HCC patients who could not be resected or who showed metastasis were studied to determine the safety of atezolizumab, which targets PD-L1^[122]. Atezolizumab (1200 mg) and bevacizumab (15 mg/kg) were injected via i.v. every 3 weeks. The results demonstrated a response rate of 62% and that this atezolizumab + bevacizumab combination was safe and well tolerated. The basic finding of this research was that the effects of atezolizumab were enhanced by anti-VEGF therapy. In addition, MEF2D can increase the expression of PD-L1 and inhibit antitumor immunity mediated by CD8⁺ T cells^[123].

Although immune checkpoint inhibitors have shown good efficacy in the treatment of HCC, they have limited efficacy in advanced HCC patients. The combined use of immune checkpoint inhibitors is expected to produce a synergistic effect and achieve better results. Selective clinical trials of combination immunotherapy agents in HCC are described in Table 3. In HCC patients with chronic HCV infection, the amount of CD8⁺ T cells increased, but cell activity remained unchanged after blocking PD-L1/PD-1 or CTLA4. However,

Table 3. Selective clinical trials of combination immunotherapy agents in HCC

Antibody features	Antibody name	Enrollment	NCT number	Status
CTLA-4 and PD1	Nivolumab, ipilimumab	45	NCT03222076	Recruiting
		50	NCT03203304	Recruiting
CTLA-4 + chemoembolization	Tremelimumab, TACE	61	NCT01853618	Completed
CTLA-4 + PD-L1	Durvalumab, remelimumab	433	NCT02519348	Ongoing
CTLA-4 + PD-1 + anti-OX40	CTLA-4, PD-1, INCAGN01949	52	NCT03241173	Completed
CTLA-4 + PD1 + ablative	Durvalumab, tremelimumab, ablative	90	NCT02821754	Recruiting
PD-1 + TIL	TIL, PD-1	332	NCT01174121	Recruiting
PD-1 + neoadjuvant	Nivolumab, cabozantinib	15	NCT03299946	Ongoing
	Pembrolizumab, sorafenib	27	NCT03211416	Recruiting
PD-1 + oncolytic	Nivolumab, Pexa Vec	30	NCT03071094	Ongoing
PD-1 + radiation	Nivolumab, SIRT	40	NCT03380130	Ongoing
PD-1 + TGF- β receptor I kinase inhibitor	Nivolumab, galunisertib	75	NCT02423343	Ongoing
PD-1 + TKI	Nivolumab, lenvatinib	30	NCT03418922	Ongoing
	Pembrolizumab, lenvatinib	104	NCT03006926	Ongoing
	Nivolumab, cabozantinib	15	NCT03299946	Ongoing
	Pembrolizumab, regorafenib	57	NCT03347292	Recruiting
PD-1 + radioembolization	Nivolumab, yttrium-90	35	NCT02837029	Recruiting
	Yttrium-90, nivolumab	40	NCT03380130	Ongoing
		40	NCT03033446	Recruiting
	Pembrolizumab, SBRT	30	NCT03316872	Recruiting
PD-1 + chemotherapy	Pembrolizumab, yttrium-90	30	NCT03099564	Recruiting
	Nivolumab, sorafenib	40	NCT03439891	Recruiting
PD-1 + immunomodulator	Nivolumab, CC-122 (avadoimide)	21	NCT02859324	Ongoing
PD-1 + transarterial chemoembolization	Nivolumab, Ddeb-TACE	14	NCT03143270	Recruiting
PD-1 + anti-CCR4 antibody	Nivolumab, mogamulizumab	114	NCT02705105	Completed
PD-1 + anti-VEGF	Nivolumab, bevacizumab	1	NCT03382886	Terminated
PD-1 + curative resection	Toripalimab, surgery	402	NCT03859128	Recruiting
PD-1 + curative resection or ablation	Pembrolizumab	950	NCT03867084	Recruiting
PD-L1 + small molecule DNA	Durvalumab, guadecitabine (SGI-110)	90	NCT03257761	Recruiting
PD-L1 + anti-VEGFR2	Durvalumab, ramucirumab (LY3009806)	114	NCT02572687	Ongoing
PD-L1 + anti-VEGF	Atezolizumab, bevacizumab	430	NCT02715531	Ongoing
PD-L1 + TKI	Avelumab, regorafenib	362	NCT03475953	Recruiting
	Avelumab, axitinib	22	NCT03289533	Completed

HCC: hepatocellular carcinoma; SIRT: selective internal radiation therapy; VEGF: vascular endothelial growth factor; VEGFR: VEGF receptors; SBRT: stereotactic body radiation therapy; CTLA-4: cytotoxic T lymphocyte-associated antigen-4; TIL: tumor infiltrating lymphocytes; OX40: tumor necrosis factor receptor superfamily member 4; TGF: transforming growth factor; TKI: tyrosine kinase inhibitor; PD-L1: programmed cell death-ligand 1; PD-1: pro-programmed cell death-1; CCR4: CC-chemokine receptor 4; TACE: transcatheter arterial chemoembolization

combined use of CTLA4 and PD-L1 inhibitors can reverse the instability of CD8⁺ T cells and enhance intrahepatic HCV-specific CD8 and CD4 T cell cytokine response. Moreover, in the acute hepatitis C phase, the combination of PD-1/CTLA-4 inhibitors can reverse HCV-specific CD8⁺ T cell dysfunction^[124]. Interestingly, after the clearance of HCV, CD8⁺ T cell proliferative ability can be restored preferentially under the action of immune checkpoint inhibitors^[125]. In addition, Stein *et al.*^[126] combined the PD-L1 monoclonal antibody atezolizumab and bevacizumab to treat patients with advanced HCC (NCT02715531). Moreover, the efficacy of immune checkpoint inhibitors (ipilimumab and nivolumab) combined with liver resection is being evaluated (NCT03682276, NCT03510871)^[98]. As new adjuvant therapies, the curative effect of checkpoint inhibitors is also being evaluated in HCC patients undergoing surgical resection (e.g., NCT03859128 and NCT03847428)^[98].

Although many studies have reported that immune checkpoint inhibitors are showing encouraging results in the treatment of HCC, there are still potentially serious adverse reactions. PD-1 inhibitors block the interaction between PD-1 and PD-L1 and also inhibit T cells and APCs. PD-1/PD-L1 monoclonal antibodies

cannot promote T cells to attack tumor cells^[127,128]. Existing studies suggest that it is only effective for some patients, and a part of patients will get worse after this kind of treatment, so the treatment strategy for advanced HCC also needs to be optimized.

Adoptive cell immunotherapy

Adoptive cell immunotherapy is a special therapy including active specific immunotherapy and passive immunotherapy. In this type of therapy, cancer cells are killed by using patients' own lymphocytes. Chimeric antigen receptor T cell immunotherapy (CAR-T), NK cells, tumor-infiltrating lymphocytes (TILs) and cytokine induced killer (CIK) cells constitute adoptive cell immunotherapy in HCC.

CAR-T

CAR-T cells are a special type of transgenic T lymphocytes that specifically recognize TAAs and improve the targeting of effector T cells and break host immune tolerance^[129]. A previous study confirmed that an intracellular signaling domain, extracellular antigen-binding domain, and an extracellular hinge area constitute the basic structure of CARs^[130]. The intracellular signaling domain and extracellular antigen-binding domain are connected by the extracellular hinge area. It is the hinge area that confers high activity on the extracellular antigen-binding domain. Although CAR-T cell immunity has become a research hotspot in the application of solid tumors, more studies on HCC are still in the basic research stage, and the key point of technology lies in the selection of tumor-specific antigens. GPC3 is located on the cell surface and is a member of the glypican family of heparan sulfate. A previous study reported that GPC3 is a TAA and overexpressed in HCC compared with normal tissues. Gao *et al.*^[131] found that GPC3-positive HCC cells could be lysed by GPC3-targeted CAR-T cells and that the number of lysed tumor cells correlated with the expression of GPC3. Liu *et al.*^[132] recently stated, "The inducibly expressed IL-12-armored GPC3-CAR-T cells could broaden the application of CAR-T-based immunotherapy to patients intolerant of lymphodepletion chemotherapy and might provide an alternative therapeutic strategy for patients with GPC3⁺ cancers".

Asialoglycoprotein receptor 1 (ASGR1) mediates the transport of targeted therapeutic molecules to the liver, specifically expressed on liver parenchymal cells. GPC3 and ASGR1 make a suitable target combination for dual-targeted CAR-T cells. Chen *et al.*^[133] surveyed data and found that the risk of on-target, off-tumor toxicity may be reduced when using T cells with two complementary CARs against GPC3 and ASGR1. Recently, 13 GPC3⁺ HCC patients were enrolled a phase I trial, where all patients who received GPC3 CAR-T treatment had no dose-limiting toxicity and tolerated the treatment well. The study results showed that GPC3 CAR-T treatment was feasible and safe for Chinese patients with GPC3⁺ HCC (NCT02395250)^[134]. With the application of CAR-T cells in the clinic, the safety of CAR-T cells has begun to attract people's attention. MacKay *et al.*^[135] noted, "Immune-cell failure is a major challenge for CAR therapies, and its mechanism remains to be investigated". In addition, immune rejection and off-target effects of CAR-T cells also need to be resolved^[136].

CIK cells

CIK cells are a new type of non-MHC-restricted immunocompetent cells mainly including CD3⁺/CD56⁻ cells, CD3⁻/CD56⁺ NK cells, CD3⁺/CD56⁺ T cells, *etc.*, among which CD3⁺/CD56⁺ T cells are the main effector cells^[137,138]. Preclinical trials have shown that CIK cells have high activity against HCC cells *in vitro*. In addition, Pan *et al.*^[139] investigated the effect of CIK as adjuvant therapy on overall survival and relapse-free survival of HCC patients who received surgical treatment. Survival analysis showed that compared with hepatectomy alone, median overall survival and PFS were clearly prolonged in the hepatectomy/CIK combination group (41, 16 months *vs.* 28, 12 months, respectively). The safety and efficacy of CIK cell immunotherapy were investigated in a phase III clinical trial in which 230 HCC patients treated with RFA, surgical resection or percutaneous ethanol injection were randomly injected with 6.4×10^9 autologous CIK cells (16 times during 60 weeks) or had no immunotherapy. The median time of recurrence-free survival in

the immunotherapy group was clearly longer than control group (44 months *vs.* 30 months). Cancer-related death was also lower in the CIK group than in the no immunotherapy group ($P = 0.02$); 17% of patients experienced adverse reactions related to CIK cytokines, but there was no difference in severe adverse events^[140].

In addition, the efficacy and safety of the combination of CIK cells with liver-directed therapies in HCC were also evaluated. In a study that evaluated CIK cells combined with TACE + RFA in HCC patients, overall efficacy in the TACE + RFA + CIK group was better than in the TACE + RFA group (76.5% *vs.* 79.8%). Kaplan-Meier analysis indicated that the overall survival rate of the TACE + RFA + CIK treatment group was significantly prolonged (56 months *vs.* 31 months, $P = 0.001$)^[141]. In another study, HCC patients with Child-Pugh scores of A or B and without prior treatment were enrolled. One group ($n = 66$) received CIK treatment and standard treatment, while another group ($n = 66$) received standard treatment only. The results showed that overall survival and PFS were significantly prolonged after treatment with CIK cells in patients who were not suitable for surgery^[142]. Furthermore, one study found that targeting MDSCs is a good strategy to enhance the antitumor efficacy of CIKs for the treatment of patients with HCC^[143].

Although more and more studies have shown that CIK cells makes up a significant part of HCC treatment, only a portion of T cells can provide a full antitumor function because of immune escape mechanisms and lack of specific tumor antigens.

TILs

TILs are isolated from tumor tissue and induced by IL-2 *in vitro*. TILs are relatively rare in HCC, but they play an important role in tumor recurrence in patients. A phase I clinical trial indicated that immunotherapy with autologous TILs could be successfully performed with low toxicity^[144]. In another randomized clinical trial, 76 HCC patients were enrolled and the ratio of postoperative recurrence was significantly lower in the group using IL-2 to activate lymphocytes compared to the control group^[145]. Furthermore, the functions of tumor-infiltrating T cells were restored via the antibodies targeting immune checkpoints^[146]. Until now, the widespread use of TIL immunotherapy has been limited, mainly because of difficulties in purification and amplification.

NK cells

NK cells belong to innate immune cells, and the main feature is that they can directly destroy tumor cells without prior stimulation. Preclinical experiments showed that CXCR6 can inhibit hepatocarcinogenesis by promoting natural killer T cell- and CD4⁺ T cell-dependent control of senescence^[147]. Another study also confirmed that expanded activated NK cells are highly cytotoxic to HCC cells. The function of NK cells against HCC were enhanced by the expression of NKG2D-CD3 ζ -DAP10^[148]. Tan *et al.*^[149] reported that LrNK cells were observed to be present in liver cancer, often showing abnormal function. Moreover, this dysfunction is caused by Tim-3-mediated PI3K/mTORC1 interference. However, there is still a lack of clinical trials of NK cells in the treatment of HCC, so more clinical studies are urgently needed.

SUMMARY AND FUTURE EXPECTATIONS OF IMMUNOTHERAPY IN HCC

With the development of molecular biotechnology and tumor immunology, immunotherapy has been an important part in the mode of combined therapy of tumors. Along with the gradually deepened study of molecular biology and molecular immunology, T cells have an important influence on tumor immunity in the hepatic microenvironment. Recently, there have been a large number of studies on HCC immunotherapy, and some of them have achieved important positive results. At present, many preclinical studies demonstrate that vaccine therapy, adoptive cell therapy and immune checkpoint inhibitors make up a significant part in inhibiting the growth and development of HCC. Moreover, more clinical trials of immunotherapy for liver cancer are being conducted. In early clinical trials, tremelimumab, nivolumab, pembrolizumab and

atezolizumab are targeting PD-1/PD-L1 or CTLA-4, and there have been encouraging results for a variety of tumors. However, phase III clinical research is still lacking, and more clinical research is urgently needed.

In addition, the results of the present study indicate that the effect of single immunotherapy on liver cancer remains limited. The indication of immunotherapy in HCC may depend on combination therapies. Combination of liver-targeted therapy and immunotherapy can enhance tumor and systemic immune response. In conclusion, the advent of immunotherapy revolutionized cancer therapy and brought treatment for HCC to a brand-new period.

DECLARATIONS

Authors' contributions

Study concept and design: Xu XD

Literature search: Qin W

Drafting of the manuscript: Qin W, Cao ZY, Liu SY

Critical revision of the manuscript for important intellectual content: Xu XD

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al.; Global Burden of Disease Liver Cancer Collaboration. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. *JAMA Oncol* 2017;3:1683-91.
2. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018;391:1301-14.
3. Fujimoto A, Totoki Y, Abe T, Borojevich KA, Hosoda F, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. *Nat Genet* 2012;44:760-4.
4. Hilmi M, Vienot A. Immune therapy for liver cancers. *Cancers (Basel)* 2019;12:77.
5. Gao B, Jeong WI, Tian Z. Liver: an organ with predominant innate immunity. *Hepatology* 2008;47:729-36.
6. Shalpour S, Lin XJ, Bastian IN, Brain J, Burt AD, et al. Inflammation-induced IgA⁺ cells dismantle anti-liver cancer immunity. *Nature* 2017;551:340-5.
7. Movahedi K, Laoui D, Gysemans C, Baeten M, Stange G, et al. Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. *Cancer Res* 2010;70:5728-39.
8. Mantovani A, Allavena P. The interaction of anticancer therapies with tumor-associated macrophages. *J Exp Med* 2015;212:435-45.
9. Ye YC, Zhao JL, Lu YT, Gao CC, Yang Y, et al. NOTCH signaling via WNT regulates the proliferation of alternative, CCR2-independent tumor-associated macrophages in hepatocellular carcinoma. *Cancer Res* 2019;79:4160-72.
10. Zhu F, Li X, Chen S, Zeng Q, Zhao Y, et al. Tumor-associated macrophage or chemokine ligand CCL17 positively regulates the tumorigenesis of hepatocellular carcinoma. *Med Oncol* 2016;33:17.
11. Zhou SL, Zhou ZJ, Hu ZQ, Huang XW, Wang, Z, et al. Tumor-associated neutrophils recruit macrophages and T-regulatory cells to

- promote progression of hepatocellular carcinoma and resistance to sorafenib. *Gastroenterology* 2016;150:1646-58.e17.
12. Li ZQ, Wang HY, Zeng QL, Yan JY, Hu YS, et al. p65/miR-23a/CCL22 axis regulated regulatory T cells recruitment in hepatitis B virus positive hepatocellular carcinoma. *Cancer Med* 2020;9:711-23.
13. Zhou J, Ding T, Pan W, Zhu LY, Li L, et al. Increased intratumoral regulatory T cells are related to intratumoral macrophages and poor prognosis in hepatocellular carcinoma patients. *Int J Cancer* 2009;125:1640-8.
14. Sharma S, Khosla R, David P, Rastogi A, Vyas A, et al. CD4⁺CD25⁺CD127(low) regulatory T cells play predominant anti-tumor suppressive role in hepatitis B virus-associated hepatocellular carcinoma. *Front Immunol* 2015;6:49.
15. Zhu Y, Yang J, Xu D, Gao XM, Zhang Z, et al. Disruption of tumour-associated macrophage trafficking by the osteopontin-induced colony-stimulating factor-1 signalling sensitises hepatocellular carcinoma to anti-PD-L1 blockade. *Gut* 2019;68:1653-66.
16. Kuang DM, Peng C, Zhao Q, Wu Y, Chen MS, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma promote expansion of memory T helper 17 cells. *Hepatology* 2010;51:154-64.
17. Fu Y, Liu S, Zeng S, Shen H. From bench to bed: the tumor immune microenvironment and current immunotherapeutic strategies for hepatocellular carcinoma. *J Exp Clin Cancer Res* 2019;38:396.
18. Darvishi B, Majidzadeh AK, Ghadirian R, Mosayebzadeh M, Farahmand L. Recruited bone marrow derived cells, local stromal cells and IL-17 at the front line of resistance development to anti-VEGF targeted therapies. *Life Sci* 2019;217:34-40.
19. Chang CP, Su YC, Lee PH, Lei HY. Targeting NFKB by autophagy to polarize hepatoma-associated macrophage differentiation. *Autophagy* 2013;9:619-21.
20. Nicolas-Avila JA, Adrover JM, Hidalgo A. Neutrophils in homeostasis, immunity, and cancer. *Immunity* 2017;46:15-28.
21. Andzinski L, Kasnitz N, Stahnke S, Wu CF, Gereke M, et al. Type I IFNs induce anti-tumor polarization of tumor associated neutrophils in mice and human. *Int J Cancer* 2016;138:1982-93.
22. Michaeli J, Shaul ME, Mishalian I, Hovav AH, Levy L. Tumor-associated neutrophils induce apoptosis of non-activated CD8 T-cells in a TNFalpha and NO-dependent mechanism, promoting a tumor-supportive environment. *Oncoimmunology* 2017;6:e1356965.
23. Margetts J, Ogle LF, Chan SL, Chan AWH, Chan KCA, et al. Neutrophils: driving progression and poor prognosis in hepatocellular carcinoma? *Br J Cancer* 2018;118:248-57.
24. Mano Y, Shirabe K, Yamashita Y, Harimoto N, Tsujita E, et al. Preoperative neutrophil-to-lymphocyte ratio is a predictor of survival after hepatectomy for hepatocellular carcinoma: a retrospective analysis. *Ann Surg* 2013;258:301-5.
25. Zhou SL, Zhou ZJ, Hu ZQ, Huang XW, Wang Z, et al. Tumor-associated neutrophils recruit macrophages and T-regulatory cells to promote progression of hepatocellular carcinoma and resistance to sorafenib. *Gastroenterology* 2016;150:1646-58.e17.
26. Zhou SL, Yin D, Hu ZQ, Luo CB, Zhou ZJ, et al. A positive feedback loop between cancer stem-like cells and tumor-associated neutrophils controls hepatocellular carcinoma progression. *Hepatology* 2019;70:1214-30.
27. Robinson MW, Harmon C, O'Farrelly C. Liver immunology and its role in inflammation and homeostasis. *Cell Mol Immunol* 2016;13:267-76.
28. Sundstrom P, Szeponik L, Ahlmanner F, Sundquist M, Wong JSB, et al. Tumor-infiltrating mucosal-associated invariant T (MAIT) cells retain expression of cytotoxic effector molecules. *Oncotarget* 2019;10:2810-23.
29. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* 2013;14:1014-22.
30. Parodi M, Raggi F, Cangelosi D, Manzini C, Balsamo M, et al. Hypoxia modifies the transcriptome of human NK cells, modulates their immunoregulatory profile, and influences NK cell subset migration. *Front Immunol* 2018;9:2358.
31. Sheppard S, Ferry A, Guedes J, Guerra N. The paradoxical role of NKG2D in cancer immunity. *Front Immunol* 2018;9:1808.
32. Chambers AM, Lupo KB, Matosevic S. Tumor microenvironment-induced immunometabolic reprogramming of natural killer cells. *Front Immunol* 2018;9:2517.
33. Dengler VL, Galbraith M, Espinosa JM. Transcriptional regulation by hypoxia inducible factors. *Crit Rev Biochem Mol Biol* 2014;49:1-15.
34. Vujanovic L, Stahl EC, Pardee AD, Geller DA, Tsung A, et al. Tumor-derived alpha-fetoprotein directly drives human natural killer-cell activation and subsequent cell death. *Cancer Immunol Res* 2017;5:493-502.
35. Sun C, Sun H, Zhang C, Tian Z. NK cell receptor imbalance and NK cell dysfunction in HBV infection and hepatocellular carcinoma. *Cell Mol Immunol* 2015;12:292-302.
36. Cai L, Zhang Z, Zhou L, Wang H, Fu J, et al. Functional impairment in circulating and intrahepatic NK cells and relative mechanism in hepatocellular carcinoma patients. *Clin Immunol* 2008;129:428-37.
37. Fan Y, Gao Y, Rao J, Wang K, Zhang F, et al. YAP-1 promotes tregs differentiation in hepatocellular carcinoma by enhancing TGFBR2 transcription. *Cell Physiol Biochem* 2017;41:1189-98.
38. Wu R, Liu C, Deng X, Chen L, Hao S, et al. Enhanced alleviation of aGVHD by TGF-beta1-modified mesenchymal stem cells in mice through shifting MPhi into M2 phenotype and promoting the differentiation of Treg cells. *J Cell Mol Med* 2020;24:1684-99.
39. Gao Q, Qiu SJ, Fan J, Zhou J, Wang XY, et al. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol* 2007;25:2586-93.
40. Fu J, Xu D, Liu Z, Shi M, Zhao P, et al. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology* 2007;132:2328-39.
41. Yang XH, Yamagiwa S, Ichida T, Matsuda Y, Sugahara S, et al. Increase of CD4⁺ CD25⁺ regulatory T-cells in the liver of patients with hepatocellular carcinoma. *J Hepatol* 2006;45:254-62.
42. Guo CL, Yang XH, Cheng W, Xu Y, Li JB, et al. Expression of Fas/FasL in CD8⁺ T and CD3⁺ Foxp3⁺ Treg cells--relationship with apoptosis of circulating CD8⁺ T cells in hepatocellular carcinoma patients. *Asian Pac J Cancer Prev* 2014;15:2613-8.
43. Huang CY, Wang H, Liao W, Han F, Li YQ, et al. Transforming growth factor beta is a poor prognostic factor and inhibits the favorable prognostic value of CD8⁺ CTL in human hepatocellular carcinoma. *J Immunother* 2017;40:175-86.
44. Sun Z, Ren Z, Yang K, Liu Z, Cao S, et al. A next-generation tumor-targeting IL-2 preferentially promotes tumor-infiltrating CD8(+)

- T-cell response and effective tumor control. *Nat Commun* 2019;10:3874.
45. Motz GT, Santoro SP, Wang LP, Garrabrant T, Lastra RR et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nat Med* 2014;20:607-15.
 46. Han Y, Chen Z, Yang Y, Jiang Z, Gu Y, et al. Human CD14⁺ CTLA-4⁺ regulatory dendritic cells suppress T-cell response by cytotoxic T-lymphocyte antigen-4-dependent IL-10 and indoleamine-2,3-dioxygenase production in hepatocellular carcinoma. *Hepatology* 2014;59:567-79.
 47. Shedlock DJ, Shen H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. *Science* 2003;300:337-9.
 48. Borst J, Ahrends T, Babala N, Melief CJM, Kastenmuller W. CD4(+) T cell help in cancer immunology and immunotherapy. *Nat Rev Immunol* 2018;18:635-47.
 49. Benites BD, Alvarez MC, Saad STO. Small particles, big effects: the interplay between exosomes and dendritic cells in antitumor immunity and immunotherapy. *Cells* 2019;8:1648.
 50. Choi YJ, Park SJ, Park YS, Park HS, Yang KM, et al. EpCAM peptide-primed dendritic cell vaccination confers significant anti-tumor immunity in hepatocellular carcinoma cells. *PloS one* 2018;13:e0190638.
 51. Dou L, Ono Y, Chen YF, Thomson AW, Chen XP. Erratum: hepatic dendritic cells, the tolerogenic liver environment, and liver disease. *Semin Liver Dis* 2018;38:298.
 52. Palucka K, Banchereau J. Dendritic-cell-based therapeutic cancer vaccines. *Immunity* 2013;39:38-48.
 53. Sun K, Wang L, Zhang Y. Dendritic cell as therapeutic vaccines against tumors and its role in therapy for hepatocellular carcinoma. *Cell Mol Immuno* 2006;3:197-203.
 54. Chen YX, Man K, Ling GS, Chen Y, Sun BS, et al. A crucial role for dendritic cell (DC) IL-10 in inhibiting successful DC-based immunotherapy: superior antitumor immunity against hepatocellular carcinoma evoked by DC devoid of IL-10. *J Immunol* 2007;179:6009-15.
 55. Beckebaum S, Zhang X, Chen X, Yu Z, Frilling A, et al. Increased levels of interleukin-10 in serum from patients with hepatocellular carcinoma correlate with profound numerical deficiencies and immature phenotype of circulating dendritic cell subsets. *Clin Cancer Res* 2004;10:7260-9.
 56. Perez CR, De Palma M. Engineering dendritic cell vaccines to improve cancer immunotherapy. *Nat Commun* 2019;10:5408.
 57. Peng M, Mo Y, Wang Y, Wu P, Zhang Y, et al. Neoantigen vaccine: an emerging tumor immunotherapy. *Mol Cancer* 2019;18:128.
 58. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* 2009;9:162-74.
 59. Xu M, Zhao Z, Song J, Lan X, Lu S, et al. Interactions between interleukin-6 and myeloid-derived suppressor cells drive the chemoresistant phenotype of hepatocellular cancer. *Exp Cell Res* 2017;351:142-49.
 60. Guha P, Gardell J, Darpolor J, Cunetta M, Lima M, et al. STAT3 inhibition induces Bax-dependent apoptosis in liver tumor myeloid-derived suppressor cells. *Oncogene* 2019;38:533-48.
 61. Sun H, Yang W, Tian Y, Zeng X, Zhou J, et al. An inflammatory-CCRk circuitry drives mTORC1-dependent metabolic and immunosuppressive reprogramming in obesity-associated hepatocellular carcinoma. *Nat Commun* 2018;9:5214.
 62. Li B, Zhang S, Huang N, Chen H, Wang P, et al. CCL9/CCR1 induces myeloid-derived suppressor cell recruitment to the spleen in a murine H22 orthotopic hepatoma model. *Oncol Rep* 2019;41:608-18.
 63. Zhou J, Liu M, Sun H, Feng Y, Xu L, et al. Hepatoma-intrinsic CCRk inhibition diminishes myeloid-derived suppressor cell immunosuppression and enhances immune-checkpoint blockade efficacy. *Gut* 2018;67:931-44.
 64. Limagne E, Richard C, Thibaudin M, Fumet JD, Truntzer C, et al. Tim-3/galectin-9 pathway and mMDSC control primary and secondary resistances to PD-1 blockade in lung cancer patients. *Oncoimmunology* 2019;8:e1564505.
 65. Hoechst B, Voigtlaender T, Ormandy L, Gamrekelashvili J, Zhao F, et al. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the Nkp30 receptor. *Hepatology* 2009;50:799-807.
 66. Hu CE, Gan J, Zhang RD, Cheng YR, Huang GJ. Up-regulated myeloid-derived suppressor cell contributes to hepatocellular carcinoma development by impairing dendritic cell function. *Scand J Gastroenterol* 2011;46:156-64.
 67. Wu CJ, Tsai YT, Lee IJ, Wu PY, Lu LS, et al. Combination of radiation and interleukin 12 eradicates large orthotopic hepatocellular carcinoma through immunomodulation of tumor microenvironment. *Oncoimmunology* 2018;7:e1477459.
 68. Liu M, Zhou J, Liu X, Feng Y, Yang W, et al. Targeting monocyte-intrinsic enhancer reprogramming improves immunotherapy efficacy in hepatocellular carcinoma. *Gut* 2020;69:365-79.
 69. Kang N, Gores GJ, Shah VH. Hepatic stellate cells: partners in crime for liver metastases? *Hepatology* 2011;54:707-13.
 70. Amann T, Bataille F, Spruss T, Muhlbauer M, Gabele E, et al. Activated hepatic stellate cells promote tumorigenicity of hepatocellular carcinoma. *Cancer Sci* 2009;100:646-53.
 71. Neaud V, Faouzi S, Guirouilh J, Le Bail B, Balabaud C, et al. Human hepatic myofibroblasts increase invasiveness of hepatocellular carcinoma cells: evidence for a role of hepatocyte growth factor. *Hepatology* 1997;26:1458-66.
 72. Amin DN, Hida K, Bielenberg DR, Klagsbrun M. Tumor endothelial cells express epidermal growth factor receptor (EGFR) but not ErbB3 and are responsive to EGF and to EGFR kinase inhibitors. *Cancer Res* 2006;66:2173-80.
 73. Rolny C, Nilsson I, Magnusson P, Armulik A, Jakobsson L, et al. Platelet-derived growth factor receptor-beta promotes early endothelial cell differentiation. *Blood* 2006;108:1877-86.
 74. Limmer A, Ohl J, Kurts C, Ljunggren HG, Reiss Y, et al. Efficient presentation of exogenous antigen by liver endothelial cells to CD8⁺ T cells results in antigen-specific T-cell tolerance. *Nat Med* 2000;6:1348-54.
 75. Bilzer M, Roggel F, Gerbes AL. Role of Kupffer cells in host defense and liver disease. *Liver Int* 2006;26:1175-86.
 76. Ramaiah SK, Rittling S. Pathophysiological role of osteopontin in hepatic inflammation, toxicity, and cancer. *Toxicol Sci* 2008;103:4-13.
 77. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, et al. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science* 1996;274:1379-83.
 78. Zhang M, Xu S, Han Y, Cao X. Apoptotic cells attenuate fulminant hepatitis by priming Kupffer cells to produce interleukin-10

- through membrane-bound TGF- β . *Hepatology* 2011;53:306-16.
79. Hong Y, Peng Y, Guo ZS, Guevara-Patino J, Pang J, et al. Epitope-optimized alpha-fetoprotein genetic vaccines prevent carcinogen-induced murine autochthonous hepatocellular carcinoma. *Hepatology* 2014;59:1448-58.
 80. Bei R, Mizejewski GJ. Alpha fetoprotein is more than a hepatocellular cancer biomarker: from spontaneous immune response in cancer patients to the development of an AFP-based cancer vaccine. *Curr Mol Med* 2011;11:564-81.
 81. Nakagawa H, Mizukoshi E, Kobayashi E, Tamai T, Hamana H, et al. Association between high-avidity T-cell receptors, induced by alpha-fetoprotein-derived peptides, and anti-tumor effects in patients with hepatocellular carcinoma. *Gastroenterology* 2017;152:1395-406.e10.
 82. Zhang W, Liu J, Wu Y, Xiao F, Wang Y, et al. Immunotherapy of hepatocellular carcinoma with a vaccine based on xenogeneic homologous alpha fetoprotein in mice. *Biochem Biophys Res Commun* 2008;376:10-4.
 83. Chen K, Wu Z, Zhao H, Wang Y, Ge Y, et al. XCL1/Glypican-3 fusion gene immunization generates potent antitumor cellular immunity and enhances anti-PD-1 efficacy. *Cancer Immunol Res* 2020;8:81-93.
 84. Sawada Y, Yoshikawa T, Nobuoka D, Shirakawa H, Kuronuma T, et al. Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival. *Clin Cancer Res* 2012;18:3686-96.
 85. Nakayama J, Tahara H, Tahara E, Saito M, Ito K, et al. Telomerase activation by hTERT in human normal fibroblasts and hepatocellular carcinomas. *Nat Genet* 1998;18:65-8.
 86. Harrington L, Zhou W, McPhail T, Oulton R, Yeung DS, et al. Human telomerase contains evolutionarily conserved catalytic and structural subunits. *Genes Dev* 1997;11:3109-15.
 87. Mizukoshi E, Nakamoto Y, Marukawa Y, Arai K, Yamashita T, et al. Cytotoxic T cell responses to human telomerase reverse transcriptase in patients with hepatocellular carcinoma. *Hepatology* 2006;43:1284-94.
 88. Mizukoshi E, Nakagawa H, Kitahara M, Yamashita T, Arai K, et al. Immunological features of T cells induced by human telomerase reverse transcriptase-derived peptides in patients with hepatocellular carcinoma. *Cancer Lett* 2015;364:98-105.
 89. Bricard G, Bouzourene H, Martinet O, Rimoldi D, Halkic N, et al. Naturally acquired MAGE-A10- and SSX-2-specific CD8⁺ T cell responses in patients with hepatocellular carcinoma. *J Immunol* 2005;174:1709-16.
 90. Dhodapkar MV, Steinman RM, Krasovsky J, Munz C, Bhardwaj N. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J Exp Med* 2001;193:233-8.
 91. Schuler-Thurner B, Schultz ES, Berger TG, Weinlich G, Ebner S, et al. Rapid induction of tumor-specific type 1 T helper cells in metastatic melanoma patients by vaccination with mature, cryopreserved, peptide-loaded monocyte-derived dendritic cells. *J Exp Med* 2002;195:1279-88.
 92. Chen C, Ma YH, Zhang YT, Zhang F, Zhou N, et al. Effect of dendritic cell-based immunotherapy on hepatocellular carcinoma: a systematic review and meta-analysis. *Cytotherapy* 2018;20:975-89.
 93. Lu Z, Zuo B, Jing R, Gao X, Rao Q, et al. Dendritic cell-derived exosomes elicit tumor regression in autochthonous hepatocellular carcinoma mouse models. *J Hepatol* 2017;67:739-48.
 94. Wilgenhof S, Corthals J, Heirman C, van Baren N, Lucas S, et al. Phase Ii study of autologous monocyte-derived mRNA electroporated dendritic cells (TriMixDC-MEL) plus ipilimumab in patients with pretreated advanced melanoma. *J Clin Oncol* 2016;34:1330-8.
 95. Lee JH, Lee Y, Lee M, Heo MK, Song JS, et al. A phase I/IIa study of adjuvant immunotherapy with tumour antigen-pulsed dendritic cells in patients with hepatocellular carcinoma. *Br J Cancer* 2015;113:1666-76.
 96. Nemunaitis J, Barve M, Orr D, Kuhn J, Magee M, et al. Summary of bi-shRNA/GM-CSF augmented autologous tumor cell immunotherapy (FANG) in advanced cancer of the liver. *Oncology* 2014;87:21-9.
 97. Pexa-Vec/Nexavar Combination Fails Phase III Trial in Liver Cancer. Available from: <https://www.genengnews.com/news/pexa-vec-nexavar-combination-fails-phase-iii-trial-in-liver-cancer/> [Last accessed on 21 Apr 2020]
 98. Mahipal A, Tella SH, Kommalapati A, Lim A, Kim R. Immunotherapy in hepatocellular carcinoma: is there a light at the end of the tunnel? *Cancers (Basel)* 2019;11:1078.
 99. Yoo SY, Jeong SN, Kang DH, Heo J. Evolutionary cancer-favoring engineered vaccinia virus for metastatic hepatocellular carcinoma. *Oncotarget* 2017;8:71489-99.
 100. Ady JW, Heffner J, Mojica K, Johnsen C, Belin LJ, et al. Oncolytic immunotherapy using recombinant vaccinia virus GLV-1h68 kills sorafenib-resistant hepatocellular carcinoma efficiently. *Surgery* 2014;156:263-9.
 101. Wang J, Xu L, Zeng W, Hu P, Zeng M, et al. Treatment of human hepatocellular carcinoma by the oncolytic herpes simplex virus G47delta. *Cancer Cell Int* 2014;14:83.
 102. Twumasi-Boateng K, Pettigrew JL, Kwok YYY, Bell JC, Nelson BH. Oncolytic viruses as engineering platforms for combination immunotherapy. *Nat Rev Cancer* 2018;18:419-32.
 103. Haanen JB, Robert C. Immune checkpoint inhibitors. *Prog Tumor Res* 2015;42:55-66.
 104. Meng X, Huang Z, Teng F, Xing L, Yu J. Predictive biomarkers in PD-1/PD-L1 checkpoint blockade immunotherapy. *Cancer Treat Rev* 2015;41:868-76.
 105. Sangro B, Gomez-Martin C, de la Mata M, Inarrairaegui M, Garraza E, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol* 2013;59:81-8.
 106. Zhang M, Pang HJ, Zhao W, Li YF, Yan LX, et al. VISTA expression associated with CD8 confers a favorable immune microenvironment and better overall survival in hepatocellular carcinoma. *BMC Cancer* 2018;18:511.
 107. Prieto J, Melero I, Sangro B. Immunological landscape and immunotherapy of hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2015;12:681-700.
 108. Li H, Wu K, Tao K, Chen L, Zheng Q, et al. Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor

- prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma. *Hepatology* 2012;56:1342-51.
109. Woo SR, Turnis ME, Goldberg MV, Bankoti J, Selby M, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res* 2012;72:917-27.
110. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med* 1995;182:459-65.
111. van der Merwe PA, Bodian DL, Daenke S, Linsley P, Davis SJ. CD80 (B7-1) binds both CD28 and CTLA-4 with a low affinity and very fast kinetics. *J Exp Med* 1997;185:393-403.
112. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* 2011;332:600-3.
113. Duffy AG, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J Hepatol* 2017;66:545-51.
114. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* 2008;26:677-704.
115. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. *Int Immunol* 2007;19:813-24.
116. Bardhan K, Anagnostou T, Boussiotis VA. The PD1:PD-L1/2 Pathway from Discovery to Clinical Implementation. *Front Immunol* 2016;7:550.
117. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, et al. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci U S A* 2002;99:12293-7.
118. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
119. Kudo M, Matilla A, Santoro A, Melero I, Gracian AC, et al. Checkmate-040: Nivolumab (NIVO) in patients (pts) with advanced hepatocellular carcinoma (aHCC) and Child-Pugh B (CPB) status. *J Clin Oncol* 2019;37:327-2.
120. Zhu AX, Finn RS, Edeline J, Cattani S, Ogasawara S, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018;19:940-52.
121. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, et al. Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: a randomized, double-blind, phase III trial. *J Clin Oncol* 2020;38:193-202.
122. Stein S, Pishvaian MJ, Lee MS, Lee KH, Hernandez S, et al. Safety and clinical activity of 1L atezolizumab + bevacizumab in a phase Ib study in hepatocellular carcinoma (HCC). *J Clin Oncol* 2018;36:4074-74.
123. Xiang J, Zhang N, Sun H, Su L, Zhang C, et al. Disruption of SIRT7 Increases the Efficacy of Checkpoint Inhibitor via MEF2D Regulation of PD-L1 in Hepatocellular Carcinoma Cells. *Gastroenterology* 2020;158:664-78.e24.
124. Nakamoto N, Cho H, Shaked A, Olthoff K, Valiga ME, et al. Synergistic reversal of intrahepatic HCV-specific CD8 T cell exhaustion by combined PD-1/CTLA-4 blockade. *PLoS Pathog* 2009;5:e1000313.
125. Aregay A, Owusu Sekyere S, Deterding K, Port K, Dietz J, et al. Elimination of hepatitis C virus has limited impact on the functional and mitochondrial impairment of HCV-specific CD8⁺ T cell responses. *J Hepatol* 2019;71:889-99.
126. Stein S, Pishvaian MJ, Lee MS, Lee KH, Hernandez S, et al. Safety and clinical activity of 1L atezolizumab plus bevacizumab in a phase Ib study in hepatocellular carcinoma (HCC). *J Clin Oncol* 2018;36.
127. Baxi S, Yang A, Gennarelli RL, Khan N, Wang Z, et al. Immune-related adverse events for anti-PD-1 and anti-PD-L1 drugs: systematic review and meta-analysis. *BMJ* 2018;360:k793.
128. Moslehi JJ, Salem JE, Sosman JA, Lebrun-Vignes B, Johnson DB. Increased reporting of fatal immune checkpoint inhibitor-associated myocarditis. *Lancet* 2018;391:933.
129. Chmielewski M, Hombach AA, Abken H. Antigen-specific T-cell activation independently of the MHC: chimeric antigen receptor-redirected T cells. *Front Immunol* 2013;4:371.
130. Ramos CA, Dotti G. Chimeric antigen receptor (CAR)-engineered lymphocytes for cancer therapy. *Expert Opin Biol Ther* 2011;11:855-73.
131. Gao HP, Li KS, Tu H, Pan XR, Jiang H, et al. Development of T cells redirected to glypican-3 for the treatment of hepatocellular carcinoma. *Clin Cancer Res* 2014;20:6418-28.
132. Liu Y, Di S, Shi B, Zhang H, Wang Y, et al. Armored inducible expression of IL-12 enhances antitumor activity of glypican-3-targeted chimeric antigen receptor-engineered T cells in hepatocellular carcinoma. *J Immunol* 2019;203:198-207.
133. Chen C, Li K, Jiang H, Song F, Gao H, et al. Development of T cells carrying two complementary chimeric antigen receptors against glypican-3 and asialoglycoprotein receptor 1 for the treatment of hepatocellular carcinoma. *Cancer Immunol Immunother* 2017;66:475-89.
134. Zhai B, Shi D, Gao H, Qi X, Jiang H, et al. A phase I study of anti-GPC3 chimeric antigen receptor modified T cells (GPC3 CAR-T) in Chinese patients with refractory or relapsed GPC3⁺ hepatocellular carcinoma (r/r GPC3⁺ HCC). *J Clin Oncol* 2017;35:3049-49.
135. MacKay M, Afshinnekoo E, Rub J, Hassan C, Khunte M, et al. The therapeutic landscape for cells engineered with chimeric antigen receptors. *Nat Biotechnol* 2020;38:233-44.
136. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat Rev Clin Oncol* 2020;17:147-67.
137. Gao XC, Mi YJ, Guo N, Xu H, Xu LX, et al. Cytokine-induced killer cells as pharmacological tools for cancer immunotherapy. *Front Immunol* 2017;8:774.
138. Mata-Molanes JJ, Gonzalez MS, Jimenez BV, Navarro EMM, Masllorens AB. Cancer immunotherapy with cytokine-induced killer cells. *Target Oncol* 2017;12:289-99.
139. Pan QZ, Wang QJ, Dan JQ, Pan K, Li YQ, et al. A nomogram for predicting the benefit of adjuvant cytokine-induced killer cell immunotherapy in patients with hepatocellular carcinoma. *Sci Rep* 2015;5:9202.
140. Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, et al. Adjuvant immunotherapy with autologous cytokine-induced killer cells for

- hepatocellular carcinoma. *Gastroenterology* 2015;148:1383-91.e6.
141. Huang ZM, Li W, Li S, Gao F, Zhou QM, et al. Cytokine-induced killer cells in combination with transcatheter arterial chemoembolization and radiofrequency ablation for hepatocellular carcinoma patients. *J Immunother* 2013;36:287-93.
 142. Yu X, Zhao H, Liu L, Cao S, Ren B, et al. A randomized phase II study of autologous cytokine-induced killer cells in treatment of hepatocellular carcinoma. *J Clin Immunol* 2014;34:194-203.
 143. Yu SJ, Ma C, Heinrich B, Brown ZJ, Sandhu M, et al. Targeting the crosstalk between cytokine-induced killer cells and myeloid-derived suppressor cells in hepatocellular carcinoma. *J Hepatol* 2019;70:449-57.
 144. Jiang SS, Tang Y, Zhang YJ, Weng DS, Zhou ZG, et al. A phase I clinical trial utilizing autologous tumor-infiltrating lymphocytes in patients with primary hepatocellular carcinoma. *Oncotarget* 2015;6:41339-49.
 145. Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, et al. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. *Lancet* 2000;356:802-7.
 146. Zhou G, Sprengers D, Boor PPC, Doukas M, Schutz H, et al. Antibodies against immune checkpoint molecules restore functions of tumor-infiltrating t cells in hepatocellular carcinomas. *Gastroenterology* 2017;153:1107-19.e10.
 147. Mossanen JC, Kohlhepp M, Wehr A, Krenkel O, Liepelt A, et al. CXCR6 inhibits hepatocarcinogenesis by promoting natural killer T- and CD4(+) T-cell-dependent control of senescence. *Gastroenterology* 2019;156:1877-89.e4.
 148. Kamiya T, Chang YH, Campana D. Expanded and activated natural killer cells for immunotherapy of hepatocellular carcinoma. *Cancer Immunol Res* 2016;4:574-81.
 149. Tan S, Xu Y, Wang Z, Wang T, Du X, et al. Tim-3 hampers tumor surveillance of liver resident and conventional NK cells by disrupting PI3K signaling. *Cancer Res* 2020;80:1130-42.

Review

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The advancement of immunotherapy in hepatocellular carcinoma

Ling Ni¹, Yu Feng¹, Chen Dong^{1,2}

¹Institute for Immunology and School of Medicine, Tsinghua University, Beijing 100084, China.

²Beijing Key Lab for Immunological Research on Chronic Diseases, Beijing 100084, China.

Correspondence to: Dr. Chen Dong, Institute for Immunology and School of Medicine, Tsinghua University, Medical Research Building, No.30 Haidian Shuangqing Road, Beijing 100084, China. E-mail: chendong@tsinghua.edu.cn

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Abstract

Most patients diagnosed with hepatocellular carcinoma (HCC) present with advanced or metastatic disease. The lack of therapeutic options in the treatment of advanced HCC accounts for its high mortality and recurrence rate. HCC is known as an immunogenic tumor, which develops in chronically inflamed livers. Anti-PD-1/PD-L1 antibodies (immune checkpoint inhibitors, ICB) were approved by the FDA to treat advanced HCC in patients previously treated with sorafenib as a second line. This has opened up a new era of anticancer treatment, although the response rate of HCC to anti-PD-1/PD-L1 antibodies is only around 20%. Other than ICB treatment, adoptive cell transfer, dendritic cell-based vaccines and oncolytic therapy are currently under clinical trials. In this review, different immunotherapy approaches for HCC is presented. Current knowledge on the mechanisms of action for each approach is discussed and relevant, ongoing clinical trials are presented. We also discuss the future of immunotherapy and combination treatment for HCC patients.

Keywords: Hepatocellular carcinoma, immunotherapy, anti-PD-1/PD-L1 antibodies

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer, and the second most common cause of cancer-related death worldwide. The incidence of HCC is higher in China and may account for 50% of new cases globally each year^[1]. Most HCCs in China and South-East Asia are caused by the hepatitis



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B virus while in the West, nonalcoholic-associated steatosis seems to be one of the main causes^[2]. In the early stages of HCC, hepatic resection or liver transplantation is first-line treatment with a high probability of a recurrence-free postoperative course. Sorafenib, a broad tyrosine kinase inhibitor, is standard treatment for advanced or metastatic HCC^[3]. HCC is also known as an immunogenic tumor, which develops in chronically inflamed livers from both viral and non-viral causes. This inflammation promotes tumor development and is related with increased tumor immunogenicity^[4]. As such, immunotherapy might be a more appropriate treatment strategy for HCC.

The liver, however, plays a pivotal role in host defense and the maintenance of immune competence. It is known to have an intrinsic, immunosuppressive microenvironment, which may serve as a major barrier to cancer immunotherapy^[4]. Moreover, the low expression levels of tumor-associated antigens result in weaker T cell activation and in turn, tumor infiltration, which results in less efficient tumor control and consequently, a poorer clinical outcome. Thus, therapeutic strategies need to be developed against the HCC tumor suppressive microenvironment, which is involved in lowering the efficacy of immunotherapy^[5].

Although treatment strategies for patients with intermediate and advanced HCC are quite limited, an attractive option is immunomodulation therapy^[6]. Current HCC immunotherapy approaches include immune checkpoint inhibitor antibodies (ICB), adoptive cell transfer (ACT), dendritic cell-based vaccines and oncolytic therapy but only ICB (anti-PD-1/PD-L1 antibodies) have been approved by the FDA as second-line treatment for HCC patients^[7]. The major impediment to the development and success of immunotherapy in HCC patients is the intrinsic immunosuppressive microenvironment^[6]. In this review article, we summarize recent advances in, and the future of immunotherapy for HCC and discuss their combination for the treatment of HCC patients.

TUMOR MICROENVIRONMENT

The tumor microenvironment (TME) is widely studied for the identification of novel therapeutic targets. The HCC TME consists of cancer and stromal cells, including hepatic stellate cells, cancer-associated fibroblasts, immune cells and endothelial cells, which interact with and affect the growth of the tumor^[8]. The immune landscape of HCC is heterogeneous. By using 10×-single cell RNA-sequencing, Zhang and colleagues found that intratumor LAMP3⁺ mature dendritic cells (DCs) had the capacity to migrate from tumors to lymph nodes^[9]. LAMP3⁺ DCs also expressed diverse immune-relevant ligands and possessed the ability to regulate several types of lymphocytes. Macrophages in tumors displayed distinct transcriptional states and tumor-associated macrophages (TAMs) were correlated with a poor prognosis. Myeloid cells in ascites were predominantly of tumor origin while lymphoid cells originated from blood^[9]. Through time-of-flight mass cytometry, Chew *et al.*^[10] found that the HCC TME was enriched in regulatory T cells, tissue resident memory CD8⁺ T cells, resident natural killer cells, and TAMs. Tissue resident memory PD-1⁺CD8⁺ T cells were the major T cell subset responsive to PD-1 blockade. Zhang *et al.*^[11] found that B cell infiltration of tumors was significantly impaired during tumor progression but tumors with a high density of B cell infiltration correlated with better clinical outcomes. It was also found that there was a high number of TIM-1⁺ Breg cell infiltrating the tumors of HCC patients^[12,13]. These infiltrating Breg cells expressed IL-10 and had high suppressive activity against CD8⁺ and CD4⁺ T cells. B cells, except Breg cells, may contribute to tumor regression. Thus, using a selective reagent to inhibit suppressive B cells may be a viable strategy. By improving understanding of the immunologic components of the TME and its corresponding interactions at the biological and molecular levels, more effective immunotherapies for HCC can be designed.

ADVANCEMENT OF ICB

The mechanism of action of ICB is to stimulate or normalize the immune system of cancer patients by targeting negative regulators of T cell signaling pathways. FDA approved PD-1/PD-L1 inhibitor antibodies

can be used to treat advanced HCC in patients previously on sorafenib as second line treatment^[7]. HCC however, shows relatively low responsiveness to PD-1/PD-L1 inhibitor antibodies with an objective response rate of only around 20%^[14].

Factors regulating and predictive of the response to anti-PD-1/PD-L1 immunotherapy in HCC are still largely unknown. To test whether PD-L1 expression on tumor cells can serve as a biomarker for PD-1 blockade therapy, Ou *et al.*^[15] used a mouse liver cancer cell line BNL-MEA transfected with a PD-L1 plasmid to establish an orthotopic HCC model. Wild-type (WT) and PD-L1-expressing tumor-bearing mice were then treated with PD-1 inhibitor antibodies. PD-L1-expressing tumors showed better response to anti-PD-1 therapy than WT tumors. In addition, depletion of CD8⁺ T cells abolished the anti-tumor responses. Thus, PD-L1 expression in HCC cells may contribute to tumor immune evasion by suppressing T cell function. In turn, PD-L1 expression on tumor cells can be a biomarker for PD-1 ICB in this rodent model of HCC. However, the use of PD-L1 expression on human HCC cells as a biomarker needs to be investigated further. In a study of HCC patients treated with anti-PD-1 antibodies, the responders' (responding to PD-1 ICB) fecal samples exhibited higher taxa richness and more gene counts compared to those from non-responders. During PD-1 inhibitor immunotherapy, dynamic analysis showed that the dissimilarity of beta diversity displayed was obvious between patients from as early as Week 6. Proteobacteria increased from week 3 and became predominant at week 12 in non-responders. The dynamic variation in characteristics of the gut microbiome may predict immunotherapy outcomes in HCC, which is important for disease-monitoring and treatment decision-making^[16].

Due to the low response rate to anti-PD-1 monotherapy, combining immunotherapy has become a new direction for future exploration in HCC treatment. Combination therapy of PD-1 ICB with targeted therapy, CTLA-4 ICB, oncolytic immunotherapy, epigenetic modulation and radiotherapy are all worthy of attention.

Recently, anti-PD-L1 therapy in combination with anti-VEGF antibodies demonstrated success based on the Phase III IMbrave150 trial in 501 patients with unresectable HCC who had not received previous systemic therapy. Participants were split 2:1 to receive the combination of anti-PD-L1 and anti-VEGF antibody versus sorafenib. Both anti-PD-L1 and anti-VEGF were administered intravenously whereas sorafenib is an oral medication. Patients in both arms received treatment until unacceptable toxicity developed or when there was no longer any clinical benefit. The trial met its co-primary endpoints, showing statistically significant and clinically meaningful improvements in overall survival (OS) and progression-free survival compared to the standard-of-care, sorafenib. Safety were consistent with those known for both anti-PD-L1 and anti-VEGF antibodies (ClinicalTrials.gov Identifier: NCT03434379). These findings indicate that the PD-L1/VEGF inhibitor combination can improve the efficacy of ICB.

The combined immunotherapy of anti-PD-1 with anti-CTLA-4 is now in a phase II trial on treating patients with resectable HCC (ClinicalTrials.gov Identifier: NCT03222076). Recently a case that achieved complete response with such combined therapy was reported. There was correlation of clinical response with increased CD8⁺ T cell infiltration, as well as increased effector T cell clusters. The study is ongoing and final results may contribute to a paradigm shift in the perioperative treatment of HCC^[17].

Enhancer of Zeste Homolog 2 (EZH2) and DNA Methyltransferase 1 (DNMT1) are two components of epigenetic modulation. In a mouse HCC model, combining anti-PD-L1 therapy with either DZNep (EZH2 inhibitor) or 5-Azacytidine (DNMT1 inhibitor) resulted in significantly increased tumor regression compared to epigenetic therapy or immunotherapy alone, which suggests that epigenetic modulation might be a potential, novel strategy to enhance immunotherapy for HCC^[18]. Mechanistically, epigenetic modulators can activate transcriptionally repressed chemokine genes and stimulate T cell trafficking into

the TME. In addition, these modulators can also induce previously silent neoantigens and elicit neoantigen-specific T cell responses to control tumors^[18].

Combination therapy with yttrium-90 radioembolization and PD-1 ICB for HCC treatment was also studied^[19]. 26 consecutive HCC patients who received PD-1 ICB and radioembolization from April 2015 to May 2018 were included in a single-center, retrospective study. The results indicate that PD-1 ICB combined with yttrium-90 radioembolization in cases of HCC appears to be well-tolerated. Future prospective studies are needed to optimize treatment protocols and evaluate the efficacy of combination therapy^[19].

ADVANCEMENT OF DENDRITIC CELL-BASED HCC VACCINES

The response of HCC to ICB has been disappointing and new strategies are being explored. Tumor-associated antigen-loaded DCs are a potential immunotherapy for cancer, since DCs are the strongest professional antigen-presenting cells, which can orchestrate the innate and adaptive immune systems. Sipuleucel-T, the first FDA-approved DC vaccine, was observed to be effective in the treatment of human prostate cancer to some extent^[20]. In the context of HCC, DC-based immunotherapy has recently been reported. Although HCC in China develops from chronic hepatitis B virus (HBV) infection, most HCC cells containing HBV-DNA fragments do not encode entire HBV antigens, but HBV epitopes can be encoded. To date, several tumor-associated antigens have been identified for DC-based vaccines for the treatment of patients with HCC including alpha-fetoprotein, glypican-3, melanoma associated antigen-1 and aspartate β -hydroxylase.

Alpha-fetoprotein (AFP) is a glycoprotein derived from embryonic endoderm tissue. Expression levels of AFP in fetal serum are high and decrease over time. While mature hepatocytes lose the ability to synthesize AFP, liver cancer cells can synthesize AFP after transformation. Hence, AFP has been detected in several malignant tumors, including that of the stomach, pancreas and liver. The majority of human HCC cells express high levels of AFP in Eastern populations^[21]. Therefore, HCC patients with negative or weakly positive AFP in serum are associated with highly differentiated cancers. Glypican-3 (GPC3), another tumor-associated antigen, is a heparan sulfate proteoglycan, which binds to glycosylphosphatidylinositol-anchored proteins and can be detected in the liver and kidneys of healthy fetuses. Adults express low or no GPC3, except in the placenta. GPC3 is also specifically expressed in several types of cancers including HCC, ovarian clear cell carcinoma, melanoma, squamous cell carcinoma of the lung, hepatoblastoma, nephroblastoma (Wilms tumor), yolk sac tumors, and some pediatric cancers. In some trials, HLA-A24- and A2-restricted GPC3-derived peptide vaccines elicited GPC3-specific cytotoxic T cells in most vaccinated patients and in turn, improved outcomes^[22]. Melanoma antigen 1 family contains a lot of chromosome X-clustered genes, including *MAGE-1*, *MAGE-B* and *MAGE-C* groups. Most of them cannot be detected in normal adult tissues except in the testis^[23]. However, various types of cancers highly express it, including HCC. *MAGE-1* protein-derived peptides are currently being studied as targets for the development of cancer vaccines^[24]. Aspartate β -hydroxylase (ASPH), a type II transmembrane protein, is a highly conserved dioxygenase enzyme, which is overexpressed in a variety of cancers, including HCC. Normal adult tissues rarely express ASPH, except in placental trophoblastic cells. In HCC patients, ASPH overexpression was significantly correlated with higher relapse and lower survival rates after surgery, and could also predict worse surgical outcomes in early-stage HCC patients^[25]. The expression pattern of ASPH makes it a potential biomarker and therapeutic target in cancer.

In phase I/IIA^[26] and phase II^[27] trials, Lee *et al.*^[26,27] showed that autologous DC vaccines prepared by loading with the three most common HCC TAAs (AFP, *MAGE-1*, and GPC-3) in order to cover HCC heterogeneity were safe and well tolerated in HCC patients. In addition, DC vaccination led to enhanced tumor-specific immune responses. Therefore, adjuvant immunotherapy with DC vaccines decreases the

risk of tumor relapse in HCC patients who have undergone standard treatment.

ASPH was also utilized to make DC-based HCC vaccines. An adenovirus vector encoding the ASPH gene (*Ad-ASPH-IRES2-EGFP*) was constructed and bone marrow-derived DCs were infected by *Ad-AAH-IRES2-EGFP* to prepare ASPH-DC vaccines. After infection, DCs showed a mature phenotype with higher expression of CD11c, CD80, and MHC-II. Co-culture of ASPH-DCs and T cells resulted in enhanced killing capacity of T lymphocytes on the HepG2 HCC cell line. In addition, this ASPH-DC vaccine also improved the killing function of cytotoxic T lymphocytes (CTLs) compared to controls in an animal model. Therefore, this finding indicates that the ASPH-DC vaccine may be a potential candidate for DC-based immunotherapy of HCC^[28]. However, for the ASPH-DC vaccine, only one molecule, ASPH, can be targeted, which cannot account fully for HCC heterogeneity.

From these studies, Chen *et al.*^[29] conducted a systematic review and meta-analysis to evaluate the clinical efficacy of DC-based vaccines in treating HCC. In total, there were 1276 cases from 19 clinical trials. They found that DC-based vaccines not only improved tumor control and increased the survival rate of HCC patients, its toxicity was also well-tolerated. These findings will provide new insight towards further development of DC-based vaccines as an adjuvant treatment strategy. However, the sample size, publication biases, varied study designs, pre-treatment and therapeutic processes of DCs have to be taken into consideration when evaluating DC-based immunotherapy.

ADVANCEMENT OF ACT

Cellular immunotherapy appears to be a promising modality for the treatment of malignant tumors. The mechanism of action in adoptive cell transfer is to treat cancer patients with their own naturally occurring or genetically modified anti-tumor lymphocytes to control tumors. Several meta-analyses have confirmed the evidence that adjuvant ACT for HCC patients after curative treatment reduces the risk of mortality and tumor relapse^[30,31].

Chimeric antigen receptor T cells

Chimeric antigen receptor T cells (CAR-T cells) are T cells that have been engineered to express an artificial T cell receptor. The FDA has approved CAR-T cells that target CD19 to treat myeloma, a hematological malignancy derived from plasma cells^[32]. However, the use of CAR-T cells to treat solid tumors is limited due to the scarcity of tumor-specific antigen targets and the poor infiltration of CAR-T cells into tumor tissue. In the context of HCC, CAR-T cells are still under investigation. Currently, these targets include GPC3, CD133, NKG2D and CD147.

GPC3-targeted CAR-T cells could be a promising therapeutic option for HCC^[33-35]. Gao *et al.*^[33] found that GPC3-CAR-T cells could efficiently eliminate GPC3 positive HCC cells but not GPC3 negative cells *in vitro*, and their cytotoxic activities might be positively associated with the expression levels of GPC3 on target cells. Moreover, third-generation GPC3-CAR-T cells could eliminate HCC xenografts with high GPC3 expression and potentially impair HCC xenograft growth with low expression levels of GPC3 *in vivo*. Treatment with third-generation GPC3-targeted CAR-T cells also significantly prolonged the survival of mice bearing established orthotopic Huh-7 xenografts. In addition, in order to reduce on-target, off-tumor toxicity by GPC3-CAR-T cells, CAR-T cells targeting GPC3 and asialoglycoprotein receptor 1 (ASGR1) (a liver tissue-specific protein) were prepared. Dual-targeted CAR-T cells carry anti-GPC3-CD3 ζ for primary signal transduction and anti-ASGR1-28BB for co-stimulatory signal transduction. The results showed that dual-targeted CAR-T cells had no cytotoxic effect on ASGR1⁺GPC3⁻ tumor cells but killed GPC3⁺ASGR1⁻ and GPC3⁺ASGR1⁺ HCC cells *in vitro*. Moreover, the dual-targeted CAR-T cells potentially suppressed the growth of GPC3⁺ASGR1⁺ HCC tumor xenografts while no obvious growth inhibition was seen with either single or double antigen-negative tumor xenografts. Additionally, the dual-targeted T cells showed higher

anti-tumor capacity and persistence than single-targeted T cells in two GPC3⁺ASGR1⁺ HCC xenograft models. Taken together, dual-targeted CAR-T cells may decrease the risk of on-target, off-tumor toxicity while maintaining relatively potent antitumor capacities in GPC3⁺ASGR1⁺ HCC^[36].

The combination of CAR-T cell therapy and knockout of endogenous inhibitory immune checkpoints on T cells might be a promising immunotherapeutic approach for cancer treatment. The *PD-1* gene in GPC3-targeted CAR-T cells was knocked out via the *CRISPR/Cas9* gene-editing system. *In vitro*, PD-1^{-/-} GPC3-CAR-T cells demonstrated higher anti-tumor activity against PD-L1-expressing HCC cell PLC/PRF/5 than WT CAR-T cells in a CAR-dependent manner. Moreover, PD-1 deficiency did not affect T cell subsets and activation status of CAR-T cells. Notably, co-culture of PD-1^{-/-} GPC3-CAR-T cells with native PD-L1-expressing HCC did not induce CAR-T cell exhaustion. Furthermore, the knockout of PD-1 led to enhanced anti-tumor activity of CAR-T cells against HCC *in vivo*, and improved persistence and infiltration of CAR-T cells in tumor-bearing NOD-SCID IL-2receptor gamma null (NSG) mice. These findings suggest the improved anti-tumor capacity of PD-1^{-/-} CAR-T cells in HCC and the potential of precise gene editing on immune checkpoints to increase the efficacy of CAR-T cells^[37]. In another study, Pan et al.^[38] introduced a fusion protein composed of a PD-1 extracellular domain and CH3 from IgG4 into GPC3-specific CAR-T cells (GPC3-28Z). GPC3-CAR-T cells carrying the PD-1-CH3 fusion protein (sPD1) specifically recognized and lysed GPC3⁺ HCC cells, while secreting soluble PD-1 to impair PD-1/PD-L1 signaling. The incorporation of soluble PD1 protected CAR-T cells from exhaustion when combating target cells. More importantly, GPC3-28Z-sPD1 T cells suppressed tumor xenograft growth significantly when compared with GPC3-28Z T cells in two HCC tumor xenograft models. The treatment of mice with GPC3-28Z-sPD1 T cells resulted in a higher number of CD3⁺ T cells in the circulation as well as in tumors, enhanced granzyme B expression and reduced Ki67 expression in tumors. These findings suggest that GPC3-targeted CAR-T cells carrying soluble PD-1 hold great potential for the treatment of HCC.

The combination of GPC3-CAR-T cells and targeted therapy (angiogenesis inhibitor) in the treatment of HCC has been investigated. In an immunocompetent mouse model, mouse GPC3-CAR (mCAR) T cells showed potent growth suppressive activity against small tumors, but did not show suppressive activity against large, established tumors. Sorafenib (angiogenesis inhibitor), at a subpharmacologic but not a pharmacologic dose, enhanced the anti-tumor activity of mCAR-T cells, partially by increasing IL-12 expression by TAMs and cancer cell apoptosis. Sorafenib, at both subpharmacologic and pharmacologic doses, had a weak effect on the function of human CAR (huCAR) T cells in an immunodeficient mouse model. However, huCAR-T cells and sorafenib together showed synergistic activities against tumor cells *in vivo*. Collectively, these findings suggest the potential of combining sorafenib with GPC3-targeted CAR-T cells in the treatment of HCC^[39].

Other than GPC3, CD133 is another promising therapeutic target for CAR-T cells, for it is expressed by stem cells of different cancer types. CD133-targeted CAR-T cells were prepared and its anti-tumor activity and toxicity were tested in a phase I clinical trial. In total, 23 patients with advanced and CD133⁺ tumors (14 HCC, 7 pancreatic carcinomas, and 2 colorectal carcinomas) were enrolled and received CD133-CAR-T cell infusion. Of 23 patients, 3 achieved partial remission, and 14 achieved stable disease. Repeat CAR-T cell infusions might provide a longer period of disease stability, especially in patients who have achieved tumor regression after the first ACT treatment. Of note, 91.3% of patients had not developed detectable *de novo* lesions during treatment. CD133⁺ cells were eliminated after CD133-CAR-T cell infusion and toxicity is well tolerated. This trial suggests that the infusion of CA133-CAR-T cells may be of value in treating CD133⁺ advanced cancers^[40].

NKG2D-NK group 2 member D (NKG2D) ligands (NKG2DL) are not expressed on the surface of normal cells but are overexpressed on malignant cells, thereby providing targets for CAR-T therapy. The expression levels of most NKG2DLs are higher in tumors than that in normal tissues. Due to this reason, NKG2D-

based CAR-T cells were designed and prepared. NKG2D-CAR-T cells had an efficient killing effect *in vitro* on HCC cell lines SMMC-7721 and MHCC97H, which express high levels of NKG2DLs. However, they were less efficient in eradicating NKG2DL-silenced SMMC-7721 cells or NKG2DL-negative Hep3B cells. The overexpression of NKG2DL (MICA or ULBP2) in Hep3B resulted in enhanced killing capacity of NKG2D-CAR-T cells. Importantly, T cells expressing the NKG2D-BBz CAR effectively killed SMMC-7721 HCC xenografts. Taken together, these findings imply that NKG2D-CAR-T cells might hold great promise for future therapeutic intervention of NKG2DL-positive HCC^[41].

Zhang *et al.*^[42] prepared CD147-CAR-T cells that were inducible. Since CD147 is expressed at a low level on non-tumor cells, the Tet-On 3G system was introduced to induce CD147CAR expression to minimize the on-target, off-tumor toxicity. Specifically, Tet-On-CD147-CAR lentiviral plasmid (LV-Tet-CD147CAR) was constructed, of which CD147-CAR was controlled by the Tet-On 3G system. The presence of doxycycline (Dox) resulted in enhanced proliferation, cytotoxicity, and cytokine secretion of Tet-CD147-CAR-T cells. Notably, (Dox⁺) Tet-CD147-CAR-T cells significantly suppressed tumor growth in nude mice through multiple intra-tumoral administrations. Collectively, these findings suggest that CD147-CAR expression and activity were inducible, which reduced the toxicity of CAR-T cell therapy. Furthermore, the study offered evidence to support the potential benefits and translation of CD147-CAR-T cells for the treatment of HCC patients.

Cytokine-induced killer

Adjuvant cytokine-induced killer (CIK) cell-based immunotherapy is a promising therapeutic approach that increases overall survival and decreases relapses in HCC patients. Yoon *et al.*^[43] performed a trial to investigate the efficacy of adjuvant immunotherapy with activated CIK cells for treating HCC patients. 59 patients with stage I or II HCC who had undergone surgery or radiofrequency ablation, followed by adjuvant CIK cell immunotherapy at two large-volume centers in Korea were paired with 59 matched control subjects. They found that adjuvant immunotherapy with autologous CIK cells after curative treatment prolonged relapse free survival (RFS) of patients with HCC. In a follow-up study of 226 patients with 114 in the immunotherapy group (treated with 6.4×10^9 CIK cells) and 112 controls (no treatment) after potentially curative treatment for HCC, the immunotherapy group was observed to have a significantly lower risk of relapse or death. The RFS rate was 44.8% in the immunotherapy group compared to 33.1% in the control group at 5 years. The risk of all-cause death was also decreased in the immunotherapy group vs. the control group. Furthermore, the significant increase in RFS and OS in the immunotherapy group lasted over 5 years^[44].

Yu *et al.*^[45] performed a systematic review and meta-analysis of published studies in order to evaluate the safety and efficacy of CIK cell-based immunotherapy as adjuvant therapy. This included eight randomized controlled trials (RCTs), six prospective studies, and three retrospective studies. The overall analysis indicated that CIK cell therapy resulted in an increased survival rate. CIK cells in non-RCTs resulted in improved progression-free survival, but in RCTs, had similar progression-free survival rates as those of controls. CIK cell-treatment also led to lower rates of relapse in RCTs and similar results were observed when non-RCTs and RCTs were combined. Collectively, these findings suggest that adjuvant CIK cell-based immunotherapy can enhance OS and decrease relapses in HCC patients.

PD-L1 expression may imply the presence of endogenous host immune responses to tumor. In one retrospective study including 448 HCC patients, 217 cases underwent hepatectomy and 231 were treated with both hepatectomy and post-operative CIK cell transfusion. CIK treatment led to a significantly improved prognosis compared to surgery alone. Higher expression levels of PD-L1 were observed in patients with long-term survival benefit in the CIK treatment group. Patients with more than 5% PD-L1 expression also had better OS and RFS than those with < 5% PD-L1 expression, particularly in the

subgroup with high hepatitis B viral load. PD-L1 expression however, did not correlate with patient survival in the surgery alone group^[46]. These findings suggest that PD-L1 expression can serve as a biomarker for predicting survival benefit from adjuvant CIK cell immunotherapy in HCC patients.

T cell receptor gene-engineered T cells

Genetic engineering of TAA-specific T cell receptor (TCR) could be an option to yield AFP-specific CTLs. AFP is overexpressed in HCC and serves both as a TAA as well as a potential target for adoptive immunotherapy. However, the low percentage and exhausted AFP-specific T cells *in vivo* impedes adoptive immunotherapy. Genetic modification with TCRs that are specific for HCC-associated antigens, such as AFP, strongly redirect human T cells to specifically eliminate HCC tumor cells. Using lentivector and peptide immunization, Zhu *et al.*^[47] identified a population of CD8⁺ T cells in HLA-A2 transgenic AAD mice that recognized the AFP158 epitope on human HCC cells. Adoptive injection of the AFP158-specific mouse CD8⁺ T cells killed HepG2 tumor xenografts in immunocompromised NSG mice. T cell hybridoma clones from the AFP158-specific mouse CD8⁺ T cells were then established and three sets of paired TCR genes were identified out of five hybridomas. The murine TCR-expressing primary human T cells can bind to HLA-A2/AFP158 tetramer. TCR gene-engineered T (TCR-T) cells also specifically recognized and eliminated HLA-A2⁺ AFP⁺HepG2 HCC tumor cells, but had no toxic effect on normal primary hepatocytes *in vitro*. Notably, AFP-specific TCR-T cells could kill HepG2 tumors in NSG mice. These findings suggest that AFP158-specific TCRs have the ability to engineer HLA-A2-positive autologous T cells to treat patients with HCC^[47]. Sun *et al.*^[48] used AFP158-166 peptide-loaded autologous DCs to stimulate AFP-specific CTLs. TCR genes of AFP-specific CTLs were then cloned into a lentiviral vector, which in turn infected nonspecific activated T cells. The specific cytotoxic activity against HpeG2 *in vitro* and in tumor-bearing NOD/SCID mice was significantly enhanced in engineered CTLs than that in AFP-specific CTLs stimulated by peptide-loaded DCs or controls. TCR gene transfer is thus a promising strategy to yield AFP-specific CTLs for HCC^[48].

In China, around 85% of HCC is associated with HBV infection. Cells from most HBV-associated HCCs contain HBV-DNA fragments that fail to encode entire HBV antigens, but do encode epitopes of HBV-specific T cells. Given that, autologous TCR-T cells that recognize epitopes from HBV-DNA in patients' metastases were infused into two patients without notable toxicity. This strategy might be exploited for a wider population of patients with HBV-related HCC^[49]. Qasim *et al.*^[50], also showed HBV antigen expression in HCC metastases. T cells were then genetically engineered to express an HBsAg specific TCR to treat chemo-resistant extrahepatic metastases. Genetically-engineered T cells reduced HBsAg levels without exacerbation of liver inflammation or other adverse effects. This study further supports this approach in treating patients with HBV-associated HCC^[50].

Gamma-delta T cells

Gamma-delta ($\gamma\delta$) T cells have been found to be promising as cellular immunotherapy in HCC patients^[51]. The amplifying ability of circulating $\gamma\delta$ T cells was related with the clinicopathological characteristics of patients, such as clinical stage, levels of AFP and albumin, duration of disease, size and number of tumors, numbers of Tregs and $\gamma\delta$ T17 cells, and levels of IL-17A. Notably, the frequency of $\gamma\delta$ T cells that were positive for IFN- γ , TNF- α , granzyme B, perforin, and lysosome-associated membrane protein remained unchanged both before and after amplification. More importantly, the *in vitro* cytotoxicity of $\gamma\delta$ T cells remained unchanged, which may render them feasible for HCC immunotherapy. However, adoptive transfer of $\gamma\delta$ T cells should be individualized based on the clinicopathological features of patients^[51].

Natural killer cells

Natural killer (NK) cells play a pivotal role in eradicating virus-infected and transformed cells in the innate immune system. Kamiya *et al.*^[52] successfully expanded NK cells from peripheral blood of healthy donors by using the K562-mb15-41BBL cell line as a stimulus. Following expansion, NK cells showed great

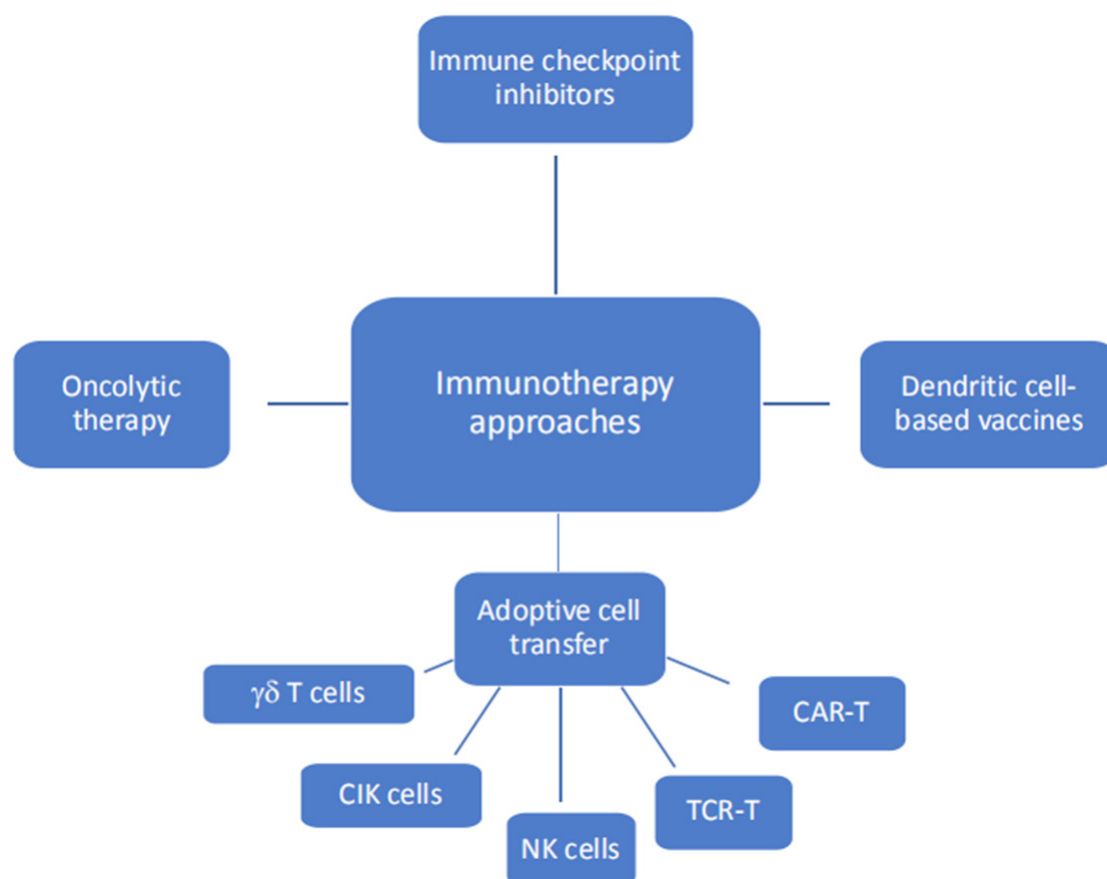


Figure 1. An overview of the different immunotherapy approaches for the treatment of hepatocellular carcinoma, which includes immune checkpoint inhibitor antibodies, adoptive cell transfer, dendritic cell-based vaccines and oncolytic therapy. CIK: cytokine-induced killer; NK: natural killer; TCR: T cell receptor; CAR: chimeric antigen receptor

cytotoxic activity against HCC cell lines *in vitro*. In immunodeficient NOD/scid IL2RG null mice engrafted with Hep3B, adoptive transfer of expanded NK cells resulted in markedly decreased tumor growth and enhanced overall survival. Expanded NK cells can further enhance the sorafenib-mediated anti-tumor effect on HCC cells. In addition, CAR-NK cells (NKG2D-CD3zeta-DAP10) were prepared and found to have increased cytotoxicity *in vitro* and in immunodeficient mice. CAR-NK cells have now been adapted for clinical-grade conditions^[52].

In one retrospective study, autologous mononuclear cells were induced into NK cells, $\gamma\delta$ T cells, and CIK cells and then infused intravenously to HCV-associated HCC patients (study group treated with a combination of ACT and conventional therapy). Compared to controls (conventional therapy), the study group showed improved prognosis and decreased virus load in HCV-related HCC patients, without impairment of liver function^[53].

ADVANCEMENT OF ONCOLYTIC IMMUNOTHERAPY

Oncolytic immunotherapy is another strategy for cancer treatment which utilizes live or heat-inactivated viruses, replicating within and destroying tumor cells. Pexastimogene devacirepvec (Pexa-Vec) is a vaccinia virus-based oncolytic immunotherapy that can destroy tumor cells and enhance anti-tumor immunity by expressing GM-CSF. A phase IIa trial in predominantly sorafenib-naïve HCC showed an OS benefit. In order to investigate whether the combination of Pexa-Vec with best supportive care (BSC) could increase OS compared to BSC alone in HCC patients who did not respond to sorafenib therapy, 129 patients were

recruited and divided into the experimental (combination of Pexa-Vec with BSC) and the control (BSC alone) groups. Although Pexa-Vec was generally well-tolerated and could induce immune responses to vaccinia and HCC associated antigens, it failed to enhance OS as a second-line treatment after sorafenib failure. This class of oncolytic immunotherapy may benefit cancer patients with earlier disease stages and the combination of this approach with other therapeutics needs to be investigated^[54].

NCT03071094 is another trial to evaluate the safety and efficacy of Pexa-Vec plus PD-1 inhibitors as first-line therapy for advanced HCC. However, no results have been released.

CONCLUSION

We have summarized the immunotherapy approaches for the treatment of HCC patients [Figure 1]. Increasing data provide evidence that HCC patients can benefit from different immunotherapies. Currently, only anti-PD-1/PD-L1 antibodies have been approved to treat advanced HCC as a first-line therapy, while other immunotherapy approaches are still in clinical trials. However, given that only a proportion of HCC patients respond to anti-PD-1/PD-L1 antibodies, combination therapy will likely be the future. Final results of ongoing trials are crucial for the design and development of further combination therapy in HCC according to its efficacy and safety profile. In addition, future studies should explore biomarkers to predict one's response to immunotherapy in HCC, other than PD-L1 expression in terms of ICB therapy, novel target antigens (neo-antigens) to prepare DC-based vaccines and CAR-T cells. Future clinical trials are necessary. Indeed, we have opened the doors to immunotherapy and complete control of HCC may not a dream.

DECLARATIONS

Authors' contributions

Wrote the review: Ni L, Dong C

Helped with review writing: Feng Y

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCE

1. Zhu RX, Seto WK, Lai CL, Yuen MF. Epidemiology of hepatocellular carcinoma in the Asia-Pacific Region. *Gut Liver* 2016;10:332-9.
2. Yang JD, Roberts LR. Hepatocellular carcinoma: a global view. *Nat Rev Gastroenterol Hepatol* 2010;7:448-58.

3. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-90.
4. Buonaguro L, Mauriello A, Cavalluzzo B, Petrizzo A, Tagliamonte M. Immunotherapy in hepatocellular carcinoma. *Ann Hepatol* 2019;18:291-7.
5. Brown ZJ, Greten TF, Heinrich B. Adjuvant treatment of hepatocellular carcinoma: prospect of immunotherapy. *Hepatology* 2019;70:1437-42.
6. Obeid JM, Kunk PR, Zaydfudim VM, Bullock TN, Slingluff CL, Jr., Rahma OE. Immunotherapy for hepatocellular carcinoma patients: is it ready for prime time? *Cancer Immunol Immunother* 2018;67:161-74.
7. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
8. Leonardi GC, Candido S, Cervello M, Nicolosi D, Raiti F, et al. The tumor microenvironment in hepatocellular carcinoma (review). *Int J Oncol* 2012;40:1733-47.
9. Zhang Q, He Y, Luo N, Patel SJ, Han Y, et al. Landscape and dynamics of single immune cells in hepatocellular carcinoma. *Cell* 2019;179:829-45.e20.
10. Chew V, Lai L, Pan L, Lim CJ, Li J, et al. Delineation of an immunosuppressive gradient in hepatocellular carcinoma using high-dimensional proteomic and transcriptomic analyses. *Proc Natl Acad Sci U S A* 2017;114:E5900-9.
11. Zhang Z, Ma L, Goswami S, Ma J, Zheng B, et al. Landscape of infiltrating B cells and their clinical significance in human hepatocellular carcinoma. *Oncoimmunology* 2019;8:e1571388.
12. Ye L, Zhang Q, Cheng Y, Chen X, Wang G, et al. Tumor-derived exosomal HMGB1 fosters hepatocellular carcinoma immune evasion by promoting TIM-1(+) regulatory B cell expansion. *J Immunother Cancer* 2018;6:145.
13. Xue H, Lin F, Tan H, Zhu ZQ, Zhang ZY, et al. Overrepresentation of IL-10-expressing B cells suppresses cytotoxic CD4+ T cell activity in HBV-induced hepatocellular carcinoma. *PLoS One* 2016;11:e0154815.
14. Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N Engl J Med* 2017;377:2500-1.
15. Ou DL, Lin YY, Hsu CL, Lin YY, Chen CW, et al. Development of a PD-L1-expressing orthotopic liver cancer model: implications for immunotherapy for hepatocellular carcinoma. *Liver Cancer* 2019;8:155-71.
16. Zheng Y, Wang T, Tu X, Huang Y, Zhang H, et al. Gut microbiome affects the response to anti-PD-1 immunotherapy in patients with hepatocellular carcinoma. *J Immunother Cancer* 2019;7:193.
17. Kaseb AO, Vence L, Blando J, Yadav SS, Ikoma N, et al. Immunologic correlates of pathologic complete response to preoperative immunotherapy in hepatocellular carcinoma. *Cancer Immunol Res* 2019;7:1390-5.
18. Hong YK, Li Y, Pandit H, Li S, Pulliam Z, et al. Epigenetic modulation enhances immunotherapy for hepatocellular carcinoma. *Cell Immunol* 2019;336:66-74.
19. Zhan C, Ruohoniemi D, Shanbhogue KP, Wei J, Welling TH, et al. Safety of combined yttrium-90 radioembolization and immune checkpoint inhibitor immunotherapy for hepatocellular carcinoma. *J Vasc Interv Radiol* 2020;31:25-34.
20. Wesley JD, Whitmore J, Trager J, Sheikh N. An overview of sipuleucel-T: autologous cellular immunotherapy for prostate cancer. *Hum Vaccin Immunother* 2012;8:520-7.
21. Gao F, Zhu HK, Zhu YB, Shan QN, Ling Q, et al. Predictive value of tumor markers in patients with recurrent hepatocellular carcinoma in different vascular invasion pattern. *Hepatobiliary Pancreat Dis Int* 2016;15:371-7.
22. Shimizu Y, Suzuki T, Yoshikawa T, Endo I, Nakatsura T. Next-generation cancer immunotherapy targeting glypican-3. *Front Oncol* 2019;9:248.
23. Sang M, Wang L, Ding C, Zhou X, Wang B, et al. Melanoma-associated antigen genes - an update. *Cancer Lett* 2011;302:85-90.
24. Castelli C, Rivoltini L, Andreola G, Carrabba M, Renkvist N, et al. T-cell recognition of melanoma-associated antigens. *J Cell Physiol* 2000;182:323-31.
25. Wang K, Liu J, Yan ZL, Li J, Shi LH, et al. Overexpression of aspartyl-(asparaginyl)-beta-hydroxylase in hepatocellular carcinoma is associated with worse surgical outcome. *Hepatology* 2010;52:164-73.
26. Lee JH, Lee Y, Lee M, Heo MK, Song JS, et al. A phase I/IIa study of adjuvant immunotherapy with tumour antigen-pulsed dendritic cells in patients with hepatocellular carcinoma. *Br J Cancer* 2015;113:1666-76.
27. Lee JH, Tak WY, Lee Y, Heo MK, Song JS, et al. Adjuvant immunotherapy with autologous dendritic cells for hepatocellular carcinoma, randomized phase II study. *Oncoimmunology* 2017;6:e1328335.
28. Zhou Y, Liu F, Li C, Shi G, Xu X, et al. Construction and characterization of adenovirus vectors encoding aspartate-beta-hydroxylase to preliminary application in immunotherapy of hepatocellular carcinoma. *J Immunol Res* 2018;2018:9832467.
29. Chen C, Ma YH, Zhang YT, Zhang F, Zhou N, et al. Effect of dendritic cell-based immunotherapy on hepatocellular carcinoma: a systematic review and meta-analysis. *Cytotherapy* 2018;20:975-89.
30. Mo HY, Liao YY, You XM, Cucchetti A, Yuan BH, et al. Timely meta-analysis on the efficacy of adoptive immunotherapy for hepatocellular carcinoma patients after curative therapy. *PLoS One* 2017;12:e0174222.
31. Ding M, Wang Y, Chi J, Wang T, Tang X, et al. Is adjuvant cellular immunotherapy essential after TACE-predominant minimally-invasive treatment for hepatocellular carcinoma? A systematic meta-analysis of studies including 1774 patients. *PLoS One* 2016;11:e0168798.
32. Liu Y, Chen X, Han W, Zhang Y. Tisagenlecleucel, an approved anti-CD19 chimeric antigen receptor T-cell therapy for the treatment of leukemia. *Drugs Today (Barc)* 2017;53:597-608.
33. Gao H, Li K, Tu H, Pan X, Jiang H, Shi B, et al. Development of T cells redirected to glypican-3 for the treatment of hepatocellular carcinoma. *Clin Cancer Res* 2014;20:6418-28.
34. Jiang Z, Jiang X, Chen S, Lai Y, Wei X, et al. Anti-GPC3-CAR T cells suppress the growth of tumor cells in patient-derived xenografts of

- hepatocellular carcinoma. *Front Immunol* 2016;7:690.
35. Wang P, Qin W, Liu T, Jiang D, Cui L, et al. PiggyBac-engineered T cells expressing a glypican-3-specific chimeric antigen receptor show potent activities against hepatocellular carcinoma. *Immunobiology* 2020;225:151850.
 36. Chen C, Li K, Jiang H, Song F, Gao H, et al. Development of T cells carrying two complementary chimeric antigen receptors against glypican-3 and asialoglycoprotein receptor 1 for the treatment of hepatocellular carcinoma. *Cancer Immunol Immunother* 2017;66:475-89.
 37. Guo X, Jiang H, Shi B, Zhou M, Zhang H, et al. Disruption of PD-1 enhanced the anti-tumor activity of chimeric antigen receptor T cells against hepatocellular carcinoma. *Front Pharmacol* 2018;9:1118.
 38. Pan Z, Di S, Shi B, Jiang H, Shi Z, et al. Increased antitumor activities of glypican-3-specific chimeric antigen receptor-modified T cells by coexpression of a soluble PD1-CH3 fusion protein. *Cancer Immunol Immunother* 2018;67:1621-34.
 39. Wu X, Luo H, Shi B, Di S, Sun R, et al. Combined antitumor effects of Sorafenib and GPC3-CAR T cells in mouse models of hepatocellular carcinoma. *Mol Ther* 2019;27:1483-94.
 40. Wang Y, Chen M, Wu Z, Tong C, Dai H, et al. CD133-directed CAR T cells for advanced metastasis malignancies: a phase I trial. *Oncoimmunology* 2018;7:e1440169.
 41. Sun B, Yang D, Dai H, Liu X, Jia R, et al. Eradication of hepatocellular carcinoma by NKG2D-based CAR-T cells. *Cancer Immunol Res* 2019;7:1813-23.
 42. Zhang RY, Wei D, Liu ZK, Yong YL, Wei W, et al. Doxycycline inducible chimeric antigen receptor T cells targeting CD147 for hepatocellular carcinoma therapy. *Front Cell Dev Biol* 2019;7:233.
 43. Yoon JS, Song BG, Lee JH, Lee HY, Kim SW, et al. Adjuvant cytokine-induced killer cell immunotherapy for hepatocellular carcinoma: a propensity score-matched analysis of real-world data. *BMC Cancer* 2019;19:523.
 44. Wang L, Simons DL, Lu X, Tu TY, Solomon S, et al. Connecting blood and intratumoral Treg cell activity in predicting future relapse in breast cancer. *Nat Immunol* 2019;20:1220-30.
 45. Yu R, Yang B, Chi X, Cai L, Liu C, et al. Efficacy of cytokine-induced killer cell infusion as an adjuvant immunotherapy for hepatocellular carcinoma: a systematic review and meta-analysis. *Drug Des Devel Ther* 2017;11:851-64.
 46. Chen CL, Pan QZ, Zhao JJ, Wang Y, Li YQ, et al. PD-L1 expression as a predictive biomarker for cytokine-induced killer cell immunotherapy in patients with hepatocellular carcinoma. *Oncoimmunology* 2016;5:e1176653.
 47. Zhu W, Peng Y, Wang L, Hong Y, Jiang X, et al. Identification of alpha-fetoprotein-specific T-cell receptors for hepatocellular carcinoma immunotherapy. *Hepatology* 2018;68:574-89.
 48. Sun L, Guo H, Jiang R, Lu L, Liu T, et al. Engineered cytotoxic T lymphocytes with AFP-specific TCR gene for adoptive immunotherapy in hepatocellular carcinoma. *Tumour Biol* 2016;37:799-806.
 49. Tan AT, Yang N, Lee Krishnamoorthy T, Oei V, Chua A, et al. Use of expression profiles of HBV-DNA integrated into genomes of hepatocellular carcinoma cells to select T cells for immunotherapy. *Gastroenterology* 2019;156:1862-76.e9.
 50. Qasim W, Brunetto M, Gehring AJ, Xue SA, Schurich A, et al. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient. *J Hepatol* 2015;62:486-91.
 51. Tian W, Ma J, Shi R, Ren C, He J, et al. gammadelta T cell-mediated individualized immunotherapy for hepatocellular carcinoma considering clinicopathological characteristics and immunosuppressive factors. *Oncol Lett* 2018;15:5433-42.
 52. Kamiya T, Chang YH, Campana D. Expanded and activated natural killer cells for immunotherapy of hepatocellular carcinoma. *Cancer Immunol Res* 2016;4:574-81.
 53. Qian L, Wang N, Tian H, Jin H, Zhao H, et al. Dual Effects of cellular immunotherapy in inhibition of virus replication and prolongation of survival in HCV-positive hepatocellular carcinoma patients. *J Immunol Res* 2016;2016:6837241.
 54. Moehler M, Heo J, Lee HC, Tak WY, Chao Y, et al. Vaccinia-based oncolytic immunotherapy Pexastimogene Devacirepvec in patients with advanced hepatocellular carcinoma after sorafenib failure: a randomized multicenter Phase IIb trial (TRAVERSE). *Oncoimmunology* 2019;8:1615817.

Review

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Treatment options for recurrence of hepatocellular carcinoma after surgical resection: review of the literature and current recommendations for management

Francesco Pasini¹, Matteo Serenari¹, Alessandro Cucchetti^{1,2}, Giorgio Ercolani^{1,2}

¹Department of Medical and Surgical Sciences, University of Bologna, Bologna 40138, Italy.

²Department of Surgery, "Morgagni - Pierantoni" Hospital, Forlì 47121, Italy.

Correspondence to: Giorgio Ercolani, Department of Medical and Surgical Sciences - DIMEC, University of Bologna; Via Massarenti 9, Bologna 40138, Italy. E-mail: giorgio.ercolani2@unibo.it

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Abstract

The recurrence rate after primary resection for hepatocellular carcinoma (HCC) has been reported to be up to 80%. There is no consensus or guideline about the best treatment option for such recurrent HCC (rHCC). It is therefore of paramount importance to select patients for suitable treatment due to the high risk of associated morbidity and mortality. In this paper, we review the literature on treatment for rHCC and propose a strategy based on the best evidence available. Even in rHCC, it is still possible to achieve cure and good survival rates through careful patient selection. Repeat hepatectomy is recognized as a feasible and safe procedure even in cirrhotic patients and should be considered as the best option with curative intent when the patient is fit enough. Greater adoption of minimally-invasive liver surgery could have the potential to increase the number of candidate patients with rHCC for repeat resection in the next few years. Liver transplantation offers longer disease-free survival compared to repeat resection, curing the underlying cirrhosis, but is not widely available due to organ shortage. When surgery is not feasible, locoregional treatments such as radiofrequency ablation and transarterial chemoembolization have an important role for patients who cannot tolerate repeat hepatectomy and are not suitable for transplantation. For advanced cases, systemic therapy could be considered.

Keywords: Recurrence, hepatocellular carcinoma, hepatic resection, second resection



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INTRODUCTION

Hepatocellular carcinoma (HCC) is the third most common cause of cancer related death worldwide^[1] and its incidence in Western countries has increased by 75% in the last three decades^[2]. Liver resection represents first-line treatment in patients with early tumors and preserved liver function, with a 5-year overall survival ranging from 60% to 80%. Nonetheless, HCC frequently arises from chronic liver disease and the recurrence rate in the remnant liver, even after R0 resection, has been reported to be up to 80%^[3]. Although guidelines from European, American and Eastern societies recommend hepatic resection as first-line treatment with curative intent for primary HCC^[3-5], none exist for recurrent liver cancers. Liver transplantation, in the case of recurrent HCC (rHCC), would offer the best chance at disease-free survival, by treating both the cancer and the underlying cirrhosis at the same time, but the scarcity of deceased organs limits its application. Furthermore, surgical treatment may not always be feasible and non-surgical options should then be considered.

To date, many papers have been published on the treatment algorithm for rHCC but evidence and consensus are still lacking. These studies are mainly from Eastern centers and almost all are retrospective data.

In this paper, we reviewed the literature on treatment for rHCC and propose our personal strategy based on the available evidence.

TREATMENT OPTIONS FOR RECURRENT HCC

Repeat hepatectomy

Liver resection is recognized as the mainstay of treatment in patients with HCC. In both Eastern and Western countries^[6], it is the first choice option in non-cirrhotic patients who can tolerate resections with low morbidity and mortality^[7,8]. On the other hand, HCC resection in cirrhotic livers requires careful patient selection and adequate surgical skills. Patient selection relies mainly on the preoperative assessment of reserve liver function and portal hypertension. Traditionally, liver function is evaluated through standard liver biochemical tests integrated into several scores such as the Child-Pugh or MELD score^[9], but nowadays, more sophisticated quantitative liver function tests such as indocyanine green retention (ICG) test^[10,11] or hepatobiliary scintigraphy^[12,13], are used in predicting post-hepatectomy liver failure. The presence of clinically relevant portal hypertension can be ruled out by measuring the hepatic vein pressure gradient, or indirectly by liver stiffness, to decrease the risk of postoperative decompensation^[14-16].

According to the BCLC algorithm, patients with a single, very early- or early-stage HCC and preserved liver function should be offered liver resection^[4]. While international guidelines tend to reserve resection for patients harboring early stage HCC, expert institutions have shown good outcomes even in patients with multinodular, large, and macrovascular, invasive HCC^[17], thus justifying expansion of HCC resectability criteria. However, even after potentially curative resections with negative margins, early recurrence accounts for more than 70% of rHCC and occurs within 2 years in 30%-50% of patients. The recurrence rate at 5 years has been reported to range from 50% to 70%^[18-20]. The key parameters related to recurrence include tumour size, multinodular tumours, serum alpha-fetoprotein and microvascular invasion^[21].

Currently, there are no guidelines or clinical algorithms for the best treatment option in rHCC^[22,23] except for expert opinion and local policies. Many authors agree with re-resection as the best therapeutic option for rHCC, when feasible. Improvement in surgical technique and implementation of the use of energy devices have led to a lower incidence of complications. Liver resections are therefore safer in patients with previous resection. Concern still exists however and is mainly related to poor remnant liver function and adhesions caused by previous surgery that could lead to iatrogenic injuries. Many studies have addressed

this topic, showing that repeat hepatectomy (RH) can be performed safely with complication rates comparable to the first resection^[24]. Another issue is the risk of further recurrence, which is dependent on the presence of multinodular cirrhosis in the remnant liver^[25,26].

Most case series of rHCC treated with RH are from Eastern Asia. Since none were randomized trials and patients were selected for resection according to different clinical criteria and hospital policies, comparison is difficult. Nagasue *et al.*^[27] first reported a small series of RH carried out in 9 of 31 patients who experienced tumor recurrence after initial resection of primary HCC. They reported that the survival rate of resected patients was significantly better than that of patients treated with palliative methods. Hu *et al.*^[28] described a retrospective analysis of 59 patients who were treated with RH. 43 had a second recurrence (median follow up 19 months) and survival at three years was 44%. Another paper from the same institution^[29] compared RH to TACE for rHCC and advocated for aggressive surgical treatment after recurrence in selected patients.

Larger series have since been published in the last 20 years, again mainly from Eastern institutions, with 5-year overall survival (OS) ranging from 30% to 60%^[30-33]. In 2011, Roayaie *et al.*^[34] presented the first and largest Western series of RH ($n = 35$), showing an overall 5-year survival rate of 67%. Only patients with a single recurrent tumor on imaging, Child's A liver disease and a platelet count $> 100,000/\text{L}$ underwent RH.

Several studies have been published in recent years and have established RH as safe in referral centers [Table 1]. The largest Eastern series by Zou *et al.*^[35] reported 635 consecutive patients who received a second resection for rHCC. The median OS was 54.8 months and the 1-, 3-, and 5-year OS rates were 96.9%, 74.8%, and 47.8%, respectively. Post-recurrence survival (i.e., calculated from the date of reoperation) rates were 75.8%, 45.7% and 37.6%, respectively. In this large, single institution series, a perioperative complication rate of 22.8% was reported with a median blood loss of 303 mL (range 100-5300). With regard to postoperative complications, a systematic review by Chan *et al.*^[36] reported a median mortality rate of 0% (ranging from 0% to 6%) and a postoperative bleeding rate (with need for transfusions) of 1%. Other postoperative complications (ascites, bile leak, liver failure) were in line with those reported in the literature for patients without preoperative liver surgery.

ROLE OF MINIMALLY-INVASIVE SURGERY

The adoption of laparoscopic liver resection (LLR) has increased over the past decade. Laparoscopy for HCC has been shown in studies to produce superior short-term and equivalent long-term outcomes compared to the open approach. However, due to the formation of intra-abdominal adhesions, LLR for rHCC after previous hepatic resection may represent a challenge. For this reason, there are only a few studies reporting a laparoscopic approach to treat liver recurrence. However, this number is believed to rise in the next few years since liver resection will increasingly be approached laparoscopically. A recent meta-analysis showed that LLR for rHCC offered a benefit in terms of lower in-hospital complication rates, blood loss and a shorter hospital stay compared to open resection, although similar 90-day mortality was observed between the two groups^[45]. This could be partially explained by the fact that, unlike conventional laparotomy, the laparoscopic approach does not require a wide surgical field, thus minimizing the freeing of adhesions and consequently, bleeding as well as other intraoperative complications. Even the scoring system for predicting complications in LLR proposed by Halls *et al.*^[46] showed that a previous open liver resection was the strongest among all independent risk factors (β coefficient = 1.401) of having high blood loss or conversion to an open approach during surgery. This finding should prompt us to at least consider more patients for minimally-invasive surgery at the time of primary resection so as to increase the number of eligible patients who may benefit from a RH in the event of rHCC. LLR has been also demonstrated to facilitate liver transplantation (LT) in terms of decreasing blood loss and transfusion requirements.

Table 1. Review of the literature on the surgical treatment of recurrent hepatocellular carcinoma

Ref.	Year	No of pts	Mortality (%)	5-year OS (%)
Zou <i>et al.</i> ^[35]	2016	635	7	47
Huang <i>et al.</i> ^[37]	2012	85	1	22
Faber <i>et al.</i> ^[38]	2011	27	0	42
Roayaie <i>et al.</i> ^[34]	2011	35	0	67
Kubo <i>et al.</i> ^[39]	2008	51	0	48
Itamoto <i>et al.</i> ^[40]	2007	84	0	50
Tralhão <i>et al.</i> ^[41]	2007	16	1	31
Kobayashi <i>et al.</i> ^[42]	2006	80	0	53
Sun <i>et al.</i> ^[31]	2005	57	0	31
Minagawa <i>et al.</i> ^[33]	2003	67	0	56
Sugimachi <i>et al.</i> ^[43]	2001	78	0	48
Shimada <i>et al.</i> ^[30]	1998	41	NR	45 (3-y)
Hu <i>et al.</i> ^[44]	1996	59	0	44 (3-y)

OS: overall survival

Nevertheless, we acknowledge that this important aspect surely requires further dedicated study and every effort should be made to minimize blood loss and the associated transfusion requirements to improve outcomes in both liver surgery and LT.

Liver transplantation (salvage)

Many transplant centres recommend LT as salvage for rHCC. Salvage LT (SLT) was proposed as an ideal treatment for patients fulfilling the Milan criteria, treating both the cancer and the underlying cirrhosis at the same time^[47]. However, many authors have questioned whether RH could be performed instead of LT. A recent meta-analysis^[48] comparing SLT with RH showed that SLT was inferior to RH with regard to operative and postoperative short-term results, but had better results in terms of overall- and disease-free survival. In fact, in SLT, a more complex operation has to be accounted for, especially in patients who are thought to have more advanced liver cirrhosis. A recent study by Lim *et al.*^[49] showed, in particular, that 90-day mortality was significantly higher in the SLT group compared to the RH group. The negative impact of resection on subsequent LT was also demonstrated in other studies comparing SLT and primary liver transplantation (PLT). Adam *et al.*^[50] first showed that LT after prior liver resection was associated with higher operative mortality and risk of intraoperative bleeding than PLT. Similarly, a recent meta-analysis has demonstrated a significantly higher rate of postoperative bleeding and operative mortality in the SLT group. However, despite the higher perioperative risk, SLT may still achieve better disease-free survival (DFS) rates compared to RH. This has to be expected, given that resection of existing distant micrometastases and removal of the underlying liver disease may prevent de novo HCC development in the remnant liver. A recent meta-analysis^[51] comparing SLT to curative locoregional treatments among seven retrospective studies showed better outcomes with SLT. In particular, SLT was associated with higher 5-year OS and DFS; when compared to RH alone, subgroup analysis still indicated a significantly higher 3- and 5-year DFS for the SLT group. Nevertheless, the authors stated that the feasibility of SLT is impaired due to donor organ shortage.

The decision to proceed with either strategy is clearly biased by institutional practices and for this reason, any comparison between SLT and RH may not be completely reliable. However, it is our opinion that patients should be listed for LT in case of worsening liver function, or any other case such that a second liver resection will not be tolerated. When feasible, any attempt to rescue these patients without affecting the donor pool should be made^[52].

Locoregional treatments

Radiofrequency ablation (RFA), applicable both via the percutaneous or open approach, is considered a safe procedure and as effective in achieving long-term survival as surgical resection, in selected patients^[53].

In particular, RFA is proposed for small primary HCC up to 3 cm in diameter^[54,55]. However, some authors have also proposed RFA for rHCC because of its low morbidity compared to surgical treatments, negligible blood loss and sparing of adjacent normal liver parenchyma^[56]. Furthermore, in patients not amenable to surgical resection due to liver dysfunction, multifocal nodules, tumour location or postsurgical adhesions, RFA represents a potentially curative alternative. In a recent metanalysis, Gavriilidis *et al.*^[57] demonstrated similar 5-year OS and DFS between RFA and RH in treating rHCC although a significant difference in morbidity was reported (2% for RFA vs. 17% for RH). However, a systematic review of 18 previous studies published by Thomasset *et al.*^[58] concluded that RFA for rHCC had a very low rate of complications but was still less efficacious than RH and thus, should be offered only to patients who cannot tolerate surgical resection.

A further treatment option for rHCC is trans-arterial chemoembolization (TACE), although it is not applied with curative intent^[26,59]. A median survival of 30 months and a 3-year survival of 29% has been reported in the treatment of primary HCC with TACE^[60,61]; outcomes reported for rHCC are arguably poorer^[26]. This interventional radiology procedure should be offered to patients who are not candidates for surgical resection, SLT or RFA. Although a recent study reported TACE to be superior to RH and RFA in cases of microvascular involvement^[62], the majority of the literature consider TACE to be palliative^[63] so it is widely used as a treatment for tumours with greater sizes or number of nodules which cannot be treated with RH or RFA. A review from Erridge *et al.*^[64] reported a 5 year survival of 15.5% in patients with rHCC treated with TACE.

Systemic therapies

When recurrence presents beyond the limits of transplantation criteria and is not amenable to locoregional treatment, survival rates are dismal but systemic therapies can still be considered in selected cases. Effective systemic treatment for HCC have been available only in recent years - since sorafenib was introduced, it has become the standard of treatment for advanced HCC^[4,65]. Several compounds have since been tested in this setting but few have proven to be effective: regorafenib was approved as a second-line treatment for patients with HCC disease progression on sorafenib^[66] and levatinib recently showed non-inferiority as first-line therapy^[67]. Since good cardiovascular status and preserved liver function are both necessary for chemotherapy to be tolerated, most reports are on the use of sorafenib in patients with rHCC after LT^[67], given the poor results obtained in the adjuvant setting after resection^[68]. On the contrary, when no clinical benefit is expected from chemotherapy, the management of end-stage rHCC is symptomatic and only supportive care should be offered^[68].

SUMMARY

Herein, we propose our recommendations for selecting the best candidate, and limiting the risk of recurrence in cases of rHCC, based on the above literature review, available guidelines on primary HCC and our personal experiences.

RH

Patients presenting with a single rHCC should undergo repeat resection whenever possible. Candidates for RH are required to have preserved liver function and no or limited signs of portal hypertension. The laparoscopic approach should be considered for RH in rHCC when the requisite expertise is available.

RFA

Patients with preserved liver function and a single rHCC ≤ 3 cm can be considered for RFA. RFA should be preferred over RH, especially in cases of patients with high surgical risk.

TACE

Patients with deteriorated liver function can be treated with TACE. Patients planned for LT should also be considered for TACE as bridging.

SLT

Patients with decompensated liver function, severe portal hypertension and single or even multiple liver recurrences, but still fulfilling LT criteria, may be referred for SLT.

Systemic therapies

In the setting of rHCC not amenable to other treatments due to disease that is far too advanced, systemic therapies can be employed in selected cases.

CONCLUSION

Recurrent HCC may occur after liver resection in up to 80% of cases. A standardized treatment algorithm for rHCC does not exist but surgical resection should be attempted whenever possible, since it may provide favourable long-term outcomes with acceptable perioperative risk comparable to primary resection. In experienced hands, laparoscopic liver resection for both primary and recurrent HCC should be considered. LT should be considered in all cases where surgical resection cannot be performed, especially in the context of underlying cirrhosis, if conventional transplantation criteria are met. The use of RFA or TACE as a bridge to LT, or for palliation represent alternatives for these patients.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception of the paper, reviewed the literature and drafted the manuscript; equally contributed to the work: Pasini F, Serenari M
Supervised the work, reviewed the manuscript, provided technical support and expert guidance: Cucchetti A, Ercolani G

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.

2. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al.; Global Burden of Disease Liver Cancer Collaboration. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level results from the global burden of disease study 2015. *JAMA Oncol* 2017;3:1683-91.
3. Kokudo N, Takemura N, Hasegawa K, Takayama T, Kubo S, et al. Clinical practice guidelines for hepatocellular carcinoma: the Japan Society of Hepatology 2017 (4th JSH-HCC guidelines) 2019 update. *Hepatol Res* 2019;49:1109-13.
4. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236.
5. Bruix J, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53:1020-2.
6. Bralet MP, Régimbeau JM, Pineau P, Dubois S, Loas G, et al. Hepatocellular carcinoma occurring in nonfibrotic liver: epidemiologic and histopathologic analysis of 80 French cases. *Hepatology* 2000;32:200-4.
7. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011;365:1118-27.
8. Llovet JM, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999;30:1434-40.
9. Cucchetti A, Ercolani G, Vivarelli M, Cescon M, Ravaioli M, et al. Impact of model for end-stage liver disease (MELD) score on prognosis after hepatectomy for hepatocellular carcinoma on cirrhosis. *Liver Transpl* 2006;12:966-71.
10. Miyagawa S, Makuuchi M, Kawasaki S, Kakazu T. Criteria for safe hepatic resection. *Am J Surg* 1995;169:589-94.
11. Seyama Y, Kokudo N. Assessment of liver function for safe hepatic resection. *Hepatol Res* 2009;39:107-16.
12. Serenari M, Collaud C, Alvarez FA, de Santibañes M, Giunta D, et al. Interstage assessment of remnant liver function in alpps using hepatobiliary scintigraphy: prediction of posthepatectomy liver failure and introduction of the HIBA index. *Ann Surg* 2018;267:1141-7.
13. Serenari M, Pettinato C, Bonatti C, Zanoni L, Odaldi F, et al. Hepatobiliary scintigraphy in the preoperative evaluation of potential living liver donors. *Transplant Proc* 2019;51:167-70.
14. Cucchetti A, Cescon M, Golfieri R, Piscaglia F, Renzulli M, et al. Hepatic venous pressure gradient in the preoperative assessment of patients with resectable hepatocellular carcinoma. *J Hepatol* 2016;64:79-86.
15. Qi X, Zhang X, Li Z, Hui J, Xiang Y, et al. HVPG signature: A prognostic and predictive tool in hepatocellular carcinoma. *Oncotarget* 2016;7:62789-96.
16. Cescon M, Colecchia A, Cucchetti A, Peri E, Montrone L, et al. Value of transient elastography measured with FibroScan in predicting the outcome of hepatic resection for hepatocellular carcinoma. *Ann Surg* 2012;256:706-12.
17. Torzilli G, Belghiti J, Kokudo N, Takayama T, Capussotti L, et al. Reply to Letter: "A snapshot of the effective indications and results of surgery for hepatocellular carcinoma in tertiary referral centers: is it adherent to the EASL/AASLD recommendations? An observational Study of the HCC East-West Study Group": when the study setting "ignores" the patients. *Ann Surg* 2015;262:e30-1.
18. Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003;38:200-7.
19. Meniconi RL, Komatsu S, Perdigao F, Boëlle PY, Soubrane O, et al. Recurrent hepatocellular carcinoma: a Western strategy that emphasizes the impact of pathologic profile of the first resection. *Surgery* 2015;157:454-62.
20. Ercolani G, Grazi GL, Ravaioli M, Del Gaudio M, Gardini A, et al. Liver resection for hepatocellular carcinoma on cirrhosis: univariate and multivariate analysis of risk factors for intrahepatic recurrence. *Ann Surg* 2003;237:536-43.
21. Xing H, Zhang WG, Cescon M, Liang L, Li C, et al. Defining and predicting early recurrence after liver resection of hepatocellular carcinoma: a multi-institutional study. *HPB (Oxford)* 2019.
22. Joliat GR, Allemann P, Labgaa I, Demartines N, Halkic N. Treatment and outcomes of recurrent hepatocellular carcinomas. *Langenbecks Arch Surg* 2017;402:737-44.
23. Erridge S, Pucher PH, Markar SR, Malietzis G, Athanasiou T, et al. Meta-analysis of determinants of survival following treatment of recurrent hepatocellular carcinoma. *Br J Surg* 2017;104:1433-42.
24. Wu CC, Cheng SB, Yeh DC, Wang J, P'eng FK. Second and third hepatectomies for recurrent hepatocellular carcinoma are justified. *Br J Surg* 2009;96:1049-57.
25. Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003;38:200-7.
26. Tabrizian P, Jibara G, Shrager B, Schwartz M, Roayaie S. Recurrence of hepatocellular cancer after resection: patterns, treatments, and prognosis. *Ann Surg* 2015;261:947-55.
27. Nagasue N, Yukaya H, Ogawa Y, Sasaki Y, Chang YC, et al. Second hepatic resection for recurrent hepatocellular carcinoma. *Br J Surg* 1986;73:434-8.
28. Hu RH, Lee PH, Yu SC, Dai HC, Sheu JC, et al. Surgical resection for recurrent hepatocellular carcinoma: prognosis and analysis of risk factors. *Surgery* 1996;120:23-9.
29. Lee PH, Lin WJ, Tsang YM, Hu RH, Sheu JC, et al. Clinical management of recurrent hepatocellular carcinoma. *Ann Surg* 1995;222:670-6.
30. Shimada M, Takenaka K, Taguchi K, Fujiwara Y, Gion T, et al. Prognostic factors after repeat hepatectomy for recurrent hepatocellular carcinoma. *Ann Surg* 1998;227:80-5.
31. Sun HC, Tang ZY, Ma ZC, Qin LX, Wang L, et al. The prognostic factor for outcome following second resection for intrahepatic recurrence of hepatocellular carcinoma with a hepatitis B virus infection background. *J Cancer Res Clin Oncol* 2005;131:284-8.
32. Huang ZY, Liang BY, Xiong M, Zhan DQ, Wei S, et al. Long-term outcomes of repeat hepatic resection in patients with recurrent hepatocellular carcinoma and analysis of recurrent types and their prognosis: a single-center experience in China. *Ann Surg Oncol*

- 2012;19:2515-25.
33. Minagawa M, Makuuchi M, Takayama T, Kokudo N. Selection criteria for repeat hepatectomy in patients with recurrent hepatocellular carcinoma. *Ann Surg* 2003;238:703-10.
34. Roayaie S, Bassi D, Tarchi P, Labow D, Schwartz M. Second hepatic resection for recurrent hepatocellular cancer: a Western experience. *J Hepatol* 2011;55:346-50.
35. Zou Q, Li J, Wu D, Yan Z, Wan X, et al. Nomograms for pre-operative and post-operative prediction of long-term survival of patients who underwent repeat hepatectomy for recurrent hepatocellular carcinoma. *Ann Surg Oncol* 2016;23:2618-26.
36. Chan DL, Morris DL, Chua TC. Clinical efficacy and predictors of outcomes of repeat hepatectomy for recurrent hepatocellular carcinoma - a systematic review. *Surg Oncol* 2013;22:e23-30.
37. Huang ZY, Liang BY, Xiong M, Zhan DQ, Wei S, et al. Long-term outcomes of repeat hepatic resection in patients with recurrent hepatocellular carcinoma and analysis of recurrent types and their prognosis: a single-center experience in China. *Ann Surg Oncol* 2012;19:2515-25.
38. Faber W, Seehofer D, Neuhaus P, Stockmann M, Denecke T, et al. Repeated liver resection for recurrent hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011;26:1189-94.
39. Kubo S, Takemura S, Uenishi T, Yamamoto T, Ohba K, et al. Second hepatic resection for recurrent hepatocellular carcinoma in patients with chronic hepatitis C. *World J Surg* 2008;32:632-8.
40. Itamoto T, Nakahara H, Amano H, Kohashi T, Ohdan H, et al. Repeat hepatectomy for recurrent hepatocellular carcinoma. *Surgery* 2007;141:589-97.
41. Tralhão JG, Dagher I, Lino T, Roudié J, Franco D. Treatment of tumour recurrence after resection of hepatocellular carcinoma. Analysis of 97 consecutive patients. *Eur J Surg Oncol* 2007;33:746-51.
42. Kobayashi A, Kawasaki S, Miyagawa S, Miwa S, Noike T, et al. Results of 404 hepatic resections including 80 repeat hepatectomies for hepatocellular carcinoma. *Hepatogastroenterology* 2006;53:736-41.
43. Sugimachi K, Maehara S, Tanaka S, Shimada M, Sugimachi K. Repeat hepatectomy is the most useful treatment for recurrent hepatocellular carcinoma. *J Hepatobiliary Pancreat Surg* 2001;8:410-6.
44. Hu RH, Lee PH, Yu SC, Dai HC, Sheu JC, et al. Surgical resection for recurrent hepatocellular carcinoma: prognosis and analysis of risk factors. *Surgery* 1996;120:23-9.
45. Cai W, Liu Z, Xiao Y, Zhang W, Tang D, et al. Comparison of clinical outcomes of laparoscopic versus open surgery for recurrent hepatocellular carcinoma: a meta-analysis. *Surg Endosc* 2019;33:3550-7.
46. Halls MC, Berardi G, Cipriani F, Barkhatov L, Lainas P, et al. Development and validation of a difficulty score to predict intraoperative complications during laparoscopic liver resection. *Br J Surg* 2018;105:1182-91.
47. Majno PE, Sarasin FP, Mentha G, Hadengue A. Primary liver resection and salvage transplantation or primary liver transplantation in patients with single, small hepatocellular carcinoma and preserved liver function: an outcome-oriented decision analysis. *Hepatology* 2000;31:899-906.
48. Kostakis ID, Machairas N, Prodromidou A, Stamopoulos P, Garoufalia Z, et al. Comparison between salvage liver transplantation and repeat liver resection for recurrent hepatocellular carcinoma: a systematic review and meta-analysis. *Transplant Proc* 2019;51:433-6.
49. Lim C, Shinkawa H, Hasegawa K, Bhangui P, Salloum C, et al. Salvage liver transplantation or repeat hepatectomy for recurrent hepatocellular carcinoma: An intent-to-treat analysis. *Liver Transpl* 2017;23:1553-63.
50. Adam R, Azoulay D, Castaing D, Eshkenazy R, Pascal G, et al. Liver resection as a bridge to transplantation for hepatocellular carcinoma on cirrhosis: a reasonable strategy? *Ann Surg* 2003;238:508-18.
51. Wang HL, Mo DC, Zhong JH, Ma L, Wu FX, et al. Systematic review of treatment strategy for recurrent hepatocellular carcinoma: Salvage liver transplantation or curative locoregional therapy. *Medicine (Baltimore)* 2019;98:e14498.
52. Lucidi V, Gustot T, Moreno C, Donckier V. Liver transplantation in the context of organ shortage: toward extension and restriction of indications considering recent clinical data and ethical framework. *Curr Opin Crit Care* 2015;21:163-70.
53. Taura K, Ikai I, Hatano E, Fujii H, Uyama N, et al. Implication of frequent local ablation therapy for intrahepatic recurrence in prolonged survival of patients with hepatocellular carcinoma undergoing hepatic resection: an analysis of 610 patients over 16 years old. *Ann Surg* 2006;244:265-73.
54. Lü MD, Kuang M, Liang LJ, Xie XY, Peng BG, et al. Surgical resection versus percutaneous thermal ablation for early-stage hepatocellular carcinoma: a randomized clinical trial. *Zhonghua Yi Xue Za Zhi* 2006;86:801-5. (in Chinese)
55. Chen MS, Li JQ, Zheng Y, Guo RP, Liang HH, et al. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006;243:321-8.
56. Chan AC, Poon RT, Cheung TT, Chok KS, Chan SC, et al. Survival analysis of re-resection versus radiofrequency ablation for intrahepatic recurrence after hepatectomy for hepatocellular carcinoma. *World J Surg* 2012;36:151-6.
57. Gavriilidis P, Askari A, Azoulay D. Survival following redo hepatectomy vs radiofrequency ablation for recurrent hepatocellular carcinoma: a systematic review and meta-analysis. *HPB (Oxford)* 2017;19:3-9.
58. Thomasset SC, Dennison AR, Garcea G. Ablation for recurrent hepatocellular carcinoma: a systematic review of clinical efficacy and prognostic factors. *World J Surg* 2015;39:1150-60.
59. Wang K, Liu G, Li J, Yan Z, Xia Y, et al. Early intrahepatic recurrence of hepatocellular carcinoma after hepatectomy treated with re-hepatectomy, ablation or chemoembolization: a prospective cohort study. *Eur J Surg Oncol* 2015;41:236-42.
60. Malagari K, Alexopoulou E, Chatzimichail K, Hall B, Koskinas J, et al. Transcatheter chemoembolization in the treatment of HCC in patients not eligible for curative treatments: midterm results of doxorubicin-loaded DC bead. *Abdom Imaging* 2008;33:512-9.

61. Llovet JM, Real MI, Montaña X, Planas R, Coll S, et al.; Barcelona Liver Cancer Group. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002;359:1734-9.
62. Jin YJ, Lee JW, Lee OH, Chung HJ, Kim YS, et al. Transarterial chemoembolization versus surgery/radiofrequency ablation for recurrent hepatocellular carcinoma with or without microvascular invasion. *J Gastroenterol Hepatol* 2014;29:1056-64.
63. Takayasu K, Arii S, Ikai I, Kudo M, Matsuyama Y, et al.; Liver Cancer Study Group of Japan. Overall survival after transarterial lipiodol infusion chemotherapy with or without embolization for unresectable hepatocellular carcinoma: propensity score analysis. *AJR Am J Roentgenol* 2010;194:830-7.
64. Erridge S, Pucher PH, Markar SR, Malietzis G, Athanasiou T, et al. Meta-analysis of determinants of survival following treatment of recurrent hepatocellular carcinoma. *Br J Surg* 2017;104:1433-42.
65. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al.; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-90.
66. Bruix J, Qin S, Merle P, Granito A, Huang YH, et al.; RESORCE Investigators. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;389:56-66.
67. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018;391:1163-73.
68. Kumar M, Panda D. Role of supportive care for terminal stage hepatocellular carcinoma. *J Clin Exp Hepatol* 2014;4:S130-9.

Review

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Mechanisms and immunotherapies of HBV- and NAFLD-related hepatocellular carcinoma

Xiao-Jia Song, Chun-Hong Ma

Key Laboratory for Experimental Teratology of Ministry of Education and Department of Immunology, School of Basic Medical Science, Shandong University, Jinan 250012, Shandong, China.

Correspondence to: Prof. Chun-Hong Ma, Key Laboratory for Experimental Teratology of Ministry of Education and Department of Immunology, School of Basic Medical Science, Shandong University, No. 44 Wenhua Xi Road, Jinan 250012, Shandong, China. E-mail: machunhong@sdu.edu.cn

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Abstract

Hepatitis B virus (HBV) infection remains the most important risk factor for hepatocellular carcinoma (HCC) worldwide and nonalcoholic fatty liver disease (NAFLD) has developed as major etiology of chronic liver diseases, cirrhosis and eventually HCC in the last decades. Although nucleos(t)ide analogs are recommended as the first-line drug for patients with chronic hepatitis B, incomplete eradication of HBV serves as an obstacle for effective cure of chronic hepatitis B and even HCC. NAFLD refers to a spectrum of hepatic metabolic disorders, compromised with multi-system diseases. Considering the specificity of hepatocytes and enrichment of immune cells in liver, this review aims to summarize the mechanisms of direct pro-tumorigenesis to hepatocytes induced by HBV infection and abnormal lipid metabolism, and indirect oncogenic processes mediated by immune cells. We also discuss similarities and differences of immune cells between HBV- and NAFLD-HCC and finally focus on the novel immunotherapies concerning preclinical and clinical studies for liver cancer.

Keywords: Hepatitis B virus, nonalcoholic fatty liver disease, hepatocellular carcinoma, immune cells, immunotherapy

INTRODUCTION

Liver cancer is one of the most common causes of cancer-related deaths worldwide, with an estimated of 1.1 million new cases to be diagnosed in 10 years (GLOBOCAN, 2018). Hepatocellular carcinoma (HCC) accounts for nearly 80% of primary liver cancer and the occurrence is globally gender and geography biased. The estimated age-standardized incidence rates in men are mostly 3 times than that of women (13.9/1,000,000 vs. 4.9/1,000,000) in 2018. The high rates in Pacific Asia, West and Central Africa have persisted for decades, although declining trends have been observed in China and Japan in the past two



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decades due to therapy advancement and control of etiology. In contrast, incidence and mortality rates in North America have been increasing since 1978, for which the main reasons are cumulative emergence of new risk factors and lack of efficient therapy strategies.

HCC is prominently induced by chronic hepatitis B virus (HBV)/hepatitis C virus (HCV) infection, alcoholic or nonalcoholic fatty liver disease, aflatoxin intake and parasitic infection, the incidence of which are related with geographic variance. About 80% of HCC arises from persistent HBV infection in Asia-Pacific and Africa countries, while non-alcoholic fatty liver disease (NAFLD) is the major contributor in North America, followed by HCV infection. The direct-acting antiviral therapy with high rates of safety and sustained virologic response (> 95%), is considered as the contributing factor for eliminating HCV^[1,2], which helps decrease the pathogenesis of HCC. However, HBV-associated HCC remains to be a global problem, because current nucleos(t)ide analogue therapy functionally reduces HBsAg level and suppresses HBV DNA but fails to control remission^[3,4]. Since obesity has emerged as a new global burden, NAFLD, which is one of the most common metabolic syndrome manifesting in liver with estimated prevalence of 20%~30% in many countries, will rank as the leading cause of HCC in future decades^[5-9].

Although patients diagnosed with early stage of HCC undergo surgical resection or radiofrequency ablation for curative intent, up to 70% of them are susceptible to recurrence within 5 years^[10]. During the last decades, conventional chemotherapy and combination of transarterial chemoembolization and sorafenib have been developed for patients at intermediate and advanced stages, however, therapy efficacy and adverse effects should be further assessed. Although sorafenib has been approved for treatment of HCC, the tyrosine kinase inhibitors-induced adverse events and drug resistance still remain an issue during clinical management^[11]. Therefore, more novel target agents and immunotherapies are being investigated and incorporated to achieve better outcomes^[12,13]. Here, this review mainly discusses the current progress in molecular and cellular mechanisms of HBV- and NAFLD-related HCC respectively, specifically the immune responses in liver, along with current immunotherapy options.

DIRECT PRO-ONCOGENIC MECHANISMS

HBV

Genomic features are significantly different in the group of HBV-HCC compared with non-infected patients. These results suggest that HBV-related HCC use alternative mechanisms for tumorigenesis to some extent. A high frequency of p53 inactivation and stem cell genes overexpression provide a potential pathogenic link between impaired cell reprogramming and HBV infection. Notably, these observations have clinical implication since TP53 mutations were associated with poor prognosis only in HBV-related tumors.

Virally oncogenic proteins

HBV genome contains four overlapping open reading frames, including preS1/preS2/S, preCore/Core, X and Pol, encoding viral proteins. Among them, hepatitis B X protein (HBx) is the most important oncogenic protein. Mutated or deleted HBx is frequently detected in HCC and plays critical role in liver carcinogenesis^[14-16]. Although it is still debated, a growing body of evidence suggests that both wild type HBx and truncated HBx promote tumorigenesis by abrogating cell-cycle arrest and apoptosis inhibition^[17-19]. HBx promotes HCC progression mainly through interaction with host factors including proteins and non-coding RNAs. HBx mutants interact with Bcl-2 and farnesoid X receptor leading to enhanced carcinogenesis^[20-22]. HBx-cortactin (CTTN) interaction promotes HCC progression by up-regulating expression of CREB1 and its downstream targets, cyclin D1 and MMP9^[23]. As a major conserved cellular pathway that controls critical cell processes, the ubiquitin proteasome system is often hijacked by HBx, leading to dysregulated ubiquitination. HBx stabilizes critical transcriptional oncoproteins Myc and PAX8 via blocking Skp2-mediated ubiquitination^[24,25]. Silencing lncRNA-MALAT1/miR-124 axis or miR-5188-FOXO1/ β -catenin-c-Jun feedback loop significantly block HBx-mediated upregulating stemness markers and reprogramming

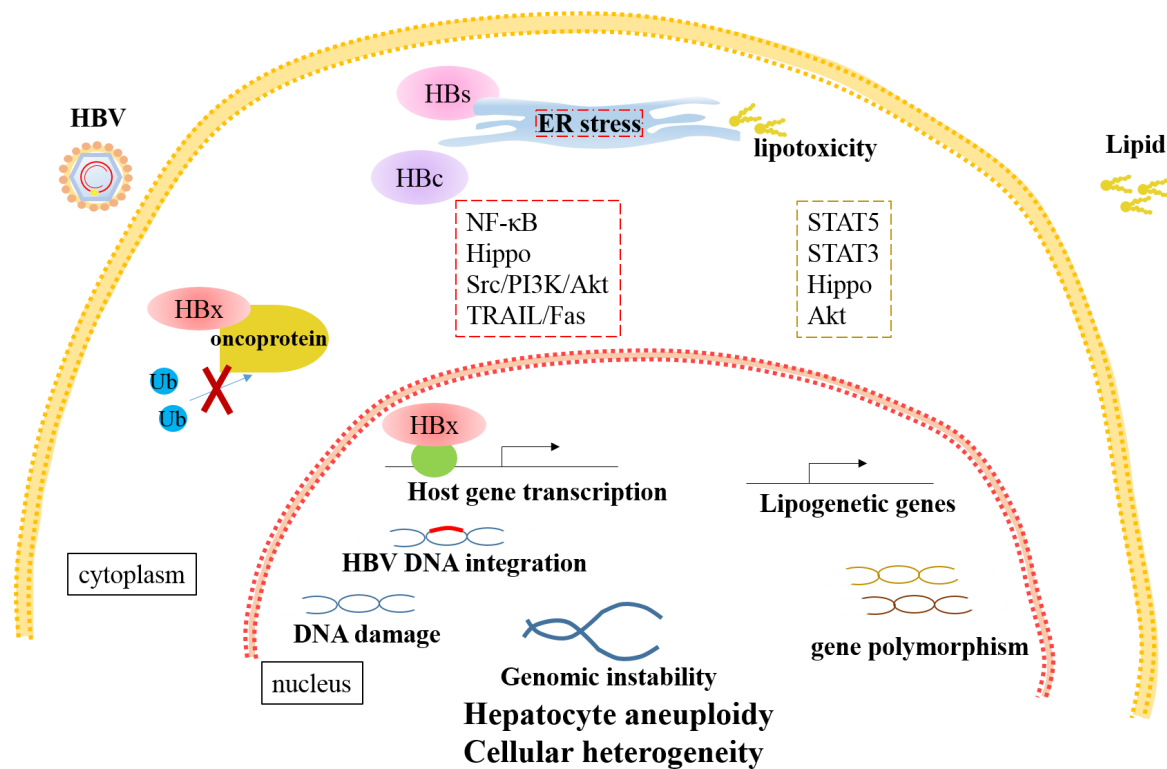


Figure 1. Direct oncogenic mechanisms of HBV- and NAFLD- HCC. HBV-encoded proteins lead to cellular heterogeneity via multiple manners. HBx stabilizes oncoproteins by suppressing ubiquitination or regulates transcription of host genes through transactivation. Cytoplasmic accumulation of HBs induces ER stress and thereby initiates cellular hippo pathways. Besides ER response, NF-κB, Src/PI3K/Akt and TRAIL/Fas pathways are involved in HBc-induced abnormal proliferation and metabolism of hepatocytes. Furthermore, HBV DNA integration and viral protein result in DNA damage and genomic instability, which drive cellular heterogeneity. Excessive lipid accumulation stimulates ER stress and dysregulates apoptosis and fibrogenesis via STAT3 and hippo pathways. The STAT5 and Akt pathways are involved in upregulation of the lipogenic genes and downstream targets. Moreover, gene polymorphism is significantly related with NAFLD progression. HBV: hepatitis B virus; NAFLD: nonalcoholic fatty liver disease; HCC: hepatocellular carcinoma; ER: endoplasmic reticulum

proteins in HCC^[26,27]. Besides, HBx targets genes at transcriptional level. HBx complexed with HDAC1 binds to glycogen synthase 2 (GYS2) and transcriptionally inhibit the expression^[28] [Figure 1].

As the most abundant proteins, HBV surface (HBs) proteins are composed of three proteins, the large (L), middle (M), and small (S) HBs, encoded by the preS1/preS2/S gene respectively^[29-31]. Particular deletion mutants in preS2 have been reported to be associated with progression of chronic liver diseases. Mutated HBs accumulated in hepatocytes induce endoplasmic reticulum (ER) stress and initiate multiple cellular signal pathways, leading to cell growth advantages^[32,33]. Intrahepatic LHBs induce DNA damage and up-regulation of Plk1 to provoke hyperploidy, which disrupts the genomic stability of host cells^[34]. Pre-S2 deletion mutant also promote carcinogenesis by up-regulating expression of various oncogenes, including hTERT, TAZ in hippo pathways, and GPC3^[35-38] [Figure 1].

As the major capsid protein and important viral replication mediator, HB core-related antigen (HBcrAg) is reported to be an independent risk factor for HCC development^[39]. Mechanistically, hepatitis B core protein (HBc) function as an immunogen as well as an important mediator of hepatocarcinogenesis via several mechanisms. HBc mutations P5T/H/L stimulate ER response, further increasing production of radical oxidative species (ROS) and activating NF-κB signaling pathway, which directly promote infected hepatocytes to malignant transformation. Furthermore, HBc promotes proliferation, glycolysis and amino acid metabolism or suppress apoptosis via regulating Src/PI3K/Akt pathway, blocking TRAIL/Fas pathway or expression of p53 in transcriptional/post-transcriptional manner^[40-44] [Figure 1].

HBV DNA integration

HBV DNA integration occurs early once HBV entering into hepatocytes and remains stable throughout tumor progression, which explains the existence of monoclonal or polyclonal origins of HCC from the view of virus infection^[45,46]. HBV DNA susceptible integrates into rare fragile sites or functional genomic regions, which are proximate to protein coding or non-coding genes. Functionally, the protein-coding genes are deeply associated with tumorigenesis and the non-coding genes are involved in telomere maintenance, protein modification processes, and chromosome localization^[47-49]. For example, integration drives mutation in tumor suppressor gene *ZNF717* and over-expression of critical oncogene *c-MYC*. Meanwhile, integration into intron of cyclin A2 generates a pseudo-exon forming a chimeric fusion with *CCNA2*, which encodes protein promoting cell cycle progression^[46,50,51]. Moreover, HBV DNA integration is closely correlated with gender-bias and recurrence of HCC. Androgen receptor, but not estrogen receptor, enhances transcription responsive of TERT promoter with HBV integration to sex hormones via hepatocyte nuclear factor 4 alpha (HNF4α)- dependent manner^[52]. Multiple studies have identified fragile integration sites more in non-cancerous tissue than those in neoplastic tissues, which implies tumor recurrence^[48,53].

Genomic instability

In infected hepatocytes, both HBV DNA integration and viral proteins lead to genomic instability. Sequencing analysis of 373 liver cancer samples demonstrated ultra-high structural instability and preserved un-methylation in HBV integrated regions^[54]. HBx directly induces genomic instability via inhibiting mono-ubiquitylation of an evolutionarily conserved E3 ligase and impairing homologous recombination, which contribute to tumorigenesis^[55]. LHBs are the major viral protein leading to genomic instability. By inducing DNA damage and G2/M checkpoint failure, LHBs promote formation of hepatocyte aneuploidy and further self-propagating cycles of chromosomal instability, which drives cellular heterogeneity and clonal cancer evolution^[56,57].

NAFLD

NAFLD and its complication nonalcoholic steatohepatitis (NASH) are becoming the leading cause of HCC. Multiple pathways, such as abnormal metabolism, dysbiosis of gut microbiota and dysregulated immune responses, are involved in NAFLD initiated hepatocarcinogenesis and have been well summarized recently^[58]. Here, we mainly focus on dysregulated pathway mediated by lipid accumulation which is fundamental in progression of NAFLD related HCC. The abnormal intrahepatic lipid metabolism invokes insulin resistance, alteration of signaling pathways and oncogenes, followed by inflammation, fibrogenesis and hepatocarcinogenesis. In clinical samples, level of p-STAT5 is positively correlated with expression of sterol regulatory element binding protein-1 (SREBP1) and further *in vivo* and *in vitro* essays demonstrate that mTORC1 interacts and phosphorylates STAT5 to upregulate expression of lipogenetic genes, including *SREBP1*, fatty acid synthase (*FASN*) and acetyl-CoA carboxylase (*ACC*)^[59]. Both oxidative and ER stress caused by chronic lipotoxicity play critical roles in NAFLD-HCC. Oxidized LDL (oxLDL) uptake triggers CEBPβ expression to directly upregulate Nogo-B, an ER-residential protein, and promote lipophagy leading to lysophosphatidic acid-enhanced YAP oncogenic activity^[60]. In addition to hippo signaling pathway, STAT3-mediated pathways are frequently involving in pathogenesis of NAFLD-HCC, which mediated lipid accumulation, apoptosis and fibrogenesis^[61-66].

Other than metabolic disorder, gene polymorphism is significantly responsible for NAFLD. Recent genome-wide association studies (GWASs) in European ancestry suggest a robust relationship of *PNPLA3* gene cluster with NAFLD activity, progression to HCC and liver-associated death^[67-69]. This study also demonstrated an association of novel loci near *IL17RA* and *ZFP90-CDH1* with NAFLD disease severity and fibrosis^[67]. rs368234815 variant in *IFNL4* and *FNDC5* rs3480 polymorphism are respectively identified to be associated with liver damage and fibrosis in patients with NAFLD^[70,71]. In addition, gender-bias is as well observed in NAFLD-HCC. As the confirmed risk factor for male HCC, androgen receptor (AR) transcription activity is enhanced by key enzymes and specific unsaturated fatty acid produced in lipogenesis via activation of Akt kinase^[72] [Figure 1].

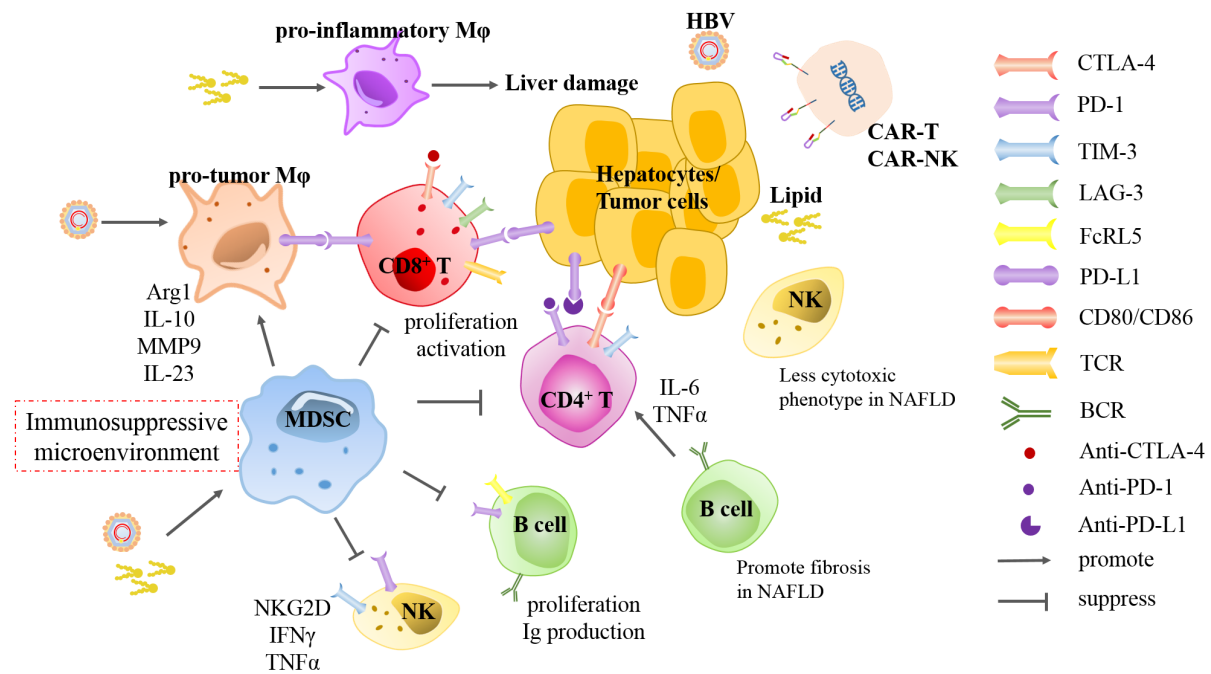


Figure 2. Alteration of immune cells in HBV- and NAFLD- HCC and corresponding immunotherapies. In HBV-infected liver microenvironment, MDSCs are recruited and macrophages are educated to polarization of pro-tumor phenotype, which constitute immunosuppressive microenvironment. However, excessive lipid accumulation and lipotoxic hepatocytes induce macrophages to pro-inflammatory phenotype which promotes liver damage. In both etiologies- associated HCC, cytotoxic T cells and NK cells inhibit progression of tumor formation through cytokines secretion or phenotype switch. B cells control progression of HBV infection by antibodies production and cytokines secretion, whereas promote NAFLD development towards fibrosis via inducing CD4⁺ T cells. Overall, activation and function of effector immune cells are suppressed by MDSCs and macrophages through interaction of checkpoint molecules, such as PD-1, PD-L1, CTLA-4, TIM-3 and LAG-3. Furthermore, several drugs blocking inhibitory checkpoint receptors have been developed and approved for treatment of cancer diseases. Collaboratively, CAR-T and CAR-NK cell therapy are investigated to be promising in treatment of liver cancer. HBV: hepatitis B virus; NAFLD: nonalcoholic fatty liver disease; HCC: hepatocellular carcinoma; MDSCs: myeloid-derived suppressor cells; CAR: chimeric antigen receptor; NK: natural killer; Mφ: macrophage; Arg1: arginase 1; MMP9: matrix metalloproteinase 9; IL: interleukin; IFN: interferon; TNF-α: tumor necrosis factor-alpha; TCR: T-cell receptor; BCR: B-cell receptor

ALTERATION OF IMMUNE MICROENVIRONMENT

Multiple resident or migratory immune cells, including innate and adaptive immune cells, are responsible for maintaining immune homeostasis in the liver. In addition, the dysregulation of hepatic immune cells play critical roles in liver cancer pathogenesis. Liver is rich of innate immune cells including myeloid cells and innate immune cells. The myeloid cells contain macrophages, dendritic cells and myeloid-derived suppressor cells (MDSCs). Liver innate lymphocytes are represented by natural killer (NK) cells, NKT cells, mucosal associated invariant T cells and $\gamma\delta$ T cells. CD4⁺ T, CD8⁺ T and B cells comprise major adaptive cells in liver^[73]. Both virus infection and metabolic disorder invoke the immune environment from tolerogenic status to active inflammation, leading to further cellular injury, fibrosis and eventually hepatocarcinogenesis. Moreover, various subtype immune cells and related markers in tumor microenvironment are significantly predictors for clinical outcome of patients^[74]. Here, this part will discuss the similarities and differences of immune cells responses between HBV and NAFLD-associated HCC [Figure 2].

Macrophages

Macrophages comprise almost 20% of overall immune cells in liver, containing residential Kupffer cells (KCs) and migratory monocyte-derived macrophages. Hepatic macrophages are extremely plastic and accommodative to signals from the microenvironment. Both inflammatory stimuli and viral proteins reprogram macrophages towards M2-like tumor macrophages, which in turn promote HCC progression^[75-77]. In chronic HBV-infected tissue, macrophages are manipulated by HBV for persistent maintenance in liver.

HBV suppresses pattern recognition receptors (PRRs) sensing or downstream interferon (IFN) response in hepatocytes but activate macrophages to secrete cytokines at high titer^[78]. Furthermore, HBc protein is detected within macrophages in liver tissues from HBV-infected patients. Macrophages exposed to HBV or HBV-producing cells are prone to secrete more IL-10, which subsequently impairs lymphocyte activation, but less pro-inflammatory cytokines such as IL-6 and IL-1 β with property of inhibition on HBV infection^[76]. Accordingly, HBV drives macrophages to be suppressive immune cells. In offspring with horizontal transmission of HBV, HBeAg induces up-regulation of checkpoint molecular PD-L1 on macrophage and polarization to M2 protumor subtype, which impairs responses of CD8⁺ T cell to HBV and lead to virus persistence^[79]. Moreover, macrophages produce matrix metalloproteinase 9 (MMP9) and IL-23 under the stimulation of HBV, which blocks binding of IFN- α to IFNAR1 and facilitate tumor angiogenesis and progression^[80,81].

Contrarily, pro-inflammatory macrophages are essential for progression of NASH-associated HCC. In a high-fat diet (HFD) zebrafish model, hepatic macrophage morphology is changed and number of TNF- α positive macrophages is increased, and liver size is subsequently reduced in macrophage-ablation NAFLD/NASH associated HCC larvae but not in HCC or NAFLD alone^[82]. The process from NAFLD to HCC is proximately accompanied by inflammation and fibrosis, in which, innate and adaptive immune cells are enriched and trafficking in liver. A recent study demonstrates that KCs induce recruitment of platelet in early stage of NAFLD, which is associated with hepatic injury. Furthermore, antiplatelet treatment abrogates infiltration of CD8⁺ T cells, Ly6G⁺ granulocytes, especially CD11b⁺ F4/80⁺ monocyte-derived macrophages and KCs, which alleviates the progression of carcinogenesis^[83]. Mechanistically, excessive toxic lipids and cholesterol crystals accumulate in macrophages, forcing cells polarization to pro-inflammatory phenotype, and depletion of KCs dampens release of pro-inflammatory cytokines and liver damage^[84-87]. Meanwhile, lipotoxic hepatocytes activate macrophages polarization via releasing exosomes containing miR-192-5p^[88]. Generally, NF- κ B and JNK signaling pathways mainly mediate the activation of macrophages towards the pro-inflammatory phenotype. Stimulator of interferon genes (STING) invokes the polarization of macrophage to produce TNF- α and IL-1 β via JNK/NF- κ B pathways, which further modulate increase lipid deposition in hepatocytes and exacerbate progression of NAFLD^[89].

MDSCs

MDSCs, generated as immature myeloid cells from bone marrow, are the major immunosuppressive cells which inhibit T cells proliferation and function and enhance induction of Treg cells and tumor-associated macrophages in chronic inflammation and cancers. Multiple studies have reported that MDSCs are closely related with HBV persistence and HCC. In patients with chronic HBV infection (CHB), HBeAg induces expansion of monocytic MDSCs (mMDSCs) via indoleamine-2,3-dioxygenase (IDO), to suppress autologous T cell proliferation and IFN- γ production, favoring the establishment of tolerant immune environment^[90]. Similarly, HBsAg promotes differentiation of mMDSCs through activation of ERK/IL-6/STAT3 signaling feedback^[91]. Mechanistically, cell cycle-related kinase (CCRK), as a direct AR-regulated oncogene, mediates virus-host signaling to promote progression of HBV-associated HCC^[92], and further induces polymorphonuclear (PMN) MDSCs in HCC^[93]. Moreover, AR-CCRK pathway is consistently shared by tumorigenesis in NASH-related HCC. Induction of CCRK activates mTORC1/4E-BP1/S6K/SREBP1 cascades and recruits PMN MDSCs to initiate metabolic reprogramming and immunosuppressive microenvironment to facilitate the progression of HCC^[94]. Concordantly, hepatic lipid deposit promotes accumulation of MDSCs and further enhances the cellular production of ROS in NASH-model mice^[95,96].

NK cells

Lymphocytes represent another crucial population in intrahepatic innate immune cells, including NKT cells, ILCs (NK cells and other ILC subpopulations) and $\gamma\delta$ T cells. NKT and NK cells, which mainly contain conventional circulating NK and liver-resident NK cells, comprise about 50% of lymphocytes in liver. Generally, hepatic NK cells recognize and restrain virus-infected cells via direct cytotoxicity or secreting

immunoregulatory cytokines, such as IFN- γ and TNF- α , to activate T cells^[97,98]. Recent studies demonstrate that liver-resident CD56 (bright) NK cells, which show increased expression of NKG2D and TRAIL and low production of IFN- γ , represent a cell population adapted for tolerogenic liver microenvironment and inducible anti-viral immunity^[99,100]. Accumulating evidence illustrates that function of NK cells is impaired in CHB and HBV progressed diseases. NK cells in blood from CHB patients express higher level of suppressive cell surface molecules and cytokines, such as Tim-3, PD-1 and IL-10, which is induced by HBsAg-mediated increase of monocytes^[101,102]. Besides, HBV nucleic acids-contained exosomes entering into NK cells inhibit expression of pattern-recognition receptors and thus NF- κ B and p38 MAPK pathways, which results in dysfunction of NK cells^[103]. Furthermore, intrahepatic NK cells-mediated apoptosis of hepatic stellate cells (HSCs) is reversed via blockade of TGF- β in HBV-infected liver cirrhosis patients, which leads to impairment of anti-fibrosis capacity in NK cells^[104]. In HBV-associated HCC, LINC01149 variant upregulates MICA expression through serving as miR-128-3p sponge to recruit NK cells to lyse infected cells, which process releases highly soluble MICA and thus induces exhaustion of NK cells^[105].

IL-15 is crucial for homeostasis of NK cells. Deficiency of IL-15 or IL-15R α inhibits high fat diet-induced accumulation of lipids and inflammation in liver^[106], which probably suggests dysregulation of NK cells involving in NAFLD progression. Although NK cells is a cell population with cytotoxicity in infected tissues, these cells convert to less cytotoxic ILC1-like phenotype in NAFLD, which probably protect obese liver from severe NAFLD but dampened capability to kill cancer cells^[107]. In advanced stages of fibrosis in NAFLD accompanied by insulin resistance, the ability of NK cells to restrain HSCs is impaired, mediated by low expression of insulin receptors, which leads to deterioration of the liver and fibrosis^[108]. Furthermore, NKp46⁺ NK cells, as immunoregulatory cells, induce polarization of hepatic macrophages towards M1-like phenotype via IFN- γ , which prevents NASH progression to fibrosis^[109].

T cells

T cells represent the major adaptive lymphocytes in transformation from inflammation to cancer. CD8⁺ T cells frequently show restricted proliferation and exhausted function with high expression of inhibitory checkpoint molecules such as CTLA-4, PD-1 and TIM-3 in CHB and HBV-HCC. High expression of PD-1 on HBV-specific T cells or B cells induces exhaustion of T cells or reduced antibodies production, which is partially reversed by PD-1 blockade^[110-112]. In HCC tissue, there exist CD8⁺ T cells expressing different levels of PD-1. The population with high-level PD-1 expresses TIM-3 and/or LAG-3 and produce limited IFN γ and TNF α when exposed to anti-CD3^[113]. Beyond, thymocyte selection associated high mobility group box (TOX) in CD8⁺ T cells promotes PD-1 translocation to cell surface by regulating endocytic recycling of PD-1^[114].

Abundant exhausted CD8⁺ T cells and Tregs exist and potentially clonally expand in cancerous tissues from HCC patients^[115], contributing to evasion of tumor cells from immune surveillance. However, several studies illustrate that CD8⁺ T cells potentiate to promote persistent hepatic inflammation susceptible to HCC development when HBV-specific CD8⁺ T cells stay in activation but lack of capability to constrain virus replication^[116]. Consistently, CD8⁺ T cells possess dual functions in progression of NAFLD-related HCC. In diet-induced obese mice, CD8⁺ T cells are recruited to liver to promote insulin resistance and glucose metabolism, leading to steatohepatitis^[117]. Meanwhile, another study demonstrates that activation of CD8⁺ T cells is suppressed by liver-resident immunoglobulin-A-producing (IgA⁺) cells, which express high levels of PD-L1 and IL-10^[118].

Compared to CD8⁺ T cells, HBV-specific CD4⁺ cytotoxic cells in PBMCs from HBV-associated HCC present at the similar level, but display weaker cytotoxicity and suppress cytotoxicity of CD8⁺ T cells in the absence of Tregs^[119]. The antigen-experienced T cells, CD4⁺ follicular helper T (Tfh) cells diminish HBV via response to HBsAg, which is impaired by CTLA-4-mediated Treg suppression^[120]. Contrary with CTLA-4, increased expression of co-stimulatory molecule OX40 on peripheral CD4⁺ T cells is associated with HBV

clearance^[121]. Furthermore, combined OX40 stimulation and PD-L1 blockade effectively activate IFN- γ producing Th1 cells and IL-21 producing Tfh cells, which potentiates the application of immunotherapeutic approaches^[122]. Consistent with anti-tumor function in HBV-related HCC, surveillance from CD4⁺ T cells are crucial for NAFLD^[123,124]. However, selective loss of CD4⁺ T cells has been recently detected in NAFLD, which promotes hepatocarcinogenesis^[125]. Mechanistically, linoleic acid (C18:2) is released from dead hepatocytes caused by lipotoxicity into CD4⁺ T cells. C18:2 then co-localizes within mitochondria, upregulating expression of carnitine palmitoyltransferase, which further promotes production of ROS and cell apoptosis^[124].

B cells

HBV viral load as well as HBsAg and HBeAg levels differed with progression of distinct phases of chronic HBV infection, accompanied by alteration of immune cells. Immunoglobulin-encoding genes and B cell function-related genes are more enriched in immune active patients than those in immune tolerant and inactive carrier patients^[126]. B-cell response and humoral immunity are essential for controlling progression of chronic HBV infection^[127,128]. However, differentiated HBsAg-specific B cells from patients with CHB are defective in antibody production and express high levels of inhibitory receptors, such as PD-1, FcRL4 and FcRL5^[112,129]. Blockade of PD-1 on B cells effectively reverses cellular dysfunction to maturation and cytokines secretion^[111]. Mechanistically, HBsAg inhibit toll-like receptor (TLR) promoter activity by suppressing phosphorylation of CAMP responsive element binding protein (CREB), thereby comprehensively inhibits expression of TLR9 in B cells, leading to reduced proliferation of B cells and decreased proinflammatory cytokines secretion^[130].

Intrahepatic B cells are highly associated with NAFLD. CD20⁺ B cells are increased in liver tissues from NAFLD patients with activity score higher than 5, compared with those of 0 or 1~4^[131]. Consistently, hepatic B cells account for more in HFD-induced NAFLD mice than in control mice. Moreover, the B cells in NAFLD liver tissues potentiate to produce higher levels of IL-6 and TNF- α , and promote differentiation of CD4⁺ T cells to Th1 cells^[132]. Functionally, the maturation of hepatic B2-cells and the increase of IgG in circulation targeting oxidative stress-derived epitopes predict the onset of steatohepatitis, and B cells-mediated activation of Th1 cells contributes to NAFLD progression toward fibrosis^[133].

IMMUNOTHERAPIES

As discussed above, a long duration is taken for both HBV- and NAFLD-related HCC, therefore, treatments aiming at eliminating HBV infection or blocking NASH progression and fibrogenesis can prevent the development of tumors. Abundant alterations of hepatic immune cells during liver carcinogenesis provide wide-range targets. Several immunotherapies have been approved for clinical application, nonetheless, the induced resistances and adverse events remain to be further investigated. The following sections will discuss immunotherapies in HCC, such as immune checkpoint inhibitors, virus or tumor vaccines, and adoptive cells transfer.

Immune checkpoint blockade

Inhibitory checkpoint receptors (ICRs) including CTLA-4, PD-1 and PD-L1, are recognized as master regulators for anti-tumor immunity. Immune checkpoint inhibition (ICI) therapy using antibody against PD-1, PD-L1 and CTLA-4 have been since been approved by the Food and Drug Administration^[134]. Moreover, function of exhausted CD8⁺ T cells is restored through implementation of anti-PD-1 therapy and even combined blockade of ICRs^[113,114]. In a cohort study with 262 patients enrolled between 2012 and 2016, nivolumab, a monoclonal antibody against PD-1, showed a decent safety profile, including acceptable tolerability^[135]. Consistently, safety and efficacy of nivolumab was comparable in Asian patients, compared to intent-to-treat overall population^[136]. However, a phase III trial showed that pembrolizumab, another PD-1 inhibitor, did not display significant increase in overall survival (OS) and progression-free survival

(PFS), compared with control groups^[137]. Since medicines based on anti-PD-L1 monoclonal antibodies have been validated to be effective for several cancer diseases, a phase Ib clinical trial of atezolizumab combined with bevacizumab, the anti-PD-L1 antibody and VEGF blocking antibody respectively (ClinicalTrials.gov NCT02715531), has announced an overall response rate of 32% among 73 patients with advanced HCC^[138]. Moreover, this combined therapy-based IMbrave150 phase III trial (ClinicalTrials.gov NCT03434379) is currently implemented and significant improvements in OS and PFS compared with sorafenib have been reported by the sponsor^[139,140] [Figure 2].

CTLA-4 is another inhibitory molecule that attenuates activation and function of T cells. Upregulation of CTLA-4 promote the apoptosis, secretion of anti-inflammatory cytokines and exhaustion of CD4⁺ Th and CD8⁺ T cells in liver tissues from CHB and HCC patients^[141-144]. Tremelimumab, which is the monoclonal antibody blocking CTLA-4, was administered in HCV-related HCC patients and assessed to possess antitumor and antiviral activity, along with good safety profile. The disease control rate was 76.4% and time to progression was 6.48 months (95%CI: 3.95-9.14)^[145]. The similar efficacy displayed in another cohort with 32 HCC patients enrolled. Combinational administration of Tremelimumab and tumor ablation results in accumulation of intratumoral CD8⁺ T cells and median OS of treated patients was 12.3 months (95%CI: 9.3 to 15.4 months), which is a potential new therapy for patients with advanced HCC^[146]. Furthermore, a preclinical study of durvalumab combined with tremelimumab was analyzed in patients with unresectable HCC (11 HBV⁺, 9 HCV⁺, 20 uninfected). Among 40 patients, 60% (24/40) had discontinued treatment. The uninfected patients after treatment showed predominantly clinical activity and no unexpected safety signals were observed. The phase II portion of this study is still ongoing^[147] [Figure 2].

Adoptive cells transfer

Chimeric antigen receptor (CAR) therapy is a promising strategy for cancer immunotherapy. Accumulating evidence has demonstrated CAR-T cell therapy as effective strategy against lymphoid leukemia and multiple myeloma^[148-150], whereas, efficacy and safety of CAR-T therapy for treatment of solid tumors remain elusive. Glypican-3 (GPC3) is broadly expressed on liver cancer cells, but minority on normal hepatocytes. GPC3-targeted CAR-T cells efficiently kill GPC3-positive hepatoma cells and the T cells expressing third-generation CAR potentiate to suppress growth of xenografts with even low GPC3 expression level in mice^[151]. Moreover, GPC3-specific CAR-T therapy combination with sorafenib could effectively promote apoptosis of liver cancer cells^[152] [Figure 2].

However, side effects to CAR-T therapy contain high manufacturing and fatal toxicities, such as cytokine release syndrome^[153]. CAR-NK therapy is under investigation and probably provide better options. Similar to CAR-T, FLT3- and CS1-targeted CAR-NK cells respectively suppress proliferation of cancer cells in myeloid leukemia and multiple myeloma^[154,155]. Furthermore, DAP12-targeted CAR-NK cells efficiently suppressed tumor cells in ascites and proliferation of liver-metastatic tumors in colorectal cancer patients^[156]. *In vivo* studies have shown that injection of ErbB2-specific NK-92/5.28.z cells possess the ability to prolong survival of glioblastoma-xenograft mice^[157] [Figure 2].

CONCLUSION

Chronic HBV infection-induced progression of HCC mainly depends on direct intrusion of viral components in hepatocytes, which disturbs its normal cellular metabolism and function. This subsequently stimulates immune imbalance in liver. Frequently accompanied by systemic metabolic syndrome, advancement of NALFD- associated HCC encompasses excessive lipid accumulation in hepatocytes and immune cells, insulin resistance and inflammation induced malignant transformation. The hepatic complexity of immune cells mediates tumorigenesis in situation of anergy of cytotoxic cells and enhancement of immunosuppressive cells. Cytotoxic T cells are the major factor which eradicates HBV-infected cells or malignant cells. The exhaustion mediated by MDSCs or Tregs leads to persistence of HBV and evasion of

cancer cells. Flexibility of macrophages facilitates themselves to polarize according to different signals from microenvironment. Macrophages mostly function as suppressive cells to inhibit proliferation and activation of CTLs in HBV-related HCC, while promotes liver damage and fibrosis via releasing pro-inflammatory cytokines.

Since there still remains lack of effective drug specifically targeting eradication of HBV or controlling development of NAFLD, illustrating molecular mechanisms of immune cells and establishing novel immunotherapies will provide promising options for treatment of HCC. Unbridling cytotoxic cells (ICI) and stimulating the cells specifically engineered with tumor-associated antigens (CAR-T and CAR-NK) mutually contribute to effectively restrain progression of HBV- and NAFLD-induced HCC. Taken together, the drug resistance and adverse effect induced by immunotherapies should be further recorded and investigated in future studies.

DECLARATIONS

Authors' contributions

Conceived of the presented idea: Ma CH, Song XJ

Performed the basic writing: Song XJ

Developed the further revision: Ma CH

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Ghany MG, Marks KM, Morgan TR, Wyles DL, Aronsohn AI, et al. Hepatitis C guidance 2019 update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. *Hepatology* 2020;71:686-721.
2. Falade-Nwulia O, Suarez-Cuervo C, Nelson DR, Fried MW, Segal JB, et al. Oral direct-acting agent therapy for hepatitis C virus infection: a systematic review. *Ann Intern Med* 2017;166:637-48.
3. Cornberg M, Lok AS, Terrault NA, Zoulim F; 2019 EASL-AASLD HBV Treatment Endpoints Conference Faculty. Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference. *J Hepatol* 2019; Epub ahead of print. doi: 10.1002/hep.31030
4. Yip TC, Wong VW, Chan HL, Tse YK, Lui GC, et al. Tenofovir is associated with lower risk of hepatocellular carcinoma than

- entecavir in patients with chronic HBV infection in China. *Gastroenterology* 2020;158:215-25.e6.
5. Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 2019;16:411-28.
 6. Kanwal F, Kramer J, Li L, Dai J, Natarajan Y, et al. Effect of metabolic traits on the risk of cirrhosis and hepatocellular cancer in non-alcoholic fatty liver disease. *Hepatology* 2020;71:808-19.
 7. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018;15:11-20.
 8. Younossi Z, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, et al. Nonalcoholic steatohepatitis is the fastest growing cause of hepatocellular carcinoma in liver transplant candidates. *Clin Gastroenterol Hepatol* 2019;17:748-55.e3.
 9. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. *J Hepatol* 2018;69:896-904.
 10. Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2016;2:16018.
 11. Rimassa L, Danesi R, Pressiani T, Merle P. Management of adverse events associated with tyrosine kinase inhibitors: Improving outcomes for patients with hepatocellular carcinoma. *Cancer Treat Rev* 2019;77:20-8.
 12. Bruix J, da Fonseca LG, Reig M. Insights into the success and failure of systemic therapy for hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2019;16:617-30.
 13. Brown ZJ, Greten TF, Heinrich B. Adjuvant treatment of hepatocellular carcinoma: prospect of immunotherapy. *Hepatology* 2019;70:1437-42.
 14. Amaddeo G, Cao Q, Ladeiro Y, Imbeaud S, Nault JC, et al. Integration of tumour and viral genomic characterizations in HBV-related hepatocellular carcinomas. *Gut* 2015;64:820-9.
 15. Hsia CC, Yuwen H, Tabor E. Hot-spot mutations in hepatitis B virus X gene in hepatocellular carcinoma. *Lancet* 1996;348:625-6.
 16. Iavarone M, Trabut JB, Delpuech O, Carnot F, Colombo M, et al. Characterisation of hepatitis B virus X protein mutants in tumour and non-tumour liver cells using laser capture microdissection. *J Hepatol* 2003;39:253-61.
 17. Ali A, Abdel-Hafiz H, Suhail M, Al-Mars A, Zakaria MK, et al. Hepatitis B virus, HBx mutants and their role in hepatocellular carcinoma. *World J Gastroenterol* 2014;20:10238-48.
 18. Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 1991;351:317-20.
 19. Kew MC. Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011;26:144-52.
 20. Jiang T, Liu M, Wu J, Shi Y. Structural and biochemical analysis of Bcl-2 interaction with the hepatitis B virus protein HBx. *Proc Natl Acad Sci U S A* 2016;113:2074-9.
 21. Niu Y, Xu M, Slagle BL, Huang H, Li S, et al. Farnesoid X receptor ablation sensitizes mice to hepatitis b virus X protein-induced hepatocarcinogenesis. *Hepatology* 2017;65:893-906.
 22. Jegal ME, Jung SY, Han YS, Kim YJ. C-terminal truncated HBx reduces doxorubicin cytotoxicity via ABCB1 upregulation in Huh-7 hepatocellular carcinoma cells. *BMB Rep* 2019;52:330-5.
 23. Li Y, Fu Y, Hu X, Sun L, Tang D, et al. The HBx-CTTN interaction promotes cell proliferation and migration of hepatocellular carcinoma via CREB1. *Cell Death Dis* 2019;10:405.
 24. Lee S, Kim W, Ko C, Ryu WS. Hepatitis B virus X protein enhances Myc stability by inhibiting SCF(Skp2) ubiquitin E3 ligase-mediated Myc ubiquitination and contributes to oncogenesis. *Oncogene* 2016;35:1857-67.
 25. Wang J, Li N, Huang ZB, Fu S, Yu SM, et al. HBx regulates transcription factor PAX8 stabilization to promote the progression of hepatocellular carcinoma. *Oncogene* 2019;38:6696-710.
 26. He B, Peng F, Li W, Jiang Y. Interaction of lncRNA-MALAT1 and miR-124 regulates HBx-induced cancer stem cell properties in HepG2 through PI3K/Akt signaling. *J Cell Biochem* 2019;120:2908-18.
 27. Lin X, Zuo S, Luo R, Li Y, Yu G, et al. HBX-induced miR-5188 impairs FOXO1 to stimulate beta-catenin nuclear translocation and promotes tumor stemness in hepatocellular carcinoma. *Theranostics* 2019;9:7583-98.
 28. Chen SL, Zhang CZ, Liu LL, Lu SX, Pan YH, et al. A GYS2/p53 negative feedback loop restricts tumor growth in HBV-related hepatocellular carcinoma. *Cancer Res* 2019;79:534-45.
 29. Liu WC, Wu IC, Lee YC, Lin CP, Cheng JH, et al. Hepatocellular carcinoma-associated single-nucleotide variants and deletions identified by the use of genome-wide high-throughput analysis of hepatitis B virus. *J Pathol* 2017;243:176-92.
 30. Bai X, Jia JA, Fang M, Chen S, Liang X, et al. Deep sequencing of HBV pre-S region reveals high heterogeneity of HBV genotypes and associations of word pattern frequencies with HCC. *PLoS Genet* 2018;14:e1007206.
 31. Huang JT, Liu YJ, Wang J, Xu ZG, Yang Y, et al. Next generation digital PCR measurement of hepatitis B virus copy number in formalin-fixed paraffin-embedded hepatocellular carcinoma tissue. *Clin Chem* 2015;61:290-6.
 32. Hsieh YH, Su IJ, Wang HC, Chang WW, Lei HY, et al. Pre-S mutant surface antigens in chronic hepatitis B virus infection induce oxidative stress and DNA damage. *Carcinogenesis* 2004;25:2023-32.
 33. Pollicino T, Cacciola I, Saffioti F, Raimondo G. Hepatitis B virus PreS/S gene variants: pathobiology and clinical implications. *J Hepatol* 2014;61:408-17.
 34. Li TN, Wu YJ, Tsai HW, Sun CP, Wu YH, et al. Intrahepatic hepatitis B virus large surface antigen induces hepatocyte hyperploidy via failure of cytokinesis. *J Pathol* 2018;245:502-13.
 35. Liu P, Zhang H, Liang X, Ma H, Luan F, et al. HBV preS2 promotes the expression of TAZ via miRNA-338-3p to enhance the tumorigenesis of hepatocellular carcinoma. *Oncotarget* 2015;6:29048-59.
 36. Luan F, Liu B, Zhang J, Cheng S, Zhang B, et al. Correlation between HBV protein preS2 and tumor markers of hepatocellular carcinoma. *Pathol Res Pract* 2017;213:1037-42.

37. Zhang R, Real CI, Liu C, Baba HA, Gerken G, et al. Hepatic expression of oncogenes Bmi1 and Dkk1 is up-regulated in hepatitis B virus surface antigen-transgenic mice and can be induced by treatment with HBV particles or lipopolysaccharides in vitro. *Int J Cancer* 2017;141:354-63.
38. Luan F, Liu H, Gao L, Liu J, Sun Z, et al. Hepatitis B virus protein preS2 potentially promotes HCC development via its transcriptional activation of hTERT. *Gut* 2009;58:1528-37.
39. Tseng TC, Liu CJ, Hsu CY, Hong CM, Su TH, et al. High level of hepatitis B core-related antigen associated with increased risk of hepatocellular carcinoma in patients with chronic HBV infection of intermediate viral load. *Gastroenterology* 2019;157:1518-29.e3.
40. Liu W, Guo TF, Jing ZT, Yang Z, Liu L, et al. Hepatitis B virus core protein promotes hepatocarcinogenesis by enhancing Src expression and activating the Src/PI3K/Akt pathway. *FASEB J* 2018;32:3033-46.
41. Liu D, Cui L, Wang Y, Yang G, He J, et al. Hepatitis B e antigen and its precursors promote the progress of hepatocellular carcinoma by interacting with NUMB and decreasing p53 activity. *Hepatology* 2016;64:390-404.
42. Xie Q, Fan F, Wei W, Liu Y, Xu Z, et al. Multi-omics analyses reveal metabolic alterations regulated by hepatitis B virus core protein in hepatocellular carcinoma cells. *Sci Rep* 2017;7:41089.
43. Du J, Liang X, Liu Y, Qu Z, Gao L, et al. Hepatitis B virus core protein inhibits TRAIL-induced apoptosis of hepatocytes by blocking DR5 expression. *Cell Death Differ* 2009;16:219-29.
44. Liu W, Lin YT, Yan XL, Ding YL, Wu YL, et al. Hepatitis B virus core protein inhibits Fas-mediated apoptosis of hepatoma cells via regulation of mFas/FasL and sFas expression. *FASEB J* 2015;29:1113-23.
45. Tu T, Budzinska MA, Vondran FWR, Shackel NA, Urban S. Hepatitis B virus DNA integration occurs early in the viral life cycle in an in vitro infection model via sodium taurocholate cotransporting polypeptide-dependent uptake of enveloped virus particles. *J Virol* 2018;92.
46. Duan M, Hao J, Cui S, Worthley DL, Zhang S, et al. Diverse modes of clonal evolution in HBV-related hepatocellular carcinoma revealed by single-cell genome sequencing. *Cell Res* 2018;28:359-73.
47. Zhao LH, Liu X, Yan HX, Li WY, Zeng X, et al. Genomic and oncogenic preference of HBV integration in hepatocellular carcinoma. *Nat Commun* 2016;7:12992.
48. Yang X, Wu L, Lin J, Wang A, Wan X, et al. Distinct hepatitis B virus integration patterns in hepatocellular carcinoma and adjacent normal liver tissue. *Int J Cancer* 2017;140:1324-30.
49. Wong DK, Cheng SCY, Mak LL, To EW, Lo RC, et al. Among patients with undetectable hepatitis B surface antigen and hepatocellular carcinoma, a high proportion has integration of HBV DNA into hepatocyte DNA and no cirrhosis. *Clin Gastroenterol Hepatol* 2020;18:449-56.
50. Yan H, Yang Y, Zhang L, Tang G, Wang Y, et al. Characterization of the genotype and integration patterns of hepatitis B virus in early- and late-onset hepatocellular carcinoma. *Hepatology* 2015;61:1821-31.
51. Chiu YT, Wong JK, Choi SW, Sze KM, Ho DW, et al. Novel pre-mRNA splicing of intronically integrated HBV generates oncogenic chimera in hepatocellular carcinoma. *J Hepatol* 2016;64:1256-64.
52. Li CL, Li CY, Lin YY, Ho MC, Chen DS, et al. Androgen receptor enhances hepatic telomerase reverse transcriptase gene transcription after hepatitis B virus integration or point mutation in promoter region. *Hepatology* 2019;69:498-512.
53. Yoo S, Wang W, Wang Q, Fiel MI, Lee E, et al. A pilot systematic genomic comparison of recurrence risks of hepatitis B virus-associated hepatocellular carcinoma with low- and high-degree liver fibrosis. *BMC Med* 2017;15:214.
54. Hama N, Totoki Y, Miura F, Tatsuno K, Saito-Adachi M, et al. Epigenetic landscape influences the liver cancer genome architecture. *Nat Commun* 2018;9:1643.
55. Ren L, Zeng M, Tang Z, Li M, Wang X, et al. The antiresection activity of the X protein encoded by hepatitis virus B. *Hepatology* 2019;69:2546-61.
56. Musa J, Li J, Grunewald TG. Hepatitis B virus large surface protein is priming for hepatocellular carcinoma development via induction of cytokinesis failure. *J Pathol* 2019;247:6-8.
57. Hsieh YH, Chang YY, Su IJ, Yen CJ, Liu YR, et al. Hepatitis B virus pre-S2 mutant large surface protein inhibits DNA double-strand break repair and leads to genome instability in hepatocarcinogenesis. *J Pathol* 2015;236:337-47.
58. Takakura K, Oikawa T, Nakano M, Saeki C, Torisu Y, et al. Recent insights into the multiple pathways driving non-alcoholic steatohepatitis-derived hepatocellular carcinoma. *Front Oncol* 2019;9:762.
59. Li T, Weng J, Zhang Y, Liang K, Fu G, et al. mTOR direct crosstalk with STAT5 promotes de novo lipid synthesis and induces hepatocellular carcinoma. *Cell Death Dis* 2019;10:619.
60. Tian Y, Yang B, Qiu W, Hao Y, Zhang Z, et al. ER-residential Nogo-B accelerates NAFLD-associated HCC mediated by metabolic reprogramming of oxLDL lipophagy. *Nat Commun* 2019;10:3391.
61. Kodama T, Yi J, Newberg JY, Tien JC, Wu H, et al. Molecular profiling of nonalcoholic fatty liver disease-associated hepatocellular carcinoma using SB transposon mutagenesis. *Proc Natl Acad Sci U S A* 2018;115:e10417-26.
62. Ye J, Li TS, Xu G, Zhao YM, Zhang NP, et al. JCAD promotes progression of nonalcoholic steatohepatitis to liver cancer by inhibiting LATS2 kinase activity. *Cancer Res* 2017;77:5287-300.
63. Jeong SH, Kim HB, Kim MC, Lee JM, Lee JH, et al. Hippo-mediated suppression of IRS2/AKT signaling prevents hepatic steatosis and liver cancer. *J Clin Invest* 2018;128:1010-25.
64. Inoue-Yamauchi A, Itagaki H, Oda H. Eicosapentaenoic acid attenuates obesity-related hepatocellular carcinogenesis. *Carcinogenesis* 2018;39:28-35.
65. Kumar DP, Santhekadur PK, Seneshaw M, Mirshahi F, Uram-Tuculescu C, et al. A regulatory role of apoptosis antagonizing transcription factor in the pathogenesis of nonalcoholic fatty liver disease and hepatocellular carcinoma. *Hepatology* 2019;69:1520-34.
66. He Y, Hwang S, Cai Y, Kim SJ, Xu M, et al. MicroRNA-223 ameliorates nonalcoholic steatohepatitis and cancer by targeting multiple inflammatory and oncogenic genes in hepatocytes. *Hepatology* 2019;70:1150-67.

67. Namjou B, Lingren T, Huang Y, Parameswaran S, Cobb BL, et al. GWAS and enrichment analyses of non-alcoholic fatty liver disease identify new trait-associated genes and pathways across eMERGE Network. *BMC Med* 2019;17:135.
68. Grimaudo S, Pipitone RM, Pennisi G, Celsa C, Camma C, et al. Association between PNPLA3 rs738409 C>G variant and liver-related outcomes in patients with non-alcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2020;18:935-44.e3.
69. Tepper CG, Dang JHT, Stewart SL, Fang DM, Wong KA, et al. High frequency of the PNPLA3 rs738409 [G] single-nucleotide polymorphism in Hmong individuals as a potential basis for a predisposition to chronic liver disease. *Cancer* 2018;124:1583-9.
70. Petta S, Valenti L, Tuttolomondo A, Dongiovanni P, Pipitone RM, et al. Interferon lambda 4 rs368234815 TT>deltaG variant is associated with liver damage in patients with nonalcoholic fatty liver disease. *Hepatology* 2017;66:1885-93.
71. Petta S, Valenti L, Svegliati-Baroni G, Ruscica M, Pipitone RM, et al. Fibronectin type III domain-containing protein 5 rs3480 A>G polymorphism, irisin, and liver fibrosis in patients with nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 2017;102:2660-9.
72. Cheng YW, Chen KW, Kuo HC, Kuo CH, Lin WH, et al. Specific diacylglycerols generated by hepatic lipogenesis stimulate the oncogenic androgen receptor activity in male hepatocytes. *Int J Obes (Lond)* 2019;43:2469-79.
73. Campisano S, La Colla A, Echarte SM, Chisari AN. Interplay between early-life malnutrition, epigenetic modulation of the immune function and liver diseases. *Nutr Res Rev* 2019;32:128-45.
74. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer* 2012;12:298-306.
75. Yan W, Liu X, Ma H, Zhang H, Song X, et al. Tim-3 fosters HCC development by enhancing TGF-beta-mediated alternative activation of macrophages. *Gut* 2015;64:1593-604.
76. Faure-Dupuy S, Delphin M, Aillot L, Dimier L, Lebosse F, et al. Hepatitis B virus-induced modulation of liver macrophage function promotes hepatocyte infection. *J Hepatol* 2019;71:1086-98.
77. Bility MT, Cheng L, Zhang Z, Luan Y, Li F, et al. Hepatitis B virus infection and immunopathogenesis in a humanized mouse model: induction of human-specific liver fibrosis and M2-like macrophages. *PLoS Pathog* 2014;10:e1004032.
78. Cheng X, Xia Y, Serti E, Block PD, Chung M, et al. Hepatitis B virus evades innate immunity of hepatocytes but activates cytokine production by macrophages. *Hepatology* 2017;66:1779-93.
79. Tian Y, Kuo CF, Akbari O, Ou JH. Maternal-derived hepatitis B virus e antigen alters macrophage function in offspring to drive viral persistence after vertical transmission. *Immunity* 2016;44:1204-14.
80. Chen J, Xu W, Chen Y, Xie X, Zhang Y, et al. Matrix metalloproteinase 9 facilitates hepatitis B virus replication through binding with type I interferon (IFN) receptor 1 to repress IFN/JAK/STAT signaling. *J Virol* 2017;91.
81. Zang M, Li Y, He H, Ding H, Chen K, et al. IL-23 production of liver inflammatory macrophages to damaged hepatocytes promotes hepatocellular carcinoma development after chronic hepatitis B virus infection. *Biochim Biophys Acta Mol Basis Dis* 2018;1864:3759-70.
82. de Oliveira S, Houseright RA, Graves AL, Golenberg N, Korte BG, et al. Metformin modulates innate immune-mediated inflammation and early progression of NAFLD-associated hepatocellular carcinoma in zebrafish. *J Hepatol* 2019;70:710-21.
83. Malehmir M, Pfister D, Gallage S, Szydłowska M, Inverso D, et al. Platelet GPIIb/IIIa is a mediator and potential interventional target for NASH and subsequent liver cancer. *Nat Med* 2019;25:641-55.
84. Eckert C, Klein N, Kornek M, Lukacs-Kornek V. The complex myeloid network of the liver with diverse functional capacity at steady state and in inflammation. *Front Immunol* 2015;6:179.
85. Walenbergh SM, Houben T, Hendriks T, Jeurissen ML, van Gorp PJ, et al. Weekly treatment of 2-hydroxypropyl-beta-cyclodextrin improves intracellular cholesterol levels in LDL receptor knockout mice. *Int J Mol Sci* 2015;16:21056-69.
86. Leroux A, Ferrere G, Godie V, Cailleux F, Renoud ML, et al. Toxic lipids stored by Kupffer cells correlates with their pro-inflammatory phenotype at an early stage of steatohepatitis. *J Hepatol* 2012;57:141-9.
87. Alisi A, Carpino G, Oliveira FL, Panera N, Nobili V, et al. The role of tissue macrophage-mediated inflammation on NAFLD pathogenesis and its clinical implications. *Mediators Inflamm* 2017;2017:8162421.
88. Liu XL, Pan Q, Cao HX, Xin FZ, Zhao ZH, et al. Lipotoxic hepatocyte-derived exosomal mir-192-5p activates macrophages via Rictor/Akt/FoxO1 signaling in NAFLD. *Hepatology* 2019; Epub ahead of print. doi: 10.1002/hep.31050
89. Luo X, Li H, Ma L, Zhou J, Guo X, et al. Expression of STING is increased in liver tissues from patients with NAFLD and promotes macrophage-mediated hepatic inflammation and fibrosis in mice. *Gastroenterology* 2018;155:1971-84.e4.
90. Yang F, Yu X, Zhou C, Mao R, Zhu M, et al. Hepatitis B e antigen induces the expansion of monocytic myeloid-derived suppressor cells to dampen T-cell function in chronic hepatitis B virus infection. *PLoS Pathog* 2019;15:e1007690.
91. Fang Z, Li J, Yu X, Zhang D, Ren G, et al. Polarization of monocytic myeloid-derived suppressor cells by hepatitis B surface antigen is mediated via ERK/IL-6/STAT3 signaling feedback and restrains the activation of T cells in chronic hepatitis B virus infection. *J Immunol* 2015;195:4873-83.
92. Yu Z, Gao YQ, Feng H, Lee YY, Li MS, et al. Cell cycle-related kinase mediates viral-host signalling to promote hepatitis B virus-associated hepatocarcinogenesis. *Gut* 2014;63:1793-804.
93. Zhou J, Liu M, Sun H, Feng Y, Xu L, et al. Hepatoma-intrinsic CCRK inhibition diminishes myeloid-derived suppressor cell immunosuppression and enhances immune-checkpoint blockade efficacy. *Gut* 2018;67:931-44.
94. Sun H, Yang W, Tian Y, Zeng X, Zhou J, et al. An inflammatory-CCRK circuitry drives mTORC1-dependent metabolic and immunosuppressive reprogramming in obesity-associated hepatocellular carcinoma. *Nat Commun* 2018;9:5214.
95. Tsunashima H, Tsuneyama K, Moritoki Y, Hara M, Kikuchi K. Accumulated myeloid-derived suppressor cells demonstrate distinct phenotypes and functions in two non-alcoholic steatohepatitis mouse models. *Hepatobiliary Surg Nutr* 2015;4:313-9.
96. Yao L, Abe M, Kawasaki K, Akbar SM, Matsuura B, et al. Characterization of liver monocytic myeloid-derived suppressor cells and their role in a murine model of non-alcoholic fatty liver disease. *PLoS One* 2016;11:e0149948.
97. Pallmer K, Oxenius A. Recognition and regulation of T cells by NK cells. *Front Immunol* 2016;7:251.
98. Biron CA, Altfield M. Is there natural killer cell memory and can it be harnessed by vaccination? Can natural killer and CD8 T cells

- switch jobs? Cold Spring Harb Perspect Biol 2018;10.
99. Harmon C, Robinson MW, Fahey R, Whelan S, Houlihan DD, et al. Tissue-resident Eomes(hi) T-bet(lo) CD56(bright) NK cells with reduced proinflammatory potential are enriched in the adult human liver. *Eur J Immunol* 2016;46:2111-20.
 100. Stegmann KA, Robertson F, Hansi N, Gill U, Pallant C, et al. CXCR6 marks a novel subset of T-bet(lo)Eomes(hi) natural killer cells residing in human liver. *Sci Rep* 2016;6:26157.
 101. Ju Y, Hou N, Meng J, Wang X, Zhang X, et al. T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) mediates natural killer cell suppression in chronic hepatitis B. *J Hepatol* 2010;52:322-9.
 102. Li H, Zhai N, Wang Z, Song H, Yang Y, et al. Regulatory NK cells mediated between immunosuppressive monocytes and dysfunctional T cells in chronic HBV infection. *Gut* 2018;67:2035-44.
 103. Yang Y, Han Q, Hou Z, Zhang C, Tian Z, et al. Exosomes mediate hepatitis B virus (HBV) transmission and NK-cell dysfunction. *Cell Mol Immunol* 2017;14:465-75.
 104. Shi J, Zhao J, Zhang X, Cheng Y, Hu J, et al. Activated hepatic stellate cells impair NK cell anti-fibrosis capacity through a TGF-beta-dependent emperipolesis in HBV cirrhotic patients. *Sci Rep* 2017;7:44544.
 105. Zhong R, Tian J, Fu M, Ma S, Liu L, et al. LINC01149 variant modulates MICA expression that facilitates hepatitis B virus spontaneous recovery but increases hepatocellular carcinoma risk. *Oncogene* 2020;39:1944-56.
 106. Cepero-Donates Y, Lacraz G, Ghobadi F, Rakotoarivelo V, Orkhis S, et al. Interleukin-15-mediated inflammation promotes non-alcoholic fatty liver disease. *Cytokine* 2016;82:102-11.
 107. Cuff AO, Sillito F, Dertschnig S, Hall A, Luong TV, et al. The obese liver environment mediates conversion of NK cells to a less cytotoxic ILC1-like phenotype. *Front Immunol* 2019;10:2180.
 108. Amer J, Salhab A, Noureddin M, Doron S, Abu-Tair L, et al. Insulin signaling as a potential natural killer cell checkpoint in fatty liver disease. *Hepatol Commun* 2018;2:285-98.
 109. Tosello-Tramont AC, Krueger P, Narayanan S, Landes SG, Leitinger N, et al. NKp46(+) natural killer cells attenuate metabolism-induced hepatic fibrosis by regulating macrophage activation in mice. *Hepatology* 2016;63:799-812.
 110. Li Y, Tang L, Guo L, Chen C, Gu S, et al. CXCL13-mediated recruitment of intrahepatic CXCR5(+)CD8(+) T cells favors viral control in chronic HBV infection. *J Hepatol* 2020;72:420-30.
 111. Salimzadeh L, Le Bert N, Dutertre CA, Gill US, Newell EW, et al. PD-1 blockade partially recovers dysfunctional virus-specific B cells in chronic hepatitis B infection. *J Clin Invest* 2018;128:4573-87.
 112. Burton AR, Pallett LJ, McCoy LE, Suveizdyte K, Amin OE, et al. Circulating and intrahepatic antiviral B cells are defective in hepatitis B. *J Clin Invest* 2018;128:4588-603.
 113. Kim HD, Song GW, Park S, Jung MK, Kim MH, et al. Association between expression level of PD1 by tumor-infiltrating CD8(+) T cells and features of hepatocellular carcinoma. *Gastroenterology* 2018;155:1936-50.e17.
 114. Wang X, He Q, Shen H, Xia A, Tian W, et al. TOX promotes the exhaustion of antitumor CD8(+) T cells by preventing PD1 degradation in hepatocellular carcinoma. *J Hepatol* 2019;71:731-41.
 115. Zheng C, Zheng L, Yoo JK, Guo H, Zhang Y, et al. Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing. *Cell* 2017;169:1342-56.e16.
 116. Kim GA, Lim YS, Han S, Choi J, Shim JH, et al. High risk of hepatocellular carcinoma and death in patients with immune-tolerant-phase chronic hepatitis B. *Gut* 2018;67:945-52.
 117. Ghazarian M, Revelo XS, Nohr MK, Luck H, Zeng K, et al. Type I interferon responses drive intrahepatic T cells to promote metabolic syndrome. *Sci Immunol* 2017;2:pil: eaai7616.
 118. Shalapour S, Lin XJ, Bastian IN, Brain J, Burt AD, et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature* 2017;551:340-5.
 119. Meng F, Zhen S, Song B. HBV-specific CD4+ cytotoxic T cells in hepatocellular carcinoma are less cytolytic toward tumor cells and suppress CD8+ T cell-mediated antitumor immunity. *APMIS* 2017;125:743-51.
 120. Wang X, Dong Q, Li Q, Li Y, Zhao D, et al. Dysregulated response of follicular helper T cells to hepatitis B surface antigen promotes HBV persistence in mice and associates with outcomes of patients. *Gastroenterology* 2018;154:2222-36.
 121. Publicover J, Gaggar A, Jespersen JM, Halac U, Johnson AJ, et al. An OX40/OX40L interaction directs successful immunity to hepatitis B virus. *Sci Transl Med* 2018;10.
 122. Jacobi FJ, Wild K, Smits M, Zoldan K, Csernalabics B, et al. OX40 stimulation and PD-L1 blockade synergistically augment HBV-specific CD4 T cells in patients with HBeAg-negative infection. *J Hepatol* 2019;70:1103-13.
 123. Walker CM, Lemon SM. Getting the skinny on CD4(+) T cell survival in fatty livers. *Immunity* 2016;44:725-7.
 124. Brown ZJ, Fu Q, Ma C, Kruhlak M, Zhang H, et al. Carnitine palmitoyltransferase gene upregulation by linoleic acid induces CD4(+) T cell apoptosis promoting HCC development. *Cell Death Dis* 2018;9:620.
 125. Ma C, Kesarwala AH, Eggert T, Medina-Echeverez J, Kleiner DE, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. *Nature* 2016;531:253-7.
 126. Vanwolleghem T, Hou J, van Oord G, Andeweg AC, Osterhaus AD, et al. Re-evaluation of hepatitis B virus clinical phases by systems biology identifies unappreciated roles for the innate immune response and B cells. *Hepatology* 2015;62:87-100.
 127. Bertoletti A, Ferrari C. Adaptive immunity in HBV infection. *J Hepatol* 2016;64:S71-83.
 128. Zhang S, Zhao J, Zhang Z. Humoral immunity, the underestimated player in hepatitis B. *Cell Mol Immunol* 2018;15:645-8.
 129. Poonia B, Ayithan N, Nandi M, Masur H, Kottilil S. HBV induces inhibitory FcRL receptor on B cells and dysregulates B cell-T follicular helper cell axis. *Sci Rep* 2018;8:15296.
 130. Tout I, Gomes M, Ainouze M, Marotel M, Pecoul T, et al. Hepatitis B virus Blocks the CRE/CREB Complex and Prevents TLR9 Transcription and Function in Human B Cells. *J Immunol* 2018;201:2331-44.
 131. Schwenger KJP, Chen L, Chelliah A, Da Silva HE, Teterina A, et al. Markers of activated inflammatory cells are associated with

- disease severity and intestinal microbiota in adults with nonalcoholic fatty liver disease. *Int J Mol Med* 2018;42:2229-37.
132. Zhang F, Jiang WW, Li X, Qiu XY, Wu Z, et al. Role of intrahepatic B cells in non-alcoholic fatty liver disease by secreting pro-inflammatory cytokines and regulating intrahepatic T cells. *J Dig Dis* 2016;17:464-74.
133. Bruzzi S, Sutti S, Giudici G, Burlone ME, Ramavath NN, et al. B2-Lymphocyte responses to oxidative stress-derived antigens contribute to the evolution of nonalcoholic fatty liver disease (NAFLD). *Free Radic Biol Med* 2018;124:249-59.
134. Zhang JC, Chen WD, Alvarez JB, Jia K, Shi L, et al. Cancer immune checkpoint blockade therapy and its associated autoimmune cardiotoxicity. *Acta Pharmacol Sin* 2018;39:1693-8.
135. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
136. Yau T, Hsu C, Kim TY, Choo SP, Kang YK, et al. Nivolumab in advanced hepatocellular carcinoma: Sorafenib-experienced Asian cohort analysis. *J Hepatol* 2019;71:543-52.
137. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, et al. Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: a randomized, double-blind, phase III trial. *J Clin Oncol* 2020;38:193-202.
138. Pishvaian MJ, Lee MS, Ryoo BY, Stein S, Lee KH, et al. Updated safety and clinical activity results from a phase Ib study of atezolizumab + bevacizumab in hepatocellular carcinoma (HCC). *Ann Oncol* 2018;29:viii718-9.
139. Roche's Tecentriq in combination with Avastin increased overall survival and progression-free survival in people with unresectable hepatocellular carcinoma. Available from: <https://www.roche.com/media/releases/med-cor-2019-10-21.htm> [Last accessed on 14 Apr 2020]
140. Faivre S, Rimassa L, Finn RS. Molecular therapies for HCC: looking outside the box. *J Hepatol* 2020;72:342-52.
141. Tang ZS, Hao YH, Zhang EJ, Xu CL, Zhou Y, et al. CD28 family of receptors on T cells in chronic HBV infection: Expression characteristics, clinical significance and correlations with PD-1 blockade. *Mol Med Rep* 2016;14:1107-16.
142. Yin Y, Wu C, Song J, Wang J, Zhang E, et al. DNA immunization with fusion of CTLA-4 to hepatitis B virus (HBV) core protein enhanced Th2 type responses and cleared HBV with an accelerated kinetic. *PLoS One* 2011;6:e22524.
143. Schurich A, Khanna P, Lopes AR, Han KJ, Peppas D, et al. Role of the coinhibitory receptor cytotoxic T lymphocyte antigen-4 on apoptosis-Prone CD8 T cells in persistent hepatitis B virus infection. *Hepatology* 2011;53:1494-503.
144. Wang X, He Q, Shen H, Lu XJ, Sun B. Genetic and phenotypic difference in CD8(+) T cell exhaustion between chronic hepatitis B infection and hepatocellular carcinoma. *J Med Genet* 2019;56:18-21.
145. Sangro B, Gomez-Martin C, de la Mata M, Inarrairaegui M, Garralda E, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol* 2013;59:81-8.
146. Duffy AG, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J Hepatol* 2017;66:545-51.
147. Kelley RK, Abou-Alfa GK, Bendel JC, Kim TY, Borad MJ, et al. Phase I/II study of durvalumab and tremelimumab in patients with unresectable hepatocellular carcinoma (HCC): Phase I safety and efficacy analyses. *J Clin Oncol* 2017;35.
148. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *New Engl J Med* 2013;368:1509-18.
149. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *New Engl J Med* 2014;371:1507-17.
150. Brudno JN, Maric I, Hartman SD, Rose JJ, Wang M, et al. T cells genetically modified to express an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of poor-prognosis relapsed multiple myeloma. *J Clin Oncol* 2018;36:2267.
151. Gao H, Li K, Tu H, Pan X, Jiang H, et al. Development of T cells redirected to glypican-3 for the treatment of hepatocellular carcinoma. *Clin Cancer Res* 2014;20:6418-28.
152. Wu X, Luo H, Shi B, Di S, Sun R, et al. Combined antitumor effects of sorafenib and GPC3-CAR T cells in mouse models of hepatocellular carcinoma. *Mol Ther* 2019;27:1483-94.
153. Maude SL, Barrett D, Teachey DT, Grupp SA. Managing cytokine release syndrome associated with novel T cell-engaging therapies. *Cancer J* 2014;20:119-22.
154. Oelsner S, Waldmann A, Billmeier A, Roder J, Lindner A, et al. Genetically engineered CAR NK cells display selective cytotoxicity against FLT3-positive B-ALL and inhibit in vivo leukemia growth. *Int J Cancer* 2019;145:1935-45.
155. Chu J, Deng Y, Benson DM, He S, Hughes T, et al. CS1-specific chimeric antigen receptor (CAR)-engineered natural killer cells enhance in vitro and in vivo antitumor activity against human multiple myeloma. *Leukemia* 2014;28:917-27.
156. Xiao L, Cen D, Gan H, Sun Y, Huang N, et al. Adoptive transfer of NKG2D CAR mRNA-engineered natural killer cells in colorectal cancer patients. *Mol Ther* 2019;27:1114-25.
157. Zhang C, Burger MC, Jennewein L, Genssler S, Schonfeld K, et al. ErbB2/HER2-specific NK cells for targeted therapy of glioblastoma. *J Natl Cancer Inst* 2016;108.

Review

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Hypoalbuminemia: an underestimated, vital characteristic of hospitalized COVID-19 positive patients?

Giuliano Ramadori

Clinic for Gastroenterology and Endocrinology, University Medical Center Göttingen, Göttingen 37075, Germany.

Correspondence to: Dr. Giuliano Ramadori, Clinic for Gastroenterology and Endocrinology, University Medical Center Göttingen, Göttingen 37075, Germany. E-mail: giulianoramadori@gmail.com

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Abstract

The COVID-19 pandemic has led to the greatest worldwide health crisis in decades. The number of infected patients with severe SARS-CoV-2 (COVID-19) disease has overwhelmed the capacity of almost all health care systems around world. Hypoalbuminemia has now been reported in patients with severe disease seeking help in the emergency room because of COVID-19 infection. In the past, hypoalbuminemia was considered to be a negative prognostic marker, not only in patients with chronic liver disease, but also in patients with SARS and MERS infections. Albumin is the major serum protein synthesized by the liver. A low serum albumin level is an ominous clinical sign. Introduction of amino acids to a patient's diet is of fundamental importance to hepatic albumin synthesis in different clinical situations. This highlights the importance of nutritional support during the early phases of COVID-19-infection. Furthermore, albumin synthesis in the hepatocyte is downregulated at a pretranslational level by the direct interaction of the major acute-phase cytokines which are released into the circulation during the cytokine "storm" induced by the viral effects on the lungs. Both mechanisms contribute to severe hypoalbuminemia which, combined with massive fluid losses due to the fever, is responsible for severe hypovolemia and shock commonly observed in patients with COVID-19 in critical care settings.

Keywords: Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, COVID-19, albumin synthesis, nutrition, acute-phase reaction, cytokines, liver, extrahepatic organs

COVID-19 INFECTION AND THE CLINICAL RELEVANCE OF HYPOALBUMINEMIA

Severe acute respiratory syndrome, coronavirus 2 (SARS-CoV-2), formally CoV-19, is a recently recognized RNA-virus which belongs to a larger family of pathogenic human viruses. Severe acute respiratory syndrome



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coronavirus-1 and Middle East respiratory syndrome coronavirus caused primarily pulmonary diseases. HuCoV 229E, oC43, NL63 and HKU1 are mainly responsible for the common cold, but can also cause lethal nonspecific pneumonias^[1]. However, SARS-CoV-2 has a wide range of clinical presentations, with acute respiratory distress syndrome being the often fatal pulmonary complication^[2-4].

Most of the publications reporting clinical characteristics for patients with SARS-CoV-2-infection originate from China, many from the city of Wuhan. These publications are descriptive retrospective case series about patients hospitalized with the virus or who died in intensive care units (ICU)^[5,6]. The symptoms reported mainly concern the reason for hospitalisation. The spectrum of all symptoms, and key timings from when patients first felt unwell is less well reported^[7,8]. In fact, far less is known about the symptomatology at the time of first appearance of the disease in hospitalized patients and in infected persons who remained at home, and who may have even died there.

Parameters indicating liver damage include prothrombin time, serum transaminase and bilirubin levels, acute-phase response markers such as leukocyte count. C-reactive protein, procalcitonin, and several serum cytokine levels have been reported in patients with SARS-CoV-2, together with changes in serum albumin levels^[2-5,9,10]. Previous experiences in patients with SARS or MERS suggested that hypoalbuminemia, lymphopenia, a serum CRP level greater than 4 mg/dL, plus elevated lactate dehydrogenase on hospital admission were predictive for pneumonia progressing to respiratory failure^[11-14]. Low serum albumin levels have now been found to be an important predictor of progression to severe disease and increased mortality in hospitalised SARS-CoV-2 positive patients of older age^[15,16].

PATHOPHYSIOLOGICAL ASPECTS OF HYPOALBUMINEMIA AND CLINICAL RELEVANCE OF ALBUMIN INFUSION

Albumin is a single chain protein with a molecular weight of 66 kDa made of 585 amino acids which represents more than 50% of the serum proteins and represents an important component of interstitial fluid. The albumin fraction was first separated from the other components of the plasma in 1944 by Edwin Cohn^[17], who also appreciated its strong oncotic properties. This characteristic of albumin was also confirmed by Scatchard *et al.*^[18] in 1944. Serum albumin levels are used as useful surrogates of liver function^[19]. Soon after the fractionation studies, intravenous albumin administration was performed in patients with advanced liver disease. This was done in the United States during the 1940's^[21,22] and also in the United Kingdom at the beginning of the 1960's by Wilkinson and Sherlock *et al.*^[22].

The beneficial effect of prolonged administration was first demonstrated in a clinical trial by the group of Paolo Gentilini in Florence^[23], and more recently by Caraceni *et al.*^[24] in Bologna.

The positive diuretic effect of albumin infusion in three patients with liver cirrhosis was published by Patek *et al.*^[25]. This finding was subsequently corroborated in a group of ten patients^[26,27], showing that albumin infusion in patients with liver cirrhosis and ascites (without spontaneous bacterial peritonitis) increased sodium excretion in the urine, and led to weight reduction and a reduction in diuretics required.

It was shown that repeated daily intravenous administration of albumin was able to avoid the requirement for transjugular stent placement into the portal tract through the hepatic vein (TIPS)^[28]. A similar experience, in a larger patient numbers, was published by Trotter *et al.*^[29].

The positive effects of albumin infusion in cirrhotic patients with low levels of serum albumin was shown by Bajaj *et al.*^[30] who observed a normalisation in serum sodium concentration in patients with liver cirrhosis and hyponatremia. Infusion of intravenous albumin solution in decompensated cirrhotic patients was also able to reduce encephalopathic episodes and associated mortality^[31].

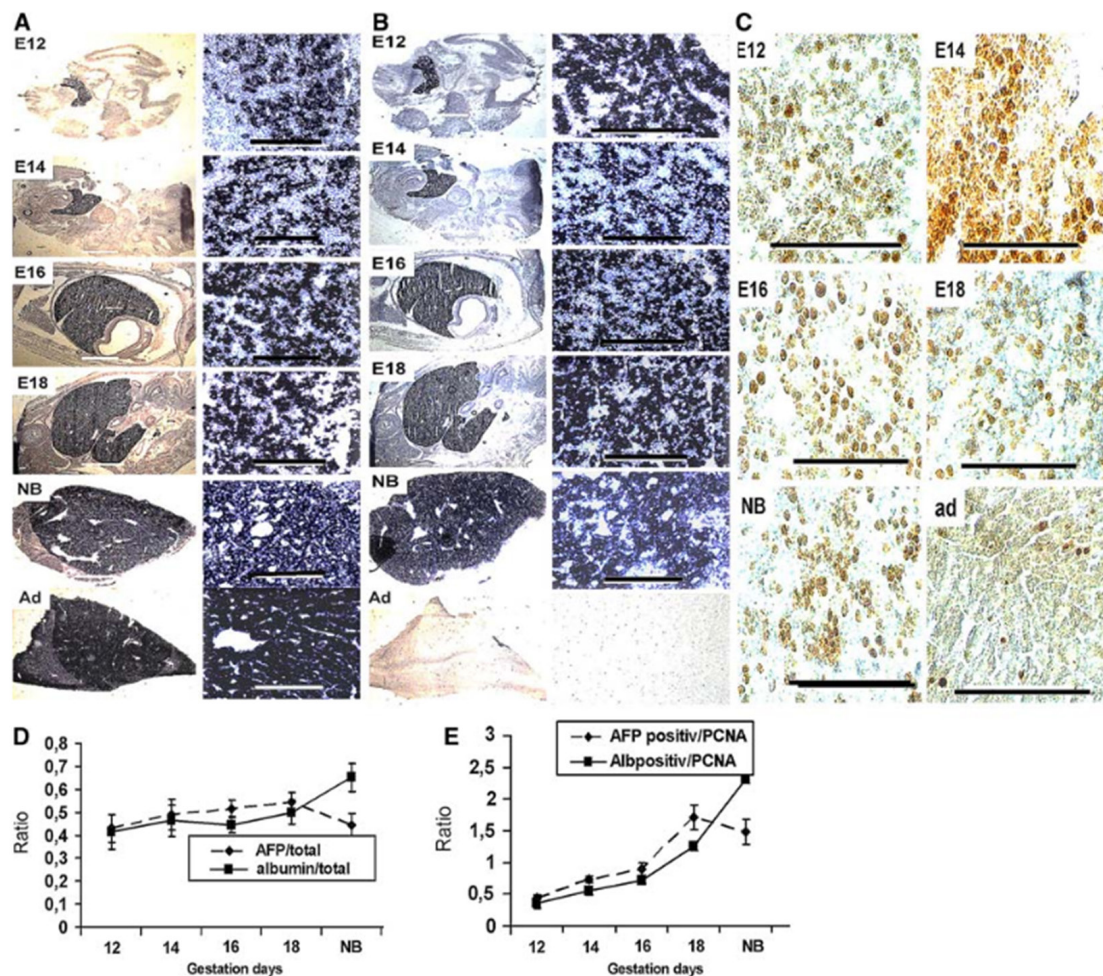


Fig. 5 Albumin- (A) and AFP- (B) mRNA-expression in developing liver assessed by in situ hybridization using DIG-labeled antisense RNA probes (Original magnification 50 \times ; 200 \times , Bars = 100 μ m). (C) Proliferation of hepatoblasts estimated by the number of cells positively stained for proliferating cell nuclear antigen (PCNA) in developing liver. Immunohistochemical reaction was detected by peroxidase-labeled secondary antibody (Original magnification 400 \times , bars = 100 μ m). (D) The ratio of albumin- and AFP-expressing cells

to total cells and (E) the ratio of albumin- and AFP-expressing cells to PCNA⁺ cells during liver development. Albumin- and AFP-positive cells were identified by in situ hybridization, and PCNA-positive cells by immunohistochemical staining. The positive cells were counted under microscope using a shaded ocular, and by application of Image J software. Error bars represent S.E.M., $n = 3$. The significance ($P < 0.05$) was analyzed by ANOVA

Figure 1. Panel A shows the results of in-situ-hybridisation analysis performed in slices of embrional liver at different stages of development in NB and Ad rats. The intensity of the reaction demonstrates an abundance of albumin-specific mRNA. NB: newborn; Ad: adult. *Histochem Cell Biol* 2007;128:431-43. (reprinted with permission)^[37]

The prognostic importance of serum albumin levels in patients with liver disease is demonstrated by the inclusion of this parameter in the Child-Turcotte-Pugh score, used to assess the prognosis of chronic liver disease, mainly cirrhosis. This score was introduced by surgeons in 1963^[32].

In addition, serum albumin level is a key nutritional parameter used to estimate the grade of malnutrition, and to predict survival in patients with liver cirrhosis. Malnutrition is an independent risk factor for transplantation, and improves the prognostic value of the Child-Turcotte-Pugh score, reported by Alberino *et al.*^[33].

While administration of albumin in patients with advanced liver disease and hypoalbuminemia is now a standard therapy, albumin administration in critically ill patients with or without liver disease in the ICU is controversial^[34-36].

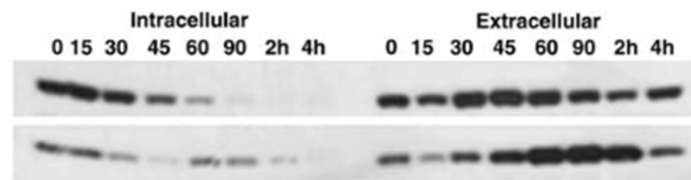


Fig. 4 Kinetics for albumin synthesis and secretion in hepatoblasts (E14) and adult hepatocytes estimated by pulse chase experiment. The secretion speed in E14 hepatoblasts (upper panel) was comparable with that of the adult hepatocytes (lower panel). At 60 min the apparent increase of labeled albumin protein in the intracellular pool of adult hepatocytes is due to the contamination of the extracellular pool, which dramatically increased at this time point

Figure 2. Autoradiograph of a SDS-PAGE-analysis of immunoprecipitates from cell culture supernatants (hepatoblasts and hepatocytes). Radioactively labelled albumin was immunoprecipitated with a specific antibody. The strong speed of the release of the newly synthesized protein is an explanation for the difficulty to detect albumin (as a protein) in the liver sections by using immunostaining techniques. *Histochem Cell Biol* 2007;128:431-43. (reprinted with permission)^[37]

The liver is the sole source of serum albumin^[37] [Figures 1 and 2] which represents more than 50% of all proteins synthesized in the liver. Under normal conditions albumin synthesis in the hepatocytes is regulated by the amount of proteins reaching the intestine after each meal, and the amount of amino acids transported into the liver through the portal system.

During fasting, reduced albumin synthesis is due to a reduced uptake of amino acids into the hepatocytes^[38], which may be in part compensated by using amino acids from muscle proteins.

During acute phase situations, characterised by tissue damage induced by different insults such as trauma, bacterial infection, or viral infections such as SARS-CoV-2, the defence mechanisms of the body concentrate on eliminating the aggressive agent at the site of tissue entry and/or the damaged tissue. The main systemic reactions during the COVID-19 illness are fever, weakness and loss of appetite. In addition vomiting, diarrhea and abdominal discomfort^[39], which may be accompanied by loss of taste^[40] and loss of smell (anosmia)^[41,42], may be also be present. At the beginning of the illness a dry cough and sometimes dyspnoea may be present. The systemic defence reaction may last for a few days and the consequences may not be clinically noted if the person continues to stay home and recovers promptly. If the symptoms last for a week or longer, two major consequences have to be considered: (1) severe fluid losses leading to dehydration and ultimately hypovolaemic shock; (2) reduction in caloric intake which worsens symptoms of weakness, and accelerates a rapid loss in body weight^[43].

These changes may be aggravated by the simultaneous intake of antihypertensive medication, including diuretics, as might be encountered in older patients and/or those patients with multiple comorbidities^[44].

The systemic reaction, a major component of body defence strategy, is induced by different cytokines that originate the main site of injury, e.g., the lungs. The so called “major acute-phase mediators” are Interleukin-6, Interleukin-1, TNF-alpha, and IFN-gamma, which are all synthesized in different amounts, depending on the quality (organ and damaging agent) and the quantity of tissue damage.

The acute phase cytokines are responsible for the central regulation of body temperature^[45], reduction in appetite, and associated adynamia and mental confusion^[46].

The reduction of appetite (anorexia) on the one hand, and abdominal discomfort on the other, can also be attributed to the direct action of the cytokines on the intestinal neurons, with alterations in the mobility

936 INTERLEUKIN 1 MODULATION OF ACUTE PHASE PROTEINS

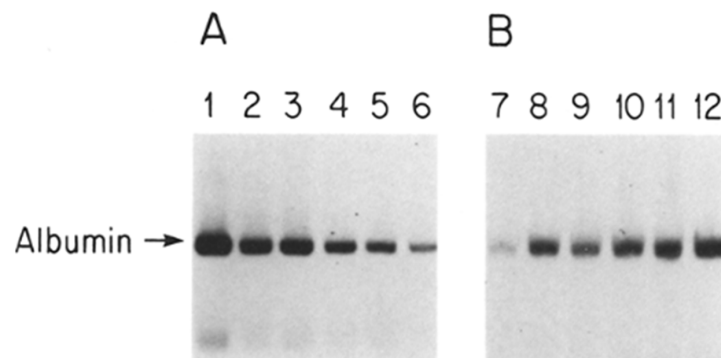


FIGURE 4. Kinetics of mouse recombinant IL-1 inhibition of (A) albumin synthesis and kinetics of release, from (B) the IL-1 effect. SDS-PAGE (7.5%) of secreted [³⁵S]methionine-labeled albumin immunoprecipitated from media of hepatocyte cultures after preincubation with mouse IL-1 (100 U/ml). A 1, control (medium alone); 2, recombinant IL-1 during the 2-h pulse-labelling period; 3, after 3 h; 4, after 7 h; 5, after 12 h; and 6, after 24 h with recombinant IL-1. B, 7, hepatocytes were pulse-labeled for 2 h, 4 h after washing out recombinant IL-1; 8, control (no IL-1 preincubation); 9, 10 h after release from recombinant IL-1; 10, 10-h control; 11, 22 h after release from IL-1; 12, 22-h control.

Figure 3. Autoradiograph of a SDS-PAGE-analysis of radioactively labelled albumin from the supernatants of hepatocytes treated with the first recombinant IL-1 for different time lengths (panel A). Panel B demonstrates that the inhibitory effect of IL-1 on albumin synthesis is reversible (kinetic of release of the effect of the cytokine). *J Exp Med* 185;168:930-42. (reprinted with permission)^[49]

of the large and small bowel^[47,48]. The liver, as the source of the majority of the serum proteins, is the main target of the acute phase cytokines. These cytokines induce pretranslational modification of gene expression through direct interaction with the hepatocytes^[49] [Figure 3]. There are positive and negative acute phase proteins^[45].

According to the variations of their serum level, the positive acute-phase proteins are defined “major”, not because of the volume of their serum level, but because of the magnitude (up to 1.000 fold) of the increase in their serum level.

CRP, Serum Amyloid A, Serum Amyloid P, lactoferrin^[50], Lipocalin-2^[51], hepcidin^[52-55], Interleukin-8^[56], and Erythropoietin^[57] all belong to the “major” acute-phase secretory protein group, while hemoxygenase-1 belongs to the positive^[58] intracellular acute-phase proteins. “Minor” acute-phase proteins are fibrinogen, fibronectin, ceruloplasmin, alpha-1-antitrypsin, complement fraction 3, Factor B, and many others.

As most of the major acute-phase proteins have a low molecular weight, measurement of their serum level may not correspond to a real increase in hepatic synthesis. This is due to the rapid elimination via the urine. Hepcidin was first identified in the urine^[59].

Albumin is the main negative secretory acute phase protein [Figure 4]^[49], whilst ferroportin-1 and hemojuvelin belong to the negative intracellular acute-phase protein group^[52-55]. In a rat model, albumin mRNA in the liver was reduced by 50%, while total mRNA was increased by 50%, 2 days after infection with live *Escherichia Coli*^[60]. During the 2 days rats ate only 5%-10% of the amount of food consumed prior to injection by the bacteria. This was followed by a further aggravation of the reduction of albumin synthesis^[60], further demonstrated in isolated liver perfusion studies^[61], and in humans under caloric restriction^[62]. The amount of the acute-phase cytokines released into the circulation, and the concentration needed for the systemic appearance of the symptoms and of the metabolic changes, are different in different patients. They may be regulated differently by the drug administered, especially in the acute diseases. However, the response is mainly proportional to the extent of the tissue damage.

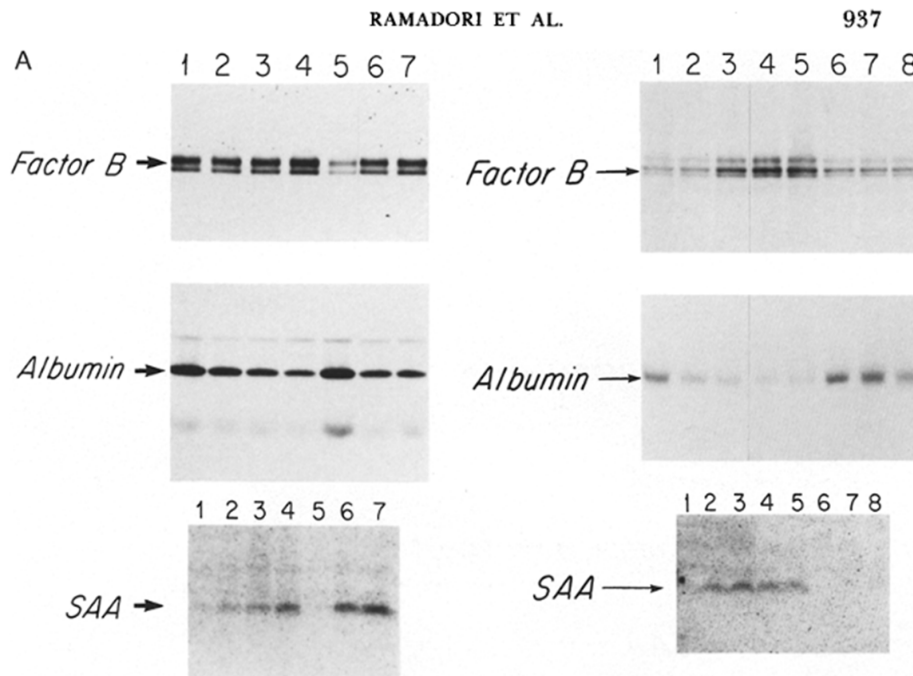


FIGURE 6. A, dose-response regulation of factor B, albumin and SAA synthesis by hepatocytes in culture. SDS-PAGE (7.5% for albumin and factor B, 15% for SAA) of extracellular factor B, albumin, and SAA immunoprecipitated from hepatocyte culture media, after a 2-h pulse with [³⁵S]methionine. Lanes 1–4, 0.5, 2.5, 5.0, and 10 U/ml human IL-1, lane 5, negative control (medium lacking human IL-1), 6 and 7, 10, and 40 U/ml of mouse recombinant IL-1, respectively. B, factor B, albumin, and SAA biosynthesis by hepatocytes in culture; dose-response to mouse recombinant IL-1. SDS-PAGE (7.5% for albumin and for factor B, 15% for SAA) of intracellular factor B, albumin, and SAA immunoprecipitated from cellular lysates after 22-h pulse with [³⁵S]methionine. Lane 1, hepatocytes incubated with medium alone; 2–4, hepatocytes incubated with medium containing 5, 20, 100 U/ml mouse recombinant IL-1; 5, 30 U/ml human IL-1; 6, nontransformed bacterial extract; 7, medium containing dilution of guanidine hydrochloride; 8, medium containing 10 µg LPS/ml. Film exposed 2 d for factor B, 24 h for albumin, and 21 d for SAA. The triplet of intracellular factor B represents different stages of glycosylation of the molecule.

Figure 4. Autoradiographs of SDS-PAGE-analysis of a biosynthetically, radio-actively labelled major positive acute-phase-protein (SAA), a minor positive acute-phase (factor B) and of the major negative acute-phase protein (albumin) immunoprecipitated from the same sample of supernatant from hepatocyte cultures treated with different amounts of recombinant IL1. Line 5 in panel A and lines 7–9 are negative controls. The relative abundance of the different proteins released into the supernatant is demonstrated by the time of exposure of the film to the filter containing the immunoprecipitated radioactive protein. The shortest time of exposure time was for albumin (24 h) and the longest was SAA (21 days). While synthesis of albumin was inhibited by increasing doses of human recombinant IL-1, synthesis of factor B and of SAA were increased at the same time in the hepatocyte reproducing the process taking place in the liver in vivo during an acute phase situation. It is understandable that the serum concentrations of the acute-phase cytokines produced at extrahepatic sites has to be quite high to induce changes of protein synthesis in the liver until these can become measurable. This is also the case for those proteins whose constitutive gene-expression is almost undetectable, as is the case for SAA or CRP in humans. SAA: serum amyloid A. 1985;162:930–42. (reprinted with permission)^[49]

In summary, two main mechanisms act in reducing albumin serum concentration in patients with severe COVID-19-infection: (1) reduction in albumin synthesis due to reduced food intake; (2) inhibition of specific mRNA-synthesis in the hepatocellular nuclei induced by the direct interaction of the cell with the acute-phase cytokines.

The acute-phase cytokines induce up-regulation of gene-expression of several positive hepatic acute-phase proteins, and in extrahepatic organs^[63] [Figure 5], but the changes in serum level are influenced by their synthesis in liver cells^[45]. This mechanism is not only active in cases of tissue damage caused by bacterial, but also by viral infections^[64]. The order of magnitude of variations in the serum level of the acute-phase proteins caused by viral infections is lower than that induced by bacterial infections.

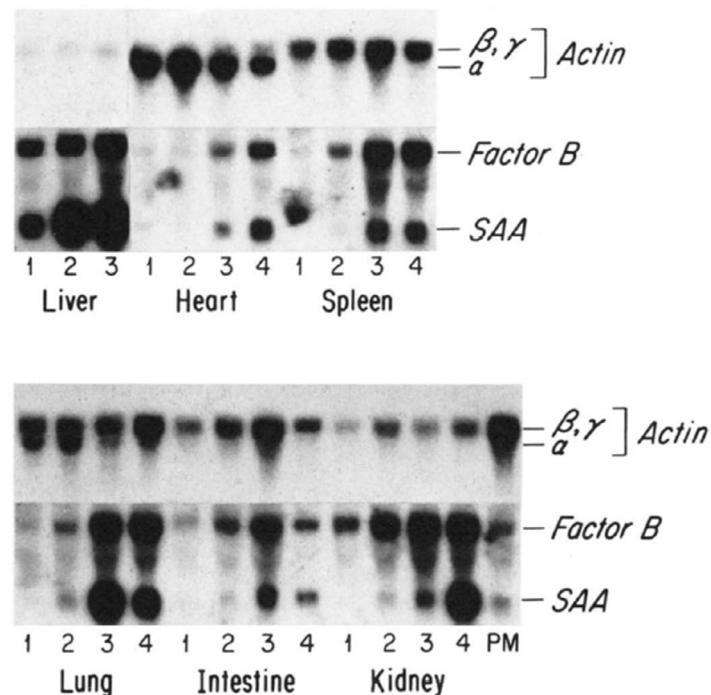


Figure 1. In vivo modulation of murine SAA, factor B, and actin gene expression by endotoxin. Animals (C3HeB/FeJ mice) were killed and liver, heart, spleen, lung, intestine, and kidney were taken 16 hr after injection of either saline (lane 1) or endotoxin at 1 μ g (lane 2), 10 μ g (lane 3), and 100 μ g (lane 4). PM = RNA isolated 2 hr after plating from peritoneal macrophages from five control animals. Fifteen micrograms of total RNA were loaded in each lane. Autoradiograms after exposure to Kodak XAR-5 films at 24 hr for the liver and 6 days for the other organs (SAA- and factor B-specific cDNA probes), and 6 hr exposure for the actin-specific cDNA probe. The signals detected between lanes 1 and 2 of heart and in lane 1 of the spleen blots represent nonspecific background. This is one of three experiments that showed similar results. In all of the experiments, a dose-related effect of endotoxin on SAA and factor B gene expression was noted.

Figure 5. Autoradiograph of results of analysis of RNA (Northern) from organs of mice treated intraperitoneally with different amounts of *E. Coli* LPS as a model to induce an acute-phase reaction. The filters containing the tissue-RNA were hybridised with radio-actively labelled cDNAs specific for factor B, for SAA and for actin as control. In all organs factor B- and SAA-gene-expression was up-regulated in a dose-dependent manner. The different time of exposure of the x-ray film demonstrate the different abundance of gene-expression of factor B and SAA in the different organs. SAA: serum amyloid A; LPS: lipopolysaccharide. *J Immunol* 1985;135:3645-7. (reprinted with permission)^[63]

Physical examination results obtained in hospitalized patients are not reported in the different publications, but most of the patients who were transferred from the emergency room to the ICU will likely have presented with clear signs of exsiccosis, hypotension and eventually malnutrition as testified by the low serum albumin levels. This should be highlighted in the guidelines for the initial supportive management of patients with COVID-19. If not recognized and promptly treated, progression to the second stage of the disease, with deterioration in respiratory function, will likely occur.

Patients suffering from mild disease who presented with normal serum albumin levels, even those who have developed a deterioration, maintained normal serum levels and could be released from the hospital^[15,16,65].

Although albumin administration is not recommended in patients with low serum albumin levels being treated in the ICU^[35,36], previous positive experiences^[66] with repeated administration of 200-400 mL of convalescent plasma showed positive effects in some critically ill COVID-19-patients^[67-70]. The positive effect of convalescent plasma infusion could be attributed not only to the COVID-19-specific immunoglobulins, but also to the other components of the plasma e.g., albumin^[71].

DECLARATIONS

Authors' contributions

The author contributed solely to the article.

Availability of data and materials

Not applicable.

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None.

Conflicts of interest

The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

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REFERENCES

1. Wang Y, Li X, Liu W, Gan M, Zhang L, et al. Discovery of a subgenotype of human coronavirus NL63 associated with severe lower respiratory tract infection in China, 2018. *Emerg Microbes Infect* 2020;9:246-55.
2. Zhou F, Yu T, Du R, Fan G, Liu Y, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395:1054-62.
3. Huang C, Wang Y, Li X, Ren L, Zhao J, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;395:497-506.
4. Shi H, Han X, Jiang N, Cao Y, Alwalid O, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect Dis* 2020;20:425-34.
5. Chen T, Wu D, Chen H, Yan W, Yang D, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ* 2020;368:m1091.
6. Wang D, Hu B, Hu C, Zhu F, Liu X, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020;323:1061-9.
7. Zhu N, Zhang D, Wang W, Li X, Yang B, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;382:727-33.
8. Li Q, Guan X, Wu P, Wang X, Zhou L, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med* 2020;382:1199-1207.
9. Liu Y, Yang Y, Zhang C, Huang F, Wang F, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci* 2020;63:364-74.
10. Zhang Y, Xiao M, Zhang S, Xia P, Cao W, et al. Coagulopathy and antiphospholipid antibodies in Patients with COVID-19. *N Engl J Med* 2020;382:e38.
11. Liu CL, Lu YT, Peng MJ, Chen PJ, Lin RL, et al. Clinical and laboratory features of severe acute respiratory syndrome vis-a-vis onset of fever. *Chest* 2004;126:509-17.
12. Leong HN, Earnest A, Lim HH, Chin CF, Tan C, et al. SARS in Singapore--predictors of disease severity. *Ann Acad Med Singapore* 2006;35:326-31.
13. Ko JH, Park GE, Lee JY, Lee JY, Cho SY, et al. Predictive factors for pneumonia development and progression to respiratory failure in MERS-CoV infected patients. *J Infection* 2016;73:468-75.
14. Leem AY, Park B, Kim YS, Jung JY, Won S. Incidence and risk of chronic obstructive pulmonary disease in a Korean community-based cohort. *Int J Chron Obstruct Pulmon Dis* 2018;13:509-17.
15. Zhang J, Wang X, Jia X, Li J, Hu K, et al. Risk factors for disease severity, unimprovement, and mortality in COVID-19 patients in Wuhan, China. *Clin Microbiol Infect* 2020; Epub ahead of print. doi: 10.1016/j.cmi.2020.04.012.
16. Gong J, Ou J, Qiu X, Jie Y, Chen Y, et al. A tool to early predict severe corona virus disease 2019 (COVID-19) : a multicenter study using

- the risk nomogram in Wuhan and Guangdong, China. *Clin Infect Dis* 2020; Epub ahead of print. doi: 10.1093/cid/ciaa443.
17. Cohn EJ, Oncley JL, Strong LE, Hughes WL, Armstrong SH. Chemical, clinical, and immunological studies on the products of human plasma fractionation. I. the characterization of the protein fractions of human plasma. *J Clin Invest* 1944;23:417-32.
18. Scatchard G, Batchelder AC, Brown A. Chemical, clinical, and immunological studies on the products of human plasma fractionation. VI. The osmotic pressure of plasma and of serum albumin. *J Clin Invest* 1944;23:458-64.
19. Post J, Patek AJ. Serum proteins in relation to liver disorders. *Bull N Y Acad Med* 1943;19:815-30.
20. Thorn GW, Armstrong SH, Davemport VD. Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXXI. The use of salt-poor concentrated human serum albumin solution in the treatment of hepatic cirrhosis. *J Clin Invest* 1946;25:304-23.
21. Kunkel HG, Labby DH, Ahrens EH, Shank RE, Hoagland CL. The use of concentrated human serum albumin in the treatment of cirrhosis of the liver. *J Clin Invest* 1948;27:305-19.
22. Wilkinson P, Sherlock S. The effect of repeated albumin infusions in patients with cirrhosis. *Lancet* 1962;2:1125-9.
23. Gentilini P, Casini-Raggi V, Di Fiore G, Romanelli RG, Buzzelli G, et al. Albumin improves the response to diuretics in patients with cirrhosis and ascites: results of a randomized, controlled trial. *J Hepatol* 1999;30:639-45.
24. Caraceni P, Riggio O, Angeli P, Alessandria C, Neri S, et al. Long-term albumin administration in decompensated cirrhosis (ANSWER): an open label randomized trial. *Lancet* 2018;391:2417-29.
25. Patek AJ, Mankin H, Colcher H, Lowell A, Earle DP. The effects of intravenous injection of concentrated human serum albumin upon blood plasma, ascites and renal functions in three patients with cirrhosis of the liver. *J Clin Invest* 1948;27:135-44.
26. Schindler C, Ramadori G. Albumin substitution improves urinary sodium excretion and diuresis in patients with liver cirrhosis and refractory ascites. *J Hepatol* 1999;31:1132.
27. Schindler C, Ramadori G. Humanalbumingaben zur Verbesserung der renalen Ausscheidungsfunktion bei Patienten mit therapierefraktärem Aszites-Ein Erfahrungsbericht. *Leber Magen Darm* 1999;4:183-7.
28. Nolte W, Ramadori G. Albumin for refractory ascites. *Gastroenterology* 2003;125:1283-4.
29. Trotter J, Pieramici E, Everson GT. Chronic albumin infusions to achieve diuresis in patients with ascites who are not candidates for transjugular intrahepatic portosystemic shunt (TIPS). *Dig Dis Sci* 2005;50:1356-60.
30. Bajaj JS, Tandon P, O'Leary JG, Biggins SW, Wong F, et al. The impact of albumin use on resolution of hyponatremia in hospitalized patients with cirrhosis. *Am J Gastroenterol* 2018;113:1339.
31. Bai Z, Bernardi M, Yoshida EM, Li H, Guo X, et al. Albumin infusion may decrease the incidence and severity of overt hepatic encephalopathy in liver cirrhosis. *Aging (Albany NY)* 2019;11:8502-25.
32. Kaplan DE, Dai F, Aytaman A, Baytarian M, Fox R, et al.; VOCAL Study Group. Development and performance of an algorithm to estimate the child-turcotte-pugh score from a national electronic healthcare database. *Clin Gastroenterol Hepatol* 2015;13:2333-41.e1-6.
33. Alberino F, Gatta A, Amodio P, Merkel C, Di Pascoli L, et al. Nutrition and survival in patients with liver cirrhosis. *Nutrition* 2001;17:445-50.
34. Paine CH, Biggins SW, Pichler RH. Albumin in cirrhosis: more than a colloid. *Curr Treat Options Gastroenterol* 2019;17:231-43.
35. Wujtewicz M, Dylczyk-Sommer A, Aszkielowicz A, Zdanowski S, Piwowarczyk S, et al. COVID-19 - what should anaesthesiologists and intensivists know about it? *Anaesthesiol Intensive Ther* 2020;52:34-41.
36. Alhazzani W, Möller MH, Arabi YM, Loeb M, Gong MN, et al. Surviving sepsis campaign: Guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). *Intensive Care Med* 2020;46:854-87.
37. Elmaouhoub A, Dudas J, Ramadori G. Kinetics of albumin- and alpha-fetoprotein-production during rat liver development. *Histochem Cell Biol* 2007;128:431-43.
38. Fanali G, di Masi A, Trezza V, Marino M, Fasano M, et al. Human serum albumin: from bench to bedside. *Mol Aspects Med* 2012;33:209-90.
39. Lin L, Jiang X, Zhang Z, Huang S, Zhang Z, et al. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut* 2020;69:997-1001.
40. Lechien JR, Chiesa-Estomba CM, De Siati DR, Horoi M, Le Bon SD, et al. Olfactory and gustatory dysfunctions as clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur Arch Otorhinolaryngol* 2020;1-11.
41. Lovato A, de Filippis C. Clinical presentation of COVID-19: a systematic review focusing on upper airway symptoms. *Ear Nose Throat J* 2020;145561320920762.
42. Russell B, Moss C, Rigg A, Hopkins C, Papa S, et al. Anosmia and ageusia are emerging as symptoms in patients with COVID-19: what does the current evidence say? *Ecanermedicalscience* 2020;14:ed98.
43. Baker T, Schell CO, Petersen DB, Sawe H, Khalid K, et al. Essential care of critical illness must not be forgotten in the COVID-19 pandemic. *Lancet* 2020;395:1253-4.
44. Dreher M, Kersten A, Bickenbach J, Balfanz P, Hartmann B, et al. Charakteristik von 50 hospitalisierten COVID-19-Patienten mit und ohne ARDS. *Deut Arch Intern Med* 2020;117:271-8.
45. Ramadori G, Christ B. Cytokines and the hepatic acute-phase response. *Semin Liver Dis* 1999;19:141-55.
46. Buckley MM, O'Brien R, Devlin M, Creed AA, Rae MG, et al. Leptin modifies the prosecretory and prokinetic effects of the inflammatory cytokine interleukin-6 on colonic function in Sprague-Dawley rats. *Exp Physiol* 2016;101:1477-91.
47. Goto J, Matsuda K, Harii N, Moriguchi T, Yanagisawa M, et al. Usefulness of a real-time bowel sound analysis system in patients with severe sepsis (pilot study). *J Artif Organs* 2015;18:86-91.
48. Groves HT, Higham SL, Moffatt MF, Cox MJ, Tregoning JS. Respiratory Viral Infection Alters the Gut Microbiota by Inducing

- Inappetence. *mBio* 2020;11:e03236-19.
49. Ramadori G, Sipe JD, Dinarello CA, Mizel SB, Colten HR. Pretranslational modulation of acute phase hepatic protein synthesis by murine recombinant interleukin 1 (IL-1) and purified human IL-1. *J Exp Med* 1985;162:930-42.
 50. Ahmad G, Sial GZ, Ramadori P, Dudas J, Batusic DS, et al. Changes of hepatic lactoferrin gene expression in two mouse models of the acute phase reaction. *Int J Biochem Cell Biol* 2011;43:1822-32.
 51. Sultan S, Pascucci M, Ahmad S, Malik IA, Bianchi A, et al. LIPOCALIN-2 is a major acute-phase protein in a rat and mouse model of sterile abscess. *Shock* 2012;37:191-6.
 52. Sheikh N, Dudas J, Ramadori G. Changes of gene-expression of iron regulatory proteins during turpentin-oil induced acute-phase response in the rat. *Lab Invest* 2007;87:713-25.
 53. Sheikh N, Batusic DS, Dudas J, Tron K, Neubauer K, et al. Hepcidin and hemojuvelin gene expression in rat liver damage: in vivo and in vitro studies. *Am J Physiol Gastrointest Liver Physiol* 2006;291:G482-90.
 54. Christiansen H, Sheikh N, Saile B, Reuter F, Rave-Fränk M, et al. x-Irradiation in rat liver: consequent upregulation of hepcidin and downregulation of hemojuvelin and ferroportin-1 gene expression. *Radiology* 2007;242:189-97.
 55. Moriconi F, Ahmad G, Ramadori P, Malik I, Sheikh N, et al. Phagocytosis of gadolinium chloride or zymosan induces simultaneous upregulation of hepcidin- and downregulation of hemojuvelin- and Fpn-1-gene expression in murine liver. *Lab Invest* 2009;89:1252-60.
 56. Sheikh N, Tron K, Dudas J, Ramadori G. Cytokine-induced neutrophil chemoattractant-1 is released by the noninjured liver in a rat acute-phase model. *Lab Invest* 2006;86:800-14.
 57. Ramadori P, Sheikh N, Ahmad G, Dudas J, Ramadori G. Hepatic changes of erythropoietin gene expression in a rat model of acute-phase response. *Liver Int* 2010;30:55-64.
 58. Tron K, Novosyadlyy R, Dudas J, Samoylenko A, Kietzmann T, et al. Upregulation of heme oxygenase-1 gene by turpentine oil-induced localized inflammation: involvement of interleukin-6. *Lab Invest* 2005;85:376-87.
 59. Krause A, Neitz S, Mägert HJ, Schulz A, Forssmann WG, et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. *FEBS Lett* 2000;480:147-50.
 60. Ruot B, Breuill   D, Rambourdin F, Bayle G, Capitan P, et al. Synthesis rate of plasma albumin is a good indicator of liver albumin synthesis in sepsis. *Am J Physiol Endocrinol Metab* 2000;279:E244-51.
 61. Flaim KE, Liao WS, Peavy DE, Taylor JM, Jefferson LS. The role of amino acids in the regulation of protein synthesis in perfused rat liver. II. Effects of amino acid deficiency on peptide chain initiation, polysomal aggregation, and distribution of albumin mRNA. *J Biol Chem* 1982;257:2939-46.
 62. Lee JL, Oh ES, Lee RW, Finucane TE. Serum albumin and prealbumin in calorically restricted, nondiseased individuals: a systematic review. *Am J Med* 2015;128:1023.e1-22.
 63. Ramadori G, Sipe JD, Colten HR. Expression and regulation of the murine serum amyloid A (SAA) gene in extrahepatic sites. *J Immunol* 1985;135:3645-7.
 64. Perez L. Acute phase protein response to viral infection and vaccination. *Arch Biochem Biophys* 2019;671:196-202.
 65. Zhou Y, Zhang Z, Tian J, Xiong S. Risk factors associated with disease progression in a cohort of patients infected with the 2019 novel coronavirus. *Ann Palliat Med* 2020;9:428-36.
 66. Chen L, Xiong J, Bao L, Shi Y. Convalescent plasma as a potential therapy for COVID-19. *Lancet Infect Dis* 2020;20:398-400.
 67. Shen C, Wang Z, Zhao F, Yang Y, Li J, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. *JAMA* 2020;323:1582-9.
 68. Duan K, Liu B, Li C, Zhang H, Yu T, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci* 2020;117:9490-6.
 69. Zhang B, Liu S, Tan T, Huang W, Dong Y, et al. Treatment with convalescent plasma for critically ill patients with SARS-CoV-2 infection. *Chest* 2020; Epub ahead of print. doi: 10.1016/j.chest.2020.03.039.
 70. Bloch EM, Shoham S, Casadevall A, Sachais BS, Shaz B, et al. Deployment of convalescent plasma for prevention and treatment of COVID-19. *J Clin Invest* 2020; Epub ahead of print. doi: 10.1172/JCI138745.
 71. Roback JD, Guarner J. Convalescent plasma to treat COVID-19: possibilities and challenges. *JAMA*. 2020; Epub ahead of print. doi: 10.1001/jama.2020.4940.

Review

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Epidemiology of hepatocellular carcinoma in metabolic liver disease

Stefano Bellentani

Consultant Gastroenterologist and Hepatologist 6600 Locarno, Ticino, Switzerland.

Correspondence to: Dr. Stefano Bellentani, Studio Medico - Via Della Pace 3, 6600 Locarno, Ticino, Switzerland.
E-mail: bellentanistefano@gmail.com

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Abstract

Nonalcoholic fatty liver disease and its evolutive form nonalcoholic steatohepatitis (NASH) are nowadays the second/third cause of chronic liver disease worldwide, and their prevalence and incidence are rapidly increasing in parallel to the burden of diabetes and obesity. Hepatocellular carcinoma (HCC) due to NASH (HCC-NASH) has become the major cause of HCC and is now one of the major indications for liver transplant in Western countries, after that due to HCV infection. NASH occurs both in the presence and absence of liver cirrhosis. In this review, we describe the epidemiology of HCC related to metabolic liver disease: not only NASH-HCC but also type 2 diabetes mellitus and obesity-related HCC. Some new practical guidelines for screening and surveillance of patients with metabolic diseases at risk for HCC are also discussed.

Keywords: Nonalcoholic steatohepatitis, hepatocellular carcinoma, metabolic syndrome, obesity, type 2 diabetes mellitus

INTRODUCTION

The prevalence of nonalcoholic steatohepatitis (NASH)-related hepatocellular carcinoma (HCC) is increasing in parallel with the prevalence of nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus (T2DM) and obesity worldwide, particularly in Western countries^[1]. HCC develops in patients with NASH and without cirrhosis in 40% of cases^[2,3]. In patients with NASH and advanced (F3) fibrosis or Child A cirrhosis, approximately 20% of patients progress to decompensated cirrhosis over a 2-year



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period^[4,5]. There are significant geographic variations in the incidence of NASH and NASH-HCC^[6,7]. HCC, in general, has the sixth highest cancer incidence, and is the fourth leading cause of cancer-related death in the world^[8]. It is already known that the most prevalent cancer in obesity and T2DM is HCC, and that the prevalence of NAFLD/NASH in obesity and T2DM is significantly higher than in the general population, being 75%, 95% and 25%, respectively^[9,10]. Obesity and high-fat diet are increasingly recognized as major risk factors for HCC-NASH, but the exact molecular mechanisms integrating these events remain unclear^[11]. Furthermore, the etiology and pathogenic link between obesity, T2DM and NASH-HCC is not completely understood, at least in humans. It could be related to STAT-1/STAT-3 intracellular signaling^[12] or, according to new pathogenetic interpretation, also to alteration of the gut microbiota^[11]. We also know that a substantial proportion of patients with NAFLD/NASH will never develop HCC, and thus, it is very important to understand the exact relationship between NASH-cirrhosis and NASH-HCC and metabolic diseases to program good prevention and treatment measures for these patients.

We try to unravel these still less known diseases in the following section.

THE NATURAL HISTORY OF NASH

NAFLD is still interpreted as a negative definition used to describe a spectrum of metabolic liver diseases due to excessive hepatic fat accumulation with associated insulin resistance, in the absence of significant alcohol consumption or other causes^[13,14]. While NAFLD can refer to simple steatosis, NASH is characterized by inflammation and is associated with an increased risk of progression to fibrosis, advanced fibrosis, cirrhosis, hepatic decompensation and HCC^[13,14]. NASH can only be reliably differentiated from NAFLD histologically, and the prevalence of NASH among biopsied NAFLD patients is estimated to be 59.1% (95%CI: 47.55%-69.73%) from pooled NASH prevalence data^[15]. Fibrosis is an important bad prognostic factor in NAFLD/NASH and in patients with NASH and advanced (F3) fibrosis or compensated cirrhosis, and it has been shown that approximately 20% of them will progress to cirrhosis/HCC, or hepatic decompensation over a 2-year period^[4,5]. As shown in [Figure 1](#) and as reported by Goah and McCollough^[5], the natural history of NASH and its passage to advanced fibrosis, and then to cirrhosis and to HCC, depends on different risk factors such as sex, age, obesity, T2DM, genetic factors, and fibrosis stage. The first steps are also driven by a genetic predisposition contributing by itself to the development of insulin resistance and hepatic steatosis. Other co-factors could determine the lower or higher rate of progression from NASH to advanced fibrosis and to cirrhosis or HCC [[Figure 1](#)].

It must also be considered that NAFLD and NASH prevalence and incidence are different according to sex, usually higher in men than in premenopausal women (or age ≤ 50 -60 years), while they tend to become more common in women after menopause (or age ≥ 50 -60 years)^[16]. This is probably due to sex hormone levels that might influence hepatic lipid and carbohydrate metabolism; particularly, it seems that estrogens may protect the liver from fibrosis in NAFLD/NASH. Estrogen seems to protect also from liver tumorigenesis, and theoretically, prolonging estrogen depletion (i.e., premature menopause) may lead to increased HCC risk in women with NASH^[16].

OBESITY, GUT MICROBIOTA AND NASH-HCC

Mechanisms of obesity induced HCC

Obesity is a major driver of cancer, especially HCC.

The prevailing view is that NASH and fibrosis or cirrhosis are required for HCC in obesity. However, they can also be dissociated in obesity. A recent work by Grohmann^[11] in mice showed a strict correlation between STAT-1 signaling in inducing NASH and fibrosis and STAT-3 in promoting HCC formation. The authors were able also to demonstrate that STAT-3 signaling can drive tumor development in a context

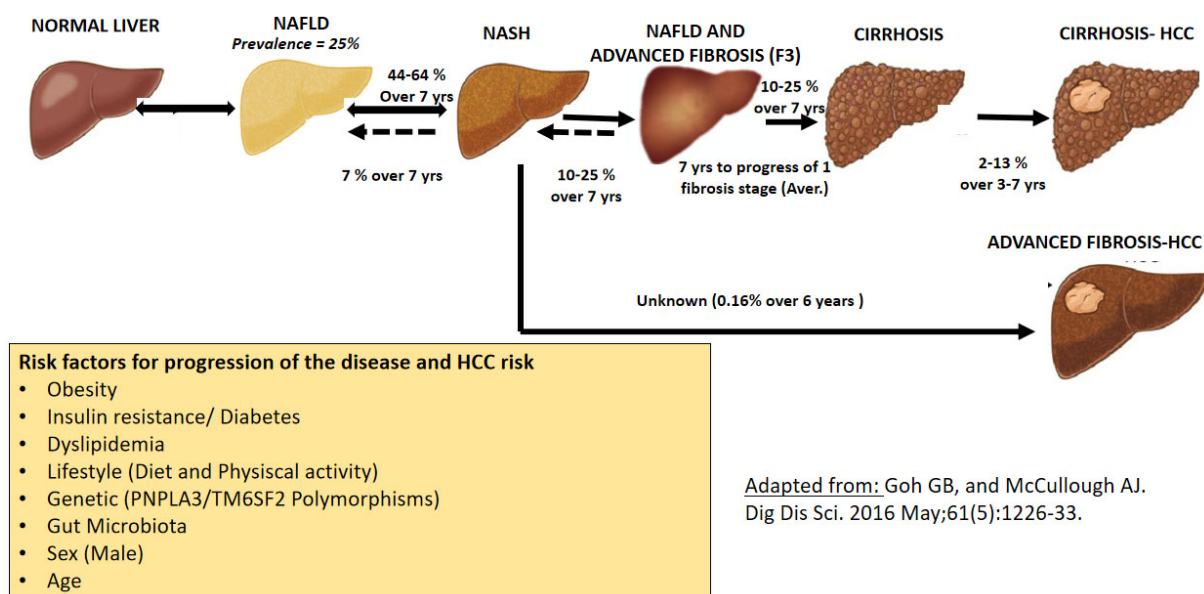


Figure 1. Natural history of NASH and risk factors for progression of NASH to HCC. Adapted from study^[5]. NASH: nonalcoholic steatohepatitis; HCC: hepatocellular carcinoma; NAFLD: nonalcoholic fatty liver disease

of obesity with NAFLD, independently of the presence of NASH and fibrosis. Their studies reveal how obesity-associated hepatic oxidative stress can independently contribute to the pathogenesis of NASH, fibrosis and HCC, but again in mice.

Furthermore, HCC exhibits several characteristic lipid metabolic changes, especially in fatty acid (FA) metabolism, which is linked to NASH and associated with the status of other metabolic pathways, such as glucose metabolism.

Accumulating evidence supports the importance of lipid metabolic reprogramming in various situations of hepatocarcinogenesis. In a recent review, the latest findings regarding the role of FA metabolism pathways in the development of HCC, focusing on reprogramming lipid metabolism, have been discussed in depth^[17].

Another recent study reported^[18] that unconventional prefoldin RPB5 interactor (URI) induces DNA damage in hepatocytes and triggers inflammation via T helper 17 (Th17) lymphocytes and interleukin-17A (IL-17A). This induces neutrophil infiltration into white adipose tissue, mediating insulin resistance (IR) and FA release, stored in liver as triglycerides, causing NASH and subsequently NASH-HCC, suggesting that IL-17A blockers may prevent IR, NASH, and HCC in high-risk patients.

Mechanisms of gut microbiota-induced obesity and HCC

Alterations of intestinal microbiota seem to play a key role in this pathogenetic mechanism. Yoshimoto *et al.*^[19] studied hepatocarcinogenesis in obese mice, showing that the administration of antibiotics and gut sterilization could decrease the development of HCC in treated mice, modulating the dysbiosis and the subsequent secretion of pro-inflammatory and pro-carcinogenetic factors. Their data suggested that gut sterilization and antibiotic treatments could prevent the development of HCC, but these treatments did not lead to the regression of already established tumors. Several other lines of research showed that the gut microbiota is also involved in the development of HCC, in particular by increasing lipopolysaccharide levels and creating a subsequent pro-inflammatory microenvironment in the liver. Another mechanism

could be the one discovered recently by Loo *et al.*^[20] involving lipoteichoic acid, a gram-positive gut microbial component that seems to promote HCC development by creating a tumor-promoting microenvironment. Lipoteichoic acid enhances the senescence-associated secretory phenotype of hepatic stellate cells (HSC) collaboratively with the obesity-induced gut microbial metabolite deoxycholic acid to upregulate the expression of senescence-associated secretory phenotype factors and COX2 through Toll-like receptor 2. Interestingly, COX2-mediated prostaglandin E2 (PGE2) production suppresses antitumor immunity through a PTGER4 receptor, thereby contributing to HCC progression.

Moreover, COX2 overexpression and excess PGE2 production have been detected in HSCs in patients with NASH-HCC. The gut microbiota-driven COX2 pathway produces the lipid mediator PGE2 in senescent HSCs, which plays a pivotal role in suppressing antitumor immunity, suggesting that also PGE2 and its receptor may be novel therapeutic targets for noncirrhotic HCC-NASH^[18].

All these new experimental findings discovering links between lipid metabolism, inflammation, insulin resistance, gut microbiome and HCC-NASH are still to be confirmed in humans, but they demonstrate that the pathogenetic pathway of NASH-HCC is multifactorial, very complex and still far away from leading to an efficacious treatment for this increasingly prevalent cancer.

T2DM AND NASH-HCC

T2DM was also found to be an independent factor associated with HCC among patients with cryptogenic NASH-cirrhosis since 2002^[21]. However, the association between diabetes and the risk of HCC in NASH patients with cirrhosis is not well quantified and completely understood. Diabetes increases the risk of liver disease progression to cirrhosis in patients with NASH. Only recently, Yang *et al.*^[22] investigated the association between diabetes and HCC in patients with NASH cirrhosis in a large cohort with a longer follow-up and were able to demonstrate, even also in humans, that T2DM is associated with an increased risk of HCC in patients with NASH cirrhosis. Their secondary aim was to investigate the association between other metabolic risk factors (body mass index, hypertension, and hyperlipidemia) and HCC. However, the above metabolic risk factors were not associated with HCC risk^[22].

Unfortunately there are not many other studies in humans that help to elucidate the molecular mechanisms underlying the pathogenesis of NASH-HCC related to T2DM. Progress in this field depends on the availability of reliable preclinical models amenable to genetic and functional analyses and exhibiting robust NASH-to-HCC progression.

GENETIC, LIVER METABOLIC DISEASE AND NASH-HCC

Only recently was the association between NASH and genetic predisposition investigated, especially in young adults with NASH without any metabolic diseases. The most recent and important study is the one by Unalp-Arida and Ruhl^[21]. They examined the relationships of liver disease markers, including patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) I148M, with mortality in a very large series (13,298 viral hepatitis-negative adults) belonging to the United States National Health and Nutrition Examination Survey, 1988-1994, with 27 years of linked mortality data. They found that *PNPLA3* I148M was associated with increased liver disease mortality, and they suggested that the genetic variant *PNPLA3* I148M may be used as a marker in patients with NAFLD/NASH for HCC surveillance.

SCREENING AND SURVEILLANCE OF HCC IN PATIENTS WITH NASH-CIRRHOSIS OR METABOLIC DISEASES

There is now substantial evidence that NAFLD/NASH-associated HCC may arise either in the presence or absence of cirrhosis^[3,4,13,14,22-24]. Limited data are available to address the clinician's need to know who is

the NASH patient or patients with metabolic disease who should undergo screening and surveillance for HCC, and many questions remain to be answered, including new and efficacious strategies for targeting high-risk subjects in the general population^[25]. A recent evidence-based expert review^[24] tries to clarify this matter, and summarizes the American Gastroenterology Association practical update on screening and surveillance for HCC in patients with NAFLD/NASH^[24]. In this review, different practical guidelines are suggested for 3 groups of patients: 1- patients with NASFLD/NASH and without advanced fibrosis; 2- patients with advanced fibrosis, and finally, 3- patients with NASH-cirrhosis^[24]. In the first group, based on a recent national Veterans Affairs study in the USA, HCC incidence rates of patients with NASFLD/NASH and not advanced fibrosis were 0.21/1000 person-years (0.02% annual risk) in NAFLD and 0.02/1000 person-years (0.002% annual) in controls^[26,27]. Although there was a higher risk of developing HCC in patients with earlier stages of NAFLD and low fibrosis than in those without NAFLD, the authors concluded that the risk estimate is likely to be too low to justify routine screening in those who have early NAFLD with no evidence of advanced fibrosis^[24].

Considering the second group, HCC screening in patients who have NAFLD/NASH with advanced fibrosis, as determined by combining at least 2 noninvasive score testing modalities suggestive of cirrhosis (in the absence of biopsy-confirmed cirrhosis or overt cirrhosis on imaging), associated with diagnosis of cirrhosis at elastography examination (i.e., with fibroscan or shear-wave elastography, or quantitative MRI), patients in whom both tests are concordant for advanced fibrosis or cirrhosis should be, on the contrary, considered for HCC screening^[24]. Finally, in the third group, the incidence rate of HCC in NAFLD cirrhosis is estimated to be > 1.5% per year, and therefore, screening for HCC in this group is justifiable, based on cost-effectiveness considerations. Therefore, the best practice guidance recommendation^[24] is to consider and offer HCC screening and surveillance to all patients with NAFLD/NASH compensated cirrhosis, in patients with NAFLD/NASH and advanced fibrosis, but not in patients with simple NAFLD or NASH and non-advanced fibrosis^[24].

CONCLUSION

NAFLD/NASH is an important cause of liver disease worldwide and is associated with an increased risk of developing HCC, either in the presence or absence of liver cirrhosis. The prevalence of metabolic liver disease and of NASH-HCC is increasing in parallel with the prevalence of obesity and T2DM worldwide. The future global burden of NAFLD-related HCC is going to become a major public health issue, so further research to identify the factors involved in promoting inflammation and hepatocarcinogenesis in NAFLD/NASH and to program cost-effective prevention and treatment strategies are urgently needed.

DECLARATIONS

Authors' contributions

The author contributed solely to the article.

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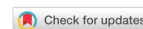
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REFERENCES

1. Bellentani S. The epidemiology of non-alcoholic fatty liver disease. *Liver Int* 2017;37:81-4.
2. Bellentani S, Baroni GS, Piscaglia F, Tiribelli C. Natural history of nonalcoholic steatohepatitis-associated hepatocellular carcinoma. *Clin Liver Dis (Hoboken)* 2016;8:105-7.
3. Piscaglia F, Svegliati-Baroni G, Barchetti A, Pecorelli A, Marinelli S, et al. Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: a multicenter prospective study. *Hepatology* 2016;63:827-38.
4. Loomba R, Adams LA. The 20% rule of NASH progression: the natural history of advanced fibrosis and cirrhosis caused by NASH. *Hepatology* 2019;70:1885-8.
5. Goh GB, McCullough AJ. Natural history of nonalcoholic fatty liver disease. *Dig Dis Sci* 2016;61:1226-33.
6. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. *J Hepatol* 2018;69:896-904.
7. Goossens N, Bellentani S, Cerny A, Dufour JF, Jornayvaz FR, et al. Nonalcoholic fatty liver disease burden - Switzerland 2018-2030. *Swiss Med Wkly* 2019;149:w20152.
8. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, et al.; Global Burden of Disease Cancer Collaboration. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. *JAMA Oncol* 2017;3:524-48.
9. Bellentani S, Saccoccio G, Masutti F, Giacca M, Miglioli L, et al. Risk factors for alcoholic liver disease. *Addict Biol* 2000;5:261-8.
10. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, et al. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005;42:44-52.
11. Brandi G, De Lorenzo S, Candela M, Pantaleo MA, Bellentani S, et al. Microbiota, NASH, HCC and the potential role of probiotics. *Carcinogenesis* 2017;38:231-40.
12. Grohmann M, Wiede F, Dodd GT, Gruzov EN, Ooi GJ, et al. Obesity drives STAT-1-dependent NASH and STAT-3-dependent HCC. *Cell* 2018;175:1289-306.e1220.
13. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1388-402.
14. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018;67:328-57.
15. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73-84.
16. Lonardo A, Nascimbeni F, Ballestri S, Fairweather D, Win S, et al. Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps. *Hepatology* 2019;70:1457-69.
17. Nakagawa H, Hayata Y, Kawamura S, Yamada T, Fujiwara N, et al. Lipid metabolic reprogramming in hepatocellular carcinoma. *Cancers (Basel)* 2018;10:447.
18. Gomes AL, Teijeiro A, Buren S, Tummala KS, Yilmaz M, et al. Metabolic inflammation-associated IL-17A causes non-alcoholic steatohepatitis and hepatocellular carcinoma. *Cancer Cell* 2016;30:161-75.
19. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013;499:97-101.
20. Loo TM, Kamachi F, Watanabe Y, Yoshimoto S, Kanda H, et al. Gut microbiota promotes obesity-associated liver cancer through PGE2-mediated suppression of antitumor immunity. *Cancer Discov* 2017;7:522-38.
21. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002;123:134-40.
22. Yang JD, Ahmed F, Mara KC, Addissie BD, Allen AM, et al. Diabetes is associated with increased risk of hepatocellular carcinoma in patients with cirrhosis from nonalcoholic fatty liver disease. *Hepatology* 2020;71:907-16.
23. Unalp-Arida A, Ruhl CE. PNPLA3 I148M and liver fat and fibrosis scores predict liver disease mortality in the United States population. *Hepatology* 2020;71:820-34.
24. Loomba R, Lim JK, Patton H, El-Serag HB. AGA clinical practice update on screening and surveillance for hepatocellular carcinoma in patients with nonalcoholic fatty liver disease: expert review. *Gastroenterology* 2020; Epub ahead of print. doi: 10.1053/j.gastro.2019.12.053
25. Younes R, Bugianesi E. Should we undertake surveillance for HCC in patients with NAFLD? *J Hepatol* 2018;68:326-34.
26. Patel YA, Gifford EJ, Glass LM, McNeil R, Turner MJ, et al. Risk factors for biopsy-proven advanced non-alcoholic fatty liver disease in the Veterans Health Administration. *Aliment Pharmacol Ther* 2018;47:268-78.
27. Reig M, Gambato M, Man NK, Roberts JP, Victor D, et al. Should patients with NAFLD/NASH be surveyed for HCC? *Transplantation* 2019;103:39-44.

Review

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Imaging biomarkers for predicting poor prognosis of hepatocellular carcinoma: a review

Yun Zhang¹, Zi-Xing Huang¹, Jie Chen¹, Yu-Jun Shi², Han-Yu Jiang¹, Li-Kun Cao³, Bin Song¹

¹Department of Radiology, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China.

²Laboratory of Pathology, Key Laboratory of Transplant Engineering and Immunology, NHC, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China.

³Department of Radiology, Peking Union Medical College Hospital (Dongdan campus), Beijing 100730, China.

Correspondence to: Prof. Bin Song, Department of Radiology, Sichuan University West China Hospital, No. 37, Guoxue Alley, Chengdu 610041, Sichuan, China. E-mail: anicesong@vip.sina.com

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Abstract

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver with a high mortality rate. Heterogeneity is the main biological characteristic of HCC, which manifests through the different biological behaviors of each phenotype and ultimately, affects patient prognosis and treatment efficacy. Various aggressive biological behaviors are considered to be associated with the poor prognosis of HCC patients including poor differentiation, microvascular invasion, intracellular fat accumulation, invasive growth, bile duct invasion or tumor thrombosis, and tumor spread and metastasis, and have been reported as prognostic biomarkers. In addition, HCC results from multifactor synergistic damage, and various factors related to genetics, molecular pathology and immunohistochemistry such as β -catenin, Ki67, cytokeratin-19, and epithelial cell adhesion molecule have an impact on HCC differentiation and prognosis. This article is an overview of the biological behaviors that lead to poor prognosis of HCC, and the roles of morphological and quantitative noninvasive imaging biomarkers in the evaluation and prediction of these behaviors. Some common biomarkers related to genetics, molecular pathology and immunohistochemistry are also briefly summarized. It is hoped that this review will provide clinicians and radiologists with an update on the development of liver imaging, and provide directions for the combination of radiology, genetics, molecular pathology and histopathology to better predict the prognosis of HCC patients.

Keywords: Hepatocellular carcinoma, poor prognosis, biological behaviors, imaging biomarkers, genetics, molecular pathology, immunohistochemistry



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INTRODUCTION

Liver cancer is the sixth most prevalent cancer and the fourth leading cause of cancer-related death worldwide. Hepatocellular carcinoma (HCC), mostly due to liver cirrhosis, accounts for 75%-80% of all liver cancers^[1]. Heterogeneity is the main biological characteristic of HCC, which manifests through different biological behaviors of each phenotype and ultimately, affects patient prognosis and treatment efficacy. Various aggressive biological behaviors including poor differentiation, microvascular invasion (MVI), intracellular fat accumulation, invasive growth, bile duct invasion or tumor thrombosis, and tumor spread and metastasis, have been reported to have an impact on clinical outcomes and the prognosis of HCC patients^[2-6].

Liver imaging, a noninvasive method for visualizing tumor morphology and function, can provide a clear diagnosis and assessment of HCC in the presence of risk factors, and has flourished in recent decades with the potential to better depict the complex biological behaviors of HCC with relevant morphological and quantitative biomarkers^[7-13]. Traditional imaging, including contrast-enhanced ultrasound, multi-phase dynamic enhanced computed tomography (CT) or magnetic resonance imaging (MRI), and fluoro-deoxy-glucose/positron emission tomography (FDG/PET), play significant roles in the characterization of HCC as most have a typical blood supply and morphological characteristics. Functional imaging, including diffusion-weighted imaging (DWI), chemical-shift MRI, magnetic resonance elastography (MRE), and MRI with liver-specific contrast, can provide cellular and metabolic information for the grading and staging of HCC^[14]. Furthermore, improvements in advanced imaging analysis, including radiomics and artificial intelligence (AI), have introduced enormous potential for assessing the aggressiveness of HCC and prognostication of patients. Table 1 shows the aggressive biological behaviors associated with poor prognosis of HCC patients and their pathological basis, as well as morphological and qualitative imaging biomarkers that can be used to evaluate and predict biological behaviors. In addition, HCC is the result of multifactor synergistic damage, and various factors related to genetics, molecular pathology and immunohistochemistry also have an impact on HCC differentiation and prognosis^[15].

This article reviews the imaging biomarkers for aggressive behaviors associated with a poor prognosis in HCC patients, and the roles of different imaging methods and related biomarkers in the evaluation and prediction of these behaviors [Table 2]. A better understanding of these imaging features and their correlation with pathology can help clinicians to design the most appropriate treatment plans for HCC. In addition, a combination or confrontation of imaging signs with other biomarkers may be a direction for future research to better predict the prognosis of HCC.

POOR DIFFERENTIATION

Differentiation refers to the dedifferentiation of premalignant nodules until the development of HCC itself. The degree of differentiation of HCC is the key to prognosis of HCC patients^[16]. Several pathological alterations are involved in loss of differentiation in HCC such as neoangiogenesis, disordered cellular structure, impairment of Kupffer cells and hepatocytes, and increased glucose metabolism in tumor cells.

NEOANGIOGENESIS

Neovascularization provides the basis for oxygen and nutrition for tumor growth, progression and metastasis. The unpaired arteries are new vessels which form through neovascularization, and play a crucial role both in the occurrence and development of HCC^[17].

As most HCCs show a typical enhancement pattern such as hyperenhancement in the hepatic arterial phase, and washout appearance in the portal venous and/or delayed phases relative to the surrounding tissue, traditional imaging technologies such as contrast-enhanced ultrasound (CEUS), dynamic contrast-enhanced

Table 1. The aggressive biological behaviors associated with poor prognosis in HCC and related imaging biomarkers

Aggressive biological behaviors	Pathological basis	Morphological imaging biomarkers	Quantitative imaging biomarkers
Poor differentiation			
Neoangiogenesis	Formation of unpaired arteries	Hyperenhancement in the hepatic arterial phase and washout appearance in the portal venous and/or delayed phases relative to the surrounding tissue. (Contrast enhanced-US, dynamic contrast enhanced-CT or MRI)	Enhancement values of tumor tissue (Dynamic contrast enhanced-CT); Wash-out rate: the interval time of wash-out after injection of contrast agent. (Time-intensity curve analysis of Contrast enhanced-US); Microvascular density: K^{trans} , K_{ep} , V_e , iAUC (Free-breathing DEC-MRI using gadoxetic acid)
Restricted diffusion movement of water molecules within tumor cells	Disordered cellular structure	High-intensity on DWI images	Value of ADC (DWI, inconsistent); Value of D and ADC (DWI, IVIM); Values of MK and ADC (DKI); Tumor stiffness (MRE)
Decreased uptake of liver-specific contrast agents	Impairment of Kupffer cells; decrease in OATPs transporters	High-intensity on contrast enhanced-MRI with SPION; low-intensity on the HBP of contrast enhanced-MRI with Gd-EOB-DTPA	High-intensity on contrast enhanced-MRI with SPION; low-intensity on the HBP of contrast enhanced-MRI with Gd-EOB-DTPA
FDG concentration	Glucose metabolism increased in tumor cells	Uptake of fluorine-18 fluorodeoxyglucose on PET imaging	Increase of SUV
Microvascular invasion	Tumor thrombi invading microvessels	Larger diameter and tumor size, multiple lesions, incomplete capsule, non-smooth tumor margins, irregular rim-like arterial phase hyperenhancement, tumor multifocality, and "mosaic" architecture; hypo-intensity on the HBP of contrast enhanced-MRI Gd-EOB-DTPA	Higher MK value of DKI; radiomics signatures related to tumor size and intra-tumoral heterogeneity; texture analysis
Intracellular fat accumulation	Steatosis occurs in the context of ischemia and hypoxia as a result of decreased portal vein and nontumoral artery flow and insufficient unpaired arteries	Intra-tumoral fat infiltration on in/out of phase (Chemical-shift MRI)	Intra-tumoral fat infiltration on in/out of phase (Chemical-shift MRI)
Invasive growth pattern	Invasion of cancer cells into adjacent tissues and the vascular lymphatic system	Infiltrative appearance; portal vein tumor thrombosis; mass with ill-defined with heterogeneous attenuation/signal intensity;	/
Bile duct invasion or tumor thrombosis	Bile duct invasion or tumor thrombosis	Frequently associated with obstructive jaundice, cholangitis, biliary bleeding; a soft tissue mass with proximal bile duct dilatation and a similar enhancement pattern to HCC; filling defect in the bile duct, unexpected obstruction of the bile duct, and cholangiectasis in MR cholangiopancreatography	/
Tumor spread and metastasis	Cell proliferation and colony formation, EMT program start-up	Intrahepatic micrometastasis; extrahepatic metastasis; [Imaging features of metastasis, (Contrast enhanced-US, dynamic contrast enhanced-CT or MRI, PET imaging)]	/

HCC: hepatocellular carcinoma; US: ultrasonography; CT: computed tomography; MRI: magnetic resonance imaging; DWI: diffusion-weighted imaging; IVIM: intravoxel incoherent motion; D: diffusion coefficient; ADC: apparent diffusion coefficient; MK: mean apparent kurtosis coefficient; DKI: diffusion kurtosis imaging; MRE: magnetic resonance elastography; OATPs: organic anionic transporting polypeptides; SPION: superparamagnetic iron-oxide nanoparticles; HBP: hepatobiliary phase; Gd-EOB-DTPA: gadolinium-ethoxy benzyl-diethylenetriamine penta-acetic acid; FDG: fludeoxyglucose; 18F-FDG: fluorine-18 fluorodeoxyglucose; PET: positron emission tomography; SUV: standardized uptake value; MRCP: magnetic resonance cholangiopancreatography; EMT: epithelial-mesenchymal transition; /: none

CT or MRI are used for the diagnosis of HCC^[18,19]. Although the typical enhancement patterns are helpful in the diagnosis of HCC, they do not provide information for the degree of differentiation in HCC. Recently, time-intensity curve analysis, a quantitative CEUS analysis method, has been used to predict the degree of HCC differentiation^[20,21]. Wash-out rate, one of the time-intensity curve-related parameters, is defined as the interval time of wash-out manifestation after injection of a contrast agent, which is recommended to identify tumor differentiation of HCC. As reported by Feng *et al.*^[20], the cut-off value of 120 s after contrast agent injection shows high accuracy for distinguishing well-differentiated HCC from poorly and

Table 2. Different imaging methods and biomarkers for predicting poor prognosis of HCC

Biological behaviors	Imaging methods	Related imaging biomarker	Roles and characteristics
Neoangiogenesis	CEUS	Typical enhancement features; wash-out rate (time-intensity curve analysis)	Surveillance and rapid diagnostic, reveal morphologic changes, convenient and radiation-free
	DCE-CT	Typical enhancement features; enhancement values of tumor tissue	Diagnosis and differential diagnosis, CT values indicate the degree of enhancement
	DCE-MRI	Typical enhancement features; microvascular density (K^{trans} , K_{ep} , V_e , iAUC)	Diagnosis and differential diagnosis, provide quantitative information of blood perfusion of tumor
	DWI	Value of ADC	Provide information regarding physiological tissue characteristics and heterogeneity
	IVIM	Value of D and ADC	
Restricted diffusion (disordered cellular structure)	DKI	Values of MK and ADC	
	MRE	Tumor stiffness	Measuring the viscoelastic properties of the liver and assessing tumor stiffness, might be associated with the grade of the tumor
Decreased uptake of liver-specific contrast agents	SPION enhanced-MRI	High-intensity (impairment of Kupffer cells)	Diagnosis and differential diagnosis; low sensitivity of characterization of HCC; can be used as a means of evaluating treatment response of HCC
FDG concentration	Gd-EOB-DTPA enhanced-MRI	Low-intensity on the HBP images (decrease of OATPs transporters)	Early diagnosis, precise characterization, follow-up and monitoring of HCC; High sensitivity
	PET imaging	Standardized uptake value	A sensitive indicator of tumor viability; limited to the level of diagnosis of HCC
	CEUS; DCE-CT/MRI	Larger diameter and tumor size, multiple lesions, incomplete capsule, non-smooth tumor margins, irregular rim-like arterial phase hyperenhancement, tumor multifocality, and 'mosaic' architecture	Diagnosis and assessment. Detection and assessment of multiple morphology imaging features
	DKI	Higher MK value	Provide quantitative information of the presence of MVI
	Gd-EOB-DTPA enhanced-MRI	Hypo-intensity on the HBP images	Assistant diagnosis; auxiliary feature (in conjunction with other clinical indicators)
Microvascular invasion	AI	Radiomics signatures related to tumor size and intra-tumoral heterogeneity; texture features	Intelligent and noninvasive means for the prediction of tumor heterogeneity
Intracellular fat accumulation	Chemical-shift MRI	Intra-tumoral fat infiltration on in/out of phase	Monitor the presence of intra-tumoral fat infiltration in tumor; related to the histological degree of HCC; optimize disease management
Invasive growth pattern	CEUS; DCE-CT/MRI	Infiltrative appearance; PVTT; mass with ill-defined and heterogeneous attenuation/signal intensity	Diagnosis and differential diagnosis; detection and assessment of multiple morphology imaging features
Bile duct invasion or tumor thrombosis	CEUS; DCE-CT/MRI	A soft tissue mass with proximal bile duct dilatation and a similar enhancement pattern to HCC	Rapid diagnosis and differential diagnosis
Tumor spread and metastasis	MRCP	Filling defect in the bile duct, unexpected obstruction of the bile duct	Noninvasive diagnosis and characterization without contrast agents
	CEUS	Imaging features of metastasis	Limited to intrahepatic metastasis, convenient and radiation-free
	DCE-CT/MRI	Imaging features of metastasis	Intrahepatic and extrahepatic metastasis
	PET imaging	Standardized uptake value	Show advantages in detecting distant metastasis and lymph node metastasis

HCC: hepatocellular carcinoma; CEUS: contrast enhanced ultrasonography; DCE-CT: dynamic contrast enhanced computed tomography; DEC-MRI: dynamic contrast enhanced magnetic resonance imaging; DWI: diffusion-weighted imaging; IVIM: intravoxel incoherent motion; D: diffusion coefficient; ADC: apparent diffusion coefficient; MK: mean apparent kurtosis coefficient; DKI: diffusion kurtosis imaging; MRE: magnetic resonance elastography; SPION: superparamagnetic iron-oxide nanoparticles; Gd-EOB-DTPA: gadolinium-ethoxy benzyl-diethylenetriamine penta-acetic acid; HBP: hepatobiliary phase; AI: artificial intelligence; PVTT: portal vein tumor thrombosis; MRCP: MR cholangiopancreatography; PET: positron emission tomography

moderately differentiated HCC. Time-intensity curve analysis is advantageous in showing the perfusion-related characteristics of different grades of HCCs, and providing quantitative indices for the assessment of hemodynamics in tumors, which is convenient and radiation-free.

Microvascular density, as assessed by immunohistochemistry, is generally considered as an indicator of angiogenesis in malignant tumors, and is an effective prognostic marker in patients with HCC^[22]. The DCE-MRI derived K^{trans} , K_{ep} and V_e , and the semi-quantitative parameter such as the initial area under the gadolinium concentration-time curve of the free-breathing DCE-MRI using gadoxetic acid, are highly associated with histological grades and microvascular density of HCC. The lower value of K^{trans} indicates the

histological grades of HCC, and the values of K_{ep} and V_e have proved to be significantly related to the tumor microvascular density. There is no difference among these DCE-MRI derived parameters for discriminating low- from high-grade HCC in terms of their performance. This means that DCE-MRI using gadoteric acid has great potential for the qualitative assessment of tumor neoangiogenesis and aggressiveness of HCC^[23].

RESTRICTED DIFFUSION MOVEMENT OF WATER MOLECULES WITHIN TUMOR CELLS

HCC is easier to recognize pathologically because of its distinctive cytological atypia and architectural abnormalities compared with other cirrhotic nodules^[24]. The early HCC nodule is well differentiated with an indistinct appearance, while HCC nodules that have progressed are mostly moderately differentiated with a distinct pattern and often, with a more disordered cellular structure^[25,26].

DWI is a noninvasive technique for visualizing the 3D microscopic movement of water molecules within cells to provide information regarding physiological tissue characteristics. HCC and other cancers usually possess higher cellularity, tissue disorganization, and less extracellular space, resulting in impedance of water diffusion; therefore, DWI shows a high-intensity signal^[27,28]. In the past few years, many studies have investigated the apparent diffusion coefficient (ADC) value of DWI in predicting the differentiation of HCC^[29-31]. The conclusions however, were not consistent. This might be due to the fact that DWI cannot simply reflect the diffusion of water molecules in living tissues and capillary perfusion affects the stability of DWI performance as well.

Intravoxel incoherent motion (IVIM), as a DWI-derived novel technique, enables monitoring of tissue diffusivity and microcapillary perfusion independently^[32]. IVIM-derived parameters such as perfusion fraction (fp) and diffusion coefficient (D) combined with ADC of DWI, are reported to correlate with HCC histological grade^[33-35]. In the study by Granata *et al.*^[33], ADC ($2.11 \times 10^{-3} \text{ mm}^2/\text{s}$), fp (47.33%) and D ($0.94 \times 10^{-3} \text{ mm}^2/\text{s}$) values were considered as the optimal cut-off points in distinguishing high- from low-grade HCCs. However, the study only included Edmondson-Steiner grade 1 to 3 HCC nodules and did not include Edmondson-Steiner grade 4 HCC, which made identification of high-grade HCCs non specific. Woo *et al.*^[34], explored the relationship of ADC and D values with the histological grade of HCC. The results showed that there was a significant negative correlation between ADC and D values with the histologic grade of HCC. Moreover, the D value had better diagnostic performance than the ADC value for differentiating low- and high-grade HCC. Similar results were reported by Sokmen *et al.*^[35]. Therefore, both studies improved the design of the experiments, and further confirmed the results on the basis of the findings by Granata *et al.*^[33], which indicated that the combination of DWI and IVIM could be used to determine the degree of HCC differentiation rather than using DWI alone. However, due to defects in the study design (i.e., retrospective study), insufficient sample size and an unclear gold standard, further prospective studies are needed.

Diffusion kurtosis imaging (DKI) is an attempt to provide a more accurate model of diffusion, and to reflect tissue heterogeneity in non-Gaussian diffusion behavior^[36]. Recently, our team validated the performance of DKI in predicting the degree of differentiation of HCC^[37]. The results showed that mean apparent kurtosis coefficient (MK) and ADC values are significantly correlated with the grade of HCC [Figure 1]. In addition, the MK value represents a higher specificity than the ADC in differentiating high-grade from low-grade HCCs. We speculated that this might be due to the fact that increased tumor cellularity results in decreased extracellular space and the restricted diffusion of water molecules, which is visually presented as decreased ADC and MD in higher-grade HCC. Furthermore, a higher MK value may be an independent risk factor for early tumor recurrence and poor prognosis in HCC patients, which might be related to the higher-grade of HCC that often tend to be more proliferative, aggressive and heterogeneous, and result in a more irregular tumor microstructure^[38].

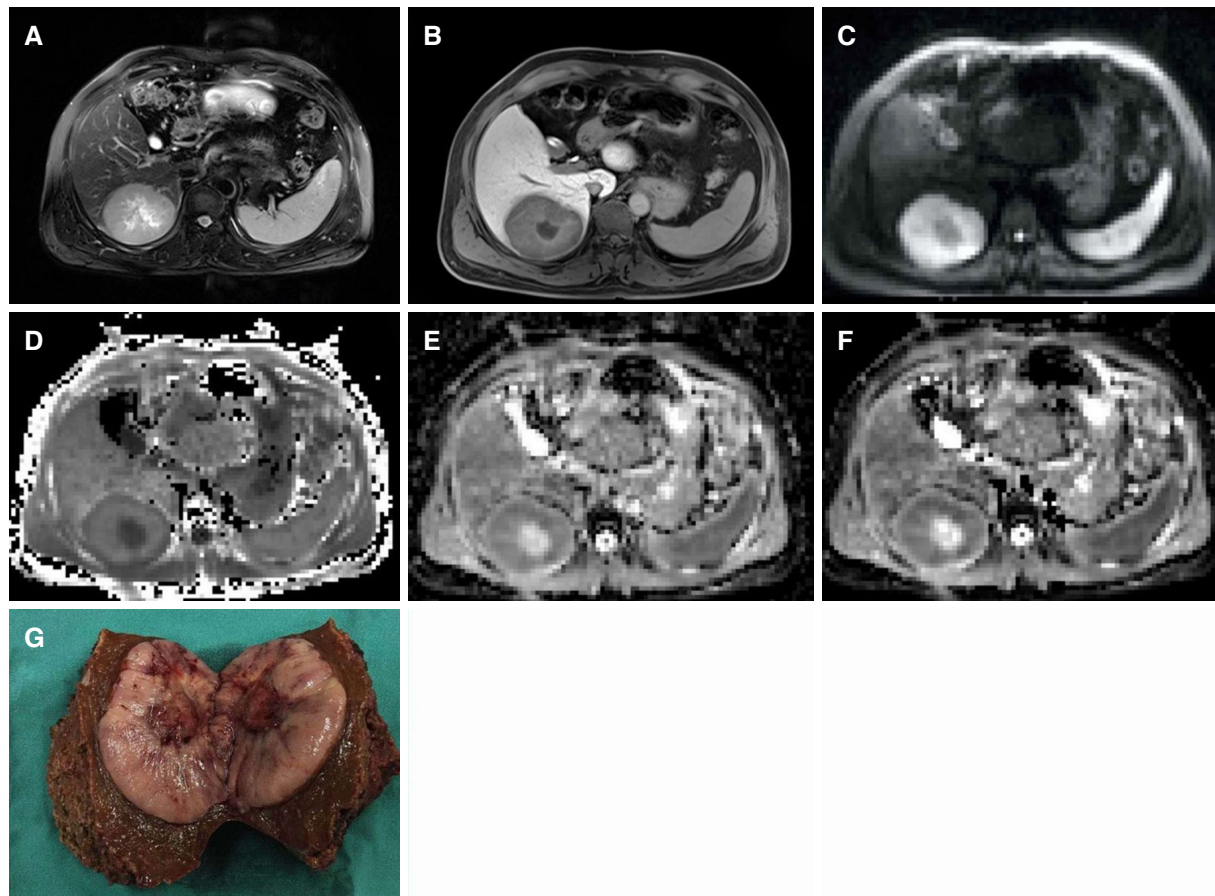


Figure 1. A 48-year-old male with a pathologically confirmed HCC with MVI. A 7.8 cm × 6.0 cm tumor in the right posterior hepatic section shows slight hyperintensity on T2-weighted image (A), hypo-intensity relative to the surrounding liver parenchyma in hepatobiliary phase (B), and restricted diffusion on the DWI with a b-value of 700 s/mm² (C). MK map (D) shows higher signal intensity of tumor compared with that of background liver parenchyma. ADC (E) and MD (F) maps show lower signal intensity compared with that of liver parenchyma. The calculated mean values of MK, ADC, and MD for the tumor were 0.99, 1.10×10^{-3} mm²/s, and 1.28 mm²/s, respectively. The pathological results after tumor resection demonstrated HCC [Edmondson-Steiner (III) with positive MVI (G)]. HCC: hepatocellular carcinoma; DWI: diffusion-weighted imaging; ADC: apparent diffusion coefficient; MD: mean diffusion; MK: mean apparent kurtosis coefficient

MRE is a noninvasive imaging technology for measuring the viscoelastic properties of the liver, and is reported to be capable of quantitatively assessing tumor stiffness, in addition to effectively identifying focal liver lesions^[39]. A preliminary study reported that tumor stiffness detected by MRE might be able to differentiate HCC tumor grade^[40]. However, further research is needed to verify the value of MRE in determining the degree of HCC differentiation.

DECREASED UPTAKE OF LIVER-SPECIFIC CONTRAST AGENTS

Kupffer cells, a type of macrophage that reside in the sinusoidal endothelial cells of the liver, are more likely to be distributed in early-stage or well-differentiated HCC than advanced HCC^[41,42]. In addition, approximately half of the administered dose of gadoxetic acid, a hepatobiliary contrast agent, is taken up by hepatocytes through the organic anionic transporting polypeptide (OATP) family and subsequently excreted into bile^[43]. Since there is progressive depletion of OATP with dedifferentiation, moderate to poorly differentiated tumors do not take up gadoxetate, and thus appear hypointense on hepatobiliary phase (HBP) of MRI.

Based on the above physiological mechanisms, liver-specific contrast agents such as superparamagnetic iron-oxide nanoparticles (SPION) and gadolinium-ethoxy benzyl-diethylenetriamine penta-acetic acid

(Gd-EOB-DTPA) have been produced and used in HCC diagnosis and characterization. However, SPION-enhanced MRI has lower sensitivity than Gd-EOB-DTPA-enhanced MRI, especially for early HCCs without early enhancement; thus, SPION-enhanced MRI is rarely used as the contrast agent of choice in Western countries. However, SPION-enhanced MRI is still used in the East as a means of evaluating treatment response in HCC^[44].

Decreased expression levels of OATP during multi-step hepatocarcinogenesis are inversely correlated with HCC tumor grade and aggressiveness^[45,46]. However, some well-differentiated HCCs and approximately 5%-12% of moderately differentiated HCCs overexpress OATP8, which is thought to be associated with a genetic alteration or of a different cellular origin^[45]. A consensus report has been reached on the application and prospects of developing Gd-EOB-DTPA enhanced MRI for the early diagnosis, follow-up and outcome monitoring of HCC patients^[47]. The report indicated that hypervascularity on the arterial phase and the low signal on HBP images of Gd-EOB-DTPA-enhanced MRI is more sensitive to the diagnosis of HCC when compared with CT. Moreover, HBP imaging provides critical information during the follow-up of cirrhosis-related nodules, especially for those with a diameter less than 2 cm, for predicting the histological grade of the tumor^[46,48]. The above research suggests that functional MRI technologies, especially the HBP scan of Gd-EOB-DTPA-enhanced-MRI, will gradually become an essential means for predicting the differentiation of tumors and precise characterization of HCC.

FDG CONCENTRATION

The upregulation of glycogen metabolism is a prominent feature of tumor cells and it promotes survival, proliferation, and resistance to antitumor therapy^[49]. Previous studies have identified that aberrant tumor cell metabolism was associated with the expression of genes encoding enzymes leading to glycogen metabolic reprogramming and promoting HCC progression^[50].

Uptake of fluorine-18 FDG (¹⁸F-FDG) based on increased glucose metabolism in tumor cells is considered as a sensitive indicator of tumor viability^[51]. PET imaging provides the metabolic status of tumor tissues with high sensitivity and specificity. Therefore, ¹⁸F-FDG/PET, as a product of the combination of the above two mechanisms, is often used as an effective means for tumor characterization and evaluation of tumor response. Standardized uptake value, a semi-quantitative index of FDG/PET, is reported to be related to HCC-histological grade and poor prognosis of patients, with poorly differentiated or high-grade tumors showing higher intensities on FDG-PET^[52]. Moreover, the advent of novel radiopharmaceuticals recently such as ¹¹C-choline has improved the diagnostic performance of HCC characterization and tumor grade assessment^[53].

In comparison with PET/CT, PET/MRI shows a higher sensitivity for displaying composition and structure of tissues. PET/MRI in combination with DWI has an advantage in the differentiation of tumor tissue from non-tumor liver parenchyma, and is also helpful for understanding tumor characteristics^[54]. However, FDG-PET/MRI is mostly limited to the diagnosis of HCC and research on the value of FDG-PET MRI in predicting poor differentiation and prognosis of HCC is still lacking.

MVI

MVI is typically correlated with aggressive biological behaviors of HCC, and is generally considered as a risk factor for early recurrence of HCC in patients^[55]. Tumor characteristics, such as a larger diameter and tumor size, multiple lesions, incomplete capsule, and an irregular tumor margin, can be used as biomarkers in predicting the presence of MVI^[56,57]. Moreover, significantly increased serum levels of parameters such as α -fetoprotein, PIVKA-II, and cytokines (e.g., interleukin-35, phospho-beta1 integrin, transforming growth factor-beta1, p-Smad-2, and E-cadherin) suggest the possibility of MVI^[58-61].

Multiple morphological imaging biomarkers including non-smooth tumor margins, irregular rim-like arterial phase hyperenhancement, tumor multifocality, and “mosaic” architecture, are reported to be highly correlated with MVI in HCC^[62-64]. DWI and its-derived technologies have also shown great potential in detecting MVI in HCC. The mean and minimum ADC values of HCCs with MVI are reported to be lower than those of HCCs without MVI^[65]. In addition, a higher MK value on DKI and irregular rim enhancement pattern are highly correlated with the presence of MVI^[66]. Similar results were reported in our previous study^[37] [Figure 1]. The mechanism underlying these results may be associated with the formation of a more complex microenvironment induced by MVI, such as the presence of tumor cell proliferation, necrosis, or inflammatory damage^[67]. Therefore, a greater packed cell structure and more irregular, heterogeneous environments are likely to occur in HCC with MVI, resulting in increased tissue diffusion, which manifest as increased MK^[66,68]. Furthermore, hypo-intensity on HBP images on Gd-EOB-DTPA enhanced-MRI in conjunction with other clinical indicators has been proven to further improve the prediction of MVI in HCC, and is superior in predicting early recurrence and the survival rate in HCC patients^[69,70].

AI, a branch of computer science, has emerged as a new technology to study and develop the theory, technology and application systems for simulating, extending and expanding human intelligence. In recent years, AI has had huge potential in medical imaging and attracted considerable attention in a range of fields from tumor diagnosis to outcomes prediction. Radiomics, and its derived analyses such as texture analysis, are noninvasive methods for the prediction of tumor heterogeneity, and have shown favorable predictive accuracy of MVI status in patients with HCC^[71-74]. Radiomics signatures associated with tumor size and intra-tumoral heterogeneity were reported to be the top-ranked indicators for MVI prediction. In addition, a radiomics nomogram incorporated with the clinical and radiological features outperform the combination of clinical and radiological features for predicting MVI and the clinical outcomes of HCC patients^[73].

INTRACELLULAR FAT ACCUMULATION

Intracellular fat accumulation, a common morphological characteristic of HCC, occurs in the context of ischemia and hypoxia due to decreased portal vein and nontumoral artery flow, and insufficient unpaired arteries^[75]. Thus, intracellular fat accumulation is gradually increased in low-grade dysplastic nodules, high-grade dysplastic nodules, and early-stage HCC^[76,77]. However, with regard to intra-tumoral fatty infiltration in poorly differentiated HCC, controversy exists^[75,78].

Chemical-shift MRI is the most commonly used technique to monitor the presence of intra-tumoral fat infiltration [Figure 2]. There is a close relationship between intracellular fatty change and MVI in HCC. MVI is more likely to occur in non-containing fatty HCCs, which means intra-tumoral fat infiltration suggests a lower risk for MVI in HCC^[79]. Moreover, Kubota *et al.*^[80], reported that macro-vesicular steatosis HCC has a better prognosis with less portal vein invasion and a lower cumulative risk of recurrence than micro-vesicular steatosis HCC. More related research is necessary for detecting intra-tumoral fat infiltration, and predicting the prognosis of HCC with different histopathological characteristics in the early stage of hepatocarcinogenesis, so as to optimize disease management and promote personalized treatment.

INVASIVE GROWTH PATTERN

Invasive growth, defined as the invasion of cancer cells into adjacent tissues and the vascular lymphatic system, is correlated with tumor metastasis and poor prognosis. Aberrant regulation of cell migration contributes to the progression of cancer cell invasion^[81].

Tumors with a permeative growth pattern on CT/MRI images are frequently termed “infiltrative”, which manifest as lesions with indistinguishable margins on CT/MRI images. The Liver Imaging Reporting and Data System was developed to standardize the interpretation, reporting, and evaluation of patients at risk of developing HCC, and provides an explicit interpretation of the features of “infiltrative appearance” in

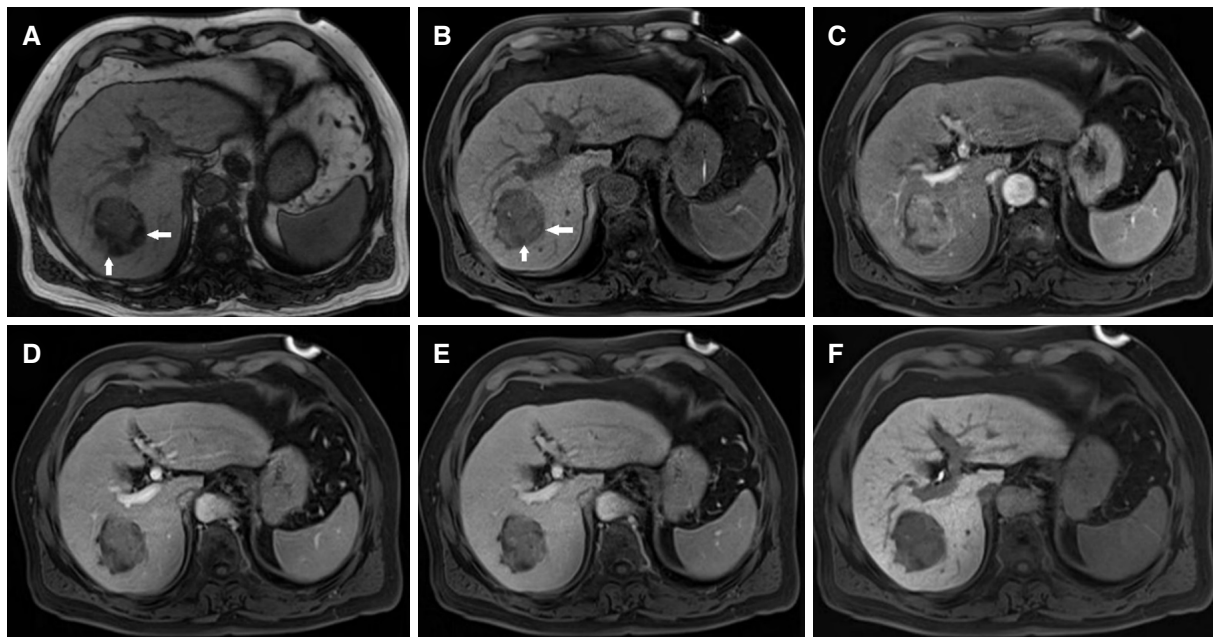


Figure 2. A 54-year-old female with hepatocellular carcinoma in segment VII of the liver. The 4.2-cm-sized mass shows focal fatty infiltration (white arrows) on opposed phase image (A) and unenhanced T1-weighted image (B), marked enhancement in arterial phase (C), wash-out appearance in portal venous phase (D) and delay phase (E), and hypo-intensity on 20 min hepatobiliary phase (F)

the latest version. It classifies a mass with infiltrative appearance as LR-M, which is probably or definitely malignant but not HCC specific. As the majority of tumors with infiltrative appearance in at-risk patients are HCC, the description of “infiltrative” is likely to indicate HCC in practice^[82]. Portal vein tumor thrombosis is the most helpful clue in the identification of infiltrative HCC, with accuracy ranging from 68% to 100%. The characteristic imaging manifestation of portal vein tumor thrombosis is the solid mass in the vascular cavity, which has similar enhancement features to the tumor itself on triphasic dynamic contrast-enhanced CT scan, especially with arterial phase enhancement and wash-out in the portal venous phase^[83,84]. Clinically, it is vital to distinguish portal vein tumor thrombosis and portal vein thrombus accurately for the intervention and treatment of patients with HCC^[85]. Imaging provides key evidence that portal vein thrombus is not enhanced in the arterial phase and occasionally, disappears after anticoagulant therapy^[86]. An ill-defined mass with heterogeneous attenuation/signal intensity is also highly indicative of the presence of an infiltrative growth pattern, which is pathologically characterized by the fusion of tumor tissue with liver parenchyma^[87].

BILE DUCT INVASION OR TUMOR THROMBOSIS

HCC with bile duct invasion has a poorer prognosis since these lesions are frequently associated with obstructive jaundice, cholangitis, and biliary bleeding, in addition to portal vein thrombosis^[88-90]. Imaging characteristics are often used to indicate the presence of tumor thrombosis in the bile duct, including a soft tissue mass with proximal bile duct dilatation and a similar enhancement pattern to HCC, since there is a possibility of necrosis and bleeding^[91]. Tumor thrombi are characterized as a filling defect in the bile duct, unexpected obstruction of the bile duct, and cholangiectasis in MR cholangiopancreatography; however, the diagnosis of both bile duct and portal vein microthrombi remains difficult^[92]. Moreover, HCC with bile duct thrombi are poorly differentiated, unencapsulated, and invasive^[93]. These findings indicate that the timely diagnosis of bile duct invasion is crucial for the management of HCC patients.

TUMOR SPREAD AND METASTASIS

Tumor metastasis accounts for the main cause of death in some cancer patients. A central pivot event of this process is the start-up of a program termed the epithelial-mesenchymal transition, which plays an important

Table 3. Genetics, molecular pathology and histopathology markers related to poor prognosis in HCC

Biomarkers	Role in occurrence, development and prognosis of HCC	Imaging biomarkers
CTNNB1	Its mutation accounts for 20%-40% of HCC; play pivotal role in the poor prognosis of HCC; HCCs with CTNNB1 mutations show a higher grade of differentiation with frequent pseudoglandular patterns and bile production. The subgroup of hepatocellular adenomas with an active β -catenin gene show high probability of malignant transformation	A high enhancement ratio on HBP images of Gadoxetic acid-enhanced-MRI; a high value of ADC on DWI images
Ki-67	Highly correlated with staging, grading and recurrence rate of HCC; high possibility of adverse pathological features and invasive behaviors	The parameter K^{trans} of free-breathing DCE-MRI using gadoxetic acid; T1 rt-20 min, tumor-to-muscle SI ratio, ADC value, and arterial inhomogeneous enhancement on gadoxetic acid-enhanced MRI; texture analysis on gadoxetic acid-enhanced-MRI
CK-19	Correlated with some clinicopathological features of tumors such as poor differentiation, metastasis, and early recurrence after resection and radiofrequency ablation	The arterial rim enhancement, targetoid appearance, non-peripheral "washout", and irregular tumor margin on gadoxetic acid-enhanced MRI; lower tumor-to-liver ADC ratio, and lower tumor-to-liver SI ratio at HBP images
EpCAM	Involved in a series of biological processes such as cell proliferation; the overexpression of EpCAM is correlated with the angiogenesis and poor outcome of HCC; in addition, EpCAM-positive HCC is considered to be a subtype of human HCC with a poor prognosis	No significant correlation between irregular rim-like hyperenhancement on gadoxetic acid-enhanced-MRI and EpCAM status in HCC. Whether imaging markers can be used to indicate the EpCAM positive HCC is still unclear

HCC: hepatocellular carcinoma; CTNNB1: β -catenin; HBP: hepatobiliary phase; Gd-EOB-DTPA: gadolinium-ethoxy benzyl-diethylenetriamine penta-acetic acid; MRI: magnetic resonance imaging; ADC: apparent diffusion coefficient; DWI: diffusion-weighted imaging; SI: signal intensity; CK-19: cytokeratin-19 (CK-19); EpCAM: epithelial cell adhesion molecule

regulatory role in embryogenesis.

Treatment approaches for cancer are largely depended on both the stage of disease at diagnosis, and the patient's performance status^[94]. Therefore, early detection of tumor spread or metastasis allows for the most appropriate treatment plans and to strive for more survival opportunities for HCC patients. Contrast-enhanced CT or MRI ensure implementation of one-step inspection and display the distinct enhancement features of metastasis better compared to ultrasound^[12]. MRI with liver-specific contrast agents improves diagnostic efficiency in the early identification of intrahepatic micrometastasis, as well as follow-up surveillance of disease development and outcomes^[95]. PET/CT has emerged as a useful modality in diagnosing extrahepatic lesions in patients with advanced HCC and assessing tumor grading^[96,97]. Furthermore, newly developed dual-tracer PET/CT improve and complement the advantages compared to single-tracer imaging in the evaluation of HCC metastasis, and has gradually become an alternative modality to PET/CT for detecting extrahepatic metastasis in HCC patients^[98].

FACTORS RELATED TO GENETICS, MOLECULAR PATHOLOGY AND IMMUNOHISTOCHEMISTRY

HCC is the result of a multifactorial, synergistic damage from various biological factors related to genetics, molecular pathology, and immunohistochemistry that impact upon HCC differentiation and prognosis. With the development of liver imaging technologies, some have been detected by imaging methods and proved to be associated with imaging biomarkers [Table 3].

β -catenin mutation

β -catenin (CTNNB1) has been recognized as the most frequently mutated oncogene during liver cancer. Its mutation accounts for around 20%-40% of all HCC cases, and is strongly associated with larger tumors, invasive growth, and vascular invasion. In addition, HCCs with the CTNNB1 mutation show a higher grade of differentiation with frequent pseudoglandular patterns and bile production^[99]. Moreover, the subgroup of hepatocellular adenomas with an active β -catenin gene shows a high probability of malignant transformation^[100]. Recently, several studies reported that gadoxetic acid-enhanced-MRI could be used to detect the presence of CTNNB1 mutations in HCC, because the mutation of CTNNB1 would promote

OATP expression^[99,101]. The results showed that a high enhancement ratio on HBP images of Gadoteric acid-enhanced-MRI indicate the mutation of CTNBB1, and is closely associated with a higher differentiation grade of HCC. Moreover, a high value of ADC on DWI images could also be used as an indicator of HCC with the CTNBB1 mutation^[101].

Ki-67

Ki-67 is highly correlated with the staging, grading and recurrence of HCCs. The overexpression of Ki-67 indicates a high possibility of adverse pathological features and invasive behaviors of the tumor^[102]. The parameter K^{trans} of free-breathing DCE-MRI using gadoteric acid is reported to be highly associated with Ki67 proliferation status, and the lower value of K^{trans} indicate higher Ki-67 indices and histological grades of HCC^[103]. In addition, the T1 rt-20 min, tumor-to-muscle signal intensity ratio, ADC value and the arterial inhomogeneous enhancement pattern on gadoteric acid-enhanced-MRI are also correlated with high-Ki-67 expression^[104-106]. Furthermore, texture analysis, on the basis of gadoteric acid-enhanced-MRI, can be applied as a tentative approach to preoperatively predict the Ki-67 status of HCC after curative resection^[107].

Cytokeratin-19

Cytokeratin-19 (CK-19) are important keratin proteins expressed in liver tissue and show differential expression at different stages of physiological development of the liver. The expression of CK-19 is correlated with some clinicopathological features of tumor such as poor differentiation, metastasis, and early recurrence after tumor resection or radiofrequency ablation^[108]. The arterial rim enhancement, targetoid appearance, non-peripheral “washout”, and irregular tumor margins on gadoteric acid-enhanced MRI are reported to be effective imaging markers for evaluating the CK-19 status of HCC^[109-112]. Moreover, lower tumor-to-liver ADC and signal intensity ratios in HBP images may be helpful for predicting CK19-positive HCC with early recurrence^[112].

Epithelial cell adhesion molecule

Epithelial cell adhesion molecule (EpCAM) is a membrane glycoprotein expressed in most normal epithelial cells, and has been proven to be involved in a series of biological processes such as cell proliferation^[113]. It has been reported that the overexpression of EpCAM is closely correlated with the angiogenesis and poor outcomes in HCC^[114]. In addition, EpCAM-positive HCC is also considered to be a subtype of human HCC with a poor prognosis^[115]. However, the imaging biomarkers that can be used to reflect EpCAM-positive status in HCC have not been described. Recently, Rhee *et al.*^[63], used the imaging feature of irregular rim-like hyperenhancement as the poor prognostic index to explore the value of gadoteric acid-enhanced MRI in predicting the overexpression of immunomarkers including EpCAM. However, results showed there is no statistically significant association between irregular rim-like hyperenhancement and EpCAM status in HCC. Therefore, whether imaging markers can be used to indicate EpCAM positive HCC is still unclear.

CONCLUSION

Hepatocarcinogenesis is a progressive and multi-stage process of molecular heterogeneity and histological dedifferentiation. The poor prognosis of HCC is strongly associated with the presence of aggressive biological behaviors in tumors. Modern noninvasive imaging technologies such as CEUS, DEC-CT/MRI and PET imaging improve the efficiency of early identification, characterization and assessment of HCC, and have been widely used for screening, diagnosis, and improving the survival of HCC patients. The increasing use of multiparametric functional methods, including DWI and its derived-technologies (IVIM, DKI), contrast enhanced-MRI with SPION or Gd-EOB-DTPA, MRE, chemical-shift MRI, radiomics and AI, for assessing tumor morphology, vascularity, cellularity, metabolic capability, texture, radiomics features, OATP function, and Kupffer cell function, thereby providing early, accurate and reliable evidence of the aggressive biological behaviors of HCC is most encouraging for clinicians and radiologists. Although liver imaging has obvious advantages in identifying the poor prognosis of HCC, currently, there is no doubt that multidisciplinary

management, including radiology, genetics, molecular pathology and histopathology would result in a more accurate and reliable diagnosis for predicting the prognosis of HCC.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and design of the study, and performed data analysis and interpretation: Zhang Y, Huang ZX, Chen J

Performed data acquisition, as well as provided administrative, technical, and material support: Shi YJ, Jiang HY, Cao LK, Song B

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Int J Cancer* 2018;68:394-424.
2. Selcuk H. Prognostic factors and staging systems in hepatocellular carcinoma. *Exp Clin Transplant* 2017;15:45-9.
3. Farinati F, Vitale A, Spolverato G, Pawlik TM, Huo TL, et al. Development and Validation of a new prognostic system for patients with hepatocellular carcinoma. *PLoS Med* 2016;13:e1002006.
4. Rodríguez-Perálvarez M, Luong TV, Andreana L, Meyer T, Dhillon AP, Burroughs AK. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. *Ann Surg Oncol* 2013;20:325-9.
5. Sohn JH, Duran R, Zhao Y, Fleckenstein F, Chapiro J, et al. Validation of the Hong Kong liver cancer staging system in determining prognosis of the North American patients following intra-arterial therapy. *Clin Gastroenterol Hepatol* 2017;15:746-55.e4.
6. Martins-Filho SN, Paiva C, Azevedo RS, Alves VAF. Histological grading of hepatocellular carcinoma-a systematic review of literature. *Front Med (Lausanne)* 2017;10:193.
7. Degasperi E, Colombo M. Distinctive features of hepatocellular carcinoma in non-alcoholic fatty liver disease. *Lancet Gastroenterol Hepatol* 2016;1:156-64.
8. Coskun M. Hepatocellular carcinoma in the cirrhotic liver: evaluation using computed tomography and magnetic resonance imaging. *Exp Clin Transplant* 2017;15:36-44.
9. Yoneda N, Matsui O, Kobayashi S, Kitao A, Kozaka K, et al. Current status of imaging biomarkers predicting the biological nature of hepatocellular carcinoma. *Jpn J Radiol* 2019;37:191-208.
10. Hu HT, Shen SL, Wang Z, Shan QY, Huang XW, et al. Peritumoral tissue on preoperative imaging reveals microvascular invasion in hepatocellular carcinoma: a systematic review and meta-analysis. *Abdom Radiol (NY)* 2018;43:3324-30.
11. Ayuso C, Rimola J, Vilana R, Burrel M, Darnell A, et al. Diagnosis and staging of hepatocellular carcinoma (HCC): current guidelines. *Eur J Radiol* 2018;101:72-81

12. Schelhorn J, Best J, Dechêne A, Göbel T, Bertram S, et al. Evaluation of combined Gd-EOB-DTPA and gadobutrol magnetic resonance imaging for the prediction of hepatocellular carcinoma grading. *Acta Radiologica* 2016;57:932-8.
13. Navin PJ, Venkatesh SK. Hepatocellular carcinoma: state of the art imaging and recent advances. *J Clin Transl Hepatol* 2019;7:72-85.
14. Jiang T, Zhu AX, Sahani DV. Established and novel imaging biomarkers for assessing response to therapy in hepatocellular carcinoma. *J Hepatol* 2013;58:169-77.
15. Clark T, Maximin S, Meier J, Pokharell S, Bhargava P. Hepatocellular carcinoma: review of epidemiology, screening, imaging diagnosis, response assessment, and treatment. *Curr Prob Diagn Radiol* 2015;44:479-86.
16. Oishi K, Itamoto T, Amano H, Fukuda S, Ohdan H, et al. Clinicopathologic features of poorly differentiated hepatocellular carcinoma. *J Surg Oncol* 2007;95:311-6.
17. Himeno H, Enzan H, Saibara T, Onishi S, Yamamoto Y. Hitherto unrecognized arterioles within hepatocellular carcinoma. *J Pathol* 1994;174:217-22.
18. Hayashi M, Matsui O, Ueda K, Kawamori Y, Gabata T, et al. Progression to hypervascular hepatocellular carcinoma: correlation with intranodular blood supply evaluated with CT during intraarterial injection of contrast material. *Radiology* 2002;225:143-9.
19. Ye XD, Yuan Z, Zhang J, Yuan Z. Radiological biomarkers for assessing response to locoregional therapies in hepatocellular carcinoma: From morphological to functional imaging (Review). *Oncol Rep* 2017;37:1337-46.
20. Feng Y, Qin XC, Luo Y, Li YZ, Zhou X. Efficacy of contrast-enhanced ultrasound washout rate in predicting hepatocellular carcinoma differentiation. *Ultrasound Med Biol* 2015;41:1553-60.
21. Lassau N, Koscielny S, Chami L, Chebil M, Benatsou B, et al. Advanced hepatocellular carcinoma: early evaluation of response to bevacizumab therapy at dynamic contrast-enhanced US with quantification—preliminary results. *Radiology* 2011;258:291-300.
22. Mann CD, Neal CP, Garcea G, Manson MM, Dennison AR, et al. Prognostic molecular markers in hepatocellular carcinoma: a systematic review. *Eur J Cancer* 2007;43:979-92.
23. Chen J, Chen C, Xia C, Huang Z, Zuo P, et al. Quantitative free-breathing dynamic contrast-enhanced MRI in hepatocellular carcinoma using gadoteric acid: correlations with Ki67 proliferation status, histological grades, and microvascular density. *Abdom Radiol (NY)* 2018;43:1393-403.
24. Jiang K, Al-Diffhala S, Centeno BA. Primary liver cancers-part 1: histopathology, differential diagnoses, and risk stratification. *Cancer Control* 2018;25:1073274817744625.
25. Park YN, Kim MJ. Hepatocarcinogenesis: imaging-pathologic correlation. *Abdom Imaging* 2011;36:232-43.
26. International Consensus Group for Hepatocellular Neoplasia. The International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 2009;49:658-64.
27. Calistri L, Castellani A, Matteuzzi B, Mazzoni E, Pradella S, et al. Focal liver lesions classification and characterization: what value do DWI and ADC have? *J Comput Assist Tomogr* 2016;40:701-8.
28. Di Pietropaolo M, Briani C, Federici GF, Marignani M, Begini P, et al. Comparison of diffusion-weighted imaging and gadoteric acid-enhanced MR images in the evaluation of hepatocellular carcinoma and hypovascular hepatocellular nodules. *Clin Imaging* 2015;39:468-75.
29. Nasu K, Kuroki Y, Tsukamoto T, Nakajima H, Mori K, et al. Diffusion-weighted imaging of surgically resected hepatocellular carcinoma: imaging characteristics and relationship among signal intensity, apparent diffusion coefficient, and histopathologic grade. *AJR Am J Roentgenol* 2009;193:438-44.
30. Heo SH, Jeong YY, Shin SS, Kim JW, Lim HS, et al. Apparent diffusion coefficient value of diffusion-weighted imaging for hepatocellular carcinoma: correlation with the histologic differentiation and the expression of vascular endothelial growth factor. *Korean J Radiol* 2010;11:295-303.
31. Nakanishi M, Chuma M, Hige S, Omatsu T, Yokoo H, et al. Relationship between diffusion-weighted magnetic resonance imaging and histological tumor grading of hepatocellular carcinoma. *Ann Surg Oncol* 2012;19:1302-9.
32. Li YT, Cercueil JP, Yuan J, Chen W, Loffroy R, et al. Liver intravoxel incoherent motion (IVIM) magnetic resonance imaging: a comprehensive review of published data on normal values and applications for fibrosis and tumor evaluation. *Quant Imaging Med Surg* 2017;7:59-78.
33. Granata V, Fusco R, Catalano O, Guarino B, Granata F, et al. Intravoxel incoherent motion (IVIM) in diffusion-weighted imaging (DWI) for Hepatocellular carcinoma: correlation with histologic grade. *Oncotarget* 2016;7:79357-64.
34. Woo S, Lee JM, Yoon JH, Joo I, Han JK, et al. Intravoxel incoherent motion diffusion-weighted MR imaging of hepatocellular carcinoma: correlation with enhancement degree and histologic grade. *Radiology* 2014;270:758-67.
35. Sokmen BK, Sabet S, Oz A, Server S, Namal E, et al. Value of intravoxel incoherent motion for hepatocellular carcinoma grading. *Transplant Proc* 2019;51:1861-6.
36. Wu EX, Cheung MM. MR diffusion kurtosis imaging for neural tissue characterization. *NMR Biomed* 2010;23:836-48.
37. Cao L, Chen J, Duan T, Wang M, Jiang H, et al. Diffusion kurtosis imaging (DKI) of hepatocellular carcinoma: correlation with microvascular invasion and histologic grade. *Quant Imaging Med Surg* 2019;9:590-602.
38. Jensen JH, Helpern JA. MRI quantification of non-Gaussian water diffusion by kurtosis analysis. *NMR Biomed* 2010;23:698-710.
39. Hennedige TP, Hallinan JT, Leung FP, Teo LL, Iyer S, et al. Comparison of magnetic resonance elastography and diffusion-weighted imaging for differentiating benign and malignant liver lesions. *Eur Radiol* 2016;26:398-406.
40. Thompson SM, Wang J, Chandan VS, Glaser KJ, Roberts LR, et al. MR elastography of hepatocellular carcinoma: correlation of tumor stiffness with histopathology features—Preliminary findings. *Magn Reson Imaging* 2017;37:41-5.

41. Toth CA, Thomas P. Liver endocytosis and Kupffer cells. *Hepatology* 1992;16:255-66.
42. Choi BI, Takayasu K, Han MC. Small hepatocellular carcinomas and associated nodular lesions of the liver: pathology, pathogenesis, and imaging findings. *AJR Am J Roentgenol* 1993;160:1177-87.
43. Nassif A, Jia J, Keiser M, Oswald S, Modess C, et al. Visualization of hepatic uptake transporter function in healthy subjects by using gadoxetic acid-enhanced MR imaging. *Radiology* 2012;264:741-50.
44. Fukuda K, Mori K, Hasegawa N, Nasu K, Ishige K, et al. Safety margin of radiofrequency ablation for hepatocellular carcinoma: a prospective study using magnetic resonance imaging with superparamagnetic iron oxide. *Jpn J Radiol* 2019;37:555-63.
45. Kitao A, Matsui O, Yoneda N, Kozaka K, Shinmura R, et al. The uptake transporter OATP8 expression decreases during multistep hepatocarcinogenesis: correlation with gadoxetic acid enhanced MR imaging. *Eur Radiol* 2011;21:2056-66.
46. Kogita S, Imai Y, Okada M, Kim T, Onishi H, et al. Gd-EOB-DTPA-enhanced magnetic resonance images of hepatocellular carcinoma: correlation with histological grading and portal blood flow. *Eur Radiol* 2010;20:2405-13.
47. Zech CJ, Ba-Ssalamah A, Berg T, Chandarana H, Chau GY, et al. Consensus report from the 8th International Forum for Liver Magnetic Resonance Imaging. *Eur Radiol* 2020;30:370-82.
48. Huang K, Dong Z, Cai H, Huang M, Peng Z, et al. Imaging biomarkers for well and moderate hepatocellular carcinoma: preoperative magnetic resonance image and histopathological correlation. *BMC Cancer* 2019;19:364.
49. Zois CE, Harris AL. Glycogen metabolism has a key role in the cancer microenvironment and provides new targets for cancer therapy. *J Mol Med (Berl)* 2016;94:137-54.
50. Cervello M, Augello G, Cusimano A, Emma MR, Balasus D, et al. Pivotal roles of glycogen synthase-3 in hepatocellular carcinoma. *Adv Biol Regul* 2017;65:59-76.
51. Chen R, Li J, Zhou X, Liu J, Huang G. Fructose-1,6-bisphosphatase 1 reduces (18)F FDG uptake in hepatocellular carcinoma. *Radiology* 2017;284:844-53.
52. Seo S, Hatano E, Higashi T, Hara T, Tada M, et al. Fluorine-18 fluorodeoxyglucose positron emission tomography predicts tumor differentiation, P-glycoprotein expression, and outcome after resection in hepatocellular carcinoma. *Clin Cancer Res* 2007;13:427-33.
53. Castilla-Lievre MA, Franco D, Gervais P, Kuhnast B, Agostini H, et al. Diagnostic value of combining ¹¹C-choline and ¹⁸F-FDG PET/CT in hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2016;43:852-9.
54. Kong E, Chun KA, Cho IH. Quantitative assessment of simultaneous F-18 FDG PET/MRI in patients with various types of hepatic tumors: correlation between glucose metabolism and apparent diffusion coefficient. *PLoS One* 2017;12:e0180184.
55. Zhang X, Li J, Shen F. Significance of presence of microvascular invasion in specimens obtained after surgical treatment of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2018;33:347-54.
56. Wu TH, Hatano E, Yamanaka K, Seo S, Taura K, et al. A non-smooth tumor margin on preoperative imaging predicts microvascular invasion of hepatocellular carcinoma. *Surg Today* 2016;46:1275-81.
57. Renzulli M, Buonfiglioli F, Conti F, Brocchi S, Serio I, et al. Imaging features of microvascular invasion in hepatocellular carcinoma developed after direct-acting antiviral therapy in HCV-related cirrhosis. *Eur Radiol* 2018;28:506-13.
58. Ma XL, Zhu J, Wu J, Tian L, Gao YY, et al. Significance of PIVKA-II levels for predicting microvascular invasion and tumor cell proliferation in Chinese patients with hepatitis B virus-associated hepatocellular carcinoma. *Oncol Lett* 2018;15:8396-404.
59. Zheng J, Seier K, Gonen M, Balachandran VP, Kingham TP, et al. Utility of serum inflammatory markers for predicting microvascular invasion and survival for patients with hepatocellular carcinoma. *Ann Surg Oncol* 2017;24:3706-14.
60. Long J, Guo H, Cui S, Zhang H, Liu X, et al. IL-35 expression in hepatocellular carcinoma cells is associated with tumor progression. *Oncotarget* 2016;7:45678-86.
61. Fransvea E, Mazzocca A, Antonaci S, Giannelli G. Targeting transforming growth factor (TGF)-betaRI inhibits activation of beta1 integrin and blocks vascular invasion in hepatocellular carcinoma. *Hepatology* 2009;49:839-50.
62. Chou CT, Chen RC, Lin WC, Ko CJ, Chen CB, et al. Prediction of microvascular invasion of hepatocellular carcinoma: preoperative CT and histopathologic correlation. *AJR Am J Roentgenol* 2014;203:W253-9.
63. Rhee H, An C, Kim HY, Yoo JE, Park YN, et al. Hepatocellular carcinoma with irregular rim-like arterial phase hyperenhancement: more aggressive pathologic features. *Liver Cancer* 2019;8:24-40.
64. Chernyak V, Fowler KJ, Kamaya A, Kielar AZ, Elsayes KM, et al. Liver imaging reporting and data system (LI-RADS) Version 2018: imaging of hepatocellular carcinoma in at-risk patients. *Radiology* 2018;289:816-30.
65. Zhao J, Li X, Zhang K, Yin X, Meng X, et al. Prediction of microvascular invasion of hepatocellular carcinoma with preoperative diffusion-weighted imaging: A comparison of mean and minimum apparent diffusion coefficient values. *Medicine* 2017;96:e7754.
66. Wang WT, Yang L, Yang ZX, Hu XX, Ding Y, et al. Assessment of microvascular invasion of hepatocellular carcinoma with diffusion kurtosis imaging. *Radiology* 2018;286:571-80.
67. Jensen JH, Helpert JA, Ramani A, Lu H, Kaczynski K. Diffusional kurtosis imaging: the quantification of non-gaussian water diffusion by means of magnetic resonance imaging. *Magn Reson Med* 2005;53:1432-40.
68. Ahn SJ, Kim JH, Park SJ, Kim ST, Han JK. Hepatocellular carcinoma: preoperative gadoxetic acid-enhanced MR imaging can predict early recurrence after curative resection using image features and texture analysis. *Abdom Radiol (NY)* 2019;44:539-48.
69. Lee S, Kim SH, Lee JE, Sinn DH, Park CK. Preoperative gadoxetic acid-enhanced MRI for predicting microvascular invasion in patients with single hepatocellular carcinoma. *J Hepatol* 2017;67:526-34.
70. Ryu T, Takami Y, Wada Y, Tateishi M, Hara T, et al. A clinical scoring system for predicting microvascular invasion in patients with hepatocellular carcinoma within the milan criteria. *J Gastrointest Surg* 2019;23:779-87.
71. Peng J, Zhang J, Zhang Q, Xu Y, Zhou J, et al. A radiomics nomogram for preoperative prediction of microvascular invasion risk in

- hepatitis B virus-related hepatocellular carcinoma. *Diagn Interv Radiol* 2018;24:121-7.
72. Xu X, Zhang HL, Liu QP, Sun SW, Zhang J, et al. Radiomic analysis of contrast-enhanced CT predicts microvascular invasion and outcome in hepatocellular carcinoma. *J Hepatol* 2019;70:1133-44.
73. Ji GW, Zhu FP. Radiomic features at contrast-enhanced CT predict recurrence in early stage hepatocellular carcinoma: a multi-institutional study. *Radiology* 2020;294:568-79.
74. Zhu YJ, Feng B, Wang S, Wang LM, Wu JF, et al. Model-based three-dimensional texture analysis of contrast-enhanced magnetic resonance imaging as a potential tool for preoperative prediction of microvascular invasion in hepatocellular carcinoma. *Oncol Lett* 2019;18:720-32.
75. Kutami R, Nakashima Y, Nakashima O, Shiota K, Kojiro M. Pathomorphologic study on the mechanism of fatty change in small hepatocellular carcinoma of humans. *J Hepatol* 2000;33:282-9.
76. Takayama T, Makuuchi M, Hirohashi S, Sakamoto M, Okazaki N, et al. Malignant transformation of adenomatous hyperplasia to hepatocellular carcinoma. *Lancet* 1990;336:1150-3.
77. Villanueva A, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. *Semin Liver Dis* 2007;27:55-76.
78. Asayama Y, Nishie A, Ishigami K, Ushijima Y, Takayama Y, et al. Fatty change in moderately and poorly differentiated hepatocellular carcinoma on MRI: a possible mechanism related to decreased arterial flow. *Clin Radiol* 2016;71:1277-83.
79. Min JH, Kim YK, Lim S, Jeong WK, Choi D, et al. Prediction of microvascular invasion of hepatocellular carcinomas with gadoxetic acid-enhanced MR imaging: Impact of intra-tumoral fat detected on chemical-shift images. *Eur J Radiol* 2015;84:1036-43.
80. Kubota N, Ojima H, Hatano M, Yamazaki K, Masugi Y, et al. Clinicopathological features of hepatocellular carcinoma with fatty change: tumors with macrovesicular steatosis have better prognosis and aberrant expression patterns of perilipin and adipophilin. *Pathol Int* 2020;70:199-209.
81. Yamaguchi H, Condeelis J. Regulation of the actin cytoskeleton in cancer cell migration and invasion. *Biochimica et biophysica acta* 2007; 1773: 642-652.
82. Kim YY, Choi JY, Sirlin CB, An C, Kim MJ. Pitfalls and problems to be solved in the diagnostic CT/MRI Liver Imaging Reporting and Data System (LI-RADS). *Eur Radiol* 2019;29:1124-32.
83. Qin S. Guidelines on the diagnosis and treatment of primary liver cancer (2011 edition). *Chin Clin Oncol* 2012;1:10.
84. Hennes T, Venkatesh SK. Advances in computed tomography and magnetic resonance imaging of hepatocellular carcinoma. *World J Gastroenterol* 2016;22:205-20.
85. Cheng S, Chen M, Cai J. Chinese expert consensus on multidisciplinary diagnosis and treatment of hepatocellular carcinoma with portal vein tumor thrombus: 2016 edition. *Oncotarget* 2017;8:8867-76.
86. Ponziani FR, Zocco MA, Campanale C, Rinninella E, Tortora A, et al. Portal vein thrombosis: insight into physiopathology, diagnosis, and treatment. *World J Gastroenterol* 2010;16:143-55.
87. Kanematsu M, Semelka RC, Leonardou P, Mastropasqua M, Lee JK. Hepatocellular carcinoma of diffuse type: MR imaging findings and clinical manifestations. *J Magn Reson Imaging* 2003;18:189-95.
88. An J, Lee KS, Kim KM. Clinical features and outcomes of patients with hepatocellular carcinoma complicated with bile duct invasion. *Clin Mol Hepatol* 2017;23:160-9.
89. Chotirosniramit A, Liwattanakun A, Lapisatepun W, Ko-Iam W, Sandhu T, et al. A single institution report of 19 hepatocellular carcinoma patients with bile duct tumor thrombus. *J Hepatocell Carcinoma* 2017;4:41-7.
90. Suh YG, Kim DY, Han KH, Seong J. Effective biliary drainage and proper treatment improve outcomes of hepatocellular carcinoma with obstructive jaundice. *Gut Liver* 2014;8:526-35.
91. Liu QY, Huang SQ, Chen JY, Li HG, Gao M, et al. Small hepatocellular carcinoma with bile duct tumor thrombi: CT and MRI findings. *Abdom Imaging* 2010;35:537-42.
92. Lu CH, Chen CL, Cheng YF, Huang TL, Tsang LL, et al. Correlation between imaging and pathologic findings in explanted livers of hepatocellular carcinoma cases. *Transplant Proc* 2010;42:830-3.
93. Liu Q, Chen J, Li H, Liang B, Zhang L, et al. Hepatocellular carcinoma with bile duct tumor thrombi: correlation of magnetic resonance imaging features to histopathologic manifestations. *Eur J Radiol* 2010;76:103-9.
94. Giannelli G, Rani B, Dituri F, Cao Y, Palasciano G. Moving towards personalised therapy in patients with hepatocellular carcinoma: the role of the microenvironment. *Gut* 2014;63:1668-76.
95. Ariizumi S, Kitagawa K, Kotera Y, Takahashi Y, Katagiri S, et al. A non-smooth tumor margin in the hepatobiliary phase of gadoxetic acid disodium (Gd-EOB-DTPA)-enhanced magnetic resonance imaging predicts microscopic portal vein invasion, intrahepatic metastasis, and early recurrence after hepatectomy in patients with hepatocellular carcinoma. *J Hepatobiliary Pancreat Sci* 2011;18:575-85.
96. Seo HJ, Kim GM, Kim JH, Kang WJ, Choi HJ. ¹⁸F-FDG PET/CT in hepatocellular carcinoma: detection of bone metastasis and prediction of prognosis. *Nucl Med Commun* 2015;36:226-33.
97. Hwang SH, Lee JW, Cho HJ, Kim KS, Choi GH, et al. Prognostic value of metabolic tumor volume and total lesion glycolysis on preoperative ¹⁸F-FDG PET/CT in patients with very early and early hepatocellular carcinoma. *Clin Nucl Med* 2017;42:34-9.
98. Ho CL, Chen S, Yeung DW, Cheng TK. Dual-tracer PET/CT imaging in evaluation of metastatic hepatocellular carcinoma. *J Nucl Med* 2007;48:902-9.
99. Kitao A, Matsui O, Yoneda N, Kozaka K, Kobayashi S, et al. Hepatocellular carcinoma with β -catenin mutation: Imaging and pathologic characteristics. *Radiology* 2015;275:708-17.
100. Zucman-Rossi J, Jeannot E, Nhieu JT, Scoazec JY, Guettier C, et al. Genotype-phenotype correlation in hepatocellular adenoma: new

- classification and relationship with HCC. *Hepatology* 2006;43:515-24.
101. Kitao A, Matsui O, Yoneda N, Kozaka K, Kobayashi S, et al. Gadoteric acid-enhanced MR imaging for hepatocellular carcinoma: molecular and genetic background. *Eur Radiol* 2020; Epub ahead of print. doi: 10.1007/s00330-020-06687-y.
 102. Guzman G, Alagiozian-Angelova V, Layden-Almer JE, Layden TJ, Testa G, et al. p53, Ki-67, and serum alpha fetoprotein as predictors of hepatocellular carcinoma recurrence in liver transplant patients. *Mod Pathol* 2005;18:1498-503.
 103. Chen J, Chen C, Xia C, Huang Z, Zuo P, et al. Quantitative free-breathing dynamic contrast-enhanced MRI in hepatocellular carcinoma using gadoteric acid: correlations with Ki67 proliferation status, histological grades, and microvascular density. *Abdom Radiol (NY)* 2018;43:1393-403.
 104. Chen Y, Qin X, Long L, Zhang L, Huang Z, et al. Diagnostic value of Gd-EOB-DTPA-enhanced MRI for the expression of Ki67 and microvascular density in hepatocellular carcinoma. *J Magn Reson Imaging* 2019; Epub ahead of print. doi: 10.1002/jmri.26974.
 105. Li Y, Chen J, Weng S, Sun H, Yan C, et al. Small hepatocellular carcinoma: using MRI to predict histological grade and Ki-67 expression. *Clin Radiol* 2019;74:653.e1-653.e9.
 106. Surov A, Meyer HJ, Wienke A. Associations between apparent diffusion coefficient (ADC) and KI 67 in different tumors: a meta-analysis. Part 1: ADCmean. *Oncotarget* 2017;8:75434-44.
 107. Ye Z, Jiang H, Chen J, Liu X, Wei Y, et al. Texture analysis on gadoteric acid enhanced-MRI for predicting Ki-67 status in hepatocellular carcinoma: a prospective study. *Chin J Cancer Res* 2019;31:806-17.
 108. Tsuchiya K, Komuta M, Yasui Y, Tamaki N, Hosokawa T, et al. Expression of keratin 19 is related to high recurrence of hepatocellular carcinoma after radiofrequency ablation. *Oncology* 2011;80:278-88.
 109. Wang W, Gu D, Wei J, Ding Y, Yang L, et al. A radiomics-based biomarker for cytokeratin 19 status of hepatocellular carcinoma with gadoteric acid-enhanced MRI. 2020;30:3004-14.
 110. Chen J, Wu Z, Xia C, Jiang H, Liu X, et al. Noninvasive prediction of HCC with progenitor phenotype based on gadoteric acid-enhanced MRI. *Eur Radiol* 2020;30:1232-42.
 111. Hu XX, Wang WT, Yang L, Yang ZX, Liang HY, et al. MR features based on LI-RADS identify cytokeratin 19 status of hepatocellular carcinomas. *Eur J Radiol* 2019;113:7-14.
 112. Choi SY, Kim SH, Park CK, Min JH, Lee JE, et al. Imaging features of gadoteric acid-enhanced and diffusion-weighted MR imaging for identifying cytokeratin 19-positive hepatocellular carcinoma: a retrospective observational study. *Radiology* 2018;286:897-908.
 113. Munz M, Baeuerle PA, Gires O. The emerging role of EpCAM in cancer and stem cell signaling. *Cancer Res* 2009;69:5627-9.
 114. Zhou L, Zhu Y. The EpCAM overexpression is associated with clinicopathological significance and prognosis in hepatocellular carcinoma patients: a systematic review and meta-analysis. *Int J Surg* 2018;56:274-80.
 115. Ji J, Yamashita T, Budhu A, Forgues M, Jia HL, et al. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. *Hepatology* 2009;50:472-80.

Review

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Impact of direct-acting antivirals on *de novo* occurrence of hepatocellular carcinoma in hepatitis C virus patients

Ming Yang^{1,#}, Ruilan Ma^{2,#}, Yuan Huang¹, Lai Wei¹

¹Hepatopancreatobiliary Center, Beijing Tsinghua Changgung Hospital, Tsinghua University, Beijing 102218, China.

²Department of radiotherapy, The Second Hospital of Dalian Medical University, Dalian, Liaoning 116027, China.

[#]Authors contributed equally.

Correspondence to: Prof. Lai Wei, Hepatopancreatobiliary Center, Beijing Tsinghua Changgung Hospital, Tsinghua University, No.168 Litang Road, Changping District, Beijing 102218, China. E-mail: weelai@163.com

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Abstract

Hepatitis C Virus (HCV) infection constitutes a significant burden to world health, leading to liver cirrhosis and hepatocellular carcinoma (HCC). In the past decades, pegylated interferon combined with ribavirin has been used extensively for HCV treatment, and interferon (IFN) is thought to have antitumor property. Direct-acting antivirals (DAAs) have fundamentally changed HCV therapy, due to their high efficacy and tolerability. However, recent studies have reported relatively high rates of HCC occurrence, and recurrence, following successful HCV treatment using DAAs. These studies were grossly underpowered due to their retrospective design, lack of untreated or IFN controls, small sample size, and limited patient follow-up time. From then, many retrospective and prospective cohort studies with larger size and longer follow-up duration after DAAs therapy have been published. These studies showed that treatment with DAAs can reduce the risk of HCC compared to no treatment, didn't increase the risk of HCC compared to IFN-based therapy after adjusting for the potential confounders of these two groups, and DAAs-induced sustained virological response decreased the risk of HCC compared to DAAs treatment failure. In conclusion, DAAs treatment doesn't appear to increase the development of HCC, even in cirrhotic patients. However, cirrhotic patients should be monitored for the development of HCC during and after DAAs treatment.

Keywords: Sustained virological response, hepatocellular carcinoma, liver cirrhosis



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INTRODUCTION

It is estimated that 71 million people have chronic hepatitis C virus (HCV) infection, which can lead to liver cirrhosis and hepatocellular carcinoma (HCC)^[1]. It seems reasonable that HCV eradication would reduce the risk of HCV-related complications including HCC. Cirrhosis is the major risk factor of HCC and about 3% patients with HCV related cirrhosis develop HCC annually^[2]. In the past decades, pegylated interferon combined with ribavirin (PR) has been widely used for the treatment of HCV. Despite the sustained virological response (SVR) rates associated with PR therapy not being high enough, and relatively more adverse events^[3] reported, studies have demonstrated that interferon (IFN)-induced SVR could reduce HCC incidence^[4]. The annual incidence of HCC is mostly less than 2% in cirrhotic patients achieving SVR after IFN-based regimens^[5,6].

Direct-acting antivirals (DAAs) have fundamentally changed HCV therapy because of their high efficacy and tolerability, even in the patients with cirrhosis^[7-9]. In these studies, patients with SVR showed improvements in disease severity and mortality. In 2016, relatively high rates of HCC occurrence and recurrence were reported after the success treatment of HCV using DAAs^[10,11]. Since then, this has been a highly controversial topic.

This review article summarizes the relevant articles focusing on the impact of DAAs on *de novo* occurrence of HCC in HCV patients. We searched the MEDLINE electronic database using the search terms ["hepatitis C" (MeSH)], ("direct-acting antivirals"), and ["hepatocellular carcinoma" (MeSH)] from the start of the database (1996 year) until April 19, 2020. Searches were limited to human studies written in English. Eligible study designs included retrospective or prospective observational cohort studies, randomized controlled trials and interventional studies. Figure 1 shows the study flow chart.

STUDIES SUPPORTING HIGH RATES OF HCC OCCURRENCE AFTER DAAS TREATMENT

Table 1 summarizes the studies supporting high rates of HCC occurrence after DAAs treatment^[11-14]. Conti *et al.*^[11] from Italy reported that HCC was detected in 3.2 % (9/285) cirrhotic patients at 24-week follow-up after DAAs therapy. Ravi *et al.*^[14] from the United States (US) reported that 9.1% (6/66 patients) of cirrhotic patients developed HCC within six months of DAAs treatment. Studies from Portugal^[12] and Austria^[13] also reported high rates of HCC occurrence after DAAs treatment. This data raised concerns that DAAs may promote the development of HCC. However, these studies were underpowered because of their retrospective design, lack of untreated or IFN controls, small sample size, and limited follow-up time.

POSSIBLE MECHANISMS OF HCC OCCURRENCE AFTER DAAS TREATMENT

There is a complex equilibrium between pro tumor factors such as HCV and inflammation and anti-tumor factors such as the immune system. A rapid reduction in the HCV viral load by DAAs treatment might impair immune surveillance, resulting in the development of HCC. Serti *et al.*^[15] reported DAAs treatment was associated with a rapid decreased activation of natural killer cells. Meissner *et al.*^[16] reported that HCV clearance by DAAs treatment was accompanied by down regulation of IFN-stimulated genes and the levels of type II and III IFNs. The serum level of microRNA-122, a regulator of HCV replication and tumor suppressor against HCC^[17], decreased in patients with IFN-free therapy induced SVR^[18]. Debes *et al.*^[19] reported a panel of cytokines, apoptosis markers, and growth factors with higher levels before DAAs therapy in patients with new HCC compared with controls. Interestingly, their results suggested that the immune background rather than DAAs mediated immune modulation would lead to HCC development.

STUDIES AGAINST HIGH RATES OF HCC DEVELOPMENT AFTER DAAS TREATMENT

From 2016, many retrospective and prospective cohort studies with larger sizes and greater follow-up duration after DAAs therapy were published. Table 2 summarizes the studies not supporting high rates

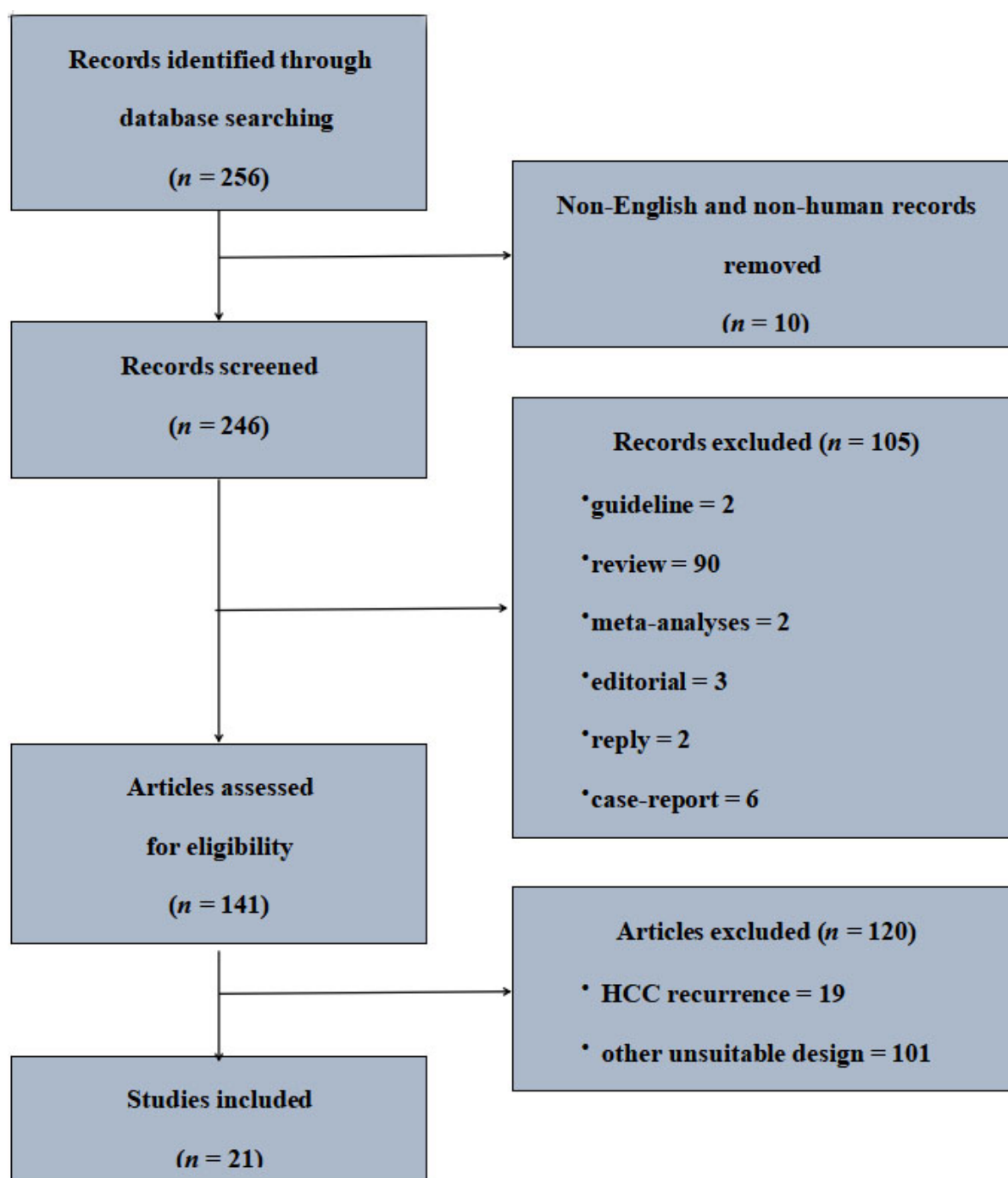


Figure 1. Study flow chart. HCC: hepatocellular carcinoma

Table 1. Studies in support of high rates of HCC odevelopment after DAAs treatment

Ref.	Nature	Patients, <i>n</i>	Liver disease stage	Median follow-up duration	HCC, <i>n</i>	Incidence of HCC
Conti <i>et al.</i> ^[11]	Retrospective	285	cirrhosis	24 weeks	9	3.16% in first 6 months
Cardoso <i>et al.</i> ^[12]	Retrospective	54	cirrhosis	1 year	4	7.4% in the first year
Kozbial <i>et al.</i> ^[13]	Retrospective	195	all stages	48 weeks	13	6.60% in the first year
Ravi <i>et al.</i> ^[14]	Retrospective	66	cirrhosis	6 months	6	9.1% in first 6 months

HCC: hepatocellular carcinoma; DAAs: direct-acting antivirals

Table 2. Studies not supporting high rates of HCC occurrence after DAAs treatment

Ref.	Nature	Patients, n	Liver disease stage	Median follow-up duration	HCC, n	Incidence rate	P value
Cheung <i>et al.</i> ^[20]	Prospective + retrospective	DAAs, 406; Untreated, 261	Decompensated cirrhosis	DAAs, 15 months; Untreated, 6 months	DAAs, 27; Untreated, 11	6-month incidence: 4% for patients with DAAs vs. 4% for untreated patients	0.98
Nagata <i>et al.</i> ^[21]	Retrospective	IFN-free, 1145; IFN-based, 752	All stages	IFN-free, 1.8 years; IFN-based, 6.8 years	-	3-year incidence: 1.4% for IFN-free patients vs. 3.3% for IFN-based patients	0.49
Kanwal <i>et al.</i> ^[22]	Retrospective	DAAs, 22,500	All stages	NA	271	Annual incidence: 0.90% for patients with SVR vs. 3.45% for patients without SVR, AHR = 0.28	< 0.001
Ioannou <i>et al.</i> ^[23]	Retrospective	DAAs-only, 21948; DAAs + IFN, 4535; IFN-only, 35,871	All stages	DAAs-only, 1.53 years	DAAs-only, 2651; DAAs+IFN, 175; IFN-only, 445	Annual incidence: 1.32% for patients with DAAs-only vs. 1.06% for patients with DAAs + IFN vs. 0.81% for patients with IFN-only	> 0.05
Singer <i>et al.</i> ^[24]	Retrospective	DAAs, 30,183; IFN, 12,948; Untreated, 137,502	All stages	DAAs, 1.05 years; IFN, 2.93 years; Untreated, 1.24 years	DAAs, 433; IFN, 388; Untreated, 1232	Annual incidence: 1.18% for patients with DAAs vs. 0.64% for untreated patients, AHR = 0.84; 1.18% for patients with DAAs vs. 0.98% for patients with IFN, AHR = 0.69	< 0.05 < 0.05
Innes <i>et al.</i> ^[25]	Retrospective	IFN-Free, 272; IFN-containing, 585	Cirrhosis and with SVR	IFN-Free, 1.7 years; IFN-containing, 3.5 years	IFN-Free, 11; IFN-containing, 30	Annual incidence: 2.53% for IFN-Free patients vs. 1.26% for IFN-containing patients, AHR = 1.15	0.744
Li <i>et al.</i> ^[26]	Retrospective	DAAs, 5834; IFN, 3534; Untreated, 8468	All stages	DAAs, 396 days; IFN, 2719 days	DAAs, 50; IFN, 196; Untreated, 436	Annual incidence: 2.12% for cirrhotic patients with DAAs vs. 2.28% for cirrhotic patients with IFN; 4.53% for untreated patients	0.78 0.03
Romano <i>et al.</i> ^[27]	Prospective	DAAs, 3917	F3 or higher	536 days	55	Annual incidence: 0.95% for cirrhotic patients with SVR vs. 7.73% for cirrhotic patients without SVR	0.0001
Nahon <i>et al.</i> ^[28]	Prospective	DAAs, 336; SVR-IFN, 495; non-SVR, 439	Cirrhosis	DAAs, 21.2 months; SVR-IFN, 64.4 months; non-SVR-IFN, 47.4 months	DAAs, 15; SVR-IFN, 31; non-SVR, 154	3-year incidence: 5.9% for patients with DAAs vs. 3.1% for patients with SVR-IFN, AHR = 0.89	0.73
Calvaruso <i>et al.</i> ^[29]	Prospective	DAAs, 2249	Cirrhosis	14 months	78	1-year incidence: 2.6% for patients with SVR vs. 8% for patients without SVR	< 0.001
Lleo <i>et al.</i> ^[30]	Prospective	DAAs, 1766	Cirrhosis	NA	50	Annual incidence: 2.1% for patients with SVR vs. 11.3% for patients without SVR, AHR = 0.21	< 0.0001
Mettke <i>et al.</i> ^[31]	Prospective + retrospective	DAAs, 158; Untreated, 184	Cirrhosis	DAAs, 440 days; Untreated, 592 days	78	Annual incidence: 2.9% for patients with DAAs vs. 4.48% for untreated patients	0.39
Carrai <i>et al.</i> ^[32]	Prospective	DAAs, 7344; Untreated, 2551	All stages	DAAs, 34.5 months; Untreated, 32.3 months	DAAs, 187; Untreated, 71	Annual incidence: 1.4% for patients with DAAs vs. 0.56% for untreated patients, AHR = 0.66	0.018

Backus <i>et al.</i> ^[33]	Retrospective	DAA, 15,059	Advanced liver disease (FIB-4 > 3.25)	1.6 years	537	Annual incidence: 1.9% for patients with SVR vs. 11.5% for patients without SVR	< 0.001
Buonomo <i>et al.</i> ^[34]	Prospective	DAA, 323	All stages	10 months	11	Per-month incidence: 0.2% for patients with SVR vs. 1.7% for patients without SVR	< 0.05
Colussi <i>et al.</i> ^[35]	Prospective	DAA, 380	All stages	58 weeks	17	Annual incidence: 1.3% for patients with SVR vs. 59% for patients without SVR	< 0.001
Ebel <i>et al.</i> ^[36]	Prospective	DAA, 158; Untreated, 184	Cirrhosis	DAA, 1109 days; Untreated, NA	DAA, 9; Untreated, 23	Annual incidence: 2.04% for patients with DAA vs. 5.04% for untreated patients	0.008

HCC: hepatocellular carcinoma; DAA: direct-acting antiviral; SVR: sustained virological response; IFN: interferon

of HCC development after DAAs treatment^[20-36]. We found that these studies could be divided into three categories: (1) DAAs-treated patients vs. untreated patients; (2) DAAs-treated patients vs. IFN-treated patients; and (3) patients with DAAs induced SVR vs. patients with DAAs failure.

A number of studies compared the risk of HCC in DAAs-treated patients with untreated patients^[20,24,26,31,32,36]. A study of decompensated cirrhotic patients from the United Kingdom reported the incidence of liver cancer was 4% at six months after DAAs treatment, comparable with untreated patients. The figures then dropped to 2.5% between month 6-15 after DAAs treatment^[20]. Similarly, a study of cirrhotic patients from Germany reported DAAs treatment did not change the short-term risk of HCC after 1.5 years of follow-up (2.9 per 100 person-years for patients with DAAs vs. 4.48 per 100 person-years for untreated patients, $P = 0.39$)^[31]. When the cohort was followed up for five years, a reduced HCC risk was confirmed (2.04 per 100 person-years for patients with DAAs vs. 5.04 per 100 person-years for untreated patients, $P = 0.008$)^[36]. Other studies showed DAAs treatment reduced *de novo* HCC directly^[24,26,32]. Singer *et al.*^[24] study from the US reported that DAAs therapy reduced risk of HCC compared to no treatment (AHR 0.84, 95%CI 0.73-0.96), and to IFN-based therapy (AHR 0.69, 95%CI: 0.59-0.81). Another large retrospective study from the US reported that among cirrhotic patients, the incidence of HCC was similar in the DAAs-induced SVR group compared to the IFN-induced SVR group (2.12 vs. 2.28 per 100 person-years, $P = 0.78$), but much higher in untreated group (4.53 per 100 person-years, $P = 0.03$)^[26]. A large prospective study from France, with a median follow-up of 33.4 months, reported that DAAs treatment was associated with a reduced risk of developing HCC, after adjusting for potential confounding factors (AHR 0.66, 95%CI 0.46-0.93)^[32].

Some studies compared the risk of HCC in DAAs-treated patients with IFN-treated patients^[21,23,25,28]. All four studies showed HCC incidence was similar between DAAs-treated patients and IFN-treated patients after adjusting for the characteristics of these two groups. One important thing we should pay attention to is that candidates for DAAs are more prone to developing HCC because of advanced liver disease. Adjustment for confounders such as patient demographics, comorbidities, health behaviors, virology, and baseline liver disease stage adequately may go some way to help address potential bias. The prior screening for HCC in DAAs group was usually suboptimal and might fail to detect the HCC that are perhaps already present before DAAs therapy. It highlighted the importance of excluding the presence of HCC before initiation of DAAs treatment.

Other studies compared the risk of HCC in DAAs-cured patients with DAAs-failed patients^[22,27,29,30,33-35]. All of these studies showed that DAAs-induced SVR decreased the incidence of HCC. The HCC risk reduction by DAAs treatment was evident not only in patients without cirrhosis, but also in patients with cirrhosis. Romano *et al.*^[27] reported that the incidence of HCC was higher during the first year (0.46% in F3, 1.49% in CTP-A and 3.61% in CTP-B cirrhosis, respectively), and declined in the second year (0% in F3, 0.2% in CTP-A, and 0.69% in CTP-B cirrhosis, respectively). But some studies reported that in cirrhotic patients with DAAs-induced SVR, the incidence of HCC was usually still higher than 1.5% above which HCC surveillance is cost-effective. Kanwal *et al.*^[37] reported the quarterly incidence rate of HCC remained stable between 1.5 to 2.3 per 100 person-years in patients with cirrhosis, which indicated HCC risk of cirrhotic patients cured with DAAs did not progress or regress during follow-up. Ide *et al.*^[38] found the one, two, and three-year cumulative incidences of HCC in patients with cirrhosis were 2.5%, 5.2%, and 10.0% respectively. Recently, Ioannou *et al.*^[39] reported that in DAAs-treated patients with cirrhosis and a fibrosis index based on four factors (FIB-4) with scores ≥ 3.25 , the annual HCC incidence decreased from 3.8% in the first year after SVR to 2.4% by the fourth year, which was still at high risk of developing HCC. Although these studies with more than three years follow-up have confirmed HCC risk is not increased after SVR by DAAs^[37-39], the changes in HCC incidence over time deserve to be further clarified in longer-term studies.

Physicians are most interested in which patients should undergo HCC surveillance after achieving SVR by DAAs. Male gender, older age, alcohol abuse, diabetes mellitus, the existence of advanced fibrosis (F3) or cirrhosis, higher alpha-fetoprotein and no SVR are risk factors for HCC occurrence. The existence of cirrhosis is the most important risk factor for HCC development. Therefore, guidelines recommend patients with advanced liver fibrosis or cirrhosis at the time of DAAs treatment should stay in HCC surveillance even after DAA-induced SVR. FIB-4 and aspartate aminotransferase to platelet ratio index (APRI) are easily accessible non-invasive indicators which can be used as stratification risk factors for HCC development, alongside cirrhosis. Kanwal *et al.*^[37] divided cirrhotic patients who achieved SVR with DAAs into three groups: patients who had persistently high FIB-4/APRI over time, patients who experienced a decline in FIB-4/APRI, and patients who had persistently low FIB-4/APRI. Annual HCC incidence remained below 1.5% in the third subgroup, suggesting that it might be possible to exclude these cirrhotic patients from HCC surveillance programs. On the contrary, the annual HCC incidence was between 0.4% to 1.6% in non-cirrhotic patients but with high FIB-4/APRI subgroup (FIB-4 >3.25 /APRI >1.5), which was high enough to recommend HCC surveillance in these patients.

Although the impact of DAAs treatment and HCC has been extensively published, our review has some strengths. Firstly, we divide the studies not supporting high rates of HCC occurrence after DAAs treatment into three categories, which facilitates easy comparison between studies. Secondly, we include the most recent published evidence on this topic up to April, 2020, with the majority of studies published in 2019 and 2020, with follow-up of three to five years. Thirdly, we discuss the changes in HCC incidence over time following DAAs-induced HCV eradication. Fourthly, we emphasize FIB-4/APRI as the stratification factors besides cirrhosis, which is more useful for the patients with F3 fibrosis.

CONCLUSION

DAAs treatment doesn't appear to increase the occurrence of HCC, even in the cirrhotic patients. DAAs treatment should be considered in all HCV patients. Cirrhotic patients should be monitored for development of HCC during and after DAAs treatment.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and performed data acquisition and interpretation: Yang M, Ma R

Made manuscript revision: Wei L, Huang Y

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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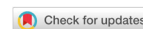
REFERENCES

1. World Health Organization. Hepatitis C. Available from: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-c> [Last accessed on 18 May 2020]
2. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011;365:1118-27.
3. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975-82.
4. Morgan RL, Baack B, Smith BD, Yartel A, Pitasi M, et al. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann Intern Med* 2013;158:329-37.
5. Bruno S, Stroffolini T, Colombo M, Bollani S, Benvegnù L, et al.; Italian Association of the Study of the Liver Disease (AISF). Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007;45:579-87.
6. Cardoso AC, Moucari R, Figueiredo-Mendes C, Ripault MP, Giuily N, et al. Impact of peginterferon and ribavirin therapy on hepatocellular carcinoma: incidence and survival in hepatitis C patients with advanced fibrosis. *J Hepatol* 2010;52:652-7.
7. Curry MP, O'Leary JG, Bzowej N, Muir AJ, Korenblat KM, et al.; ASTRAL-4 Investigators. Sofosbuvir and velpatasvir for HCV in patients with decompensated cirrhosis. *N Engl J Med* 2015;373:2618-28.
8. Foster GR, Irving WL, Cheung MC, Walker AJ, Hudson BE, et al.; HCV Research, UK. Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. *J Hepatol* 2016;64:1224-31.
9. Crespo G, Trota N, Londoño MC, Mauro E, Baliellas C, et al. The efficacy of direct anti-HCV drugs improves early post-liver transplant survival and induces significant changes in waiting list composition. *J Hepatol* 2018;69:11-7.
10. Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016;65:719-26.
11. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016;65:727-33.
12. Cardoso H, Vale AM, Rodrigues S, Gonçalves R, Albuquerque A, et al. High incidence of hepatocellular carcinoma following successful interferon-free antiviral therapy for hepatitis C associated cirrhosis. *J Hepatol* 2016;65:1070-1.
13. Kozbial K, Moser S, Schwarzer R, Laferl H, Al-Zoairy R, et al. Unexpected high incidence of hepatocellular carcinoma in cirrhotic patients with sustained virologic response following interferon-free direct-acting antiviral treatment. *J Hepatol* 2016;65:856-8.
14. Ravi S, Axley P, Jones D, Kodali S, Simpson H, et al. Unusually high rates of hepatocellular carcinoma after treatment with direct-acting antiviral therapy for hepatitis C related cirrhosis. *Gastroenterology* 2017;152:911-2.
15. Serti E, Chepa-Lotrea X, Kim YJ, Keane M, Fryzek N, et al. Successful interferon-free therapy of chronic hepatitis C virus infection normalizes natural killer cell function. *Gastroenterology* 2015;149:190-200.e2.
16. Meissner EG, Wu D, Osinusi A, Bon D, Virtaneva K, et al. Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome. *J Clin Invest* 2014;124:3352-63.
17. Tsai WC, Hsu PW, Lai TC, Chau GY, Lin CW, et al. MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. *Hepatology* 2009;49:1571-82.
18. Waring JF, Dumas EO, Abel S, Coakley E, Cohen DE, et al. Serum miR-122 may serve as a biomarker for response to direct acting antivirals: Effect of paritaprevir/R with dasabuvir or ombitasvir on miR-122 in HCV-infected subjects. *J Viral Hepat* 2016;23:96-104.
19. Debes JD, van Tilborg M, Groothuismink ZMA, Hansen BE, Schulze Zur Wiesch J, et al. Levels of cytokines in serum associate

- with development of hepatocellular carcinoma in patients with HCV infection treated with direct-acting antivirals. *Gastroenterology* 2018;154:515-517.e3.
20. Cheung MCM, Walker AJ, Hudson BE, Verma S, McLauchlan J, et al.; HCV Research UK. Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. *J Hepatol* 2016;65:741-7.
 21. Nagata H, Nakagawa M, Asahina Y, Sato A, Asano Y, et al.; Ochanomizu Liver Conference Study Group. Effect of interferon-based and -free therapy on early occurrence and recurrence of hepatocellular carcinoma in chronic hepatitis C. *J Hepatol* 2017;67:933-9.
 22. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, et al. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. *Gastroenterology* 2017;153:996-1005.e1.
 23. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol* 2017;25-32.
 24. Singer AW, Reddy KR, Telep LE, Osinusi AO, Brainard DM, et al. Direct-acting antiviral treatment for hepatitis C virus infection and risk of incident liver cancer: a retrospective cohort study. *Aliment Pharmacol Ther* 2018;47:1278-87.
 25. Innes H, Barclay ST, Hayes PC, Fraser A, Dillon JF, et al. The risk of hepatocellular carcinoma in cirrhotic patients with hepatitis C and sustained viral response: role of the treatment regimen. *J Hepatol* 2018;68:646-54.
 26. Li DK, Ren Y, Fierer DS, Rutledge S, Shaikh OS, et al. The short-term incidence of hepatocellular carcinoma is not increased after hepatitis C treatment with direct-acting antivirals: an ERCHIVES study. *Hepatology* 2018;67:2244-53.
 27. Romano A, Angeli P, Piovesan S, Noventa F, Anastassopoulos G, et al. Newly diagnosed hepatocellular carcinoma in patients with advanced hepatitis C treated with DAAs: a prospective population study. *J Hepatol* 2018;69:345-52.
 28. Nahon P, Layese R, Bourcier V, Cagnot C, Marcellin P, et al.; ANRS CO12 CirVir Group. Incidence of hepatocellular carcinoma after direct antiviral therapy for HCV in patients with cirrhosis included in surveillance programs. *Gastroenterology* 2018;155:1436-50.e6.
 29. Calvaruso V, Cabibbo G, Cacciola I, Petta S, Madonia S, et al.; Rete Sicilia Selezione Terapia-HCV (RESIST-HCV). Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. *Gastroenterology* 2018;155:411-21.e4.
 30. Lleo A, Aglitti A, Aghemo A, Maisonneuve P, Bruno S, et al.; collaborators. Predictors of hepatocellular carcinoma in HCV cirrhotic patients treated with direct acting antivirals. *Dig Liver Dis* 2019;51:310-7.
 31. Mettke F, Schlevogt B, Deterding K, Wranke A, Smith A, et al. Interferon-free therapy of chronic hepatitis C with direct-acting antivirals does not change the short-term risk for de novo hepatocellular carcinoma in patients with liver cirrhosis. *Aliment Pharmacol Ther* 2018;47:516-25.
 32. Carrat F, Fontaine H, Dorival C, Simony M, Diallo A, et al. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. *Lancet* 2019;393:1453-64.
 33. Backus LI, Belperio PS, Shahoumian TA, Mole LA. Impact of sustained virologic response with direct-acting antiviral treatment on mortality in patients with advanced liver disease. *Hepatology* 2019;69:487-97.
 34. Buonomo AR, Scotto R, Coppola C, Pinchera B, Viceconte G, et al. Direct acting antivirals treatment for hepatitis C virus infection does not increase the incidence of de novo hepatocellular carcinoma occurrence: Results from an Italian real-life cohort (LINA cohort). *Medicine (Baltimore)* 2020;99:e18948.
 35. Colussi G, Donnini D, Brizzi RF, Maier S, Valenti L, et al. Sustained virologic response to direct-acting antiviral agents predicts better outcomes in hepatitis C virus-infected patients: a retrospective study. *World J Gastroenterol* 2019;25:6094-106.
 36. Ebel F, Deterding K, Port K, Schlevogt B, Manns MP, et al. Letter: a 5-year long-term follow-up study after DAA treatment confirms a reduced HCC risk in a central European cohort of HCV patients with liver cirrhosis. *Aliment Pharmacol Ther* 2020;51:194-5.
 37. Kanwal F, Kramer JR, Asch SM, Cao Y, Li L, et al. Long-term risk of hepatocellular carcinoma in HCV patients treated with direct acting antiviral agents. *Hepatology* 2020;71:44-55.
 38. Ide T, Koga H, Nakano M, Hashimoto S, Yatsushashi H, et al. Direct-acting antiviral agents do not increase the incidence of hepatocellular carcinoma development: a prospective, multicenter study. *Hepatol Int* 2019;13:293-301.
 39. Ioannou GN, Beste LA, Green PK, Singal AG, Tapper EB, et al. Increased risk for hepatocellular carcinoma persists up to 10 years after HCV eradication in patients with baseline cirrhosis or high FIB-4 scores. *Gastroenterology* 2019;157:1264-78.e4.

Review

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Non-invasive tests for the prediction of post-hepatectomy liver failure in the elderly

Giovanni Marasco¹, Antonio Colecchia², Matteo Milandri³, Benedetta Rossini¹, Luigina Vanessa Alemanni¹, Elton Dajti¹, Federico Ravaioli¹, Matteo Renzulli³, Rita Golfieri³, Davide Festi¹

¹Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna 40138, Italy.

²Unit of Gastroenterology, Borgo Trento University Hospital of Verona, P.le Aristide Stefani 1, Verona 37126, Italy.

³Radiology Unit, Sant'Orsola Malpighi Hospital, Bologna 40138, Italy.

Correspondence to: Dr. Antonio Colecchia, Unit of Gastroenterology, Borgo Trento University Hospital of Verona, P.le Aristide Stefani 1, Verona 37126, Italy. E-mail: antonio.colecchia@aovr.veneto.it

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Abstract

Post-hepatectomy liver failure (PHLF) is associated with great morbidity and mortality after resection of hepatocellular carcinoma. Previous studies have underlined that advanced age could be a potential factor influencing post-operative complications and long-term survival.

In the past, candidates for resection were based on the Child-Pugh classification, the predictive value of which was rather low. The selection of patients undergoing resection in Western countries is based on the assessment of portal hypertension (PH), which is clinically assessed by measurement of the hepatic venous pressure gradient, an invasive and costly process. Thus, there have been several attempts to identify the best non-invasive test (NIT) to accurately predict PHLF. Most biochemical NITs for the prediction of PHLF are focused on evaluation of underlying liver cirrhosis and PH. Amongst them, FIB-4, which also includes the patient's age, seems to have more robust supporting results. In Europe and the USA., the most tested and reliable NIT for predicting PHLF is the evaluation of liver stiffness measurement, which is also influenced by age. Imaging parameters are promising tools which are used only in specialized centers however, and when available. Liver volume parameters, as well as contrast-enhanced data, demonstrate good accuracy in predicting PHLF. In this scenario, the evaluation of sarcopenia and bone mineral density through contextual imaging allows the delineation of PHLF in at-risk elderly patients. Further studies focused on parameters for the evaluation of PHLF in elderly patients are needed.

Keywords: Post-hepatectomy liver failure, liver resection, elderly, liver stiffness measurement, indocyanine green retention test



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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common cause of death in patients with cirrhosis^[1]. Despite numerous therapeutic options, the only curative treatments are liver transplantation and hepatectomy^[2]. Patients with a single HCC nodule, Child-Pugh class A, normal bilirubin (< 1 mg/dL) and without portal hypertension have the best prognosis and are ideal candidates for liver resection.

The presence of clinically significant portal hypertension (CSPH) (port-hepatic pressure gradient greater than 12 mmHg) or clinical manifestation (platelet count < 100,000/mL, associated with splenomegaly or esophageal varices) appears to be associated with a worse prognosis, but does not preclude resection in selected patients^[3,4]. Thus, patients with cirrhosis should be carefully selected to reduce the risk of postoperative liver failure and death. Post-hepatectomy liver failure (PHLF) is still a major concern for liver surgeons^[2]. In the last few years, there has been an increasing need for a simple and accurate tool for evaluating liver function before surgery to minimize PHLF and postoperative mortality^[5]. It is difficult to define PHLF exactly since it manifests with one or more of these features: ascites, jaundice, coagulopathy or kidney failure. The most commonly used criteria for defining PHLF are the 50-50 Criteria where PHLF is defined as total serum bilirubin > 50 mmol/L 5 days after surgery or thereafter and an international normalized ratio (INR) > 1.7^[6]. Other diagnostic criteria are the peak bilirubin > 7 mg/dL in any postoperative period, in the absence of cholestasis, and the International Study Group of Liver Surgery (ISGLS) criteria that define PHLF by an increased INR and concomitant hyperbilirubinemia on or after postoperative day 5^[7].

In the past, the selection of candidates for resection was based on the Child-Pugh classification but its predictive value has been determined to be insufficient. Japanese groups use the indocyanine green retention test (ICG), which has proved to be more reliable than Child-Pugh and the Model for End-stage Liver Disease (MELD) to predict PHLF^[8,9]. In Western countries, the selection of candidates for resection is usually based on the assessment of portal hypertension, which is clinically assessed by measurement of the hepatic venous pressure gradient (HVPG > 10 mmHg)^[10]. However, these methods are invasive and costly. Thus, several authors have tried to evaluate other non-invasive methods including biochemical scores, the measurement of liver and spleen stiffness (LSM and SSM) and imaging patterns as predictors of PHLF^[8,11-14].

With an increase in life expectancy and improvement in operative safety and efficacy of hepatic resection techniques, surgeons should also evaluate the best surgical option in elderly patients^[15]. Indeed, previous studies have implicated older age as a potential factor influencing post-resection complications and survival, and are summarized in a recent systematic review and meta-analysis^[15] which concluded that age > 70 was associated with increased 30-day and overall mortality when compared with non-elderly cohorts. A promising factor that also influences post-operative outcomes is patient frailty, defined as a syndrome characterized by decreased physiological reserves. Only one study has reported a specific association between frailty and PHLF^[16]. However, frailty assessment is based on a self-reporting questionnaire, which could be affected by several biases.

Thus, this review aims to summarize the recent advances on objective parameters such as non-invasive tests (NITs) for predicting PHLF, particularly in elderly patients.

BIOCHEMICAL SCORES

Fibrosis and liver functional reserve scores

FIB-4

The Fibrosis (FIB)-4 index was first proposed by Sterling and colleagues^[17] and is based on four factors included in the following equation: $[\text{age}(\text{years}) \times \text{AST}(\text{UI/L})] / [\text{platelet count} \times \text{ALT}(\text{UI/L})]$. It is a non-

invasive predictor of the progression of fibrosis in patients with chronic viral hepatitis. Over the years, several studies have validated its role as a marker of hepatic fibrosis^[18,19].

An important study^[20] indicated that the Fib-4 index not only demonstrated the best predictive ability for cirrhosis, but it was also an independent prognostic factor for postoperative hepatic insufficiency, overall survival, and disease-free survival for HCC patients with radical liver resection. They also stratified patients, with the cut-off value of 3.15, into Low and High fibrosis-4 groups. The High fibrosis-4 group had a higher mean age (56.3 ± 10.4 vs. 47.7 ± 11.7 , $P < 0.01$), higher MELD score ($P = 0.001$), and more patients with Child-Pugh grade B (7.4% vs. 4.0%, $P = 0.037$).

These results have also been confirmed by other studies^[21]. Other groups have tried to use the ratio of the remaining liver volume (FLRVR) and FIB-4 to predict PHLF and showed that FLRVR/FIB-4 was an independent predictive factor of outcomes after liver resection in cirrhotic patients^[22]. The most recent study by Zhou *et al.*^[23] demonstrated that the FIB-4 index was a more accurate predictive factor for PHLF and survival than the Child-Pugh score; the authors thus proposed the use of the FIB-4 index to perform pre-hepatectomy assessment citing a low incidence of PHLF in patients with $FIB-4 \leq 4.16$. On the other hand, Zhang *et al.*^[24] showed that FIB-4 was an independent predictor of PHLF only in minor hepatectomy patients. Multivariate analysis in this subgroup of patients revealed that age (the older the patient, the more the risk), Child-Pugh score and Albumin-Bilirubin score/spleen thickness ratio (ALBI/ST) were predictors of PHLF in the APRI model, while Child-Pugh score, FIB-4, and ALBI/ST were found to be significant risk factors of PHLF in the FIB-4 model. Thus, it is possible to conclude that FIB-4 is able to predict PHLF since it is related not only to the degree of liver fibrosis but also, to the general performance of the patient since it includes age.

Lok Index and Forns Index

The Lok Index and Forns Index are two other non-invasive markers of fibrosis. The Lok index is a non-invasive tool introduced by an American research group^[25] as a predictor of cirrhosis development in patients with chronic HCV-hepatitis. It is based on simple laboratory parameters and calculated through the following formula: $\log \text{odds (predicting cirrhosis)} = 5.56 - 0.0089 \times \text{platelet count } (\times 10^3/\text{mm}^3) + 1.26 \times \text{AST/ALT ratio} + 5.27 \times \text{INR}$. Some studies have correlated the value of the Lok index with the grade of fibrosis. For example, Ma *et al.*^[26] showed that FIB-4 and Lok's model were the most effective models for distinguishing significant and extensive fibrosis. Zhou *et al.*^[27] found that a Lok index cut-off value of 0.4531 could further spare 24.2% of gastroscopies without missing high-risk varices (HRVs)^[27]. Since this is a fibrosis marker, Mobarak *et al.*^[28] found that it was also able to predict HCC development. To date, the Lok-index is predominantly used for the prediction of fibrosis and cirrhosis but not PHLF.

The Forns Index was developed by Forns *et al.*^[29] in 2002, before the introduction of transient elastography techniques. It was first proposed as a non-invasive tool for the detection of patients with non-significant liver fibrosis. It is calculated using four variables (age, gamma glutamyl transferase, total cholesterol and platelet count) with the following formula: $7.811 - 3.131 \times \ln [\text{platelet count } (10^9/\text{L})] + 0.781 \times \ln [\text{gamma GT (IU/L)}] + 3.467 \times \ln [\text{age (years)}] - 0.014 \times [\text{cholesterol (mg/dL)}]$. The first studies on the Forns Index (FI) highlighted its accuracy in identifying patients with different stages of fibrosis and cirrhosis^[29,30]. A recent study showed that the Forns index is also useful in evaluating liver functionality and the degree of liver fibrosis, so it is able to predict HCC recurrence and patient survival^[31]. However, to date, little is known on the use of the Forns index to predict PHLF.

ALBI score

Another biochemical index used in clinical practice is the Albumin-Bilirubin (ALBI) score, which was introduced by Johnson *et al.*^[32] to evaluate liver function in patients with hepatocellular carcinoma. It was

Table 1. Studies evaluating ALBI score in predicting PHLF

Authors	Country	Population	Etiology	Outcome	Nr. cases	ALBI cut-off	AUROC
Toyoda <i>et al.</i> ^[38] , 2016	Asia/Europe	1,148	Mixed	Death	N/A	-2.60	N/A
Wang <i>et al.</i> ^[39] , 2016	China	1,242	85% HBV	PHLF	166	-2.77	0.723
Ke <i>et al.</i> ^[40] , 2016	China	372	80% HBV	Postoperative complications	166	N/A	0.721
Andreatos <i>et al.</i> ^[41] , 2017	USA	2,659	N/A	PHLF	149	-2.60/-1.39	N/A
Li <i>et al.</i> ^[42] , 2017	China	491	83% HBV	Postoperative complications	270	-2.45	0.647
Russolillo <i>et al.</i> ^[43] , 2019	Italy	400	40% HCV	Overall morbidity, PHLF	176 82	-2.60/-1.39	N/A
Božin <i>et al.</i> ^[44] , 2018	Croatia	38	84% ALD	Death	24	N/A	N/A
Zhang <i>et al.</i> ^[5] , 2018	China	338	82% HBV	PHLF	26	-2.44	0.782
Zou <i>et al.</i> ^[37] , 2018	China	473	85% HBV	PHLF	50	-2.303	0.745
Mai <i>et al.</i> ^[45] , 2019	China	1,055	HBV	PHLF	151	-2.77	0.717

ALBI: Albumin-bilirubin; ALD: alcoholic liver disease; HBV: hepatitis B virus; HCV: hepatitis C virus; N/A: not available; PHLF: post-hepatectomy liver failure

initially proposed as an alternative to the Child-Pugh score, which has some limitations, such as the inclusion of non-objective parameters (ascites, encephalopathy). However, the ALBI score was also able to predict the severity and long-term prognosis of patients with chronic liver disease^[33]. In particular, it was found to be a reliable prognostic tool in assessing short-term outcomes in hepatic decompensation^[34], in predicting in-hospital mortality in patients with acute upper gastrointestinal bleeding^[35] and as a prognostic factor in HCC patients^[36]. The ALBI score is based on serum levels of albumin and total bilirubin and can be calculated through the following formula: $(\log_{10} \text{bilirubin } [\mu\text{mol/L}] \times 0.66) + (\text{albumin } [\text{g/L}] \times -0.0852)$. It was further categorized into three different grades: grade 3 (> -1.39), grade 2 (> -2.60 to ≤ -1.39), and grade 1 (≤ -2.60)^[32].

The ALBI grade was also a significant prognostic factor for PHLF in HCC patients^[5]. In a comparative study^[37], the ALBI score showed superior predictive value of PHLF over the Child-Pugh score, MELD score and ICG R15. The area under the ROC curve (AUC) of the ALBI score (AUC 0.745) for predicting PHLF was significantly higher than that of the Child-Pugh score (AUC 0.665), MELD score (AUC 0.649) and ICG R15 (AUC 0.668). With a cut-off value of the ALBI score of -2.303, it was possible to reach a sensitivity of 77.3% and a specificity of 64.0% for predicting PHLF^[37]. Another group^[24] associated the ALBI score with spleen thickness (ST) as a surrogate of portal hypertension. In this study, they compared the predictive ability of ALBI/ST with FIB-4 and APRI and found that ALBI/ST had a higher diagnostic accuracy for PHLF than FIB-4 and APRI. The AUC for the ALBI/ST ratio (AUC = 0.774, $P < 0.001$) was larger than that of FIB-4 (AUC = 0.696, $P < 0.001$), APRI (AUC = 0.697, $P < 0.001$), ALBI (AUC = 0.701, $P < 0.001$), and ST (AUC = 0.710, $P < 0.001$)^[24]. Also, in this study, multivariate analysis in the minor hepatectomy subgroup revealed that age, Child-Pugh score and ALBI/ST and Child-Pugh score, FIB-4, and ALBI/ST were significant predictors of PHLF in the APRI and FIB-4 models respectively. However, a study conducted by Zhang *et al.*^[5] showed that the ALBI grade was a good predictor of overall survival in BCLC stage 0/A patients but not in other BCLC stages. Thus, it is possible to conclude that the novel ALBI score is certainly one of the most validated scores for predicting PHLF, as described in Table 1^[5,33,45,37-44].

Indocyanine Green Retention Test

The clearance of indocyanine green (ICG) is a test used to assess liver excretory function quantitatively. ICG is a water-soluble fluorescent dye, which totally binds to albumin and β -lipoprotein in the blood and is exclusively taken up by hepatocytes and excreted unmodified in bile, without entero-hepatic circulation^[46,47]. Thus, its clearance depends on several factors: hepatic blood flow, the function of the hepatocytes and biliary excretion^[46,47]. However, this test takes time and is uncomfortable for patients. Thus, a faster sampling method was subsequently introduced, the ICG 15-min retention test (ICG-r15), consisting of an injection of an ICG bolus and peripheral venous blood sampling every 5 min for 20 min^[48,49]. In the last few decades, a noninvasive ICG measurement by spectrophotometry, called LiMON® (Pulsion Medical System, Munich,

Table 2. Studies evaluating ICG in predicting PHLF

Authors	Country	Population	Etiology	Outcome	Nr. cases	Technique	ICG cut-off	AUROC
Kitano <i>et al.</i> ^[63] , 1997	Japan	54	N/A	Hospital mortality	7	ICG-r15	14%	N/A
Lau <i>et al.</i> ^[58] , 1997	Hong Kong	127	N/A	Death	14	ICG-r15	14% (major hep.) 23% (minor hep.)	N/A
Lam <i>et al.</i> ^[57] , 1999	Hong Kong	117	N/A	Postoperative complications	N/A	ICG-r15	14%	N/A
Hsia <i>et al.</i> ^[64] , 2000	Taiwan	168	Mixed	Morbidity Death	51 3	ICG-r15	<10% / >20%	N/A
Lao <i>et al.</i> ^[65] , 2005	China	255	N/A	Decompensation	N/A	ICG-r15	10-20%	N/A
Zou <i>et al.</i> ^[37] , 2018	China	473	85% HBV	PHLF	50	ICG-r15	N/A	0.668
Hwang <i>et al.</i> ^[66] , 2015	South Korea	723	81% HBV	Death from PHLF	6	FRL-kICG	<0.05	N/A
Wang <i>et al.</i> ^[8] , 2018	China	185	83% HBV	Severe PHLF	23	ICG-r15	7.1%	0.724
Kim DK <i>et al.</i> ^[67] , 2018	South Korea	73	Mixed	PHLF	18	ICG-PDR	N/A	0.748
Wang <i>et al.</i> ^[68] , 2019	China	35	Mixed	PHLF Day 1-3-5	16	Intra-operative ICG-r15	13.8% (day 1) 13.8% (day 3) 22.7% (day 5)	0.540 0.800 0.910

ICG: Clearance of Indocyanine green; ICG-r15: ICG 15 min retention test; FRL-kICG: ICG constant fraction of future remnant liver; ICG-PDR: ICG-plasma disappearance rate; hep: hepatectomy; HBV: hepatitis B virus; N/A: not available; PHLF: post-hepatectomy liver failure

Germany), was developed. The device uses a finger optical probe, which detects, after ICG infusion, the fractional pulsatile changes in optical absorption. The device has already been validated in several studies^[50,51] with good correlation with ICG-r15 results, comparable with correction of a mathematical formula^[50].

Since ICG clearance depends on blood flow, it was associated with portal hypertension^[48] and liver function for its pharmacokinetics (uptake and excretion through the hepatocytes) as well^[47,52-54]. Thus, in Eastern countries it is considered an accurate method to assess liver functional reserve pre-operatively and has been used for almost 30 years^[47]; on the other hand, in Western countries, it is not widely used because it is highly influenced by hepatic blood flow^[47] and thus, by other conditions that could affect it.

The normal ICG-r15 value is about 10%^[55]. The ICG-r15 reported cut-off for performing a safe major hepatectomy is between 14% and 17%, the latter in younger patients with milder liver disease^[56,57]. Other authors have reported different cut-offs of 14% and 23% for safe major and minor hepatectomy respectively^[55,58]. In another previous study^[57] with age and sex-matched patients, the authors found no difference in terms of PHLF and mortality between patients with ICG-r15 of more than, and less than 14% who have undergone major hepatectomy. However, to date, the reported upper limit of ICG-r15 for considering liver resection is 40%^[59]. The accuracy of ICG-r15 in predicting PHLF could be increased with the combination of bilirubin levels and ascites^[59]. Several authors comparing the performance of ICG-r15 with other parameters found that it was superior to MELD^[60] and that the combination with platelet count, portal hypertension (ICG-r15 cut-off value of 7.1%, sensitivity 52.2% and specificity 89.5%)^[8] and Child-Pugh stage^[61] was able to improve its accuracy. Moreover, liver stiffness measurement (LSM) was also found to correlate with ICG-r15 and to provide additional information on the prognosis of the patient^[62]. Other authors have found good correlation when comparing ICG-r15 with the degree of portal hypertension^[48,54].

In conclusion, no definitive lower ICG-r15 cut-offs for distinguishing between safe minor or major hepatectomy are currently available, as shown in Table 2^[8,37,57,58,63-67]; major hepatectomy in the presence of unsatisfactory ICG-r15 results should be performed only in high-volume centers. ICG-r15 could be considered a good marker of liver function and indirectly, of the degree of portal hypertension. Further studies are needed however, for this latter association.

Portal hypertension scores

Plated to spleen stiffness ratio PSR

Another widely used biochemical score is the PSR (platelet count-to-spleen ratio), which consists of the ratio between PLT (expressed in number/mm³) and spleen diameter (mm)^[69]. The PSR value is strictly

correlated with the degree of portal hypertension and, since it was first proposed, this score has shown good performance in predicting the development of esophageal varices in cirrhotic patients^[69-71]. A recent Chinese study^[72] evaluated the accuracy of PSR, calculated using platelet count and spleen volume (expressed in mm³), as a diagnostic index for the stage of liver fibrosis in patients with HCC and compared PSR with other currently-used scores. Among patients with severe fibrosis, AUROC was significantly higher for PSR (0.808) than for other NITs, except for APRI (0.739, $P=0.215$). Peng *et al.*^[12], instead, conducted a study on the risk factors for PHLF in which they used, among various markers, a variant of PSR based on spleen stiffness measurement (SSM), instead of the spleen diameter or volume. On multivariate analysis, PSR seemed to be an independent prognostic index for the development of hepatic decompensation ($P < 0.001$, odds ratio [OR] = 0.622, 95%CI: 0.493~0.784). The PSR thus represents a promising prognostic index for the post-resection outcome.

APRI score

The APRI score (aspartate aminotransferase [AST] to platelet ratio index) was introduced in a study by Wai *et al.*^[73] and can be calculated using the following formula: $\text{AST (UI/L)} \times [100/(\text{platelet count } 10^3/\text{mm}^3)]$. It was developed as a non-invasive predictor for progression of fibrosis in patients with chronic viral hepatitis. In 2019, a study by Mai *et al.*^[74] found that the APRI score (AUC 0.743, 95%CI: 0.706-0.780; $P < 0.001$) had greater accuracy for predicting PHLF than the Child-Pugh, MELD and ALBI scores in the entire cohort of patients with HCC. The APRI-score cut-off value of 0.55 was able to reach a sensitivity of 72.2% and a specificity of 68.0% on PHLF prediction. However, Zhang *et al.*^[24] observed that the APRI score showed a predictive significance only in the major hepatectomy subgroup. The Chinese group of Mai *et al.*^[45] used a new combination of ALBI and APRI scores with the following formula: $\text{ALBI-APRI score} = 5.280 \times \text{ALBI} + 1.583 \times \text{APRI}$. The AUC of the ALBI-APRI model (AUC 0.766, 95%CI: 0.739-0.791) for predicting the risk of PHLF was significantly higher than the single ALBI ($P < 0.001$) or APRI scores ($P = 0.047$). The ALBI-APRI score cut-off value of -13.10 had a sensitivity of 78.1% and a specificity of 62.2% for predicting the risk of PHLF^[45]. Thus, the APRI score in combination with other NITs could represent a good surrogate of portal hypertension and should be further investigated for predicting PHLF.

LSPS

The LSPS (LSM-spleen to platelet ratio score) is a biochemical index derived from the following formula: $\text{LSM (kPa)} \times [\text{spleen diameter (mm)}/\text{platelet count } (10^3/\text{mm}^3)]$. This score was first proposed as a predictive tool for high-risk esophageal varices in patients with HBV-related cirrhosis^[75]. In a study by Chon and colleagues^[76], LSPS was found to be an independent risk factor for both HCC (HR = 1.001) and hepatic decompensation (HR = 1.002) in patients with HBV-related hepatitis. Only a single report on 38 patients highlighted a potential predictive role of LSPS on univariate analysis^[77]. However, not much is known about the role of LSPS in predicting PHLF.

Other liver function tests

Over time, other tests for estimating liver function have been developed. These tests use different substrates such as lidocaine, galactose, aminopyrine, amino acid, and methacetin. However, none have been shown to be superior to the ICG clearance test in the prediction of PHLF^[55]. Other tests are based on the liver's energy production (arterial ketone body ratio; AKBR) and the number of receptors for asialo-glycoprotein (ASGP-R; technetium-99m-galactosyl human serum albumin; 99mTc-GSA scan) but they are expensive and less common than ICG^[55]. Of course, in the pre-operative assessment, other well-validated tools such as the Child-Pugh and MELD scores continue to be considered. Both have been used widely to predict the outcomes of cirrhotic patients in many different contexts; they showed similar prognostic significance in most cases, even with slight differences in accuracy due to specific settings, as described in a recent, comprehensive meta-analysis^[78]. However, as described above, nowadays several NITs (such as FIB-4^[23], APRI score^[74], ALBI score^[37,39,41,42] or ICG-r15^[8]) appear to be better predictors of PHLF, and warrant further study.

Table 3. Studies evaluating LSM by elastography in predicting PHLF

Authors	Country	Population	Etiology	Outcome	Nr. cases	Technique	LSM cut-off	AUROC
Kim <i>et al.</i> ^[83] 2008	South Korea	72	83% HCV	PHLF	7	TE	25.6 kPa	0.824
Cescon <i>et al.</i> ^[84] 2012	Italy	90	66% HCV	PHLF	26	TE	15.7 kPa	0.865
Harada <i>et al.</i> ^[85] 2012	Japan	50	68% HCV	Ascites	19	ARFI	1.68 m/s	0.900
Wong <i>et al.</i> ^[86] 2013	China	105	67% HBV	Major complications	15	TE	12 kPa	0.790
Zhang <i>et al.</i> ^[87] 2015	China	75	HBV	Ascites	13	TE	15.6 kPa	0.902
				PHLF	4		14.3 kPa	0.915
Nishio <i>et al.</i> ^[88] 2016	Japan	177	Mixed	PHLF B or C	21	ARFI	1.61 m/s	0.780
Cucchetti <i>et al.</i> ^[11] 2017	Italy	202	64% HCV	PHLF	60	TE	N/A	N/A
Chong <i>et al.</i> ^[89] 2017	China	255	82% HBV	PHLF B or C	46	TE	11.5 kPa	0.650
							20 kPa	0.825
Han <i>et al.</i> ^[90] 2017	China	77	90% HBV	PHLF	27	2D-SWE	6.9 kPa	0.843
Abe <i>et al.</i> ^[91] 2017	Japan	175	Mixed	Major complications	28	MRE	5.3 kPa	0.810
Shen <i>et al.</i> ^[92] 2017	China	280	HBV	PHLF	55	2D-SWE	11.8 kPa	0.720
Rajakannu <i>et al.</i> ^[93] 2017	France	106	Mixed	Decompensation	9	TE	22 kPa	0.810
Donadon <i>et al.</i> ^[94] 2017	Italy	340	Mixed	Complications	95	TE	9.7 kPa	0.728
Wu <i>et al.</i> ^[95] 2017	China	54	65% HBV	PHLF	7	TE	16.2 kPa	0.760
Lei <i>et al.</i> ^[96] 2017	China	247	HBV	PHLF	37	TE	14 kPa	0.860
Hu <i>et al.</i> ^[97] 2018	China	216	88% HBV	PHLF	64	SWE	N/A	0.850
Sato <i>et al.</i> ^[98] 2018	Japan	96	Mixed	Major complications	15	MRE	4.3 kPa	0.813
Procopet <i>et al.</i> ^[99] 2018	Romania	51	65% Viral	Decompensation	15	TE	13.6/21 kPa	0.780
				PHLF	20		N/A	NS

ARFI: Acoustic radiation force impulse; HBV: hepatitis B virus; HCV: hepatitis C virus; LSM: liver stiffness measurement; MRE: magnetic resonance elastography; N/A: not available; PHLF: post-hepatectomy liver failure; SWE: Shear wave elastography; TE: transient elastography

ULTRASOUND-BASED AND OTHER IMAGING PREDICTORS

Liver stiffness measurement

In the last few years, the liver stiffness measurement (LSM) has been proposed as a practical and widely validated surrogate of liver fibrosis and portal hypertension, able to accurately predict the risk of cirrhosis^[79], CSPH^[80] and its complications, such as the development of varices^[81] and hepatic decompensation^[82]. Given that these attributes are major determinants of the risk of PHLF development, LSM has been investigated as a predictor of decompensation and other complications after hepatic resection with several methods that are mainly ultrasound-based^[11,83-99] [Table 3].

The study by Cescon *et al.*^[84] was one of the first papers to demonstrate that LSM, evaluated by transient elastography (TE), was an independent predictor of PHLF, together with histological cirrhosis and lower sodium levels. Since then, numerous studies have confirmed that LSM by TE is an important prognostic pre-operative variable that is able to stratify the risk of decompensation, PHLF and overall complications after liver resection^[83,93,94,96,100]. Different cut-offs have been proposed for this purpose, ranging from 9.7 to 22 kPa^[93,94], which correspond to current cut-offs for advanced chronic liver disease and CSPH respectively^[80]. Positive findings have also been described for LSM evaluated by other elastosonography techniques. For instance, Hu *et al.*^[97] developed and validated a nomogram including LSM assessment by shear-wave elastography, which is able to accurately predict the risk of any grade of PHLF (c-statistic 0.825). More recently, Sato *et al.*^[98] reported in a series of 96 consecutive patients who underwent liver resection, that LSM by magnetic resonance elastography (MRE), with a best-cut-off of 4.3 kPa, was an independent predictor of major surgical complications. When compared to other indices of portal hypertension, the accuracy of LSM was found to be non-inferior to that of the gold standard, hepatic venous pressure gradient (HVPG)^[93], and superior to that of ICG-r15^[83]. Some preliminary studies have reported a promising role of the spleen stiffness measurement (SSM)^[12,77], which is known to be a more accurate surrogate of portal hypertension^[101] but its prognostic role and accuracy in this context have yet to be established.

All the above-mentioned evidence supports the fact that LSM provides valuable prognostic information in patients undergoing liver resection^[102]. Indeed, in the last European guidelines on HCC, LSM was included among the pre-operative tools to assess liver reserve before surgery. However, most of the prognostic models including LSM have not been validated externally, the proposed cut-offs differ among studies and differ too, for the elastosonography technique applied. Therefore, LSM is still not routinely used in pre-operative risk stratification of patients undergoing surgery.

Noteworthy, in none of these studies was age an independent predictor of PHLF. However, a recent paper showed that the risk of PHLF development after right hepatectomy rapidly increased in patients over 75 (incidence 35% > 75 years *vs.* 7% < 75 years, OR = 8.8, 95%CI: 3.6-21)^[103]. Considering that older age is a known risk factor for unreliable LSM measurement^[104,105] and that this category of patients might have been underrepresented in the previously published cohorts, future studies are needed to investigate the prognostic role of LSM in elderly patients undergoing liver resection.

Computed tomography

Several computed tomography (CT) signatures have been reported in association with PHLF^[106,107]. With regard to liver volumetry, this is performed using CT imaging, preferably utilizing the images obtained during the venous phase. Liver volumetry is obtained by contouring the liver boundaries and segments, with semi-automated methods or manually, on dedicated software. PHLF occurrence is closely related to the volume and functional capacity of the remnant liver. Patients with a small future liver remnant (FLR) are at higher risk of developing PHLF. Shoup *et al.*^[108] demonstrated that the remnant liver volume (RLV) correlates with post-operative prothrombin time and bilirubin levels. In their analysis, a RLV < 25% was more predictive of PHLF than the anatomical extent of resection^[108]. There is no consensus about “how much is enough” but, in general, a FLR of about 20%-30% has been reported as representing the limit of safety in hepatectomy, in non-cirrhotic livers, by some authors^[109-112].

Remnant liver function, estimated with CT volumetry, is reliable only when liver function is assumed homogeneous in the entire organ^[113]. In cirrhotic patients, the small liver volume suggests the severity of cirrhosis and poor function of the liver. Indeed, cirrhotic livers have lower levels of hepatocyte growth factor and slower and less complete regeneration, compared with non-cirrhotic livers^[114]. Therefore, in these patients, the hepatectomy-associated risk cannot be accurately determined with volumetry alone. In different published series, the critical minimum FLR for a safe hepatectomy was estimated to be approximately 40% in patients with cirrhosis^[115,116].

Spleen Volume (Sp) can also be a critical factor for the outcome of patients undergoing major liver resection. An increased Sp/RLV ratio (> 0.199) correlates with PHLF^[114].

Another imaging pre-operative evaluation that should be assessed is the quantification of hepatic steatosis, which is shown on pre-contrast CT images as lower attenuation of the liver than that of the spleen^[117]. Steatosis contributes to post-operative liver dysfunction, especially in diabetic patients and in patients with chemotherapy-associated steatohepatitis undergoing major hepatic resection^[117]. The effect of steatosis is explained by the higher incidence of ischemia-reperfusion injury due to altered sinusoidal microcirculation. A recent study found a significantly higher incidence of hepatic decompensation, 90-day post-operative morbidity and surgical hepatic complications in patients with steatohepatitis than in patients without^[118].

Among other conditions that could be associated with older age and contribute to the development of PHLF, it is widely known that primary sarcopenia is strongly associated with age. Therefore, elderly patients have less skeletal muscle mass than younger patients^[119,120] and this loss of muscle mass is accelerated due to chronic medical illnesses and malnutrition^[121]. At the same time, nutritional status is a major concern

in liver disease. Cirrhotic patients often develop protein-energy malnutrition (PEM), as a result of poor dietary intake, malabsorption, increased intestinal protein loss, decreased hepatic protein synthesis, abnormal substrate utilization and hypermetabolism^[122]. Malnutrition in liver disease is also associated with worse outcomes, increased complications and mortality^[123,124], and leads to a high prevalence of secondary sarcopenia^[125].

Muscular mass can be evaluated by CT, using different methods such as calculating the area (cm²) and density of the psoas muscle at the level of the third lumbar vertebrae, or calculating the ratio between the muscular surface area (external and internal oblique, transverse, psoas and paravertebral muscles) and the square of height^[126].

Recent studies have investigated the effect of sarcopenia on the morbidity of patients undergoing liver surgery, both in cases of colon cancer metastases and of HCC. They have shown that sarcopenia is an independent risk factor for increased post-operative morbidity^[120,121,127-129]. Indeed, sarcopenia is associated with a lower functional liver reserve; therefore, the average RLV of sarcopenic patients is statistically and significantly less than that of non-sarcopenic patients^[130]. Obese patients can also be sarcopenic if they have increased fatty mass (BMI ≥ 30) but a loss of muscular mass^[131]. Peng *et al.*^[121] showed that sarcopenic obesity multiplied the risk of major complications five-fold after hepatectomy in patients with liver metastases.

In addition, on CT, is possible to calculate the intra-muscular adipose tissue content (IMAC) at the level of L3 (i.e., IMAC = CT attenuation value of the multifidus muscles [HU]/CT attenuation value of the subcutaneous fat [HU]). A recent paper demonstrated that muscle steatosis is associated with significantly lower overall survival and recurrence-free survival, and it is an independent risk factor for increased major post-operative complications in patients undergoing hepatectomy for HCC. Moreover, patients with high IMAC are older and have a higher mass index^[132].

Another comorbidity parameter that can be evaluated with pre-operative CT scan is the bone mineral density (BMD), which is classically defined as a “T-score”, evaluated by dual X-ray absorptiometry (DXA) of the spine or hip. Of note, BMD has a significant negative correlation with age, especially in female patients^[67]. Sharma *et al.*^[133] reported BMD by measuring the CT attenuation of the trabecular bone of the eleventh thoracic vertebral body and found that BMD < 160HU was an independent predictor of post-liver transplant mortality in HCC patients. Miyachi *et al.*^[134] demonstrated however, that low BMD (< 160 HU) has a strong correlation with a poor outcome post-hepatectomy only for male patients. Thus, it is possible to utilize peri-operative imaging parameters to assess the future liver remnant and the remnant liver volume, which are strictly correlated with the risk of PHLF; other imaging parameters associated with both the elderly and the health status of the patient, such as the presence of sarcopenia and low bone mineral density, are also associated with PHLF.

Magnetic resonance imaging

Both CT and magnetic resonance imaging (MRI) show excellent accuracy and quantification of hepatic volume^[106]. Volumetry assessment by MRI is preferable to be performed on the hepato-biliary phase (HBP, about 30 min after hepatospecific contrast agent injection). Diffusion Weighted Imaging (DWI) measures the apparent diffusion coefficient (ADC) of water, a parameter that is dependent on tissue structure^[135]. Several reports suggest a lower ADC value in cirrhosis than in normal livers^[136,137], probably due to the restricted water diffusion in fibrotic tissue. Chuang *et al.*^[114] reported that pre-operative liver ADC values $\leq 1.34 \times 10^{-3}$ significantly predicted PHLF in patients undergoing hepatectomy.

The administration of hepato-specific contrast agents can help the radiologist and the clinician to evaluate the liver's reserve function and thus, predicts the occurrence of PHLF. Gadolinium, Gd-EOB-DTPA is a hepato-

specific contrast agent that shows up to 50% hepatocyte uptake and is then excreted into the bile ducts. In non-cirrhotic livers, it has peak enhancement on T1-W images at about 20-30 min after injection^[138,139]. Uptake and metabolism of this contrast agent is related to hepatocyte function^[140,141]. Therefore, hepatic parenchymal enhancement is affected by the severity of cirrhosis^[138]. The mean signal intensity (SI) of liver parenchyma on HBP reflects a quantitative measure of hepatocyte contrast agent uptake^[114]. Watanabe *et al.*^[142] found that liver SI on Gd-EOB-DTPA MRI is strongly correlated with fibrosis stage and concluded that it is more reliable for staging hepatic fibrosis than DWI or hematologic and clinical parameters. Moreover, many recent studies^[143,144] have reported the usefulness of relative liver enhancement [RLE = (SI HBP - SI PRE)/SI PRE] in predicting PHLF in patients with hepatic metastases or with HCC because of the superiority of pre-operative RLE over both the 50-50 criteria and ISGLS grading system^[67,143]. Pre-operative RLE measurement is considered reliable and reproducible with high inter-observer variability^[145]. However, further studies are necessary to understand the real role of RLE to predict PHLF.

Other parameters derived from Gd-EOB-DTPA MRI have been evaluated as predictors of PHLF with modest success. Contrast enhancement ratio (CER= [(SIHBP - SIPRE)/(SITP - SIPRE] where SITP is measured on transitional phases, about 3 min post-contrast injection) is less affected by the hemodynamics of a patient than RLE, and better reflects Gd-EOB-DTPA uptake by hepatocytes. CER can also be multiplied by TLV/SLV ratio (total CER, tCER) and by RLV/SLV ratio (remnant CER, rCER)^[138]. A recent study demonstrated that rCER correlates with the development of PHLF better than volumetry (cut off ≤ 1.23) and that tCER is an independent predictive factor for PHLF (cut off ≤ 1.42)^[114]. The prognostic value of CER, in predicting PHLF, seems to be stronger than the ADC value and TVL/SLV ratio in cirrhotic patients^[114]. Therefore, patients with a relatively small tCER should preferably go under local treatment rather than resection^[114]. Asenbaum *et al.*^[146] combined functional and morphological parameters (functional FLR, functFLR) by measuring remnant RLE on Gd-EOB-DTPA MRI and the RLV by the formula: (RLV*remnantRLE)/body weight (BW). A decreased functFLR (< 8.73 mL/kg) demonstrated a strong correlation with the development of PHLF in patients that underwent major liver resection^[146].

Kim *et al.*^[67] verified the correlation between the remnant hepatocellular uptake index (rHUI = $RVL \times [(L20/S20)-1]$) and PHLF, where L20 is the mean SI of the FLR, and S20 is the mean SI of the spleen on HBP images. A lower rHUI (< 0.89) and a lower body weighted and corrected rHUI (rHUI-BW < 12.38) showed a statistically significant correlation with the development of PHLF in patients undergoing major liver resection, and predicted PHLF better than ICG related parameters. In this study, the severity of PHLF also showed a statistically significant association with rHUI-BW^[67]. Nevertheless, despite numerous promising findings, MRI still represents an expensive, not immediate and not widely available technique, and careful evaluation about its use needs to be performed according to each hospital setting. Thus, pre-operative MRI parameters could be useful in predicting PHLF when available, otherwise, cheaper and faster techniques should be used.

Single photon emission computed tomography

Single photon emission computed tomography (SPECT) using 99 metastable technetium diethylenetriamine-pentaacetic acid-galactosyl human serum albumin (99mTc-GSA) is of increasing interest for the pre-operative evaluation of cirrhotic patients. The molecule 99mTc-GSA is taken up rapidly by the liver, reflecting accurately the volume of functional liver and FLR; indeed, it is correlated to bilirubin levels, INR, and ICG clearance^[147]. Liver 99mTc-GSA SPECT has been reported to be more useful than CT in predicting remnant liver function before hepatic resection^[148]. This technique is thought to be a substitute for ICG rate. It can be used for patients whose liver function cannot be fully estimated using multimodal algorithms, such as patients with jaundice, portal hypertension, or ICG intolerance^[149]. However, as for MRI, there has not been real-life application of this technique for predicting PHLF to date.

Interventional radiology

Portal vein embolization (PVE) is an interventional radiological procedure. It consists of embolization of portal branches in the future resected liver, thus shifting blood flow to the FLR, allowing its hypertrophy before major hepatectomy. By increasing the volume of FLR, the risk for PHLF is decreased, even after extended liver resection^[117]. Furthermore, preoperative PVE reduces intra-operative hepatocyte injury caused by the sudden increase in portal pressure at resection. Current guidelines recommend PVE for cirrhotic patients and an estimated FLR of $\leq 40\%$, or normal patients with an intended FLR of $< 20\%$ ^[150].

CT volumetry should be performed 3-4 weeks after PVE to assess the degree of hypertrophy, which if $> 5\%$, is associated with improved patient outcomes^[115]. A study by Capussotti *et al.*^[151] reported a FLR hypertrophy of 30%-40% in 4-6 weeks in more than 80% of patients, and was therefore able to prepare patients for hepatectomy after that period. Hepatic arterial buffer response, after reduction of portal blood flow post-PVE, can increase the size of the tumor. However, PVE preceded by trans-arterial chemoembolization (TACE) may prevent this by causing tumour necrosis^[152]. RLE on Gd-EOB-DTPA-MRI has also been evaluated both pre- and post-PVE. In particular, the corresponding increase in RLE of the remnant liver at 14 and 28 days after PVE is significantly lower in patients who develop PHLF than in those who do not. Similar results were found comparing patients without or with mild PHLF versus those with severe PHLF^[67].

CONCLUSION

PHLF is still an event associated with major concerns by surgeons, especially in elderly patients. Several attempts have been made to identify the best non-invasive predictor of PHLF, in order to introduce a pre-operative tool for the assessment of such risk in routine clinical practice. Particularly, and when available, imaging parameters allow the identification of peri-operative risk factors related to the underlying cirrhosis, the volume of the liver remnant and patient related characteristics, mainly associated with the elderly such as sarcopenia and low bone mineral density. Otherwise, in other settings, LSM as well as ICG-r15 and the ALBI score are useful NITs able to mirror hepatic dysfunction and portal hypertension, and are thus being recommended before surgery for PHLF risk assessment. However, there is still poor evidence for their application in older patients. Further prospective and well-designed studies evaluating the ability of these NITs in predicting PHLF in the elderly are thus needed.

DECLARATIONS

Authors' contributions

Conceptualized and designed the review: Marasco G, Colecchia A

Wrote, reviewed and edited the manuscript: Marasco G, Milandri M, Rossini B, Alemanni LV, Dajti E, Ravaioli F

Provided the tables: Alemanni LV, Dajti E

Reviewed and approved the final manuscript as submitted: Colecchia A, Renzulli M, Golfieri R, Festi D

Read and approved the final manuscript: All authors

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REFERENCES

1. Therapy S, Resection L. Hepatocellular carcinoma (liver cancer) therapy. Johns Hopkins; 2012. pp. 2-9.
2. Dufour JF, Greten TF, Raymond E, Roskams T, De T, et al. Clinical practice guidelines EASL - EORTC clinical practice guidelines: management of hepatocellular carcinoma European organisation for research and treatment of cancer. *J Hepatol* 2012;56:908-43.
3. Cucchetti A, Ercolani G, Vivarelli M, Cescon M, Ravaioli M, et al. Is portal hypertension a contraindication to hepatic resection? *Ann Surg* 2009;250:922-8.
4. Anna Elisabetta Brunetti S, Francesco Fiore Gennaro Daniele Radiologia Interventistica -Istituto Nazionale Tumori E, Pascale F, Sperimentazioni cliniche -Istituto Nazionale Tumori -Napoli, Giuseppe Cabibbo -Napoli, Casadei Gardini A, et al. Coordinatore Bruno Daniele UOC Oncologia-Ospedale del Mare-Napoli. 2018. Available from: https://www.aiom.it/wp-content/uploads/2018/11/2018_LG_AIOM_Epatocarcinoma.pdf
5. Zhang ZQ, Xiong L, Zhou JJ, Miao XY, Li QL, et al. Ability of the ALBI grade to predict posthepatectomy liver failure and long-term survival after liver resection for different BCLC stages of HCC 11 Medical and Health Sciences 1112 Oncology and Carcinogenesis. *World J Surg Oncol* 2018;16.
6. Balzan S, Belghiti J, Farges O, Ogata S, Sauvanet A, et al. The “50-50 Criteria” on postoperative day 5 an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005;242:824-9.
7. Rahbari NN, Garden OJ, Padbury R, Brooke-Smith M, Crawford M, et al. Posthepatectomy liver failure: a definition and grading by the international study group of liver surgery (ISGLS). *Surgery* 2011;149:713-24.
8. Wang YY, Zhao XH, Ma L, Ye JZ, Wu FX, et al. Comparison of the ability of Child-Pugh score, MELD score, and ICG-R15 to assess preoperative hepatic functional reserve in patients with hepatocellular carcinoma. *J Surg Oncol* 2018;118:440-5.
9. Schneider PD. Preoperative assessment of liver function. *Surg Clin North Am* 2004;84:355-73.
10. Bosch J, Abraldes JG, Berzigotti A, García-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. *Nat Rev Gastroenterol Hepatol* 2009;6:573-82.
11. Cucchetti A, Cescon M, Colecchia A, Neri F, Cappelli A, et al. Adding liver stiffness measurement to the routine evaluation of hepatocellular carcinoma resectability can optimize clinical outcome. *Eur J Ultrasound* 2017;38:515-22.
12. Peng W, Li JW, Zhang XY, Li C, Wen TF, et al. A novel model for predicting posthepatectomy liver failure in patients with hepatocellular carcinoma. *PLoS One* 2019;14:e0219219.
13. Cucchetti A, Cescon M, Golfieri R, Piscaglia F, Renzulli M, et al. Hepatic venous pressure gradient in the preoperative assessment of patients with resectable hepatocellular carcinoma. *J Hepatol* 2016;64:79-86.
14. Zhang ZQ, Xiong L, Zhou JJ, Miao XY, Li QL, et al. Ability of the ALBI grade to predict posthepatectomy liver failure and long-term survival after liver resection for different BCLC stages of HCC. *World J Surg Oncol* 2018;16:208.
15. Phan K, An VVG, Ha H, Phan S, Lam V, et al. Hepatic resection for malignant liver tumours in the elderly: a systematic review and meta-analysis. *ANZ J Surg* 2015;85:815-22.
16. Tanaka S, Ueno M, Iida H, Kaibori M, Nomi T, et al. Preoperative assessment of frailty predicts age-related events after hepatic resection: a prospective multicenter study. *J Hepatobiliary Pancreat Sci* 2018;25:377-87.
17. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006;43:1317-25.
18. Mallet V, Dhalluin-Venier V, Roussin C, Bourliere M, Pettinelli ME, et al. The accuracy of the FIB-4 index for the diagnosis of mild fibrosis in chronic hepatitis B. *Aliment Pharmacol Ther* 2009;29:409-15.
19. Kim BK, Kim DY, Park JY, Ahn SH, Chon CY, et al. Validation of FIB-4 and comparison with other simple noninvasive indices for predicting liver fibrosis and cirrhosis in hepatitis B virus-infected patients. *Liver Int* 2010;30:546-53.
20. Wang H, Liu A, Bo W, Feng X, Hu Y, et al. Fibrosis-4 model influences results of patients with hepatocellular carcinoma undergoing hepatectomy. *Biomed Res Int* 2018;2018.
21. Toyoda H, Kumada T, Tada T, Kaneoka Y, Maeda A. A laboratory marker, FIB-4 index, as a predictor for long-term outcomes of hepatocellular carcinoma patients after curative hepatic resection. *Surgery* 2015;157:699-707.
22. Dong J, Zhang XF, Zhu Y, Ma F, Liu C, et al. The value of the combination of fibrosis index based on the four factors and future liver remnant volume ratios as a predictor on posthepatectomy outcomes. *J Gastrointest Surg* 2015;19:682-91.
23. Zhou P, Chen B, Miao XY, Zhou JJ, Xiong L, et al. Comparison of FIB-4 index and child-pugh score in predicting the outcome of hepatic resection for hepatocellular carcinoma. *J Gastrointest Surg* 2019;10-3.
24. Zhang ZQ, Yang B, Zou H, Xiong L, Miao XY, et al. ALBI/ST ratio versus FIB-4 and APRI as a predictor of posthepatectomy liver failure in hepatocellular carcinoma patients. *Medicine (Baltimore)* 2019;98:e15168.
25. Lok ASF, Ghany MG, Goodman ZD, Wright EC, Everson GT, et al. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. *Hepatology* 2005;42:282-92.
26. Ma J, Jiang Y, Gong G. Evaluation of seven noninvasive models in staging liver fibrosis in patients with chronic hepatitis B virus

- infection. *Eur J Gastroenterol Hepatol* 2013;25:428-34.
27. Zhou H, Long J, Hu H, Tian CY, Lin SD. Liver stiffness and serum markers for excluding high-risk varices in patients who do not meet Baveno VI criteria. *World J Gastroenterol* 2019;25:5323-33.
 28. Mobarak L, Omran D, Nabeel MM, Zakaria Z. Fibro markers for prediction of hepatocellular carcinoma in Egyptian patients with chronic liver disease. *J Med Virol* 2017;89:1062-8.
 29. Forns X, Ampurdanès S, Llovet JM, Aponte J, Quintó L, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002;36:986-92.
 30. Bourliere M, Penaranda G, Renou C, Botta-Fridlund D, Tran A, et al. Validation and comparison of indexes for fibrosis and cirrhosis prediction in chronic hepatitis C patients: proposal for a pragmatic approach classification without liver biopsies. *J Viral Hepat* 2006;13:659-70.
 31. Choi WM, Lee JH, Ahn H, Cho H, Cho YY, et al. Forns index predicts recurrence and death in patients with hepatitis B-related hepatocellular carcinoma after curative resection. *Liver Int* 2015;35:1992-2000.
 32. Johnson PJ, Berhane S, Kagebayashi C, Satomura S, Teng M, et al. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. *J Clin Oncol* 2015;33:550-8.
 33. Wang J, Zhang Z, Yan X, Li M, Xia J, et al. Albumin-Bilirubin (ALBI) as an accurate and simple prognostic score for chronic hepatitis B-related liver cirrhosis. *Dig Liver Dis* 2019;51:1172-8.
 34. Wan SZ, Nie Y, Zhang Y, Liu C, Zhu X. Assessing the prognostic performance of the Child-Pugh, model for end-stage liver disease, and albumin-bilirubin scores in patients with decompensated cirrhosis: a large asian cohort from gastroenterology department. *Dis Markers* 2020;2020:5193028.
 35. Zou D, Qi X, Zhu C, Ning Z, Hou F, et al. Albumin-bilirubin score for predicting the in-hospital mortality of acute upper gastrointestinal bleeding in liver cirrhosis: a retrospective study. *Turkish J Gastroenterol* 2016;27:180-6.
 36. Xu YX, Wang YB, Tan YL, Xi C, Xu XZ. Prognostic value of pretreatment albumin to bilirubin ratio in patients with hepatocellular cancer: a meta-analysis. *Medicine (Baltimore)* 2019;98:e14027.
 37. Zou H, Yang X, Li QL, Zhou QX, Xiong L, et al. A comparative study of albumin-bilirubin score with Child-Pugh score, model for end-stage liver disease score and indocyanine green R15 in predicting posthepatectomy liver failure for hepatocellular carcinoma patients. *Dig Dis* 2018;36:236-43.
 38. Toyoda H, Lai PBS, O'Beirne J, Chong CC, Berhane S, et al. Long-term impact of liver function on curative therapy for hepatocellular carcinoma: application of the ALBI grade. *Br J Cancer* 2016;114:744-50.
 39. Wang YY, Zhong JH, Su ZY, Huang JF, Lu SD, et al. Albumin-bilirubin versus Child-Pugh score as a predictor of outcome after liver resection for hepatocellular carcinoma. *Br J Surg* 2016;103:725-34.
 40. Ke M, Xu T, Li N, Ren Y, Shi A, et al. Prognostic nutritional index predicts short-term outcomes after liver resection for hepatocellular carcinoma within the Milan criteria. *Oncotarget* 2016;7:81611-20.
 41. Andreatos N, Amini N, Gani F, Margonis GA, Sasaki K, et al. Albumin-bilirubin score: predicting short-term outcomes including bile leak and post-hepatectomy liver failure following hepatic resection. *J Gastrointest Surg* 2017;21:238-48.
 42. Li MX, Zhao H, Bi XY, Li ZY, Huang Z, et al. Prognostic value of the albumin-bilirubin grade in patients with hepatocellular carcinoma: validation in a chinese cohort. *Hepatol Res* 2017;47:731-41.
 43. Russolillo N, Forchino F, Conci S, Mele C, Langella S, et al. Validation of the albumin-indocyanine green evaluation model in patients with resected hepatocellular carcinoma and comparison with the albumin-bilirubin score. *J Hepatobiliary Pancreat Sci* 2019;26:51-7.
 44. Božin T, Mustapić S, Bokun T, Patrlj L, Rakić M, et al. Albi score as a predictor of survival in patients with compensated cirrhosis resected for hepatocellular carcinoma: exploratory evaluation in relationship to palbi and meld liver function scores. *Acta Clin Croat* 2018;57:292-300.
 45. Mai RY, Wang YY, Bai T, Chen J, Xiang BD, et al. Combination of ALBI and APRI to predict posthepatectomy liver failure after liver resection for HBV-related HCC patients. *Cancer Manag Res* 2019;11:8799-806.
 46. De Gasperi A, Mazza E, Prosperi M. Indocyanine green kinetics to assess liver function: ready for a clinical dynamic assessment in major liver surgery? *World J Hepatol* 2016;8:355-67.
 47. Levesque E, Martin E, Dudau D, Lim C, Dhonneur G, et al. Current use and perspective of indocyanine green clearance in liver diseases. *Anaesth Crit Care Pain Med* 2016;35:49-57.
 48. Lisotti A, Azzaroli F, Buonfiglioli F, Montagnani M, Cecinato P, et al. Indocyanine green retention test as a noninvasive marker of portal hypertension and esophageal varices in compensated liver cirrhosis. *Hepatology* 2014;59:643-50.
 49. Kim HJ, Kim CY, Park EK, Hur YH, Koh YS, et al. Volumetric analysis and indocyanine green retention rate at 15 min as predictors of post-hepatectomy liver failure. *HPB* 2015;17:159-67.
 50. Cheung TT, Chan SC, Chok KSH, Chan ACY, Yu WC, et al. Rapid measurement of indocyanine green retention by pulse spectrophotometry: a validation study in 70 patients with Child-Pugh A cirrhosis before hepatectomy for hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2012;11:267-71.
 51. Purcell R, Kruger P, Jones M. Indocyanine green elimination: a comparison of the LiMON and serial blood sampling methods. *ANZ J Surg* 2006;76:75-7.
 52. Lisotti A, Azzaroli F, Cucchetti A, Buonfiglioli F, Cecinato P, et al. Relationship between indocyanine green retention test, decompensation and survival in patients with Child-Pugh A cirrhosis and portal hypertension. *Liver Int* 2016;36:1313-21.
 53. Zipprich A, Kuss O, Rogowski S, Kleber G, Lotterer E, et al. Incorporating indocyanin green clearance into the model for end stage liver disease (MELD-ICG) improves prognostic accuracy in intermediate to advanced cirrhosis. *Gut* 2010;59:963-8.

54. Marie-louise L. Pind FBTKSM. Indocyanine green retention test (icg-r15) as a noninvasive predictor of portal hypertension in patients with different severity of cirrhosis. *Eur J Gastroenterol* 2016;28:948-54.
55. Fan ST. Liver functional reserve estimation: state of the art and relevance for local treatments. *J Hepatobiliary Pancreat Sci* 2010;17:380-4.
56. Yau T, Tang VYF, Yao TJ, Fan ST, Lo CM, et al. Development of Hong Kong liver cancer staging system with treatment stratification for patients with hepatocellular carcinoma. *Gastroenterology* 2014;146:1691-700.e3.
57. Lam CM, Fan ST, Lo CM, Wong J. Major hepatectomy for hepatocellular carcinoma in patients with an unsatisfactory indocyanine green clearance test. *Br J Surg* 1999;86:1012-7.
58. Lau H, Man K, Fan ST, Yu WC, Lo CM, et al. Evaluation of preoperative hepatic function in patients with hepatocellular carcinoma undergoing hepatectomy. *Br J Surg* 1997;84:1255-9.
59. Imamura H, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Assessment of hepatic reserve for indication of hepatic resection: decision tree incorporating indocyanine green test. *J Hepatobiliary Pancreat Surg* 2005;12:16-22.
60. Kim JM, Kwon CHD, Joh JW, Park JB, Lee JH, et al. Can the model for end-stage liver disease score replace the indocyanine green clearance test in the selection of right hemihepatectomy in Child-Pugh class A? *Ann Surg Treat Res* 2014;86:122-9.
61. Au KP, Chan SC, Chok KSH, Chan ACY, Cheung TT, et al. Child-Pugh parameters and platelet count as an alternative to ICG test for assessing liver function for major hepatectomy. *HPB Surg* 2017;2017.
62. Fung J, Poon RTP, Yu WC, Chan SC, Chan ACY, et al. Use of liver stiffness measurement for liver resection surgery: correlation with indocyanine green clearance testing and post-operative outcome. *PLoS One* 2013;8.
63. Kitano S, Kim YI. ICG clearance in assessing cirrhotic patients with hepatocellular carcinoma for major hepatic resection. *HPB Surg* 1997;10:182-3.
64. Hsia CY, Lui WY, Chau GY, King KL, Loong CC, et al. Perioperative safety and prognosis in hepatocellular carcinoma patients with impaired liver function. No competing interests declared. *J Am Coll Surg* 2000;190:574-9.
65. Lao XM, Zhang YQ, Lin XJ, Guo RP, Chen MS, et al. Estimation of hepatic resection volume in hepatocellular carcinoma by ICG(R15) and its relation with postoperative liver failure. *Ai Zheng* 2005;24:337-40.
66. Hwang S, Ha TY, Song GW, Jung DH, Ahn CS, et al. Quantified risk assessment for major hepatectomy via the indocyanine green clearance rate and liver volumetry combined with standard liver volume. *J Gastrointest Surg* 2015;19:1305-14.
67. Kim DK, Choi JI, Choi MH, Park MY, Lee YJ, et al. Prediction of posthepatectomy liver failure: MRI with hepatocyte-specific contrast agent versus indocyanine green clearance test. *AJR Am J Roentgenol* 2018;211:580-7.
68. Wang L, Xie L, Zhang N, Zhu W, Zhou J, et al. Predictive value of intraoperative indocyanine green clearance measurement on postoperative liver function after anatomic major liver resection. *J Gastrointest Surg* 2019; doi: 10.1007/s11605-019-04262-5.
69. Giannini E, Botta F, Borro P, Risso D, Romagnoli P, et al. Platelet count/spleen diameter ratio: proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis. *Gut* 2003;52:1200-5.
70. Giannini EG, Zaman A, Kreil A, Floreani A, Dulbecco P, et al. Platelet count/spleen diameter ratio for the noninvasive diagnosis of esophageal varices: results of a multicenter, prospective, validation study. *Am J Gastroenterol* 2006;101:2511-9.
71. Ying L, Lin X, Xie ZL, Hu YP, Shi KQ. Performance of platelet count/spleen diameter ratio for diagnosis of esophageal varices in cirrhosis: a meta-analysis. *Dig Dis Sci* 2012;57:1672-81.
72. Ouyang GX, Zhang YM, Zhu SL, Wang P, Ren Y, et al. A novel, non-invasive model for diagnosing liver fibrosis stage in patients with hepatocellular carcinoma. *Sci Rep* 2018;8:13074.
73. Wai CT, Greenon JK, Fontana RJ, Kalbfleisch JD, Marrero JA, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*. 2003;38:518-26.
74. Mai RY, Ye JZ, Long ZR, Shi XM, Bai T, et al. Preoperative aspartate aminotransferase-to-platelet-ratio index as a predictor of posthepatectomy liver failure for resectable hepatocellular carcinoma. *Cancer Manag Res* 2019;11:1401-14.
75. Kim BK, Han KH, Park JY, Ahn SH, Kim JK, et al. A liver stiffness measurement-based, noninvasive prediction model for high-risk esophageal varices in B-viral liver cirrhosis. *Am J Gastroenterol* 2010;105:1382-90.
76. Chon YE, Jung ES, Park JY, Kim DY, Ahn SH, et al. The accuracy of noninvasive methods in predicting the development of hepatocellular carcinoma and hepatic decompensation in patients with chronic hepatitis B. *J Clin Gastroenterol* 2012;46:518-25.
77. Marasco G, Colecchia A, Dajti E, Ravaioli F, Cucchetti A, et al. Prediction of posthepatectomy liver failure: role of SSM and LSPS. *J Surg Oncol* 2019;119:400-1.
78. Peng Y, Qi X, Guo X. Child-pugh versus MELD score for the assessment of prognosis in liver cirrhosis a systematic review and meta-analysis of observational studies. *Medicine (Baltimore)* 2016;95:e2877.
79. Castera L. Non-invasive tests for liver fibrosis progression and regression. *J Hepatol* 2016;64:232-3.
80. de Franchis R, Baveno VI Faculty. Expanding consensus in portal hypertension: report of the baveno VI consensus workshop: stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015;63:743-52.
81. Colecchia A, Ravaioli F, Marasco G, Colli A, Dajti E, et al. A combined model based on spleen stiffness measurement and Baveno VI criteria to rule out high-risk varices in advanced chronic liver disease. *J Hepatol* 2018;69:308-17.
82. Singh S, Fujii LL, Murad MH, Wang Z, Asrani SK, et al. Liver stiffness is associated with risk of decompensation, liver cancer, and death in patients with chronic liver diseases: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013;11:1573-84.e1-2; quiz e88-9.
83. Kim SU, Ahn SH, Park JY, Kim DY, Chon CY, et al. Prediction of postoperative hepatic insufficiency by liver stiffness measurement (FibroScan®) before curative resection of hepatocellular carcinoma: a pilot study. *Hepatol Int* 2008;2:471-7.

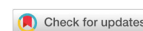
84. Cescon M, Colecchia A, Cucchetti A, Peri E, Montrone L, et al. Value of transient elastography measured with fibroscan in predicting the outcome of hepatic resection for hepatocellular carcinoma. *Ann Surg* 2012;256:706-13.
85. Harada N, Shirabe K, Ijichi H, Matono R, Uchiyama H, et al. Acoustic radiation force impulse imaging predicts postoperative ascites resulting from curative hepatic resection for hepatocellular carcinoma. *Surgery* 2012;151:837-43.
86. Wong JSW, Wong GLH, Chan AWH, Wong VWS, Cheung YS, et al. Liver stiffness measurement by transient elastography as a predictor on posthepatectomy outcomes. *Ann Surg* 2013;257:922-8.
87. Zhang ZM, Zhang YM, Yao F, Yi P, Huang S, et al. Analysis on postoperative efficacy of radical hepatectomy for patients with Non-HBV/HCV hepatocellular carcinoma. *Asian Pac J Cancer Prev* 2015;16:3479-83.
88. Nishio T, Taura K, Koyama Y, Tanabe K, Yamamoto G, et al. Prediction of posthepatectomy liver failure based on liver stiffness measurement in patients with hepatocellular carcinoma. *Surgery* 2016;159:399-408.
89. Chong CCN, Wong GLH, Chan AWH, Wong VWS, Fong AK, et al. Liver stiffness measurement predicts high-grade post-hepatectomy liver failure: a prospective cohort study. *J Gastroenterol Hepatol* 2017;32:506-14.
90. Han H, Yang J, Zhuze Y, Zhang M, Wu M. Point shear wave elastography to evaluate and monitor changing portal venous pressure in patients with decompensated cirrhosis. *Ultrasound Med Biol* 2017;43:1134-40.
91. Abe H, Midorikawa Y, Mitsuka Y, Aramaki O, Higaki T, et al. Predicting postoperative outcomes of liver resection by magnetic resonance elastography. *Surgery* 2017;162:248-55.
92. Shen Y, Zhou C, Zhu G, Shi G, Zhu X, et al. Liver stiffness assessed by shear wave elastography predicts postoperative liver failure in patients with hepatocellular carcinoma. *J Gastrointest Surg* 2017;21:1471-9.
93. Rajakannu M, Cherqui D, Ciacio O, Golse N, Pittau G, et al. Liver stiffness measurement by transient elastography predicts late posthepatectomy outcomes in patients undergoing resection for hepatocellular carcinoma. *Surgery* 2017;162:766-74.
94. Donadon M, Fontana A, Palmisano A, Viganò L, Procopio F, et al. Individualized risk estimation for postoperative morbidity after hepatectomy: the Humanitas score. *HPB* 2017;19:910-8.
95. Wu D, Chen E, Liang T, Wang M, Chen B, et al. Predicting the risk of postoperative liver failure and overall survival using liver and spleen stiffness measurements in patients with hepatocellular carcinoma. *Medicine (Baltimore)* 2017;96:e7864.
96. Lei J, Ji X, Hong J, Li W, Chen Y, et al. Prediction of posthepatectomy liver failure using transient elastography in patients with hepatitis B related hepatocellular carcinoma. *BMC Gastroenterol* 2017;17:171.
97. Hu H, Han H, Han XK, Wang WP, Ding H. Nomogram for individualised prediction of liver failure risk after hepatectomy in patients with resectable hepatocellular carcinoma: the evidence from ultrasound data. *Eur Radiol* 2018;28:877-85.
98. Sato N, Kenjo A, Kimura T, Okada R, Ishigame T, et al. Prediction of major complications after hepatectomy using liver stiffness values determined by magnetic resonance elastography. *Br J Surg* 2018;105:1192-9.
99. Procopet B, Fischer P, Horhat A, Mois E, Stefanescu H, et al. Good performance of liver stiffness measurement in the prediction of postoperative hepatic decompensation in patients with cirrhosis complicated with hepatocellular carcinoma. *Med Ultrason* 2018;20:272.
100. Huang Z, Huang J, Zhou T, Cao H, Tan B. Prognostic value of liver stiffness measurement for the liver-related surgical outcomes of patients under hepatic resection: a meta-analysis. *PLoS One* 2018;13:e0190512.
101. Ma X, Wang L, Wu H, Feng Y, Han X, et al. Spleen stiffness is superior to liver stiffness for predicting esophageal varices in chronic liver disease: a meta-analysis. *PLoS One* 2016;11:e0165786.
102. Galle PR, Forner A, Llovet JM, Mazzaferro V, Piscaglia F, et al. EASL clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236.
103. Longbotham D, Young A, Nana G, Feltbower R, Hidalgo E, et al. The impact of age on post-operative liver function following right hepatectomy: a retrospective, single centre experience. *HPB (Oxford)* 2020;22:151-60.
104. Şirli R, Sporea I, Bota S, Jurchiş A. Factors influencing reliability of liver stiffness measurements using transient elastography (M-probe) - Monocentric experience. *Eur J Radiol* 2013;82:e313-6.
105. Varbobitis IC, Siakavellas SI, Koutsounas IS, Karagiannakis DS, Ioannidou P, et al. Reliability and applicability of two-dimensional shear-wave elastography for the evaluation of liver stiffness. *Eur J Gastroenterol Hepatol* 2016;28:1204-9.
106. D'Onofrio M, De Robertis R, Demozzi E, Crosara S, Canestrini S, et al. Liver volumetry: is imaging reliable? Personal experience and review of the literature. *World J Radiol* 2014;6:62-71.
107. Cescon M, Cucchetti A, Grazi GL, Ferrero A, Viganò L, et al. Indication of the extent of hepatectomy for hepatocellular carcinoma on cirrhosis by a simple algorithm based on preoperative variables. *Arch Surg* 2009;144:57-63; discussion 63.
108. Shoup M. Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection. *J Gastrointest Surg* 2003;7:325-30.
109. Kim HJ, Kim CY, Hur YH, Koh YS, Kim JC, et al. Comparison of remnant to total functional liver volume ratio and remnant to standard liver volume ratio as a predictor of postoperative liver function after liver resection. *Korean J Hepato-Biliary-Pancreatic Surg* 2013;17:143.
110. Abdalla EK, Vauthey JN, Ellis LM, Ellis V, Pollock R, et al. Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases. *Ann Surg* 2004;239:818-25; discussion 825-7.
111. Vauthey JN, Abdalla EK, Doherty DA, Gertsch P, Fenstermacher MJ, et al. Body surface area and body weight predict total liver volume in Western adults. *Liver Transpl* 2002;8:233-40.
112. Kishi Y, Abdalla EK, Chun YS, Zorzi D, Madoff DC, et al. Three hundred and one consecutive extended right hepatectomies: evaluation of outcome based on systematic liver volumetry. *Ann Surg* 2009;250:540-8.
113. Schindl MJ, Redhead DN, Fearon KCH, Garden OJ, Wigmore SJ, et al. The value of residual liver volume as a predictor of hepatic

- dysfunction and infection after major liver resection. *Gut* 2005;54:289-96.
114. Chuang YH, Ou HY, Lazo MZ, Chen CL, Chen MH, et al. Predicting post-hepatectomy liver failure by combined volumetric, functional MR image and laboratory analysis. *Liver Int* 2018;38:868-74.
 115. Ribero D, Abdalla EK, Madoff DC, Donadon M, Loyer EM, et al. Portal vein embolization before major hepatectomy and its effects on regeneration, resectability and outcome. *Br J Surg* 2007;94:1386-94.
 116. Guglielmi A, Ruzzenente A, Conci S, Valdegamberi A, Iacono C. How much remnant is enough in liver resection? *Dig Surg* 2012;29:6-17.
 117. Kauffmann R, Fong Y. Post-hepatectomy liver failure. *Hepatobiliary Surg Nutr* 2014;3:238-46.
 118. Reddy SK, Marsh JW, Varley PR, Mock BK, Chopra KB, et al. Underlying steatohepatitis, but not simple hepatic steatosis, increases morbidity after liver resection: a case-control study. *Hepatology* 2012;56:2221-30.
 119. Kaido T, Hamaguchi Y, Uemoto S. Significance of preoperative sarcopenia to liver surgery. *HepatoBiliary Surg Nutr* 2019;8:59-62.
 120. Valero V, Amini N, Spolverato G, Weiss MJ, Hirose K, et al. Sarcopenia adversely impacts postoperative complications following resection or transplantation in patients with primary liver tumors. *J Gastrointest Surg* 2015;19:272-81.
 121. Peng PD, Van Vledder MG, Tsai S, De Jong MC, Makary M, et al. Sarcopenia negatively impacts short-term outcomes in patients undergoing hepatic resection for colorectal liver metastasis. *HPB* 2011;13:439-46.
 122. Toshikuni N, Arisawa T, Tsutsumi M. Nutrition and exercise in the management of liver cirrhosis. *World J Gastroenterol* 2014;20:7286-97.
 123. Maharshi S, Sharma BC, Srivastava S. Malnutrition in cirrhosis increases morbidity and mortality. *J Gastroenterol Hepatol* 2015;30:1507-13.
 124. Buchard B, Boirie Y, Cassagnes L, Lamblin G, Coilly A, et al. Assessment of malnutrition, sarcopenia and frailty in patients with cirrhosis: which tools should we use in clinical practice? *Nutrients* 2020;12.
 125. Sinclair M, Gow PJ, Grossmann M, Angus PW. Review article: Sarcopenia in cirrhosis - Aetiology, implications and potential therapeutic interventions. *Aliment Pharmacol Ther* 2016;43:765-77.
 126. Chen LK, Liu LK, Woo J, Assantachai P, Auyeung TW, et al. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *J Am Med Dir Assoc* 2014;15:95-101.
 127. Harimoto N, Shirabe K, Yamashita YI, Ikegami T, Yoshizumi T, et al. Sarcopenia as a predictor of prognosis in patients following hepatectomy for hepatocellular carcinoma. *Br J Surg* 2013;100:1523-30.
 128. Voron T, Tselikas L, Pietrasz D, Pigneur F, Laurent A, et al. Sarcopenia impacts on short- and long-term results of hepatectomy for hepatocellular carcinoma. *Ann Surg* 2015;261:1173-83.
 129. Hamaguchi Y, Kaido T, Okumura S, Kobayashi A, Shirai H, et al. Preoperative visceral adiposity and muscularity predict poor outcomes after hepatectomy for hepatocellular carcinoma. *Liver Cancer* 2019;8:92-109.
 130. Dello SAWG, Lodewick TM, Van Dam RM, Reisinger KW, Van Den Broek MAJ, et al. Sarcopenia negatively affects preoperative total functional liver volume in patients undergoing liver resection. *HPB* 2013;15:165-9.
 131. Stenholm S, Harris TB, Rantanen T, Visser M, Kritchevsky SB, et al. Sarcopenic obesity: definition, cause and consequences. *Curr Opin Clin Nutr Metab Care* 2008;11:693-700.
 132. Hamaguchi Y, Kaido T, Okumura S, Kobayashi A, Fujimoto Y, et al. Muscle steatosis is an independent predictor of postoperative complications in patients with hepatocellular carcinoma. *World J Surg* 2016;40:1959-68.
 133. Sharma P, Parikh ND, Yu J, Barman P, Derstine BA, et al. Bone mineral density predicts posttransplant survival among hepatocellular carcinoma liver transplant recipients. *Liver Transplant* 2016;22:1092-8.
 134. Miyachi Y, Kaido T, Yao S, Shirai H, Kobayashi A, et al. Bone mineral density as a risk factor for patients undergoing surgery for hepatocellular carcinoma. *World J Surg* 2019;43:920-8.
 135. Shen YN, Zheng ML, Guo CX, Bai XL, Pan Y, et al. The role of imaging in prediction of post-hepatectomy liver failure. *Clin Imaging* 2018;52:137-45.
 136. Taouli B, Vilgrain V, Dumont E, Daire JL, Fan B, et al. Evaluation of liver diffusion isotropy and characterization of focal hepatic lesions with two single-shot echo-planar MR imaging sequences: prospective study in 66 patients. *Radiology* 2003;226:71-8.
 137. Ichikawa T, Haradome H, Hachiya J, Nitatori T, Araki T. Diffusion-weighted MR imaging with a single-shot echoplanar sequence: detection and characterization of focal hepatic lesions. *AJR Am J Roentgenol* 1998;170:397-402.
 138. Tamada T, Ito K, Higaki A, Yoshida K, Kanki A, et al. Gd-EOB-DTPA-enhanced MR imaging: evaluation of hepatic enhancement effects in normal and cirrhotic livers. *Eur J Radiol* 2011;80:e311-6.
 139. Schuhmann-Giampieri G, Schmitt-Willich H, Press WR, Negishi C, Weinmann HJ, et al. Preclinical evaluation of Gd-EOB-DTPA as a contrast agent in MR imaging of the hepatobiliary system. *Radiology* 1992;183:59-64.
 140. Frericks BB, Loddenkemper C, Huppertz A, Valdeig S, Stroux A, et al. Qualitative and quantitative evaluation of hepatocellular carcinoma and cirrhotic liver enhancement using Gd-EOB-DTPA. *AJR Am J Roentgenol* 200;193:1053-60.
 141. Kukuk GM, Schaefer SG, Fimmers R, Hadizadeh DR, Ezziddin S, et al. Hepatobiliary magnetic resonance imaging in patients with liver disease: correlation of liver enhancement with biochemical liver function tests. *Eur Radiol* 2014;24:2482-90.
 142. Watanabe H, Kanematsu M, Goshima S, Kondo H, Onozuka M, et al. Staging hepatic fibrosis: comparison of gadoxetate disodium-enhanced and diffusion-weighted MR imaging--preliminary observations. *Radiology* 2011;259:142-50.
 143. Wibmer A, Prusa AM, Nolz R, Gruenberger T, Schindl M, et al. Liver failure after major liver resection: risk assessment by using preoperative gadoxetic acid-enhanced 3-T MR imaging. *Radiology* 2013;269:777-86.
 144. Jin YJ, Lee SH, Cho SG, Kim JH, Lee JW, et al. Prediction of postoperative liver failure using gadoxetic acid-enhanced magnetic

- resonance imaging in patients with hepatocellular carcinoma. *J Gastroenterol Hepatol* 2016;31:1349-56.
145. Theilig D, Steffen I, Malinowski M, Stockmann M, Seehofer D, et al. Predicting liver failure after extended right hepatectomy following right portal vein embolization with gadoxetic acid-enhanced MRI. *Eur Radiol* 2019;29:5861-72.
146. Asenbaum U, Kaczirek K, Ba-Ssalamah A, Ringl H, Schwarz C, et al. Post-hepatectomy liver failure after major hepatic surgery: not only size matters. *Eur Radiol* 2018;28:4748-56.
147. Hoekstra LT, de Graaf W, Nibourg GAA, Heger M, Bennink RJ, et al. Physiological and biochemical basis of clinical liver function tests: a review. *Ann Surg* 2013;257:27-36.
148. Iimuro Y, Kashiwagi T, Yamanaka J, Hirano T, Saito S, et al. Preoperative estimation of asialoglycoprotein receptor expression in the remnant liver from CT/^{99m}Tc-GSA SPECT fusion images correlates well with postoperative liver function parameters. *J Hepatobiliary Pancreat Sci* 2010;17:673-81.
149. Okabayashi T, Shima Y, Morita S, Shimada Y, Sumiyoshi T, et al. Liver Function assessment using technetium 99m-galactosyl single-photon emission computed tomography/CT Fusion imaging: a prospective trial. *J Am Coll Surg* 2017;225:789-97.
150. May BJ, Madoff DC. Portal vein embolization: rationale, technique, and current application. *Semin Intervent Radiol* 2012;29:81-9.
151. Capussotti L, Muratore A, Baracchi F, Lelong B, Ferrero A, et al. Portal vein ligation as an efficient method of increasing the future liver remnant volume in the surgical treatment of colorectal metastases. *Arch Surg* 2008;143:978-82; discussion 982.
152. Ray S, Mehta NN, Golhar A, Nundy S. Post hepatectomy liver failure - a comprehensive review of current concepts and controversies. *Ann Med Surg* 2018;34:4-10.

Review

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Predictive factors for hepatocellular carcinoma recurrence after curative treatments

Giovanni Marasco¹, Federico Ravaioli¹, Amanda Vestito¹, Benedetta Rossini¹, Elton Dajti¹, Luigi Colecchia¹, Kamela Gjini³, Matteo Renzulli², Rita Golfieri², Davide Festi¹, Antonio Colecchia³

¹Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna 40138, Italy.

²Radiology Unit, Sant'Orsola Malpighi Hospital, Bologna 40138, Italy.

³Unit of Gastroenterology, Borgo Trento University Hospital of Verona, Verona 37126, Italy.

Correspondence to: Dr. Antonio Colecchia, Unit of Gastroenterology, Borgo Trento University Hospital, Verona 37126, Italy.
E-mail: antonio.colecchia@aovr.veneto.it

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm worldwide. Recurrence of HCC after resection or loco-regional therapies represents an important clinical issue as it affects up to 70% of patients. This can be divided into early or late, if it occurs within or after 24 months after treatment, respectively. While the predictive factors for early recurrence are mainly related to tumour biology (local invasion and intrahepatic metastases), late recurrences are mainly related to de novo tumour formation. Thus, it is important to recognize these factors prior to any treatment in each patient, in order to optimize the treatment strategy and follow-up after treatment. The aim of this review is to summarize the current evidence available regarding predictive factors for the recurrence of HCC, according to the different therapeutic strategies available. In particular, we will discuss the role of new ultrasound-based techniques and biological features, such as tumor-related and circulating biomarkers, in predicting HCC recurrence. Recent advances in imaging-related parameters in computed-tomography scans and magnetic resonance imaging will also be discussed.

Keywords: Liver resection, trans-arterial chemoembolization, radiofrequency ablation, liver stiffness measurement, indocyanine green retention test

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common tumor and one of the leading causes of cancer mortality worldwide^[1,2]. To date, the numbers of HCC has not dropped, despite the introduction of new direct antiviral agents for hepatitis virus C (HCV) eradication^[3] to lower the risk of developing HCC in these



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patients^[3]. HCC remains a widespread tumor due to the persistence of a high prevalence of hepatitis B virus (HBV) infection in African and Asian countries, and from the increasing incidence of non-alcoholic fatty liver disease, alcoholic and non-alcoholic steatohepatitis (ASH/NASH) patients in Western countries.

There are different therapeutic strategies to manage HCC according to both the underlying extent of liver disease and tumor-related factors, as stated by the different guidelines available^[2,4-6]. The recommended treatment of choice in these guidelines is mainly an adaptation from the BCLC classification^[2,7]. Patients with HCC are classified into five stages by tumor related factors (size, number, vascular invasion, N1, M1), liver function (bilirubin, portal hypertension, liver function preservation) and health status (ECOG)^[2]. These guidelines were developed to provide the best treatment to maximize overall survival (OS) according to tumor characteristics listed above. Liver resection (LR) is recommended mainly for single HCCs of any size and in particular, for size > 2 cm, with preserved hepatic function and sufficient residual liver volume. In patients with BCLC 0 and A, or in single tumors 2 to 3 cm in size not suitable for surgery, the treatment of choice is thermal ablation with radiofrequency (RFA) as an alternative to surgical resection. In patients with BCLC B, trans-arterial chemoembolization (TACE) is recommended^[2]. Even though treatment choice is tailored for individual patients, HCC recurrence remains the most important concern and can occur regardless of treatment^[8].

In fact, in curative treatments such as liver resection, HCC recurrence develops in up to 70% of patients at 5 years after hepatic resection, both within (early recurrence) or after 24 months (late recurrence)^[1,2,9]. Several authors^[10-13] have explored the differences between early and late recurrence and investigated the risk factors for each. Predictive factors for early recurrence are well-known^[14]. On the other hand, the prediction of late recurrences is limited by poor data; it can also be considered a 'de novo' tumour and thus, has been associated mainly with the degree of fibrosis and extent of portal hypertension (PH)^[15-17].

The aim of this review is to summarize the most recent advances in the role of predictive factors for HCC recurrence in patients undergoing curative HCC treatment beyond liver transplantation such as LR, RFA and TACE. Furthermore, when possible, biomarkers and imaging predictors are also differentiated.

LIVER RESECTION

LR is the standard of care of patients with solitary tumors and preserved liver function^[4,5]. The major complication after LR is HCC recurrence which reaches an incidence of more than 70% at 5 years^[14]. As stated, HCC recurrence can be classified as early or late^[11,18]. Early recurrences have well-established predictive factors which are mainly related to tumor biology (i.e., tumor size, tumor number, presence of microsatellites and vascular invasion) and the treatment modality used^[14]. In particular, vascular invasion (both macroscopic and microscopic) is one of the most reliable predictors of recurrence and survival and strictly associated with histological differentiation and tumor size^[14]. On the other hand, the development of late recurrences is widely considered a de novo HCC affected by the underlying liver status^[19,20]. Thus, the presence and degree of PH could play an important role in predicting late recurrences. Indeed, the extent of PH is directly correlated with the risk of developing complications^[21-24], including HCC^[25]. Recently, several studies have highlighted the role of non-invasive tests such as liver (LSM) and spleen stiffness measurements (SSM) as predictors of late recurrence of HCC^[16,17,26]. The predictive factors for early and late HCC recurrence after LR are summarized in [Table 1](#).

Early HCC recurrence

Tumor-related factors

A clear correlation has already been highlighted between tumor size, number and HCC recurrence^[27]. These factors remain the best pre-operative prognostic factors such that both the American and European Association for the Study of the Liver endorsed these criteria in their staging systems^[2,4]. Indeed, HCC

Table 1. Predictive factors for early and late HCC recurrence after liver resection

	Early recurrence	Late recurrence
Tumor related factors		
Tumor size	[27-29]	[27-29]
Tumor grade	[29-32]	[29]
Macrovascular invasion	[19,33-35]	[19]
Microvascular invasion	[19,36-39]	[19,37]
Satellite nodules	[40]	-
Tumor-free margins	[41-43]	[41,43]
Biomarkers		
AFP	[32,35,44-46]	[27,28]
Immunomarkers	[47-54]	-
ERASL-pre score	[55]	-
REACH score	[56]	-
SVR	[57]	[58-60]
HBV replication	[61]	[15,61]
Alcohol intake		[62,63]
Others		
MicroRNAs	[64,65]	-
Imaging factors	[66-75]	
IGC15	-	[16]
Sarcopenia	[76]	-
NITs		
LSM	-	[16,26,77]
SSM	-	[17]
FIB-4	-	[78]
ALBI > 2	[55,79-82]	[78,83,84]
Platelet/spleen length ratio	-	[17]

HCC: hepatocellular carcinoma; AFP: alpha-fetoprotein; ERASL: early recurrence after surgery for liver tumor; REACH-B: estimates risk of HCC in patients with chronic hepatitis B; SVR: sustained virological response; HBV: hepatitis B virus; IGC15: indocyanine green retention rate at 15 min; NITs: non-invasive tests; LSM: liver stiffness measurement; SSM: spleen stiffness measurement; FIB4: fibrosis-4 index; ALBI: albumin-bilirubin grade

nodules ≥ 5 cm are associated with an increased recurrence rate due to the higher risk of portal vein^[28] and micro-vascular invasion (MVI)^[36]. Besides, vascular invasion represents another good predictor of tumor recurrence^[19,27]. It could be defined as macroscopic, when it is visible on imaging or even on gross examination, and microscopic, when seen only on histological examination. The presence of macrovascular invasion is able to reduce the time to recurrence by 4-fold^[19]. Moreover, extension of portal vein thrombosis is directly related to poor prognosis^[33,34].

With regard to MVI, it is usually defined as the presence of tumour emboli within the central hepatic vein, the portal vein, or the large capsular vessels^[12]. Unfortunately, this evaluation is subject to great variability which affects the true incidence of this condition^[37]. The presence of MVI is related to an increased risk of HCC recurrence^[19,38,39]. The main limitation of MVI assessment depends on its timing since it is often obtained on resected specimens. MVI can be accurately assessed only on resected specimens, which constitutes a strong limitation to such assessment^[29,30,35]. Tumor grade (grade 3/3) and tumor size have also been associated with early HCC recurrence and are strictly related to MVI^[29-32].

Several authors have tried to evaluate the usefulness of other pre-operative parameters beyond tumor grade in predicting MVI, such as increased alpha-fetoprotein (AFP), L3-AFP and prothrombin induced by vit K absence-II (PIVKA-II)^[30,31,35]. Recently, a low concentration of the autophagy-related marker LC3^[85] on HCC and adjacent non-tumor tissues has also been found to be a significant predictor of both early and late HCC recurrence. A further recent study^[47] evaluated the role of immunohistochemical markers in a large cohort of resected HCC, concluding that 14 factors showed a prognostic role for predicting recurrence, including

6 clinico-pathological characteristics (clinical stage, differentiation level, capsular invasion, tumor number, tumor diameter, and AFP level) and 8 immunomarkers (CD34, CDKN1A, E-cadherin, HRas, PCNA, p53, TGF- β and VEGF). Other authors^[86] evaluated the Epstein-Barr virus-induced gene 3 (*EBI3*) which encodes a secretory glycoprotein which was previously found to be upregulated in different tumors; they found that *EBI3* was a predictor for tumor recurrence. Among the myriad of other tumoral-tissue related markers, a prognostic role was also reported for the Peroxiredoxin 1 (PRDX1)^[87]; the divalent metal-ion transporter-1 (DMT1)^[88]; the cell cycle factor NIMA-related kinase 2 (NEK2)^[89]; among the non-coding tumoral RNAs, the miR-210 and miR-550a-1 were associated with a high risk of recurrence^[64], similar to miR-483-3p^[65]; low c-Myc protein expression^[90]; low MHC class I chain-related gene A (*MICA*)^[91]; the long noncoding RNA (lncRNA) expression signature^[92]; and the NUF2^[93]. However, all these tumoral-tissue related markers have not found clinical application yet.

Circulating biomarkers

Several circulating markers have been identified to be able to predict early HCC recurrence. Certainly, AFP is used not only for diagnosing HCC but also plays a key role in predicting recurrence^[44-46] with a cut-off that has recently been lowered from 200 ng/mL to 100 ng/mL^[46]. Since the liver is the organ that synthesizes lipoprotein (a) [Lp (a)], reduced levels of this lipoprotein could be a sign of liver dysfunction. Thus, as Lp (a) mirrors hepatic function and the degree of underlying disease, it has recently been found^[94] in a cohort of HCC patients that low Lp (a) levels (≤ 20 mg/dL) significantly correlated with low time to recurrence ($P = 0.009$) and low OS ($P = 0.007$). Among other circulating markers or composite scores, the albumin-bilirubin (ALBI) score has been validated as a predictor of survival in HCC^[79]. Recently, an Asiatic study^[80] evaluated the role of ALBI in predicting early recurrence. It was found that an ALBI grade ≥ 2 ($P = 0.003$) in addition to HBV surface antigen (HBsAg)-positive status ($P < 0.001$), tumor size ≥ 3.5 cm ($P \leq 0.001$), lympho-vascular invasion ($P = 0.001$), and the presence of satellite lesions ($P = 0.009$) were the only predictors of early HCC recurrence on multivariate analysis. The same results on ALBI were recently reported by other authors^[55,81,82], among whom Chan *et al.*^[55] developed the ERASL-pre score, which included male gender, large tumor size, multinodular tumors, high ALBI grade and high serum AFP. More recently, an elevated fibrinogen/albumin ratio was found^[95] to be significantly correlated with shorter survival and an increase risk of HCC recurrence. On the same line, the ratio between high-sensitivity C-reactive protein (hsCRP) and albumin was associated with a 1.19-fold increase in the risk of HCC recurrence^[96]. In an additional study on hsCRP, it was found to be associated with lymphocyte ratio (HCLR) and positively correlated with large tumor size, TNM stage, MVI, and HCC recurrence^[48]. A further composite score is represented by REACH^[56] which includes NxS factor, MVI, differentiation, serum albumin, platelet count and indocyanine green retention rate at 15 min and was able to predict the risk of HCC recurrence.

Other simple factors that are able to predict early recurrence of HCC that should be taken into account are the lymphocyte and neutrophil levels computed into the NLR^[49], which mirrors a pro-tumor inflammatory environment and the activity of host immunity. In fact, these authors found that patients with pre-operative NLR < 2.5 were at lower risk of recurrence. Thus, others have tried to refine the predictive value of these scores by building a composite score with ALBI and platelet to-lymphocyte ratio^[97], which was able to predict the outcome after liver resection, including HCC recurrence. Platelet to-lymphocyte ratio was further associated with NLR with good results^[50]. Finally, other immune-biomarkers that have promising results are the lymphocyte-to-monocyte ratio^[51], gamma-glutamyl transpeptidase to lymphocyte count ratio^[52] and systemic inflammation score^[53]. A Chinese group^[54] recently demonstrated that IgG4/IgG ratio is an independent indicator of tumor recurrence and a high ratio is associated with a shorter time to recurrence.

Circulating microparticles are novel biomarkers with a potential prognostic role in patients with cancer. Their role in HCC has been investigated by Abbate *et al.*^[98] and it was found that the number of circulating HepPar1+ microparticles before resection was higher in patients with early recurrence compared to those

without ($P = 0.02$). With regard to other cancer biomarkers, platelet derived growth factor-BB has been claimed to be one of the key cytokines in malignant transformation of different cells and recently^[99], diminished perioperative platelet derived growth factor-BB has been linked to HCC recurrence. Another potential biomarker concerns serum proteome alterations^[100]: PGK1, which is directly involved with carcinogenesis and the intracellular inflammation cascade, was found to be increased in patients with early tumor recurrence. Recently, a glycan-based immunoassay targeting Wisteria floribunda agglutinin-positive human Mac-2 binding protein (WFA⁺-M2BP) was evaluated as a noninvasive biomarker of liver fibrosis and predictor of HCC and HCC recurrence, as it was associated with pro-carcinogenic activity in patients with chronic liver disease^[101]. In a further study, it was associated with both early (HR = 1.667) and late recurrence (HR = 1.416) with multivariate analysis^[102].

In conclusion, some circulating biomarkers have gradually been endorsed in clinical practice in several centers for their simplicity and real-time application based on standard liver tests, and are able to give reliable prognostic information on these patients.

Imaging predictive factors

Pre-operative imaging signatures have been proposed in the last decade for predicting HCC recurrence before resection. With regard to computed-tomography (CT) scan prediction, beyond the classic HCC features associated with HCC recurrence, a HCC texture based study found good correlation with histological grade and thus, the risk of disease recurrence^[66].

As for magnetic resonance imaging (MRI), correlation between the preoperative diffusion-weighted imaging and early recurrence has been found^[67,68], specifically^[67] with minimal apparent diffusion coefficient values. Several studies reported that imaging findings based on peri-tumoral tissue enhancement and hypointensity in the hepatobiliary phase were useful for predicting MVI and early recurrence in HCC^[68-70].

A Korean study carried out in 2017^[70], which is one of the largest among those stated above, included 197 patients and using gadoteric acid-enhanced MRI for predicting MVI, highlighted that the combination of at least two of the following - arterial peri-tumoral enhancement, non-smooth tumor margin, and peri-tumoral hypointensity on hepatobiliary phase - was able to predict MVI with > 90% specificity and associated with early recurrence after a single HCC nodule resection. These results have been further confirmed by other groups worldwide^[68,69]. To enhance the predictive accuracy of these findings, a recent study^[71] used Radiomics on CT-scans for this purpose. Radiomics is a new method for medical image analysis^[72], defined as the high-throughput extraction of quantitative metric features that result in the conversion of images into mineable data. These authors^[71] found that peri-tumoral radiomics was better in predicting HCC early recurrence than tumoral radiomics^[73]. Other authors using radiomics on pre-operative CT-scans found a good correlation with MVI (AUC 0.80)^[74]. Beyond texture, using 3D MRI was also possible to evaluate tissue stiffness; a multicenter study^[75] recently found that HCCs with subsequent recurrence had higher tumor stiffness.

Finally, an additional prognostic role has also been investigated for fluoro-deoxyglucose (FDG)-positron emission tomoscintigraphy (PET). Indeed, a recent study^[103] with FDG-PET before surgery concluded that a larger tumor size and serum AFP were correlated with higher SUV max (≥ 4.9), which was able to distinguish between patients with or without HCC recurrence after resection.

Beyond the standard evaluation of HCC (tumor number, size, location, vascular invasion) however, the above evaluation methods remain as research and are only applied in highly specialized centers.

Resection-related factors

Strong evidence is available on the role of tumor-free margins in HCC^[104]. Most previous studies have stated that tumor margins should be at least 1 cm^[42,43]. A randomized controlled trial showed however, that in order to improve survival margins should be at least 2 cm^[104].

On the other hand, the extent of resection is another key factor to take into account when predicting tumor recurrence. If anatomical resection (whole hepatic segment) allows reduction of risk for intrahepatic metastasis due to microsatellite nodules and segmental neoplastic thrombi, most surgeons would prefer non-anatomical resections instead in order to reduce the risk of post-hepatectomy liver failure^[40]. Most studies on this topic are affected by much heterogeneity amongst the patients enrolled, since non-anatomical resections are performed mainly in patients with small HCC nodules and with a higher degree of liver dysfunction. A large series has demonstrated that non-anatomical resection is equally safe in terms of recurrence for HCC nodules less than 2 cm^[105]. On the other hand, for larger tumors, anatomical resection is equally able to guarantee a lower rate of early HCC recurrence^[8].

Late HCC recurrence

Late HCC recurrence is currently not considered a true recurrence of the primary HCC since it seems to be a “*de novo*” tumor and thus, dependent on the degree of underlying liver cirrhosis^[106]. Most studies on this topic highlight predictive factors for late recurrence including the severity of liver cirrhosis, presence of active hepatitis and the degree of PH^[11,16,17,26,57,61,77,107]. Indeed, the sole presence of liver cirrhosis itself leads to a doubling of risk for late recurrences^[11]. In the specific setting of HBV, Ishak activity > 6, an indocyanine green clearance (ICG-15) > 10% and HBsAg > 250 IU/mL were found to be predictors of late HCC recurrence^[15,61]. With regard to HCV etiology, a recent study^[58] demonstrated that HCV-eradication was able to reduce the recurrence of HCC, independent of HCC treatment and the HCV-treatment regimen administered. Further confirmation of the importance of viral eradication came from a recent North American study^[59] on new direct antiviral agents and regimens, which demonstrated that the risk of HCC recurrence was not increased by this treatment, as previously postulated^[60]. Even continuous alcohol intake in patients with Alcohol-related Liver Disease seems to be a HCC risk factor for both the occurrence of primary HCC and late recurrences, since the development of HCC depends both on direct (genotoxic) and indirect factors (cirrhosis development)^[62,108]. Indeed, a recent study by Kudo *et al.*^[63] found that preoperative excessive alcohol intake was related to decreased disease-free survival rate of HCC recurrence after surgery. Similarly, the presence of obesity at the time of LR has been reported as a risk factor for HCC recurrence^[109].

Going back to liver cirrhosis, also in this setting there is an increasing need for non-invasive tests to stratify late HCC recurrences. One of the most frequently used and non-invasive test is the ALBI grade^[83], which is an objective and discriminatory method for assessing liver function in HCC, and is gradually replacing the Child-Pugh score. The ALBI has also been found to be a predictor of late HCC recurrence after resection ALBI grades 2 and 3 ($P < 0.001$)^[84]. Recently, a composite score^[110] for predicting both early and late HCC recurrences in HBV has been developed and validated; this DFT score includes liver function through the use of FIB-4, which is a surrogate marker of liver fibrosis, tumor burden and grade of differentiation. A combination of ALBI and FIB-4 has also been proposed with good accuracy in predicting HCC recurrence^[78].

In line with these efforts, Jung *et al.*^[26] found that patients with LSM values > 13.4 kilopascal (kPa) were at increased risk for late HCC recurrence with a HR of 1.9. Another research group followed up patients with HCC after treatment and found that a decrease in LSM < 8 kPa suggested a reduced risk of late recurrence^[77]. In a subsequent study by Jung *et al.*^[16], the LSM value, together with activity grade II-III, the presence of multiple tumours, and ICG R15 achieved good accuracy in predicting late HCC recurrence. Another non-invasive test capable of mirroring the degree of PH is the evaluation of SSM^[23,24,111,112], which has been demonstrated to be associated with post-hepatectomy liver failure too^[113]. We recently demonstrated^[17] in a cohort of compensated advanced chronic liver disease patients undergoing LR for primary HCC, that univariate analyses of late HCC recurrences were associated with esophageal varices, spleen length, platelet/spleen length ratio, LSM and SSM. Multivariate analyses however, showed that SSM was the only predictor of late recurrence (HR = 1.046). Thus, it is possible to conclude that NITs focused on the evaluation of the

Table 2. Predictive factors for HCC recurrence stratified by ethanol percutaneous injection, radiofrequency ablation and trans-arterial chemoembolization

	Ethanol percutaneous injection	Radiofrequency ablation	Trans-arterial chemoembolization
Tumor related factors			
Tumor size	[114,116,118]	[130,131]	[132]
Tumor grade	[121]	[130]	[133]
Macrovascular invasion			[132]
Satellite nodules	[120]		
Intra-tumoral septa	[121,122]		
Tumor-free margins		[131,134]	
Angiogenic factor			[135-138]
Biomarkers		[139]	
AFP	[116,121]	[130]	[140-142]
Immunomarkers			[143,144]
ALBI			[145,146]
HBV replication		[147,148]	
Genetic factors			
MicroRNAs			[149-152]
Imaging factors			[153-163]
Sarcopenia			[76,164]

AFP: alpha-fetoprotein; ALBI: albumin-bilirubin grade; HBV: hepatitis B virus; HCC: hepatocellular carcinoma

degree of liver fibrosis and function and thus, also of PH, are capable of predicting the future development of HCC nodules in patients who have undergone LR for primary HCC.

ETHANOL PERCUTANEOUS INJECTION

Patients with early stage HCC, who are not suitable for resection or transplantation, are ideal candidates for percutaneous ablation. Guidelines for locoregional therapy include patients with a single HCC nodule ≤ 5 cm or up to 3 nodules ≤ 3 cm, even if minor discrepancies exist between different investigators and studies^[114]. Percutaneous ethanol injection (PEI) is a percutaneous, ultrasound-guided ablative procedure involving the injection of 95% absolute alcohol which induces coagulative necrosis of the lesion due to protein denaturation, cellular dehydration and chemical occlusion of small tumor vessels^[1]. PEI was first described in the early 1980s^[115] and had long been the standard in ablation. Indeed, this technique is the most studied type of percutaneous ablation^[116]. PEI is also an inexpensive and well-tolerated procedure with few adverse effects, and has been considered the standard against which any new ablation therapy should be compared to^[117]. Predictors of HCC recurrence after PEI are summarized in Table 2.

The most important predictor of treatment efficacy and HCC recurrence in this setting is tumor size. Indeed, tumors less than 2 cm in diameter have more than 90% tumor necrosis rate. As the tumor size increases however, the necrosis rate decreases and for tumors 3 to 5 cm in size, this rate is only 50%^[114]. Notably, the major limitation of PEI is the high local recurrence rate, particularly for lesions larger than 3 cm^[118]. Other potential factors affecting tumor recurrence are the total number of treated lesions, satellite nodules, the presence of a halo and an intra-tumoral heterogeneous echo pattern or intra-tumoral septa and AFP levels > 20 mg/dL^[119-121]. When the size of the nodule increases, intra-tumoral septation increases, which is mainly composed of collagen and lipid matrix^[122]. A possible explanation is that ethanol diffusion is blocked either by intra-tumoral fibrotic septa and/or the tumor capsule, limiting its curative effect in lesions larger than 2 cm^[122,123]. In addition, the OS is modified by the size of the tumor. Patients with Child-Pugh class A function and a solitary HCC smaller than 2 cm have 3- and 5-year OS rates of 70% to 80% and $\geq 50\%$, respectively. For HCCs 2 to 3 cm in diameter, the 3-year OS rate ranges from 47% to 64%^[124,125]. To overcome the limits of conventional PEI in patients with tumors larger than 2 cm that cannot be treated with other procedures, a retractable multipronged injection needle was developed^[126]. Chemical ablation with 15%

acetic acid with the use of a multiple-tine infusion device resulted in larger diameters of contiguous tumor coagulation and enabled greater volumes of infusion than the standard technique^[126]. However, as this is associated with higher recurrence rates and inferior OS compared to hyper-thermic ablation^[127,128], it only plays a secondary role in HCC treatment today, having widely been replaced by more modern techniques such as RFA^[129], mainly because it has to be performed repetitively compared to RFA. Furthermore, it is difficult to obtain complete necrosis for tumors larger than 3 cm^[114].

RADIOFREQUENCY ABLATION

RFA is a non-surgical, curative treatment for HCC^[165] which is designed to destroy the tumor by heating^[166]. The heat (above 60 °C) generated by alternating current passing down from the tip of an electrode into the surrounding tissues induces changes in ionic agitation and drives extracellular and intracellular water out of tissues, resulting in their destruction by coagulative necrosis^[166,167]. Heat is administered by probes that are inserted through the skin (percutaneously), laparoscopically or with open surgery^[168]. In cirrhotic patients treated with RFA for HCC, the 5-year OS reached 74%^[169]. Thus, RFA is considered a viable and curative alternative treatment to LR in these patients^[170]. Based on current guidelines, RFA is performed on single lesions < 5 cm in diameter or ≤ 3 lesions < 3 cm in largest diameter, Child-Pugh class A or B, and ECOG 0^[2]. The high rates of post-procedural recurrence, which might be up to 70% at 5 years, remain a major challenge for long-term survival^[130]. Recurrence after RFA for HCC occurs as a result of local tumor progression (LTP) or intrahepatic distant recurrence. LTP occurs along the peripheral margin of the ablative zone when the primary tumor had not been controlled completely after RFA^[131]. Several risk factors have been associated with local recurrence including tumor size more than 2 cm, poorly differentiated carcinoma, advanced tumor stage, high AFP levels, and an insufficient safety margin^[13]. An ablative margin of at least 5 mm is required to avoid the risk of LTP because microsatellite lesions are frequently present and surround the HCC nodule^[134]. The risk of local recurrence is also closely related to the location of the tumor: HCCs next to the portal vein or major hepatic veins were associated with a higher risk for local recurrence (HR = 1.70-2.81) because the patient's blood flow reduces elevation of the tumor's temperature during RFA^[171]. Several studies have reported the ability of AFP levels to predict response to ablation: serum AFP increase have been shown to predict a higher risk of HCC recurrence after ablation treatment^[130]. The heterogeneity of the studies precludes the formulation of a definite magnitude level, but it is suggested that AFP cut off levels of > 200 and/or > 400 ng/mL are associated with poor outcomes^[2]. In contrast to local recurrence, distant intrahepatic recurrence is observed far from the ablation zone and corresponds usually to *de novo* hepatocarcinogenesis on cirrhosis or metastatic dissemination^[172]. Similar to LR, HCC recurrence following RFA occurring early - within 2 years of follow-up - is considered the result of an intrahepatic metastatic process from the primary tumor (related to tumor biology), whereas late recurrence after 2 years would result only from *de novo* carcinogenesis in cirrhosis^[170]. The Child-Pugh score is associated with distant HCC recurrence; this would suggest that the severity of liver disease is a risk factor not only for HCC occurrence but also for distant HCC recurrence^[173]. The link between HBV replication (high pre-procedural serum viral load ≥ 2000 UI/mL) and the recurrence of HCC after RFA suggests that secondary chemoprevention with nucleos(t)ide analogues could improve the prognosis following percutaneous ablation^[147,148,174]. Similarly, several studies found that patients with HCV related cirrhosis who have achieved sustained response to antiviral therapy have a substantially lower rate of HCC recurrence after percutaneous ablation in cirrhotic patients with HCC^[175]. The development of non-invasive methods to assess the degree of liver fibrosis including blood marker tests and transient elastography has revolutionized the assessment of liver fibrosis over the last decade^[176]. Recent data reported the role of transient elastography in predicting intrahepatic distant recurrence of HCC following RFA^[177]. In conclusion, RFA is a potential curative modality for cirrhotic patients with early HCC. Predictors of HCC recurrence after RFA are summarized in Table 2. Additional studies are needed to identify patients with a higher risk of early and late recurrence to improve disease control.

TRANS-ARTERIAL CHEMOEMBOLIZATION

TACE, a direct therapy with a minimally invasive catheter, is the most commonly used interventional radiology technique for the first-line treatment of intermediate stage (BCLC-B) and unresectable HCC^[178,179]. The TACE procedure works on the pathophysiological principle that malignant hepatic lesions receive blood supply from the hepatic artery. Thus, the intra-arterial infusion of a cytotoxic agent followed by embolisation of the blood vessels that feed the tumor leads to a direct cytotoxic and ischemic effect on the tumor mass. HCC, indeed, tends to be fed entirely through the arterial supply, unlike the surrounding parenchyma which receives most of its inflow through the portal system. During TACE procedures, a catheter super-selectively places an emulsion of the water-soluble antitumor agent mixed usually with ethiodized oil^[180]. The effectiveness of TACE is through providing highly concentrated doses of chemotherapy to the tumor bed, while sparing the surrounding liver parenchyma. Conventional TACE, also known as Lipiodol TACE, consists of catheter delivery of the chemotherapeutic emulsified with Lipiodol, followed by vascular stagnation obtained with embolisation of the particles^[181]. During these procedures, the most commonly used chemotherapy drugs are epirubicin, doxorubicin, miriplatin or cisplatin^[181]. Besides conventional TACE, other image-guided transcatheter techniques have been developed recently (chemo-lipiodolisation, bland transcatheter embolisation and intra-arterial chemotherapy) but not recommended clinically yet^[179]. Recently, drug-eluting beads transcatheter arterial chemoembolization (DEB-TACE) has become a routinely used locoregional treatment for unresectable HCC. DEB-TACE have the same clinical outcomes and reduced procedure-related side effects compared to conventional transcatheter arterial chemoembolization^[182,183]. Many potential factors affecting HCC recurrence after TACE treatment have been investigated in the last decades [Table 2].

Tumor-related factors

HCC is a hypervascular tumour such that tumour angiogenesis may be essential to its growth, invasion, or metastasis^[184,185]. The changes induced by TACE in the expression of angiogenic and invasiveness factors [basic fibroblast growth factor (b-FGF), vascular endothelial growth factor (VEGF), urokinase-type plasminogen activator (uPA) and mammalian chitinase-like proteins without chitinase activity (YKL-40 -CHI3L1)] have been investigated. These markers work synergically and seem to be overexpressed after TACE as a result of the related ischemic damage, which renders them useful for predicting treatment response to TACE. In fact, when TACE is not adequate, a significant neoangiogenesis reaction happens, as suggested by an increase in VEGF, uPA, b-FGF and YKL-40 following treatment, and affects patient survival^[135-138,186]. Moreover, the systemic inflammatory response^[187] reflects the state of angiogenesis, DNA damage and tumor invasion^[188]. Among these, systemic immune-inflammation index^[143], aspartate aminotransferase-lymphocyte ratio index and CRP/Alb ratio^[144] are useful non-invasive biological markers with high negative predictive values for HCC OS after TACE. As a matter of fact, tumor size and portal invasion represent the most well-validated HCC prognostic factors after TACE treatment^[132]. In 87% of patients, the low-grade tumors (grade 0, 1, or 2) have shown encouraging long-term treatment response (49%; stable disease or local disease progression, 13%; partial response, 38%; complete response) vs. 33% of high-grade tumors (grade 3 or 4) (stable disease or disease progression, 67%; partial response, 33%; complete response, 0%) after TACE^[133].

Circulating biomarkers and patients' characteristics

Several laboratory markers have been associated with prognostic outcomes in HCC patients undergoing TACE. AFP levels and changes^[140-142], low pretreatment platelets^[189], low baseline serum 25-hydroxyvitamin (D25-OHD) levels^[190], high values of CRP^[138], high levels of serum gamma-glutamyl transferase^[191] and high levels of bilirubin^[132] are all potentially useful biomarkers to predict the poor prognosis in patients with HCC treated with TACE. Moreover, procalcitonin is a precursor of the hormone calcitonin and usually rises in bacterial infections; it was recently proposed as an important prognostic factor for foretelling the prognosis of patients treated with TACE with unresectable HCC^[192].

More than a dozen staging systems have been described and some have been put into clinical practice for either HCC prognostication or as treatment guidelines^[193]. Amongst them, the Cancer of the Liver Italian Program (CLIP) score (takes into account liver function reserves and tumor characteristics), and the ALBI-CLIP grade (consisting of a formula based on albumin and bilirubin) were good predictors of survival up to 2-years after TACE^[145,146]. The CLIP system was superior to the Okuda system for predicting the survival of patients with unresectable HCC treated with TACE^[194]. MELD is inferior to the Child-Pugh system in predicting patient survival in those with unresectable HCC after TACE^[195]. Instead, the CLIP and MELD systems are superior to the Okuda system in predicting the survival of patients with viral hepatitis and unresectable HCC treated with TACE^[196]. A nomogram based on AFP, ICG15, portal vein invasion, tumor capsule, AST and the tumor number has been developed and validated in a precise prognostic model in patients treated with TACE for unresectable HCC^[197]. Finally, sarcopenia has also been correlated with disease-free survival and poorer OS in patients with HCC^[76]. Recently, the rate of change ($< -4.6\%$) in skeletal muscle mass ($\Delta L3$ SMI) over six months after TACE has been associated with a poor prognosis^[164]. Venous ketone bodies, which mirrors muscles status and hepatic reserve function, has been negatively correlated with survival and is thus, a useful predictor of HCC treatment response and prognosis^[198].

Molecular and genetics biomarkers

It has been reported that some patients have developed resistance to chemotherapy drugs in which efficacy becomes greatly reduced and toxicity to normal hepatocytes has grown. Therefore, molecular biomarkers capable of predicting treatment response have been studied to improve chemotherapy efficacy.

Many single nucleotide polymorphism (SNP) have been evaluated as an independent prognosis biomarker for HCC after TACE treatment. The main recently studied SNPs were: SNP rs1126497 in the epithelial cell adhesion/activating molecule (EPCAM) gene^[199], multidrug resistance gene 1 (MDR1) C1236T and C3435T^[200], isocitrate dehydrogenase (IDH) gene^[201], polypeptide N-Acetylgalactosaminyltransferase 14 (GALNT14) “TT” genotype^[202] and pri-let-7a-1^[203]. Moreover, recently a TACE-specific 14-gene signature has been independently related to early disease-free survival and OS in an Asian cohort of HCC patients and further validated in a European cohort^[204].

MicroRNAs in circulating blood have also been studied as prognostic markers in HCC^[149,150].

Imaging predictive factors

There is a notion that imaging techniques (ultrasound, CT and MRI) might facilitate the visualisation and characterization of HCC nodules clearly and accurately. Additionally, it may also aid in displaying perfusion differences between residual carcinoma and necrotic tissue following TACE ablation. Ultrasound techniques, mainly contrast-enhanced ultrasound, can act as a valuable tool to assess the results of TACE and exhibit mostly optimal effects in the early and very early evaluation of TACE^[153,205]. More recently, Xuan *et al.*^[138] showed that four contrast-enhanced ultrasound parameters prior to TACE, including time to peak, maximum tumor intensity, washout time, and rise time, were associated with the recurrence and prognosis of HCC after TACE. Time to peak tumor reflected the structure of the blood supply in tumor lesions and was correlated with enhanced tumor metastasis and invasion that can lead to worse survival rates and an unfavourable prognosis^[138].

Multi-detector CT is the most commonly used imaging technology for assessing therapeutic response to TACE^[154,156]. Patients with hypervascular HCC (defined by an enhancement pattern on the arterial-phase of a CT scan) were more likely to respond to TACE with a reduction in tumor size and increased survival than patients with a less vascular tumor^[139]; it is also notable that patients with hypervascular HCCs have a survival benefit from TACE, even if they are classified as non-responders by size criteria^[157]. Other criteria such as higher arterial enhancement and grey-level co-occurrence matrix moments (by dynamic CT texture

analysis), lower homogeneity, and smaller tumor size are all significant predictors of complete response after TACE^[158].

Recently, in patients with very early and early HCC, the presence of hypovascular hepatic nodules represents a significant risk factor for recurrence and a bad prognosis after treatment; this is a contraindication to the procedure in these patients^[159]. Moreover, a CT image analysis method known as the parametric response map approach is more sensitive for finding changes in the response to treatment than the conventional approach based on the recap of statistics assessed on a region of interest. Briefly, parametric response map aligns spatially the longitudinal images before and after treatment and classifies the patient's images into three categories: reduced, unchanged, and increased intensity^[160].

MRI has a central role in the identification of focal liver lesions. With technical progress, liver MRI has improved with many imaging modalities now for the diagnosis of HCC^[161]. Indeed, gadoxetic acid (Primovist, Bayer Schering Pharma) provides dynamic perfusion imaging and also, evaluation of delayed hepatocyte uptake and biliary excretion which concurs with the precise detection and characterisation of HCC^[206]. Gadoxetic acid-enhanced liver MRI is now widely used and plays a crucial role, not just in the initial diagnosis of HCC, but also in the evaluation of therapeutic efficacy and early diagnosis of residual or recurrent tumor after TACE^[207]. Indeed, HCCs showing high uptake of gadoxetic acid appear to be susceptible to TACE with increasing HCC free-survival in these patients^[162]. Combined diffusion-weighted imaging and choline levels measured at hydrogen-1 magnetic resonance spectroscopy can be used as an early imaging biomarker of treatment response in HCC patients after DEB-TACE^[163]. Moreover, in unresectable HCC, baseline early apparent diffusion coefficients $< 0.83 \times 10^{-3} \text{ mm}^2/\text{s}$ is a predictor of treatment response at 1 and 3 months after DEB-TACE and OS with high specificity and sensitivity^[155]. Finally, a sophisticated 3D MRI and CT method based on quantitative tumor response (volumetric Response Evaluation Criteria in Solid Tumors and the European Association for Study of the Liver guidelines) were early response markers that can be used to predict survival after initial TACE and allow univocal identification of responders and non-responders in terms of median OS^[208].

CONCLUSION

HCC characteristics and the severity of the underlying liver disease are the main considerations in the decision-making process for the best therapeutic strategy for each patient, in order to improve survival and reduce recurrence rates. HCC early recurrence remains related to the aggressiveness of the treated HCC and the technique used. HCC late recurrence, being a “*de novo*” tumor, is mainly predicted by markers of severity of liver fibrosis and the degree of portal hypertension.

Beyond liver disease etiology and the continuous presence of pro-carcinogenetic factors that are etiology-related, the most reliable markers for predicting HCC recurrence after LR are the presence of macro- and micro-vascular invasion, tumor size and the assessment of liver disease severity through the use of LSM. For the same purpose, no definitive data are available in the setting of RFA, even if most studies seem to support a role for LSM. On the other hand, one of the most reliable predictive factors for HCC recurrence after TACE is tumor hypervascularity. The novel circulating, genetic and imaging related markers still need additional validation. Thus, further prospective and well-designed studies are needed to discover new and reliable predictive markers for HCC recurrence after treatments with curative intent.

DECLARATIONS

Authors' contributions

Conceptualized and designed the review: Marasco G, Colecchia A

Wrote, reviewed and edited the manuscript: Marasco G, Ravaioli F, Vestito A, Rossini B, Dajti E, Renzulli M

Provided the tables: Rossini B, Colecchia L, Gjini K

Reviewed and approved the final manuscript as submitted: Marasco G, Colecchia A, Golfieri R, Festi D
All authors read and approved the final manuscript.

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All authors declared that there are no conflicts of interest.

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REFERENCES

1. Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999;19:329-38.
2. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236.
3. Kanwal F, Kramer JR, Asch SM, Cao Y, Li L, et al. Long-term risk of hepatocellular carcinoma in HCV patients treated with direct acting antiviral agents. *Hepatology* 2020;71:44-55.
4. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358-80.
5. Dufour JF, Greten TF, Raymond E, Roskams T, De T, et al. Clinical practice guidelines EASL - EORTC clinical practice guidelines: management of hepatocellular carcinoma European Organisation for Research and Treatment of Cancer. *J Hepatol* 2012;56:908-43.
6. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017;11:317-70.
7. Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. *Gastroenterology* 2016;150:835-53.
8. Colecchia A, Schiumerini R, Cucchetti A, Cescon M, Taddia M, et al. Prognostic factors for hepatocellular carcinoma recurrence. *World J Gastroenterol* 2014;20:5935-50.
9. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006;44:217-31.
10. Zhou JY, Zhang L, Li L, Gu GY, Zhou YH, et al. High hepatitis B virus load is associated with hepatocellular carcinomas development in Chinese chronic hepatitis B patients: a case control study. *Viral J* 2012;9:16.
11. Poon RT, Fan ST, Ng IO, Lo CM, Liu CL, et al. Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. *Cancer* 2000;89:500-7.
12. Tung-Ping Poon R, Fan ST, Wong J. Risk factors, prevention, and management of postoperative recurrence after resection of hepatocellular carcinoma. *Ann Surg* 2000;232:10-24.
13. Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003;38:200-7.
14. Llovet JM, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. *Semin Liver Dis* 2005;25:181-200.
15. Qu LS, Liu JX, Zhu J, Lu CH. Risk factors for prognosis of hepatocellular carcinoma after curative resection in patients with low hepatitis B viral load. *Ann Hepatol* 2017;16:412-20.
16. Jung KS, Kim JH, Kim SU, Song K, Kim BK, et al. Liver stiffness value-based risk estimation of late recurrence after curative resection of hepatocellular carcinoma: Development and validation of a predictive model. *PLoS One* 2014;9:e99167.
17. Marasco G, Colecchia A, Colli A, Ravaioli F, Casazza G, et al. Role of liver and spleen stiffness in predicting the recurrence of hepatocellular carcinoma after resection. *J Hepatol* 2019;70:440-8.

18. Yamamoto Y, Ikoma H, Morimura R, Konishi H, Murayama Y, et al. Optimal duration of the early and late recurrence of hepatocellular carcinoma after hepatectomy. *World J Gastroenterol* 2015;21:1207-15.
19. Roayaie S, Blume IN, Thung SN, Guido M, Fiel MI, et al. A system of classifying microvascular invasion to predict outcome after resection in patients with hepatocellular carcinoma. *Gastroenterology* 2009;137:850-5.
20. Pawlik TM, Esnaola NF, Vauthey JN. Surgical treatment of hepatocellular carcinoma: similar long-term results despite geographic variations. *Liver Transpl* 2004;10:S74-80.
21. Ripoll C, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, et al. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* 2007;133:481-8.
22. Colecchia A, Colli A, Casazza G, Mandolesi D, Schiumerini R, et al. Spleen stiffness measurement can predict clinical complications in compensated HCV-related cirrhosis: a prospective study. *J Hepatol* 2014;60:1158-64.
23. Colecchia A, Marasco G, Taddia M, Montrone L, Eusebi LH, et al. Liver and spleen stiffness and other noninvasive methods to assess portal hypertension in cirrhotic patients: a review of the literature. *Eur J Gastroenterol Hepatol* 2015;27:992-1001.
24. Colecchia A, Ravaioli F, Marasco G, Festi D. Spleen stiffness by ultrasound elastography. In: Berzigotti A, Bosch J, editors. *Diagnostic methods for cirrhosis and portal hypertension*. Cham, Springer International Publishing; 2018. pp. 113-37.
25. Ripoll C, Groszmann RJ, Garcia-Tsao G, Bosch J, Grace N, et al. Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis. *J Hepatol* 2009;50:923-8.
26. Jung KS, Kim SU, Choi GH, Park JY, Park YN, et al. Prediction of recurrence after curative resection of hepatocellular carcinoma using liver stiffness measurement (FibroScan®). *Ann Surg Oncol* 2012;19:4278-86.
27. Kamiyama T, Nakanishi K, Yokoo H, Kamachi H, Tahara M, et al. Analysis of the risk factors for early death due to disease recurrence or progression within 1 year after hepatectomy in patients with hepatocellular carcinoma. *World J Surg Oncol* 2012;10:107.
28. Liu L, Miao R, Yang H, Lu X, Zhao Y, et al. Prognostic factors after liver resection for hepatocellular carcinoma: a single-center experience from China. *Am J Surg* 2012;203:741-50.
29. Pawlik TM, Delman KA, Vauthey JN, Nagorney DM, Ng IOL, et al. Tumor size predicts vascular invasion and histologic grade: Implications for selection of surgical treatment for hepatocellular carcinoma. *Liver Transplant* 2005;11:1086-92.
30. Colecchia A, Scafoli E, Montrone L, Vestito A, Di Biase AR, et al. Pre-operative liver biopsy in cirrhotic patients with early hepatocellular carcinoma represents a safe and accurate diagnostic tool for tumour grading assessment. *J Hepatol* 2011;54:300-5.
31. Pawlik TM, Gleisner AL, Anders RA, Assumpcao L, Maley W, et al. Preoperative assessment of hepatocellular carcinoma tumor grade using needle biopsy. *Ann Surg* 2007;245:435-42.
32. Jung SM, Kim JM, Choi GS, Kwon CHD, Yi NJ, et al. Characteristics of early recurrence after curative liver resection for solitary hepatocellular carcinoma. *J Gastrointest Surg* 2019;23:304-11.
33. Lau WY, Sangro B, Chen PJ, Cheng SQ, Chow P, et al. Treatment for hepatocellular carcinoma with portal vein tumor thrombosis: the emerging role for radioembolization using yttrium-90. *Oncology* 2013;84:311-8.
34. Mazzaferro V, Sposito C, Bhoori S, Romito R, Chiesa C, et al. Yttrium-90 radioembolization for intermediate-advanced hepatocellular carcinoma: a phase 2 study. *Hepatology* 2013;57:1826-37.
35. Hirokawa F, Hayashi M, Miyamoto Y, Asakuma M, Shimizu T, et al. Outcomes and predictors of microvascular invasion of solitary hepatocellular carcinoma. *Hepatol Res* 2014;44:846-53.
36. Kaibori M, Ishizaki M, Matsui K, Kwon AH. Predictors of microvascular invasion before hepatectomy for hepatocellular carcinoma. *J Surg Oncol* 2010;102:462-8.
37. Rodríguez-Perálvarez M, Luong TV, Andreana L, Meyer T, Dhillon AP, et al. A systematic review of microvascular invasion in hepatocellular carcinoma: Diagnostic and prognostic variability. *Ann Surg Oncol* 2013;20:325-39.
38. Kim JM, Joh JW, Yi NJ, Choi GS, Kim K, et al. Predicting hepatocellular carcinoma recurrence beyond milan criteria after liver resection for solitary hepatocellular carcinoma. *J Gastrointest Surg* 2019; Epub ahead of print. doi: 10.1007/s11605-019-04363-1.
39. Zhao H, Hua Y, Lu Z, Gu S, Zhu L, et al. Prognostic value and preoperative predictors of microvascular invasion in solitary hepatocellular carcinoma ≤ 5 cm without macrovascular invasion. *Oncotarget* 2017;8:61203-14.
40. Cucchetti A, Cescon M, Ercolani G, Bigonzi E, Torzilli G, et al. A comprehensive meta-regression analysis on outcome of anatomic resection versus nonanatomic resection for hepatocellular carcinoma. *Ann Surg Oncol* 2012;19:3697-705.
41. Shi M, Guo RP, Lin XJ, Zhang YQ, Chen MS, et al. Partial hepatectomy with wide versus narrow resection margin for solitary hepatocellular carcinoma. *Ann Surg* 2007;245:36-43.
42. Lai ECS, You KT, Ng IOL, Shek TWH. The pathological basis of resection margin for hepatocellular carcinoma. *World J Surg* 1993;17:786-90.
43. Tsilimigras DI, Sahara K, Moris D, Hyer JM, Paredes AZ, et al. Effect of surgical margin width on patterns of recurrence among patients undergoing R0 hepatectomy for T1 hepatocellular carcinoma: an international multi-institutional analysis. *J Gastrointest Surg* 2019; Epub ahead of print. doi: 10.1007/s11605-019-04275-0.
44. Belghiti J, Panis Y, Farges O, Benhamou JP, Fekete F. Intrahepatic recurrence after resection of hepatocellular carcinoma complicating cirrhosis. *Ann Surg* 1991;214:114-7.
45. Guo Y, Chua DW, Koh YX, Lee SY, Cheow PC, et al. Preoperative predictors including the role of inflammatory indices in predicting early recurrence after re-resection for recurrent hepatocellular carcinoma. *World J Surg* 2019;43:2587-94.
46. Tabrizian P, Jibara G, Shrager B, Schwartz M, Roayaie S. Recurrence of hepatocellular cancer after resection: patterns, treatments, and prognosis. *Ann Surg* 2015;261:947-55.
47. Zhang X, Bai Y, Xu L, Zhang B, Feng S, et al. Clinical and morpho-molecular classifiers for prediction of hepatocellular carcinoma

- prognosis and recurrence after surgical resection. *Hepatol Int* 2019;13:715-25.
48. Liao M, Chen P, Liao Y, Li J, Yao W, et al. Preoperative high-sensitivity C-reactive protein to lymphocyte ratio index plays a vital role in the prognosis of hepatocellular carcinoma after surgical resection. *Onco Targets Ther* 2018;11:5591-600.
 49. Hung HC, Lee JC, Cheng CH, Wu TH, Wang YC, et al. Impact of neutrophil to lymphocyte ratio on survival for hepatocellular carcinoma after curative resection. *J Hepatobiliary Pancreat Sci* 2017;24:559-69.
 50. Zheng J, Cai J, Li H, Zeng K, He L, et al. Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio as prognostic predictors for hepatocellular carcinoma patients with various treatments: a meta-analysis and systematic review. *Cell Physiol Biochem* 2017;44:967-81.
 51. Itoh S, Yugawa K, Shimokawa M, Yoshiya S, Mano Y, et al. Prognostic significance of inflammatory biomarkers in hepatocellular carcinoma following hepatic resection. *BJS Open* 2019;3:500-8.
 52. Liao M, Qin W, Liao Y, Yao R, Yu J, et al. Prognostic value of gamma-glutamyl transpeptidase to lymphocyte count ratio in patients with single tumor size ≤ 5 cm hepatocellular carcinoma after radical resection. *Front Oncol* 2019;9:347.
 53. Shi S, Chen Q, Ye L, Yin D, Li X, et al. Prognostic value of systemic inflammation score in patients with hepatocellular carcinoma after hepatectomy. *Oncotarget* 2017;8:79366-75.
 54. Wu J, Ma XL, Tian L, Zhang CY, Wang BL, et al. Serum IgG4: IgG ratio predicts recurrence of patients with hepatocellular carcinoma after curative resection. *J Cancer* 2017;8:1338-46.
 55. Chan AWH, Zhong J, Berhane S, Toyoda H, Cucchetti A, et al. Development of pre and post-operative models to predict early recurrence of hepatocellular carcinoma after surgical resection. *J Hepatol* 2018;69:1284-93.
 56. Tokumitsu Y, Sakamoto K, Tokuhisa Y, Matsui H, Matsukuma S, et al. A new prognostic model for hepatocellular carcinoma recurrence after curative hepatectomy. *Oncol Lett* 2018;15:4411-22.
 57. Cabibbo G, Petta S, Calvaruso V, Cacciola I, Cannavò MR, et al.; Rete Sicilia Selezione Terapia - HCV (RESIST-HCV). Is early recurrence of hepatocellular carcinoma in HCV cirrhotic patients affected by treatment with direct-acting antivirals? A prospective multicentre study. *Aliment Pharmacol Ther* 2017;46:688-95.
 58. Petta S, Cabibbo G, Barbara M, Attardo S, Bucci L, et al. Hepatocellular carcinoma recurrence in patients with curative resection or ablation: impact of HCV eradication does not depend on the use of interferon. *Aliment Pharmacol Ther* 2017;45:160-8.
 59. Singal AG, Rich NE, Mehta N, Branch A, Pillai A, et al. Direct-acting antiviral therapy not associated with recurrence of hepatocellular carcinoma in a multicenter North American cohort study. *Gastroenterology* 2019;156:1683-92.e1.
 60. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016;65:727-33.
 61. Wu JC, Huang YH, Chau GY, Su CW, Lai CR, et al. Risk factors for early and late recurrence in hepatitis B-related hepatocellular carcinoma. *J Hepatol* 2009;51:890-7.
 62. Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. *Am J Epidemiol* 2002;155:323-31.
 63. Kudo A, Tanaka S, Ban D, Matsumura S, Irie T, et al. Alcohol consumption and recurrence of non-B or non-C hepatocellular carcinoma after hepatectomy: a propensity score analysis. *J Gastroenterol* 2014;49:1352-61.
 64. Bai F, Zhou H, Ma M, Guan C, Lyu J, et al. A novel RNA sequencing-based miRNA signature predicts with recurrence and outcome of hepatocellular carcinoma. *Mol Oncol* 2018;12:1125-37.
 65. Vasuri F, Fittipaldi S, De Pace V, Gramantieri L, Bertuzzo V, et al. Tissue miRNA 483-3p expression predicts tumor recurrence after surgical resection in histologically advanced hepatocellular carcinomas. *Oncotarget* 2018;9:17895-905.
 66. Oh J, Lee JM, Park J, Joo I, Yoon JH, et al. Hepatocellular carcinoma: Texture analysis of preoperative computed tomography images can provide markers of tumor grade and disease-free survival. *Korean J Radiol* 2019;20:569-79.
 67. Lee S, Kim SH, Hwang JA, Lee JE, Ha SY. Pre-operative ADC predicts early recurrence of HCC after curative resection. *Eur Radiol* 2019;29:1003-12.
 68. Rimola J, Forner A, Sapena V, Llarch N, Darnell A, et al. Performance of gadoxetic acid MRI and diffusion-weighted imaging for the diagnosis of early recurrence of hepatocellular carcinoma. *Eur Radiol* 2020;30:186-94.
 69. Ahn SJ, Kim JH, Park SJ, Kim ST, Han JK. Hepatocellular carcinoma: preoperative gadoxetic acid-enhanced MR imaging can predict early recurrence after curative resection using image features and texture analysis. *Abdom Radiol* 2019;44:539-48.
 70. Lee S, Kim SH, Lee JE, Sinn DH, Park CK. Preoperative gadoxetic acid-enhanced MRI for predicting microvascular invasion in patients with single hepatocellular carcinoma. *J Hepatol* 2017;67:526-34.
 71. Shan Q, Hu H, Feng S, Peng Z, Chen S, et al. CT-based peritumoral radiomics signatures to predict early recurrence in hepatocellular carcinoma after curative tumor resection or ablation. *Cancer Imaging* 2019;19:11.
 72. Lambin P, Rios-Velazquez E, Leijenaar R, Carvalho S, Van Stiphout RGPM, et al. Radiomics: extracting more information from medical images using advanced feature analysis. *Eur J Cancer* 2012;48:441-6.
 73. Kim S, Shin J, Kim DY, Choi GH, Kim MJ, et al. Radiomics on gadoxetic acid-enhanced magnetic resonance imaging for prediction of postoperative early and late recurrence of single hepatocellular carcinoma. *Clin Cancer Res* 2019;25:3847-55.
 74. Zheng J, Chakraborty J, Chapman WC, Gerst S, Gonen M, et al. Preoperative prediction of microvascular invasion in hepatocellular carcinoma using quantitative image analysis. *J Am Coll Surg* 2017;225:778-88.e1.
 75. Wang J, Shan Q, Liu Y, Yang H, Kuang S, et al. 3D MR elastography of hepatocellular carcinomas as a potential biomarker for predicting tumor recurrence. *J Magn Reson Imaging* 2019;49:719-30.
 76. Fujiwara N, Nakagawa H, Kudo Y, Tateishi R, Taguri M, et al. Sarcopenia, intramuscular fat deposition, and visceral adiposity independently predict the outcomes of hepatocellular carcinoma. *J Hepatol* 2015;63:131-40.

77. Lee JI, Lee HW, Kim SU, Ahn SH, Lee KS. Follow-up liver stiffness measurements after liver resection influence oncologic outcomes of hepatitis-b-associated hepatocellular carcinoma with liver cirrhosis. *Cancers (Basel)* 2019;11:425.
78. Zhang J, Luo Y, Li C, Liu J, Xiang H, et al. The combination of the preoperative albumin-bilirubin grade and the fibrosis-4 index predicts the prognosis of patients with hepatocellular carcinoma after liver resection. *Biosci Trends* 2019;13:351-7.
79. Johnson PJ, Berhane S, Kagebayashi C, Satomura S, Teng M, et al. A nsessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach - the albi grade. *J Clin Oncol* 2015;33:550-8.
80. Lee YH, Koh YS, Hur YH, Cho CK, Kim HJ, et al. Effectiveness of the albumin-bilirubin score as a prognostic factor for early recurrence after curative hepatic resection for hepatocellular carcinoma. *Ann Hepato-Biliary-Pancreatic Surg* 2018;22:335.
81. Amisaki M, Uchinaka E, Morimoto M, Tokuyasu N, Sakamoto T, et al. Post-operative albumin-bilirubin grade predicts long-term outcomes among Child-Pugh grade A patients with hepatocellular carcinoma after curative resection. *Hepatobiliary Pancreat Dis Int* 2018;17:502-9.
82. Matsushima H, Takami Y, Ryu T, Yoshitomi M, Tateishi M, et al. Prognosis of hepatocellular carcinoma patients who achieved long-term recurrence-free survival after curative therapy: impact of the ALBI grade. *J Gastrointest Surg* 2018;22:1230-8.
83. Johnson PJ, Berhane S, Kagebayashi C, Satomura S, Teng M, et al. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. *J Clin Oncol* 2015;33:550-8.
84. Lin CY, Lin CC, Wang CC, Chen CL, Hu TH, et al. The ALBI grade is a good predictive model for very late recurrence in patients with hepatocellular carcinoma undergoing primary resection. *World J Surg* 2020;44:247-57.
85. Lin CW, Chen YS, Lin CC, Lee PH, Lo GH, et al. Clinical and translational gastroenterology autophagy-related gene LC3 expression in tumor and liver microenvironments significantly predicts recurrence of hepatocellular carcinoma after surgical resection. *Clin Transl Gastroenterol* 2018;9:166.
86. Song Q, Chen X, Hu W, Mei G, Yang X, et al. Downregulation of epstein-barr virus-induced gene 3 is associated with poor prognosis of hepatocellular carcinoma after curative resection. *Oncol Lett* 2018;15:7751-9.
87. Fang Y, He J, Janssen HLA, Wu J, Dong L, et al. Peroxiredoxin 1, restraining cell migration and invasion, is involved in hepatocellular carcinoma recurrence. *J Dig Dis* 2018;19:155-69.
88. Hoki T, Katsuta E, Yan L, Takabe K, Ito F. Low DMT1 expression associates with increased oxidative phosphorylation and early recurrence in hepatocellular carcinoma. *J Surg Res* 2019;234:343-52.
89. Chang YY, Yen CJ, Chan SH, Chou YW, Lee YP, et al. NEK2 promotes hepatoma metastasis and serves as biomarker for high recurrence risk after hepatic resection. *Ann Hepatol* 2018;17:843-56.
90. Ji F, Zhang Z, Zhang Y, Shen SL, Cao QH, et al. Low expression of c-Myc protein predicts poor outcomes in patients with hepatocellular carcinoma after resection. *BMC Cancer* 2018;18:460.
91. Huang CF, Wang SC, Chang WT, Yeh ML, Huang CI, et al. Lower protein expression levels of MHC class I chain-related gene A in hepatocellular carcinoma are at high risk of recurrence after surgical resection. *Sci Rep* 2018;8:15821.
92. Lv Y, Wei W, Huang Z, Chen Z, Fang Y, et al. Long non-coding RNA expression profile can predict early recurrence in hepatocellular carcinoma after curative resection. *Hepatol Res* 2018;48:1140-8.
93. Wang Y, Tan PY, Handoko YA, Sekar K, Shi M, et al. NUF2 is a valuable prognostic biomarker to predict early recurrence of hepatocellular carcinoma after surgical resection. *Int J Cancer* 2019;145:662-70.
94. Gao XH, Zhang SS, Chen H, Wang K, Xie W, et al. Lipoprotein (A): a promising prognostic biomarker in patients with hepatocellular carcinoma after curative resection. *Onco Targets Ther* 2018;11:5917-24.
95. Xu Q, Yan Y, Gu S, Mao K, Zhang J, et al. A novel inflammation-based prognostic score: The fibrinogen/albumin ratio predicts prognoses of patients after curative resection for hepatocellular carcinoma. *J Immunol Res* 2018;2018:4925498.
96. Oh TK, Choi YR, Cho JY, Yoon YS, Han HS, et al. The high-sensitivity C-reactive protein/albumin ratio predicts long-term oncologic outcomes after curative resection for hepatocellular carcinoma. *J Clin Med* 2018;7:139.
97. Li C, Zhang XY, Peng W, Wen TF, Yan LN, et al. Preoperative albumin-bilirubin grade plus platelet-to-lymphocyte ratio predict the outcomes of patients with BCLC stage A hepatocellular carcinoma after liver resection. *Medicine (Baltimore)* 2018;97:e11599.
98. Abbate V, Marcantoni M, Giuliani F, Vecchio FM, Gatto I, et al. HepPar1-positive circulating microparticles are increased in subjects with hepatocellular carcinoma and predict early recurrence after liver resection. *Int J Mol Sci* 2017;18:1043.
99. Aryal B, Yamakuchi M, Shimizu T, Kadono J, Furoi A, et al. Predictive value of diminished serum PDGF-BB after curative resection of hepatocellular cancer. *J Oncol* 2019;2019:1925315.
100. Liu H, Chen H, Wu X, Sun Y, Wang Y, et al. The serum proteomics tracking of hepatocellular carcinoma early recurrence following radical resection. *Cancer Manag Res* 2019;11:2935-46.
101. Toyoda H, Kumada T, Tada T, Kaneoka Y, Maeda A, et al. Serum WFA+ -M2BP levels as a prognostic factor in patients with early hepatocellular carcinoma undergoing curative resection. *Liver Int* 2016;36:293-301.
102. Kim HS, Kim SU, Kim BK, Park JY, Kim DY, et al. Serum Wisteria floribunda agglutinin-positive human Mac-2 binding protein level predicts recurrence of hepatitis B virus-related hepatocellular carcinoma after curative resection. *Clin Mol Hepatol* 2020;26:33-44.
103. Lee JH, Park JY, Kim DY, Ahn SH, Han KH, et al. Prognostic value of 18F-FDG PET for hepatocellular carcinoma patients treated with sorafenib. *Liver Int* 2011;31:1144-9.
104. Cescon M, Colecchia A, Cucchetti A, Peri E, Montrone L, et al. Value of transient elastography measured with fibroscan in predicting the outcome of hepatic resection for hepatocellular carcinoma. *Ann Surg* 2012;256:706-13.
105. Eguchi S, Kanematsu T, Arii S, Okazaki M, Okita K, et al.; Liver Cancer Study Group of Japan. Comparison of the outcomes between an anatomical subsegmentectomy and a non-anatomical minor hepatectomy for single hepatocellular carcinomas based on a Japanese

- nationwide survey. *Surgery* 2008;143:469-75.
106. Xu XF, Xing H, Han J, Li ZL, Lau WY, et al. Risk factors, patterns, and outcomes of late recurrence after liver resection for hepatocellular carcinoma: a multicenter study from China. In: *JAMA Surg* 2019;154:209-17.
 107. Chan EEH, Chow PKH. A review of prognostic scores after liver resection in hepatocellular carcinoma: the MSKCC, SLICER and SSCLIP scores. *Jpn J Clin Oncol* 2016;47:287-93.
 108. Testino G, Leone S, Borro P. Alcohol and hepatocellular carcinoma: a review and a point of view. *World J Gastroenterol* 2014;20:15943-54.
 109. Shinkawa H, Tanaka S, Takemura S, Ito T, Aota T, et al. Obesity and recurrence-free survival in patients with hepatocellular carcinoma after achieving sustained virological response to interferon therapy for chronic hepatitis C. *Ann Gastroenterol Surg* 2018;2:319-26.
 110. Qin W, Wang L, Hu B, Leng S, Tian H, et al. A novel score predicts HBV-related hepatocellular carcinoma recurrence after hepatectomy: a retrospective multicenter study. *J Gastrointest Surg* 2019;23:922-32.
 111. Colecchia A, Montrone L, Scaioi E, Bacchi-Reggiani ML, Colli A, et al. Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis. *Gastroenterology* 2012;143:646-54.
 112. Festi D, Dajti E, Ravaioli F, Marasco G, Colecchia A. Role and clinical meaning of non-invasive tests in the evaluation of portal hypertension in patients with advanced chronic liver disease. *Recent Prog Med* 2018;109:574-84.
 113. Marasco G, Colecchia A, Dajti E, Ravaioli F, Cucchetti A, et al. Prediction of posthepatectomy liver failure: role of SSM and LSPS. *J Surg Oncol* 2019;119:400-1.
 114. Korean Liver Cancer Association (KLCA); National Cancer Center (NCC). 2018 Korean liver cancer association-national cancer center korea practice guidelines for the management of hepatocellular carcinoma. *Korean J Radiol* 2019;20:1042-113.
 115. Livraghi T, Festi D, Monti F, Salmi A, Vettori C. US-guided percutaneous alcohol injection of small hepatic and abdominal tumors. *Radiology* 1986;161:309-12.
 116. Shiina S, Tateishi R, Imamura M, Teratani T, Koike Y, et al. Percutaneous ethanol injection for hepatocellular carcinoma: 20-year outcome and prognostic factors. *Liver Int* 2012;32:1434-42.
 117. Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005;42:1208-36.
 118. Khan KN, Yatsushashi H, Yamasaki K, Yamasaki M, Inoue O, et al. Prospective analysis of risk factors for early intrahepatic recurrence of hepatocellular carcinoma following ethanol injection. *J Hepatol* 2000;32:269-78.
 119. Germani G, Pleguezuelo M, Gurusamy K, Meyer T, Isgrò G, et al. Clinical outcomes of radiofrequency ablation, percutaneous alcohol and acetic acid injection for hepatocellular carcinoma: a meta-analysis. *J Hepatol* 2010;52:380-8.
 120. Ebara M, Okabe S, Kita K, Sugiura N, Fukuda H, et al. Percutaneous ethanol injection for small hepatocellular carcinoma: Therapeutic efficacy based on 20-year observation. *J Hepatol* 2005;43:458-64.
 121. Koda M, Murawaki Y, Mitsuda A, Ohya K, Horie Y, et al. Predictive factors for intrahepatic recurrence after percutaneous ethanol injection therapy for small hepatocellular carcinoma. *Cancer* 2000;88:529-37.
 122. Ohnishi K, Yoshioka H, Ito S, Fujiwara K. Prospective randomized controlled trial comparing percutaneous acetic acid injection and percutaneous ethanol injection for small hepatocellular carcinoma. *Hepatology* 1998;27:67-72.
 123. Shiina S, Tagawa K, Unuma T, Takanashi R, Yoshiura K, et al. Percutaneous ethanol injection therapy for hepatocellular carcinoma. a histopathologic study. *Cancer* 1991;68:1524-30.
 124. Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma ≤ 4 cm. *Gastroenterology* 2004;127:1714-23.
 125. Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. *Gut* 2005;54:1151-6.
 126. Hines-Peralta A, Liu ZJ, Horkan C, Solazzo S, Goldberg SN. Chemical tumor ablation with use of a novel multiple-tine infusion system in a canine sarcoma model. *J Vasc Interv Radiol* 2006;17:351-8.
 127. Livraghi T, Meloni F, Di Stasi M, Rolle E, Solbiati L, et al. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: is resection still the treatment of choice? *Hepatology* 2008;47:82-9.
 128. Lencioni R, Crocetti L. Local-regional treatment of hepatocellular carcinoma. *Radiology* 2012;262:43-58.
 129. Lurje I, Czigany Z, Bednarsch J, Roderburg C, Isfort P, et al. Treatment strategies for hepatocellular carcinoma - a multidisciplinary approach. *Int J Mol Sci* 2019;20:1465.
 130. Ganne-Carrié N, Nault JC, Ziol M, N'Kontchou G, Nahon P, et al. Predicting recurrence following radiofrequency percutaneous ablation for hepatocellular carcinoma. *Hepatic Oncol* 2014;1:395-408.
 131. Zytoun AA, Ishii H, Murakami K, El-kholy MR, Furuse J, et al. Recurrence-free survival after radiofrequency ablation of hepatocellular carcinoma. A registry report of the impact of risk factors on outcome. *Jpn J Clin Oncol* 2007;37:658-72.
 132. Bruix J, Sala M, Llovet JM. Chemoembolization for hepatocellular carcinoma. *Gastroenterology* 2004;127:S179-88.
 133. Sandow TA, Arndt SE, Albar AA, DeVun DA, Kirsch DS, et al. Assessment of response to transcatheter arterial chemoembolization with doxorubicin-eluting microspheres: tumor biology and hepatocellular carcinoma recurrence in a 5-year transplant cohort. *Radiology* 2018;286:1072-83.
 134. Yamakado K, Takaki H, Nakatsuka A, Yamaknaka T, Fujimori M, et al. Radiofrequency ablation for hepatocellular carcinoma. *Gastrointest Interv* 2014;3:35-9.
 135. Poon RTP, Lau C, Yu WC, Fan ST, Wong J. High serum levels of vascular endothelial growth factor predict poor response to transarterial chemoembolization in hepatocellular carcinoma: a prospective study. *Oncol Rep* 2004;11:1077-84.

136. Liao X, Yi J, Li X, Yang Z, Deng W, et al. Expression of angiogenic factors in hepatocellular carcinoma after transcatheter arterial chemoembolization. *J Huazhong Univ Sci Technolog Med Sci* 2003;23:280-2.
137. Sergio A, Cristofori C, Cardin R, Pivetta G, Ragazzi R, et al. TTranscatheter arterial chemoembolization (TACE) in hepatocellular carcinoma (HCC): the role of angiogenesis and invasiveness. *Am J Gastroenterol* 2008;103:914-21.
138. Xuan ZD, Zhou L, Wang Y, Zheng X. Prognostic value of the combination of serum levels of vascular endothelial growth factor, C-reactive protein and contrast-enhanced ultrasound in patients with primary liver cancer who underwent transcatheter arterial chemoembolization. *Expert Rev Anticancer Ther* 2017;17:1169-78.
139. Katyal S, Oliver JH, Peterson MS, Chang PJ, Baron RL, et al. Prognostic significance of arterial phase CT for prediction of response to transcatheter arterial chemoembolization in unresectable hepatocellular carcinoma: a retrospective analysis. *Am J Roentgenol* 2000;175:1665-72.
140. Lee YK, Kim SU, Kim DY, Ahn SH, Lee KH, et al. Prognostic value of α -fetoprotein and des- γ -carboxy prothrombin responses in patients with hepatocellular carcinoma treated with transarterial chemoembolization. *BMC Cancer* 2013;13:5.
141. Kim BK, Ahn SH, Seong JS, Park JY, Kim DY, et al. Early α -fetoprotein response as a predictor for clinical outcome after localized concurrent chemoradiotherapy for advanced hepatocellular carcinoma. *Liver Int* 2011;31:369-76.
142. Riaz A, Ryu RK, Kulik LM, Mulcahy MF, Lewandowski RJ, et al. Alpha-fetoprotein response after locoregional therapy for hepatocellular carcinoma: oncologic marker of radiologic response, progression, and survival. *J Clin Oncol* 2009;27:5734-42.
143. Yang Z, Zhang J, Lu Y, Xu Q, Tang B, et al. Aspartate aminotransferase-lymphocyte ratio index and systemic immune-inflammation index predict overall survival in HBV-related hepatocellular carcinoma patients after transcatheter arterial chemoembolizations. *Oncotarget* 2015;6:43090-8.
144. Shen Y, Wang H, Li W, Chen J. Prognostic significance of the CRP/Alb and neutrophil to lymphocyte ratios in hepatocellular carcinoma patients undergoing TACE and RFA. *J Clin Lab Anal* 2019;33:e22999.
145. Cai XR, Chen ZH, Liu MM, Lin JX, Zhang XP, et al. Modified CLIP score with the albumin-bilirubin grade retains prognostic value in HBV-related hepatocellular carcinoma patients treated with trans-catheter arterial chemoembolization therapy. *J Cancer* 2018;9:2380-8.
146. Kim JH, Sinn DH, Lee JH, Hyun D, Cho SK, et al. Novel albumin-bilirubin grade-based risk prediction model for patients with hepatocellular carcinoma undergoing chemoembolization. *Dig Dis Sci* 2018;63:1062-71.
147. Chung GE, Kim W, Lee JH, Kim YJ, Yoon JH, et al. Negative hepatitis B envelope antigen predicts intrahepatic recurrence in hepatitis B virus-related hepatocellular carcinoma after ablation therapy. *J Gastroenterol Hepatol* 2011;26:1638-45.
148. Sohn W, Paik YH, Lee MW, Rhim H, Lim HK, et al. Predisposing factors for recurrence of HBV-related small hepatocellular carcinoma after percutaneous radiofrequency ablation. *Scand J Gastroenterol* 2014;49:373-80.
149. Kim SS, Nam JS, Cho HJ, Won JH, Kim JW, et al. Plasma microRNA-122 as a predictive marker for treatment response following transarterial chemoembolization in patients with hepatocellular carcinoma. *J Gastroenterol Hepatol* 2017;32:199-207.
150. Cui L, Hu Y, Bai B, Zhang S. Serum miR-335 level is associated with the treatment response to trans-arterial chemoembolization and prognosis in patients with hepatocellular carcinoma. *Cell Physiol Biochem* 2015;37:276-83.
151. Lu YL, Yao JG, Huang XY, Wang C, Wu XM, et al. Prognostic significance of miR-1268a expression and its beneficial effects for post-operative adjuvant transarterial chemoembolization in hepatocellular carcinoma. *Sci Rep* 2016;6:36104.
152. Ali HEA, Emam AA, Zeeneldin AA, Srour R, Tabashy R, et al. Circulating miR-26a, miR-106b, miR-107 and miR-133b stratify hepatocellular carcinoma patients according to their response to transarterial chemoembolization. *Clin Biochem* 2019;65:45-52.
153. Gao Y, Zheng DY, Cui Z, Ma Y, Liu YZ, et al. Predictive value of quantitative contrast-enhanced ultrasound in hepatocellular carcinoma recurrence after ablation. *World J Gastroenterol* 2015;21:10418-26.
154. Kim SH, Lee WJ, Lim HK, Lim JH. Prediction of viable tumor in hepatocellular carcinoma treated with transcatheter arterial chemoembolization: usefulness of attenuation value measurement at quadruple-phase helical computed tomography. *J Comput Assist Tomogr* 2007;31:198-203.
155. Kokabi N, Ludwig JM, Camacho JC, Xing M, Mittal PK, et al. Baseline and early MR apparent diffusion coefficient quantification as a predictor of response of unresectable hepatocellular carcinoma to doxorubicin drug-eluting bead chemoembolization. *J Vasc Interv Radiol* 2015;26:1777-86.
156. Kim HC, Kim AY, Han JK, Chung JW, Lee JY, et al. Hepatic arterial and portal venous phase helical CT in patients treated with transcatheter arterial chemoembolization for hepatocellular carcinoma: added value of unenhanced images. *Radiology* 2002;225:773-80.
157. Ebied OM, Federle MP, Carr BI, Pealer KM, Li W, et al. Evaluation of responses to chemoembolization in patients with unresectable hepatocellular carcinoma. *Cancer* 2003;97:1042-50.
158. Park HJ, Kim JH, Choi SY, Lee ES, Park SJ, et al. Prediction of therapeutic response of hepatocellular carcinoma to transcatheter arterial chemoembolization based on pretherapeutic dynamic CT and textural findings. *Am J Roentgenol* 2017;209:W211-20.
159. Maesaka K, Sakamori R, Yamada R, Tahata Y, Urabe A, et al. Hypovascular hepatic nodules as a predictive factor for transcatheter arterial chemoembolization refractoriness in hepatocellular carcinoma. *Hepatol Res* 2020;50:365-73.
160. Choi SJ, Kim J, Seo J, Kim HS, Lee JM, et al. Parametric response mapping of dynamic CT as an imaging biomarker to distinguish viability of hepatocellular carcinoma treated with transcatheter arterial chemoembolization. *Abdom Imaging* 2014;39:518-25.
161. Huppertz A, Haralda S, Kraus A, Zech CJ, Scheidler J, et al. Enhancement of focal liver lesions at gadoxetic acid-enhanced MR imaging: correlation with histopathologic findings and spiral CT-initial observations. *Radiology* 2005;234:468-78.
162. Ishimaru H, Nakashima K, Sakugawa T, Sakamoto A, Matsuoka Y, et al. Local recurrence after chemoembolization of hepatocellular carcinoma: uptake of gadoxetic acid as a new prognostic factor. *Am J Roentgenol* 2014;202:744-51.
163. Ou HY, Cheng YF, Chuang YH, Hsu HW, Chen CL, et al. Quantification of functional MR predicts early response in post-doxorubicin

- drug-eluting beads chemoembolization for hepatocellular carcinoma. *Dig Dis Sci* 2019; Epub ahead of print. doi: 10.1007/s10620-019-05951-6.
164. Kobayashi T, Kawai H, Nakano O, Abe S, Kamimura H, et al. Rapidly declining skeletal muscle mass predicts poor prognosis of hepatocellular carcinoma treated with transcatheter intra-arterial therapies. *BMC Cancer* 2018;18:756.
 165. Eggert T, Greten TF. Current standard and future perspectives in non-surgical therapy for hepatocellular carcinoma. *Digestion* 2017;96:1-4.
 166. Minami Y. Radiofrequency ablation of hepatocellular carcinoma: current status. *World J Radiol* 2010;2:417.
 167. Lau WY, Lai ECH. The current role of radiofrequency ablation in the management of hepatocellular carcinoma: a systematic review. *Ann Surg* 2009;249:20-5.
 168. Na BG, Kim JM, Oh DK, Lee KW, Kang TW, et al. Clinical outcomes of laparoscopic radiofrequency ablation of single primary or recurrent hepatocellular carcinoma (≤ 3 cm). *Ann Surg Treat Res* 2017;92:355-60.
 169. Lencioni R, Cioni D, Crocetti L, Franchini C, Della Pina C, et al. Early-stage hepatocellular carcinoma in patients with cirrhosis: Long-term results of percutaneous image-guided radiofrequency ablation. *Radiology* 2005;234:961-7.
 170. Nault JC, Sutter O, Nahon P, Ganne-Carrié N, S  r O. Percutaneous treatment of hepatocellular carcinoma: State of the art and innovations. *J Hepatol* 2018;68:783-97.
 171. Toshimori J, Nouse K, Nakamura S, Wada N, Morimoto Y, et al. Local recurrence and complications after percutaneous radiofrequency ablation of hepatocellular carcinoma: a retrospective cohort study focused on tumor location. *Acta Med Okayama* 2015;69:205-12.
 172. Tiong L, Maddern GJ. Systematic review and meta-analysis of survival and disease recurrence after radiofrequency ablation for hepatocellular carcinoma. *Br J Surg* 2011;98:1210-24.
 173. Sparchez Z, Mocan T, Radu P, Mocan LP, Sparchez M, et al. Prognostic factors after percutaneous radiofrequency ablation in the treatment of hepatocellular carcinoma. Impact of incomplete ablation on recurrence and overall survival rates. *J Gastrointest Liver Dis* 2018;27:399-407.
 174. Wu CY, Chen YJ, Ho HJ, Hsu YC, Kuo KN, et al. Association between nucleoside analogues and risk of hepatitis B virus-related hepatocellular carcinoma recurrence following liver resection. *JAMA* 2012;308:1906-13.
 175. N'Kontchou G, Mahamoudi A, Aout M, Ganne-Carri   N, Grando V, et al. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 western patients with cirrhosis. *Hepatology* 2009;50:1475-83.
 176. European Association for Study of Liver; Asociacion Latinoamericana para el Estudio del H  gado. EASL-ALEH Clinical Practice Guidelines: non-invasive tests for evaluation of liver disease severity and prognosis. *J Hepatol* 2015;63:237-64.
 177. Rekik S, Allaire M, Mumana A, Guyot E, Nkontchou G, et al. Transient elastography predicts survival after radiofrequency ablation of hepatocellular carcinoma developing on cirrhosis. *J Gastroenterol Hepatol* 2020;35:142-50.
 178. Llovet JM, Real MI, Mont  a X, Planas R, Coll S, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002;359:1734-9.
 179. Kong J, Li S, Fan H, Zhang L, Zhao H, et al. Transarterial chemoembolization extends long-term survival in patients with unresectable hepatocellular carcinoma. *Medicine (Baltimore)* 2018;97:e11872.
 180. Liapi E, Georgiades CC, Hong K, Geschwind JFH. Transcatheter arterial chemoembolization: current technique and future promise. *Tech Vasc Interv Radiol* 2007;10:2-11.
 181. Sieghart W, Hucke F, Peck-Radosavljevic M. Transarterial chemoembolization: modalities, indication, and patient selection. *J Hepatol* 2015;62:1187-95.
 182. Dhanasekaran R, Kooby DA, Staley CA, Kauh JS, Khanna V, et al. Comparison of conventional transarterial chemoembolization (TACE) and chemoembolization with doxorubicin Drug Eluting Beads (DEB) for unresectable Hepatocellular Carcinoma (HCC). *J Surg Oncol* 2010;101:476-80.
 183. Poon RTP, Tso WK, Pang RWC, Ng KKC, Woo R, et al. A phase I/II trial of chemoembolization for hepatocellular carcinoma using a novel intra-arterial drug-eluting bead. *Clin Gastroenterol Hepatol* 2007;5:1100-8.
 184. Yamaguchi R, Yano H, Iemura A, Ogasawara S, Haramaki M, et al. Expression of vascular endothelial growth factor in human hepatocellular carcinoma. *Hepatology* 1998;28:68-77.
 185. Ogasawara S, Yano H, Iemura A, Hisaka T, Kojiro M. Expressions of basic fibroblast growth factor and its receptors and their relationship to proliferation of human hepatocellular carcinoma cell lines. *Hepatology* 1996;24:198-205.
 186. Zhu CB, Wang C, Chen LL, Ma GL, Zhang SC, et al. Serum YKL-40 independently predicts outcome after transcatheter arterial chemoembolization of hepatocellular carcinoma. *PLoS One* 2012;7:e44648.
 187. Casadei Gardini A, Conti F, Foschi FG, Brillanti S, Andreone P, et al. Imbalance of neutrophils and lymphocyte counts can be predictive of hepatocellular carcinoma occurrence in hepatitis C-related cirrhosis treated with direct-acting antivirals. *Gastroenterology* 2018;154:2281-2.
 188. Casadei Gardini A, Foschi FG, Conti F, Petracci E, Vukotic R, et al. Immune inflammation indicators and ALBI score to predict liver cancer in HCV-patients treated with direct-acting antivirals. *Dig Liver Dis* 2019;51:681-8.
 189. Wang B, Li F, Cheng L, Zhan Y, Wu B, et al. The pretreatment platelet count is an independent predictor of tumor progression in patients undergoing transcatheter arterial chemoembolization with hepatitis B virus-related hepatocellular carcinoma. *Futur Oncol* 2019;15:827-39.
 190. Wu YQ, Fan WZ, Xue M, Guo J, Wei JL, et al. 25-OH-vitamin D deficiency identifies poor tumor response in hepatocellular carcinoma treated with transarterial chemoembolization. *Clin Transl Oncol* 2020;22:70-80.
 191. Zhang JB, Chen Y, Zhang B, Xie X, Zhang L, et al. Prognostic significance of serum gamma-glutamyl transferase in patients with

- intermediate hepatocellular carcinoma treated with transcatheter arterial chemoembolization. *Eur J Gastroenterol Hepatol* 2011;23:787-93.
192. Shen H, Zheng S, Chen R, Jin X, Xu X, et al. Prognostic significance of serum procalcitonin in patients with unresectable hepatocellular carcinoma treated with transcatheter arterial chemoembolization. *Medicine (Baltimore)* 2017;96:e7438.
193. Liu PH, Hsu CY, Hsia CY, Lee YH, Su CW, et al. Prognosis of hepatocellular carcinoma: assessment of eleven staging systems. *J Hepatol* 2016;64:601-8.
194. Biselli M, Andreone P, Gramenzi A, Trevisani F, Cursaro C, et al. Transcatheter arterial chemoembolization therapy for patients with hepatocellular carcinoma: a case-controlled study. *Clin Gastroenterol Hepatol* 2005;3:918-25.
195. Brown DB, Fundakowski CE, Lisker-Melman M, Crippin JS, Pilgram TK, et al. Comparison of MELD and Child-Pugh scores to predict survival after chemoembolization for hepatocellular carcinoma. *J Vasc Interv Radiol* 2004;15:1209-18.
196. Testa R, Testa E, Giannini E, Botta F, Malfatti F, et al. Trans-catheter arterial chemoembolisation for hepatocellular carcinoma in patients with viral cirrhosis: Role of combined staging systems, Cancer Liver Italian Program (CLIP) and Model for End-stage Liver Disease (MELD), in predicting outcome after treatm. *Aliment Pharmacol Ther* 2003;17:1563-9.
197. Xu L, Peng ZW, Chen MS, Shi M, Zhang YJ, et al. Prognostic nomogram for patients with unresectable hepatocellular carcinoma after transcatheter arterial chemoembolization. *J Hepatol* 2015;63:122-30.
198. Sasaki R, Taura N, Miyazoe Y, Yamamichi S, Nakashiki S, et al. Ketone bodies as a predictor of prognosis of hepatocellular carcinoma after transcatheter arterial chemoembolization. *Nutrition* 2018;50:97-103.
199. Yu X, Ge N, Guo X, Shen S, Liang J, et al. Genetic variants in the EPCAM gene is associated with the prognosis of transarterial chemoembolization treated hepatocellular carcinoma with portal vein tumor thrombus. *PLoS One* 2014;9:e93416.
200. Hu W, Huang S, Dong L, Yu C, Li C, et al. MDR1 gene polymorphism correlated with pathological characteristics and prognosis in patients with primary hepatocellular carcinoma receiving interventional therapy. *Anticancer Drugs* 2019;30:233-40.
201. Zhang H, Guo X, Dai J, Wu Y, Ge N, et al. Genetic variations in IDH gene as prognosis predictors in TACE-treated hepatocellular carcinoma patients. *Med Oncol* 2014;31:1-8.
202. Liang KH, Lin CL, Chen SF, Chiu CW, Yang PC, et al. GALNT14 genotype effectively predicts the therapeutic response in unresectable hepatocellular carcinoma treated with transcatheter arterial chemoembolization. *Pharmacogenomics* 2016;17:353-66.
203. Wang G, Bi C. Correlations of pri-Let-7 gene polymorphisms with the recurrence and metastasis of primary liver cancer after transcatheter arterial chemoembolization. *Pathol Res Pract* 2018;214:667-72.
204. Fako V, Martin SP, Pomyen Y, Budhu A, Chaisaingmongkol J, et al. Gene signature predictive of hepatocellular carcinoma patient response to transarterial chemoembolization. *Int J Biol Sci* 2019;15:2654-63.
205. Spârchez Z, Mocan T, Radu P, Anton O, Comsa M, et al. Contrast enhanced ultrasonography in assessing the treatment response to transarterial chemoembolization in patients with hepatocellular carcinoma. *Med Ultrason* 2016;18:96-102.
206. Sung SA, Kim MJ, Joon SL, Hong HS, Yong EC, et al. Added value of gadoxetic acid-enhanced hepatobiliary phase MR imaging in the diagnosis of hepatocellular carcinoma. *Radiology* 2010;255:459-66.
207. Hwang J, Kim SH, Kim YS, Lee MW, Woo JY, et al. Gadoxetic acid-enhanced MRI versus multiphase multidetector row computed tomography for evaluating the viable tumor of hepatocellular carcinomas treated with image-guided tumor therapy. *J Magn Reson Imaging* 2010;32:629-38.
208. Tacher V, Lin M, Duran R, Yarmohammadi H, Lee H, et al. Comparison of existing response criteria in patients with hepatocellular carcinoma treated with transarterial chemoembolization using a 3D quantitative approach. *Radiology* 2016;278:275-84.

Meta-Analysis

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Role of laparoscopic and robotic liver resection compared to open surgery in elderly hepatocellular carcinoma patients: a systematic review and meta-analysis

Alberto Brolese¹, Marta Rigoni^{2,8}, Alessandro Vitale³, Giovanni de Pretis⁴, Ivo Avancini⁴, Cecilia Pravadelli⁴, Michela Frisinghelli⁵, Umberto Rozzanigo⁶, Giacomo Luppi⁶, Francesco Dionisi⁷, Stefano Marcucci¹, Giovanni Viel¹, Paolo Beltempo¹, Cristina Prezzi¹, Marco Frisini¹, Marco Brolese⁹, Giandomenico Nollo^{2,8}, Francesco A. Ciarleglio¹

¹Department of General Surgery & HPB Unit (Chief: Alberto Brolese, MD), Azienda Provinciale per i Servizi Sanitari (APSS), Santa Chiara Hospital, Trento 38122, Italy.

²IRCS - Innovation and Clinical Health Research - Bruno Kessler Foundation (FBK), Trento 38123, Italy.

³Department of Surgical, Oncological & Gastroenterological Sciences, Padua University Hospital, Padua 35128, Italy.

⁴Department of Hepatology & Gastroenterology, APSS, Trento 38122, Italy.

⁵Department of Oncology, Azienda Provinciale per i Servizi Sanitari (APSS), Trento 38122, Italy.

⁶Department of Radiology, Azienda Provinciale per i Servizi Sanitari (APSS), Trento 38122, Italy.

⁷Proton therapy Unit, Azienda Provinciale per i Servizi Sanitari (APSS), Trento 38122, Italy.

⁸Department of Industrial Engineering, BIOTech Lab, University of Trento, Trento 38122, Italy.

⁹School of Medicine, Padua University, Padua 35128, Italy.

Correspondence to: Dr. Alberto Brolese, Department of General Surgery & HPB Unit, Azienda Provinciale per i Servizi Sanitari (APSS), Santa Chiara Hospital, Largo Medaglie d'oro, 1, Trento 38122, Italy. E-mail: alberto.brolese@apss.tn.it

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Abstract

Aim: This study aimed to compare mini-invasive liver resection (MILR) (laparoscopic/robotic approach) and open liver resection (OLR) for hepatocellular carcinoma (HCC) in elderly patients with regard to clinical and oncological outcomes through a comprehensive systematic review.

Methods: The MEDLINE and Cochrane Library electronic databases were systematically searched from 2009 to December 2019 to identify relevant English written studies comparing MILR and OLR. The main endpoints were Child-Pugh score, serum total bilirubin level, comorbidity, presence/absence of cirrhosis, minor/major resection, challenge segment approach, operative time, estimated intraoperative blood loss, liver failure rate, morbidity according to the Clavien-Dindo classification, length of hospital stay (LOS), postoperative mortality, number of lesions, tumor size, readmission rate, recurrence rate and survival at 1, 3 and 5 years after operation. Meta-



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analyses provided pooled relative risks and mean differences for these outcomes. Cut-off for “elderly age” was set at 65 years old.

Results: Eight studies that evaluated 3051 patients who underwent liver resection for HCC, with 950 undergoing MILR and 2101 OLR, were included after the screening process. Blood loss, morbidity, and LOS showed statistical significance in favor of MILR. In particular, with respect to OLR, MILR decreased on average blood loss by 161.43 mL (95%CI: 250.24-72.61), risk of morbidity by 42% ($P < 0.01$), LOS by 4 days (95%CI: 7-2), postoperative mortality risk by 47% (although not significantly, $P = 0.06$). Major resections were significantly more common in the OLR group ($P < 0.0001$). Recurrence, although not significant ($P = 0.06$), must also be emphasized. The two surgical approaches were comparable with regard to the other outcomes investigated.

Conclusion: Meta-analyses confirmed the advantages of MILR in terms of short perioperative outcomes, where it may promote the extension of liver resection to HCC patients with borderline liver function. MILR may be considered an important treatment option with significant benefits in the elderly and fragile patients. However, large well-designed prospective comparative studies or randomized controlled trials would be necessary to further confirm our conclusions.

Keywords: Hepatocellular carcinoma, HCC, mini-invasive liver resection, laparoscopic liver surgery, robotic liver surgery, open liver surgery, meta-analysis

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver neoplasm: it is the second leading cause of cancer-related deaths worldwide and sixth for cancer-related deaths in developed countries^[1,2]. With regard to Italian data, HCC accounts for 79% of primary liver cancer, and it is among the first five causes of cancer-related deaths (7% global population)^[3].

The elderly rate in Italian and Western populations has increased for reduced newborn/year and the progressive increasing of mean age. The risk of developing cancer is age-dependent. In Italy, patients over 75 years old have a 25% higher relative risk than the 60-74 age group (147/100,000 vs. 106/100,000), and it is 5 times higher than the 45-59 age group^[4]. In the next years, the true incidence of HCC will be directly related to population age up to rates of 51 cases/100,000 in males and 119 cases/100,000 in females^[5], according to EURO CARE report^[6]. Therefore, the number of elderly patients requiring treatment for primary and metastatic liver cancer is constantly rising and, despite a limited life expectancy, the use of liver surgery has been found by many authors to be a safe and effective treatment for these patients^[7,8].

Laparoscopic liver resection for HCC in selected patients has shown very good results^[9,10] with regard to oncological outcomes, morbidity, mortality, length of hospital stay (LOS) and fast postoperative recovery. This is important after oncological surgery, because complications may negatively impact on short-term outcomes, long-term survival and recurrence^[11]. The robotic approach has been introduced to overcome some limitations of conventional laparoscopy, such as improved range of movements and enhanced instrument dexterity, a 3-dimensional view of the surgical field, a reduction in surgeon tremors and shortened learning curve.

The effect of age on cancer treatment allocation is controversial^[12]. Mini-invasive surgery is a new goal in the treatment of HCC because it has made a great impact on surgical practice and on liver surgery. The management of elderly patients with HCC is becoming routine in clinical practice, but it is substantially more complicated than with younger patients because of comorbidities such as cardiovascular and respiratory disease, diabetes, renal failure and fragility. Age may not represent a limiting factor for liver resection, but it is still unclear if elderly patients can benefit from minimally invasive surgery. The most common concerns

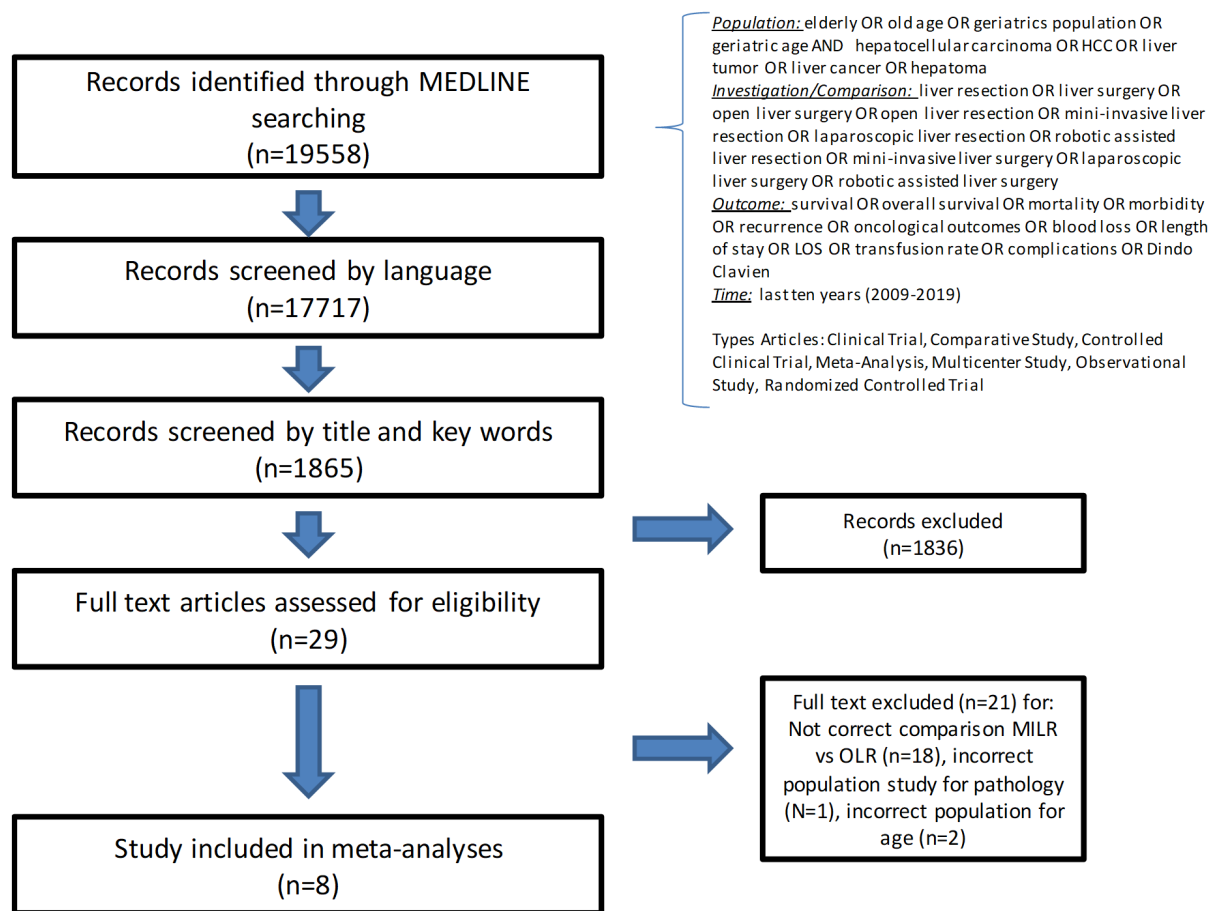


Figure 1. Flow chart of literature selection and PICOT description. MILR: mini-invasive liver resection; OLR: open liver resection

for surgeons and anesthesiologists in this regard are as follows: longer operative times, pneumoperitoneum and its physiological consequences, diminished functional reserve, and pre- or postoperative comorbidities.

In the last 10 years, only some single-center retrospective studies have analyzed the results of mini-invasive surgery in elderly patients, and very few reports have focused on topics about mini-invasive surgical treatment of the elderly with HCC.

The objective of this study was to perform a systematic review to compare mini-invasive liver resection (MILR) (laparoscopic/robotic approach) and open liver resection (OLR) for HCC in the elderly, across a comprehensive range of outcomes reported from both randomized and observational studies.

METHODS

Literature search strategy

Literature documenting a comparison of clinical and oncological outcomes in elderly patients who underwent MILR vs. OLR therapy for HCC was analyzed by searching PubMed and Cochrane Library from 2009 to December 2019. The search terms, either independently or in combination, were used according to PICOT framework [Figure 1]. A systematic search was conducted for relevant systematic reviews, randomized controlled trials, and observational studies (prospective or retrospective cohort and case-control or case-match studies) using a search strategy guided by oncological or surgical information, abstract and keywords related to our research question. Only English language published articles were screened. When more than one article was reported by the same institution or author, we selected either the one with the largest series or the most recent, with the exception of multicenter studies.

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement checklist was used to report selection^[13].

Study inclusion criteria

In this section, we report the selection criteria to identify eligible studies for this review that aimed to compare studies on the effects of MILR vs. OLR for HCC in elderly patients. Different cut-offs for “elderly age” were considered in most studies. In the present study, the cut-off was set at 65 years old. Different resections, major or minor, were included. Study selection criteria were defined according to the PICOT framework [Figure 1]. Three different searches were identified: type 1, comparing open and laparoscopic liver resections; type 2, comparison in elderly between open and robotic liver resections; and type 3, comparison between laparoscopic and robotic liver resections. The following studies or data were excluded: case report, abstract, review, editorial letter, study without control group, and comparative study with population less than 10 patients for each group. The quality assessment of the primary studies did not represent exclusion criteria.

Outcomes

Primary outcomes of all eligible studies included Child-Pugh score, serum total bilirubin level, comorbidities, presence/absence of cirrhosis, minor/major resection, challenge segment approach, operative time, estimated intraoperative blood loss, liver failure rate, morbidity according to the Clavien-Dindo classification^[14], LOS, and postoperative mortality. Operative time was defined as the time from skin incision to wound closure. Postoperative mortality was defined as death during the same hospital admission or within 30 days after liver resection. Major resection was defined as a liver resection of three or more contiguous segments in all papers under investigation. Challenge segments were posterolateral segments (Sg6 and Sg7), posterosuperior segments (Sg8 and Sg4a) and caudate lobe. Secondary outcomes included tumor size, number of lesions (single/multiple), readmission rate, recurrence rate and survival at 1, 3 and 5 years after operation.

Data extraction and quality assessment

Two reviewers (F.C. and M.R.) independently screened titles, abstracts, full texts, and extracted the demographic and clinical outcome data from the selected studies. When disagreement occurred, they reviewed the papers together to reach joint conclusions. The methodological quality of the studies was evaluated by applying the Critical Appraisal Skills Program - CASP Checklists for Case Control Study (Critical Appraisal Skills Programme (2018). CASP Case Control Study Checklist. Available at: <https://casp-uk.net/casp-tools-checklists/>). The overall quality of the primary studies was assessed as low, moderate or high quality.

Statistical analyses

All the analyses were performed with the data originating from the included studies. When available, patient characteristics and outcomes were expressed as numbers or percentages, mean \pm SD or median (interquartile range or range), as reported in primary studies.

Some of the included studies reported the continuous variables with means and standard deviation, other studies with median and range or interquartile range. For continuous outcomes (i.e., operative time, estimated blood loss, and LOS), mean \pm SD for some primary studies were estimated from median, range, and interquartile range following the approach by Hozo *et al.*^[15] and Deeks *et al.*^[16]. For mortality at 1, 3 and 5 years, row data (i.e., counts) were calculated by simple proportions by the given percentages of survivors in whole population of each primary study.

Eleven meta-analyses were performed, one for every outcome considered. A random effects model based on the method used by DerSimonian and Laird^[17] was used to estimate pooled risk ratios (RRs), pooled mean differences (MDs) and 95% confidence intervals (95% CIs). Data heterogeneity between studies was estimated

Table 1. Characteristics and quality assessment of studies included in the systematic review

Study	Year	Country	Study design	Surgical group	Number	Quality assessment**
Badawy <i>et al.</i> ^[18]	2017	Japan	Retro + PMS*	MILR OLR	40 40	M
Chan <i>et al.</i> ^[19]	2014	China	Retro	MILR OLR	17 34	L
Amato <i>et al.</i> ^[20]	2016	Italy	Retro	MILR OLR	11 18	M
Nomi <i>et al.</i> ^[21]	2020	Japan	Retro-multicenter	MILR OLR	221 409	H
Wang <i>et al.</i> ^[22]	2015	China	Retro	MILR OLR	30 60	M
Tee <i>et al.</i> ^[23]	2019	USA	Retro-multicenter	MILR OLR	487 1282	H
Wang <i>et al.</i> ^[24]	2018	Taiwan	Retro	MILR OLR	63 177	M
Chen <i>et al.</i> ^[25]	2017	Taiwan	Retro + PMS*	MILR OLR	81 81	H

*PMS: propensity match score; **quality assessment evaluated by Critical Appraisal Skills Programme (2018). CASP Case Control Study Checklist (L: low quality; M: medium quality; H: high quality). MILR: mini-invasive liver resection; OLR: open liver resection

by Chi2, I2, and Tau2 statistics, which were determined by an inverse-variance fixed-effect model. Funnel plots graphically assessed publication bias.

A 2-tailed *P* value < 0.05 indicated statistical significance. All analyses were performed using the Cochrane Collaboration Software Review Manager 5 (version 5.2).

RESULTS

Study characteristics and population

The flow diagram for article selection for systematic review is shown in Figure 1 according to the PRISMA guidelines. The initial search yielded 19,558 reports but only 17,717 were in English. After examining the titles and key words, we excluded 15,852 citations because of irrelevance, and after abstract screening, we removed 1836 other records because of incongruences on population or outcomes. The 29 remaining studies were assessed for eligibility by a full-text examination. Finally, eight studies^[18-25] were included in this systematic review for the quantitative synthesis, five of which compared laparoscopic liver resection and open approach, two robotic *vs.* open liver resection and one both laparoscopic and robotic *vs.* open approach [Table 1].

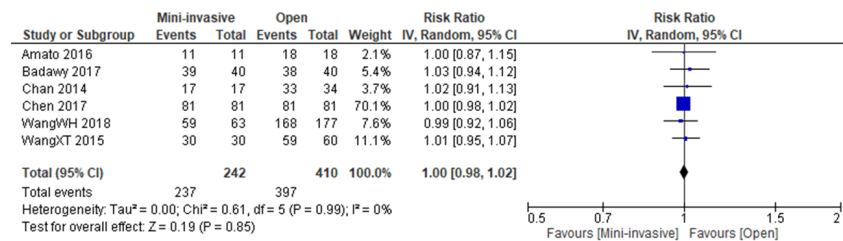
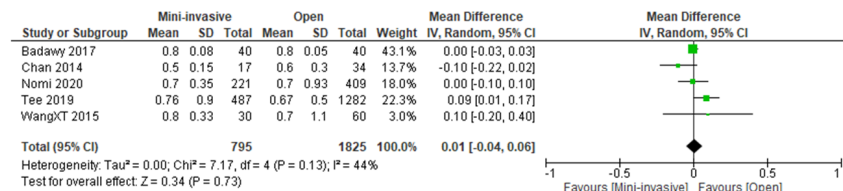
A total of 3051 patients who underwent liver resection for HCC from 8 studies were included, with 950 undergoing MILR and 2101 OLR. All the selected studies were retrospective (5 case-control and 3 case-matched). The cut-off age for elderly was 75 years old in 2 studies and 70 for 5 studies and median age was > 65 for 1 study. Percentages of HCC patients were 100% for all the included studies with exception of Badawy *et al.*^[18] and Chan *et al.*^[19]. For these two studies percentages of HCC patients in both mini-invasive and open groups were greater than 50%, therefore we included the studies in the review and in the statistical analyses. The overall quality assessment of each of the studies included is given in Table 1. One study was assessed as low quality^[19], four studies as moderate quality^[18,20,22,24] and three studies as high quality^[21,23,25].

Primary outcomes

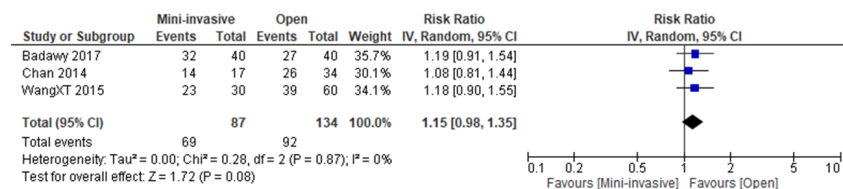
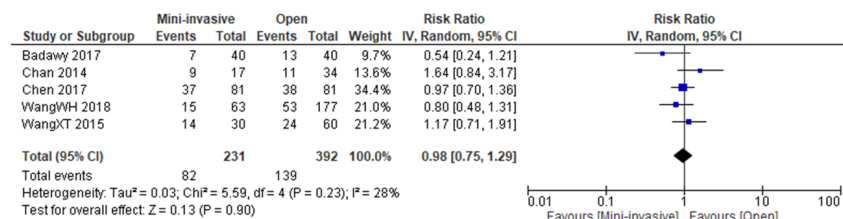
Meta-analyses of the considered outcomes are reported in Figure 2A and B, Table 2 and Table 3. No significant differences in preoperative characteristics were noted between the groups for liver assessment and function, including Child-Pugh score, serum total bilirubin level, comorbidities, presence/absence of cirrhosis. Major resections were significantly more common in the OLR group compared to the MILR group; indeed, the relative risk for MILR was reduced by 42% (RR = 0.58, 95%CI: 0.34-0.97), but this result was affected by substantial heterogeneity ($I^2 = 86\%$). Segmentectomies and wedge resections were significantly

A

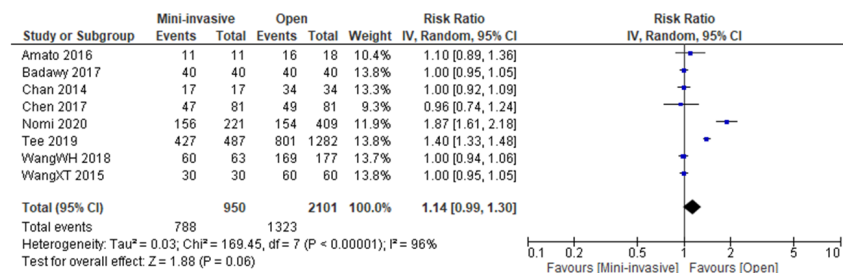
Child-Plug score

Total bilirubin value
(mg/dl)

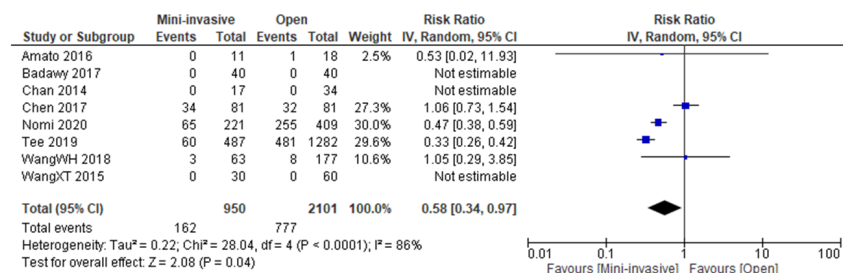
Comorbidity

Presence/absence of
Cirrhosis

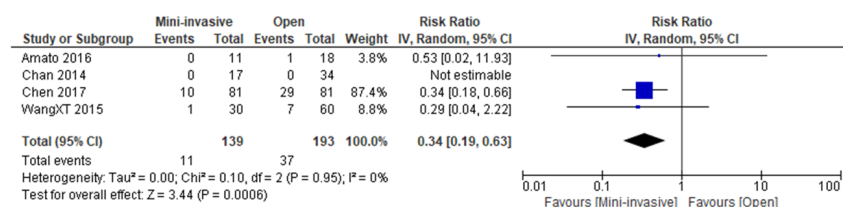
Minor resections



Major resections



Challenge segments



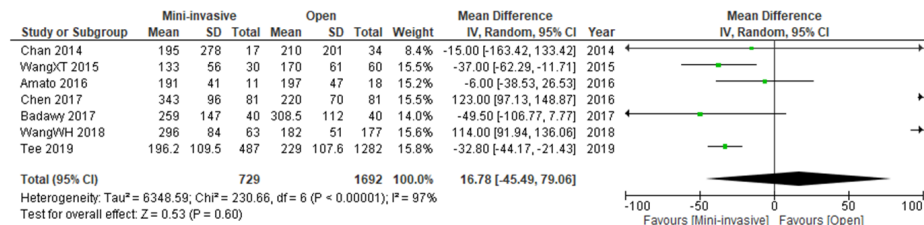
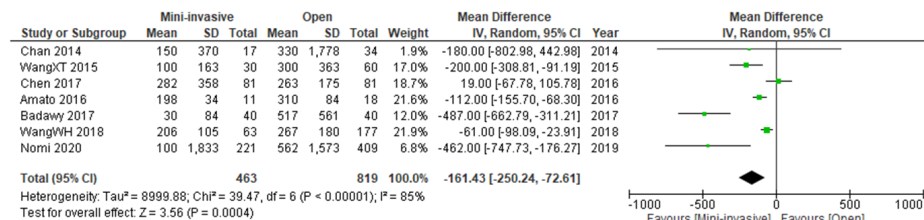
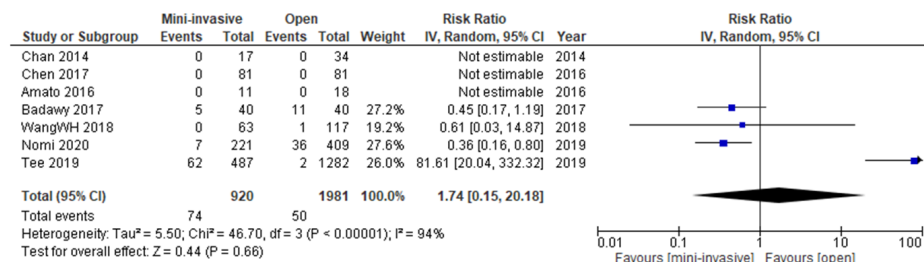
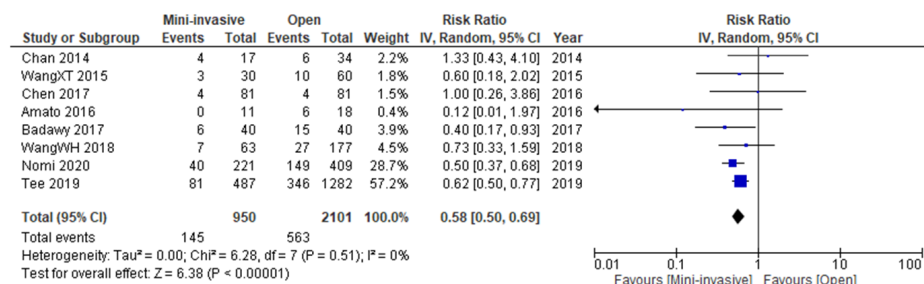
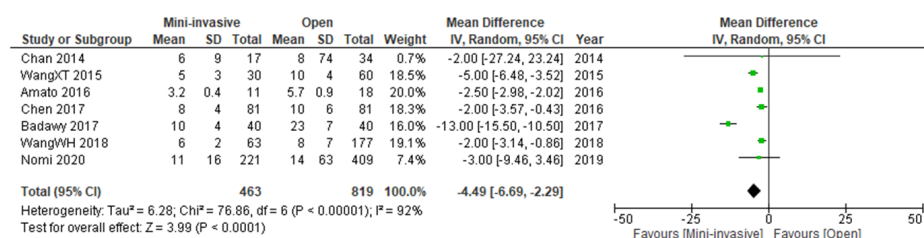
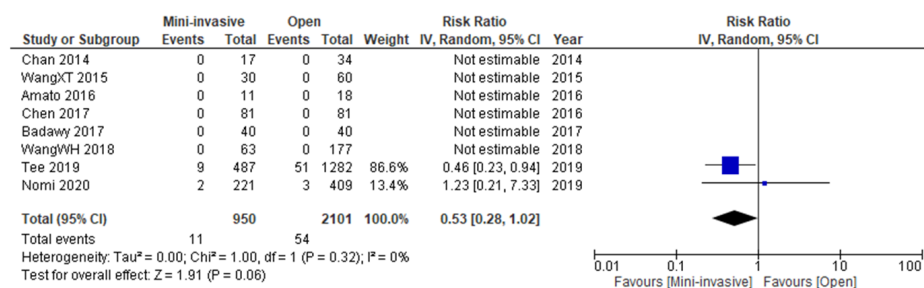
B**Operative time****Blood loss****Liver failure rate****Clavien-Dindo morbidity****Length of hospital stay (LOS)****Post-operative mortality****Figure 2.** A: Meta-analyses of included studies on primary outcomes; B: Meta-analyses of included studies on primary outcomes

Table 2. Clinical characteristics and primary outcomes of studies included in the meta-analysis

Study	Year	CP score A		Mean total bilirubin (mg/dL)		Comorbidity (pathological history)		Cirrhosis (presence)		Minor resection		Major resection	
		MILR	OLR	MILR	OLR	MILR	OLR	MILR	OLR	MILR	OLR	MILR	OLR
Badawy <i>et al.</i> ^[18]	2017	39/40	38/40	0.8	7/40	40/40	40/40	40/40	40/40	6/40	6/40	22	23.5
Chan <i>et al.</i> ^[19]	2014	17/17	33/34	0.5	9/17	17/17	34/34	17/17	34/34	3/17	12/34	30	30
Amato <i>et al.</i> ^[20]	2016	11/11	18/18	na	11/11	11/11	16/18	11/11	16/18	0	0	35.45	39.83
Nomi <i>et al.</i> ^[21]	2020	na	na	0.7	na	156/221	154/409	156/221	154/409	na	na	25	38
Wang <i>et al.</i> ^[22]	2015	30/30	59/60	0.8	14/30	30/30	60/60	30/30	60/60	3/30	7/60	40	50
Tee <i>et al.</i> ^[23]	2019	na	na	0.76	na	427/487	801/1282	427/487	801/1282	na	na	na	na
Wang <i>et al.</i> ^[24]	2018	59/63	168/177	na	15/63	60/63	169/177	60/63	169/177	8/63	35/177	36.3	30.5
Chen <i>et al.</i> ^[25]	2017	81/81	81/81	na	37/81	47/81	49/81	47/81	49/81	na	na	na	na

MILR: mini-invasive liver resection; OLR: open liver resection; CP: Child-Pugh

Table 3. Further primary outcomes of studies included in the meta-analysis

Study	Year	Challenge segment resection		Operative time (min)		Liver failure		Blood loss (mL)		Morbidity		Mortality		LOS (Day)	
		MILR	OLR	MILR	OLR	MILR	OLR	MILR	OLR	MILR	OLR	MILR	OLR	MILR	OLR
Badawy <i>et al.</i> ^[18]	2017	na	na	259	308.5	5/40	11/40	30	517	6/40	15/40	0/40	0/40	10	23
Chan <i>et al.</i> ^[19]	2014	0/17	0/34	195	210	0/17	0/34	150	330	4/17	6/34	0/17	0/34	6	8
Amato <i>et al.</i> ^[20]	2016	0/11	1/18	190.9	196.9	0/11	0/18	198	310	0/11	6/18	0/11	0/18	3.18	5.7
Nomi <i>et al.</i> ^[21]	2020	na	na	na	na	7/221	36/409	100	562	40/221	149/409	2/221	3/409	11	14
Wang <i>et al.</i> ^[22]	2015	1/30	7/60	133	170	na	na	100	300	3/30	10/60	0/30	0/60	5	10
Tee <i>et al.</i> ^[23]	2019	na	na	196.2	229.0	62/487	2/1282	na	na	81/487	346/1282	9/487	51/1282	na	na
Wang <i>et al.</i> ^[24]	2018	na	na	296	182	0/63	1/177	206	267	7/63	27/177	0/63	0/177	6.21	8.18
Chen <i>et al.</i> ^[25]	2017	10/81	29/81	343	220	0/81	0/81	282	263	4/81	4/81	0/81	0/81	7.5	10.1

MILR: mini-invasive liver resection; LOS: length of hospital stay; OLR: open liver resection

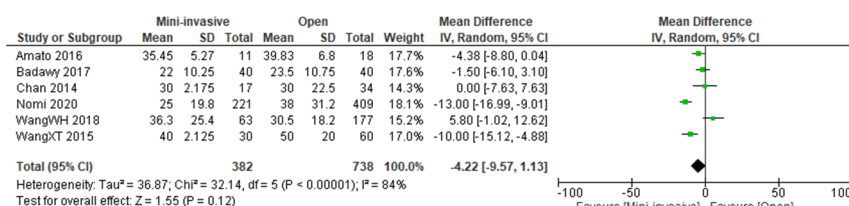
more common in the MILR than in the OLR group (RR = 0.34, 95%CI: 0.19-0.63). The risk for the mini-invasive group with respect to the open group was reduced by 66%, but the result showed considerable heterogeneity ($I^2 = 95\%$) and these results were however related to only one study^[25].

Among the thirteen outcomes, estimated blood loss, morbidity according to Clavien-Dindo classification, and LOS showed statistical significance in favor of the mini-invasive approach. In particular, on average, mini-invasive intervention decreased blood loss by 161.43 (95%CI: 250.24-72.61) mL, although this result showed a substantial percentage of statistical heterogeneity ($I^2 = 85\%$) between studies. The mini-invasive approach decreased the risk of morbidity by 42% with respect to open resection ($P < 0.01$), and these pooled data were strengthened by no important heterogeneity between studies ($I^2 = 0\%$). LOS indicated an average decrease of 4 (95%CI: 7-2) days for mini-invasive with respect to open surgery, even if this effect showed considerable heterogeneity ($I^2 = 92\%$) between studies. Finally, postoperative mortality showed a risk reduction of 47% for mini-invasive compared to open surgery, although not significant ($P = 0.06$). Due to zero events both in the mini-invasive and open groups, 6 out of 8 studies were not informative for this outcome. Consequently, this outcome was estimated by 2 out of 8 studies, that demonstrated no important statistical heterogeneity ($I^2 = 0\%$). Funnel plots of each outcome showed no graphical asymmetry, indicating no publication bias, although the number of studies was too low to support strong deductions.

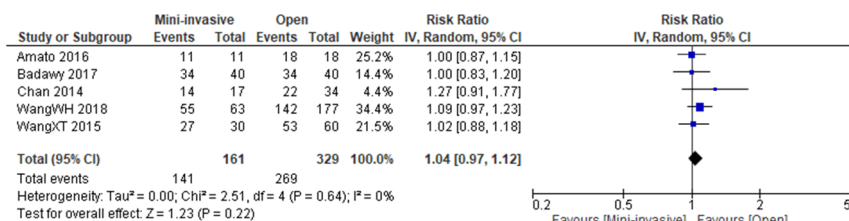
Secondary outcomes

Meta-analyses of the outcomes considered are shown in Figure 3. Number of lesions (single/multiple), readmission rate, recurrence rate, survival at 1, 3, and 5 years showed no statistical differences between the mini-invasive and open groups. Tumor size plotting analysis reported a mean pooled size reduction of 4.22 mm in the MILR group, although this result was not statistically significant (95%CI: 9.57-1.13, $P = 0.12$), and heterogeneity was substantial ($I^2 = 84\%$). In particular, the recurrence outcome, estimated by two studies,

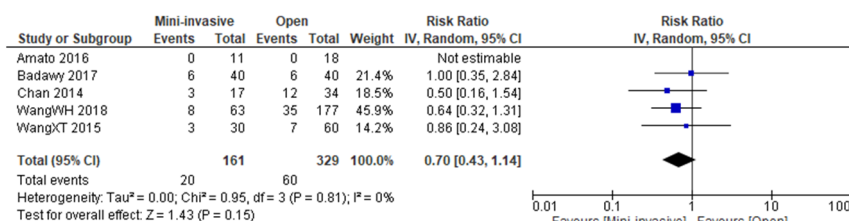
Tumor size (mm)



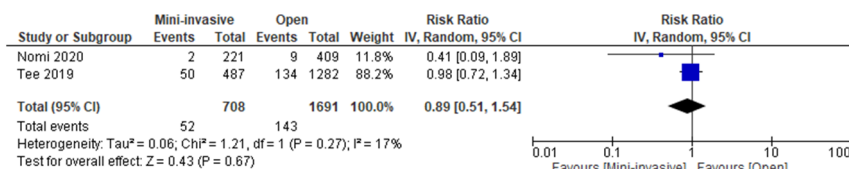
Patients with one lesion



Patients with multiple lesions



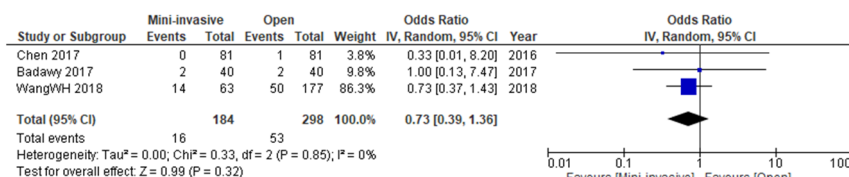
Readmission



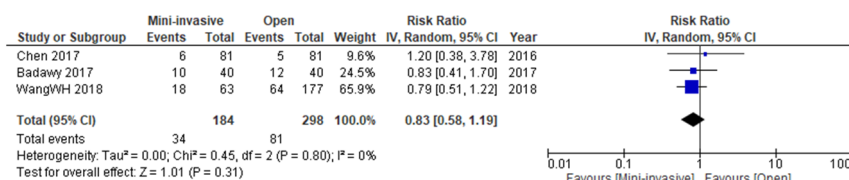
Recurrence



Survival at 1 year



Survival at 3 years



Survival at 5 years

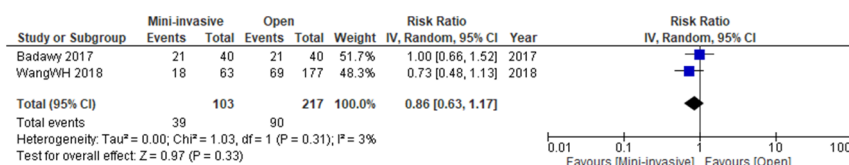


Figure 3. Meta-analyses of included studies on secondary outcomes

demonstrated a risk reduction of 45% for the mini-invasive group with respect to the open one, although not significant ($P = 0.06$) and with moderate heterogeneity ($I^2 = 57\%$). However, the number of studies for each outcome was too low to evaluate publication bias.

DISCUSSION

The management of HCC in elderly patients is multidisciplinary with a wide range of treatment options ranging from liver resection, liver transplantation, loco-regional therapies including ablation and transarterial-chemoembolization, to molecular-targeting therapies^[26]. The right patient allocation is determined by many factors including clinical characteristics, tumor burden, and multidisciplinary staff expertise^[27]. Elderly patients have increased comorbidities including cardiovascular disease, pulmonary disease, diabetes mellitus, and renal insufficiency: these are conditional factors for outcome after surgical therapy as compared to the younger population^[28]. Mini-invasive liver surgery represents a particular challenge for elderly patients affected by cardiopulmonary disease. Carbon dioxide pneumoperitoneum may result in acid-base disturbance with acidosis^[29] and the increase of intra-abdominal pressure may result in a decrease in lung compliance, vital capacity, venous return and vascular perfusion of intra-abdominal organs^[30].

In the last 10 years, improvement of perioperative care, careful patient selection and the presence of strong clinical evidence of benefits have increased the application of laparoscopic procedures in elderly patients. Several studies have reported on the safety and reduced postoperative morbidity and mortality in laparoscopic surgery in elderly patients^[31,32]. Randomized trials, multicenter trials, systematic reviews, and meta-analyses about laparoscopic colorectal resection in the elderly indicate a real benefit in terms of lower risk of blood transfusion, postoperative complications and oncological outcome. Longer operative time and pneumoperitoneum seem to promote short-term pulmonary and/or cardiac complications^[33-36].

Surgical resection is a potentially curative option for the elderly patient. Several meta-analyses^[37,38] have shown that laparoscopic and robotic liver resection is associated with faster recovery, less postoperative pain and shorter hospital stay when compared with open liver resection.

Although the elderly could have a more complex clinical profile and a number of fragilities, age is not an absolute contraindication to liver surgery. The Barcelona Clinic Liver Cancer staging and treatment algorithm recommend surgical resection as elective treatment without difference between young or elderly^[39]. Nevertheless, the correct determination of which patients in the elderly group would benefit from surgical therapy is the most important clinical challenge. Poor liver function, portal hypertension, important comorbidities and cirrhosis stage are the true selection criteria for the right therapy and are helpful for identifying unfit patients.

Many studies have already demonstrated the feasibility of liver resections by the open approach in elderly patients including those suffering from other concomitant diseases^[40], but the role of the mini-invasive approach (laparoscopic or robotic) in the surgical management of HCC is under investigation.

This systematic review focused on the elderly population affected by HCC to assess if MILR may be safe and feasible in this group of fragile patients. In this study, we included eight primary studies with a total of 3051 patients undergoing liver resection; 950 were treated by MILR and 2101 by OLR. Using these data, we performed twenty-one meta-analyses investigating the main clinical and oncological outcomes of relevance. Regarding the functional selection criteria for MILR or OLR in HCC patients, all papers^[18-25] reported that the only patients considered eligible were those with well-compensated cirrhosis or liver function without severe portal hypertension or bilirubin level out of normal range. They were essentially identical in both groups (OLR and MILR), because a careful patient selection and a complete liver function assessment were mandatory in these patients. Of all meta-analyses investigated, only 8 patients in the OLR group^[18,19,22,24] and only 2

patients in the MILR group^[18,24] were identified as Child-Pugh score B. These data, as shown in Figure 2A, however represented a study limitation since the present meta-analysis was not able to find statistically significant results.

Meta-analyses demonstrated that in elderly patients MRL had similar organ failure, mortality and readmission rate as compared to the open approach. MILR can be safe in the elderly because it requires less sacrifice of liver tissue and has better bleeding control and lower rate of intermittent Pringle maneuver and because it can treat multiple lesions at the same time, especially in anterior segments. However, there are cases where complete MILR is not possible and use of ablative therapy combined with surgery increase oncological outcome^[41].

Nomi *et al.*^[21] demonstrated in their series that MILR was safer and more feasible when compared with OLR, even in octogenarian patients. This study was the first multicenter, propensity score-matched study to show better short-term outcomes with MILR than with OLR in elderly patients with HCC. These authors performed a subgroup analysis according to patient's age (group 75-79 compared with group > 80) and dividing patients in relation to treatment (MILR - 78 patients and OLR - 147 patients). In the cohort > 80, the major complication rate and LOS were significantly lower in the MILR group than OLR group. Furthermore, in the MILR group, the study reported both a 90-day mortality rate and transfer to rehabilitation facility rate of 0% in the MILR group. These data suggested that mini-invasive surgery was less invasive and was associated with early recovery in elderly patients.

In our analysis, morbidity rate according to the Clavien-Dindo classification, LOS and intraoperative blood loss were lower in the mini-invasive group with high statistical impact. These findings were consistent with many studies and meta-analyses on major resection^[42-44]. The Southampton Guidelines reported that the laparoscopic approach was found to be the only independent factor to reduce the complication rate in resections for HCC^[45]. In cirrhotic patients, the laparoscopic approach reduces the incidence of postoperative ascites, liver failure and morbidity assessed in terms of "Comprehensive Complication Index"^[45-47]. Blood loss and transfusion rate are very important prognostic factors in liver surgery^[48]. Morbidity rate reduction demonstrated by our meta-analysis in patients undergoing MRL could be explained by many factors. First, pneumoperitoneum with abdominal negative pressure decreased portal flow rate and reduced the small and continuous venous bleeding during the parenchymal transection phase^[49]. Second, the use of an energy instrument for transection of liver parenchyma has proved to be highly effective for hemostasis^[50]. Moreover, the absence of a large abdominal skin incision reduces muscle wall bleeding, and finally, laparoscopy and robotic technology offer an optimal magnified and three-dimensional view, which are important surgical factors for meticulous hemostasis as well as for greatly facilitating parenchymal transection in cirrhotic livers^[51].

However, one of the major limitations of our meta-analysis could be that surgical indications to MILR were selected at the center's discretion according to surgical procedure complexity rather than by defined criteria. All authors included in this meta-analysis always reported the principles guiding patient selection to undergo MRL were according to the International Position on Mini-Invasive Liver Surgery agreement of Louisville (2008) or Morioka Guidelines (2014)^[9,10,24], tumor size^[24] and tumor location^[18,19,20,22,24]. An important point that needs to be investigated is that all papers reported many minor liver resections in the MILR group rather than in the OLR group. However, it remains uncertain if the same short and long benefits could be extended to elderly patients with major anatomical resection involving larger parenchymal transection area or longer operative time. Wang *et al.*^[24] found in their study that 38% of HCC cases in the robotic assisted group were located in challenge segments, but they never performed a major hepatectomy in the MILR group. The large number of minor resections, wedge or segmentectomies, suggested that a parenchymal sparing strategy and R0 resections are however basic and main guidelines for treatment when using a mini-invasive technique. This means that the mini-invasive cohort included in this paper was certainly not previously highly selected because all authors, as stated, followed international guidelines.

Amato *et al.*^[20] wrote that the main factor that would contribute to decreased blood loss might be the tumor position in anterior segments. Challenge segment resection in their series was performed only with the open approach. This selection might have had significant effects in reducing severe bleeding risks, but the robotic approach can represent the ideal overlap technique to overcome the bias in their study^[52].

Pulmonary and cardiovascular failure after liver resection might be very dangerous in the elderly. The incidence range has been reported to be from 10% to 20%^[53], and they are related to functional changes in old age^[54,55] but also to intraoperative fluid overload^[53]. Some conditions such as a lower morbidity rate or a lower intraoperative blood loss in the MILR group might contribute to reduced fluid administration during liver resection. Thus, the absence of large abdominal incision might increase thoracic cage excursion and decrease the pain without respiratory distress. This might be associated with enhanced postoperative recovery and shorter hospital stay.

MILR reduces LOS rate because the absence of large abdominal incision and preservation of postoperative pulmonary function may explain less minor postoperative complications in the MILR group^[56]. However, careful patient selection about assessment of liver function is the most important factor in morbidity prevention.

This report reveals that operative time in the MILR group was longer than OLR group. The learning curve was associated with experience of surgeons and might be a significant factor contributing to the difference in operative time for the mini-invasive group. The robotic approach, in the MILR group, was associated with longer operative time. This can be explained by the large proportion of major hepatectomy or challenge segment approach, and especially for additional time required for docking and de-docking of robotic system. Tsung *et al.*^[57] found that operative time decreased significantly as the number of cases accumulated and increase of experience with robotic liver surgery.

Oncological outcome such as tumor recurrence and survival did not differ significantly between the two groups, but this outcome was investigated in only half of studies^[18,24,25]. Recurrence rate is a very important prognostic element. It is essential for improving long-term prognosis, and it is related to tumor-free margins in oncological surgeries, because histologically negative margins could result in a better outcome after HCC resection^[38]. For patients with HCC, clinical and oncological outcomes are conditioned by tumor invasiveness and underlying liver disease^[58]. The risk of recurrence of HCC after liver resection is always a concern and is common with the diseased liver remaining in situ. Perhaps not surprisingly, recurrence and survival after surgery for HCC has been shown to be shorter in patients with advanced cirrhosis compared with patients with early disease. The higher recurrence rate during the worsening of the disease probably reflects the carcinogenic effect of advanced cirrhosis, being more prominent than in less cirrhotic livers or in chronic hepatitis, which is well established in the literature^[59]. Therefore, MILR for HCC provided long-term outcomes that were comparable with OLR and did not generate unusual HCC recurrence patterns.

Study limitation

There were several limitations in this systematic review. First, the literature search was only done on the two most relevant scientific databases for medical practice (PubMed and Cochrane Library). Second, the review was limited by the lack of randomized controlled studies or prospective studies regarding comparable populations. Indeed most of the studies on this topic were observational and retrospective, although some of them^[18,21,23,25] minimized selection bias, performing a matching of the populations studied.

Due to no events in small sample size papers, or outcomes not available in the primary studies, few studies were available in some of the meta-analyses, thus limiting the strength and trustworthiness of our results.

Meta-analyses are characteristically limited by the presence of heterogeneity between studies. Sources of heterogeneity in this review were different patient's age cut-off, different percentages of HCC patients, and

different countries of studies. We incorporated heterogeneity by performing random effect model meta-analyses. On the contrary, the small numbers of the studies included did not allow us to further explore heterogeneity with subgroup analyses and meta-regression.

Moreover, due to the limited number of the studies included in the quantitative analyses, we were unable to properly verify if publication bias was present. Finally, we observed that our systematic review pooled papers with different study populations. Indeed, two reports included a small number of patients^[19,20]; however, on the other hand, two main studies analyzed very large populations^[21,23].

To conclude, the scientific literature shows the presence of other systematic reviews on this topic. However, all these secondary studies are characterized by different study selection criteria, outcomes, and populations. Consequently, we believe that our paper could add value to the HCC surgical literature, especially because it assessed a very large number of key outcomes.

In conclusion, this study provides an overview of the last ten years about the comparison between MILR and OLR for HCC treatment in elderly patients. Meta-analyses confirmed the advantages of MILR, both laparoscopic and robotic, in terms of perioperative outcomes, where it may promote the extension of liver resection to HCC patients with borderline liver function. Specifically, our results showed shorter LOS, less intraoperative blood loss and lower morbidity rate in MILR. Moreover, major resections were significantly more common in the OLR group compared to the MILR group. There were no significant differences in survival and recurrence outcomes between the two groups.

According to our results, MILR, which minimizes surgical trauma, must be considered as an important treatment option with significant quality of life benefits in the elderly, showing hopefully one of its best advantages in this fragile population. Efforts should be made to avoid as much as possible OLR in this population. However, randomized controlled trials or well-designed large prospective comparative studies would be necessary to definitely support the superiority of MILR in elderly patients with HCC.

DECLARATIONS

Authors' contributions

Study conception and design of the work: Brolese A, Ciarleglio FA

Literature search, acquisition, selection and reading: Brolese A, Rigoni M, Vitale A, de Pretis G, Avancini I, Pravadelli C, Frisinghelli M, Rozzanigo U, Luppi G, Dionisi F, Marcucci S, Viel G, Beltempo P, Prezzi C, Frisini M, Brolese M, Nollo G, Ciarleglio FA

Screening of the papers and data extraction from the selected studies: Rigoni M, Ciarleglio FA

Data analysis and statistical evaluation: Rigoni M, Nollo G

Interpretation of data for the work: Brolese A, Rigoni M, Vitale A, Nollo G, Ciarleglio FA

Drafting the work or revising it critically for important intellectual content: Brolese A, Rigoni M, Ciarleglio FA

Final approval of the version to be published: Brolese A, Rigoni M, Nollo G, Ciarleglio FA

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the cited current literature or websites (PubMed and Cochrane).

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Benson AB, Abrams TA, Ben-Josef E, Bloomston PM, Botha JF, et al. NCCN clinical practice guidelines in oncology: hepatobiliary cancers. *J Natl Compr Canc Netw* 2009;7:350-91.
2. Kabbach G, Assi HA, Bolotin G, Schuster M, Lee HJ, et al. Hepatobiliary tumors: update on diagnosis and management. *J Clin Transl Hepatol* 2015;3:169-81.
3. Italian Association of Medical Oncologist (AIOM) Guidelines 2019- Hepatocellular Carcinoma (HCC) (in Italian). Available from: <https://www.aiom.it/linee-guida-aiom-epatocarcinoma-2019/> [Last accessed on 25 May 2020]
4. Italian Association Of Tumor Registry (AIRTUM) - Cancer Numer in Italy 2019 (in Italian). Available from: https://www.aiom.it/wp-content/uploads/2019/09/2019_Numeri_Cancro-operatori-web.pdf [Last accessed on 25 May 2020]
5. Italian Association of Medical Oncologist (AIOM) Guidelines 2019 - Cancer in Elderly (in Italian). Available from: <https://www.aiom.it/linee-guida-aiom-tumori-dellanziano-2019/> [Last accessed on 25 May 2020]
6. De Angelis R, Sant M, Coleman MP, Francisci S, Baili P, et al. Cancer Survival in Europe 1999-2007 by country and age: results of EURO-CARE -5- a population based study. *Lancet Oncol* 2014;15:23-34.
7. Yamada S, Shimada M, Miyake H, Utsunomiya T, Morine Y, et al. Outcome of hepatectomy in super-elderly patients with hepatocellular carcinoma. *Hepatol Res* 2012;42:454-8.
8. Nguyen KT, Marsh JW, Tsung A, Steel JJ, Gamblin TC, et al. Comparative benefits of laparoscopic vs open hepatic resection: a critical appraisal. *Arch Surg* 2011;146:348-56.
9. Buell JF, Cherqui D, Geller DA, O'Rourke N, Iannitti D, et al. The international position on laparoscopic liver surgery: the Louisville Statement, 2008. *Ann Surg* 2009;250:825-30.
10. Wakabayashi G, Cherqui D, Geller DA, Buell JF, Kaneko H, et al. Recommendations for laparoscopic liver resection: a report from the second international consensus conference held in Morioka. *Ann Surg* 2015;261:619-29.
11. Doussot A, Lim C, Lahat E, Salloum C, Osseis M, et al. Complications after hepatectomy for hepatocellular carcinoma independently shorten survival: a western, single-center audit. *Ann Surg Oncol* 2017;24:1569-78.
12. Nishikawa H, Kimura T, Kita R, Osaki Y. Treatment for hepatocellular carcinoma in elderly patients: a literature review. *J Cancer* 2013;4:635-43.
13. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* 2010;8:336-41.
14. Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, et al. The Clavien-Dindo classification of surgical complications: five-year experience. *Ann Surg* 2009;250:187-96.
15. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol* 2005;5:13.
16. Deeks JJ, Higgins JPT, Altman DJ. Analysing data and undertaking meta-analyses. In: Higgins JPT, Green S, editors. *Cochrane Handbook for Systematic Reviews of Interventions*. Chichester: Wiley & Sons Ltd; 2008. p. 243-93.
17. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Controlled Clinical Trials* 1986;7:177-88.
18. Badawy A, Seo S, Toda R, Fuji H, Fukumitsu K, et al. A propensity score-based analysis of laparoscopic liver resection for liver malignancies in elderly patients. *J Invest Surg* 2017;32:75-82.
19. Chan ACY, Poon RTP, Cheung TT, Chok KSH, Dai WC, et al. Laparoscopic versus open liver resection for elderly patients with malignant liver tumors: a single-center experience. *J Gastroenterol Hepatol* 2014;29:1279-83.
20. Amato B, Aprea G, De Rosa D, Milone M, di Domenico L, et al. Laparoscopic hepatectomy for HCC in elderly patients: risks and feasibility. *Aging Clin Exp Res* 2016;29:179-83.
21. Nomi T, Hirokawa F, Kaibori M, Ueno M, Tanaka S, et al. Laparoscopic versus open liver resection for hepatocellular carcinoma in elderly patients: a multi-centre propensity score-based analysis. *Surg Endosc* 2020;34:658-66.
22. Wang XT, Wang HG, Duan WD, Wu CY, Chen MY, et al. (2015) Pure laparoscopic versus open liver resection for primary liver carcinoma in elderly patients: a single-center, case-matched study. *Medicine (Baltimore)* 2015;94:e1854.
23. Tee MC, Chen L, Peightal D, Franko J, Kim PT, et al. Minimally invasive hepatectomy is associated with decreased morbidity and resource utilization in the elderly. *Surg Endosc* 2019; Epub ahead of print. doi: 10.1007/s00464-019-07298-5.
24. Wang WH, Kuo KK, Wang SN, Lee KT. Oncological and surgical result of hepatoma after robot surgery. *Surg Endosc* 2018;32:3918-24.
25. Chen PD, Wu CY, Hu RH, Chou WH, Lai HS, et al. Robotic versus open hepatectomy for hepatocellular carcinoma: a matched comparison. *Ann Surg Oncol* 2017;24:1021-8.
26. Hung AK, Guy J. Hepatocellular carcinoma in the elderly: meta-analysis and systematic literature review. *World J Gastroenterol* 2015;21:12197-210.
27. Chen KW, Ou TM, Hsu CW, Horng CT, Lee CC, et al. Current systemic treatment of hepatocellular carcinoma: a review of the literature.

- World J Hepatol 2015;7:1412-20.
28. Tsujita E, Utsunomiya T, Yamashita Y, Ohta M, Tagawa T, et al. Outcome of hepatectomy in hepatocellular carcinoma patients aged 80 years and older. *Hepatogastroenterology* 2012;59:1553-5.
 29. O'Malley C, Cunningham AJ. Physiologic changes during laparoscopy. *Anesthesiol Clin N Am* 2001;19:1-19.
 30. Demyttenaere S, Feldman LS, Fried GM. Effect of pneumoperitoneum on renal perfusion and function: a systematic review. *Surg Endosc* 2007;21:152-60.
 31. Antoniou SA, Antoniou GA, Koch OO, Pointner R, Granderath FA. Meta-analysis of laparoscopic vs open cholecystectomy in elderly patients. *World J Gastroenterol* 2014;20:17626-34.
 32. Kunisaki C, Makino H, Takagawa R, Oshima T, Nagano Y, et al. Efficacy of laparoscopy-assisted distal gastrectomy for gastric cancer in the elderly. *Surg Endosc* 2009;23:377-83.
 33. Fujii S, Ishibe A, Ota M, Yamagishi S, Watanabe K, et al. Short-term results of a randomized study between laparoscopic and open surgery in elderly colorectal cancer patients. *Surg Endosc* 2014;28:466-76.
 34. Hinoi T, Kawaguchi Y, Hattori M, Okajima M, Ohdan H, et al.; Japan Society of Laparoscopic Colorectal Surgery. Laparoscopic versus open surgery for colorectal cancer in elderly patients: a multicenter matched case-control study. *Ann Surg Oncol* 2015;22:2040-50.
 35. Fujii S, Tsukamoto M, Fukushima Y, Shimada R, Okamoto K, et al. Systematic review of laparoscopic vs open surgery for colorectal cancer in elderly patients. *World J Gastrointest Oncol* 2016;8:573-82.
 36. Devoto L, Celentano V, Cohen R, Khan J, Chand M. Colorectal cancer surgery in the very elderly patient: a systematic review of laparoscopic versus open colorectal resection. *Int J Colorectal Dis* 2017;32:1237-42.
 37. Yin Z, Fan X, Ye H, Yin D, Wang J. Short and long term outcomes after laparoscopic and open hepatectomy for hepatocellular carcinoma: a global systematic review and meta-analysis. *Ann Surg Onc* 2013;20:1203-15.
 38. Guan R, Chen Y, Yang K, Ma D, Gong X, et al. Clinical efficacy of robot assisted versus laparoscopic liver resection: a meta analysis. *Asian J Surg* 2019;42:19-31.
 39. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53:1020-2.
 40. Adam R, Frilling A, Elias D, Laurent C, Ramos E, et al. Liver resection of colorectal metastases in elderly patients. *Br J Surg* 2010;97:366-76.
 41. Glassberg MB, Ghosh S, Clymer JW, Wright GWJ, Ferko N, et al. Microwave ablation compared with hepatic resection for the treatment of hepatocellular carcinoma and liver metastases: a systematic review and meta-analysis. *World J Surg Oncol* 2019;17:98.
 42. Xiong JJ, Altaf K, Javed MA, Huang W, Mukherjee R, et al. Meta-analysis of laparoscopic vs open liver resection for hepatocellular carcinoma. *World J Gastroenterol* 2012;18:6657-68.
 43. Takahara T, Wakabayashi G, Beppu T, Aihara A, Hasegawa K, et al. Long-term and perioperative outcomes of laparoscopic versus open liver resection for hepatocellular carcinoma with propensity score matching: a multi-institutional Japanese study. *J Hepatobiliary Pancreat Sci* 2015;22:721-7.
 44. Yoon YI, Kim KH, Kang SH, Kim WJ, Shin MH, et al. Pure laparoscopic versus open right hepatectomy for hepatocellular carcinoma in patients with cirrhosis: a propensity score matched analysis. *Ann Surg* 2016;265:856-63.
 45. Abu Hilal M, Aldrighetti L, Dagher I, Edwin B, Troisi RI, et al. The southampton consensus guidelines for laparoscopic liver surgery: from indication to implementation. *Ann Surg* 2018;268:11-8.
 46. Zhang Y, Huang J, Chen XM, Sun DL. A comparison of laparoscopic versus open left hemihepatectomy for hepatocellular carcinoma. *Surg Laparosc Endosc Percutan Tech* 2016;26:146-9.
 47. Morise Z, Ciria R, Cherqui D, Chen KH, Belli G, et al. Can we expand the indications for laparoscopic liver resection? A systematic review and meta-analysis of laparoscopic liver resection for patients with hepatocellular carcinoma and chronic liver disease. *J Hepatobiliary Pancreat Sci* 2015;22:342-52.
 48. Liu L, Wang Z, Jiang S, Shao B, Liu J, et al. Perioperative allogeneic blood transfusion is associated with worse clinical outcomes for hepatocellular carcinoma: a meta-analysis. *PLoS One* 2013;8:e64261.
 49. Morise Z. Developments and perspectives of laparoscopic liver resection in the treatment of hepatocellular carcinoma. *Surg Today* 2019;49:649-55.
 50. Miyazawa M, Aikawa M, Okada K, Watanabe Y, Okamoto K, et al. Laparoscopic liver resection using a monopolar soft-coagulation device to provide maximum intraoperative bleeding control for the treatment of hepatocellular carcinoma. *Surg Endosc* 2018;32:2157-8.
 51. Levi Sandri GB, Ettorre GM, Aldrighetti L, Cillo U, Dalla Valle R, et al.; I Go MILS Group on HCC. Laparoscopic liver resection of hepatocellular carcinoma located in unfavorable segments: a propensity score-matched analysis from the I Go MILS (Italian Group of Minimally Invasive Liver Surgery) Registry. *Surg Endosc* 2019;33:1451-8.
 52. Nota C, Molenaar IQ, van Hillegeersberg R, Borel Rinckes IHM, Hagendoorn J. Robotic liver resection including the posteriosuperior segments: initial experience. *J Surg Res* 2016;206:133-8.
 53. Fuks D, Cauchy F, F  riche S, Nomi T, Schwarz L, et al. Laparoscopy decreases pulmonary complications in patients undergoing major liver resection: a propensity score analysis. *Ann Surg* 2016;263:353-61.
 54. Lentschener C, Ozier Y. Anaesthesia for elective liver resection: some points should be revisited. *Eur J Anaesthesiol* 2002;19:780-8.
 55. Ford GT, Rosenal TW, Clergue F, Whitelaw WA. Respiratory physiology in upper abdominal surgery. *Clin Chest Med* 1993;14:237-52.
 56. Nanashima A, Abo T, Nonaka T, Fukuoka H, Hidaka S, et al. Prognosis of patients with hepatocellular carcinoma after hepatic resection: are elderly patients suitable for surgery? *J Surg Oncol* 2011;104:284-91.
 57. Tsung A, Geller DA, Sukato DC, Sabbaghian S, Tohme S, et al. Robotic versus laparoscopic hepatectomy: a matched comparison. *Ann Surg* 2014;259:549-55.
 58. Bruix J, Reig M, Sherman M. Evidence-based diagnosis staging, and treatment of patients with hepatocellular carcinoma. *Gastroenterology* 2016;150:835-53.
 59. Taura K, Ikai I, Hatano E, Yasuchika K, Nakajima A, et al. Influence of coexisting cirrhosis on outcomes after partial hepatic resection for hepatocellular carcinoma fulfilling the Milan criteria: an analysis of 293 patients. *Surgery* 2007;142:685-94.

Review

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Emerging risk factors for nonalcoholic fatty liver disease associated hepatocellular carcinoma

Jihane N. Benhammou¹, Jonathan Lin², Shehnaz K. Hussain^{3,4}, Mohamed El-Kabany¹

¹Pfleger Liver Institute, University of California Los Angeles, Los Angeles, CA 90095, USA.

²Vatche and Tamar Manoukian Division of Digestive Diseases, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA.

³Department of Epidemiology, Fielding School of Public Health, University of California, CA 90095, USA.

⁴Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA.

Correspondence to: Dr. Mohamed El-Kabany, Pfleger Liver Institute, 200 Medical Plaza, Los Angeles, CA 90095, USA.
E-mail: melkabany@mednet.ucla.edu

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Abstract

Worldwide, nonalcoholic fatty liver disease (NAFLD) has reached epidemic proportions and in parallel, hepatocellular carcinoma (HCC) has become one of the fastest growing cancers. Epidemiological studies have not only shed light on the prevalence and incidence of the disease but have also unmasked important environmental risk factors, including the role of diabetes and dyslipidemia in disease pathogenesis. Genetic association studies have identified single nucleotide polymorphisms implicated in NAFLD-HCC, many of which are part of lipid metabolism pathways. Through these clinical studies and subsequently, translational and basic research, the role of statins as a chemoprotective agent has also emerged with ongoing clinical trials assessing their utility in HCC prevention and treatment. In this review, we summarize the recent epidemiological studies describing the burden of NAFLD-HCC in different patient populations and countries. We discuss the genetic and environmental risk factors for NAFLD-HCC and highlight the chemoprotective role of statins and aspirin. We also summarize what is known about NAFLD-HCC in the cirrhosis and non-cirrhosis populations and briefly address the role of surveillance in NAFLD-HCC patients.

Keywords: Nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, hepatocellular carcinoma, statins, metabolic syndrome

INTRODUCTION

The metabolic syndrome (MetS), defined by the clustering of biochemical and clinical features, which includes type 2 diabetes (T2D), hypertension, dyslipidemia and obesity, has reached epidemic proportions^[1].



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Table 1. NAFLD-associated HCC epidemiology and burden

Country	Incidence and prevalence	Population	Study period	Ref.
United States	0.21 per 1000 person-years	Veterans Affairs	2003-2011	Kanwal <i>et al.</i> ^[22]
	14.1% of all cases	SEER registries	2004-2009	Younossi <i>et al.</i> ^[136]
	8% of all HCC cases	Veterans Affairs	2005-2011	Mittal <i>et al.</i> ^[23]
	1.56% of HCC cases	Veterans Affairs	2012-2018	Ioannou <i>et al.</i> ^[24]
	5.29 per 1000 person-years	Meta-analysis	1989-2015	Younossi <i>et al.</i> ^[3]
	13.5% of all liver transplants	United Network Organ for Sharing	2000-2012	Wong <i>et al.</i> ^[26]
Spain, Italy, the Netherlands, United Kingdom	0.3 per 1000 person-years	European primary care databases	2016	Alexander <i>et al.</i> ^[30]
United Kingdom	35% of all HCC referrals	National Health Services	2010	Dyson <i>et al.</i> ^[137]
Japan	6% incidence	Single hospital in Tokyo	1994-2007	Arase <i>et al.</i> ^[138]
South Korea	12.2% incidence	South Korean hospital	2006-2010	Cho <i>et al.</i> ^[34]

NAFLD: nonalcoholic fatty liver disease; HCC: hepatocellular carcinoma; SEER: surveillance, epidemiology and end results

Non-alcoholic fatty liver disease (NAFLD), the liver manifestation of MetS, has increased in parallel and is now the most common cause of liver disease in the United States^[2]. Although the true prevalence of NAFLD remains unknown given the lack of validated and/or recommended screening practices, it is estimated that the disease affects about a quarter of the world's population, depending on geographical differences^[3]. NAFLD can progress to nonalcoholic steatohepatitis (NASH)^[3] (characterized by $\geq 5\%$ of hepatic steatosis with lobular inflammation and hepatocyte ballooning^[4]), cirrhosis and hepatocellular carcinoma (HCC)^[5,6]. Given the estimated increase in NAFLD, NASH and NAFLD-associated HCC^[7] and the anticipated burden on health care costs^[8-10], several studies have focused on understanding the clinical and biological drivers of NAFLD-associated HCC and its potential treatment options.

Understanding this disease process is especially relevant since NAFLD-associated HCC can occur in a non-cirrhotic background^[11-13]. This poses a clinical dilemma given the lack of screening guidelines for this subgroup of patients, thus prompting the need for further understanding of the natural history of NAFLD-HCC and identifying at-risk populations who would benefit from screening. More recently, studies have also identified the protective effects of statins and aspirin on fibrosis progression and HCC^[14-17], providing an avenue for further research in this group of patients who are also at high risk for cardiovascular disease.

A full review and discussion of the pathophysiology of NAFLD and NASH is beyond the scope of this report and has been summarized by Anstee *et al.*^[18]. In this review, we explore what is known about the genetic (non-modifiable) and environmental (modifiable) risk factors of NAFLD-associated HCC, examine the role of statins and aspirin and what microbiome research has to offer in the field of NAFLD-related HCC.

BURDEN OF NAFLD-ASSOCIATED HCC

HCC is a lethal cancer with a rising incidence over the last 30 years^[19]. Its incidence is increasing most rapidly of any cancer, with an age-adjusted annual increase of 3.8% and 2.8% in men and women in the U.S., respectively^[20]. The rising HCC burden has largely been attributed to the rise in obesity and diabetes^[21]. As follows, several epidemiological studies have specifically examined the incidence and risk of NAFLD-associated HCC [Table 1]. The results have been varied however, due to differences between the studies in patient population, time-period, and NAFLD and/or NASH ascertainment. For example, in a large Veterans Affairs (VA) Health System study between 2003-2011, the incidence of HCC in a NAFLD cohort was 0.21 per 1000 person-years^[22]. A separate study within the VA further demonstrated that of the 1500 HCC cases identified from 2005-2011, NAFLD was the underlying risk factor in 8% of all cases with an annual proportion of NAFLD-related HCC ranging from 7.5%-12.0%^[23]. Ioannou *et al.*^[24] also reported that the incidence of NAFLD-associated HCC was 1.56% within the VA from 2012-2018 over a 3.7 years follow up period. In non-VA populations, the incidence rate for NAFLD-associated HCC and NASH-associated HCC were 0.44 and 5.29 per 1000 person-years, respectively^[3].

Changes in liver transplantation (LT) indications are also reflective of the increasing rates of NAFLD. For instance, Younossi *et al.*^[25] demonstrated that of 158,347 LT candidates from 2002-2016, the prevalence of

NAFLD-associated HCC increased by 11.8 fold, which was higher than hepatitis B, hepatitis C and alcoholic liver disease. Other studies have also corroborated that NAFLD and NAFLD-associated HCC are the most rapidly growing indications for LT^[26,27].

Other countries in Europe and Asia have made similar observations to these U.S.-based studies^[28,29]. In a European Electronic Health Record study of patients seen in primary care in Spain, Italy, the Netherlands and the United Kingdom, the incidence of NAFLD-associated HCC diagnosis was 0.3 per 1000 person-years over a median follow up of 3.3 years^[30]. A study of the European Liver Transplant Registry database from 2002-2016 also showed that among the 68,950 transplant patients, 8.4% were for NASH in 2016 (compared to 1.3% in 2002), 39% of whom had HCC^[31]. In Asian countries, where hepatitis B has been the main driver of liver disease and HCC, the prevalence of NAFLD is also about 25% depending on the country studied, ranging from 6.2% in South China to 51% in Indonesia^[3,32,33]. NAFLD-associated HCC has also increased in countries such as South Korea where its prevalence rose from 3.8% (2001-2005) to 12.2% (2006-2010)^[34].

Although a rise in sedentary life styles leading to increase in MetS and diabetes are thought to be the culprits in NAFLD-associated HCC, Asian patients are more likely to have “lean” or “non-obese” NAFLD, potentially representing a pathophysiologically different group of patients from those seen in Western countries. Few studies have addressed this although a recent European study assessing the outcomes of biopsy-proven lean NAFLD (BMI < 25 kg/cm²; *n* = 123) patients compared to healthy controls, overweight NAFLD (BMI 25-30 kg/cm²; *n* = 335) and obese NAFLD patients (BMI > 30 kg/cm²; *n* = 188) demonstrated that lean NAFLD patients had a tendency towards more liver-related complications including cirrhosis, decompensated cirrhosis and HCC over a follow-up period of 19.9 years^[35].

In summary, the burden of NAFLD-HCC is on the rise with the prevalence of NAFLD, depending on geographical and ethnic differences, affecting about a quarter of the world's population. Although NASH is thought to be a more severe form of NAFLD that more commonly progresses to chronic liver disease and HCC, the true prevalence of NAFLD (6.2%-51%) and NASH (10%-20%), based on large epidemiological data, remains unclear given that no specific biomarkers exist to differentiate the two other than a liver biopsy, which is scarcely performed, yet the gold standard for diagnosis.

GENETIC RISK FACTORS FOR NAFLD-HCC

There is a large body of literature linking genetic polymorphisms to the development of NAFLD and NASH but few have addressed the genetic contributions to NAFLD-associated HCC [Table 2]. Early studies in the field of NAFLD identified ethnic differences in disease prevalence whereby Hispanics were the most commonly affected group followed by Caucasians and African Americans^[36-38], suggesting a genetic predisposition to NAFLD. Consistent with these findings, twin studies and phenotypic clustering of fatty liver were also more commonly seen in patients in the same family, suggesting heritability of the disease, which has been reported to range from 38%-50%, depending on the modality used for phenotyping of NAFLD (biopsy, MRI or abdominal ultrasound)^[39,40]. In recent years, large genome-wide studies have followed and revolutionized what is known about NAFLD and NASH, and possibly the risk for HCC development.

Romeo *et al.*^[41] conducted the first genome-wide association study in the Dallas Heart Study using proton magnetic resonance spectroscopy to quantitate hepatic steatosis as the phenotype. Patatin-like phospholipase domain 3, PNPLA3 (rs738409), was the single variant strongly associated with hepatic steatosis. Although the mechanism by which PNPLA3 leads to hepatic steatosis accumulation remains unknown, it was shown to play a role in hepatocellular lipid droplet remodeling and very low-density lipoprotein secretion^[42,43]. Interestingly, subsequent association studies of PNPLA3 also demonstrated that the variant was associated with histological severity of the disease, thus suggesting that it may influence HCC development, where GG sequence carriers had more necroinflammation and fibrosis compared to CC carriers^[44]. Indeed, Liu *et al.*^[45],

Table 2. Summary of NAFLD HCC polymorphisms

SNP	Clinical significance	Location (human chromosome)	Associated gene	Role of the gene	Ref.
rs738409 C>G	Associated with increased liver fat accumulation and a higher risk for developing liver cirrhosis. Given the increased severity of necroinflammation in GG sequence carriers, these individuals may be at higher risk for developing HCC	chr22:43928847 (GRCh38.p12)	<i>PNPLA3</i> ; Patatin-like phospholipase domain-containing protein 3	The <i>PNPLA3</i> gene encodes the protein Adiponutrin, which is thought to help regulate the development of adipocytes as well as lipogenesis and lipolysis in the liver	Sookoian <i>et al.</i> ^[44] Shen <i>et al.</i> ^[139]
rs58542926 C>T	Associated with an increased risk of developing diabetes and NAFLD. The polymorphism results in a loss of function and individuals with the CT phenotype have a reduced hepatic capability to secrete LDLs and are at higher risk for developing liver inflammation and potentially HCC	chr19:19268740 (GRCh38.p12)	<i>TM6SF2</i> ; Transmembrane 6 Superfamily Member 2	The <i>TM6SF2</i> gene is involved in regulating liver fat metabolism, lipoprotein secretion and hepatic lipid droplet content	Kozlitina <i>et al.</i> ^[46] Falleti <i>et al.</i> ^[47] Vespasiani-Gentilucci <i>et al.</i> ^[140]
rs641738 C>T	Associated with an increased risk of hepatic fat accumulation, fibrosis, and potentially HCC	chr19:54173068 (GRCh38.p12)	<i>MBOAT7</i> ; Membrane Bound O-Acyltransferase Domain Containing 7	The <i>MBOAT7</i> gene encodes a protein known as Lysophospholipid acyltransferase 7 that is involved in the re-acylation of phospholipids as part of the phospholipid remodeling pathway	Mancina <i>et al.</i> ^[48] Luukkonen <i>et al.</i> ^[49] Donati <i>et al.</i> ^[50]

NAFLD: nonalcoholic fatty liver disease; HCC: hepatocellular carcinoma; LDLs: low density lipoproteins

demonstrated that in a Northern European Caucasian cohort of patients with primary HCC attributed to NAFLD, carriage of the *PNPLA3* rs738409 polymorphism was associated with NAFLD fibrosis and HCC, where GG carriers had a 5-fold increase in HCC (95%CI: 1.47-17.29) compared to CC carriers.

In subsequent studies, the transmembrane 6 superfamily member 2, *TM6SF2* (rs58542926), followed suit and was identified in an exome-wide association study of fatty liver and serum aminotransferases^[46]. Although the association of *TM6SF2* with NAFLD is well established, its association with HCC development is disputed^[45,47]. More recently, the *MBOAT7* (Membrane Bound O-Acetyltransferase Domain Containing 7) variant rs641738 has also been associated with NAFLD and its histological severity^[48,49]. An Italian study of 132 NAFLD-associated HCC cases also linked the *MBOAT4* variant to non-cirrhosis NAFLD HCC^[50]. In another European study, Pelusi *et al.*^[51] identified rare variants of candidate genes (*SMAD4*, *SQSTM1*, *TEL*, *RB1*, *TSC1*), including *APOB* (Apolipoprotein B) which is involved in very low-density lipoprotein secretion and therefore export of lipids using whole exome sequencing methods. Noteworthy of these findings is the common thread of lipid metabolism genes being identified in genetic NAFLD and NAFLD-related HCC association studies. Although intrahepatic steatosis alone was thought to be benign and the hallmark of NAFLD, lipid dysregulation may play an important role in promoting carcinogenesis independently of NAFLD disease pathogenesis.

In addition to being at risk for NAFLD and NASH, Hispanic patients have been shown to be at risk of NAFLD-associated HCC^[22,52,53], which has been attributed largely to an increase in the incidence of cirrhosis^[22]. Similar to what has been reported in the literature, we found in our local cohort of 125 NAFLD-associated HCC cases, of whom > 85% had histological data available for review, that of the 20% of patients who did not have underlying cirrhosis or advanced fibrosis, none were of Hispanic background. However, the remaining cohort that had NAFLD-associated HCC cases in cirrhotic livers was Hispanic (manuscript submitted). Our findings and that of others suggest that although there are genetic predispositions to NAFLD and NASH, NAFLD-associated HCC may have independent mechanisms other than those that play for NAFLD disease progression and fibrosis. Further studies that are more inclusive of patients of non-European descent are needed to determine if these associations remain true in other populations.

Beyond germ-like mutations that may predispose to disease, which has largely been the focus of genetic studies in NAFLD, heritable epigenetic changes also have an important role in NAFLD-associated HCC which include but are not limited to DNA methylation, chromosomal looping interactions, RNA modifications and the emerging role of non-coding RNAs^[54-57]. Additionally, several studies have applied circulating tumor DNA (ctDNA) methods to further study HCC^[58,59], which has not only shed some light on the biology of HCC but is also promising for use as a biomarker in the future. The identification of these epigenetic modifications points to further understanding of gene regulation changes, which are influenced by their environment^[54].

NON-GENETIC ENVIRONMENTAL FACTORS FOR NAFLD-HCC: CLINICAL, PHARMACOLOGICAL AND LIFESTYLE FACTORS

Diabetes

NAFLD is a complex trait with common and rare variants^[51] that are influenced by the environment. Clinical studies have demonstrated that the features of MetS affect NAFLD-associated HCC development. T2D has been known to affect the risk of HCC as early as when NAFLD and NASH were starting to become more recognized. The seminal VA study by El-Serag *et al.*^[60] demonstrated in a cohort of 173,643 Veterans followed for 10 years that T2D significantly increased the risk of HCC. Studies that followed not only corroborated these findings^[61,62] but also demonstrated an increase in the risk of HCC when more features of MetS were present^[63]. Using a prospectively collected cohort in the Nurses' Health Study and Health Professionals' Health Study, Simon *et al.*^[63] found that the adjusted hazard ratio (HR) for HCC in patients with diabetes was 5.8 (95%CI: 3.49-9.64) and 5.49 (95%CI: 3.16-9.51) compared to non-diabetic patients in women and men after adjusting for baseline characteristics, respectively. Interestingly, the risk of HCC was also dependent on the duration of diabetes, solidifying the effects of T2D and insulin resistance on hepatocellular carcinogenesis. Compared to patients without diabetes, diabetic patients had an adjusted HR of 7.52 (95%CI: 3.88-14.6) if they have had the disease for 10 years or more.

Obesity

Obesity has been associated with an increased risk of developing many cancers and this association is strongest for HCC^[64,65]. By convention, BMI has been used to measure obesity in epidemiological studies. Although readily available in the clinical setting, BMI does not inform adipose distribution, specifically visceral versus peripheral, which have different implications on metabolic health. Early studies in patients with cirrhosis demonstrated that those with visceral adiposity were at higher risk of death compared to those with peripheral adipose tissue. Ioannou *et al.*^[66] elegantly demonstrated these associations using the National Health and Nutritional Examination Survey where patients were categorized based on central or peripheral adipose distribution. Among patients with central adipose distribution, cirrhosis-related death and hospitalizations were more common in the obese group ($BMI \geq 30 \text{ kg/m}^2$) (adjusted HR = 2.2, 95%CI: 1.1-4.6) compared to normal-weight individuals ($BMI < 25 \text{ kg/m}^2$), which was not observed in patients with increased peripheral adipose distribution. In NAFLD and NAFLD-associated HCC, central obesity, a key feature of MetS, is also more physiologically informative of metabolic health^[67,68]. This is also relevant in the setting of studying NAFLD in groups of patients that may not have similar body compositions such as the Asian population, which tends to have a higher percentage of body fat compared to White patients^[69].

Hypertension and dyslipidemia

The evidence for hypertension, which is included in many definitions of MetS, is inconsistent and has been shown to be a risk factor in some studies but not others^[70,71]. Many studies also use collective features of MetS to assess the attendant risk. Thus, the true effects of hypertension in isolation without other features of MetS are unclear. Similarly, the data on dyslipidemia is conflicting. For instance, Welzel *et al.*^[70] demonstrated that patients with a diagnosis of dyslipidemia based on ICDs demonstrated an adjusted odds ratio (OR) of 1.35 (95%CI: 1.26-1.45) for NAFLD-HCC development, similar to others^[63]. Other studies have shown

the opposite effect however, in which a diagnosis of dyslipidemia was protective against HCC^[72]. One potential reason for these conflicting reports is the definitions used to identify patients (i.e. ICDs versus lipid measurements versus medication use). These data must be interpreted in the context of statin use, which has more recently been shown to have chemoprotective effects against fibrosis progression and HCC^[14,15].

Statins

Early animal studies showed that statins, which inhibit the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), cause hepatotoxicity. The level of hepatic injury did not translate to humans clinical trials however, and mostly caused asymptomatic abnormal aminotransferases that would resolve with time^[73]. Over the years, research and subsequently clinical practice has changed to favor the use of statins in NAFLD and NASH patients for the cardiovascular protective effects, which are closely linked to NAFLD^[74,75]. Independent of its association with cardiometabolic disease, plasma lipidomic studies in NAFLD and NASH patients have also shown that lipid dysregulation is important in NAFLD pathogenesis, where levels of palmitoleic and oleic acids are increased^[76-78]. These plasma levels were reflective of an increase in the activity of certain lipid enzymes, such as stearoyl-CoA desaturase 1 (SCD1), the rate limiting enzyme in monounsaturated fatty acids^[77,78], which is now being targeted in phase 3 clinical trials for the treatment of NAFLD^[76,79,80]. Plasma (whole body) and liver lipid compositions also do not always correlate such that understanding lipid dysregulation becomes even more complex^[78].

Over the years studies have showed the benefits of statins for patients with cirrhosis. In a retrospective cohort analysis conducted within the VA from 2008-2016 that included 21,921 patients on statins and 51,023 controls without statins, Kaplan *et al.*^[14] demonstrated that for every year of statin exposure, the associated adjusted HR was 0.92 (95%CI: 0.89-0.94) for mortality. This was seen in all etiologies of cirrhosis including NAFLD and NASH, which comprised 23% of the cohort. These benefits of statins have also been reported in HCC. In another retrospective cohort within the VA from 2002-2016, Thrift *et al.*^[15] reported that after a diagnosis of HCC, statin users had a decreased risk of cancer-specific death with an adjusted HR of 0.85 (95%CI: 0.77-0.93). Their sub-group analysis of NAFLD-associated HCCs did not demonstrate a decrease in cancer-specific (adjusted HR = 1.80, 95%CI: 0.59-1.08) or all-cause (adjusted HR = 0.90, 95%CI: 0.73-1.10) mortalities. The benefits were evident in the group of patients with cirrhosis however, for both cancer-specific (adjusted HR = 0.80, 95%CI: 0.68-0.94) and all-cause (adjusted HR = 0.88, 95%CI: 0.79-0.98) mortalities, respectively, suggesting a fibrosis or cirrhosis-specific effect^[15]. A nested case-control study conducted from 2002-2013 in the Republic of Korea also demonstrated a benefit from statin use with a reduced risk of HCC development (adjusted OR = 0.44, 95%CI: 0.33-0.58)^[81]. Several meta-analyses have also confirmed these findings across different study populations, health care systems and etiologies of HCC^[82-84]. The anti-fibrotic and chemoprotective effects of statins are thought to be potentially independent of their lipid-lowering mechanisms and through other pleiotropic effects mediated by the mevalonate pathway^[85,86]. Among the statins, those that are lipophilic (atorvastatin and simvastatin) have been linked to reduced HCC incidence and mortality outcomes compared to hydrophilic statins (pravastatin and rosuvastatin) in patients with chronic hepatitis B or C-related HCC^[17]. To the best of our knowledge, the differentiating effects of the type of statin used in NAFLD and NAFLD-associated HCC have not been studied.

Aspirin

Chronic inflammation is a known risk factor for fibrosis. Non-steroidal anti-inflammatory drugs, including aspirin, have been associated with having chemoprotective effects in other malignancies including colorectal, breast, prostate and other gastrointestinal cancers^[87,88]. Early *in vitro* studies demonstrated that aspirin had chemoprotective effects against HCC^[89]. Sahasrabudde *et al.*^[90] assessed the association between aspirin use in a prospectively collected data of 300,504 patients in the National Institutes of Health-AARP Diet and Health Study cohort, where patients had used aspirin over the previous 12 months (73% used aspirin). Of the 250 patients who developed HCC, those on aspirin had decreased risk for both HCC (adjusted relative risk = 0.49,

95%CI: 0.39-0.61) and chronic liver disease mortality (adjusted relative risk = 0.55, 95%CI: 0.45-0.67)^[91]. In an observational study using the Liver Cancer Pooling Project which consisted of US cohorts from the National Cancer Institute Cohort Consortium, of the 679 patients who developed HCC, those who were on aspirin also had a 32% reduction in the risk of HCC (adjusted HR = 0.68, 95%CI: 0.57-0.81) over the follow-up period of 11.9 years^[92]. Although there was no mention of NAFLD patients (mostly hepatitis B and C), this early study demonstrated a potential benefit of aspirin in other cirrhosis populations at risk for HCC. These data led to a more recent study using the Nurses' Health Study and the Health Professionals Follow-up Study, where over a median follow-up of 8 years, 133,371 patients were assessed for HCC development. Regular users of aspirin (≥ 2 doses; 325 mg per week) had a reduced risk of HCC (adjusted HR = 0.51, 95%CI: 0.34-0.77), which appeared to be dose and time-dependent^[16]. Patients who used aspirin for 5 years or more had the lowest risk of HCC development (adjusted HR = 0.41, 95%CI: 0.21-0.77). Again, the etiology of HCC and cirrhosis was not evident in the study, although one would expect these data to extrapolate to NAFLD-associated HCC^[16]. This is further supported by NASH animal models treated with aspirin demonstrating a decrease in hepatic fibrosis through decreased activation of pro-inflammatory pathways, potentially by altering the microbiome^[93]. Further studies will be needed to ascertain this however, especially given the benefit of aspirin in cardiovascular disease, which is one of the leading causes of mortality in that patient population^[94].

Microbiome

Advancements in technology, accessibility to sequencing and manipulation of big data has introduced tools to start understanding how the microbiome contributes to NAFLD, NASH and HCC progression. There is increasing evidence that gut dysbiosis plays a key role in driving the progression of NAFLD to NASH and liver cirrhosis by creating a micro-environment which supports: (1) altered energy absorption^[95], (2) modification of gut permeability^[96,97], (3) promotion of chronic low-level inflammation^[98] and, (4) dysregulation of bile acid signaling^[96,99-101]. There is however, a limited understanding of the unique relationship between intestinal microbiota, dysbiosis and the development of HCC.

Studies have consistently shown that two phyla of bacteria are dominant within the gut flora, Firmicutes and Bacteroidetes and that their ratios are altered in NAFLD/NASH patients when compared to healthy controls^[100,102-105]. In a recent study using whole-genome shotgun sequencing of DNA extracted from stool samples, Loomba *et al.*^[106] analyzed the microbiota of subjects with worsening degrees of fibrosis. The authors discovered that in patients with mild/moderate (F0-F1) fibrosis, the gut flora is dominated by Firmicutes and Bacteroidetes followed by Proteobacteria and Actinobacteria. In contrast, Proteobacteria levels were augmented in patients with severe fibrosis ($F \geq 2$) while levels of Firmicutes were diminished. In patients with advanced fibrosis, *Bacteroides vulgatus* and *Escherichia coli* (*E.coli*) were the most abundant organisms. Given that advanced fibrosis is a risk factor for HCC, it is conceivable that similar differences may be found in patients with HCC. Indeed, a study performed by Grat *et al.*^[107] comparing the stool composition of patients with and without HCC (matched by etiology of cirrhosis and MELD scores) discovered that patients with HCC had significantly higher levels of *E. coli* in their stools.

Studies have also shown that alcohol espouses gut permeability through the alteration of tight junctions in gut epithelium^[108]. With the liver being a first-pass organ, it is likely that subjects with gut dysbiosis are predisposed to inappropriate translocation of gut bacteria and their endotoxins, leading to a chronic state of inflammation. This is supported by data from Ponziani *et al.*^[109] who showed that compared to patients with NAFLD cirrhosis without HCC, those with HCC have higher levels of fecal calprotectin - a surrogate measure of gastrointestinal inflammation. Furthermore, Ponziani *et al.*^[109] demonstrated that independent of HCC, patients with compensated liver cirrhosis have higher levels of plasma lipopolysaccharide (LPS). LPS is a well-known endotoxin that simulates toll-like (TLR) and nod-like receptors in the intestinal epithelium. NASH patients have been shown to have higher expression of circulating LPS levels as well as localization to

hepatocytes, which subsequently leads to activation of TLR4 and pro-fibrotic pathways^[93]. Overexpression of TLRs leads to overproduction of chemokine ligand (CCL) 3, CCL4, CCL5 and interleukin (IL) 4, proinflammatory proteins known to be elevated in the presence of HCC, with IL-8 known specifically to be a hepatocarcinogen^[109].

Beyond modification of intestinal microbiota and overstimulation of the host's innate immune system, dysbiosis has also been shown to disrupt bile acid regulation, where under physiological conditions, the microbiome metabolizes primary bile acids to secondary bile acids that are recirculated through the enterohepatic circulation^[110]. When comparing patients with NAFLD to healthy controls and those with simple steatosis, Mouzaki *et al.*^[100] discovered that NAFLD patients had higher fecal levels of primary bile acids, specifically colic acid, chenodeoxycholic acid and lithocholic acid. Others have also shown, using animal models, that elevated levels of lithocholic acid may be carcinogenic^[100,102]. Bile acid dysbiosis in patients with cirrhosis is also well illustrated by Jacobs *et al.*^[111] using duodenal aspirate analyses. Although NASH only comprised 13% of the cohort of patients with cirrhosis, the study identified microbial differences based on the etiology of the cirrhosis, cirrhosis complications (specifically patients with hepatic encephalopathy) and ethnic differences where Hispanics were found to have lower levels of two conjugated forms of ursodeoxycholic acid. This is especially interesting given the ethnic differences previously described in Hispanics and the observation that ursodeoxycholic acid may have anti-carcinogenic effects on HCC^[112-114].

The role of gut dysbiosis in creating a pro-carcinogenic environment is further supported by animal studies. Specifically, in mice models, researchers have shown that the administration of antibiotics/probiotics disrupts the development of new HCC lesions. The replacement of the pro-inflammatory gut milieu, either with sterilization or bacterial replacement, highlights once again the role of the gut-hepatic axis in HCC development, and brings to light the potential role of therapeutics in patients at high risk of HCC^[115-117].

Overall, gut dysbiosis has been repeatedly shown to promote metabolic diseases and accelerate the progression of fatty liver disease. There is now increasing evidence however, that the gut microbiome acts as an independent risk factor for HCC development. By promoting an environment that espouses intestinal permeability, hepatic inflammation and bile acid dysregulation, the gut microbiome creates a pro-carcinogenic environment that may allow for the development of HCC, potentially by working as an epigenetic regulator of gene expression. Most studies in the literature are currently limited to animal models, and of those involving human subjects, samples sizes are often limited by their understandably strict inclusion criteria. Thus, further studies are needed to elucidate the intricate relationship between gut dysbiosis and HCC. Although far from clinical application, this also opens up new avenues for biomarker discovery and potentially, therapies.

CIRRHOSIS VS. NON-CIRRHOSIS

As highlighted above, work remains to be done to further understand the prevalence, causes and outcomes of NAFLD-associated HCC given its complexities with many environmental contributors. Complicating the presentation of NAFLD-associated HCC is that it can occur in a non-cirrhosis background, which causes a clinical dilemma to providers given the lack of current screening guidelines for this patient population. It is estimated that the prevalence of NASH-associated HCC in patients without cirrhosis to be 38% based on a recent meta-analysis^[11], which differs based on the population, country and how the study was conducted. In Japan, where patients can also have "lean" NAFLD, studies have reported that NASH-related HCC can occur without cirrhosis in 38%-49% of cases^[118-120]. A multi-center prospective study conducted in Spain reported a prevalence of 50% of HCC in NAFLD patients without cirrhosis^[12]. Similarly, a retrospective study in France assessing the prevalence of non-cirrhosis HCC in a cohort of 323 HCCs over a 20-year span (of which 12% were due to NAFLD) determined that 63% of the cases occurred in the absence of bridging fibrosis/cirrhosis, although this was biased towards patients without advanced liver disease who could undergo hepatic

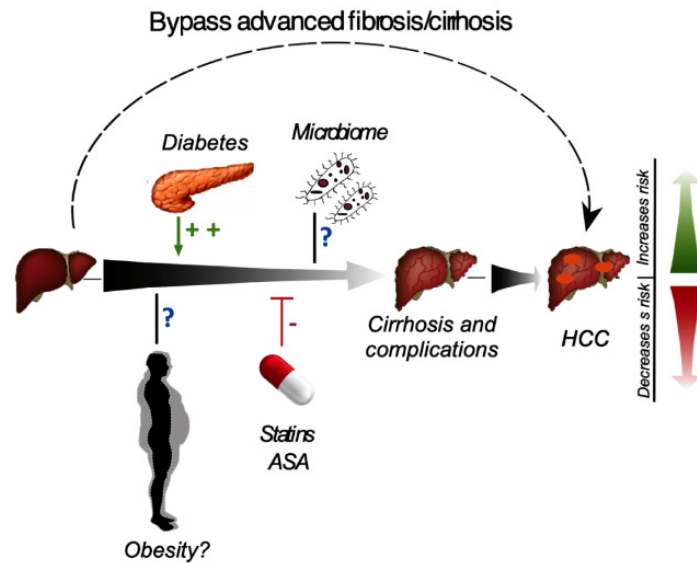


Figure 1. Schematic diagram of the spectrum of NAFLD to NAFLD-associated HCC and the clinical factors implicated in its pathogenesis or prevention. NAFLD: nonalcoholic fatty liver disease; HCC: hepatocellular carcinoma; ASA: aspirin

resection instead of liver transplantation^[121].

Interestingly, statin use or dyslipidemia also appears to have different effects on non-cirrhosis HCC patients. For instance, using Taiwan's National Health Insurance Research Database that included 31,751 NAFLD patients from 1998-2012, Lee *et al.*^[13] demonstrated that patients on statin therapy (35% of all patients) had a decreased risk for HCC (adjusted HR = 0.29, 95%CI: 0.21-0.68). Few other studies have addressed the effects of statins between the cirrhosis and non-cirrhosis NAFLD patient, which necessitates future work in this area.

Animal studies have shed some light on the pathogenesis of NAFLD and NAFLD-associated HCC. Through a series of detailed experiments, Grohmann *et al.*^[122] demonstrate that while obesity promotes NASH pathogenesis through STAT-1 (signal transducer and activator of transcription 1) activation, NASH-associated HCC is mostly driven by STAT-3 (signal transducer and activator of transcription 3), a transcription factor implicated in the immune system. They further corroborated this using human samples as proof of concept. Their work was complimentary to previous reports showing that STAT-3 activation correlates with tumor aggressiveness and prognosis^[123,124]. As our knowledge of NAFLD and NAFLD-associated HCC advances, it is becoming evident that the two diseases are divergent and should, potentially, be thought of as separate entities and not on the same spectrum [Figure 1]. This is also apparent in our epidemiological studies where we found that NAFLD-associated HCC tended to occur in non-Hispanic patients, suggesting different mechanisms of action between the cirrhosis and non-cirrhosis patient populations (manuscript submitted).

SCREENING?

Expert societies recommend HCC screening with bi-annual ultrasounds with or without alpha-fetal protein (AFP) or other biomarkers, based on its cost-effectiveness and benefits^[125,126]. Although the incidence of NAFLD-associated HCC remains low overall, given the magnitude of MetS and the NAFLD epidemic, NAFLD-HCC cases are expected to increase. Since HCC can also occur in a non-cirrhotic background, future research is imperative in this field to identify at-risk patient populations that would benefit from screening programs, which have largely operated under a "one-size-fits-all" model. Unlike hepatitis C, B and

alcohol, NAFLD HCC screening has several challenges. Obesity, common in this patient population, has been shown to decrease the effectiveness of ultrasound screening^[127-129]. The effects of visceral adipose tissue or intrahepatic steatosis in lesions are also unclear. The use of other imaging modalities has been proposed including abbreviated MRI scans, which are promising given their sensitivity and specificity, although their cost-effectiveness remains to be evaluated^[130,131].

Over the years, new biomarkers have been introduced including the Lens culinaris lectin-binding subfraction of the AFP (AFP-L 3%)^[132] and des gamma carboxy prothrombin^[133]. The clinical utility of these biomarkers in NAFLD-associated HCC patients remains unknown. In our studies, we found that compared to hepatitis B and C, patients with NAFLD-associated HCC were less likely to be AFP producers (AFP < 10 ng/mL) (manuscript submitted). Due to the complex nature of NAFLD, the use of a combination of markers will likely be more clinically relevant. This is demonstrated by Best *et al.*'s^[134] study using the GALAD (Gender, Age, AFP-L3, and Des-carboxy-prothormbin) score to predict early detection of NAFLD-associated HCC cases in Europe and Japan, including the sub-group of patients without cirrhosis. These results will need to be validated in the US patient population given the heterogeneity of NAFLD across different ethnic groups, especially given the lower performance of the GALAD score in the sub-group of US-American cohort. Risk stratification calculators have been developed to assess the risk of HCC and the utility of HCC screening, including in the NAFLD patient population, however these have focused on the cirrhosis group which is at higher risk of HCC and have not entered clinical practice yet^[24].

Clinical practices therefore vary due to the limited data, which has prompted expert societies to provide some guidance in the form of expert opinion^[135]. In our local liver transplantation institution, we choose to screen patients yearly with an abdominal ultrasound and blood work, especially if they have a diagnosis of T2D.

CONCLUSIONS AND OUTSTANDING QUESTIONS

NAFLD-associated HCC is on the rise and will continue to create a large economic burden on health care, prompting essential research. Its heterogenous clinical presentation is reflective of its complex traits with important genetic and non-genetic, as well as environmental risk factors. Although a combination of basic, translational and clinical research has shed some light on its distinct presentations, many questions remain unanswered. The lack of consistent clinical definitions to identify NAFLD and NASH patients when using electronic medical records in research over the years has made comparison between studies challenging. This has been recognized in clinical trials where a larger effort has been made on using consistent and reproducible definitions to identify patients and characterize responses to NASH treatment. Bigger public health questions remain unanswered including who to screen and how to use modifiable factors such as statins and aspirin to mitigate the risk of liver disease progression and HCC, where liver transplantation remains scarce and not available to all patients. In the era of precision healthcare and medicine, identifying “at-risk” populations within the larger NAFLD group will be key to tailoring screening and treatment options and will help providers identify patients in need of close monitoring under the care of subspecialists versus primary care. In light of the complex and heterogenous nature of the disease, identifying “at-risk” patients will likely require a combination of clinical characteristics, biomarker discovery and risk-stratification calculators.

DECLARATIONS

Authors' contributions

Involved in conception of the study, drafting of the manuscript and critical revision for important intellectual content: Benhammou JN, Hussain SK, El-Kabany M

Conducted the literature review, drafted the section on the microbiome and provided critical revision of the manuscript for intellectual content: Lin J

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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REFERENCES

1. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013;10:686-90.
2. Adams LA, Lindor KD. Nonalcoholic fatty liver disease. *Ann Epidemiol* 2007;17:863-9.
3. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73-84.
4. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41:1313-21.
5. Page JM, Harrison SA. NASH and HCC. *Clin Liver Dis* 2009;13:631-47.
6. Sanyal AJ; American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002;123:1705-25.
7. Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* 2018;67:123-33.
8. Allen AM, Van Houten HK, Sangaralingham LR, Talwalkar JA, McCoy RG. Healthcare cost and utilization in nonalcoholic fatty liver disease: real-world data from a large U.S. claims database. *Hepatology* 2018;68:2230-8.
9. Younossi ZM, Henry L, Bush H, Mishra A. Clinical and economic burden of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Clin Liver Dis* 2018;22:1-10.
10. Younossi ZM, Zheng L, Stepanova M, Henry L, Venkatesan C, et al. Trends in outpatient resource utilizations and outcomes for Medicare beneficiaries with nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2015;49:222-7.
11. Stine JG, Wentworth BJ, Zimmet A, Rinella ME, Loomba R, et al. Systematic review with meta-analysis: risk of hepatocellular carcinoma in non-alcoholic steatohepatitis without cirrhosis compared to other liver diseases. *Aliment Pharmacol Ther* 2018;48:696-703.
12. Piscaglia F, Svegliati-Baroni G, Barchetti A, Pecorelli A, Marinelli S, et al. Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: a multicenter prospective study. *Hepatology* 2016;63:827-38.
13. Lee TY, Wu JC, Yu SH, Lin JT, Wu MS, et al. The occurrence of hepatocellular carcinoma in different risk stratifications of clinically noncirrhotic nonalcoholic fatty liver disease. *Int J Cancer* 2017;141:1307-14.
14. Kaplan DE, Serper MA, Mehta R, Fox R, John B, et al. Effects of hypercholesterolemia and statin exposure on survival in a large national cohort of patients with cirrhosis. *Gastroenterology* 2019;156:1693-706.e12.
15. Thrift AP, Natarajan Y, Liu Y, El-Serag HB. Statin use after diagnosis of hepatocellular carcinoma is associated with decreased mortality. *Clin Gastroenterol Hepatol* 2019;17:2117-25.e3.
16. Simon TG, Ma Y, Ludvigsson JF, Chong DQ, Giovannucci EL, et al. Association between aspirin use and risk of hepatocellular carcinoma. *JAMA Oncol* 2018;4:1683-90.
17. Simon TG, Duberg AS, Aleman S, Hagstrom H, Nguyen LH, et al. Lipophilic statins and risk for hepatocellular carcinoma and death in patients with chronic viral hepatitis: results from a nationwide Swedish population. *Ann Intern Med* 2019;171:318-27.
18. Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 2019;16:411-28.
19. Singal AG, El-Serag HB. Hepatocellular carcinoma from epidemiology to prevention: translating knowledge into practice. *Clin Gastroenterol Hepatol* 2015;13:2140-51.

20. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017;67:7-30.
21. Welzel TM, Graubard BI, Quraishi S, Zeuzem S, Davila JA, et al. Population-attributable fractions of risk factors for hepatocellular carcinoma in the United States. *Am J Gastroenterol* 2013;108:1314-21.
22. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, et al. Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease. *Gastroenterology* 2018;155:1828-37.e2.
23. Mittal S, Sada YH, El-Serag HB, Kanwal F, Duan Z, et al. Temporal trends of nonalcoholic fatty liver disease-related hepatocellular carcinoma in the veteran affairs population. *Clin Gastroenterol Hepatol* 2015;13:594-601.e1.
24. Ioannou GN, Green P, Kerr KF, Berry K. Models estimating risk of hepatocellular carcinoma in patients with alcohol or NAFLD-related cirrhosis for risk stratification. *J Hepatol* 2019;71:523-33.
25. Younossi Z, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, et al. Nonalcoholic steatohepatitis is the fastest growing cause of hepatocellular carcinoma in liver transplant candidates. *Clin Gastroenterol Hepatol* 2019;17:748-55.e3.
26. Wong RJ, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014;59:2188-95.
27. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015;148:547-55.
28. Petrick JL, Braunlin M, Laversanne M, Valery PC, Bray F, et al. International trends in liver cancer incidence, overall and by histologic subtype, 1978-2007. *Int J Cancer* 2016;139:1534-45.
29. Kawamura Y, Arase Y, Ikeda K, Seko Y, Imai N, et al. Large-scale long-term follow-up study of Japanese patients with non-alcoholic fatty liver disease for the onset of hepatocellular carcinoma. *Am J Gastroenterol* 2012;107:253-61.
30. Alexander M, Loomis AK, van der Lei J, Duarte-Salles T, Prieto-Alhambra D, et al. Risks and clinical predictors of cirrhosis and hepatocellular carcinoma diagnoses in adults with diagnosed NAFLD: real-world study of 18 million patients in four European cohorts. *BMC Med* 2019;17:95.
31. Haldar D, Kern B, Hodson J, Armstrong MJ, Adam R, et al. Outcomes of liver transplantation for non-alcoholic steatohepatitis: a European Liver Transplant Registry study. *J Hepatol* 2019;71:313-22.
32. Fan JG, Kim SU, Wong VW. New trends on obesity and NAFLD in Asia. *J Hepatol* 2017;67:862-73.
33. Wong MCS, Huang JLW, George J, Huang J, Leung C, et al. The changing epidemiology of liver diseases in the Asia-Pacific region. *Nat Rev Gastroenterol Hepatol* 2019;16:57-73.
34. Cho EJ, Kwack MS, Jang ES, You SJ, Lee JH, et al. Relative etiological role of prior hepatitis B virus infection and nonalcoholic fatty liver disease in the development of non-B non-C hepatocellular carcinoma in a hepatitis B-endemic area. *Digestion* 2011;84:17-22.
35. Hagström H, Nasr P, Ekstedt M, Hammar U, Stål P, et al. Risk for development of severe liver disease in lean patients with nonalcoholic fatty liver disease: a long-term follow-up study. *Hepatol Commun* 2018;2:48-57.
36. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;40:1387-95.
37. Caldwell SH, Harris DM, Patrie JT, Hespeneheide EE. Is NASH underdiagnosed among African Americans? *Am J Gastroenterol* 2002;97:1496-500.
38. Browning JD, Kumar KS, Saboorian MH, Thiele DL. Ethnic differences in the prevalence of cryptogenic cirrhosis. *Am J Gastroenterol* 2004;99:292-8.
39. Schwimmer JB, Celedon MA, Lavine JE, Salem R, Campbell N, et al. Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009;136:1585-92.
40. Loomba R, Schork N, Chen CH, Bettencourt R, Bhatt A, et al. Heritability of hepatic fibrosis and steatosis based on a prospective twin study. *Gastroenterology* 2015;149:1784-93.
41. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40:1461-5.
42. Dongiovanni P, Donati B, Fares R, Lombardi R, Mancina RM, et al. PNPLA3 I148M polymorphism and progressive liver disease. *World J Gastroenterol* 2013;19:6969-78.
43. Smagris E, BasuRay S, Li J, Huang Y, Lai KM, et al. Pnpla3I148M knockin mice accumulate PNPLA3 on lipid droplets and develop hepatic steatosis. *Hepatology* 2015;61:108-18.
44. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011;53:1883-94.
45. Liu YL, Patman GL, Leathart JB, Piguet AC, Burt AD, et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014;61:75-81.
46. Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014;46:352-6.
47. Falletti E, Cussigh A, Cmet S, Fabris C, Toniutto P. PNPLA3 rs738409 and TM6SF2 rs58542926 variants increase the risk of hepatocellular carcinoma in alcoholic cirrhosis. *Dig Liver Dis* 2016;48:69-75.
48. Mancina RM, Dongiovanni P, Petta S, Pingitore P, Meroni M, et al. The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. *Gastroenterology* 2016;150:1219-30.e6.
49. Luukkainen PK, Zhou Y, Hyötyläinen T, Leivonen M, Arola J, et al. The MBOAT7 variant rs641738 alters hepatic phosphatidylinositols and increases severity of non-alcoholic fatty liver disease in humans. *J Hepatol* 2016;65:1263-5.
50. Donati B, Dongiovanni P, Romeo S, Meroni M, McCain M, et al. MBOAT7 rs641738 variant and hepatocellular carcinoma in non-

- cirrhotic individuals. *Sci Rep* 2017;7:4492.
51. Pelusi S, Baselli G, Pietrelli A, Dongiovanni P, Donati B, et al. Rare pathogenic variants predispose to hepatocellular carcinoma in nonalcoholic fatty liver disease. *Sci Rep* 2019;9:3682.
 52. El-Serag HB, Lau M, Eschbach K, Davila J, Goodwin J. Epidemiology of hepatocellular carcinoma in Hispanics in the United States. *Arch Intern Med* 2007;167:1983-9.
 53. Rich NE, Hester C, Odewole M, Murphy CC, Parikh ND, et al. Racial and ethnic differences in presentation and outcomes of hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2019;17:551-9.e1.
 54. Garske KM, Pan DZ, Miao Z, Bhagat YV, Comenho C, et al. Reverse gene-environment interaction approach to identify variants influencing body-mass index in humans. *Nat Metab* 2019;1:630-42.
 55. Benhammou JN, Ko A, Alvarez M, Kaikkonen MU, Rankin C, et al. Novel lipid long intervening noncoding RNA, oligodendrocyte maturation-associated long intergenic noncoding RNA, regulates the liver steatosis gene stearyl-coenzyme a desaturase as an enhancer RNA. *Hepatol Commun* 2019;3:1356-72.
 56. Villanueva A, Portela A, Sayols S, Battiston C, Hoshida Y, et al. DNA methylation-based prognosis and epdrivers in hepatocellular carcinoma. *Hepatology* 2015;61:1945-56.
 57. Fei Q, Shang K, Zhang J, Chuai S, Kong D, et al. Histone methyltransferase SETDB1 regulates liver cancer cell growth through methylation of p53. *Nat Commun* 2015;6:8651.
 58. Xu RH, Wei W, Krawczyk M, Wang W, Luo H, et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. *Nat Mater* 2017;16:1155-61.
 59. Ng CKY, Di Costanzo GG, Terracciano LM, Piscuoglio S. Circulating cell-free DNA in hepatocellular carcinoma: current insights and outlook. *Front Med (Lausanne)* 2018;5:78.
 60. El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004;126:460-8.
 61. Lai SW, Chen PC, Liao KF, Muo CH, Lin CC, et al. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. *Am J Gastroenterol* 2012;107:46-52.
 62. Chen HF, Chen P, Li CY. Risk of malignant neoplasms of liver and biliary tract in diabetic patients with different age and sex stratifications. *Hepatology* 2010;52:155-63.
 63. Simon TG, King LY, Chong DQ, Nguyen LH, Ma Y, et al. Diabetes, metabolic comorbidities, and risk of hepatocellular carcinoma: results from two prospective cohort studies. *Hepatology* 2018;67:1797-806.
 64. Cancer Prevention Study II. The American Cancer Society Prospective Study. *Stat Bull Metrop Insur Co* 1992;73:21-9.
 65. Karagozian R, Derdak Z, Baffy G. Obesity-associated mechanisms of hepatocarcinogenesis. *Metabolism* 2014;63:607-17.
 66. Ioannou GN, Weiss NS, Boyko EJ, Kowdley KV, Kahn SE, et al. Is central obesity associated with cirrhosis-related death or hospitalization? A population-based, cohort study. *Clin Gastroenterol Hepatol* 2005;3:67-74.
 67. Schlesinger S, Aleksandrova K, Pischon T, Fedirko V, Jenab M, et al. Abdominal obesity, weight gain during adulthood and risk of liver and biliary tract cancer in a European cohort. *Int J Cancer* 2013;132:645-57.
 68. Pang Q, Zhang JY, Song SD, Qu K, Xu XS, et al. Central obesity and nonalcoholic fatty liver disease risk after adjusting for body mass index. *World J Gastroenterol* 2015;21:1650-62.
 69. Consultation WE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004;363:157-63.
 70. Welzel TM, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, et al. Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the SEER-Medicare database. *Hepatology* 2011;54:463-71.
 71. Borena W, Strohmaier S, Lukanova A, Bjørge T, Lindkvist B, et al. Metabolic risk factors and primary liver cancer in a prospective study of 578,700 adults. *Int J Cancer* 2012;131:193-200.
 72. Chiang CH, Lee LT, Hung SH, Lin WY, Hung HF, et al. Opposite association between diabetes, dyslipidemia, and hepatocellular carcinoma mortality in the middle-aged and elderly. *Hepatology* 2014;59:2207-15.
 73. Chalasani N. Statins and hepatotoxicity: focus on patients with fatty liver. *Hepatology* 2005;41:690-5.
 74. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012;142:1592-609.
 75. Bays H, Cohen DE, Chalasani N, Harrison SA, The national lipid association's Statin safety task F. An assessment by the Statin Liver Safety Task Force: 2014 update. *J Clin Lipidol* 2014;8:S47-57.
 76. Puri P, Wiest MM, Cheung O, Mirshahi F, Sargeant C, et al. The plasma lipidomic signature of nonalcoholic steatohepatitis. *Hepatology* 2009;50:1827-38.
 77. Chiappini F, Coilly A, Kadar H, Gual P, Tran A, et al. Metabolism dysregulation induces a specific lipid signature of nonalcoholic steatohepatitis in patients. *Sci Rep* 2017;7:46658.
 78. Walle P, Takkunen M, Männistö V, Vaittinen M, Lankinen M, et al. Fatty acid metabolism is altered in non-alcoholic steatohepatitis independent of obesity. *Metabolism* 2016;65:655-66.
 79. Safadi R, Konikoff FM, Mahamid M, Zelber-Sagi S, Halpern M, et al. The fatty acid-bile acid conjugate Aramchol reduces liver fat content in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2014;12:2085-91.e1.
 80. Ajmera VH, Cachay E, Ramers C, Vodkin I, Bassirian S, et al. MRI assessment of treatment response in HIV-associated NAFLD: a randomized trial of a stearyl-coenzyme-A-desaturase-1 inhibitor (ARRIVE Trial). *Hepatology* 2019;70:1531-45.

81. Kim G, Jang SY, Nam CM, Kang ES. Statin use and the risk of hepatocellular carcinoma in patients at high risk: a nationwide nested case-control study. *J Hepatol* 2018;68:476-84.
82. Friedman GD, Achacoso N, Fireman B, Habel LA. Statins and reduced risk of liver cancer: evidence for confounding. *J Natl Cancer Inst* 2016;108.
83. Yi C, Song Z, Wan M, Chen Y, Cheng X. Statins intake and risk of liver cancer: a dose-response meta analysis of prospective cohort studies. *Medicine (Baltimore)* 2017;96:e7435.
84. Shi M, Zheng H, Nie B, Gong W, Cui X. Statin use and risk of liver cancer: an update meta-analysis. *BMJ Open* 2014;4:e005399.
85. Ogura S, Yoshida Y, Kurahashi T, Egawa M, Furuta K, et al. Targeting the mevalonate pathway is a novel therapeutic approach to inhibit oncogenic FoxM1 transcription factor in human hepatocellular carcinoma. *Oncotarget* 2018;9:21022-35.
86. Gruenbacher G, Thurnher M. Mevalonate metabolism governs cancer immune surveillance. *Oncoimmunology* 2017;6:e1342917.
87. Cuzick J, Otto F, Baron JA, Brown PH, Burn J, et al. Aspirin and non-steroidal anti-inflammatory drugs for cancer prevention: an international consensus statement. *Lancet Oncol* 2009;10:501-7.
88. Bosetti C, Gallus S, La Vecchia C. Aspirin and cancer risk: a summary review to 2007. *Recent Results Cancer Res* 2009;181:231-51.
89. Leng J, Han C, Demetris AJ, Michalopoulos GK, Wu T. Cyclooxygenase-2 promotes hepatocellular carcinoma cell growth through Akt activation: evidence for Akt inhibition in celecoxib-induced apoptosis. *Hepatology* 2003;38:756-68.
90. Sahasrabudhe VV, Gunja MZ, Graubard BI, Trabert B, Schwartz LM, et al. Nonsteroidal anti-inflammatory drug use, chronic liver disease, and hepatocellular carcinoma. *J Natl Cancer Inst* 2012;104:1808-14.
91. Schatzkin A, Subar AF, Thompson FE, Harlan LC, Tangrea J, et al. Design and serendipity in establishing a large cohort with wide dietary intake distributions : the National Institutes of Health-American Association of Retired Persons Diet and Health Study. *Am J Epidemiol* 2001;154:1119-25.
92. Petrick JL, Sahasrabudhe VV, Chan AT, Alavanja MC, Beane-Freeman LE, et al. NSAID use and risk of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: the liver cancer pooling project. *Cancer Prev Res (Phila)* 2015;8:1156-62.
93. Carpino G, Del Ben M, Pastori D, Carnevale R, Baratta F, et al. Increased liver localization of lipopolysaccharides in human and experimental non-alcoholic fatty liver disease. *Hepatology* 2019; Epub ahead of print. doi: 10.1002/hep.31056.
94. Paik JM, Henry L, De Avila L, Younossi E, Racila A, et al. Mortality related to nonalcoholic fatty liver disease is increasing in the United States. *Hepatol Commun* 2019;3:1459-71.
95. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, et al. Metagenomic analysis of the human distal gut microbiome. *Science* 2006;312:1355-9.
96. Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009;49:1877-87.
97. Volynets V, Küper MA, Strahl S, Maier IB, Spruss A, et al. Nutrition, intestinal permeability, and blood ethanol levels are altered in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Dis Sci* 2012;57:1932-41.
98. Noverr MC, Huffnagle GB. Does the microbiota regulate immune responses outside the gut? *Trends Microbiol* 2004;12:562-8.
99. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016;65:1038-48.
100. Mouzaki M, Wang AY, Bandsma R, Comelli EM, Arendt BM, et al. Bile acids and dysbiosis in non-alcoholic fatty liver disease. *PLoS One* 2016;11:e0151829.
101. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012;482:179-85.
102. Boursier J, Diehl AM. Implication of gut microbiota in nonalcoholic fatty liver disease. *PLoS Pathog* 2015;11:e1004559.
103. Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* 2013;57:601-9.
104. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006;444:1022-3.
105. Boursier J, Mueller O, Barret M, Machado M, Fizanne L, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016;63:764-75.
106. Loomba R, Seguritan V, Li W, Long T, Klitgord N, et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metab* 2019;30:607.
107. Grąt M, Wronka KM, Krasnodebski M, Masior Ł, Lewandowski Z, et al. Profile of gut microbiota associated with the presence of hepatocellular cancer in patients with liver cirrhosis. *Transplant Proc* 2016;48:1687-91.
108. Chassaing B, Etienne-Mesmin L, Gewirtz AT. Microbiota-liver axis in hepatic disease. *Hepatology* 2014;59:328-39.
109. Ponziani FR, Bhoori S, Castelli C, Putignani L, Rivoltini L, et al. Hepatocellular carcinoma is associated with gut microbiota profile and inflammation in nonalcoholic fatty liver disease. *Hepatology* 2019;69:107-20.
110. Roberts MS, Magnusson BM, Burczynski FJ, Weiss M. Enterohepatic circulation: physiological, pharmacokinetic and clinical implications. *Clin Pharmacokinet* 2002;41:751-90.
111. Jacobs JP, Dong TS, Agopian V, Lagishetty V, Sundaram V, et al. Microbiome and bile acid profiles in duodenal aspirates from patients with liver cirrhosis: the Microbiome, Microbial Markers and Liver Disease Study. *Hepatol Res* 2018;48:1108-17.
112. Chung GE, Yoon JH, Lee JH, Kim HY, Myung SJ, et al. Ursodeoxycholic acid-induced inhibition of DLC1 protein degradation leads to suppression of hepatocellular carcinoma cell growth. *Oncol Rep* 2011;25:1739-46.
113. Liu H, Xu HW, Zhang YZ, Huang Y, Han GQ, et al. Ursodeoxycholic acid induces apoptosis in hepatocellular carcinoma xenografts in mice. *World J Gastroenterol* 2015;21:10367-74.

114. Zhu L, Shan LJ, Liu YJ, Chen D, Xiao XG, et al. Ursodeoxycholic acid induces apoptosis of hepatocellular carcinoma cells in vitro. *J Dig Dis* 2014;15:684-93.
115. Li J, Sung CY, Lee N, Ni Y, Pihlajamäki J, et al. Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. *Proc Natl Acad Sci U S A* 2016;113:E1306-15.
116. Zhang HL, Yu LX, Yang W, Tang L, Lin Y, et al. Profound impact of gut homeostasis on chemically-induced pro-tumorigenic inflammation and hepatocarcinogenesis in rats. *J Hepatol* 2012;57:803-12.
117. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013;499:97-101.
118. Tokushige K, Hashimoto E, Horie Y, Taniai M, Higuchi S. Hepatocellular carcinoma in Japanese patients with nonalcoholic fatty liver disease, alcoholic liver disease, and chronic liver disease of unknown etiology: report of the nationwide survey. *J Gastroenterol* 2011;46:1230-7.
119. Yasui K, Hashimoto E, Komorizono Y, Koike K, Arii S, et al. Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2011;9:428-33.
120. Kawada N, Imanaka K, Kawaguchi T, Tamai C, Ishihara R, et al. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. *J Gastroenterol* 2009;44:1190-4.
121. Pais R, Fartoux L, Goumard C, Scatton O, Wendum D, et al. Temporal trends, clinical patterns and outcomes of NAFLD-related HCC in patients undergoing liver resection over a 20-year period. *Aliment Pharmacol Ther* 2017;46:856-63.
122. Grohmann M, Wiede F, Dodd GT, Gutzov EN, Ooi GJ, et al. Obesity drives STAT-1-dependent NASH and STAT-3-dependent HCC. *Cell* 2018;175:1289-306.e20.
123. Calvisi DF, Ladu S, Gorden A, Farina M, Conner EA, et al. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. *Gastroenterology* 2006;130:1117-28.
124. He G, Yu GY, Temkin V, Ogata H, Kuntzen C, et al. Hepatocyte IKK β /NF- κ B inhibits tumor promotion and progression by preventing oxidative stress-driven STAT3 activation. *Cancer Cell* 2010;17:286-97.
125. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;68:723-50.
126. European Association for the Study of the Liver. Corrigendum to "EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma" [*J Hepatol* 69 (2018) 182-236]. *J Hepatol* 2019;70:817.
127. Del Poggio P, Olmi S, Ciccarese F, Di Marco M, Rapaccini GL, et al. Factors that affect efficacy of ultrasound surveillance for early stage hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2014;12:1927-33.e2.
128. Simmons O, Fetzter DT, Yokoo T, Marrero JA, Yopp A, et al. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. *Aliment Pharmacol Ther* 2017;45:169-77.
129. Simmons O, Fetzter DT, Yokoo T, Marrero JA, Yopp A, et al. Editorial: ultrasound surveillance of hepatocellular carcinoma in the 21st century - authors' reply. *Aliment Pharmacol Ther* 2017;45:562-63.
130. Zhang E, Wartelle-Bladou C, Lepanto L, Lachaine J, Cloutier G, et al. Cost-utility analysis of nonalcoholic steatohepatitis screening. *Eur Radiol* 2015;25:3282-94.
131. Khatri G, Pedrosa I, Ananthakrishnan L, de Leon AD, Fetzter DT, et al. Abbreviated-protocol screening MRI vs. complete-protocol diagnostic MRI for detection of hepatocellular carcinoma in patients with cirrhosis: an equivalence study using LI-RADS v2018. *J Magn Reson Imaging* 2020;51:415-25.
132. Leerapun A, Suravarapu SV, Bida JP, Clark RJ, Sanders EL, et al. The utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. *Clin Gastroenterol Hepatol* 2007;5:394-402.
133. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med* 1984;310:1427-31.
134. Best J, Bechmann LP, Sowa JP, Sydor S, Dechêne A, et al. GALAD score detects early hepatocellular carcinoma in an international cohort of patients with nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2020;18:728-35.e4.
135. Loomba R, Lim JK, Patton H, El-Serag HB. AGA clinical practice update on screening and surveillance for hepatocellular carcinoma in patients with nonalcoholic fatty liver disease: expert review. *Gastroenterology* 2020;158:1822-30.
136. Younossi ZM, Otgonsuren M, Henry L, Venkatesan C, Mishra A, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015;62:1723-30.
137. Dyson J, Jaques B, Chattopadhyay D, Lochan R, Graham J, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014;60:110-7.
138. Arase Y, Kobayashi M, Suzuki F, Suzuki Y, Kawamura Y, et al. Difference in malignancies of chronic liver disease due to non-alcoholic fatty liver disease or hepatitis C in Japanese elderly patients. *Hepatol Res* 2012;42:264-72.
139. Shen JH, Li YL, Li D, Wang NN, Jing L, et al. The rs738409 (I148M) variant of the PNPLA3 gene and cirrhosis: a meta-analysis. *J Lipid Res* 2015;56:167-75.
140. Vespasiani-Gentilucci U, Gallo P, Dell'Unto C, Volpentesta M, Antonelli-Incalzi R, et al. Promoting genetics in non-alcoholic fatty liver disease: combined risk score through polymorphisms and clinical variables. *World J Gastroenterol* 2018;24:4835-45.

Review

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Intraarterial and intravenous contrast enhanced CT and MR imaging of multi-step hepatocarcinogenesis defining the early stage of hepatocellular carcinoma development

Satoshi Kobayashi^{1,2}, Kazuto Kozaka², Toshifumi Gabata², Osamu Matsui², Tetsuya Minami³

¹Department of Quantum Medical Technology, Kanazawa University Graduate School of Medical Sciences, Kanazawa 920-0942, Japan.

²Department of Radiology, Kanazawa University Graduate School of Medical Sciences, Kanazawa 920-8641, Japan.

³Department of Radiology, Kanazawa Medical University, Uchinada 920-0293, Japan.

Correspondence to: Dr. Satoshi Kobayashi, Department of Radiology, Kanazawa University Graduate School of Medical Sciences, 13-1, Takara-machi, Kanazawa 920-8641, Japan. E-mail: satoshik@staff.kanazawa-u.ac.jp

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Abstract

Liver cancer is the second leading cause of cancer deaths in men worldwide, and the 6th and 7th cause of cancer deaths in men and women in developed countries. 70%-90% of primary liver cancer is hepatocellular carcinoma. Hepatitis B or C viruses and chronic inflammation due to alcohol intake are the main risk factors for hepatocellular carcinoma. One of the key approaches for the early detection of hepatocellular carcinoma is to understand the specific imaging findings of liver nodules in the multi-step hepatocarcinogenesis process. In this article, we review the imaging findings of multistep hepatocarcinogenesis, with a focus on the early detection of malignant, cirrhotic nodules with CT and MRI.

Keywords: Hepatocellular carcinoma, multistep hepatocarcinogenesis, early hepatocellular carcinoma, dysplastic nodule, CT, MRI

INTRODUCTION

Liver cancer is the second and 6th leading cause of cancer deaths in men and women worldwide, and the 6th and 7th cause of cancer deaths in men and women in developed countries. In 2012, the annual incidence and mortality rates were approximately 780,000 and 750,000 respectively^[1].



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70%-90% of primary liver cancer is hepatocellular carcinoma (HCC). Hepatitis B or C virus infections and chronic inflammation due to alcohol intake are the main risk factors of HCC. In Japan, such high-risk patients have been identified and early detection and treatment systems have been established.

One of the key approaches for the early detection of HCC is to understand its characteristic imaging findings of nodules undergoing the multi-step hepatocarcinogenesis process. In other words, picking up early HCC within various types of pre-malignant nodules in cirrhotic livers is essential for the early detection of clinically significant HCC.

For such early detection, contrast enhanced CT, MRI and contrast enhanced ultrasound (CE-US) might be used as imaging modalities. In particular, recent advancements in ultrasonographic contrast agents have enabled monitoring of detailed haemodynamics and Kupffer cell function of liver nodules. Compared to CT and MRI, CE-US is a powerful tool for the analysis of multistep hepatocarcinogenesis because it enables real time monitoring of the dynamics of the contrast agent within the liver. However, we do not have enough experience to discuss the advantages of CE-US in multi-step hepatocarcinogenesis. Therefore, in this article, we will review the imaging findings of multi-step hepatocarcinogenesis on CT and MRI instead, and to focus on the early detection of malignant cirrhotic nodules.

MULTI-STEP HEPATOCARCINOGENESIS

Hepatocarcinogenesis exhibits a multi-step process. It starts from a regenerative nodule in cirrhosis or as a dysplastic nodule (DN), which is a precancerous condition, and progresses to advanced HCC [Figure 1]^[2]. A considerable proportion of HCC arise through multi-step hepatocarcinogenesis while other HCCs develop *de-novo*^[2,3].

Therefore, in high-risk patients, it is important to monitor the process of malignant transformation from precancerous nodules to overt malignant nodule in HCC. Treatment of malignant nodules in early stages might thus be expected to lead to an improved prognosis for HCC patients if detected early.

PATHOLOGICAL DIFFERENTIATION OF MALIGNANT FROM BENIGN NODULES

Histopathologically, the multi-step hepatocarcinogenesis process is observed when moderately or poorly differentiated HCC (advanced HCC) develops in the nodule of well differentiated HCC [Figure 1]^[4]. This nodule-in-nodule appearance differs however, when considering definitions in pathology (malignant focus in premalignant nodule) and imaging (hypervascular focus in nonhypervascular nodule). Similar nodule-in-nodule structures are also observed in diagnostic imaging although the detection rate is not high in routine CT and MRI. However, when the characteristics of the inner nodule are different from the surrounding nodule, such as a hypervascular foci within a hypovascular nodule (as discussed later), or the absence of a superparamagnetic iron oxide (SPIO) uptake foci within the SPIO-uptake nodule, these different findings within the same nodule would represent imaging findings of multi-step hepatocellular carcinogenesis [Figure 2].

Pathologically, DNs are classified into low grade DN (LGDN), which is close to normal, and high grade DN (HGDN), which is close to malignancy. The nodule that is histologically more malignant than HGDN is called early HCC. The clinical and pathological characteristics of the nodules which develop in multi-step hepatocarcinogenesis in cirrhosis are shown in Figure 3^[5,6].

To differentiate HGDN from early HCC histologically, the findings of tumor cell invasion in the remaining portal tract (stromal invasion) is an important hallmark of early HCC^[5,7].

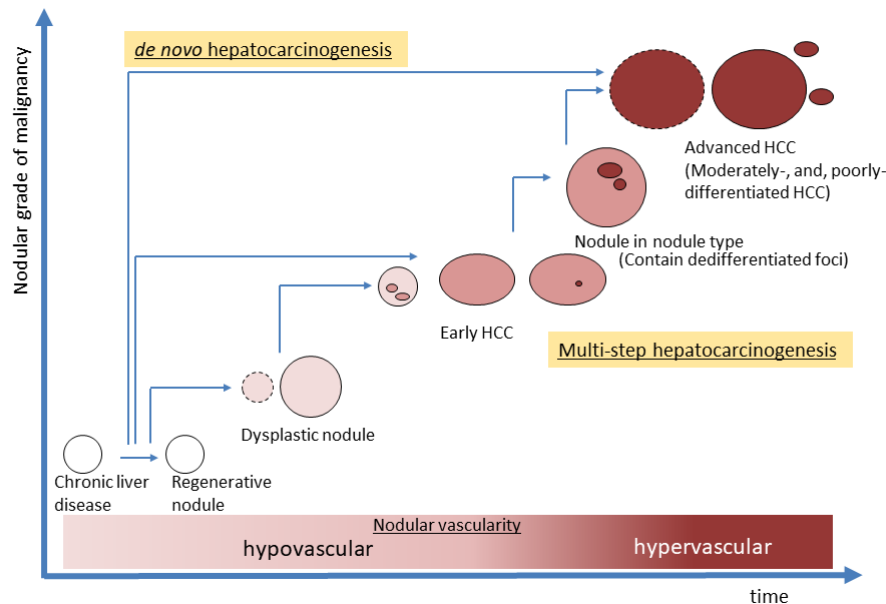


Figure 1. Schematic diagram of multistep hepatocarcinogenesis. Two types of human hepatocarcinogenesis are currently considered, one is multistep- and the other is de novo-hepatocarcinogenesis. In multistep hepatocarcinogenesis, the nodular grade of malignancy changes from dysplastic nodule to advanced HCC stepwisely, and nodular vascularity also gradually change from hypovascular to hypervascular. HCC: hepatocellular carcinoma

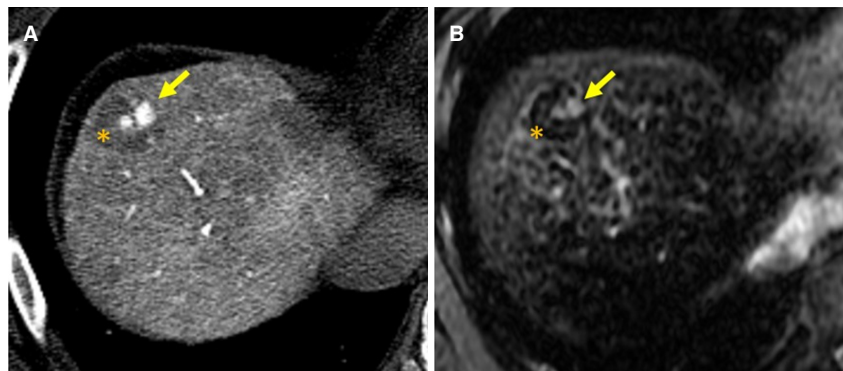


Figure 2. Example of nodule in nodule appearance lesion in alcoholic cirrhosis. A: CT during hepatic arteriography image of nodule in nodule lesion. Some hypervascular foci (arrow) are observed within hypovascular nodule (*); B: SPIO enhanced T2-MR image of nodule in nodule lesion. Small hyperintense spots, which do not uptake SPIO (arrow), are observed within the nodule which shows hypointensity because of uptake of SPIO (*). These findings represent the visualization of multistep hepatocarcinogenesis within the nodule. SPIO: superparamagnetic iron oxide

Macroscopically, HGDN and early HCC both show ill-defined nodules and are difficult to differentiate. Histologically, both show increased cell density but are scarce in structural atypia or cellular atypia. Therefore, stromal invasion of atypical cells is important for differentiating HGDN and early HCC on histopathology^[5].

When a histological study is performed in the portal tract of a completely excised nodule, differentiation of HGDN or early HCC is possible [Figure 4]. In a biopsy specimen however, misdiagnosis of the early HCC nodule as HGDN can happen because the specimen is small and does not contain sufficient portal tracts [Figure 4]. In diagnosis based on imaging, findings reflect the total image of the nodule and the presence of stromal invasion of atypical cells might not be shown.

For these reasons, it is inevitable that borderline cases on histology between HCC and DN is different from that seen on imaging.

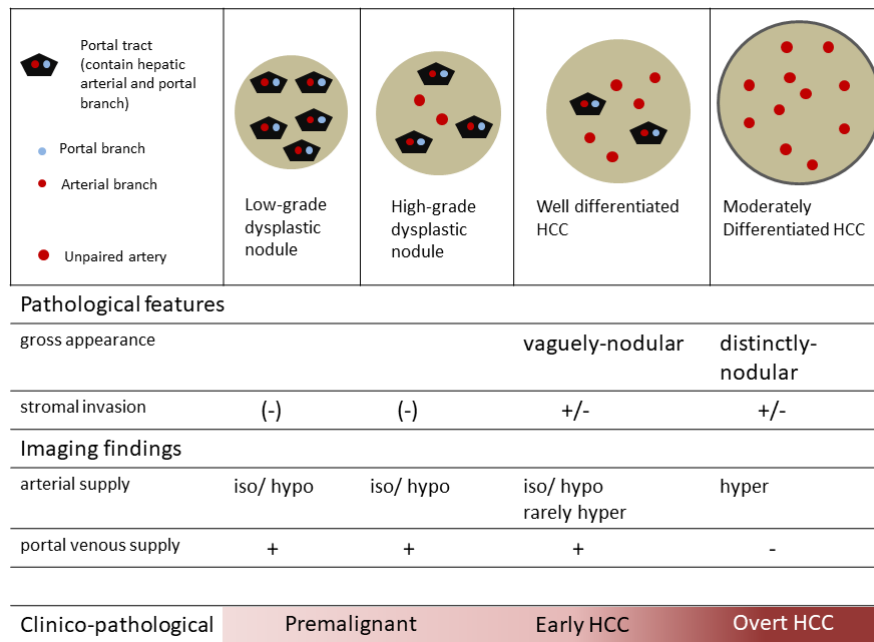


Figure 3. Clinical and pathological characteristics of small nodular lesions during multistep hepatocarcinogenesis step in cirrhotic liver. HCC: hepatocellular carcinoma

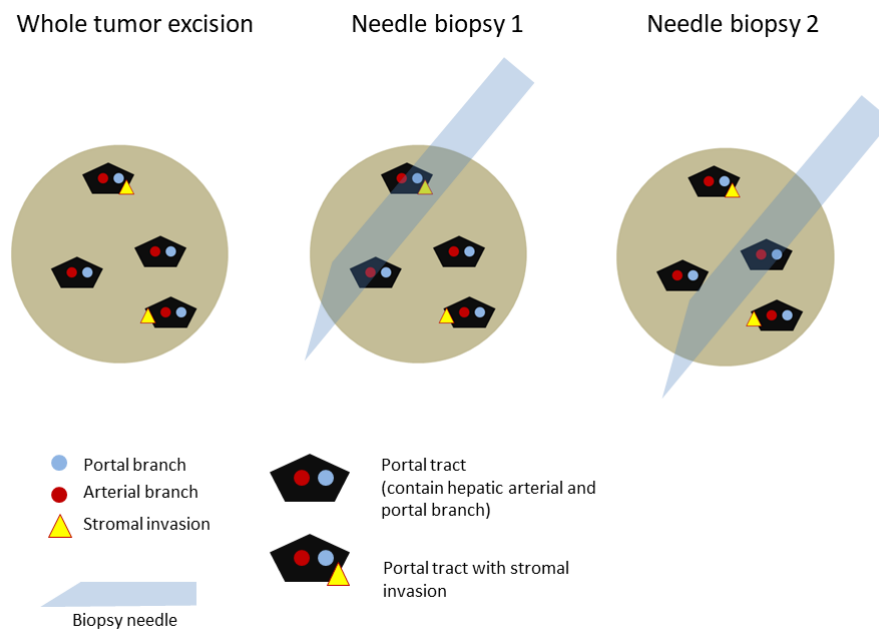


Figure 4. Problem in histological diagnosis of early HCC with needle biopsy specimen. Histopathologically, the presence of the stromal invasion of tumor cell to the portal tract is the most reliable marker for the diagnosis of early HCC. In whole tumor excision specimen, and needle biopsy 1 specimen, pathologist can find out the stromal invasion within the specimen and diagnose the nodule as early HCC. However, in needle biopsy 2, the stromal invasion finding is not contained within the specimen and pathologist diagnose the nodule as high-grade dysplastic nodule, not early HCC. This kind of sampling error issue is possible in histopathological diagnosis of the nodule during multistep hepatocarcinogenesis. HCC: hepatocellular carcinoma

It is impossible however, to excise all nodules in liver cirrhosis to identify HGDN or early HCC. In addition, biopsies could be misdiagnosed due to sampling error. Therefore, it is reasonable to attempt differentiation between benign and malignant nodules through imaging with CT and MRI, which are relatively minimally invasive compared to histopathology.

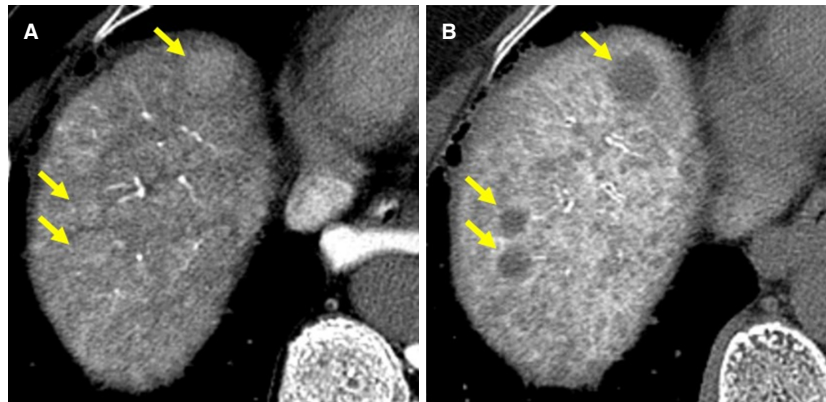


Figure 5. Example of high grade dysplastic nodule in cirrhotic liver. A: on CT during arterial portography, there are isodense nodule compared to background liver (arrows), which represent nodular portal flow is preserved; B: on CT during hepatic arteriography, these nodules show hypodensity compared to the background liver (arrows), which indicate the nodular arterial supply is decreased. This hypovascular pattern represents relatively benign nature nodules under multistep hepatocarcinogenesis

The different imaging modalities however, reflect different pathophysiological features between precancerous conditions (benign) and cancerous nodules (malignant), so the same nodule may be assessed differently through different modalities. Therefore, sound judgment is necessary based on the findings of multiple modalities when there is discrepancy.

VISUALIZATION OF MULTI-STEP HEPATOCARCINOGENESIS ON IMAGING AND DIFFERENTIATION OF BENIGN AND MALIGNANT NODULES

Approach by haemodynamic imaging

Histological studies have found that during multi-step hepatocarcinogenesis, the number of portal tracts in nodules gradually decreases as the nodular grade of malignancy changes from DN to hypervascular, classical HCC, and the number of arteries which do not run with portal veins (unpaired artery) increases [Figure 3]^[8].

Angiography-assisted CT, which selectively infuses a contrast agent into the hepatic artery or portal vein during CT scan of the liver, can evaluate the degree of arterial and portal blood supply to the nodule. Angiography-assisted CT consists of the following two methods: one is CT during hepatic arteriography (CTHA), which evaluates hepatic arterial blood supply, and the other is CT during arterial portography (CTAP), which evaluates portal venous blood supply. We can evaluate the degree of hepatic arterial flow and portal venous flow of the nodules undergoing multi-step hepatocarcinogenesis on CTHA and CTAP. With angiography-assisted CT, we have found that LGDN, HGDN, and early HCC, which are all relatively benign nodules, were hypovascular in nature with low arterial and preserved portal blood flow [Figure 5]. In contrast, more malignant nodules, such as well differentiated HCC and moderately differentiated HCC, showed a hypervascular nature with low portal blood flow and high arterial blood flow [Figure 6]^[9].

These imaging findings reflect the histological changes of the nodules during multistep hepatocarcinogenesis: the number of portal tracts, which contain hepatic arteries and portal veins, decreases as the nodular grade of malignancy increases, and at some point, unpaired arteries starts to develop within the nodule. Figure 7 shows the relationship between the nodular grade of malignancy and the degree of hepatic arterial and portal blood supply of nodules.

Recently, Kitao *et al.*^[10] showed that drainage vessels of the nodules during multistep hepatocarcinogenesis process transform as the nodular grade of malignancy changes [Figure 8]. They compared angiography-assisted CT findings with histological findings of the nodules during multistep hepatocarcinogenesis and

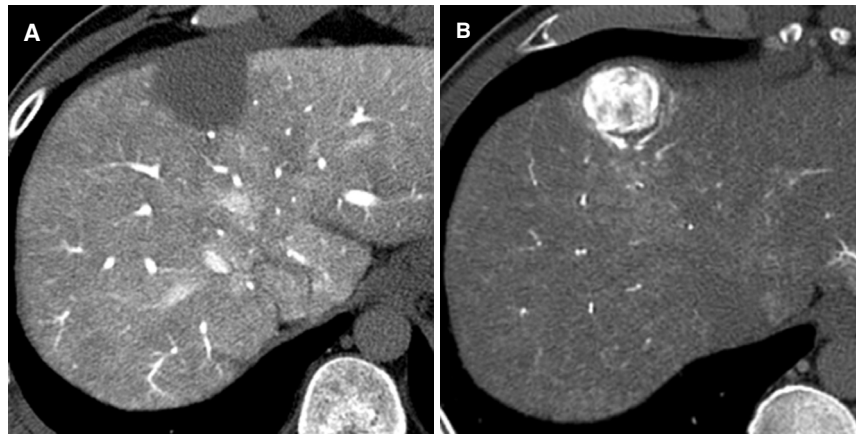


Figure 6. Example of advanced (moderately differentiated) hepatocellular carcinoma in cirrhotic liver. A: on CT during arterial portography, there are hypodense nodule compared to background liver, which represent loss of nodular portal flow; B: on CT during hepatic arteriography, the lesion shows marked hyperdensity compared to the background liver, which indicate increased nodular arterial flow. This hypervascular pattern indicates definite malignant nodule such as moderately differentiated hepatocellular carcinoma

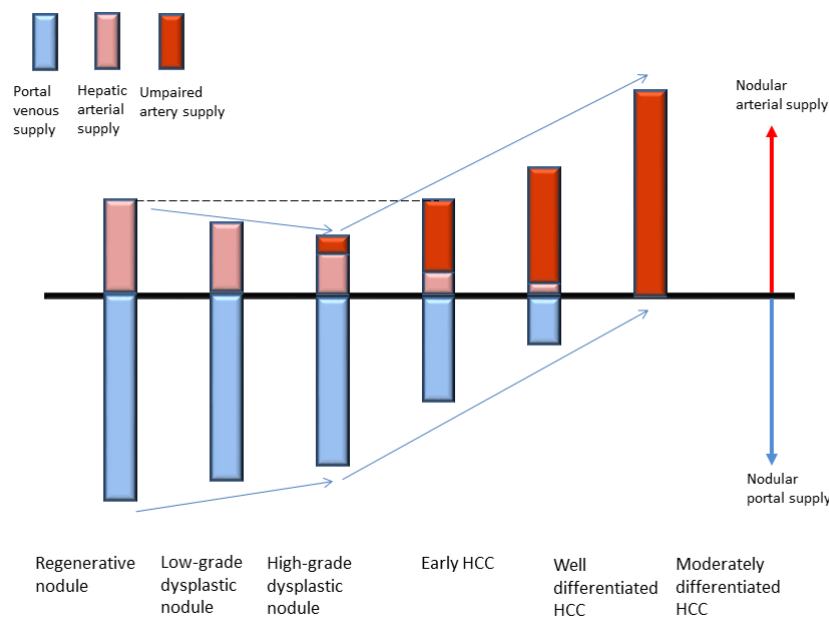


Figure 7. Relationship between the nodular grade of malignancy and the degree of intranodular blood supply of the nodule. HCC: hepatocellular carcinoma

found that in hypovascular nodules, such as DN and early HCC in which portal blood flow is maintained and the increase in arterial blood supply not seen yet, blood that drains from the tumor flows from tumor sinusoids into intranodular hepatic venules and then into extranodular hepatic veins. In contrast, in hypervascular nodules, especially in HCC without capsules, blood flows from within tumor sinusoids into the surrounding hepatic sinusoids; and in hypervascular HCC with capsules, blood flows from within tumor sinusoids into intracapsule portal venules, and then into surrounding hepatic sinusoids.

These findings show that during hepatocarcinogenesis, the hepatic vein is initially occluded by increased intratumoral pressure caused by increase in cell density, and direct connections between tumor sinusoids and surrounding hepatic sinusoids work as the tumor drainage route because of occlusion of the hepatic vein. Then, as the nodular grade of malignancy progresses, the tumor becomes encapsulated and the direct

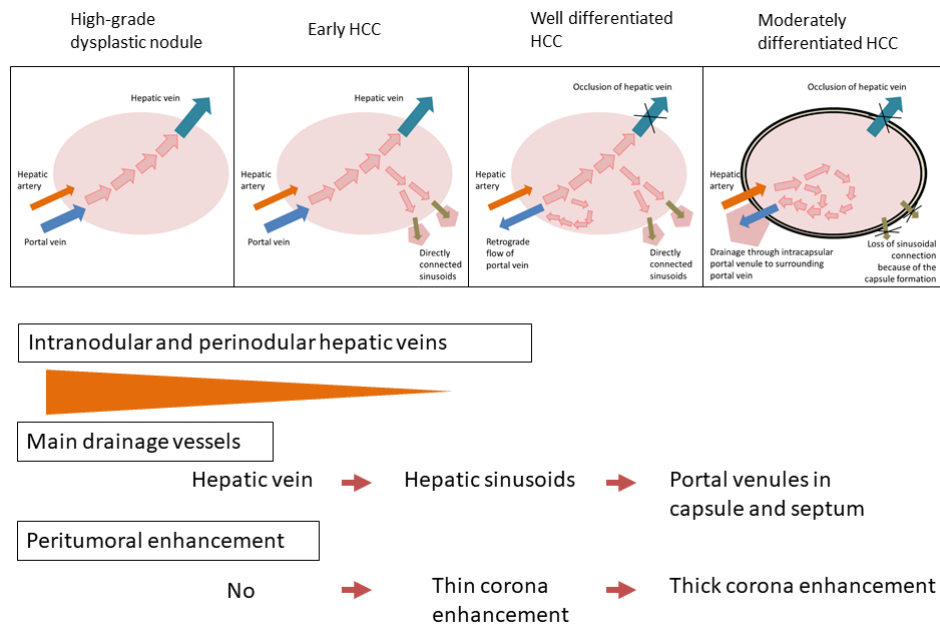


Figure 8. Multistep changes of drainage vessels and peritumoral enhancement during hepatocarcinogenesis. HCC: hepatocellular carcinoma

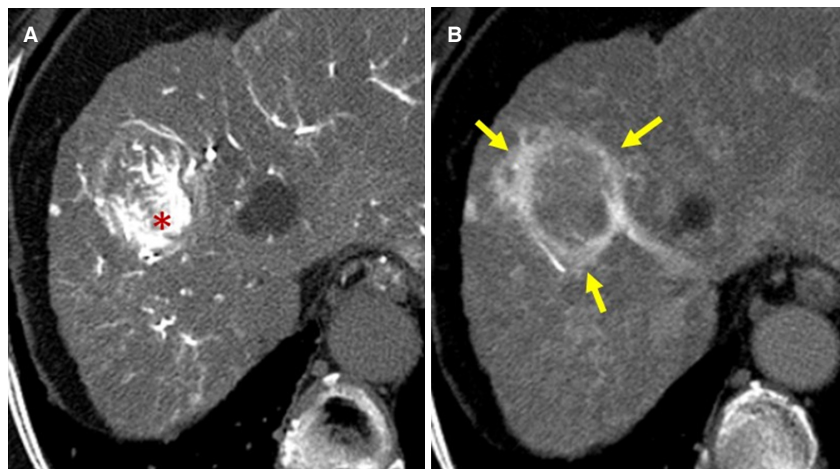


Figure 9. Example of corona enhancement caused by drainage flow from hypervascular HCC. A: on early phase image of CT during hepatic arteriography, HCC shows marked enhancement because of hypervascularity (*); B: on late phase image of CT during hepatic arteriography, since the intraarterial contrast injection is stopped, the enhancement of HCC decreases (*) and peritumoral normal liver parenchyma is enhanced by drainage of contrast material from HCC (arrow). HCC: hepatocellular carcinoma

connection between tumor sinusoids and surrounding hepatic sinusoids is lost, such that drainage flow is retrograde through the intracapsular portal venules. On CTHA, this retrograde flow of contrast medium stains surrounding hepatic parenchyma around the tumor and is identified as corona enhancement [Figure 9]^[11].

On angiography-assisted CT, LGDN, early HCC, and part of well-differentiated HCC are all observed as hypovascular nodules. In contrast, the presence of a nodule-in-nodule appearance, which has a partly hypervascular foci within a hypovascular nodule, is a definite hallmark to discriminate between hypovascular, relatively benign nodules and more malignant nodules since the latter display hypervascularity [Figure 10]. In hypovascular nodules, most show iso-density on CTAP and hypo-density on CTHA since nodular portal blood flow is preserved and increase in hepatic arterial flow has not started, which represents

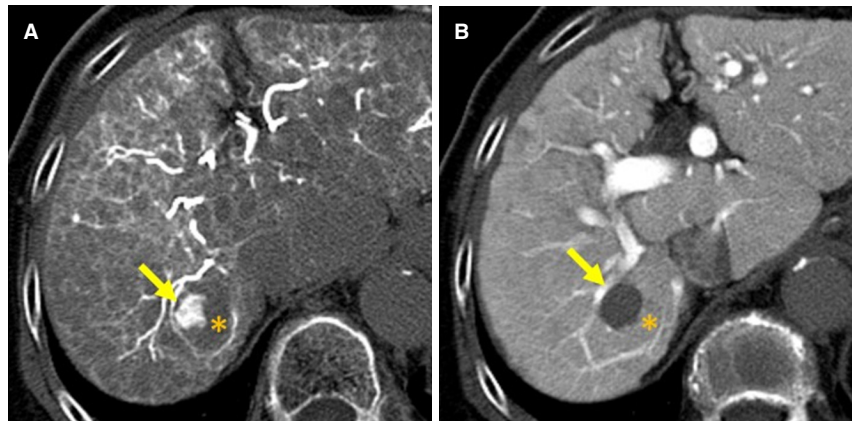


Figure 10. Hypovascular nodule with hypervascular foci (nodule in nodule appearance lesion). A: on CT during hepatic arteriography, hypervascular foci (arrow) is observed within hypovascular nodule (*); B: on CT during arterial portography, only hypervascular portion on CT during hepatic arteriography (arrow) shows hypointensity and other portion of the nodule show isointensity to the background liver (*). In this nodule hypovascular portion of the nodule (*) are relatively benign nature and hypervascular foci represents dedifferentiated more malignant nature

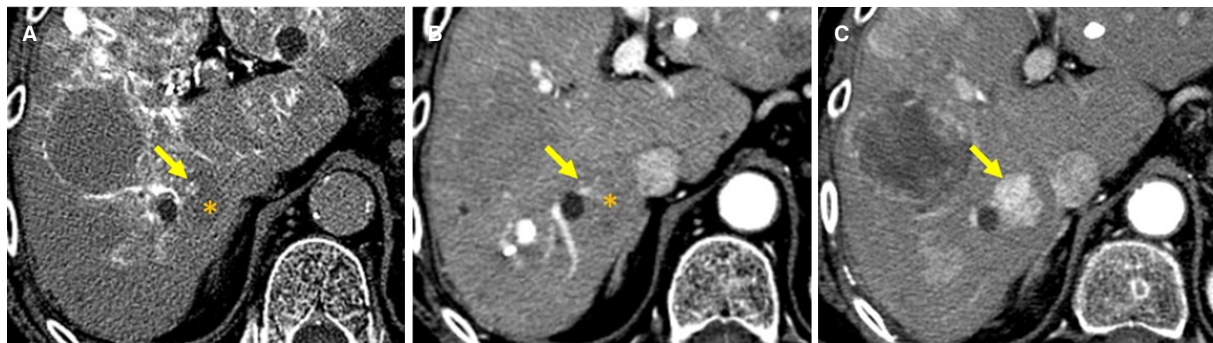


Figure 11. Development of hepatocellular carcinoma from hypovascular nodule with hypervascular foci. A, B: on CT during hepatic arteriography (A) and arterial phase of dynamic CT (B), small hypervascular foci (arrow) is observed within hypovascular nodule (*); C: eleven months after, on arterial phase of dynamic CT, the hypovascular nodule with hypervascular foci transformed to hypervascular hepatocellular carcinoma (arrow)

the relatively benign nature of most of the nodule. However, if nodules contain foci that show low density on CTAP but a high density on CTHA, this would represent hypervascularity and likely malignant nature of the affected portion. This type of nodule is considered visualization of the multi-step hepatocarcinogenesis process within the tumor, namely a more hypervascular and thus malignant foci develops within a hypovascular and relatively benign nodule. To find out this kind of dedifferentiated hypervascular foci within hypovascular nodule might be easy and reliable method to pick up high-risk precancerous nodule among hypovascular cirrhotic nodules.

Nodules with relatively benign features including preserved portal blood flow on CTAP and hypovascularity on CTHA vary from LGDN to early HCC that are relatively benign. In contrast, nodules that lack portal blood flow on CTAP and have an area of high density on CTHA are moderately differentiated and hypervascular HCC, which are definitely malignant nodules. The hypovascular nodule with a hypervascular foci is considered the transitional stage nodule between more benign and definitely malignant nodules [Figure 11].

Nodules with such hypervascular foci within hypovascular nodules, which is the visualization of multi-step hepatocarcinogenesis, are relatively easy to pick up on dynamic contrast enhanced CT/MR imaging [Figure 12],



Figure 12. Visualization of hypervascular foci on dynamic contrast enhanced CT and MRI. A: on CT during hepatic arteriography, hypervascular foci (arrow) is observed within hypovascular nodule (*); B: on arterial phase of dynamic CT, small early enhancement (arrow) is observed within hypovascular nodule; C: on arterial phase of dynamic MRI with Gd-EOB-DTPA, small early enhancement (arrow) is observed within hypovascular nodule

and can be used as a kind of potential biomarker to differentiate between benign and malignant nodules on imaging.

Until now, we have mainly reviewed the imaging findings of angiography-assisted CT. To acquire angiography-assisted CT images however, angiography, which is relatively invasive and an unusual procedure in daily clinical practice, is necessary. By comparison, intravenous contrast enhanced dynamic CT or dynamic MRI, which is usually performed in clinical practice, can also detect hypervascular foci in hypovascular nodules.

Shinmura *et al.*^[12] reported in 2008 that when the size of the hypervascular foci was 5 mm or more, dynamic CT and dynamic MRI could detect 83% and 64% of hypervascular foci in hypovascular nodules respectively. Detection rates have improved on the latest CT and MR equipment but further examination is needed to clarify this issue.

Approach from MRI signal intensity

On correlation studies of MRI signal intensities with histopathological findings, DN and early HCC have iso- to high-intensity on T1-weighted images and a iso- to low-intensity on T2-weighted images^[13], while moderately differentiated HCC has low-, iso-, and high-intensity on T1-weighted images and a high-intensity on T2-weighted images [Figure 13]^[14]. One of the reasons for various signal intensities on T1-weighted images in HCC is a higher protein content due to an increase in cell density and/or N-C ratio.

In terms of the relationship between nodular arterial and portal blood supply and MR signal intensity, nodules with normal portal blood flow but low hepatic arterial flow (estimated to be DN to early HCC) had 63% high-intensity on T1-weighted images and 72% low-intensity on T2-weighted images; nodules which had mildly decreased portal blood flow and still low hepatic arterial flow (estimated to be early HCC to well differentiated HCC) had 39% iso-intensity on T1-weighted images and 52% iso-intensity on T2-weighted images; and nodules which lacked portal blood flow and had markedly high hepatic arterial flow (estimated to be moderately differentiated HCC) had 48% low-intensity on T1-weighted images and 85% high-intensity on T2-weighted images^[15].

These studies show that for estimation of the grade of malignancy in hepatocellular nodules on MRI signal intensity, T2-weighted images are useful, and the low- to iso-intensity of the nodules suggest relatively benign pathology such as RN, DN to early HCC, and high-intensity would suggest moderately differentiated HCC.

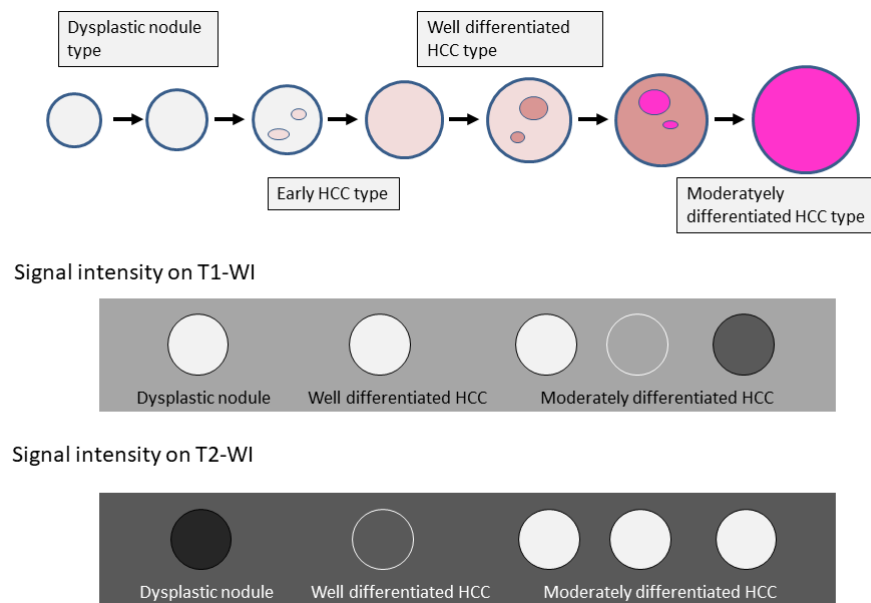


Figure 13. MR signal intensity change during multistep hepatocarcinogenesis. HCC: hepatocellular carcinoma

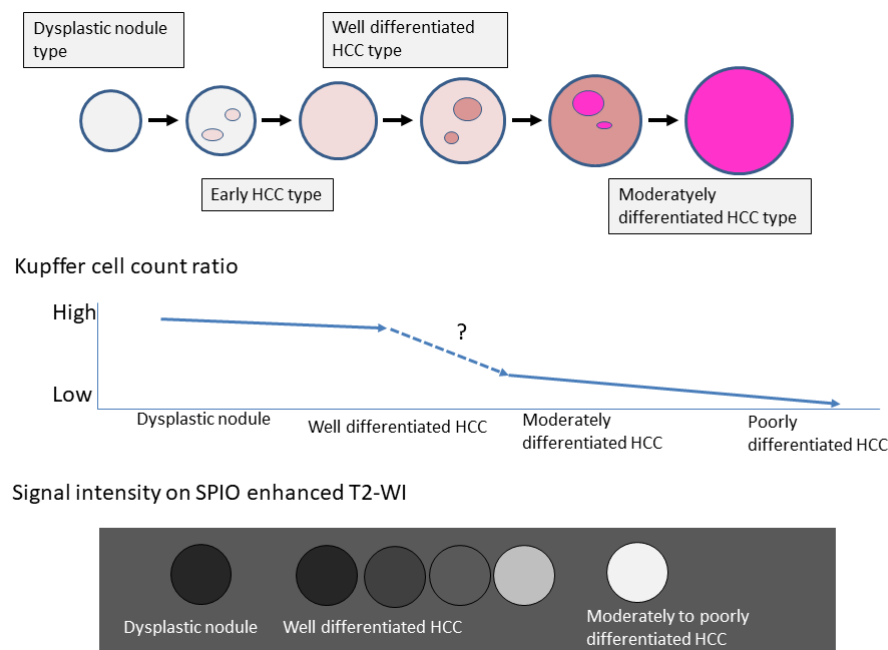


Figure 14. SPIO enhanced T2 weighted MR signal intensity change during multistep hepatocarcinogenesis. HCC: hepatocellular carcinoma; SPIO: superparamagnetic iron oxide

Approach from functional evaluation of Kupffer cells

Sinusoids are where blood flows through the liver cords. It consists of sinusoidal endothelial cells, Kupffer cells, stellate cells, and pit cells^[16]. Kupffer cells are intravascular resident macrophages in liver sinusoids. They are capable of phagocytosis and eliminate foreign bodies, bacteria, and broken hepatocytes.

When SPIO is administered intravenously in the normal liver, Kupffer cells in sinusoids take it up. SPIO shortens the transverse relaxation time of protons of tissues, which results in low signal intensities on T2-

weighted MR images. In cases where the normal structure of sinusoids is lost (metastatic liver cancer *etc.*), signal intensity changes do not occur because there are no Kupffer cells. Similarly, the function of Kupffer cells are decreased despite their presence due to irradiation *etc.*, the degree of SPIO uptake is lowered, and such areas show relatively high signal intensities on T2-weighted images compared to the surrounding normal liver where normal Kupffer cells are present and take up SPIO^[17].

On histopathological analysis, Imai *et al.*^[18] found that in multi-step hepatocarcinogenesis, as the nodular degree of differentiation is lowered, the number of Kupffer cells within the nodule is decreased, and there was a significant difference between the number of Kupffer cells in DN, well differentiated HCC, moderately differentiated HCC, and poorly differentiated HCC. In most DN and 46% of well differentiated HCC, the number of Kupffer cells was increased compared with the surrounding liver; the number of Kupffer cells decreased to 30% compared with the surrounding liver in moderately differentiated HCC; and the number of Kupffer cells decreased to 13% on average in poorly differentiated HCC [Figure 14].

On SPIO-enhanced MRI, DN and well-differentiated HCC present with iso- to slightly low-intensity on T2 weighted MR images compared to the surrounding liver. In well-differentiated HCC especially, 46% of the nodules presented with a low-intensity compared to the surrounding liver. Moderately and poorly differentiated HCC though presented with a high-intensity compared with the surrounding liver. There was a significant difference in signal intensity between DN with well-differentiated HCC, and moderately and poorly differentiated HCC [Figure 14]^[18]. These results show that SPIO-enhanced MRI is a good tool to estimate the degree of differentiation in HCC, but it has a limit in differentiating DN from well-differentiated HCC.

Approach from the function of hepatocyte membrane transporter

Approximately 50% of Gd-EOB-DTPA (EOB), as well as other extracellular contrast agents, is excreted from the kidneys and the remaining 50% is taken up by hepatocytes and then excreted into the biliary tract, hence, it is called hepatocyte-specific contrast agent. The advantage of this MRI contrast agent is it can evaluate both blood flow information and hepatocyte function simultaneously. However, in EOB-enhanced MRI, there are some drawbacks of low accumulation in hepatocytes depending on liver function and the low degree of arterial enhancement, and there are also limitations in viewing true washout or enhancement of capsules, which are major features characterizing overt HCC.

After rapid intravenous injection of EOB, blood flow evaluation by dynamic study is performed similarly to other extracellular contrast agents. In the hepatobiliary phase after 15-20 min of injection, the liver parenchyma presents high signal intensity on T1-weighted images because of EOB uptake by normal hepatocytes. Areas that do not have normal hepatocytes (metastatic liver cancer *etc.*) or areas where hepatocytes have poor EOB uptake ability (irradiated area *etc.*) present with low intensities compared to the surrounding normal liver.

EOB is taken up into the intracellular space of hepatocytes by organic anion transporting polypeptide (OATP) 1B3, which is expressed on the sinusoidal side of hepatocyte membranes and excreted in the biliary tract through multidrug resistance associated protein 2 (MRP2), which is expressed on the bile canalicular side of hepatocyte membrane^[19]. Under the conditions of bile duct obstruction, MRP3, which is expressed on the sinusoidal side of hepatocyte membranes, increase and EOB is excreted into the sinusoids^[19]. Kitao *et al.*^[20] compared the degree of OATP1B3 expression in hepatocellular nodules undergoing multi-step hepatocarcinogenesis with the surrounding normal liver, and found that it was preserved in LGDN but decreased in approximately 30% of HGDN and approximately 70% of early HCC [Figure 15].

In most moderately differentiated HCC, there was decreased expression of OATP1B3. As well, in the hepatobiliary phase of EOB-enhanced MRI, the signal enhancement ratio was significantly lowered in well to

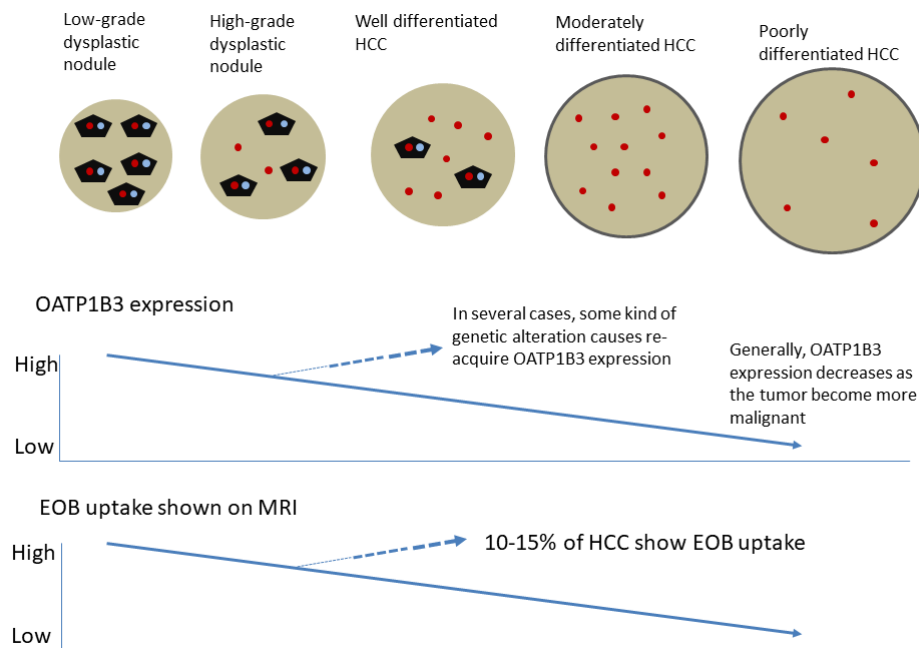


Figure 15. Relationship between OATP1B3 expression, EOB uptake and multistep hepatocarcinogenesis. HCC: hepatocellular carcinoma; EOB: Gd-EOB-DTPA

moderately differentiated HCC compared to the surrounding liver. As the grade of malignancy increases from well-differentiated to moderately- and poorly-differentiated HCC, the signal enhancement ratio decreases. However, in 10%-15% of moderately differentiated HCC, there was increased expression of OATP1B3 and these HCC present a high signal in the hepatobiliary phase of EOB-enhanced MRI [Figure 15].

Sano *et al.*^[21] compared the ability of various imaging modalities, including dynamic CT, angiography-assisted CT, and EOB-enhanced MRI, in differentiating between histologically confirmed DN and early HCC. They showed that in the hepatobiliary phase of EOB-enhanced MRI, there were no DN nodules which presented with low-intensity whereas 97% of early HCC presented with low-intensity, and concluded this was best suited for the differential diagnosis of DN and early HCC. We surmise then that before starting histopathological and/or morphological malignant transformation observed as microscopic stromal invasion, functional alterations of cellular components such as membranous transporter functional states, which is regulated by genome level alterations, are already advanced within the nodule and can be detected by EOB. Further study is needed though to clarify this issue.

We also studied the relationship between the signal intensity of nodules during the hepatobiliary phase of EOB-enhanced MRI with the prognosis of these nodules that were smaller than 20 mm (average size and SD; 11.0 ± 2.8 mm), both in hypovascular ones (relatively more benign nature nodule) in multi-step hepatocarcinogenesis and in hypovascular ones with hypervascular foci (hypervascular foci-containing nodules) that are more representative of malignancy. Hypovascular nodules with a low-intensity in the hepatobiliary phase of EOB-enhanced MRI, progressed to hypervascular HCC in 17%, 28% and 41% after 1, 2 and 3 years respectively, while those with iso-intensity only progressed to hypervascular HCC in 7% after 1 year and those with high-intensity did not progress at all^[22].

On the other hand, in hypervascular foci-containing nodules, whether the signal intensity was low or iso during the hepatobiliary phase of EOB-enhanced MRI, 50% progressed to hypervascular HCC after 1 year^[23]. This suggests that hypervascular foci in nodules can exacerbate multi-step hepatocarcinogenesis regardless of the decrease in EOB uptake.

CONCLUSION

In this article, we have reviewed how malignant nodules can be differentiated from benign ones in multi-step hepatocarcinogenesis using various imaging techniques. It is difficult to draw a clear line between them however, because different imaging modalities reflect different pathophysiology and biopsies could have sampling errors. However, this review would have helped in understanding of the current status in diagnosing hepatocellular nodules, whether benign or malignant, in various imaging modalities.

DECLARATIONS

Authors' contributions

Design of the review: Matsui O, Kobayashi S

Literature review and Manuscript writing: Kobayashi S, Kozaka K, Minami T

Manuscript revision: Kobayashi S, Gabata T

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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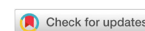
REFERENCES

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, et al. Global cancer statistics. 2012. *CA Cancer J Clin* 2015;65:87-108.
2. Ichikawa S, Ichikawa T, Motosugi U, Sano K, Morisaka H, et al. Was hypervascular hepatocellular carcinoma visible on previous gadoxetic acid-enhanced magnetic resonance images? *Liver Cancer* 2015;4:154-62.
3. Tamada T, Korenaga M, Yamamoto A, Higaki A, Kanki A, et al. Assessment of clinical and magnetic resonance imaging features of de novo hypervascular hepatocellular carcinoma using gadoxetic acid-enhanced magnetic resonance imaging. *Hepatol Res* 2017;47:E152-60.
4. Sakamoto M, Effendi K, Masugi Y. Molecular diagnosis of multistage hepatocarcinogenesis. *Jpn J Clin Oncol* 2010;40:891-6.
5. International Consensus Group for Hepatocellular Neoplasia. The International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 2009;49:658-64.
6. Torbenson MS, Ng IOL, Park YN, Sakamoto M. Hepatocellular carcinoma. In: edited by the WHO Classification of Tumours Editorial Board. WHO Classification of Tumours. 5th edition. Digestive System Tumours. Lyon, France: International Agency for Research on Cancer; 2019. pp. 229-39.
7. Nakano M, Saito A, Yamamoto M, Doi M, Takasaki K. Stromal and blood vessel wall invasion in well-differentiated hepatocellular carcinoma. *Liver* 1997;17:41-6.
8. Ueda K, Terada T, Nakanuma Y, Matsui O. *Hum Pathol* 1992;23:619-26.
9. Hayashi M, Matsui O, Ueda K, Kawamori Y, Kadoya M, et al. Correlation between the blood supply and grade of malignancy of hepatocellular nodules associated with liver cirrhosis: evaluation by CT during intraarterial injection of contrast medium. *Am J Roentgenol* 1999;172:969-76.
10. Kitao A, Zen Y, Matsui O, Gabata T, Nakanuma Y. Hepatocarcinogenesis: multistep changes of drainage vessels at CT during arterial

- portography and hepatic arteriography--radiologic-pathologic correlation. *Radiology* 2009;252:605-14.
11. Ueda K, Matsui O, Kawamori Y, Nakanuma Y, Kadoya M, et al. Hypervascular hepatocellular carcinoma: evaluation of hemodynamics with dynamic CT during hepatic arteriography. *Radiology* 1998;206:161-6.
 12. Shinmura R, Matsui O, Kadoya M, Kobayashi S, Terayama N, et al. Detection of hypervascular malignant foci in borderline lesions of hepatocellular carcinoma: comparison of dynamic multi-detector row CT, dynamic MR imaging and superparamagnetic iron oxide-enhanced MR imaging. *Eur Radiol* 2008;18:1918-24.
 13. Matsui O, Kadoya M, Kameyama T, Yoshikawa J, Arai K, et al. Adenomatous hyperplastic nodules in the cirrhotic liver: differentiation from hepatocellular carcinoma with MR imaging. *Radiology* 1989;173:123-6.
 14. Kadoya M, Matsui O, Takashima T, Nonomura A. Hepatocellular carcinoma: correlation of MR imaging and histopathologic findings. *Radiology* 1992;183:819-25.
 15. Shinmura R, Matsui O, Kobayashi S, Terayama N, Sanada J, et al. Cirrhotic nodules: association between MR imaging signal intensity and intranodular blood supply. *Radiology* 2005;237:512-9.
 16. Abu Rmilah A, Zhou W, Nelson E, Lin L, Amiot B, et al. Understanding the marvels behind liver regeneration. *Wiley Interdiscip Rev Dev Biol* 2019;8:e340.
 17. Kawamori Y, Matsui O, Kadoya M, Yoshikawa J, Demachi H, et al. Differentiation of hepatocellular carcinomas from hyperplastic nodules induced in rat liver with ferrite-enhanced MR imaging. *Radiology* 1992;183:65-72.
 18. Imai Y, Murakami T, Yoshida S, Nishikawa M, Ohsawa M, et al. Superparamagnetic iron oxide-enhanced magnetic resonance images of hepatocellular carcinoma: correlation with histological grading. *Hepatology* 2000;32:205-12.
 19. Kitao A, Zen Y, Matsui O, Gabata T, Kobayashi S, et al. Hepatocellular carcinoma: signal intensity at gadoxetic acid-enhanced MR Imaging--correlation with molecular transporters and histopathologic features. *Radiology* 2010;256:817-26.
 20. Kitao A, Matsui O, Yoneda N, Kozaka K, Shinmura R, et al. The uptake transporter OATP8 expression decreases during multistep hepatocarcinogenesis: correlation with gadoxetic acid enhanced MR imaging. *Eur Radiol* 2011;21:2056-66.
 21. Sano K, Ichikawa T, Motosugi U, Sou H, Muhi AM, et al. Imaging study of early hepatocellular carcinoma: usefulness of gadoxetic acid-enhanced MR imaging. *Radiology* 2011;261:834-44.
 22. Kobayashi S, Matsui O, Gabata T, Koda W, Minami T, et al. Relationship between signal intensity on hepatobiliary phase of gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA)-enhanced MR imaging and prognosis of borderline lesions of hepatocellular carcinoma. *Eur J Radiol* 2012;81:3002-9.
 23. Kobayashi S, Matsui O, Gabata T, Koda W, Minami T, et al. Gadolinium ethoxybenzyl diethylenetriamine pentaacetic Acid-enhanced magnetic resonance imaging findings of borderline lesions at high risk for progression to hypervascular classic hepatocellular carcinoma. *J Comput Assist Tomogr* 2011;35:181-6.

Review

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Risk factors of portal vein thrombosis after splenectomy in patients with liver cirrhosis

Ze-long Yang¹, Ting Guo², Dong-Lie Zhu¹, Shi Zheng¹, Dan-Dan Han¹, Yong Chen¹

¹Department of Hepatobiliary Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, Shaanxi, China.

²Department of Obstetrics, Huaxi Hospital, Sichuan University, Cheng Du 610011, Si Chuan, China.

Correspondence to: Yong Chen, PhD, Chief Physician, Department of Hepatobiliary Surgery, Xijing Hospital, Fourth Military Medical University, 127 West Changle Road, Xincheng District, Xi'an 710032, Shaanxi, China. E-mail: gdwkcy@fmmu.edu.cn

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Abstract

Portal vein thrombosis (PVT) is a common complication after splenectomy, causing a possible negative impact on the prognosis of patients with liver cirrhosis. However, the risk factors of PVT are not completely clear. Many factors are related to the occurrence of postoperative PVT, such as hemodynamic changes, splenomegaly, splenectomy, coagulation and anticoagulation disorder, liver cirrhosis, platelet count, D-dimer level, infection, inflammation, and other factors. Hemodynamic changes are mainly caused by thicker portal and splenic vein diameters, larger spleen, slower portal vein blood flow rate, lower portal vein pressure before and after surgery, etc. It is timely detection and advanced prevention that really matter in reducing PVT incidence and improving patient prognosis. We systematically reviewed the researches on the risk factors and therapies of PVT to provide useful information on a comprehensive understanding for researchers.

Keywords: Liver cirrhosis, splenectomy, portal vein thrombosis, risk factors, treatments, prophylaxis

INTRODUCTION

Portal vein thrombosis (PVT) after splenectomy is a common postoperative complication, which has non-specific clinical manifestation^[1], including fever, anorexia, abdominal pain, abnormal liver function, elevated C-reactive protein level, etc. As PVT progresses, complications can be worse and finally result in liver function deterioration, intestinal infarction and even intestinal necrosis^[2-4], making the mortality rate of PVT even up to 10%^[5]. The incidence of PVT after splenectomy in patients with cirrhosis and portal hypertension is reported to range 24% to 29%^[6,7]. For those who undergo splenectomy or accepted liver transplantation, early prevention of PVT is quite important to improve prognosis, which requires a full understanding of the risk factors of PVT. But so far, the risk factors of PVT still remain controversial.



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RISK FACTORS

Hemodynamic changes

Hemodynamic changes are considered to be the most important risk factor in the pathogenesis of PVT. The formation of pseudolobules in the liver can reduce portal blood flow velocity markedly, causing obstructed portal blood flow, increased portal pressure, and decreased blood flow velocity^[8]. It can be said that the hemodynamic changes caused by the primary disease lays the foundation for the development of PVT.

Splenic vein diameter (SVD) is thought to be the most influential risk factor. A retrospective trail conducted in China found that 8 factors including the SVD are associated with postoperative PVT formation^[9]. SVD greater than 10 mm has been a cut-off for predictors of PVT development and those greater than 14 mm has been a cut-off for predictors of PVT which develops from splenic vein^[10]. Some authors even suggest that preoperative SVD greater than 8 mm is an independent risk factor for predicting PVT^[11]. SVD is inversely related to the rate of change in portal blood flow, and its sensitivity, specificity and efficiency vary with the study's setting^[6]. In addition, spleen weight, discussed below, shows a significant correlation with SVD, where removal of an enlarged spleen may cause a sudden decrease in splenic vein flow, forming and pushing the thrombus to migrate to the portal vein. But compared with the weight of the spleen, SVD plays a greater role in predicting PVT. Some researchers recommend the measurement of SVD preoperatively, and a close follow-up of patients with SVD greater than 8 mm^[12].

Generally, for patients with decompensated cirrhosis, PVD is proportional to the degree of portal hypertension, and once it is determined that PVD is greater than 13 mm, portal hypertension is considered to exist, and PVD is the independent risk factor of PVT^[9]. Multivariate analysis in some studies certify the significant relationship between pathogenesis of PVT and a series factors, including wider preoperative PVD, of which the cut-off width was determined to be 13 mm^[13]. Similarly, in another study, the incidence of PVT in patients with a PVD > 13 mm was 35 times that of others with PVD < 13 mm^[14]. When PVD becomes larger, blood flow velocity decreases, and the formation of blood clot is easier.

But some researchers believe that portal blood flow velocity is the only independent risk factor for PVT formation, and when velocity is lower than 15 cm/s, the risk of PVT increases significantly^[15]. By dividing cirrhotic patients into two groups according to portal vein flow velocity (≤ 15 cm/s or > 15 cm/s), the incidence of PVT within one year after surgery for the two groups turns out to be 47.8% and 2.0%, respectively^[14]. In a study examining the risk factors of PVT after the Hassab procedure, the cut-off points for portal vein flow, SVD and PVD were 1822.32 mL/min, 1.37 cm, and 1.56 cm, respectively^[16]. Besides, varicose veins of the esophagus and stomach caused by portal hypertension are also independently associated with the development of PVT^[17], and the wider portal collaterals can shunt more blood from the portal vein, slowing portal vein blood flow, and may increase the risk of PVT^[14]. Slow blood flow not only causes the procoagulant substances in the blood to be slowly concentrated locally^[18], but also causes an increase in pressure on the blood vessel wall according to Bernoulli's principle, which will damage the endothelial cells and initiate the coagulation mechanism^[3,19]. Therefore, it can be concluded that the wider PVD and SVD, the slower the blood flow of the portal vein system after operation and the greater the possibility of thrombosis^[20].

Splenomegaly

Splenomegaly is common in patients with liver cirrhosis, and patients with splenomegaly are at high risk of PVT; splenomegaly was previously thought to be related to passive congestion caused by portal hypertension^[21]. However, there is no necessary correlation between portal vein pressure and spleen size, because the grown spleen does not retract, and hypersplenism is not relieved after transjugular intrahepatic portal body shunt (TIPS) or splenorenal shunt^[22]. During years of follow-up of liver transplant recipients, portal pressure remained normal, but spleen enlargement persisted^[22]. Despite that passive congestion may

be one cause of the early stage of splenomegaly, there are still many causes to maintain splenomegaly, such as abnormal hyperplasia of white pulp, red pulp and fibrous tissues^[23].

Splenomegaly reduces the greater portal vein return flow after operation, inducing platelet aggregation^[18]. It has been reported that spleen weight (> 650 g) is associated with the development of PVT^[24] and that spleen weight (≥ 1311.5 g) is a significant independent predictor of PVT^[25]. Patients with PVT had a significantly greater splenic weight (median = 216 g) than those without PVT (median = 82 g)^[26], and some authors suggest that spleen weight is the only predictive factor of postoperative thrombosis after laparoscopic splenectomy (LS)^[27]. Here, there is a consensus among researchers that an increasingly larger and heavier spleen would finally be able to cause PVT after splenectomy if there is no medical intervention^[28]. Splenomegaly is a useful indicator of PVT, which can be conveniently evaluated before splenectomy by computed tomography^[10].

Splenectomy

Splenectomy is a major risk factor of PVT. Patients after splenectomy have a significantly higher incidence rate of PVT than those who have not undergone splenectomy^[29]. During the operation, the use of cutting tools and ligation causes thermal or mechanical damage to vascular endothelial cells, inducing thrombus formation^[30,31]. Different surgical procedures cause different levels of PVT risk. In China, the Hassab operation has been adopted widely because of the benefits it brings and less tissue damage compared with shunt surgery^[32], but some clinicians believe that selective decongestive devascularization combined with gastrosplenic shunt is superior to the Hassab operation, because this surgical method results in a lower long-term incidence of re-bleeding and PVT compared with the Hassab group^[33]. However, some studies have found that there is no relationship between different surgical methods and PVT formation^[12].

After splenectomy, reflux blood through the splenic vein almost disappears, and portal vein pressure and portal vein blood flow decrease by 20%-35%^[13]. If portal vein blood flow decreases sharply in the short-term, it will be easy for the portal vein to form eddies or other hemodynamic abnormalities, which is more likely to form a blood thrombus. In addition, after splenectomy, the distal end of the splenic vein becomes a dead end, which fosters blood retention, and the splenic vein thrombus is inclined to spread to the portal vein trunk^[34]. Although devascularization blocks the collateral circulation of the portal vein and draws some of the blood back to the portal vein system, it is often not enough to make up for the blood loss of the portal vein after splenectomy. Some researchers recommend partial splenectomy (PS) or partial splenic embolization instead of splenectomy, because after PS or embolization, the blood flow velocity of the portal vein is higher in comparison with whole splenectomy, and besides, PS can retain the immune function of the spleen to some degree^[35,36]. Some authors used an animal model of portal hypertension to determine the effect of PS, and after PS and intramuscular spleen transposition, portocaval collaterals developed and the portal venous pressure was reduced, while portocaval pressure difference was maintained to prevent deprivation of the liver. In a case report on the treatment of pediatric patients with portal hypertension, an 11-year-old girl underwent one-stage surgery, that is, PS with a view of transferring the remaining spleen to the thoracic cavity in the future. During follow-up, it was found that the patient's collateral circulation was well formed and that portal hypertension disappeared. The researchers believed that this case demonstrated that PS can be used as an alternative therapy to whole splenectomy^[37]. Another clinical study on the treatment of extrahepatic portal vein occlusion in children showed that through distal spleen and kidney shunt and PS with 20%-30% spleen retention, the platelet and white blood cell counts of the patients returned to normal after operation, which indicates that shunt surgery plus PS is an effective and safe method for treating portal hypertension and hypersplenism^[38].

Nowadays, LS combined with devascularization or LS combined with shunt surgery is carried out in many hospitals. And, LS is considered to be more likely to cause PVT than open splenectomy. A clinical

study revealed that PVT occurred in 12 (55%) patients of the LS group, but in only 4 (19%) of the open splenectomy group, and that LS leads to a higher incidence rate of PVT than does open splenectomy^[39,40]. Due to hypercapnia caused by CO₂, blood viscosity increases; in addition, blood flow velocity decreases with the positive pressure caused by pneumoperitoneum during the laparoscopic operation^[13,41]. Consequently, from a certain perspective, open surgery has a relative preventive effect on PVT itself compared with LS^[42].

Coagulation and anticoagulation disorder

In the coagulation system of patients with liver cirrhosis, procoagulant factors and anticoagulant factors are in a dangerous equilibrium^[43]; they are too complex and delicate to strike a balance: bleeding or thrombosis^[44], which may be disrupted by splenectomy, infection, acute renal failure, *etc.*^[45]. Patients with cirrhosis are not sensitive or even resistant to thrombomodulin, and the blood coagulation state is higher in patients with Child-Pugh C than in patients with A or B^[46]. It has been reported that a decrease in the levels of anticoagulant protein C and protein S can promote the pathogenesis of thrombosis, and in cirrhosis, the synthesis of protein C and S is impaired, so as cirrhosis worsens, factor VIII (procoagulant) increases, while protein C, one of the anticoagulants decreases^[46]; increased levels of factor VIII and decreased levels of protein C may be the major factors for PVT^[47]. However, protein C and protein S in PVT might not be associated with PVT in liver cirrhosis, especially when the impact of liver function is excluded^[48,49].

Preoperative antithrombin III (AT-III) is an important risk factor for PVT, where the synthesis of it is reduced because of cirrhosis, and it is further reduced after splenectomy, leading to overconsumption of anticoagulants^[50,51]. Some authors have demonstrated that prophylaxis with AT-III concentrates and danaparoid sodium after splenectomy can dramatically reduce the incidence of PVT^[51].

Prolonged prothrombin time (PT) is thought to be an independent factor in the occurrence of postoperative PVT^[29], but other researchers hold a different opinion that the formation of PVT has nothing to do with PT^[52], which may be so because traditional coagulation indicators do not reflect the true coagulation status of patients with liver cirrhosis^[53].

Fibrinogen can participate in the development of thrombosis^[54], and increased fibrinogen indicates a decrease in fibrinolytic activity and increases the incidence of thrombus^[55].

Liver cirrhosis

Different causes of liver cirrhosis lead to different PVT risks^[56]. As decreased liver synthesis results in hypoproteinemia, extravasation of plasma water, and hypercoagulable blood concentration, it is easy to form thrombus in the portal vein system^[57]. Because of the special pathophysiology of patients with liver cirrhosis, the incidence of PVT after splenectomy is higher^[58]. A study showed that the incidence of PVT in patients with and without cirrhosis was 32.0% and 9.5%^[59]. Liver dysfunction can affect the formation of PVT by affecting the synthesis of coagulation factors, thrombin, albumin, *etc.*^[51]. Studies show that Child-Pugh scores are significantly higher in patients with postoperative PVT than in patients without PVT^[60]. Meanwhile, incidence of PVT increases when MELD score is ≥ 13 points^[15]. Studies have shown the portal vein system rapidly forms a thrombus long before the improvement of liver function after splenectomy^[41], and some authors believe that traditional serological indicators (ALT, AST, *etc.*) have no relationship with PVT formation^[52], but ascites may be an important predictor of PVT, and albumin and hemoglobin decrease in cirrhotic patients significantly, which have potential value for the prediction^[18,61].

The occurrence and development of PVT has been shown to be related to the degree of esophageal varices^[17], which is caused by portal hypertension. Another study showed that the portal venous blood flow velocity in the severe gastroesophageal varices group was (12.13 ± 2.59) cm/s, while in the non-severe gastroesophageal varices group, velocity was (15.26 ± 5.06) cm/s^[62]. As is known to all, cirrhosis is an irreversible disease that

continues to progress. The degree of cirrhosis worsens with time, and the occurrence of PVT is related to the severity of cirrhosis directly or indirectly.

Platelet counts

The enlarged spleen secretes related factors that inhibit platelet release from the marrow and reduces thrombopoietin during cirrhosis^[63]. Once the spleen is removed, the suppression and clearance of platelets disappear, megakaryocytes proliferate in the marrow, causing platelets to skyrocket transiently. The formation of PVT might be associated with the soaring platelet count after operation^[64].

Preoperative decrease in platelet numbers is related to PVT^[11], the incidence of PVT in patients with PLT less than $50 \times 10^9/L$ before surgery is significantly different from patients with PLT greater than $50 \times 10^9/L$ ^[11]. And PLT is considered to be an independent risk factor for PVT after splenectomy^[6,13,29].

PLT often peaks at 3 to 20 days after splenectomy and gradually decreases to normal level. In one study, PVT was detected on the 6th day after surgery in patients whose PLT exceeded $200 \times 10^9/L$ ^[29]. Researchers indicate that when the postoperative PLT exceeds $1000 \times 10^9/L$, PVT is almost sure to take place^[65]. The ratio of maximum postoperative PLT to preoperative PLT ($r = 1.144$; $P = 0.007$) and PLT increasing to > 8 times baseline levels after surgery are risk factors for PVT after LS^[59]. Moreover, elevated platelets and D-dimers can cause atherosclerotic changes in blood vessels, and make the smooth muscles thick in the intima of the vein wall, thus causing fiber breakage, blood cell adhesion and formation of thrombi^[11].

However, thrombosis does not always occur in high PLT patients after splenectomy. Some studies have suggested that the function and quality of platelets have a greater impact on thrombosis than simple PLT elevation^[64]. But this view is currently being challenged. In a prospective study, patients with PLT $> 300 \times 10^9/L$ after splenectomy were given antiplatelet therapy, namely aspirin, while the rest $< 300 \times 10^9/L$ were not given any antiplatelet therapy, and the results showed that there was no significant difference between the two groups (7.0% vs. 16.1%, $P = 0.858$), so the pathogenesis of PVT may have nothing to do with the function of platelets^[29]. Some researchers regard primary thrombocytosis as the cause of thrombosis, rather than secondary thrombocytosis, and thrombocytosis after splenectomy is secondary thrombocytosis^[66].

Elevated PLT and mean platelet volume (MPV) after splenectomy have been considered to be the main cause of PVT^[67-69], and average platelet volume may also be a risk for PVT after surgery^[69]. As for the large platelets, they are more active in metabolism and enzymatic reactions, and they contain more dense particles, α -particles and highly active proteases. When larger platelets are activated, they can release more thrombus precursor material to induce thrombosis. Platelet membrane surface protein CD62P, also known as P-selectin, is an indicator of the degree of platelet activation and functional status. Some researchers have found that CD62P can be used as a sensitive high-risk indicator of PVT^[70]. Antiplatelet therapy should be adopted when PLT exceeds $1000 \times 10^9/L$ ^[66], and the consensus about this aspect has not been well established. Some clinicians believe that when the patient's PLT is $> 400 \times 10^9/L$, anticoagulation therapy should be started immediately^[71]. But for safety reasons, $> 600 \times 10^9/L$ may be a more suitable PLT to start the treatment.

Level of D-dimer

D-dimer mainly reflects lytic function. It is used in the diagnosis and prevention of many thrombotic diseases. The increase in D-dimer level is often associated with the enhanced lytic activity of secondary fibrin^[11]. D-dimer has been reported as a diagnostic marker for PVT^[69]. In comparison with PLT, D-dimer performs better in predicting thrombosis. Some studies suggest that the combined application of D-dimer and P-selectin can effectively diagnose PVT after splenectomy^[72]. Like platelets, increased D-dimer after surgery can damage endothelial cells and cause PVT^[11]. Based on the views of some authors, the evaluation of D-dimer serologic levels is considered to own the adequate sensitivity and high negative predictive value

in diagnosing thrombotic events^[24,73], but the reported low specificity rate is expected because D-dimer is elevated in different conditions, such as disseminated intravascular coagulation and sepsis^[42,74,75]. However, the negative predictive value of the test is significant^[42]. Negative test results will strengthen the diagnostic value of other aspects and the need for more specific studies will be minimal. D-dimer only reflects secondary fibrinolysis, and therefore, it can only have diagnostic value for the thrombosis that has occurred, and cannot be used as a predictor of PVT.

Infection and inflammation

The spleen can produce immune substances such as immunoglobulins and complements to play an immune role. In addition, the spleen also has the function of producing lymphocytes^[76]. Splenectomized patients are at increased risk for infection, in particular, overwhelming post-splenectomy infection.

Infection and inflammation can cause a hypercoagulable state in the blood^[77]. Infection-related and inflammation-induced coagulation activation is characterized by enhanced fibrin formation and impaired fibrin degradation, where enhanced fibrin formation is caused by tissue factor (TF)-mediated thrombin generation and inhibition of the anticoagulant system including proteins C and S. Inflammation increases circulating levels of plasminogen activator inhibitor type 1, which inhibits endogenous thrombolytic reactions, mediated by various pro-inflammatory cytokines^[78].

Studies have shown that inhibiting IL-6 almost eliminates the coagulation effect of the TF-dependent coagulation pathway^[79]. Monocytes stimulated by pro-inflammatory cytokines increase expression of TF in sepsis^[80,81]. In inflammation, platelets can be directly activated by endotoxin or proinflammatory mediators (e.g., platelet activating factor). Platelets and granulocytes also can stimulate TF expression of monocytes by activated NFκB^[82]. The interactions between inflammatory cells promote the expression of IL-1b, IL-8, TNF-α, and P-selectin, and they have a role in mediating the adhesion of platelets to endothelial cells.

Other factors

Among all cirrhotic patients, 80%-100% of them have malnutrition. Malnutrition will further increase liver damage, and exacerbate liver cirrhosis, so it can be seen as an indirect risk factor for PVT^[83].

Among patients with liver cirrhosis, 70% of them have gene mutations before or after splenectomy. It mainly includes prothrombin genes, coagulation factor genes and so on, which eventually make patients more prone to thrombosis^[84]. For example, the G20210A mutation of the prothrombin gene has been found to play an important role in forming PVT^[85]. Another study discovered that mutation rates of V-Leiden factor gene and prothrombin gene were higher in patients with PVT than in controls^[86], indicating that these genes may be related to the onset and development of PVT.

In addition, low white blood cell count ($\leq 2 \times 10^3/L$), upper gastric bleeding history, and some underlying diseases, such as hematologic diseases, are also thought to be independent risk factors for PVT^[6,42,52].

Pathological changes occur in the vessel wall because of intravascular pressure, leading to “atherosclerosis-like” changes. It is an important factor that leads to PVT. As endothelial lesions are gradually aggravated, the age of patients appears to be positively correlated with thrombosis. Older individuals are at increased risk for PVT, which is linked to age-specific pathophysiological characteristics, including decreased blood flow, endothelial injury, and hypercoagulability. Some authors have pointed out that > 50 years old might be an independent risk factor for PVT. Patients who are over 50 are more than 20 times likely to suffer from PVT than those younger^[13].

Pancreatic fistula was reported as an independent risk factor for the development of PVT after the Hassab operation^[87]. The reason may be that pancreatic fistula causes prolonged hospital stay for the patient and increases the patient's bed time.

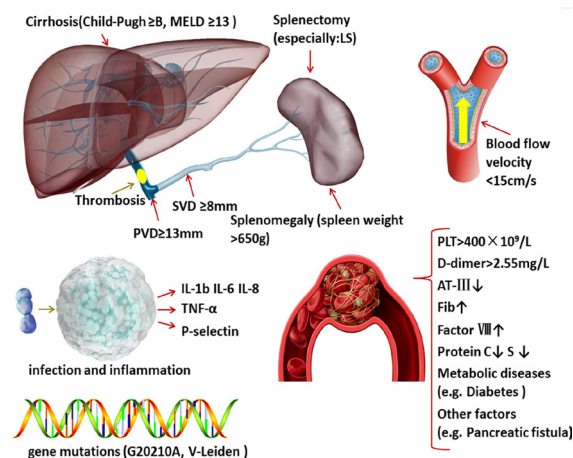


Figure 1. A preliminary summary of risk factors for portal vein thrombosis after splenectomy in patients with liver cirrhosis. SVD: splenic vein diameter; LS: laparoscopic splenectomy

A series of diseases, such as high blood lipids, high cholesterol and obesity, may also be risk factors for PVT formation. Still, some authors do not consider obesity to be associated with PVT^[88]. Another metabolic disease, diabetes, may be an independent risk factor for PVT in cirrhosis^[89]. This may be due to excessive blood glucose concentration, damaging the vascular endothelium. Therefore, blood glucose should also be regularly screened for thrombosis perioperatively.

There are many other medical factors that promote PVT, for instance, esophageal gastric varices sclerosis treatment, use of postoperative diuretics, and percutaneous transhepatic portal vein puncture.

Moreover, primary liver cancer can aggravate portal hypertension and change portal vein hemodynamics^[90]. In summary, the relevant risk factors are listed in Figure 1.

PROPHYLAXIS AND TREATMENTS

Today, we have clear guidelines or consensus on the prevention of deep vein thrombosis and pulmonary embolism after operation^[11]. However, there is no clear effective guidelines or consensus on PVT. Our management of PVT mainly relies on our clinical experience. Once the diagnosis is clear, all patients should be proactively treated except for asymptomatic incomplete embolism.

Medical treatment

Since the occurrence and development of PVT is likely to start before the operation, attention should be paid to early preventive anticoagulation. Anticoagulation plays a key role in preventing the formation of PVT, improving liver function, and reducing mortality^[91]. Low-molecular weight heparin (LMWH), warfarin, low-molecular weight dextran and bayaspirin are used to treat PVT in clinical practice. Some researchers have proposed that AT-III is also an approach to prevent PVT^[51]. Currently, interventional infusion thrombolytic drugs for thrombolysis have been used for PVT^[92].

LMWH can inhibit the activation of factor Xa and the formation of thrombus by binding AT-III^[93], and LMWH has a strong antithrombotic effect with less effect on platelet function, and does not prolong bleeding time^[94]. Studies have suggested that the use of LMWH at an early stage can significantly and safely reduce the incidence of PVT after splenectomy^[95]. Early use of LMWH contributes to thrombosis recanalization, and treatment should be started within 14 days of the discovery of thrombus^[96]. Some researchers have even suggested that all patients undergoing splenectomy should be prophylactically given low-molecular weight heparin^[42]. In authors' view, routine use of anticoagulant or antiplatelet drugs in the short-term after

splenectomy for cirrhosis does not increase the risk of postoperative bleeding and can effectively prevent PVT. At the same time, antiplatelet drugs should be used until PLT has fallen to normal levels. The specific application methods are described as follows. Anticoagulation should be applied at 72 h after surgery for safety concerns. Either LMWH-calcium or LMWH-sodium can be used. Taking enoxheparin (0.4 mL, 4000 AXa IU, ih, qd) as an example, the duration of treatment is 5 days. After discontinuation of enoxaparin, oral warfarin is used for continuous anticoagulation for 6 months. INR should be maintained between 2 and 3. When platelets exceed $400 \times 10^9/L$, antiplatelet drugs, such as aspirin, ticlopidine or dipyridamole should be used. After the platelets have returned to normal values, they should be discontinued. PLT and coagulation states of patients should be monitored every other day in the first week, and then monthly in the first year after surgery. Color Doppler ultrasound should be repeated monthly in the first year. It is worth noting that for PVT patients with mild to moderate renal dysfunction, the dose of enoxaparin should be halved, and liver and kidney function should be closely monitored. In patients with severe renal dysfunction or renal failure, enoxaparin must be used with caution according to the specific situation.

Warfarin inhibits the synthesis of vitamin K-dependent coagulation factors II, VII, IX, and X^[41]. Studies have shown that prophylactic anticoagulation is effective and safe in patients undergoing splenectomy, and that warfarin is effective in patients undergoing LS^[41,97]. But warfarin can also inhibit the synthesis of proteins C and S, and thus, it has the potential to increase thrombosis^[98]. Rivaroxaban and Dabigatran are approved for clinical use, and these drugs are widely used since there is no need for dose adjustment. Rivaroxaban has been successfully used to treat PVT according to some reports^[99,100]. Studies have shown that for patients with cirrhosis, the anticoagulant effect of dabigatran is very considerable, while the anticoagulant effect of rivaroxaban is slightly weaker^[101].

However, some researchers warn that considering that the rethrombosis rate after anticoagulation therapy is still high (52%), long-term anticoagulation should be applied more carefully^[102]. At present, most of the research on the prevention of PVT focuses on LMWH because it has a good anticoagulant effect, but to date, there is no uniform standard for applying LMWH to PVT^[103].

Surgery and interventional treatment

For PVT after splenectomy, when non-invasive treatment of thrombosis fails and causes portal vein stenosis, invasive treatment can be considered: surgery and interventional treatment.

Surgical treatments are mostly performed with clear serious complications, but there is still no unified standard for the indications, surgical choices, contraindications and complications of surgery. There are three main types of interventional therapies for PVT: TIPS, percutaneous transhepatic portal vein thrombolysis or thrombectomy. One of the most common treatments is TIPS, where it can improve portal vein hemodynamics and dissolve fluid clot^[104]. Portal vein flow increases more than 5 times after TIPS^[105]. Moreover, PVT can also be treated directly by intravascular techniques (balloon angioplasty, stent placement, thrombectomy, and thrombolytic drugs) when the catheter is inserted into the portal vein^[106]. TIPS can play an active role in resolving portal hypertension and preventing the recurrence of blood clots by creating venous shunts^[107]. Portal recanalization can be achieved in 87% to 100% of patients by TIPS^[106,108]. However, the timing of TIPS treatment is a tough issue. A complete occlusion of the thrombus will significantly increase the technical difficulty and surgical risk of TIPS^[109]. Further studies are required to solve this problem.

In addition, some researchers have proposed autologous spleen transplantation combined with esophageal transection anastomosis to treat cirrhotic portal hypertension. After 2 months of operation, patients with autologous spleen transplantation had significantly higher levels of tuftsin and IgM than splenectomy patients, and there was no significant difference in liver function, which proves that autologous spleen transplantation combined with esophageal transection anastomosis is a safe and effective treatment strategy for the treatment

of cirrhotic portal hypertension, and that the transplantation of spleen tissue into the retroperitoneal cavity can partially retain immune function^[110]. The cited researchers believe that this operation can not only retain the function of the spleen, but also has a combined effect of blood flow cut-off and diversion when the collateral circulation is well formed^[111]. Some authors believe that the autotransplantation of the spleen must weigh over 50 g to retain the immune function of the spleen, but when the spleen was cut into 8 small pieces, 23-28 g/piece, and transplanted in the omentum, the postoperative infection rate did not increase, and complement and immunoglobulin were normal, indicating that this procedure also has a certain therapeutic effect^[112].

CONCLUSION

PVT is the most important complication that affects the prognosis of patients after splenectomy, which may further aggravate portal hypertension and cause postoperative bleeding. Therefore, attention should be paid to preoperative prediction and evaluation, intraoperative improvement of surgical methods, timely postoperative monitoring and prevention. Clinicians should be fully aware of all aspects of a patient's illness, such as the etiology, classification, degree of obstruction, comorbidities of postoperative PVT, *etc.* Of course, there is still much controversy about the understanding of risk factors of PVT after splenectomy. Therefore, multidisciplinary randomized controlled trials are needed.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and design of the study and interpretation of results: Yang ZL

Contributed to the article and Figure: Guo T

Performed literature collection and provided technical suggestions as well: Zhu DL, Zheng S, Han DD

Put forward targeted opinions on the content, and control the quality of manuscripts: Chen Y

Availability of data and materials

All data are fully available without restriction.

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Zhang X, Wang Y, Yu M, Huang J, Deng D, et al. Effective prevention for portal venous system thrombosis after splenectomy: a meta-analysis. *J Laparoendosc Adv Surg Tech A* 2017;27:247-52.
2. Hashizume M, Tomikawa M, Akahoshi T, Tanoue K, Gotoh N, et al. Laparoscopic splenectomy for portal hypertension. *Hepatogastroenterology* 2002;49:847-52.

3. Raja K, Jacob M, Asthana S. Portal vein thrombosis in cirrhosis. *J Clin Exp Hepatol* 2014;4:320-31.
4. Targarona EM. Portal vein thrombosis after laparoscopic splenectomy: the size of the risk. *Surg Innov* 2008;15:266-70.
5. Krauth MT, Lechner K, Neugebauer EA, Pabinger I. The postoperative splenic/portal vein thrombosis after splenectomy and its prevention - an unresolved issue. *Haematologica* 2008;93:1227-32.
6. Kinjo N, Kawanaka H, Akahoshi T, Tomikawa M, Yamashita N, et al. Risk factors for portal venous thrombosis after splenectomy in patients with cirrhosis and portal hypertension. *Br J Surg* 2010;97:910-6.
7. Yoshida M, Watanabe Y, Horiuchi A, Yamamoto Y, Sugishita H, et al. Portal and splenic venous thrombosis after splenectomy in patients with hypersplenism. *Hepatogastroenterology* 2009;56:538-41.
8. Xu X, Guo X, De Stefano V, Silva-Junior G, Goyal H, et al. Nonselective beta-blockers and development of portal vein thrombosis in liver cirrhosis: a systematic review and meta-analysis. *Hepatol Int* 2019;13:468-81.
9. Huang XL, Wang FH, Zhang JK. Risk factors of portal vein thrombosis after surgery for advanced schistosomiasis portal hypertension. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi* 2015;27:177-9. (in Chinese)
10. Kuroki T, Kitasato A, Tokunaga T, Takeshita H, Taniguchi K, et al. Predictors of portal and splenic vein thrombosis after laparoscopic splenectomy: a retrospective analysis of a single-center experience. *Surg Today* 2018;48:804-9.
11. Wu S, Wu Z, Zhang X, Wang R, Bai J. The incidence and risk factors of portal vein system thrombosis after splenectomy and pericardial devascularization. *Turk J Gastroenterol* 2015;26:423-8.
12. Harding DJ, Perera MT, Chen F, Olliff S, Tripathi D. Portal vein thrombosis in cirrhosis: controversies and latest developments. *World J Gastroenterol* 2015;21:6769-84.
13. Jiang GQ, Bai DS, Chen P, Qian JJ, Jin SJ, et al. Risk factors for portal vein system thrombosis after laparoscopic splenectomy in cirrhotic patients with hypersplenism. *J Laparoendosc Adv Surg Tech A* 2016;26:419-23.
14. Qi X, Li H, Liu X, Yao H, Han G, et al. Novel insights into the development of portal vein thrombosis in cirrhosis patients. *Expert Rev Gastroenterol Hepatol* 2015;9:1421-32.
15. Zocco MA, Di Stasio E, De Cristofaro R, Novi M, Ainora ME, et al. Thrombotic risk factors in patients with liver cirrhosis: correlation with MELD scoring system and portal vein thrombosis development. *J Hepatol* 2009;51:682-9.
16. Huang L, Yu Q, Wang J. Association between changes in splanchnic hemodynamics and risk factors of portal venous system thrombosis after splenectomy with periesophagogastric devascularization. *Med Sci Monit* 2018;24:4355-62.
17. Nery F, Chevret S, Condat B, de Raucourt E, Boudaoud L, et al. Groupe d'Etude et de Traitement du Carcinome H. Causes and consequences of portal vein thrombosis in 1,243 patients with cirrhosis: results of a longitudinal study. *Hepatology* 2015;61:660-7.
18. Abdel-Razik A, Mousa N, Elhelaly R, Tawfik A. De-novo portal vein thrombosis in liver cirrhosis: risk factors and correlation with the model for end-stage liver disease scoring system. *Eur J Gastroenterol Hepatol* 2015;27:585-92.
19. Trebicka J, Strassburg CP. Etiology and complications of portal vein thrombosis. *Viszeralmedizin* 2014;30:375-80.
20. Dai J, Qi X, Peng Y, Hou Y, Chen J, et al. Association between D-dimer level and portal venous system thrombosis in liver cirrhosis: a retrospective observational study. *Int J Clin Exp Med* 2015;8:15296-301.
21. Bolognesi M, Merkel C, Sacerdoti D, Nava V, Gatta A. Role of spleen enlargement in cirrhosis with portal hypertension. *Dig Liver Dis* 2002;34:144-50.
22. McCormick PA, Murphy KM. Splenomegaly, hypersplenism and coagulation abnormalities in liver disease. *Baillieres Best Pract Res Clin Gastroenterol* 2000;14:1009-31.
23. Romano A, Hou X, Sertorio M, Dessein H, Cabantous S, et al. FOXP3+ regulatory T cells in hepatic fibrosis and splenomegaly caused by schistosoma japonicum: the spleen may be a major source of tregs in subjects with splenomegaly. *PLoS Negl Trop Dis* 2016;10:e0004306.
24. Stamou KM, Toutouzas KG, Kekis PB, Nakos S, Gafou A, et al. Prospective study of the incidence and risk factors of postsplenectomy thrombosis of the portal, mesenteric, and splenic veins. *Arch Surg* 2006;141:663-9.
25. Wang M, Zhang M, Li J, Zhou J, Wu Z, et al. Risk factors of portal vein thrombosis in patients with beta thalassemia major after splenectomy: laparoscopic versus open procedure. *Hepatogastroenterology* 2014;61:48-54.
26. Ikeda M, Sekimoto M, Takiguchi S, Kubota M, Ikenaga M, et al. High incidence of thrombosis of the portal venous system after laparoscopic splenectomy: a prospective study with contrast-enhanced CT scan. *Ann Surg* 2005;241:208-16.
27. Pietrabissa A, Moretto C, Antonelli G, Morelli L, Marciano E, et al. Thrombosis in the portal venous system after elective laparoscopic splenectomy. *Surg Endosc* 2004;18:1140-3.
28. Danno K, Ikeda M, Sekimoto M, Sugimoto T, Takemasa I, et al. Diameter of splenic vein is a risk factor for portal or splenic vein thrombosis after laparoscopic splenectomy. *Surgery* 2009;145:457-64; discussion 65-6.
29. Li MX, Zhang XF, Liu ZW, Lv Y. Risk factors and clinical characteristics of portal vein thrombosis after splenectomy in patients with liver cirrhosis. *Hepatobiliary Pancreat Dis Int* 2013;12:512-9.
30. Sun L, Zhou H, Gu L, Jiang C, Liu Y, et al. Effects of surgical procedures on the occurrence and development of postoperative portal vein thrombosis in patients with cirrhosis complicated by portal hypertension. *Int J Surg* 2015;16:31-5.
31. Bai DS, Xia BL, Zhang C, Ye J, Qian JJ, et al. Warfarin versus aspirin prevents portal vein thrombosis after laparoscopic splenectomy and azygoportal disconnection: A randomized clinical trial. *Int J Surg* 2019;64:16-23.
32. Zheng S, Sun P, Liu X, Li G, Gong W, et al. Efficacy and safety of laparoscopic splenectomy and esophagogastric devascularization for portal hypertension: a single-center experience. *Medicine* 2018;97:e13703.
33. Bao H, He Q, Dai N, Ye R, Zhang Q. Retrospective study to compare selective decongestive devascularization and gastrosplenic shunt versus splenectomy with pericardial devascularization for the treatment of patients with esophagogastric varices due to cirrhotic portal hypertension. *Med Sci Monit* 2017;23:2788-95.

34. de'Angelis N, Abdalla S, Lizzi V, Esposito F, Genova P, et al. Incidence and predictors of portal and splenic vein thrombosis after pure laparoscopic splenectomy. *Surgery* 2017;162:1219-30.
35. Eyraud D, Granger B, Ionescu C, Fratea S, Darnat S, et al. Thrombocytopenia, splenomegaly, and portal blood flow in patients who have undergone liver transplantation for cirrhosis. *Liver Transpl* 2012;18:340-6.
36. Guan YS, Hu Y. Clinical application of partial splenic embolization. *Sci World J* 2014;2014:961345.
37. Kheradpir MH. Partial splenectomy and partial splenic attachment for the treatment of portal hypertension. *Z Kinderchir* 1990;45:98-9.
38. Sretenovic A, Perisic V, Vujovic D, Opacic D, Vukadinovic V, et al. Warren shunt combined with partial splenectomy in children with extra-hepatic portal hypertension, massive splenomegaly and severe hypersplenism. *Srp Arh Celok Lek* 2014;142:419-23.
39. Zhe C, Jian-Wei L, Jian C, Yu-dong F, Ping B, et al. Laparoscopic versus open splenectomy and esophagogastric devascularization for bleeding varices or severe hypersplenism: a comparative study. *J Gastrointest Surg* 2013;17:654-9.
40. Stine JG, Shah PM, Cornella SL, Rudnick SR, Ghabril MS, et al. Portal vein thrombosis, mortality and hepatic decompensation in patients with cirrhosis: a meta-analysis. *World J Hepatol* 2015;7:2774-80.
41. Jiang GQ, Xia BL, Chen P, Qian JJ, Jin SJ, et al. Anticoagulation therapy with warfarin versus low-dose aspirin prevents portal vein thrombosis after laparoscopic splenectomy and azygoportal disconnection. *J Laparoendosc Adv Surg Tech A* 2016;26:517-23.
42. Vecchio R, Cacciola E, Cacciola RR, Marchese S, Intagliata E. Portal vein thrombosis after laparoscopic and open splenectomy. *J Laparoendosc Adv Surg Tech A* 2011;21:71-5.
43. Leonardi F, Maria N, Villa E. Anticoagulation in cirrhosis: a new paradigm? *Clin Mol Hepatol* 2017;23:13-21.
44. Monroe DM, Hoffman M. The coagulation cascade in cirrhosis. *Clin Liver Dis* 2009;13:1-9.
45. Primignani M, Tosetti G, La Mura V. Therapeutic and clinical aspects of portal vein thrombosis in patients with cirrhosis. *World J Hepatol* 2015;7:2906-12.
46. Tripodi A, Primignani M, Chantarangkul V, Dell'Era A, Clerici M, et al. An imbalance of pro- vs anti-coagulation factors in plasma from patients with cirrhosis. *Gastroenterology* 2009;137:2105-11.
47. Tripodi A, Primignani M, Lemma L, Chantarangkul V, Mannucci PM. Evidence that low protein C contributes to the procoagulant imbalance in cirrhosis. *J Hepatol* 2013;59:265-70.
48. Qi X, Chen H, Han G. Effect of antithrombin, protein C and protein S on portal vein thrombosis in liver cirrhosis: a meta-analysis. *Am J Med Sci* 2013;346:38-44.
49. Rodriguez-Leal GA, Moran S, Corona-Cedillo R, Brom-Valladares R. Portal vein thrombosis with protein C-S deficiency in a non-cirrhotic patient. *World J Hepatol* 2014;6:532-7.
50. Kawanaka H, Akahoshi T, Kinjo N, Konishi K, Yoshida D, et al. Impact of antithrombin III concentrates on portal vein thrombosis after splenectomy in patients with liver cirrhosis and hypersplenism. *Ann Surg* 2010;251:76-83.
51. Kawanaka H, Akahoshi T, Itoh S, Iguchi T, Harimoto N, et al. Optimizing risk stratification in portal vein thrombosis after splenectomy and its primary prophylaxis with antithrombin III concentrates and danaparoid sodium in liver cirrhosis with portal hypertension. *J Am Coll Surg* 2014;219:865-74.
52. Han J, Yi Y, Ding H, Liu J, Zhang Y, et al. Preoperative risk factors of portal venous thrombosis after splenectomy and gastric pericardial devascularization for portal hypertension. *Zhonghua Gan Zang Bing Za Zhi* 2014;22:739-43. (in Chinese)
53. Zhang Y, Wen TF, Yan LN, Yang HJ, Deng XF, et al. Preoperative predictors of portal vein thrombosis after splenectomy with periesophagogastric devascularization. *World J Gastroenterol* 2012;18:1834-9.
54. Rossetto V, Spiezia L, Senzolo M, Rodriguez-Castro KI, Gavasso S, et al. Does decreased fibrinolysis have a role to play in the development of non-neoplastic portal vein thrombosis in patients with hepatic cirrhosis? *Intern Emerg Med* 2014;9:397-403.
55. Smalberg JH, Koehler E, Darwish Murad S, Plessier A, Seijo S, et al. European Network for Vascular Disorders of the L. Fibrinogen gamma' and variation in fibrinogen gamma genes in the etiology of portal vein thrombosis. *Thromb Haemost* 2013;109:558-60.
56. Kinjo N, Kawanaka H, Akahoshi T, Matsumoto Y, Kamori M, et al. Portal vein thrombosis in liver cirrhosis. *World J Hepatol* 2014;6:64-71.
57. Qi X, Su C, Ren W, Yang M, Jia J, et al. Association between portal vein thrombosis and risk of bleeding in liver cirrhosis: a systematic review of the literature. *Clin Res Hepatol Gastroenterol* 2015;39:683-91.
58. Zhang N, Yao Y, Xue W, Wu S. Early prophylactic anticoagulation for portal vein system thrombosis after splenectomy: a systematic review and meta-analysis. *Biomed Rep* 2016;5:483-90.
59. Huang D, Tao M, Cao L, Wang X, Zheng S, et al. Risk factors and anticoagulation effects of portal vein system thrombosis after laparoscopic splenectomy in patients with or without cirrhosis. *Surg Laparosc Endosc Percutan Tech* 2019;29:498-502.
60. Nadinskaia MY, Kodzoeva KB, Ulyanova KA, Volkova AS, Rogacheva SI, et al. Risk factors associated with portal vein thrombosis in liver cirrhosis: a case-control study. *Ter Arkh* 2019;91:73-81.
61. Hernandez Conde M, Llop Herrera E, de la Revilla Negro J, Pons Renedo F, Fernandez Puga N, et al. Prevalence and outcome of portal thrombosis in a cohort of cirrhotic patients undergoing liver transplantation. *Rev Esp Enferm Dig* 2016;108:716-20.
62. Tarzamni MK, Somi MH, Farhang S, Jalilvand M. Portal hemodynamics as predictors of high risk esophageal varices in cirrhotic patients. *World J Gastroenterol* 2008;14:1898-902.
63. Chawla YK, Bodh V. Portal vein thrombosis. *J Clin Exp Hepatol* 2015;5:22-40.
64. Francoz C, Valla D, Durand F. Portal vein thrombosis, cirrhosis, and liver transplantation. *J Hepatol* 2012;57:203-12.
65. Machado NO, Chopra PJ, Sankhla D. Portal vein thrombosis postlaparoscopic splenectomy presenting with infarction of gut: review of risk factors, investigations, postoperative surveillance, and management. *Surg Laparosc Endosc Percutan Tech* 2010;20:273-7.
66. Griesshammer M, Bangerter M, Sauer T, Wennauer R, Bergmann L, et al. Aetiology and clinical significance of thrombocytosis: analysis

- of 732 patients with an elevated platelet count. *J Intern Med* 1999;245:295-300.
67. Coppinger JA, Cagney G, Toomey S, Kislinger T, Belton O, et al. Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. *Blood* 2004;103:2096-104.
 68. Ghanima W, Godeau B, Cines DB, Bussel JB. How I treat immune thrombocytopenia: the choice between splenectomy or a medical therapy as a second-line treatment. *Blood* 2012;120:960-9.
 69. He S, He F. Predictive model of portal venous system thrombosis in cirrhotic portal hypertensive patients after splenectomy. *Int J Clin Exp Med* 2015;8:4236-42.
 70. Wang L, Liu GJ, Chen YX, Dong HP, Zhang YQ, et al. Combined use of D-dimer and P-selectin for the diagnosis of splenic or portal vein thrombosis following splenectomy. *Thromb Res* 2010;125:e206-9.
 71. Hirashita T, Ohta M, Kai S, Masuda T, Eguchi H, et al. Implications of portal vein thrombosis after splenectomy for patients with idiopathic portal hypertension. *Surg Today* 2011;41:1475-80.
 72. Qi X, De Stefano V, Li H, Dai J, Guo X, et al. Anticoagulation for the treatment of portal vein thrombosis in liver cirrhosis: a systematic review and meta-analysis of observational studies. *Eur J Intern Med* 2015;26:23-9.
 73. Bates SM, Grand'Maison A, Johnston M, Naguit I, Kovacs MJ, et al. A latex D-dimer reliably excludes venous thromboembolism. *Arch Intern Med* 2001;161:447-53.
 74. Shorr AF, Thomas SJ, Alkins SA, Fitzpatrick TM, Ling GS. D-dimer correlates with proinflammatory cytokine levels and outcomes in critically ill patients. *Chest* 2002;121:1262-8.
 75. Kinasewitz GT, Yan SB, Basson B, Comp P, Russell JA, et al. Universal changes in biomarkers of coagulation and inflammation occur in patients with severe sepsis, regardless of causative micro-organism [ISRCTN74215569]. *Crit Care* 2004;8:R82-90.
 76. Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. *Lancet* 2011;378:86-97.
 77. Levi M, Sivapalaratnam S. Disseminated intravascular coagulation: an update on pathogenesis and diagnosis. *Expert Rev Hematol* 2018;11:663-72.
 78. Levi M, van der Poll T, ten Cate H, van Deventer SJ. The cytokine-mediated imbalance between coagulant and anticoagulant mechanisms in sepsis and endotoxaemia. *Eur J Clin Invest* 1997;27:3-9.
 79. van der Poll T, Levi M, Hack CE, ten Cate H, van Deventer SJ, et al. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. *J Exp Med* 1994;179:1253-9.
 80. Osterud B, Rao LV, Olsen JO. Induction of tissue factor expression in whole blood: lack of evidence for the presence of tissue factor expression in granulocytes. *Thromb Haemost* 2000;83:861-7.
 81. Franco RF, de Jonge E, Dekkers PE, Timmerman JJ, Spek CA, et al. The in vivo kinetics of tissue factor messenger RNA expression during human endotoxemia: relationship with activation of coagulation. *Blood* 2000;96:554-9.
 82. Furie B, Furie BC. Role of platelet P-selectin and microparticle PSGL-1 in thrombus formation. *Trends Mol Med* 2004;10:171-8.
 83. Campillo B, Richardet JP, Bories PN. Validation of body mass index for the diagnosis of malnutrition in patients with liver cirrhosis. *Gastroenterol Clin Biol* 2006;30:1137-43.
 84. Ponziani FR, Zocco MA, Campanale C, Rinninella E, Tortora A, et al. Portal vein thrombosis: insight into physiopathology, diagnosis, and treatment. *World J Gastroenterol* 2010;16:143-55.
 85. Amitrano L, Guardascione MA, Brancaccio V, Margaglione M, Manguso F, et al. Risk factors and clinical presentation of portal vein thrombosis in patients with liver cirrhosis. *J Hepatol* 2004;40:736-41.
 86. Dentali F, Galli M, Gianni M, Ageno W. Inherited thrombophilic abnormalities and risk of portal vein thrombosis: a meta-analysis. *Thromb Haemost* 2008;99:675-82.
 87. Zhou JB, Luo BY, Liu CW, Zhu F. Effects of early antiplatelet therapy after splenectomy with gastro-oesophageal devascularization. *ANZ J Surg* 2018;88:E725-9.
 88. Zakko A, Kroner PT, Nankani R, Karagozian R. Obesity is not associated with an increased risk of portal vein thrombosis in cirrhotic patients. *Gastroenterol Hepatol Bed Bench* 2018;11:153-8.
 89. Eshraghian A, Nikeghbalian S, Kazemi K, Mansoorian M, Shamsaeefar A, et al. Portal vein thrombosis in patients with liver cirrhosis and its impact on early and long-term outcomes after liver transplantation. *Int J Clin Pract* 2018;e13309.
 90. Hu KQ, Yu AS, Tiyyagura L, Redeker AG, Reynolds TB. Hyperfibrinolytic activity in hospitalized cirrhotic patients in a referral liver unit. *Am J Gastroenterol* 2001;96:1581-6.
 91. Villa E, Camma C, Marietta M, Luongo M, Critelli R, et al. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. *Gastroenterology* 2012;143:1253-60.e4.
 92. Valentin N, Korrapati P, Constantino J, Young A, Weisberg I. The role of transjugular intrahepatic portosystemic shunt in the management of portal vein thrombosis: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2018;30:1187-93.
 93. Romualdi E, Ageno W. Investigational factor Xa inhibitors for thrombosis and acute coronary syndromes. *Expert Opin Investig Drugs* 2011;20:495-505.
 94. Millan M, Caicedo LA, Villegas JI, Serrano O, Caicedo L, et al. Case report of cadaveric kidney transplantation with renal-portal venous drainage: a feasible way for a venous drainage in a complex generalized thrombosed vessels setting. *Int J Surg Case Rep* 2016;28:192-5.
 95. Lai W, Lu SC, Li GY, Li CY, Wu JS, et al. Anticoagulation therapy prevents portal-splenic vein thrombosis after splenectomy with gastroesophageal devascularization. *World J Gastroenterol* 2012;18:3443-50.
 96. Delgado MG, Seijo S, Yepes I, Achecar L, Catalina MV, et al. Efficacy and safety of anticoagulation on patients with cirrhosis and portal vein thrombosis. *Clin Gastroenterol Hepatol* 2012;10:776-83.
 97. Cheng Z, Yu F, Tian J, Guo P, Li J, et al. A comparative study of two anti-coagulation plans on the prevention of PVST after laparoscopic

- splenectomy and esophagogastric devascularization. *J Thromb Thrombolysis* 2015;40:294-301.
98. Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. *N Engl J Med* 2011;365:147-56.
99. Lenz K, Dieplinger B, Buder R, Piringer P, Rauch M, et al. Successful treatment of partial portal vein thrombosis (PVT) with low dose rivaroxaban. *Z Gastroenterol* 2014;52:1175-7.
100. Yang H, Kim SR, Song MJ. Recurrent acute portal vein thrombosis in liver cirrhosis treated by rivaroxaban. *Clin Mol Hepatol* 2016;22:499-502.
101. Potze W, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, et al. Differential in vitro inhibition of thrombin generation by anticoagulant drugs in plasma from patients with cirrhosis. *PLoS One* 2014;9:e88390.
102. Artaza T, Lopes M, Romero M, Gomez AZ, de la Cruz G, et al. Efficacy and safety of anticoagulation in non-malignant portal vein thrombosis in patients with liver cirrhosis. *Gastroenterol Hepatol* 2018;41:611-7.
103. Qi X, Han G, Wang J, Wu K, Fan D. Degree of portal vein thrombosis. *Hepatology* 2010;51:1089-90.
104. Senzolo M, Sartori T, Rossetto V, Burra P, Cillo U, et al. Prospective evaluation of anticoagulation and transjugular intrahepatic portosystemic shunt for the management of portal vein thrombosis in cirrhosis. *Liver Int* 2012;32:919-27.
105. Gaba RC, Parvinian A. Transjugular intrahepatic portosystemic shunt for maintenance of portal venous patency in liver transplant candidates. *J Clin Imaging Sci* 2013;3:28.
106. Lakhoo J, Gaba RC. Outcomes of transjugular intrahepatic portosystemic shunt creation for flow-enabled dissolution of spleno-mesenterico-portal venous thrombosis. *Diagn Interv Imaging* 2016;97:1085-93.
107. Riggio O, Ridola L, Lucidi C, Angeloni S. Emerging issues in the use of transjugular intrahepatic portosystemic shunt (TIPS) for management of portal hypertension: time to update the guidelines? *Dig Liver Dis* 2010;42:462-7.
108. Inagaki Y, Sugimoto K, Shiraki K, Tameda M, Kusagawa S, et al. The long-term effects of splenectomy and subsequent interferon therapy in patients with HCV-related liver cirrhosis. *Mol Med Rep* 2014;9:487-92.
109. Yang S, He C, Fan X, Ding W, Wu X, et al. Early prophylactic anticoagulation via transjugular intrahepatic route for portal vein thrombosis after splenectomy in cirrhotic portal hypertension. *J Vasc Interv Radiol* 2015;26:1009-17.
110. Shang CZ, Chen YJ, Zhang HW, Zhang L, Cao J, et al. Splenic autotransplantation combined with lower esophagus transaction in the treatment of hepatic cirrhosis induced portal hypertension. *Zhonghua Wai Ke Za Zhi* 2009;47:83-5. (in Chinese)
111. Shan HJ, Ji-Sheng C, Zhuo W, Rufu C, Zhihao Z, et al. Portal venous hemodynamics after subtotal splenectomy and portaazygous devascularization. *Zhongguo Putong Waik Zazhi* 2010;25:20-3.
112. Zhu Y. Analysis of 26 cases of autotransplantation of spleen tissue after splenectomy. *Clin Med Chin* 2004;20:459-60. (in Chinese)

Review

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Conventional type 1 dendritic cells in protective antitumor immunity and its potential in hepatocellular carcinoma

Chunfeng Qu, Kun Chen, Shao-Yan Cheng

State Key Lab of Molecular Oncology & Immunology Department, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China.

Correspondence to: Prof. Chunfeng Qu, State Key Lab of Molecular Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 17 Panjiayuan South lane, Chaoyang District, Beijing 100021, China. E-mail: quchf@cicams.ac.cn

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Abstract

Immunotherapy is revolutionizing the clinical management of cancer patients by modulating T cells and natural killer cells. Dendritic cells (DCs) have the capacity to orchestrate the expansion and function of these effector cells both in lymphoid and non-lymphoid tissues of cancer patients. Distinct subtypes of DCs have various capacities to prime and activate different T cell responses. Here, we review conventional type 1 dendritic cells (cDC1s) and their crucial role in protective anti-tumor immunity. Targeting cDC1s as a cancer vaccine against the development of hepatocellular carcinoma will be discussed.

Keywords: Conventional type 1 dendritic cells, antitumor immunity, hepatocellular carcinoma, cancer vaccine

INTRODUCTION

Immunotherapy is now widely considered as an important tool for the treatment of individuals with cancer. Several effective immunotherapy approaches have been developed over the past decade, including adoptive cell transfer (e.g., CAR-T therapy) and immune checkpoint blockade (e.g., anti-PD1/PDL1 antibodies)^[1,2]. In solid tumors including hepatocellular carcinoma (HCC), the tumor microenvironment contains a large amount of stromal cells and immune cells, which shape cancer development and impact



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upon the response to tumor therapy. The effectiveness of immune-based therapeutic strategies clearly demonstrates the possibility of eradicating cancer through cellular immunity, particularly when active T cells recognize cognate tumor antigens^[1,3]. Dendritic cells (DCs) have the capacity to prime naïve T cells by inducing functional polarization, and are in charge of orchestrating the expansion and functions of T cells and natural killer (NK) cells in lymphoid and non-lymphoid tissues of cancer patients. About 25 years ago, some investigators put forward the concept of harnessing DC immunogenicity to induce protective responses in cancer patients^[4,5]. Nevertheless, results have been far below expectations. These failures occurred due to the almost exclusive usage of monocyte-derived cells (MoDCs) and tumor-associated antigens. The advancement in basic understanding of the heterogeneity and functional plasticity of different DC subsets suggests that conventional (classical) type 1 DCs (cDC1s) other than MoDCs may be better suited for this purpose.

DENDRITIC CELL SUBSETS

Shortly after the discovery of DCs by Steinman in 1973, van Furth grouped them under the mononuclear phagocyte system. Since then monocytes, macrophages and DCs have been grouped together, and they are distinguished on the basis of their morphology, function and origin^[6]. However, lineage-tracing studies by different groups demonstrate that most macrophages in adults are maintained independently of blood monocytes and rely on self-renewal of embryonically derived macrophages under steady-state conditions^[7,8]. The circulating monocytes could contribute to the expanding pool of macrophages in the liver under inflammation status^[9,10]. DCs arise from a common DC precursor in the bone marrow, and their development depends on the cytokine Flt3L^[11,12]. Although monocytes in some inflammation develop to inflammatory DCs, termed IDCs^[13], DCs are now generally grouped into conventional (classical) type 1 DCs (cDC1s), conventional type 2 DCs (cDC2s) and plasmacytoid DCs (pDCs)^[14].

In mice, cDC1s include murine lymphoid CD8 α ⁺ DCs, migratory CD103^{high} DCs, and dermal CD207⁺ DCs; cDC2s include lymphoid CD4⁺ DCs and migratory CD11b⁺ DCs; pDCs are IFN-producing DCs with the cell surface markers B220, PDCA.1 and Ly6C^[14]. DCs have been divided into many different subsets based on the expression of surface markers including CD40, CD11c, CD103 (integrin α E), CD11b (integrin α M), F4/80, CD8 α , CD24, CD172a (SIRP α and SHPS1), CX₃C-chemokine receptor 1 (CX₃CR1), XC-chemokine receptor 1 (XCR1), CLEC9A (DNDR1), E-cadherin (cadherin 1) and CD64 (Fc γ RI)^[14]. Inflammation further complicates the picture as mononuclear phagocytes in inflamed tissues undergo phenotypical changes. Some researchers consider monocyte-derived cells as MoDCs or macrophages, based on CD11c expression^[13-15]. The analysis of gene expression profiles revealed that the gene transcripts in different populations of macrophages are diverse, and some mRNA transcripts and surface proteins were selectively expressed by macrophages but not DCs^[16]. Therefore, macrophages, monocytes and DCs are different cell types with distinct ontogeny^[7-14,16].

DC-BASED CANCER VACCINES

DC-based cancer vaccines typically culture DCs with various tumor associated antigens *ex vivo*, such as pulsing with peptides, whole proteins, tumor lysates, or fusion of DCs with entire tumor cells^[1,4,5]. Most DCs were generated from peripheral blood mononuclear cells (PBMCs)-derived CD14⁺ monocytes or CD34⁺ hematopoietic stem and progenitor cells via culturing with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 4 (IL-4). The efficacy of such vaccine formulations is suboptimal since the macrophages were generated in culture with GM-CSF and its migratory capacity limited to lymph nodes^[1,5].

To overcome these tedious processes and the uncertainty of quality, approaches that home antigens directly to DCs *in vivo* via DC receptors were developed, such as antibodies against DEC-205, Clec9A and Clec12A^[1,4,17,18]. By using model antigens, i.e., OVA, or antigens from microbial products, direct targeting

strategies showed some protective effects on tumor growth^[4,18,19]. However, these effects were suboptimal in clinical trials when the antibody against DEC-205, which is expressed in different subsets of DCs in human, was conjugated with the tumor antigen NY-ESO^[20]. Therefore, when contemplating DCs for *in vivo* targeting for tumor vaccination, it is important to consider the differences of targeting DC receptors and their function^[1,5].

THE cDC1s AND THEIR ROLES AGAINST CANCERS

The “cross-presentation” by DCs of tumor antigens that are expressed in solid tumors is crucial for generating effective CD8⁺ cytotoxic T lymphocytes (CTLs)^[1,21]. Accumulated experimental evidence revealed that distinct subtypes of DCs show various capacities to prime and activate different T cells^[1,5]. Mouse cDC1s were identified as the most efficient cells in cross-presenting cellular-associated antigens to initiate CD8⁺ T cells^[22-25]. The following transcription factors *ICSBP* (*Irf8*)^[26], *Id2*^[27], *Batf3*^[28], *Nfil3*^[29] were reported to control the development of cDC1s. Further epistasis analysis after single-cell RNA sequencing revealed a genetic hierarchy. The *Nfil3* induces a transition from common DC precursors to express high levels of *Id2* and low levels of *Zeb2*. Upon *Id2* induction, *Batf3* expression is increased and *Zeb2* is repressed. Meanwhile, *Id2* extinguished E-protein activity at the +41-kb *Irf8* enhancer, and the expressed BATF3 acted at the +32-kb *Irf8* enhancer to maintain *Irf8* activation for the commitment of cDC1 clonogenic progenitors^[30,31]. Currently, the homolog and functional equivalent of mouse cDC1s have been identified as CD141 (BDCA3)⁺ XCR1⁺ DCs in humans^[14,32,33]. Both mouse and human cDC1s selectively express the chemokine receptor XCR1 and C-type lectin endocytic receptor CLEC9A^[4,14,34,35].

The role of cDC1s in antitumor immunity was investigated in different animal models. In the *Batf3*^{-/-} mouse, the rejection of highly immunogenic syngeneic tumors was impaired^[28]. Using 2-photon intravital imaging, the spatial organization of DCs and macrophages within the tumor and their dynamics with T cells were analyzed. It was found that macrophage populations were preferentially marginating tightly on tumoral lesions, DC populations were typically in separate collagen-rich zones distal to tumor lesions. Stable T cell interactions were largely confined to tumor margins, where macrophage populations dominated and DC numbers were little^[36]. Analyzing the different types of human tumors in TCGA data revealed that patients with a high ratio of the CD103⁺/CD103⁻ gene showed better survival compared to those with a low ratio^[37]. In mouse melanoma models, it was found that CD103⁺ DCs were the only antigen-presenting cells to transport intact antigens to lymph nodes for priming tumor-specific CD8⁺ T cells. When CD103⁺ DC progenitors in the tumor were expanded and activated, the effects of anti-checkpoint inhibitor were enhanced for anti-tumor responses^[38].

In the context of cancer, there are many different cell populations. In addition to cDC1s, several myeloid cell populations, including tumor-associated macrophages, are also able to acquire tumor material. However, cDC1s have been demonstrated to be superior to other cells in stimulating T cell activation and proliferation^[36-38]. The stimulatory function of cDC1s in tumors is not restricted to T cells only. IL-12 production by these cells support IFN- γ production from NK cells for eradicating established tumor cells^[39,40]. More importantly, activated NK cells can generate the chemokines CCL5 and XCL1 to recruit cDC1s into the tumor microenvironment and promote cancer immune control^[25]. In melanoma patients, it was found that the abundance of cDC1s is associated with intra-tumor gene expression of the cytokine FLT3LG, which is predominantly produced by NK cells in tumors. The numbers of cDC1 is correlated with NK frequency and the patients' response to anti-PD1 immunotherapy^[41]. It was found that cDC1s might be the main source of CXCL9 and CXCL10, and these chemokines are able to recruit CXCR3⁺ effector T cells^[24,40,42,43]. Therefore, the cDC1s in tumors and their interactions with NK and effector T cells establish local anti-tumor immunity to control and eventually, eradicate established tumors^[24,40].

TARGETING CDC1S FOR HCC INTERVENTION

Some patients with a variety of cancers, including HCC, benefit from immune checkpoint inhibitors^[44,45]. Since not all HCC patients are sensitive to this therapy, research has found that WNT activation correlated with T cell exclusion and resistance to anti-PD1 therapy^[46,47]. By using a murine autochthonous liver cancer model based on hydrodynamic tail vein delivery of different genetic elements, it was found that WNT activation led to defective DC recruitment, mainly cDC1s. As a consequence, the anti-tumor immune response was impaired. The impaired immunity in HCC from WNT activation might be due to decreased CCL5 secretion by the tumor^[48]. These investigations pointed to the importance of cDC1 recruitment in HCC immunotherapy.

HCCs arising in cirrhosis are usually preceded by the appearance of malignant precancerous nodules^[49]. The liver of a cirrhotic patient may harbor either a single, benign or precancerous malignant nodule, or even both. HCC progression could be interfered with if the malignant progenitors in cirrhosis were eliminated. At this stage, it is difficult to treat with conventional means such as surgery, radiation, and chemotherapy. However, it may still be controllable by stimulating the immune response through cancer vaccines. Tumor-associated antigens, which are re-expressed in tumor and not/lowly expressed in normal tissues, might potentially be appropriate targets^[50]. Previous studies have documented that inducing the generation of specific T cells to alpha-fetoprotein (AFP), which is re-expressed in most HCCs, led to tumor regression^[50] and prevented carcinogen-induced murine autochthonous HCC^[51]. However, in regenerating mouse liver significant hepatocyte damage was observed^[52]. Two decades ago, glypican-3 (GPC3) was identified as a new HCC-associated antigen^[53]. Different from AFP, it is undetectable in cirrhotic livers, or even in benign hepatic lesions. Tissue expression of GPC3 was used to discriminate the nature of a < 2 cm hepatocellular lesion lacking HCC radiological features detected in a cirrhotic patient under surveillance. Up to 60% of early HCCs showed immunoreactivity to GPC3, either as membranous and/or cytoplasmic staining in biopsy material^[53]. We therefore hypothesized that eliciting the host's own specific T cell immunity against GPC3 could interfere with disease progression in cirrhosis patients.

With the growing importance of cDC1s in initiating effective anti-tumor immunity, and the selective expression of XCR1 on these cDC1s, the XCL1-GPC3 fusion protein may be an efficient cancer vaccine. We have linked the XCL1 chemokine to GPC3 for *in vivo* targeting to induce GPC3-specific CTLs for eliminating GPC3-positive HCC. Our results showed that the expressed XCL1-GPC3 chemoattracted murine XCR1⁺CD8 α ⁺DCs and human XCR1⁺CD141⁺DCs *in vitro* and promoted IL-12 production. After the *mXCL1-GPC3* plasmid was injected subcutaneously, the expressed *mXCL1-GPC3* protein was detected mainly in CD8 α ⁺DCs of mice draining lymph nodes. XCL1-GPC3-targeted DCs enhanced antigen-specific CD8⁺T cell proliferation and induced the *de novo* generation of GPC3-specific CD8⁺T cells, which abolished GPC3-expressing tumor cells both in the murine and human systems. In a murine autochthonous liver cancer model of AlBIHBV mice, the 3-dose (30- μ g in total) immunization suspended tumor development with significantly reduced tumor incidence and tumor load compared with GPC3-immunized mice. Notably, the mouse livers have increased infiltration of GPC3-specific CD8⁺T cells, activated NK cells and NKT cells after *mXCL1-GPC3* immunization. The antitumor effects of these cells were further enhanced by the administration of anti-PD-1^[40]. Therefore, the XCL1-GPC3, which targets cDC1s, might be a promising cancer vaccine to compensate for the deficiency of checkpoint blockades in HCC immunotherapy.

SUMMARY

The presence of cDC1s in the tumor microenvironment is often associated with a good prognosis in cancer patients and better response to immune checkpoint inhibitors. This cell population is an attractive target for delivering HCC tumor antigens to induce antitumor specific T cell responses. The combination of cDC1-

targeting cancer vaccines and checkpoint inhibitors will improve the efficacy of immunotherapy. Murine models have proven the effect of HCC cancer vaccines in compensating for anti-PD-1 by targeting cDC1s. However, clinical trials of such cancer vaccine are still required. It is also necessary to understand how cDC1s regulate anti-tumor immunity.

DECLARATIONS

Authors' contributions

Manuscript draft and finalization: Qu C

Participated in drafting of the manuscript: Chen K, Cheng SY

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature* 2017;541:321-30.
2. Friedrich MJ. Immunotherapy 2.0: improving the response to checkpoint inhibitors. *JAMA* 2019;321:131-3.
3. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, et al. IFN γ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 2001;410:1107-11.
4. van Dinther D, Stolk DA, van de Ven R, van Kooyk Y, de Gruijl TD, et al. Targeting C-type lectin receptors: a high-carbohydrate diet for dendritic cells to improve cancer vaccines. *J Leukoc Biol* 2017;102:1017-34.
5. Romero P, Banchereau J, Bhardwaj N, Cockett M, Disis ML, et al. The Human Vaccines Project: A roadmap for cancer vaccine development. *Sci Transl Med* 2016;8:334ps9.
6. Muller WA, Randolph GJ. Migration of leukocytes across endothelium and beyond: molecules involved in the transmigration and fate of monocytes. *J Leukoc Biol* 1999;66:698-704.
7. Jakubzick C, Gautier EL, Gibbings SL, Sojka DK, Schlitzer A, et al. Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity* 2013;39:599-610.
8. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 2013;38:79-91.
9. Scott CL, Zheng F, De Baetselier P, Martens L, Saeys Y, et al. Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. *Nat Commun* 2016;7:10321.
10. Ramachandran P, Dobie R, Wilson-Kanamori JR, Dora EF, Henderson BEP, et al. Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature* 2019;575:512-8.
11. Naik SH, Sathe P, Park HY, Metcalf D, Proietto AI, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol* 2007;8:1217-26.
12. Onai N, Obata-Onai A, Schmid MA, Ohteki T, Jarrossay D, et al. Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nat Immunol* 2007;8:1207-16.
13. Palomino-Segura M, Perez L, Farsakoglu Y, Virgilio T, Latino I, et al. Protection against influenza infection requires early recognition by

- inflammatory dendritic cells through C-type lectin receptor SIGN-R1. *Nat Microbiol* 2019;4:1930-40.
14. Guillems M, Ginhoux F, Jakubzick C, Naik SH, Onai N, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* 2014;14:571-8.
 15. Menezes S, Melandri D, Anselmi G, Perchet T, Loschko J, et al. The Heterogeneity of Ly6Chi Monocytes Controls Their Differentiation into iNOS+ Macrophages or Monocyte-Derived Dendritic Cells. *Immunity* 2016;45:1205-18.
 16. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, et al; Immunological Genome Consortium. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* 2012;13:1118-28.
 17. Caminschi I, Proietto AI, Ahmet F, Kitsoulis S, Shin Teh J, et al. The dendritic cell subtype-restricted C-type lectin Clec9A is a target for vaccine enhancement. *Blood* 2008;112:3264-73.
 18. Bonifaz LC, Bonnyay DP, Charalambous A, Darguste DI, Fujii S, et al. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J Exp Med* 2004;199:815-24.
 19. Neubert K, Lehmann CH, Heger L, Baranska A, Staedtler AM, et al. Antigen delivery to CD11c+CD8- dendritic cells induces protective immune responses against experimental melanoma in mice in vivo. *J Immunol* 2014;192:5830-8.
 20. Dhodapkar MV, Sznol M, Zhao B, Wang D, Carvajal RD, et al. Induction of antigen-specific immunity with a vaccine targeting NY-ESO-1 to the dendritic cell receptor DEC-205. *Sci Transl Med* 2014;6:232ra51.
 21. Cruz FM, Colbert JD, Merino E, Kriegsman BA, Rock KL. The Biology and Underlying Mechanisms of Cross-Presentation of Exogenous Antigens on MHC-I Molecules. *Annu Rev Immunol* 2017;35:149-76.
 22. den Haan JM, Lehar SM, Bevan MJ. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med* 2000;192:1685-96.
 23. Kitano M, Yamazaki C, Takumi A, Ikeno T, Hemmi H, et al. Imaging of the cross-presenting dendritic cell subsets in the skin-draining lymph node. *Proc Natl Acad Sci U S A* 2016;113:1044-9.
 24. Cancel JC, Crozat K, Dalod M, Mattiuz R. Are Conventional Type 1 Dendritic Cells Critical for Protective Antitumor Immunity and How? *Front Immunol* 2019;10:9.
 25. Böttcher JP, Bonavita E, Chakravarty P, Brees H, Cabeza-Cabrero M, et al. NK Cells Stimulate Recruitment of cDC1 into the Tumor Microenvironment Promoting Cancer Immune Control. *Cell* 2018;172:1022-1037.e14.
 26. Schiavoni G, Mattei F, Sestili P, Borghi P, Venditti M, et al. ICSBP is essential for the development of mouse type I interferon-producing cells and for the generation and activation of CD8alpha(+) dendritic cells. *J Exp Med* 2002;196:1415-25.
 27. Hacker C, Kirsch RD, Ju XS, Hieronymus T, Gust TC, et al. Transcriptional profiling identifies Id2 function in dendritic cell development. *Nat Immunol* 2003;4:380-6.
 28. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science* 2008;322:1097-100.
 29. Kashiwada M, Pham NL, Pewe LL, Harty JT, Rothman PB. NFIL3/E4BP4 is a key transcription factor for CD8alpha+ dendritic cell development. *Blood* 2011;117:6193-7.
 30. Bagadia P, Huang X, Liu TT, Durai V, Grajales-Reyes GE, et al. An Nfil3-Zeb2-Id2 pathway imposes Irf8 enhancer switching during cDC1 development. *Nat Immunol* 2019;20:1174-85.
 31. Grajales-Reyes GE, Iwata A, Albring J, Wu X, Tussiwand R, et al. Batf3 maintains autoactivation of Irf8 for commitment of a CD8alpha(+) conventional DC clonogenic progenitor. *Nat Immunol* 2015;16:708-17.
 32. Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. *J Exp Med* 2010;207:1261-71.
 33. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J Exp Med* 2010;207:1247-60.
 34. Dorner BG, Dorner MB, Zhou X, Opitz C, Mora A, et al. Selective expression of the chemokine receptor XCR1 on cross-presenting dendritic cells determines cooperation with CD8+ T cells. *Immunity* 2009;31:823-33.
 35. Crozat K, Guiton R, Contreras V, Feuillet V, Dutertre CA, et al. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. *J Exp Med* 2010;207:1283-92.
 36. Engelhardt JJ, Boldajipour B, Beemiller P, Pandurangi P, Sorensen C, et al. Marginating dendritic cells of the tumor microenvironment cross-present tumor antigens and stably engage tumor-specific T cells. *Cancer Cell* 2012;21:402-17.
 37. Broz ML, Binnewies M, Boldajipour B, Nelson AE, Pollack JL, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell* 2014;26:638-52.
 38. Salmon H, Idoyaga J, Rahman A, Leboeuf M, Remark R, et al. Expansion and Activation of CD103(+) Dendritic Cell Progenitors at the Tumor Site Enhances Tumor Responses to Therapeutic PD-L1 and BRAF Inhibition. *Immunity* 2016;44:924-38.
 39. Mittal D, Vijayan D, Putz EM, Aguilera AR, Markey KA, et al. Interleukin-12 from CD103+ Batf3-Dependent Dendritic Cells Required for NK-Cell Suppression of Metastasis. *Cancer Immunol Res* 2017;5:1098-108.
 40. Chen K, Wu Z, Zhao H, Wang Y, Ge Y, et al. XCL1/Glypican-3 Fusion Gene Immunization Generates Potent Antitumor Cellular Immunity and Enhances Anti-PD-1 Efficacy. *Cancer Immunol Res* 2020;8:81-93.
 41. Barry KC, Hsu J, Broz ML, Cueto FJ, Binnewies M, et al. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments. *Nat Med* 2018;24:1178-91.
 42. Kastenmüller W, Brandes M, Wang Z, Herz J, Egen JG, et al. Peripheral prepositioning and local CXCL9 chemokine-mediated guidance orchestrate rapid memory CD8+ T cell responses in the lymph node. *Immunity* 2013;38:502-13.
 43. Spranger S, Dai D, Horton B, Gajewski TF. Tumor-residing Batf3 dendritic cells are required for effector T cell trafficking and adoptive T

- cell therapy. *Cancer Cell* 2017;31:711-23.e4.
44. Zhu AX, Finn RS, Edeline J, Cattani S, Ogasawara S, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018;19:940-52.
45. El-khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
46. Sia D, Jiao Y, Martinez-Quetglas I, Kuchuk O, Villacorta-Martin C, et al. Identification of an immune-specific class of hepatocellular carcinoma, based on molecular features. *Gastroenterology* 2017;153:812-26.
47. Harding JJ, Nandakumar S, Armenia J, Khalil DN, Albano M, et al. Prospective genotyping of hepatocellular carcinoma: clinical implications of next-generation sequencing for matching patients to targeted and immune therapies. *Clin Cancer Res* 2019;25:2116-26.
48. Ruiz de Galarreta M, Bresnahan E, Molina-Sánchez P, Lindblad KE, Maier B, et al. β -catenin activation promotes immune escape and resistance to anti-PD-1 therapy in hepatocellular carcinoma. *Cancer Discov* 2019;9:1124-41.
49. Di Tommaso L, Sangiovanni A, Borzio M, Park YN, Farinati F, et al. Advanced precancerous lesions in the liver. *Best Pract Res Clin Gastroenterol* 2013;27:269-84.
50. Grimm CF, Ortmann D, Mohr L, Michalak S, Krohne TU, et al. Mouse alpha-fetoprotein-specific DNA-based immunotherapy of hepatocellular carcinoma leads to tumor regression in mice. *Gastroenterology* 2000;119:1104-12.
51. Hong Y, Peng Y, Guo ZS, Guevara-Patino J, Pang J, et al. Epitope-optimized alpha-fetoprotein genetic vaccines prevent carcinogen-induced murine autochthonous hepatocellular carcinoma. *Hepatology* 2014;59:1448-58.
52. Geissler M, Mohr L, Weth R, Kohler G, Grimm CF, et al. Immunotherapy directed against alpha-fetoprotein results in autoimmune liver disease during liver regeneration in mice. *Gastroenterology* 2001;121:931-9.
53. Filmus J, Capurro M. Glypican-3: a marker and a therapeutic target in hepatocellular carcinoma. *FEBS J* 2013;280:2471-6.

Review

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Stereotactic body radiation therapy for the management of HCC

Jia-Xin Li, Hong Wu, Yong Zeng

Department of Liver Surgery, West China Hospital of Sichuan University, Chengdu 610041, Sichuan, China.

Correspondence to: Prof. Yong Zeng, Department of Liver Surgery, West China Hospital of Sichuan University, Chengdu 610041, Sichuan, China. E-mail: zengyong@medmail.com.cn

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Abstract

Hepatocellular carcinoma (HCC) is a common malignant tumor in China. After years of efforts, there has been great progress in the management of liver cancer, but overall, it is still not ideal. At present, there are many therapies for liver cancer, including surgical resection, transcatheter arterial chemoembolization (TACE), ablation, molecular targeted therapy, stereotactic body radiation therapy, chemotherapy, immunotherapy, and so on. Studies have reported that TACE combined with radiotherapy can shrink the tumor, and some of the remainder will be resectable, resulting in cure. For HCC with tumor thrombus, the tumor thrombus was reduced and then resected after neoadjuvant radiotherapy. The survival time of the patients with portal vein tumor thrombus was significantly longer than that of the patients without neoadjuvant radiotherapy. Large liver cancer will be reduced to small liver cancer after comprehensive treatment, which can be transformed into stereotactic radiotherapy or radiofrequency ablation, and can also be palliative to radical treatment. Individualized and multidisciplinary therapy for liver cancer is the direction of future development. More clinical evidence-based level of radiotherapy treatment of liver cancer should be done in the future.

Keywords: Hepatocellular carcinoma, stereotactic body radiation therapy, tumor thrombus, combined therapy

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignant tumor that seriously endangers human health. At present, the main treatment for small HCC is surgical resection and radiofrequency ablation, but some patients are not suitable for surgery or radiofrequency ablation. In recent years, with the development of radiotherapy technology, the application of stereotactic body radiation therapy (SBRT) is increasing. Some retrospective studies have shown that its therapeutic effect on small liver tumors is equivalent to that of traditional surgery or radiofrequency ablation, and that it can also be used as a radical treatment



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for small liver tumors. Some patients with unresectable tumor thrombus can be converted to surgical resection after radiotherapy, and external radiotherapy can also be applied for bridging treatment before liver transplantation. A narrow margin with a distance of less than 1 cm from tumor resection edge is possible with radiotherapy after liver resection, to reduce local recurrence or distant metastasis of HCC and to prolong disease-free survival (DFS) of patients. The mechanism of killing tumor cell by SBRT may be different from conventional fractionated radiotherapy. With the increase of fractionated dose, double-strand DNA breaks increase, and the repair of sublethal damage decreases. Moreover, when the single fractionated dose is more than 8-10 Gy, the tumor vascular endothelial cells rapidly wither after 1-6 h of irradiation, and blood vessels are occluded, leading to a secondary tumor killing effect, which is mainly through the acid sphingomyelinase pathway-mediated apoptosis of vascular endothelial cells^[1-3], but conventional radiotherapy does not have the above effect. After conventional fractionated radiotherapy, tumor cell death is mainly achieved by cell apoptosis, while after high dose irradiation, tumor cell death is mainly in the form of “swelling death”^[4], and tumor cell membrane is destroyed, releasing tumor specific antigen, becoming “*in situ* tumor vaccine”, which can stimulate the immune system to kill residual tumor^[5].

EFFECT OF SBRT ON LIVER FUNCTION

Cárdenes *et al.*^[6] conducted a phase I clinical study of dose escalation in patients with liver cancer: patients with a tumor diameter less than 6 cm and Child-Pugh (CP) Class A could tolerate 48 Gy in three fractions, and the patients with CP class B could not tolerate 42 Gy in three fractions, while no adverse effects occurred after adjustment to 40 Gy in 5 fractions. The CP score was the only risk factor related to hepatotoxicity or death within six months of SBRT treatment ($P = 0.03$). Three patients had classic radiation-induced liver disease, all of which were above CP class B7. We believe that good liver function was needed for SBRT treatment of liver cancer. Furthermore, CP score must be ≤ 7 , and patients with CP score > 8 have high risk of liver failure after SBRT treatment.

SBRT FOR PRIMARY LIVER CANCER

Many studies in the literature have focused on the study of liver cancer lesions with the diameter of less than 6 cm. The 1- and 2-year local control (LC) rates after SBRT were 72%-100% and 54.4%-100%, respectively, and the 1-year survival rates were 72.7%-100% and 45.3%-87.9%, respectively^[6-17], but the dose fractionation of radiotherapy used in various clinical studies was not the same. Patients with CP class A or tumor diameter less than 3 cm usually undergo irradiation more than 10 Gy each time, total for 3-5 times. Because of the good LC rates and tolerability, SBRT is recognized as an alternative for small liver tumors unsuitable for surgery. Yoon *et al.*^[7] applied SBRT to treat 93 cases of inoperable liver cancer with median diameter of 2 cm (1-6 cm), given 30-60 Gy or 10-20 Gy in 3-4 fractions, and the 1- and 3-year overall survival (OS) rates were 86 and 53.8%, respectively. LC failure was mainly seen in patients with liver cancer ≥ 3 cm, and the 3-year LC rates of liver cancer with diameter ≥ 3.0 , 2.1-3.0 and ≤ 2.0 cm were 76.3, 93.3 and 100%, respectively, the intrahepatic 3-year recurrence-free survival (RFS) rate was 32.4%. Six patients (6.5%) had \geq grade 3 hepatotoxicity. Similarly, Kwon *et al.*^[8] reported that 42 cases of liver cancer were treated with CyberKnife at a dose of 30-39 Gy in 3 fractions; the complete response (CR) rate was 59.6% and the partial response (PR) rate was 26.2%, while the 1- and 3-year OS rates were 92.9 and 58.6%, respectively. Kimura *et al.*^[9] used 48 Gy in 4 fractions to treat 65 patients with CP class A/B and median tumor diameter of 1.6 cm; 2-year OS, RFS and LC were 76, 40 and 100%. Fifteen patients (23%) had \geq grade 3 adverse reactions 6-12 months after SBRT, and the level ≥ 3 adverse reactions in CP class B were higher than those in CP class A ($P = 0.0127$)^[9].

There is no prospective study comparing SBRT with surgery or TACE in the treatment of primary liver cancer. Honda *et al.*^[15] applied sequential SBRT and TACE compared with TACE alone in the treatment of primary liver cancer with diameter less than 3 cm; of the 30 cases in the SBRT group, 29 cases (96.3%) had a CR, but only 1 in 38 cases in the TACE group had a CR (3.3%). There was no radiation-related injury in

Table 1. Studies of stereotactic body radiation therapy in hepatocellular carcinoma

Authors	Cases	CP class A/ B/C	Tumor diameter or GTV	Dose/times (Gy/ time)	CR/PR/SD (%)	1/2/3-year LC (%)	1/2/3-year OS (%)
Cárdenes <i>et al.</i> ^[6]	17	A6/B11	2-6cm or 8-95 cm ³	36-48/3;36-42/3;40/5	25/56/19	100/N/N	75/60/N
Yoon <i>et al.</i> ^[7]	93	A69/B24	1-6 cm	30-60/3-4	15.5/45.7/36.9	94.8/N/92.1	86/N/53.8
Kimura <i>et al.</i> ^[9]	65	A56/B9	0.5-5.4 cm ³	48/4 or 60/8	100/100/N	92.3/76/N	N
Andolino <i>et al.</i> ^[12]	60	A36/B24	1-6.5 cm	m44/m3;m40/m5	30/40/25	N/90/N	N/67/N
Takeda <i>et al.</i> ^[13]	63	A44/B19	1-5 cm	35-40/5	100/95/92	100/87/73	N
Park <i>et al.</i> ^[16]	26	A19/B7	1.1-5.7 cm	40-50/10	25/42.9/32.1	N/87.6/N	88.5/67.2/N
Yuan <i>et al.</i> ^[17]	22	A10/B10/C2	1.6-9.5 cm	39-54/3-8	50/41/9	92.9/90/67.7	N

CP: Child-Pugh Class; GTV: gross tumor volume; m: median; N: not applicable; CR: complete response; PR: partial response; SD: stable disease; LC: local control; OS: overall survival

the SBRT group. Another retrospective study compared CyberKnife with surgical resection in the treatment of stage I liver cancer; 22 cases were treated with SBRT, and 26 patients achieved R0 resection. There was no difference in 3-year survival rate (69.2% vs. 57.1%, $P = 0.49$). Although the sample size was limited, the authors believed that SBRT is effective in the treatment of early stage liver cancer and that the curative effect is not inferior to that of surgical resection^[17] [Table 1].

SBRT IN THE TREATMENT OF PORTAL/VENA CAVA TUMOR THROMBUS

Tumor thrombus is an unavoidable difficulty in the treatment of liver cancer. The tumor thrombus will aggravate the occurrence of portal hypertension, ascites, liver failure, intrahepatic and extrahepatic dissemination. Choi *et al.*^[18] analyzed the treatment of advanced liver cancer with portal vein tumor thrombus (PVT) by SBRT combined with TACE; 9 cases with PVT treated with TACE at a median dose of 36 Gy (30-39 Gy in 3 fractions), and after a median follow-up time of 10.5 months, achieved PVT CR rate of 11.1% (1/9), PR rate of 33.3% (3/9), objective response rate (ORR) of 44.4% (4/9) and median OS of 8 months in advanced liver cancer^[18]. Xi *et al.*^[19] applied a median dose of 36 Gy in 6 fractions to treat primary liver cancer with portal/vena cava tumor thrombus, and 25 of 41 patients had received TACE, with a median follow-up of 10 months. The CR, PR, stable disease (SD), progressive disease (PD) of tumor thrombus were 36.6, 39, 17 and 7%, respectively. The 1-year OS was 50.3%, and the median OS was 13 months. Only one patient had an increased bilirubin level.

Wu *et al.*^[20] reported TACE combined with SBRT with 4-8 Gy per fraction for advanced liver cancer with portal/vena cava tumor thrombus. After 4-6 weeks, CR was 8.6%, PR 42.8%, SD 48.6%, and 1-, 2-, 3-year OS were 59.3, 31.6 and 26.6%, respectively, while median survival time was 11 months. Tse *et al.*^[21] recruited 16 cases of primary liver cancer with PVT, applied an average dose of 36 Gy in 6 fractions, achieved CR of 6%, PR of 19% and SD of 38%. Bujold *et al.*^[22] reported on 56 patients with PVT who were treated with an average dose of 36 Gy (24-54 Gy in 6 fractions), where the 1- and 2-year OS rates were 44 and 27%, respectively. Multivariate analysis showed that venous tumor thrombus was the worst prognostic factor, HR = 2.47 ($P = 0.01$).

PREOPERATIVE NEOADJUVANT SBRT FOR HCC

Patients with HCC (China liver cancer staging, CNLC IIIa) undergo surgical resection, but only a small part of the patients have long-term survival, and most of the patients have recurrence or metastasis in the short term, leading to death. Radiotherapy alone for PVT is a palliative therapy. Kamiyama *et al.*^[23] reported on patients with HCC complicated with tumor thrombus who received preoperative neoadjuvant radiotherapy, which was more effective than simple operation. The first branch or main tumor thrombus of portal vein was treated with radiotherapy at 30-36 Gy/10-12 times. After radiotherapy, the thrombus and intrahepatic lesions were removed simultaneously within 2 weeks. Intervention, radiofrequency, anhydrous

alcohol injection and other treatments were given during the follow-up periods. The results showed that the median survival time was 19.6 months for the patients who received surgery combined with external radiotherapy, and 9.1 months for the patients who did not undergo surgery. There was a significant difference between the two groups ($P = 0.036$). The pathology of the surgical specimens showed that 83% of the tumor thrombi were completely necrotic (completely relieved of disease). Therefore, the main purpose of surgery is to improve the control rate of primary HCC and dredge the portal vein. For patients with tumor thrombus, preoperative radiotherapy combined with surgical removal of thrombus is an effective method of comprehensive treatment. A randomized, prospective, multicenter clinical study was conducted in Shanghai Oriental Hepatobiliary Surgery Hospital to compare the survival of patients with HCC and PVTT before operation with or without neoadjuvant radiotherapy^[24]. A total of 82 patients in the radiotherapy group received 18 Gy/6 times of neoadjuvant external radiotherapy; 82 patients in the control group did not receive neoadjuvant radiotherapy. The results showed that the OS rates of radiotherapy group and operation group were 89.0% vs. 81.7%, 75.2% vs. 43.1%, 43.9% vs. 16.7% and 27.4% vs. 9.4% at 6, 12, 18 and 24 months, respectively ($P < 0.001$). The DFS rates of the two groups were 56.9% vs. 42.1%, 33.0% vs. 14.9%, 20.3% vs. 5.0% and 13.3% vs. 3.3% ($P < 0.001$)^[24]. Neoadjuvant radiotherapy can significantly improve the postoperative survival of patients with HCC.

TRANSFORMATION SBRT FOR HCC

Kim *et al.*^[25] of Yonsei University in South Korea reviewed the effect of concurrent radiotherapy and chemotherapy on patients with HCC. A total of 264 patients were selected, most of whom received three-dimensional conformal radiotherapy because of PVTT or residual liver volume insufficiency. Most of them received radiotherapy with a single dose of 1.8 Gy and a total dose of 45 Gy, and 5-fluorouracil (5-FU) was infused in the first and fifth week simultaneous with radiotherapy. One month after radiotherapy, 5-FU and cisplatin were infused once every 4 weeks for 3-12 cycles. Among them, 18 cases were converted into resectable cases. Postoperative pathology showed that 4 cases (22.2%) were completely necrotic and 7 cases (38.9%) were 70%-99% necrotic. Among the 18 patients who underwent surgery, the median survival time and median progression time were 40 and 24 months, respectively, and the median DFS time of the 4 patients with complete tumor necrosis was 54.6 months. Therefore, after conformal radiotherapy, some patients with unresectable HCC could be converted to resectable HCC.

BRIDGING SBRT BEFORE LIVER TRANSPLANTATION

Orthotopic liver transplantation is the most effective treatment for HCC patients with liver transplantation indications. However, due to the limited number of liver donors, many patients may have tumor progression while on the long waiting list, thus losing the best opportunity for liver transplantation. Therefore, bridging therapy to delay tumor progression is very important. SBRT can be used as a bridge treatment for liver cancer patients waiting for liver transplantation. It is neither a new adjuvant radiotherapy nor a transformation radiotherapy. Because SBRT belongs to radical treatment, the majority of HCC patients with T1 or T2 can be reduced to T0. The purpose of liver transplantation is to replace the decompensated liver with normal liver function. The purpose of radiotherapy is to control tumor progression while lacking a liver donor.

Eighteen patients with liver cancer who received SBRT before transplantation were reported by Rochester University Medical Center and William Beaumont Hospital in Michigan^[26,27]. The median dose of radiotherapy was 50 Gy/10 times. There was no serious gastrointestinal adverse reactions or radiation hepatitis. The median waiting period after radiotherapy was 6.3 months. Twelve patients received liver transplantation successfully, and 10 patients showed complete pathological necrosis. The median follow-up period was 19.6 months, and all patients survived. Therefore, SBRT is a safe and effective treatment for liver cancer patients waiting for liver transplantation. It can control the tumor and relieve the pressure of a liver donor before liver transplantation. A study reported^[28] on 379 patients treated with pre-transplantation

bridging therapy, including 36 patients with SBRT, 99 patients with TACE and 244 patients with radiofrequency ablation (RFA). Finally, 312 patients received liver transplantation, 30 in the SBRT group, 79 in the TACE group, and 203 in the RFA group. The 1-, 3- and 5-year survival rates were 83%, 61% and 61% in the SBRT group, 86, 61 and 56% in the TACE group, and 86%, 72% and 61% in the RFA group, respectively ($P = 0.4$). SBRT is as safe and effective as TACE and RFA in the treatment of HCC before transplantation. Compared with TACE and RFA, SBRT has more advantages in the treatment of HCC patients with ascites and prolonged prothrombin time.

POSTOPERATIVE ADJUVANT SBRT FOR HCC

Liver cancer with the distance of less than 1 cm from the bifurcation of the portal vein, the confluence of three main hepatic veins and inferior vena cava, is at high risk of recurrence after liver resection. The 5-year recurrence rate is reported to be more than 90%. At present, there is no effective adjuvant therapy to reduce such a high recurrence rate of this kind of liver cancer. Wang *et al.*^[29] reported the results of radiotherapy for the first time in patients with narrow margin surgery of central type liver cancer, who had retained a silver positioning mark in the operation area guided by postoperative adjuvant radiotherapy.

From 2007 to 2010, 181 patients were analyzed retrospectively. They were divided into three groups: group A: narrow margin surgery combined with postoperative radiotherapy (33 patients), group B: narrow margin surgery (83 patients) and group C: wide margin surgery with cutting edge more than 1 cm (65 patients), and postoperative radiotherapy dose was 46-60 Gy/23-30 times, with a median dose of 56 Gy; the 3-year OS of groups A, B and C was 89.1, 67.7 and 86.0%, respectively, while DFS was 64.2, 52.2 and 60.1%, respectively. Group A had similar OS ($P = 0.957$) and DFS ($P = 0.972$) as group C. Compared with group B, the OS ($P = 0.009$) and DFS ($P = 0.038$) of group A showed significant advantages. The incidence of grade-3 adverse reactions in group A was 12.1% and no more than grade-4 adverse reactions occurred. The study showed that postoperative radiotherapy can make up for the deficiency of narrow margin surgery, and that the effect of narrow margin surgery combined with postoperative radiotherapy can achieve a similar effect as wide margin radical surgery without serious adverse reactions.

Microvascular invasion (MVI) is the most important risk factor for early postoperative recurrence of HCC, which has been proved to be an independent predictor of OS and DFS^[30]. Even for patients with small liver cancer, MVI can increase tumor recurrence rate and significantly reduce OS, and MVI can only be detected by postoperative histological examination.

Wang *et al.*^[31] reviewed and compared the results of conservative therapy (CT), TACE and radiotherapy in 136 HCC patients with MVI. Narrow margin rate in the radiotherapy group was significantly higher than in the other two groups ($P = 0.010$). Postoperative radiotherapy dose was 54-60 Gy/23-30 times, where the results showed that there were significant differences between the radiotherapy group and TACE group and the radiotherapy group and CT group in DFS ($P < 0.05$), and the radiotherapy group and TACE group and the RT group and CT group in OS ($P < 0.05$). Subgroup analysis based on MVI degree and surgical margin showed that RFS and OS of patients in the radiotherapy group with narrow surgical margin were significantly longer than those of the TACE and CT groups, but not related to MVI degree. There was no significant difference in survival outcome between the three groups with wide surgical margin.

SUMMARY

To sum up, surgery is an effective way to cure HCC in the early and middle stages. The high recurrence rate and the lack of effective rescue treatment make the curative effect not very ideal. Although the related research results of surgery combined with SBRT are limited, they all show that it is a safe and effective treatment. As a new way of radiotherapy, preoperative SBRT has shown its unique advantages in the adjuvant

treatment of liver cancer. In a word, it is safe and feasible to combine preoperative and postoperative radiotherapy in the era of multidisciplinary comprehensive treatment, and it has also improved the curative effect. However, more in-depth research is needed to further determine whether it can make HCC patients benefit more from it.

DECLARATIONS

Authors' contributions

Data analysis and interpretation and paper writing: Li JX

Conception and design of the study: Wu H, Zeng Y

Availability of data and materials

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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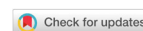
REFERENCES

1. Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, et al. Tumor response to radiotherapy regulated by endothelial cell apoptosis. *Science* 2003;300:1155-9.
2. Kolesnick R, Fuks Z. Radiation and ceramide-induced apoptosis. *Oncogene* 2003;22:5897-906.
3. Park HJ, Griffin RJ, Hui S, Levitt SH, Song CW. Radiation-induced vascular damage in tumors: implications of vascular damage in ablative hypofractionated radiotherapy (SBRT and SRS). *Radiat Res* 2012;177:311-27.
4. Würthwein G, Klingebiel T, Krümpelmann S, Metz M, Schwenker K, et al. Population pharmacokinetics of high-dose etoposide in children receiving different conditioning regimens. *Anticancer Drugs* 2002;13:101-10.
5. Lee Y, Auh SL, Wang Y, Burnette B, Wang Y, et al. Therapeutic effects of ablative radiation on local tumor require CD8⁺ T cells: changing strategies for cancer treatment. *Blood* 2009;114:589-95.
6. Cárdenes HR, Price TR, Perkins SM, Maluccio M, Kwo P, et al. Phase I feasibility trial of stereotactic body radiation therapy for primary hepatocellular carcinoma. *Clin Transl Oncol* 2010;12:218-25.
7. Yoon SM, Lim YS, Park MJ, Kim SY, Cho B, et al. Stereotactic body radiation therapy as an alternative treatment for small hepatocellular carcinoma. *PLoS One* 2013;8:e79854.
8. Kwon JH, Bae SH, Kim JY, Choi BO, Jang HS, et al. Long-term effect of stereotactic body radiation therapy for primary hepatocellular carcinoma ineligible for local ablation therapy or surgical resection. *Stereotactic radiotherapy for liver cancer. BMC Cancer* 2010;10:475.
9. Kimura T, Aikata H, Takahashi S, Takahashi I, Nishibuchi I, et al. Stereotactic body radiotherapy for patients with small hepatocellular carcinoma ineligible for resection or ablation therapies. *Hepatol Res* 2015;45:378-86.
10. Scorsetti M, Comito T, Cozzi L, Clerici E, Tozzi A, et al. The challenge of inoperable hepatocellular carcinoma (HCC): results of a single-institutional experience on stereotactic body radiation therapy (SBRT). *J Cancer Res Clin Oncol* 2015;141:1301-9.
11. Sanuki N, Takeda A, Oku Y, Mizuno T, Aoki Y, et al. Stereotactic body radiotherapy for small hepatocellular carcinoma: a retrospective outcome analysis in 185 patients. *Acta Oncol* 2014;53:399-404.
12. Andolino DL, Johnson CS, Maluccio M, Kwo P, Tector AJ, et al. Stereotactic body radiotherapy for primary hepatocellular carcinoma. *Int J Radiat Oncol Biol Phys* 2011;81:e447-53.
13. Takeda A, Sanuki N, Eriguchi T, Kobayashi T, Iwabuchi S, et al. Stereotactic ablative body radiotherapy for previously untreated solitary

- hepatocellular carcinoma. *J Gastroenterol Hepatol* 2014;29:372-9.
14. Bae SH, Park HC, Lim DH, Lee JA, Gwak GY, et al. Salvage treatment with hypofractionated radiotherapy in patients with recurrent small hepatocellular carcinoma. *Int J Radiat Oncol Biol Phys* 2012;82:e603-7.
 15. Honda Y, Kimura T, Aikata H, Kobayashi T, Fukuhara T, et al. Stereotactic body radiation therapy combined with transcatheter arterial chemoembolization for small hepatocellular carcinoma. *J Gastroenterol Hepatol* 2013;28:530-6.
 16. Park JH, Yoon SM, Lim YS, Kim SY, Shim JH, et al. Two-week schedule of hypofractionated radiotherapy as a local salvage treatment for small hepatocellular carcinoma. *J Gastroenterol Hepatol* 2013;28:1638-42.
 17. Yuan Z, Tian L, Wang P, Song Y, Dong Y, et al. Comparative research on the efficacy of CyberKnife® and surgical excision for Stage I hepatocellular carcinoma. *Onco Targets Ther* 2013;6:1527-32.
 18. Choi BO, Choi IB, Jang HS, Kang YN, Jang JS, et al. Stereotactic body radiation therapy with or without transarterial chemoembolization for patients with primary hepatocellular carcinoma: preliminary analysis. *BMC Cancer* 2008;8:351.
 19. Xi M, Zhang L, Zhao L, Li QQ, Guo SP, et al. Effectiveness of stereotactic body radiotherapy for hepatocellular carcinoma with portal vein and/or inferior vena cava tumor thrombosis. *PLoS One* 2013;8:e63864.
 20. Wu DH, Chen LH. Efficacy of 3 dimensional conformal hypofractionated single high dose radiotherapy combined with transcatheter arterial chemoembolization for portal vein tumor thrombus in patients with hepatocellular carcinoma. *Chin J Cancer* 2004;23:3.825s.
 21. Tse RV, Hawkins M, Lockwood G, Kim JJ, Cummings B, et al. Phase I study of individualized stereotactic body radiotherapy for hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *J Clin Oncol* 2008;26:657-64.
 22. Bujold A, Massey CA, Kim JJ, Brierley J, Cho C, et al. Sequential phase I and II trials of stereotactic body radiotherapy for locally advanced hepatocellular carcinoma. *J Clin Oncol* 2013;31:1631-9.
 23. Kamiyama T, Nakanishi K, Yokoo H, Tahara M, Nakagawa T, et al. Efficacy of preoperative radiotherapy to portal vein tumor thrombus in the main trunk or first branch in patients with hepatocellular carcinoma. *Int J Clin Oncol* 2007;12:363-8.
 24. Wei X, Jiang Y, Zhang X, Feng S, Zhou B, et al. Neoadjuvant three-dimensional conformal radiotherapy for resectable hepatocellular carcinoma with portal vein tumor thrombus: a randomized, open-label, multicenter controlled study. *J Clin Oncol* 2019;37:2141-51.
 25. Kim J, Lee I, Han K, Kim J, Kim K, et al. Clinical features of hepatocellular carcinoma patients undergoing resection after concurrent chemoradiation therapy. *Int J Radiat Oncol Biol Phys* 2012;84:S336-7.
 26. Katz AW, Chawla S, Qu Z, Kashyap R, Milano MT, et al. Stereotactic hypofractionated radiation therapy as a bridge to transplantation for hepatocellular carcinoma: clinical outcome and pathologic correlation. *Int J Radiat Oncol Biol Phys* 2012;83:895-900.
 27. O'Connor JK, Trotter J, Davis GL, Dempster J, Klintmalm GB, et al. Long-term outcomes of stereotactic body radiation therapy in the treatment of hepatocellular cancer as a bridge to transplantation. *Liver Transpl* 2012;18:949-54.
 28. Sapisochin G, Barry A, Doherty M, Fischer S, Goldaracena N, et al. Stereotactic body radiotherapy vs. TACE or RFA as a bridge to transplant in patients with hepatocellular carcinoma. An intention-to-treat analysis. *J Hepatol* 2017;67:92-9.
 29. Wang WH, Wang Z, Wu JX, Zhang T, Rong WQ, et al. Survival benefit with IMRT following narrow-margin hepatectomy in patients with hepatocellular carcinoma close to major vessels. *Liver Int* 2015;35:2603-10.
 30. Rodríguez-Perálvarez M, Luong TV, Andreana L, Meyer T, Dhillon AP, et al. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. *Ann Surg Oncol* 2013;20:325-39.
 31. Wang L, Wang W, Yao X, Rong W, Wu F, et al. Postoperative adjuvant radiotherapy is associated with improved survival in hepatocellular carcinoma with microvascular invasion. *Oncotarget* 2017;8:79971-81.

Review

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The advances in immunotherapy for hepatocellular carcinoma

Fan Zhang^{1,2}, Yumin Li^{1,2}

¹Department of Oncology Surgery, Lanzhou University Second Hospital, Lanzhou 730030, China.

²Key Laboratory of Digestive System Tumors of Gansu Province, Lanzhou University Second Hospital, Lanzhou 730030, Gansu, China.

Correspondence to: Dr. Yumin Li, Key Laboratory of Digestive System Tumors of Gansu Province, Lanzhou University Second Hospital, 82 Cuiyingmen, Lanzhou 730030, Gansu, China. E-mail: liym@lzu.edu.cn

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Abstract

Hepatocellular carcinoma (HCC) is one of the malignant tumors with higher incidence and mortality worldwide. Recently, significant progress has been made in uncovering immunotherapy in HCC, for instance programmed death-1, cytotoxic T-lymphocyte antigen 4, chimeric antigen receptor T-cell therapy, T cell receptor T cell therapy, dendritic cell vaccine, and cytokine-induced killer cells. This paper reviews the advances in immunotherapy and focuses on the results of many of preclinical studies and clinical trials in the field, as well as some of the promising therapeutic strategies for HCC in the future.

Keywords: HCC, PD-1, PD-L1, CTLA-4, CAR-T, T cell receptor, DC, CIK

INTRODUCTION

Hepatocellular carcinoma (HCC) was predicted to have the sixth highest incidence and the second highest mortality of malignant tumors worldwide in 2018^[1]. The risk factors for HCC are closely related with lifestyle, chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, fatty liver disease, and cirrhosis^[2-4]. The management of HCC involves a multidisciplinary team approach, considering not only the tumor stage and patient complications but also the seriousness of damaged liver function, as most HCC treatments can aggravate the severity of disease^[5]. Although surgical resection remains the cornerstone of HCC therapy, limitations are caused by high recurrence rates after surgery because HCC is often diagnosed at advanced stage^[6]. Liver transplantation (LT) is the optimal treatment means for early-stage HCC, but limitations of LT are caused by organ shortage, tumor recurrences, and low-ratio eligibility. Comprehensive therapies for advanced HCC patients, such as radiotherapy, chemotherapy, interventional therapy, and targeted therapy, have been developed, but the 5-year survival rate remains low^[7].



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Cancer immunotherapy was selected as its annual breakthrough in Science journal in 2013. Following the advancements of immunotherapy in solid tumors over the last few years, as shown by the results of immune checkpoint inhibitors (ICIs) in lung cancer, renal cell cancer and melanoma^[8], in recent years, ICIs with anti-CTLA-4 antibodies and anti-PD-1/PD-L1 antibodies have been utilized to treat advanced melanoma^[9]. In 2018, because of the achievements in the treatment of cancer with ICIs of CTLA-4 and PD-1/PD-L1, James P. Allison and Tasuku Honjo were awarded the Nobel prize.

Chimeric antigen receptor T-cell (CAR-T) immunotherapy has become more popular in the last decade as an antitumor therapy. Anti-CD19 CAR-T cell was approved by the FDA for treatment of subjects up to 25 years of age with B-cell acute lymphoblastic leukemia in 2017^[10]. This article mainly summarizes the advances in ICIs and cellular immunotherapy for HCC.

PD-1/PD-L1

PD-1 is expressed on a subset of thymocytes and is upregulated on activated T cell, B cell, and myeloid cells^[11]. Two ligands for PD-1 were identified in 2000 and 2001 and named PD-L1 and programmed death ligand 2 (PD-L2), respectively^[12,13]. PD-L1 is mainly expressed on stationary T cells, B cells, DC, and hepatoma cells, while PD-L2 is only expressed on DC and macrophages^[14-16]. In theory, the interaction of PD-1 and PD-L1 expressed on immature T cells can interfere with activation. Similarly, if PD-L1 is highly expressed on tumor cell, the ligand receptor interactions between tumor cells and activated T cells triggers the immunosuppressive response, leading to immune tolerance^[17]. It provides a theoretical basis for the treatment of PD-L1 in HCC.

It has been demonstrated that PD-L1 is overexpressed in HCC tissues; however, the results are controversial with respect to PD-L1 as predictive biomarkers for HCC^[18]. Several studies have reported that the higher PD-L1 expression on tumor cell in HCC patients were related with worse prognosis and tumor recurrence, and the studies also showed PD-L1 expression on macrophages was associated with favorable survival rate^[19-23]. However, two studies suggested that the expression of PD-L1 was not significantly correlated with survival outcomes in HCC^[24,25]. Both soluble PD-1 (sPD-1) and soluble PD-L1 (sPD-L1) were prognostic factors with opposite prognostic values for HCC patients, while sPD-1 and sPD-L1 were not significantly related with PD-L1 expression in tumor^[26]. However, two studies suggested plasma sPD-1 was associated with HBV activity and increased risk of HCC^[27,28].

Liang *et al.*^[29] illustrated that inhibition of PD-1 can suppress the growth of hepatoma and promote the apoptosis of hepatoma. Increased expression levels of PD-1 were detected in peripheral blood and tumor infiltrating lymphocytes (TILs) of recurrent HCC patients^[30]. Blockade of PD-1 on TILs can restore anti-tumor effects of TILs^[31]. However, sPD-1/sPD-L1 was not associated with either PD-L1 expression of tumor cell or the numbers of CD4-positive TILs and CD8-positive TILs^[26]. Tumor infiltrating neutrophils as a new target of immunotherapy participate in tumor progression, while the tumor microenvironment (TME) induces impaired antitumor immunity via the modulation of PD-L1 expression on tumor infiltrating neutrophils^[32]. PD-L1 was positively associated with expression of CD3 and CD8 in HCC samples^[23]. PD-L1 expression on macrophages is also a prognostic factor for HCC patients, and it could activate high levels of CD8(+) cytotoxic T-lymphocyte (CTL) infiltration and immune related gene expression^[21].

Since immune checkpoint molecules are recognized as vital indicators of HCC progress, series of clinical trials with ICIs have been implemented to confirm their potential function for advanced HCC. Nivolumab was approved by the FDA as immunotherapy for advanced stage HCC in 2017^[33]. The efficiency of nivolumab was observed in a Phase I/II non-comparative trial (CheckMate 040) of patients with HCC and prior sorafenib treatment^[33]. Forty-eight patients were treated with nivolumab in a dose-escalation phase. Then, since nivolumab showed adequate safety and feasibility, 214 patients from 39 sites in 11 countries received

nivolumab in a dose-expansion phase. The objective response rates (ORR) of nivolumab were 15% in the dose-escalation phase and 20% in the dose-expansion phase, suggesting that efficacy of nivolumab is not efficient. Twelve of 48 patients had Grade 3/4 treatment-associated adverse events. Although the study was positive in favor of anti-PD-1 treatment, it is worth corroborating the efficacy of nivolumab in a therapeutic schedule. Pre-treatment of sorafenib might potentiate the therapeutic response to subsequent treatment with nivolumab. In real-life experience from three German centers, Grade 3 treatment-associated events occurred in two patients (5.9%), and the partial response rate and stable disease rate in 34 patients with advanced HCC and nivolumab treatment were 11.8% and 23.5% in line with data from the CheckMate 040 trial^[34].

Pembrolizumab is also an antibody against PD-1. In a Phase II open-label non-randomized trial (KEYNOTE-224) to assess the efficacy of pembrolizumab as an alternative second-line treatment for HCC patients, the median overall survival (OS) was 12.9 months with a disease control rate of 61% and ORR of 17%^[35]. Grade 3 toxicities arose in 25 (24%) of the 104 patients. Hence, pembrolizumab is temporarily approved by the FDA as a second-line therapy for advanced HCC, but it still needs to be verified by the results of more Phase III trials^[36]. A Phase III randomized, double-blind trial to further assess the efficacy of pembrolizumab versus placebo in HCC patient is still ongoing (NCT02702401). A Phase II study evaluating camrelizumab for HCC patients with resistance to systemic treatment displayed ORR of 13.8% and acceptable treatment-related adverse events in Chinese advanced HCC patients^[37]. In addition to monotherapy, possible multimodality therapeutic options involving ICIs are under investigation. Some research has observed that ICIs of PD-L1 in combination with sorafenib, lenvatinib, rapamycin, and histone deacetylase inhibitor may enhance therapeutic benefit^[38-41].

Clinical trials of PD-1 antibodies combined with other adjuvant therapy, e.g., transarterial chemoembolization (TACE) and selective internal radiation treatment, are currently in progress. In addition, different combination regimens, which depend on understanding of the actual immune mechanisms in the various combinations, could help us select the optimal therapeutic option for advanced HCC.

CTLA-4

CTLA-4 downregulates activation of T cells by interacting with CD80/CD86 on the surface of DCs^[42]. For naive T cell activation, CD28 on T cells provides the second activation signal by binding to CD80/CD86 on DCs^[43]. CTLA-4 has a greater affinity for interacting with CD80/CD86 than CD28 so that it interferes in T cell activation^[44]. Various single nucleotide polymorphisms (SNP) in CTLA-4 have been well-studied. Several studies observed that polymorphism of CTLA-4 was associated with increased susceptibility to HCC and haplotypes of CTLA-4 may affect the risk of HCC^[45-48].

In 2013, the first CTLA-4 blocking inhibitor in practical HCC treatment was tremelimumab, which displayed promising antitumor activity and acceptable safety^[49]. In a clinical trial to validate efficacy of tremelimumab in patients with HCC and HCV infection, partial response rate and disease control rate were 17.6% and 76.4%, respectively^[49]. Duffy *et al.*^[50] attempted to combine tremelimumab with ablation as an expected therapeutic option for patients with advanced HCC (NCT01853618). Five partial responses were observed in 19 patients, with median OS of 12.3 months. Tremelimumab is a human IgG2 monoclonal antibody that blocks the binding of CTLA-4 on the surface of activated T cell^[51]. It has been reported that tremelimumab could induce tumor responses in a subset of patients with non-small cell lung cancer and refractory biliary tract cancer^[52,53]. Tremelimumab therapy could elevate the amount of T cells in the peripheral blood and TILs, and CD4(+) PD-1(+) cells were more likely to be activated by tremelimumab^[54]. An important adverse effect of tremelimumab is transaminitis, as a high proportion of reversible Grade 3/4 transaminitis was observed in both the above-mentioned studies.

Preclinical data based on series of solid tumors indicate that dual immune checkpoint blockade is synergistic and leads to higher response rates and improved treatment outcomes compared to monotherapy. Most

clinical data suggest that both CTLA-4 and PD-1/PD-L1 blockade a portion of HCC patients. Compared to CTLA-4 blockade, PD-1 and PD-L1 blockade showed relatively higher ORR, which could reach 10%-20% in advanced HCC patients. PD-1/PD-L1 blockade agents were more tolerable and less hepatotoxic. Further studies for combined PD-1/PD-L1 and CTLA-4 blockades in HCC treatment are still expected, which may help to mitigate the adverse effects of the treatment. Immune checkpoint blockade in advanced HCC combined with other conventional ablative treatments, such as radiofrequency ablation (RFA) or microwave, TACE, chemotherapy, targeted medicine, or surgery would be the most promising approach for HCC patients. However, for unresectable advanced HCC, it is more appropriate to search for other combination strategies, such as the combination with multi-kinase inhibitors, vaccines, and oncolytic viruses, as well as dual inhibition of two immune checkpoint molecules.

Based on current evidence, combination therapies with CTLA-4 are now an expected direction for the immunotherapy of advanced HCC patients in the future. A Phase III study (NCT03298451) of durvalumab with or without tremelimumab *vs.* sorafenib in patients of advanced HCC enrolled about 1,350 patients and explored two treatment schedules. Given the limited data to date, further testing of this combination is ongoing in a Phase II expansion. Most ongoing clinical trials have been designed to assess the efficiency of the combination strategies.

CAR-T CELL THERAPY

CD19 targeted CAR-T immunotherapy is an expecting therapeutic option that has shown high efficacy in treating hematologic malignancies^[55]. Moreover, a great number of CAR-T cell products in solid tumors has also been investigated in preclinical and clinical studies. In 2008, Wilkie *et al.*^[56] reported for the first time that MUC1 targeted CAR-T could significant delay tumor growth in solid tumor^[56]. The basic principle of CAR-T cell therapy is the modification of T cells with CARs, so that they can identify tumor cells, and then the retransfusion of these CAR-T cells into the human body to fight against the target cells^[57,58]. Several studies have found that GPC3-targeted CAR-T cell therapy can eliminate HCC cells in preclinical research^[59-61]. GPC3 is a 70-kDa heparan oncofetal proteoglycans that is located on the tumor cell membrane^[62]. It has been demonstrated that GPC3 is detected in HCC tissues with higher expression but not in normal tissues^[63]. A Phase I trial (NCT02395250) of 13 Chinese GPC3-positive HCC patients illustrated the safety and preliminary efficacy of GPC3 CAR-T cells in 2017^[64]. According to the patient's tolerance, the preliminary analysis showed that GPC3 targeted CAR-T combined with the lymphodepleting conditioning had a certain efficacy^[64]. The pre-clinical studies for dual-targeted CAR-T cells co-expressing GPC3 CARs and GPC3-specific CAR-modified T cells fusing a soluble PD1-CH3 fusion protein showed promising results^[60,61].

α -fetoprotein (AFP) has been used not only as a biomarker for surveillance and diagnosis of HCC, but also as a target for immunotherapy^[65]. In a clinical trial of 15 HCC patients who were given a subcutaneous injection of AFP-derived peptides, 1 patient had a complete response and the disease stabilized in 8 patients^[66]. AFP, an intracellular/secreted protein, can generate AFP peptide-major histocompatibility complex (MHC) complexes as targets for CAR T-cell therapy for solid tumors. Liu *et al.*^[67] detected that AFP-targeted CAR-T cells showed significant antitumor capacity in a mouse model. Additionally, AFP-derived vaccines can augment the activity of ICIs, leading to deterioration of HCC.

The experience from successful clinical studies of hematologic malignancies provides us with the understanding that, although selection of the specific antigen to avoid off-target or on-target/off-tumor toxicity is a primary task to be tackled, for HCC, the challenge of CAR-T is the need to ascertain a specific neoantigen and overcome the TME, gut microbiome, and HCC genomic features. Furthermore, the activation, proliferation, and persistence of CAR-T are more important for therapy. In addition, standardization in the production of CAR-T and achieving individualized treatment should be considered.

TCR-T CELL THERAPY

TCR-T cell immunotherapy, as one of the novel and effective antitumor treatment means, has been widely studied in oncotherapy. In 2011, Parkhurst *et al.*^[68] firstly reported that human carcinoembryonic antigen (CEA)-targeted TCR-T cell therapy could induce objective regression of metastatic colorectal cancer^[68]. The mechanisms of TCR-T cell therapies are similar to CAR-T immunotherapy. TCR-T therapy also modifies the autologous T cells with TCR, and then retransfusion expands TCR-T cell back into the patient to recognize and eliminate tumor cell, but the mechanisms for identifying antigens are quite different from CAR-T cell therapies^[57]. The specific antigens recognized by CAR-T cell are all cell membrane antigens, while TCR-T cell can identify intracellular and cell membrane antigen peptides presented by MHC molecules^[69]. In HBV-related HCC, by performing the high-throughput TCR sequence of TILs in tumor and matched adjacent normal tissues, Lin *et al.*^[70] found that the combination of TCR repertoire overlap and TNM stage showed a better prognostic effect for HCC than TNM stage. Qasim *et al.*^[71] firstly reported an HBV-related end-stage HCC case treated with HBV surface antigen as a target for HBV-specific TCR T cell therapy in 2015. In most HBV-related HCC, HBV integrations have been observed and can result in the expression of HCC cells^[72]. HCC cells comprise fragments of integrated HBV-DNA that encodes peptides, which can be identified by T cells^[73]. Another trial was conducted in two advanced HCCs patients who underwent liver transplantation with HCC relapses^[74]. During the one-year period of follow up, the volume of 5/6 pulmonary metastases was decreased in one patient receiving HBV-specific TCR T cell therapy^[74]. Basic studies of TCR-T cells therapy with specific targets, such as HCV, AFP, and GPC, may be a promising immunotherapy strategy for HCC in the future^[75-77]. With TCR-T immunotherapy, the efficacy and side effects seem to mainly depend on the quality of the specific target and the TCR structure. The primary challenge is the discovery of new targets, particularly in the promising field of neoantigens. However, it should be emphasized that neoantigens may be expressed on a subset of tumor cells due to heterogeneity of tumor cell; otherwise, it may cause immune escape.

DENDRITIC CELL VACCINE

DCs are powerful antigen-presenting cells that can stimulate T cells to induce antitumor activity. The infiltration of DCs in tumor tissue was closely associated to the improved clinical prognosis in HCC patients^[78,79]. In 2002, Ladhams *et al.*^[80] firstly reported two patients with end-stage HCC treated with autologous DCs vaccination co-cultured with autologous HCC antigens. The efficacy of DC vaccination loaded with tumor antigens from different sources has been investigated in clinical studies. Lee *et al.*^[81] reported a trial which enrolled 31 advanced HCC patients receiving DC vaccine pulsed with autologous tumor lysates in 2005. They reported that rates of partial response and stable disease were 12.9% and 54.8%, respectively. A Phase II clinical trial reported disease control rate was 28% for advanced HCC patients with DC vaccination pulsed HepG2 lysate^[82]. In another study of note, El Ansary *et al.*^[83], also using DC vaccine pulsed with HepG2 lysate, showed that DC vaccination could partially improve survival outcome. DC vaccination loaded with autologous tumor lysates or ex vivo HepG2 cell lysate were feasible and effective.

However, the efficacy of DCs vaccination pulsed with tumor cell lysate is not satisfactory, and thus the use of specific antigen-modified DC vaccination has been attempted. Kakumu *et al.*^[84] suggested that the depressed function of DCs is associated with pathogenesis of HCC with HBV or HCV infection. Several pre-clinical studies indicated that DCs infected with AFP or HBV antigen or both were effective strategies to enhance efficacy of DC-based vaccine^[85-87]. GPC3-modified DCs were potent in inducing T cell proliferation and interferon (IFN)- γ production^[88]. Tada *et al.*^[89] reported a clinical effect was observed in one of the five patients receiving DC vaccination pulsed with AFP, GPC3, and MAGE-1 fusion proteins in 2012. Subsequently, a large sample study confirmed that the median time of progression of HCC patients with DC vaccination pulsed with AFP, GPC3 and MAGE-1 fusion proteins was longer than the control group (36.6 months vs. 11.8 months)^[90].

DCs pulsed with Hsp70 peptide and OK-432 can enhance efficacy of vaccine inducing T cell proliferation and CTL response^[91,92]. In a clinical trial using Hsp70-DC vaccination, 2/12 patients demonstrated complete response and 5/12 patients demonstrated stable disease^[93]. In our previous meta-analysis, we concluded DC-based therapy could prolong the median progression free survival (PFS) time and median OS time^[94].

However, the maturation of DC was closely associated with efficacy of DC immunotherapy. The stimulatory capacity of dendritic cells from HCC patients was significantly lower than dendritic cells from liver cirrhosis tissue and normal samples^[95]. Meanwhile, the numbers of CD83-positive DCs in HCC specimens were significantly lower compared with liver cirrhosis samples^[96]. Therefore, it is very important to improve the maturation of DC, increase antigen source, and depress TME. Various stimuli, such as tumor necrosis factor alpha, lipopolysaccharide, IFN gamma, CD40-ligand, PEG10, IL-12, EpCAM, and HCA661, can significantly increase the stimulatory capacity of DCs^[97-102]. Tumor endothelial marker 8 modified DCs could stimulate antitumor immunity by disrupting tumor vasculature, and DCs loaded with specific peptide, such as FoxM1, could significantly inhibit tumor growth and metastasis^[103,104]. In addition, RFA can create an antigenic source with stimuli appropriate for maturation of DCs^[105]. Regulatory T cells, producing immunosuppressive cytokine IL-10, were concentrated within HCC tissue and were induced by local TME to interfere the differentiation and maturation of DC^[7]. To overcome the immunosuppressive TME, Hu *et al.*^[106] introduced a promising vaccine candidate, which combine the DC/tumor cell fusion vaccine with nanoparticles of folate-modified chitosan carrying interferon-induced protein-10, which could effectively inhibit tumor cell proliferation and significantly reduce myeloid-derived suppressor cells in mouse immune organs.

CIK/DC-CIK

CIK are a subset of non-MHC-restricted T lymphocytes with immune modulatory effects and a crucial role in anti-tumor immunotherapy^[107]. Several studies suggested that CIK cells co-cultured with DCs can significantly enhance antitumor efficiency^[108,109]. Qiu *et al.*^[110] reported that alpha-Gal epitope-pulsed DC-CIK therapy remarkably prolonged the survival of patients with stage III primary HCC as compared to the controls (17.1 months vs. 10.1 months). In a retrospective study from 45 patients with metastatic HCC, median OS of DC-CIK immunotherapy plus ablation (32 months) or ablation (17.5 months) was higher than untreated group (3 months)^[111]. In a propensity score-matched analysis, autologous CIK immunotherapy showed significantly longer RFS than the control group^[112]. After 5-year follow-up, CIK immunotherapy show a significant reduction in the risk of recurrence or death^[113]. The combination therapies DC-CIK with other therapeutic options, such as TACE, could improve the antitumor efficacy. Guo *et al.*^[114] reported that DC-CIK therapy combined with TACE can improve the PFS but not the OS outcomes. However, TACE plus DC-CIK therapy for HCC patients is superior to TACE alone in improving median OS and PFS in a meta-analysis^[115]. Zhou *et al.*^[116] analyzed that clinical benefit rate of sorafenib combined with DC-CIK is higher than oral administration of sorafenib (88.6% vs. 41.9%) in a meta-analysis.

To enhance the therapeutic efficacy of CIK cells, several pre-clinical studies suggested that co-culture of modified DCs, such as IL24-modified DCs, AFP-modified DCs, and GPC3-modified DCs, with CIKs can significantly promote CIKs differentiation and enhance lytic activity of CIK cells^[117-119]. They provided a promising DC-CIK vaccine candidate for further clinical trials of HCC patients. Indeed, CIK or DC-CIK immunotherapies from autologous or allogeneic donors have already been extensively used in solid tumor patients. In our clinical center, we have experience with more than 100 gastric cancer patients with DC-CIK immunotherapy and have found that treatment outcomes were effective, safe, and feasible for gastric cancer patients. The standardization in the preparation and criteria of indication are progressing; several clinical trials are registered and ongoing. CIK or DC-CIK immunotherapies, combined with other antitumor agents, should be considered.

CONCLUSION AND OUTLOOK

Immunotherapies appear to be a promising treatment for advanced HCC. Multiple prospective studies are attempting to validate the therapy outcomes with PD-1/PD-L1 and/or CTLA-4 blockade. However, only a small proportion of HCC patients effectively respond to immunotherapies and much research is still needed. One of the future directions for immunotherapies is combination therapies with other ICIs, TKIs, vaccines, and oncolytic viruses, as well as conventional treatments in various stages of patients to improve the antitumor efficacy. In addition, it is important to research how to elevate immunotherapy efficacy and ascertain the biomarkers of predictive therapeutic response to immunotherapy. For example, TMB has been used in several tumor types to predict therapeutic response to anti-PD-1 therapy. In the future, we expect to identify more predictive biomarker subsets which can be used to accurately evaluate the efficacy of immunotherapy.

CAR-T technology and its application has been hailed as a scientific breakthrough in the field of hematological tumors. Application of CAR-T therapy and TCR to treat HCC is expected to be a promising therapeutic method. The crucial challenge is the need to identify specific antigens; overcome the TME, gut microbiome, and HCC genomic features; and guard against adverse effects. Furthermore, the activation, proliferation, and persistence of CAR-T immunotherapy should be considered with the outcomes of treatments for HCC. In addition, standardization in the production of CAR-T and achieving individualized treatment should be considered.

Different modifications of DC vaccine or DC-CIK therapy, such as selection of specific antigen targets and appropriate immunologic adjuvant, may elevate the effectiveness and safety in further studies. Dendritic cells lead to an increase in the naturally occurring neoantigen-specific immune response as well as the diversity of neoantigens. The combination of DC vaccination with other immunotherapies, e.g., TCR-T, may be a novel treatment modality in the future.

Because of the heterogeneity of tumor cells and the complexity of immuno-regulatory mechanisms, multimodality therapies based on immunotherapy represent the next step in clinical antitumor efficacy, which will enable advancing the field and improving the outcomes of HCC patients.

DECLARATIONS

Authors' contributions

Planned and designed of the study: Li Y

Searched the literature and wrote the manuscript, performed revisions, read and approved the final manuscript for publication: Zhang F, Li Y

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that they have no conflicts of interest.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCE

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
- Tian Y, Yang B, Qiu W, Hao Y, Zhang Z, et al. ER-residential Nogo-B accelerates NAFLD-associated HCC mediated by metabolic reprogramming of oxLDL lipophagy. *Nat Commun* 2019;10:3391.
- Papatheodoridis GV, Chan HL, Hansen BE, Janssen HL, Lampertico P. Risk of hepatocellular carcinoma in chronic hepatitis B: assessment and modification with current antiviral therapy. *J Hepatol* 2015;62:956-67.
- Singal AG, Rich NE, Mehta N, Branch AD, Pillai A, et al. Direct-acting antiviral therapy for hepatitis C virus infection is associated with increased survival in patients with a history of hepatocellular carcinoma. *Gastroenterology* 2019;157:1253-63.e2.
- Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, et al. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019;16:589-604.
- Desert R, Rohart F, Canal F, Sicard M, Desille M, et al. Human hepatocellular carcinomas with a periportal phenotype have the lowest potential for early recurrence after curative resection. *Hepatology* 2017;66:1502-18.
- Bruix J, da Fonseca LG, Reig M. Insights into the success and failure of systemic therapy for hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2019;16:617-30.
- Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012;366:2443-54.
- Mason R, Au L, Ingles Garcés A, Larkin J. Current and emerging systemic therapies for cutaneous metastatic melanoma. *Expert Opin Pharmacother* 2019;20:1135-52.
- Administration UFA. FDA approves tisagenlecleucel for B-cell ALL and tocilizumab for cytokine release syndrome. 2017. Available from: <https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm606540.htm> [Last accessed on 16 Jun 2020]
- Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, et al. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol* 1996;8:765-72.
- Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2001;2:261-8.
- Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000;192:1027-34.
- Finkelmeier F, Waidmann O, Trojan J. Nivolumab for the treatment of hepatocellular carcinoma. *Expert Rev Anticancer Ther* 2018;18:1169-75.
- Yarchoan M, Xing D, Luan L, Xu H, Sharma RB, et al. Characterization of the Immune Microenvironment in Hepatocellular Carcinoma. *Clin Cancer Res* 2017;23:7333-9.
- Mocan T, Sparchez Z, Craciun R, Bora CN, Leucuta DC. Programmed cell death protein-1 (PD-1)/programmed death-ligand-1 (PD-L1) axis in hepatocellular carcinoma: prognostic and therapeutic perspectives. *Clin Transl Oncol* 2019;21:702-12.
- Shi F, Shi M, Zeng Z, Qi RZ, Liu ZW, et al. PD-1 and PD-L1 upregulation promotes CD8(+) T-cell apoptosis and postoperative recurrence in hepatocellular carcinoma patients. *Int J Cancer* 2011;128:887-96.
- Long J, Qu T, Pan XF, Tang X, Wan HH, et al. Expression of programmed death ligand-1 and programmed death 1 in hepatocellular carcinoma and its clinical significance. *J Cancer Res Ther* 2018;14:S1188-92.
- Ma LJ, Feng FL, Dong LQ, Zhang Z, Duan M, et al. Clinical significance of PD-1/PD-Ls gene amplification and overexpression in patients with hepatocellular carcinoma. *Theranostics* 2018;8:5690-702.
- Jung HI, Jeong D, Ji S, Ahn TS, Bae SH, et al. Overexpression of PD-L1 and PD-L2 is associated with poor prognosis in patients with hepatocellular carcinoma. *Cancer Res Treat* 2017;49:246-54.
- Liu CQ, Xu J, Zhou ZG, Jin LL, Yu XJ, et al. Expression patterns of programmed death ligand 1 correlate with different microenvironments and patient prognosis in hepatocellular carcinoma. *Br J Cancer* 2018;119:80-8.
- Chang H, Jung W, Kim A, Kim HK, Kim WB, et al. Expression and prognostic significance of programmed death protein 1 and programmed death ligand-1, and cytotoxic T lymphocyte-associated molecule-4 in hepatocellular carcinoma. *Apmis* 2017;125:690-8.
- Gabrielson A, Wu Y, Wang H, Jiang J, Kallakury B, et al. Intratumoral CD3 and CD8 T-cell densities associated with relapse-free survival in HCC. *Cancer Immunol Res* 2016;4:419-30.
- Huang CY, Wang Y, Luo GY, Han F, Li YQ, et al. Relationship between PD-L1 expression and CD8+ T-cell immune responses in hepatocellular carcinoma. *J Immunother* 2017;40:323-33.
- Umemoto Y, Okano S, Matsumoto Y, Nakagawara H, Matono R, et al. Prognostic impact of programmed cell death 1 ligand 1 expression in human leukocyte antigen class I-positive hepatocellular carcinoma after curative hepatectomy. *J Gastroenterol* 2015;50:65-75.
- Chang B, Huang T, Wei H, Shen L, Zhu D, et al. The correlation and prognostic value of serum levels of soluble programmed death

- protein 1 (sPD-1) and soluble programmed death-ligand 1 (sPD-L1) in patients with hepatocellular carcinoma. *Cancer Immunol Immunother* 2019;68:353-63.
27. Cheng HY, Kang PJ, Chuang YH, Wang YH, Jan MC, et al. Circulating programmed death-1 as a marker for sustained high hepatitis B viral load and risk of hepatocellular carcinoma. *PLoS One* 2014;9:e95870.
 28. Li N, Zhou Z, Li F, Sang J, Han Q, et al. Circulating soluble programmed death-1 levels may differentiate immune-tolerant phase from other phases and hepatocellular carcinoma from other clinical diseases in chronic hepatitis B virus infection. *Oncotarget* 2017;8:46020-33.
 29. Liang L, Ge K, Zhang F, Ge Y. The suppressive effect of co-inhibiting PD-1 and CTLA-4 expression on H22 hepatomas in mice. *Cell Mol Biol Lett* 2018;23:58.
 30. Song B, Zhen S, Meng F. T cell inflammation profile after surgical resection may predict tumor recurrence in HBV-related hepatocellular carcinoma. *Int Immunopharmacol* 2016;41:35-41.
 31. Liu F, Zeng G, Zhou S, He X, Sun N, et al. Blocking Tim-3 or/and PD-1 reverses dysfunction of tumor-infiltrating lymphocytes in HBV-related hepatocellular carcinoma. *Bull Cancer* 2018;105:493-501.
 32. He G, Zhang H, Zhou J, Wang B, Chen Y, et al. Peritumoural neutrophils negatively regulate adaptive immunity via the PD-L1/PD-1 signalling pathway in hepatocellular carcinoma. *J Exp Clin Cancer Res* 2015;34:141.
 33. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
 34. Finkelmeier F, Czauderna C, Perkhofer L, Ettrich TJ, Trojan J, et al. Feasibility and safety of nivolumab in advanced hepatocellular carcinoma: real-life experience from three German centers. *J Cancer Res Clin Oncol* 2019;145:253-9.
 35. Zhu AX, Finn RS, Edeline J, Cattani S, Ogasawara S, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018;19:940-52.
 36. Marquardt JU, Saborowski A, Czauderna C, Vogel A. The changing landscape of systemic treatment of advanced hepatocellular carcinoma: new targeted agents and immunotherapies. *Target Oncol* 2019;14:115-23.
 37. Qin SK, Ren ZG, Meng ZQ, Chen ZD, Chai XL, et al. A randomized multicentered phase II study to evaluate SHR-1210 (PD-1 antibody) in subjects with advanced hepatocellular carcinoma (HCC) who failed or intolerable to prior systemic treatment. *Ann Oncol* 2018;29:viii719-20.
 38. Llopiz D, Ruiz M, Villanueva L, Iglesias T, Silva L, et al. Enhanced anti-tumor efficacy of checkpoint inhibitors in combination with the histone deacetylase inhibitor Belinostat in a murine hepatocellular carcinoma model. *Cancer Immunol Immunother* 2019;68:379-93.
 39. Li H, Li X, Liu S, Guo L, Zhang B, et al. Programmed cell death-1 (PD-1) checkpoint blockade in combination with a mammalian target of rapamycin inhibitor restrains hepatocellular carcinoma growth induced by hepatoma cell-intrinsic PD-1. *Hepatology* 2017;66:1920-33.
 40. Chen ML, Yan BS, Lu WC, Chen MH, Yu SL, et al. Sorafenib relieves cell-intrinsic and cell-extrinsic inhibitions of effector T cells in tumor microenvironment to augment antitumor immunity. *Int J Cancer* 2014;134:319-31.
 41. Kimura T, Kato Y, Ozawa Y, Kodama K, Ito J, et al. Immunomodulatory activity of lenvatinib contributes to antitumor activity in the Hepa1-6 hepatocellular carcinoma model. *Cancer Sci* 2018;109:3993-4002.
 42. Butty V, Roy M, Sabeti P, Besse W, Benoist C, et al. Signatures of strong population differentiation shape extended haplotypes across the human CD28, CTLA4, and ICOS costimulatory genes. *Proc Natl Acad Sci USA* 2007;104:570-5.
 43. Esensten JH, Helou YA, Chopra G, Weiss A, Bluestone JA. CD28 costimulation: from mechanism to therapy. *Immunity* 2016;44:973-88.
 44. Valk E, Rudd CE, Schneider H. CTLA-4 trafficking and surface expression. *Trends Immunol* 2008;29:272-9.
 45. Yang J, Liu J, Chen Y, Tang W, Liu C, et al. Association of CTLA-4 tagging polymorphisms and haplotypes with hepatocellular carcinoma risk: a case-control study. *Medicine (Baltimore)* 2019;98:e16266.
 46. Wang C, Liu W, Zhao L, Dong Z. Association of cytotoxic T-lymphocyte antigen-4 + 49A/G gene polymorphism with hepatocellular carcinoma risk in Chinese. *J Cancer Res Ther* 2018;14:S1117-20.
 47. Hu L, Liu J, Chen X, Zhang Y, Liu L, et al. CTLA-4 gene polymorphism +49 A/G contributes to genetic susceptibility to two infection-related cancers-hepatocellular carcinoma and cervical cancer. *Hum Immunol* 2010;71:888-91.
 48. Gu X, Qi P, Zhou F, Ji Q, Wang H, et al. +49G > A polymorphism in the cytotoxic T-lymphocyte antigen-4 gene increases susceptibility to hepatitis B-related hepatocellular carcinoma in a male Chinese population. *Hum Immunol* 2010;71:83-7.
 49. Sangro B, Gomez-Martin C, de la Mata M, Inarrairaegui M, Garralda E, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol* 2013;59:81-8.
 50. Duffy AG, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J Hepatol* 2017;66:545-51.
 51. Ribas A, Camacho LH, Lopez-Berestein G, Pavlov D, Bulanhagui CA, et al. Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675,206. *J Clin Oncol* 2005;23:8968-77.
 52. Antonia S, Goldberg SB, Balmanoukian A, Chaft JE, Sanborn RE, et al. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. *Lancet Oncol* 2016;17:299-308.
 53. Xie C, Duffy AG, Mabry-Hrones D, Wood B, Levy E, et al. Tremelimumab in combination with microwave ablation in patients with refractory biliary tract cancer. *Hepatology* 2019;69:2048-60.
 54. Agdashian D, ElGindi M, Xie C, Sandhu M, Pratt D, et al. The effect of anti-CTLA4 treatment on peripheral and intra-tumoral T cells in patients with hepatocellular carcinoma. *Cancer Immunol Immunother* 2019;68:599-608.
 55. Mohty M, Gautier J, Malard F, Aljurf M, Bazarbachi A, et al. CD19 chimeric antigen receptor-T cells in B-cell leukemia and lymphoma: current status and perspectives. *Leukemia* 2019;33:2767-78.

56. Wilkie S, Picco G, Foster J, Davies DM, Julien S, et al. Retargeting of human T cells to tumor-associated MUC1: the evolution of a chimeric antigen receptor. *J Immunol* 2008;180:4901-9.
57. Zhao L, Cao YJ. Engineered T cell therapy for cancer in the clinic. *Front Immunol* 2019;10:2250.
58. Sadelain M, Riviere I, Riddell S. Therapeutic T cell engineering. *Nature* 2017;545:423-31.
59. Gao H, Li K, Tu H, Pan X, Jiang H, et al. Development of T cells redirected to glypican-3 for the treatment of hepatocellular carcinoma. *Clin Cancer Res* 2014;20:6418-28.
60. Chen C, Li K, Jiang H, Song F, Gao H, et al. Development of T cells carrying two complementary chimeric antigen receptors against glypican-3 and asialoglycoprotein receptor 1 for the treatment of hepatocellular carcinoma. *Cancer Immunol Immunother* 2017;66:475-89.
61. Pan Z, Di S, Shi B, Jiang H, Shi Z, et al. Increased antitumor activities of glypican-3-specific chimeric antigen receptor-modified T cells by coexpression of a soluble PD1-CH3 fusion protein. *Cancer Immunol Immunother* 2018;67:1621-34.
62. Zhou F, Shang W, Yu X, Tian J. Glypican-3: a promising biomarker for hepatocellular carcinoma diagnosis and treatment. *Med Res Rev* 2018;38:741-67.
63. Baumhoer D, Tornillo L, Stadlmann S, Roncalli M, Diamantis EK, et al. Glypican 3 expression in human nonneoplastic, preneoplastic, and neoplastic tissues: a tissue microarray analysis of 4,387 tissue samples. *Am J Clin Pathol* 2008;129:899-906.
64. Zhai B, Shi D, Gao H, Qi X, Jiang H, et al. A phase I study of anti-GPC3 chimeric antigen receptor modified T cells (GPC3 CAR-T) in Chinese patients with refractory or relapsed GPC3 + hepatocellular carcinoma (t/r GPC3 + HCC). *J Clin Oncol* 2017;35:3049.
65. He Y, Hong Y, Mizejewski GJ. Engineering α -fetoprotein-based gene vaccines to prevent and treat hepatocellular carcinoma: review and future prospects. *Immunotherapy* 2014;6:725-36.
66. Nakagawa H, Mizukoshi E, Kobayashi E, Tamai T, Hamana H, et al. Association between high-avidity T-cell receptors, induced by α -fetoprotein-derived peptides, and anti-tumor effects in patients with hepatocellular carcinoma. *Gastroenterology* 2017;152:1395-406.e10.
67. Liu H, Xu Y, Xiang J, Long L, Green S, et al. Targeting alpha-fetoprotein (AFP)-MHC complex with CAR T-cell therapy for liver cancer. *Clin Cancer Res* 2017;23:478-88.
68. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan DA, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. *Mol Ther* 2011;19:620-6.
69. Xu Y, Yang Z, Horan LH, Zhang P, Liu L, et al. A novel antibody-TCR (AbTCR) platform combines Fab-based antigen recognition with gamma/delta-TCR signaling to facilitate T-cell cytotoxicity with low cytokine release. *Cell Discov* 2018;4:62.
70. Lin KR, Deng FW, Jin YB, Chen XP, Pan YM, et al. T cell receptor repertoire profiling predicts the prognosis of HBV-associated hepatocellular carcinoma. *Cancer Med* 2018;7:3755-62.
71. Qasim W, Brunetto M, Gehring AJ, Xue SA, Schurich A, et al. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient. *J Hepatol* 2015;62:486-91.
72. Brechot C, Pourcel C, Louise A, Rain B, Tiollais P. Presence of integrated hepatitis B virus DNA sequences in cellular DNA of human hepatocellular carcinoma. *Nature* 1980;286:533-5.
73. Gehring AJ, Xue SA, Ho ZZ, Teoh D, Ruedl C, et al. Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines. *J Hepatol* 2011;55:103-10.
74. Tan AT, Yang N, Lee Krishnamoorthy T, Oei V, Chua A, et al. Use of expression profiles of HBV-DNA integrated into genomes of hepatocellular carcinoma cells to select T cells for immunotherapy. *Gastroenterology* 2019;156:1862-76.e9.
75. Spear TT, Callender GG, Roszkowski JJ, Moxley KM, Simms PE, et al. TCR gene-modified T cells can efficiently treat established hepatitis C-associated hepatocellular carcinoma tumors. *Cancer Immunol Immunother* 2016;65:293-304.
76. Zhu W, Peng Y, Wang L, Hong Y, Jiang X, et al. Identification of alpha-fetoprotein-specific T-cell receptors for hepatocellular carcinoma immunotherapy. *Hepatology* 2018;68:574-89.
77. Dargel C, Bassani-Sternberg M, Hasreiter J, Zani F, Bockmann JH, et al. T cells engineered to express a T-cell receptor specific for glypican-3 to recognize and kill hepatoma cells in vitro and in mice. *Gastroenterology* 2015;149:1042-52.
78. Yin XY, Lu MD, Lai YR, Liang LJ, Huang JF. Prognostic significances of tumor-infiltrating S-100 positive dendritic cells and lymphocytes in patients with hepatocellular carcinoma. *Hepatogastroenterology* 2003;50:1281-4.
79. Cai XY, Gao Q, Qiu SJ, Ye SL, Wu ZQ, et al. Dendritic cell infiltration and prognosis of human hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2006;132:293-301.
80. Ladhams A, Schmidt C, Sing G, Butterworth L, Fielding G, et al. Treatment of non-resectable hepatocellular carcinoma with autologous tumor-pulsed dendritic cells. *J Gastroenterol Hepatol* 2002;17:889-96.
81. Lee WC, Wang HC, Hung CF, Huang PF, Lia CR, et al. Vaccination of advanced hepatocellular carcinoma patients with tumor lysate-pulsed dendritic cells: a clinical trial. *J Immunother* 2005;28:496-504.
82. Palmer DH, Midgley RS, Mirza N, Torr EE, Ahmed F, et al. A phase II study of adoptive immunotherapy using dendritic cells pulsed with tumor lysate in patients with hepatocellular carcinoma. *Hepatology* 2009;49:124-32.
83. El Ansary M, Mogawer S, Elhamid SA, Alwakil S, Aboelkasem F, et al. Immunotherapy by autologous dendritic cell vaccine in patients with advanced HCC. *J Cancer Res Clin Oncol* 2013;139:39-48.
84. Kakumu S, Ito S, Ishikawa T, Mita Y, Tagaya T, et al. Decreased function of peripheral blood dendritic cells in patients with hepatocellular carcinoma with hepatitis B and C virus infection. *J Gastroenterol Hepatol* 2000;15:431-6.
85. Tan XH, Zhu Q, Liu C, Liu XL, Shao XT, et al. Immunization with dendritic cells infected with human AFP adenovirus vector effectively elicits immunity against mouse hepatocellular carcinomas. *Zhonghua Zhong Liu Za Zhi* 2006;28:13-6.

86. Yang JY, Cao DY, Xue Y, Yu ZC, Liu WC. Improvement of dendritic-based vaccine efficacy against hepatitis B virus-related hepatocellular carcinoma by two tumor-associated antigen gene-infected dendritic cells. *Hum Immunol* 2010;71:255-62.
87. Shi M, Qian S, Chen WW, Zhang H, Zhang B, et al. Hepatitis B virus (HBV) antigen-pulsed monocyte-derived dendritic cells from HBV-associated hepatocellular carcinoma patients significantly enhance specific T cell responses in vitro. *Clin Exp Immunol* 2007;147:277-86.
88. Guo DW, Zhang SY, Hou XZ, Li HW, Jiang XF, et al. Glypican3 in genetically modified human monocyte-derived dendritic cells induced specific cytotoxicity against glypican3 overexpressing human hepatocellular carcinoma cells in vitro. *Saudi Med J* 2008;29:1235-40.
89. Tada F, Abe M, Hirooka M, Ikeda Y, Hiasa Y, et al. Phase I/II study of immunotherapy using tumor antigen-pulsed dendritic cells in patients with hepatocellular carcinoma. *Int J Oncol* 2012;41:1601-9.
90. Lee JH, Lee Y, Lee M, Heo MK, Song JS, et al. A phase I/IIa study of adjuvant immunotherapy with tumour antigen-pulsed dendritic cells in patients with hepatocellular carcinoma. *Br J Cancer* 2015;113:1666-76.
91. Ge C, Xing Y, Wang Q, Xiao W, Lu Y, et al. Improved efficacy of therapeutic vaccination with dendritic cells pulsed with tumor cell lysate against hepatocellular carcinoma by introduction of 2 tandem repeats of microbial HSP70 peptide epitope 407-426 and OK-432. *Int Immunopharmacol* 2011;11:2200-7.
92. Nakamoto Y, Mizukoshi E, Kitahara M, Arihara F, Sakai Y, et al. Prolonged recurrence-free survival following OK432-stimulated dendritic cell transfer into hepatocellular carcinoma during transarterial embolization. *Clin Exp Immunol* 2011;163:165-77.
93. Maeda Y, Yoshimura K, Matsui H, Shindo Y, Tamesa T, et al. Dendritic cells transfected with heat-shock protein 70 messenger RNA for patients with hepatitis C virus-related hepatocellular carcinoma: a phase I dose escalation clinical trial. *Cancer Immunol Immunother* 2015;64:1047-56.
94. Chen C, Ma YH, Zhang YT, Zhang F, Zhou N, et al. Effect of dendritic cell-based immunotherapy on hepatocellular carcinoma: a systematic review and meta-analysis. *Cytotherapy* 2018;20:975-89.
95. Ninomiya T, Akbar SM, Masumoto T, Horiike N, Onji M. Dendritic cells with immature phenotype and defective function in the peripheral blood from patients with hepatocellular carcinoma. *J Hepatol* 1999;31:323-31.
96. Chen S, Akbar SM, Tanimoto K, Ninomiya T, Iuchi H, et al. Absence of CD83-positive mature and activated dendritic cells at cancer nodules from patients with hepatocellular carcinoma: relevance to hepatocarcinogenesis. *Cancer Lett* 2000;148:49-57.
97. Iwashita Y, Goto S, Tahara K, Kawano K, Chen CL, et al. Potent stimuli combined with lipopolysaccharide and IFN γ may improve immunotherapy against HCC by increasing the maturation and subsequent immune response of the dendritic cells. *Cancer Biother Radiopharm* 2003;18:1-6.
98. Gonzalez-Carmona MA, Lukacs-Kornek V, Timmerman A, Shabani S, Kornek M, et al. CD40ligand-expressing dendritic cells induce regression of hepatocellular carcinoma by activating innate and acquired immunity in vivo. *Hepatology* 2008;48:157-68.
99. Chan RC, Pang XW, Wang YD, Chen WF, Xie Y. Transduction of dendritic cells with recombinant adenovirus encoding HCA661 activates autologous cytotoxic T lymphocytes to target hepatoma cells. *Br J Cancer* 2004;90:1636-43.
100. Peng W, Zhao G, Ma Y, Yu H, Wang X. Dendritic cells transfected with PEG10 recombinant adenovirus elicit anti-tumor immune response in vitro and in vivo. *Vaccine* 2011;29:3501-6.
101. Vogt A, Sievers E, Lukacs-Kornek V, Decker G, Raskopf E, et al. Improving immunotherapy of hepatocellular carcinoma (HCC) using dendritic cells (DC) engineered to express IL-12 in vivo. *Liver Int* 2014;34:447-61.
102. Choi YJ, Park SJ, Park YS, Park HS, Yang KM, et al. EpCAM peptide-primed dendritic cell vaccination confers significant anti-tumor immunity in hepatocellular carcinoma cells. *PLoS One* 2018;13:e0190638.
103. Yang X, Zhu H, Hu Z. Dendritic cells transduced with TEM8 recombinant adenovirus prevents hepatocellular carcinoma angiogenesis and inhibits cells growth. *Vaccine* 2010;28:7130-5.
104. Su H, Li B, Zheng L, Wang H, Zhang L. Immunotherapy based on dendritic cells pulsed with CTPFoxM1 fusion protein protects against the development of hepatocellular carcinoma. *Oncotarget* 2016;7:48401-11.
105. Zerbini A, Pilli M, Fagnoni F, Pelosi G, Pizzi MG, et al. Increased immunostimulatory activity conferred to antigen-presenting cells by exposure to antigen extract from hepatocellular carcinoma after radiofrequency thermal ablation. *J Immunother* 2008;31:271-82.
106. Hu Z, Chen J, Zhou S, Yang N, Duan S, et al. Mouse IP-10 gene delivered by folate-modified chitosan nanoparticles and dendritic/tumor cells fusion vaccine effectively inhibit the growth of hepatocellular carcinoma in mice. *Theranostics* 2017;7:1942-52.
107. Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, et al. Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. *Gastroenterology* 2015;148:1383-91.e6.
108. Jung NC, Lee JH, Choi HJ, Hwang SU, Song JY, et al. Dendritic cell immunotherapy combined with cytokine-induced killer cells effectively suppresses established hepatocellular carcinomas in mice. *Immunol Invest* 2016;45:553-65.
109. Chen BA, Li M, Sun ZY, Li CP, Gao C, et al. Killing activity in DC and CIK co-culture against hepatocarcinoma cells. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2006;14:543-6.
110. Qiu Y, Xu MB, Yun MM, Wang YZ, Zhang RM, et al. Hepatocellular carcinoma-specific immunotherapy with synthesized α 1,3-galactosyl epitope-pulsed dendritic cells and cytokine-induced killer cells. *World J Gastroenterol* 2011;17:5260-6.
111. Niu LZ, Li JL, Zeng JY, Mu F, Liao MT, et al. Combination treatment with comprehensive cryoablation and immunotherapy in metastatic hepatocellular cancer. *World J Gastroenterol* 2013;19:3473-80.
112. Yoon JS, Song BG, Lee JH, Lee HY, Kim SW, et al. Adjuvant cytokine-induced killer cell immunotherapy for hepatocellular carcinoma: a propensity score-matched analysis of real-world data. *BMC Cancer* 2019;19:523.
113. Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, et al. Sustained efficacy of adjuvant immunotherapy with cytokine-induced killer cells for hepatocellular carcinoma: an extended 5-year follow-up. *Cancer Immunol Immunother* 2019;68:23-32.
114. Guo W, Liu L, Wu D. Dendritic cell-cytokine induced killer cell immunotherapy combined with transcatheter arterial chemoembolization

- for hepatocellular carcinoma: safety and efficacy. *Nan Fang Yi Ke Da Xue Xue Bao* 2014;34:674-8.
115. He G, Zheng C, Huo H, Zhang H, Zhu Z, et al. TACE combined with dendritic cells and cytokine-induced killer cells in the treatment of hepatocellular carcinoma: a meta-analysis. *Int Immunopharmacol* 2016;40:436-42.
116. Zhou Z, Qin H, Weng L, Ni Y. Clinical efficacy of DC-CIK combined with sorafenib in the treatment of advanced hepatocellular carcinoma. *J Buon* 2019;24:615-21.
117. Gonzalez-Carmona MA, Marten A, Hoffmann P, Schneider C, Sievers E, et al. Patient-derived dendritic cells transduced with an a-fetoprotein-encoding adenovirus and co-cultured with autologous cytokine-induced lymphocytes induce a specific and strong immune response against hepatocellular carcinoma cells. *Liver Int* 2006;26:369-79.
118. Yu X, Xia W, Zhang T, Wang H, Xie Y, et al. Enhanced cytotoxicity of IL-24 gene-modified dendritic cells co-cultured with cytokine-induced killer cells to hepatocellular carcinoma cells. *Int J Hematol* 2010;92:276-82.
119. Wang Y, Wang Y, Mu H, Liu T, Chen X, et al. Enhanced specific antitumor immunity of dendritic cells transduced with the glypican 3 gene and co-cultured with cytokine-induced killer cells against hepatocellular carcinoma cells. *Mol Med Rep* 2015;11:3361-7.

Original Article

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β -catenin in intranuclear inclusions of hepatocellular carcinoma

Suzan Schwertheim¹, Holger Jastrow², Julia Kälsch^{1,3}, Thomas Herold¹, Sarah Theurer¹, Saskia Ting¹, Kurt Werner Schmid^{1,4}, Hideo Andreas Baba¹

¹Institute of Pathology, University Hospital of Essen, University of Duisburg-Essen, Essen 45147, Germany.

²Institute of Anatomy and Electron Microscopy Unit of Imaging Center Essen, University Hospital of Essen, University of Duisburg-Essen, Essen 45147, Germany.

³Department of Gastroenterology and Hepatology, University Hospital of Essen, University of Duisburg-Essen, Essen 45147, Germany.

⁴Member of the West German Cancer Centre Essen (WTZE), University Hospital of Essen, University of Duisburg-Essen, Essen 45147, Germany.

Correspondence to: Dr. Hideo Andreas Baba, Institute of Pathology, University Hospital of Essen, University of Duisburg-Essen, Hufelandstr 55, Essen 45147, Germany. E-mail: hideo.baba@uk-essen.de

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Abstract

Aim: β -catenin activation is known to promote liver regeneration and play a role in the pathogenesis of liver cancer. Recently, we detected intranuclear inclusions (NI) in hepatocellular carcinoma (HCC) containing degenerated cell organelles and lysosomal proteins and delimited by a completely closed nuclear membrane. The presence of NI was positively associated with patient survival. The aim of the current study was to investigate a possible association between proteins of the Wnt/ β -catenin pathway with NI morphology and survival.

Methods: We examined NI in 72 paraffin-embedded specimens of HCC. Immunohistochemistry (IHC) and immunofluorescence (IF) were performed to investigate the content and shape of NI. β -catenin gene (*CTNNB1*) mutations were analyzed by next generation sequencing.

Results: We detected the accumulation of β -catenin and glutamine synthetase (a target gene of β -catenin) proteins within NI. Further, we found immunopositivity for the lysine demethylase KDM2A in NI. KDM2A is known to be involved in β -catenin degradation. We detected significant associations between the presence of β -catenin and autophagy-associated proteins in NI. Double-IF revealed co-localization of β -catenin and p62 in the same NI. Kaplan-Meier survival analysis showed that the presence of NI containing KDM2A protein accumulations displayed a significant benefit in overall survival.



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Conclusion: We detected accumulations of β -catenin and proteins associated with the Wnt/ β -catenin pathway partly together with autophagy-associated proteins in the same inclusion. Our finding that KDM2A immunopositivity within NIs was associated with favorable clinical outcomes and suggests a biological significance of NI.

Keywords: Wnt/ β -catenin pathway, KDM2A, intranuclear inclusions, hepatocellular carcinoma

INTRODUCTION

The Wnt/ β -catenin signaling pathway is regarded as playing the most important role in hepatocellular regeneration^[1-4]. Spee *et al.*^[4] reported that the Wnt/ β -catenin signaling pathway is active in the ductular reaction of acute liver failure patients^[4,5]. Apte *et al.*^[1] demonstrated a significant correlation between nuclear/cytoplasmic- β -catenin staining and hepatocellular regeneration. Recently, we published a detailed study on intranuclear inclusions (NI) in hepatocellular carcinoma (HCC). In general, NI are considered to be a morphological feature, without any notable function or influence on disease. On the contrary, our earlier studies revealed that these NI are surrounded by a completely closed nuclear membrane and contain degenerated organelles, autophagy-associated proteins and lysosomes, suggesting a biological function of NI. The presence of NI was also significantly correlated with survival in HCC^[6]. However, the factors that play a role in the occurrence of NI are mostly still unknown. Thus, we questioned whether proteins of the Wnt/ β -catenin pathway are involved in the functioning of NI.

We performed immunohistochemical analyses to investigate the presence of β -catenin, glutamine synthetase and KDM2A in NI. Glutamine synthetase, an enzyme involved in the removal of ammonia in the liver^[7], nitrogen balance and pH regulation^[8] is also a target of the Wnt/ β -catenin pathway in the liver^[9]. Long *et al.*^[8] showed increased levels of expression of glutamine synthetase in HCC and in liver tissue with cirrhosis and chronic hepatitis B. KDM2A (FBXL11) is known to demethylate histone H3K36^[10], which contains an F-box, a JmjC domain, a CxxC zinc finger, a PHD domain, and three leucine-rich repeat elements^[10-13]. KDM2A is involved in various signaling pathways including NF- κ B signaling, p53 activity and WNT/ β -catenin pathway; demethylation of β -catenin with consequent degradation has been reported^[14]. Thus, to investigate a possible correlation of KDM2A expression levels on survival in HCC patients, we performed Kaplan-Meier survival studies. Further, we examined if positive immunoreactivity for β -catenin within NI is associated simultaneously with the presence of the autophagy-associated proteins p62/sequestosome1, ubiquitin, LC3B, cathepsin B and cathepsin D in NI.

The goal of the current study was to investigate whether proteins of the Wnt/ β -catenin pathway are associated with NI morphology and disease survival.

METHODS

Patients

Seventy two patients with HCC diagnosed at the Institute of Pathology, University Hospital of Essen, Germany between 1999 and 2005 were included in this study. Formalin-fixed and paraffin-embedded (FFPE) material from untreated patients were provided in all cases, prepared according to institutional standards and stained with H&E as described before^[6]. The current WHO criteria^[15] were used for the diagnosis of all tumors and the classification of tumors was based on the TNM system (8th edition). Table 1 provides an overview of patient data and tumor characteristics. In 70 HCC cases, complete clinical records and follow-up data were available.

Informed consent was obtained from each patient. The study complied with the Helsinki Declaration of 1975 and the Ethics Committee (Institutional Review Board) of the University Hospital Essen (reference number: 16-6917-BO).

Table 1. Clinical and pathological parameters of the study group with 72 HCC cases

	All (n = 72)
Mean age (years) at diagnosis (range)	62 (17-99)
Gender (Male/Female)	55/17
Liver morphology	
Non-cirrhotic	44
Cirrhotic	22
Fibrotic	6
Background	
Underlying disease unknown	43
Alcohol abuse	2
Hepatitis B	12
Hepatitis C	14
Hepatitis B + C	0
Alpha-1-antitrypsin deficiency	1
Primary biliary cirrhosis	0
Autoimmunhepatitis	0
Tumor staging	
pT1a/b	35
pT2	24
pT3	8
pT4	5
Grading	
G1	9
G2	40
G3/G4	23
Nodal status	
pN0	68
pN1	4
Lymph vessel infiltration	
L0	72
Blood vessel infiltration	
V0	41
V1	31
Resection status	
R0	61
R1	10
R2	1
Observation period (in days) post surgery	
Minimum	55
Maximum	3009

HCC: Hepatocellular carcinoma

Tissue microarray construction and immunohistochemistry

We investigated the expression of selected candidate proteins immunohistochemically using tissue microarrays (TMAs). Regions of tumors were selected with matching H&E stained slides and marked on the donor block. Construction of the TMAs was performed by using a manual tissue-array instrument (Beecher Instruments, Silver Spring, MD, USA) as described before^[6]. Briefly, we took three 1-mm-thick tissue cores from each specimen. Each TMA contained three corresponding tumor-free liver tissue cores as controls and cores with myocardial tissue for TMA orientation and 10 sections of about 3 µm each were cut from each TMA. Immunohistochemistry (IHC) of the paraffin sections for the antibodies β-catenin, KDM2A, glutamine synthetase, ubiquitin, p62, LC3B, cathepsin B and cathepsin D was conducted as described before^[6] by using an automated staining device (Dako Autostainer, Dako, Glostrup, Denmark). Further, we stained one section from each TMA with H&E; [Supplementary Table 1](#) provides detailed information on the antibodies used and staining protocols. Additionally, we included negative controls in every run: slides were

incubated with non-immune immunoglobulin at the same concentration as the primary antibody instead of the primary antibody as described before^[6]. We counted the membrane-bound intranuclear inclusions (NI) in all 10 serial sections of each case; cases lacking evaluable material on all 10 serial sections were excluded as described before^[6]. We defined an inclusion only as positive if it was delimited by an intact membrane and completely closed. In a standardized area, inclusions were counted and calculated by: $10 \times (3 \times 1 \text{ mm tissue cores}) = 10 \times (3 \times 0.78 \text{ mm}^2) = 23.5 \text{ mm}^2$. The qualitative detection of membrane-bound nuclear inclusions was recorded as positive (1) or negative (0). We defined cases containing at least one membrane-bound nuclear inclusion as positive; haematoxylin counterstaining enabled the enumeration of the inclusions. We analyzed the immunostainings within NI. Quantitative analysis of immunopositive NI was performed for β -catenin, KDM2A, ubiquitin, p62, LC3B, cathepsin B and cathepsin D. Due to the small size of the NI and staining surface, we did not quantify immunostaining intensity. Cases with positively stained membrane-bound nuclear inclusions were classified as positive (1) or negative (0).

Next generation sequencing

FFPE tumor tissue of all cases was analyzed by next generation sequencing (NGS) with the Illumina MiSeq sequencer (Illumina, San Diego, CA, USA) following the manufacturer's instructions as described before^[16]. Briefly, 45 ng of DNA was used to perform multiplex-PCR and Biomedical Genomics Workbench (CLC Bio, Qiagen, USA) was used for analysis. We designed a customized HCC-panel containing regions of interest: 23 genes of the Wnt pathway including the *CTNNB1* gene.

Transmission electron microscopy

Ultrastructural analysis of NI was carried out as described previously^[6,16]. Briefly, for transmission electron microscopy (TEM), fresh tissue from liver biopsy of a representative HCC patient was fixed in 2% glutaraldehyde in 0.1M cacodylate buffer (cb), pH 7.3, for 4 h at room temperature. Afterwards it was washed in cb, post-fixed with 1% osmium tetroxide in cb, dehydrated in a graded series of alcohol and embedded in epoxy resin. In order to determine blocks of adequate quality, semi-thin sections were stained with basic fuchsin and methylene blue. Ultrathin sections of selected blocks were mounted on copper grids, and then treated with uranyl acetate (1%) and lead citrate (0.4%) for contrast enhancement. Digital TEM images were acquired on the Zeiss EM 902A (Zeiss, Oberkochen, Germany) equipped with a Morada slow-scan CCD camera using ITEM 5.2 software (both Olympus Soft-Imaging-Systems, Münster, Germany).

Double immunofluorescence of tissue sections

Spatial localization of β -catenin and p62 was investigated by double immunofluorescence staining. One- μm -thick FFPE tissue sections (HCC) were cut, dewaxed, rehydrated and pretreated with Target Retrieval Solution (Agilent Technologies, Ratingen, Germany) at pH 9.0 for 20 min at 97 °C. For double labeling immunofluorescence, the primary antibodies anti- β -catenin (Transduction) and anti-p62 (Enzo) were used. Anti-p62 antibody was labeled with Donkey Anti Rabbit AF555. For labeling of anti- β -catenin antibody Goat Anti Mouse HRP and Goat Anti HRP AF488 were used. Details are provided in [Supplementary Table 2](#). DNA was stained with DAPI; image analysis and microscopy was performed by using an Olympus BX43 (Olympus Deutschland, Hamburg, Germany).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 24.0, Chicago, IL, USA) program. Relationships between categorical parameters were investigated using the two-sided Fisher's exact test. Overall survival (OS) curves were performed using the Kaplan-Meier method, and differences in survival curves were compared by the log-rank test. Mann-Whitney *U*-test was used to assess whether positive KDM2A immunostaining in NI correlates with the number of NI; $P \leq 0.05$ was defined as statistically significant.

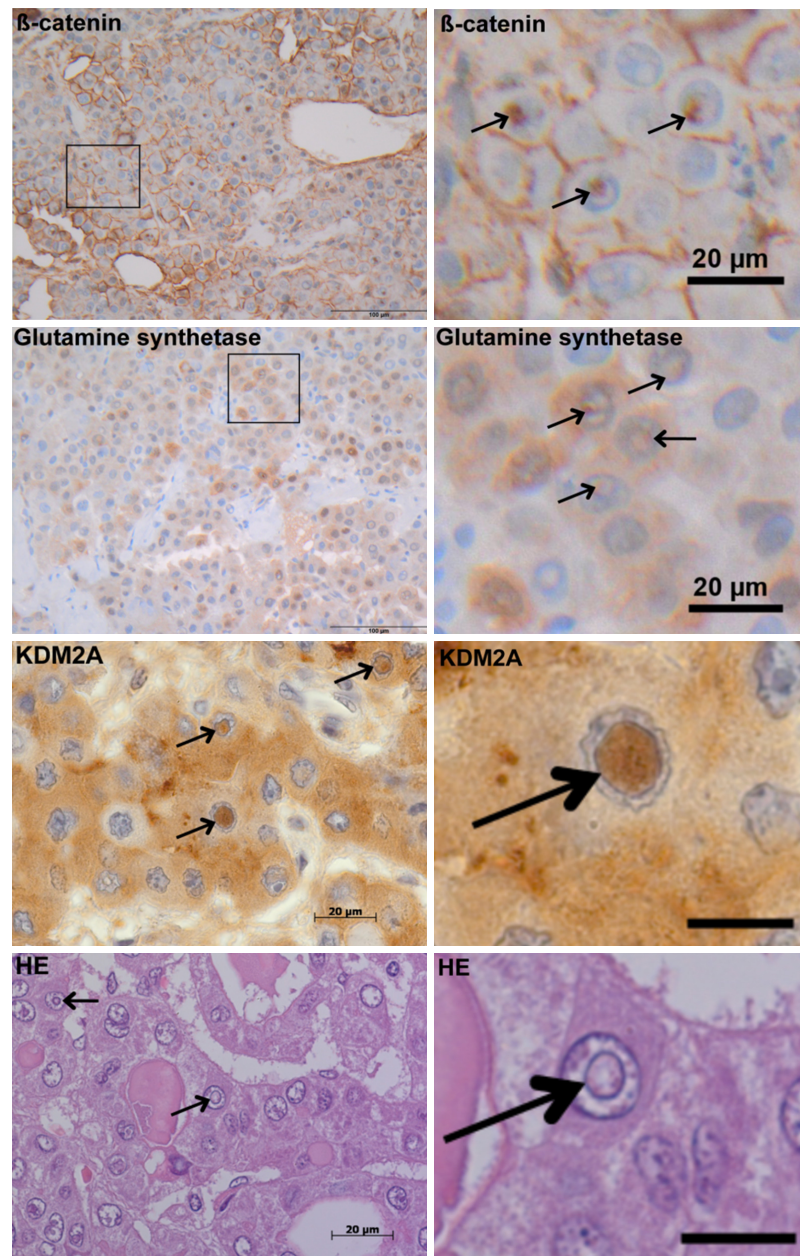


Figure 1. Intranuclear inclusions in hepatocellular carcinoma. Positive immunoreactivity for β -catenin, glutamine synthetase and KDM2A in intranuclear inclusions (NI). The images depict NI (arrows) containing accumulations of β -catenin, glutamine synthetase and KDM2A. For β -catenin and glutamine synthetase IHC increased magnification of the marked area shows positive immunostainings within NI. Further, HE-staining (bottom-left image) reveals that NI (arrows) are completely closed with no contact to the cytoplasm on this plane. Additionally increased magnification of the intranuclear inclusion in the center of the HE and KDM2A-images reveal the bordering membrane (right images; arrow). The black bars at the right (third and fourth row) equal 10 μ m. First and second row images are original magnifications: 400 \times ; third and fourth row images are original magnifications: 1,000 \times

RESULTS

Accumulation of β -catenin and proteins associated with Wnt/ β -catenin signalling in NI

Our IHC analysis of NI in HCC showed positive immunoreactivity for β -catenin within NI and in the surrounding cytoplasm [Figure 1]. Further, we observed an accumulation of glutamine synthetase, a target gene of β -catenin in NI. Additionally, we detected immunopositivity for the lysine demethylase KDM2A, which is involved in β -catenin degradation. We performed serial tissue sections for β -catenin, glutamine synthetase and KDM2A IHC staining [Supplementary Figure 1]. The number of cases containing at least one

Table 2. NGS study: *CTNNB1* mutations in the HCC cohort¹

Gene	AA mutation Cosmic_v70	n cases (%)
<i>CTNNB1</i> ²	D32G	1/72 (1.4%)
<i>CTNNB1</i> ²	D32N	1/72 (1.4%)
<i>CTNNB1</i> ²	D32V	0/72 (0%)
<i>CTNNB1</i> ²	D32Y	3/72 (4.2%)
<i>CTNNB1</i>	S33A	1/72 (1.4%)
<i>CTNNB1</i> ²	S33C	3/72 (4.2%)
<i>CTNNB1</i> ²	S33F	2/72 (2.8%)
<i>CTNNB1</i> ²	G34E	1/72 (1.4%)
<i>CTNNB1</i>	G34R	1/72 (1.4%)
<i>CTNNB1</i> ²	G34V	1/72 (1.4%)
<i>CTNNB1</i>	I35S	1/72 (1.4%)
<i>CTNNB1</i>	H36P	1/72 (1.4%)
<i>CTNNB1</i>	S37A	1/72 (1.4%)
<i>CTNNB1</i> ²	S37Y	1/72 (1.4%)
<i>CTNNB1</i> ²	T41A	1/72 (1.4%)
<i>CTNNB1</i> ²	T41I	2/72 (2.8%)
<i>CTNNB1</i> ²	S45P	2/72 (2.8%)
<i>CTNNB1</i> ²	S45Y	1/72 (1.4%)
<i>CTNNB1</i>	K335T	1/72 (1.4%)
<i>CTNNB1</i>	N387K	2/72 (2.8%)

¹All mutations found in COSMIC with the prevalence of minimum 5% are listed; ²mutations listed additionally in the ClinVar database

intranuclear inclusion with positive immunoreactivity was analyzed for β -catenin and KDM2A. We detected in 19 of 72 (26.4%) HCCs at least one membrane-bounded intranuclear inclusion with positive β -catenin immunostaining; for KDM2A 19 of 71 (26.8%) valid HCC cases showed at least one membrane-bounded intranuclear inclusion with positive immunostaining. β -catenin immunohistochemistry was available for all cases (72/72) while for KDM2A, suitable material for immunohistochemistry was available in 71 of 72 cases.

NGS study of *CTNNB1* mutations

To clarify the reason for the high number of NI containing β -catenin, we investigated HCCs with possible mutations of the β -catenin gene *CTNNB1* by NGS. We found up to twenty different *CTNNB1* mutations in our cases. We have listed all mutations found in COSMIC with a prevalence of at least 5%. The mutations that are also recorded in the ClinVar database are marked [Table 2]. Chi-square cross table analysis of the HCC cases demonstrated that a positive *CTNNB1* mutation status was significantly associated with the occurrence of positive nuclear β -catenin immunostaining ($P \leq 0.001$). Additionally, we performed cross table analysis to examine a possible association between the occurrence of NI and the presence of *CTNNB1* mutations; no significant association was found.

TEM analysis of NI

We performed ultrastructural analysis in order to investigate the content and shape of NI in more detail [Figure 2]. Our TEM studies showed that NI in HCC in most cases was completely enclosed by the nuclear membrane. NI contained degenerated cell material, lysosomes and heterolysosomes. In general, the content of the inclusions had a higher electron density than the cytoplasm, suggesting that NI are not simply passive invaginations of the cytoplasm into the nucleus [Figure 2].

Immunohistochemical analysis of NI

Association of β -catenin with autophagy-associated proteins in NI

We analysed NI by immunohistochemistry to clarify whether there was an association between the occurrence of intranuclear inclusions with β -catenin immunopositivity and autophagy [Figure 3]. Recently, we have examined the autophagy-associated proteins p62, ubiquitin, LC3B, cathepsin B and cathepsin D

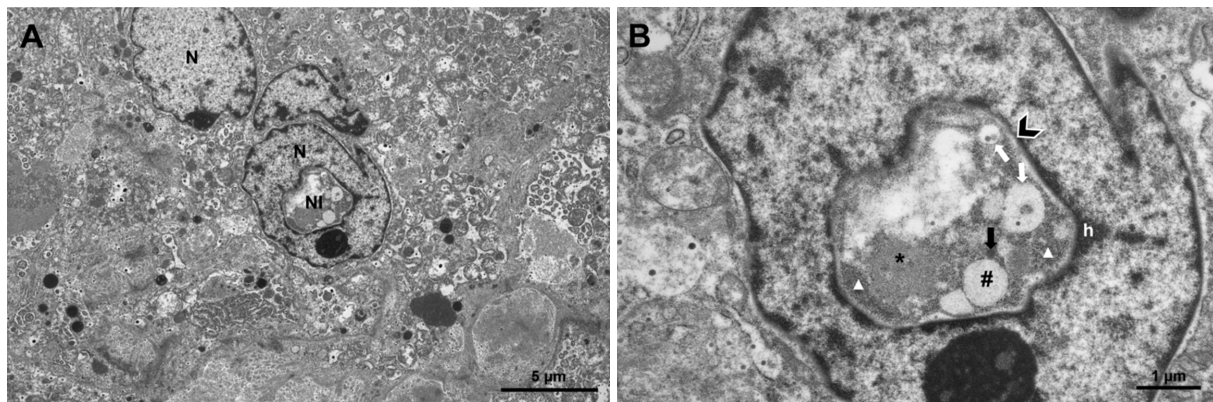


Figure 2. Ultrastructural analysis of intranuclear inclusions (NI) in hepatocellular carcinoma. A: The image depicts two nuclei, one of which contains a large NI, which we analyzed in more detail at higher magnification; B: NI is bordered by the two (inner and outer) nuclear membranes (arrowhead) with attached heterochromatin (h); degraded cellular material resembling fine filamentous material (asterisk), a small vesicle with homogenous content of high electron-density, most probably a lysosome (black arrow) and heterolysosomes (white arrows) are seen. In addition, the NI shows fine granular, ribosome-like material (triangles) and a larger round area with no clearly detectable membrane (#) and hardly electron-dense content, which might be a lipid droplet

in HCC demonstrating positive immunoreactivity for all investigated proteins in NI^[6]. In the current study [Figure 3], we used chi-square cross table analysis to investigate if positive β -catenin immunostaining in NI is associated with immunoreactivity for the autophagy-associated proteins p62, ubiquitin, LC3B, cathepsin B and cathepsin D. We defined cases as positive (1) if at least one membrane-bound inclusion showed positive immunostaining, whereas cases lacking stained inclusions were classified as negative (0) according to the same system as described before^[6]. Analysis of 19 cases with positive β -catenin immunostaining revealed that 15 also had nuclear inclusions with positive ubiquitin immunostaining ($P < 0.001$), 16 showed p62 and 17 had cathepsin B immunoreactivity ($P \leq 0.001$). We also revealed a significant relationship for LC3B ($P = 0.041$) and cathepsin D ($P = 0.005$); details are listed in Table 3.

Correlation of β -catenin immunopositivity within NI with β -catenin immunopositivity in the surrounding cytoplasm

Further chi-square cross table analysis was used to investigate the relationships between β -catenin immunoreactivity within NI and in the surrounding cytoplasm. We found that of the 19 cases that contained positive β -catenin immunostaining within NI, 11 also showed β -catenin immunopositivity in the surrounding cytoplasm ($P = 0.002$; Table 3).

Spatial co-localization of β -catenin with p62 in nuclear inclusions

To analyse if these autophagy-associated proteins are located within the same inclusion, we performed double immunofluorescence labeling. Figure 4 shows a DAPI-stained nucleus with NI. Co-localization of β -catenin with p62 in the same inclusion is proven by the formation of the merged color yellow [Figure 4, arrows].

The occurrence of NI with KDM2A immunopositivity correlates with recurrence-free and overall survival

We performed Kaplan-Meier survival curves to check if disease specific overall survival (OS) and recurrence-free survival depends on the accumulation of KDM2A protein within the inclusions.

We analyzed 10 serial sections from each TMA. Data on OS were available for 69 valid HCC cases. 19 of these 69 patients had at least one membrane-bound intranuclear inclusion showing immunoreactivity for KDM2A. We observed that most of them (16/19, 84%) survived whereas 26 (52%) of 50 patients who contained no KDM2A protein within NI died during the observation period. Kaplan-Meier survival

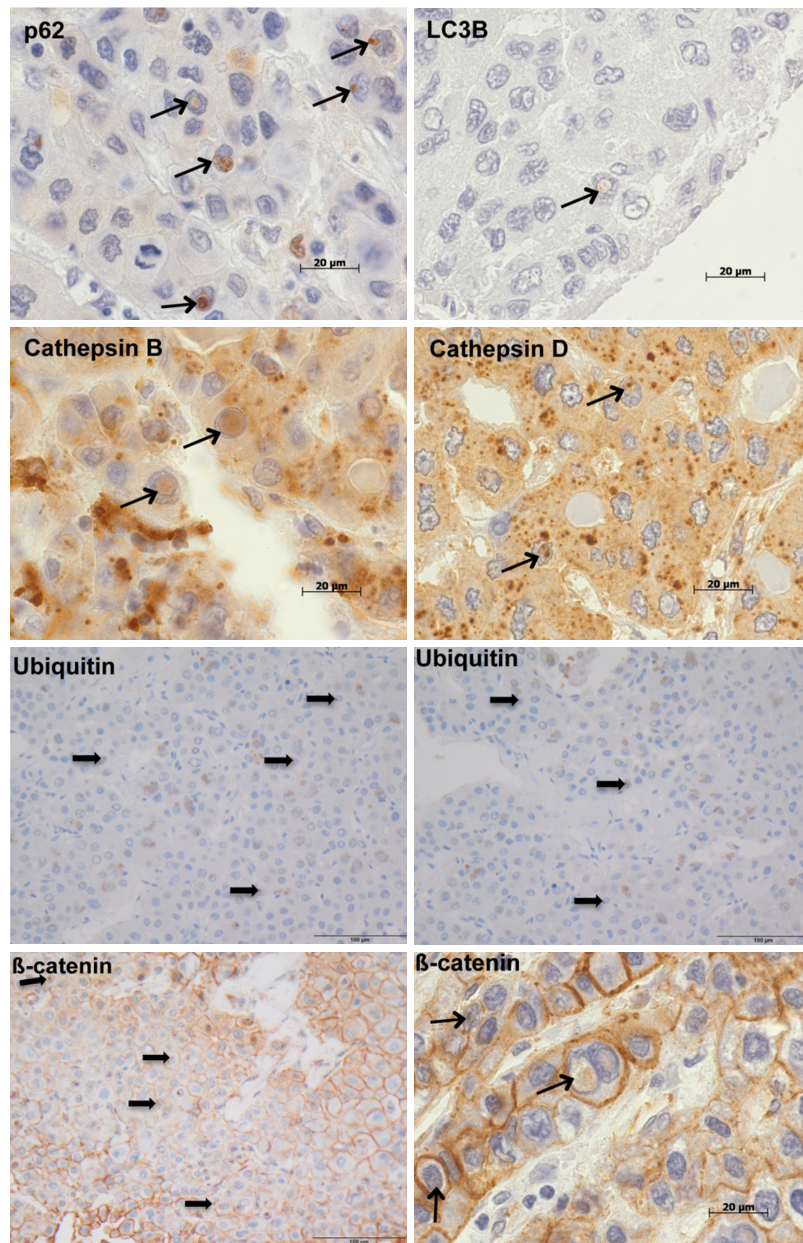


Figure 3. Immunohistochemical analysis of β -catenin and autophagy-associated proteins in hepatocellular carcinoma. The images depict intranuclear inclusions (arrows) containing positive immunoreactivity for β -catenin, p62, LC3B, ubiquitin, cathepsin B and cathepsin D. Original magnifications for ubiquitin and β -catenin (left image bottom) images: 400 \times and for p62, LC3B, ubiquitin, cathepsin B, cathepsin D, β -catenin (right image bottom) images: 1,000 \times

analysis depicted that patients with NI containing KDM2A protein accumulations displayed a lower risk for death compared to patients with lower number of KDM2A immunopositive NI ($P = 0.014$; [Figure 5A](#)). We also found that patients with KDM2A immunopositivity in the cytoplasm show a significant survival benefit compared to patients with KDM2A negative cytoplasm ($P = 0.009$; [Figure 5B](#)). Briefly, 42 of 69 patients showed immunopositive KDM2A cytoplasm and 30 of 42 (71.4%) survived compared to 17 of 27 patients with no immunoreactivity for KDM2A in the cytoplasm who died. Further, we examined recurrence-free survival in dependence on KDM2A immunoreactivity in the cytoplasm. Data on recurrence-free survival was available for 47 valid HCC cases. We found that significantly more patients with KDM2A immunopositive cytoplasm showed recurrence-free survival than patients with a lack of KDM2A in the

Table 3. Correlation of β -catenin immunopositivity within NI with autophagy associated proteins in NI and with cytoplasmic β -catenin

Cross Tabs		Tumor tissue	P value
Antibody		n	
β -catenin	p62	16/72	0.001
	LC3B	7/72	0.041
	Ubiquitin	15/71	< 0.001
	Cathepsin B	17/72	0.001
	Cathepsin D	13/72	0.005
	Cytoplasmic β -catenin	11/72	0.002

P values were calculated using two-sided Fisher's exact test. n: Number of IHC positive intranuclear inclusions/valid cases; NI: intranuclear inclusions

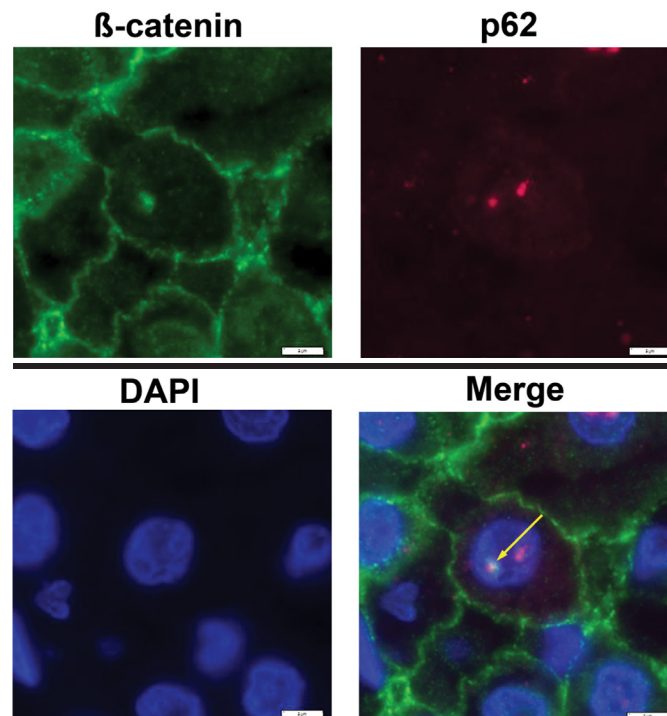


Figure 4. Double-IF studies demonstrate co-localization of β -catenin with p62 in intranuclear inclusions (NI) of hepatocellular carcinoma sections. Nuclei were stained with DAPI (blue) to detect NI. The merged images show superimposition of the signals for β -catenin (green staining) with p62 (red staining) in the same inclusion; the merged color yellow (arrow) proves co-localizations

cytoplasm ($P = 0.009$; [Figure 5C](#)); briefly 27 (84.4%) of 32 KDM2A immunopositive patients showed no local recurrence compared to 7 (46.7%) of 15 patients with lack of KDM2A in the cytoplasm and developed recurrence. Additionally, we studied the adjacent normal tissue sections (NTS) on all serial sections of the HCC patient cohort by Kaplan-Meier survival curves. We also detected in the adjacent normal tissue that the occurrence of KDM2A in the cytoplasm was associated with a significant benefit in recurrence-free survival ($P = 0.027$; [Figure 5D](#)).

Increased occurrence of NI in HCC cases with KDM2A immunopositivity

Mann-Whitney *U* Test analysis showed a significant positive correlation between the number of NI and positive KDM2A immunostaining in HCC ($P \leq 0.001$; [Figure 6](#)). Our results revealed that cases with at least one KDM2A immunopositive inclusion had, at the same time, a significantly higher number of NI than HCCs lacking KDM2A immunoreactivity within NI.

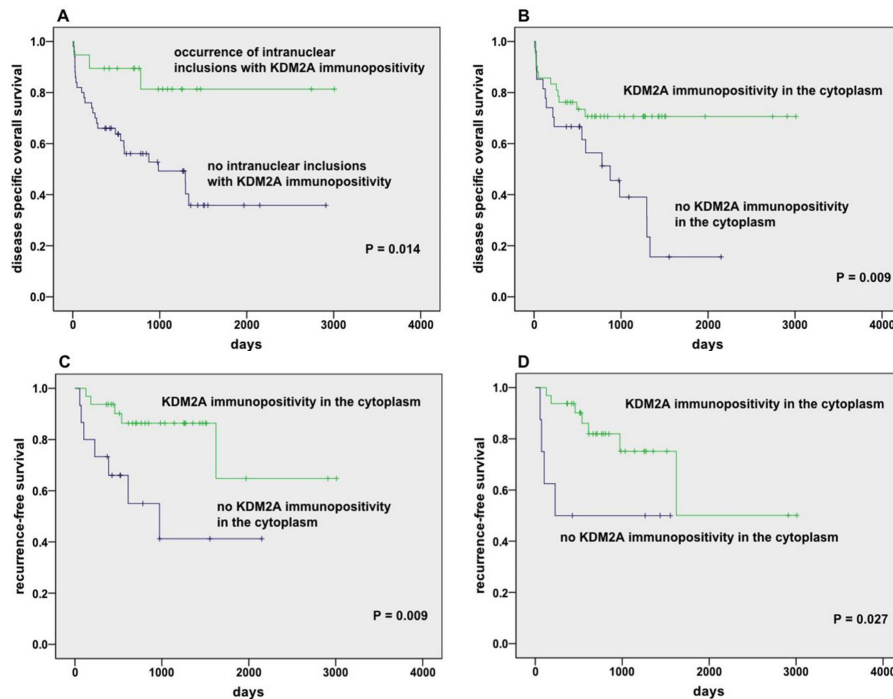


Figure 5. Kaplan-Meier survival studies. Disease specific OS was analyzed in 69 valid hepatocellular carcinoma (HCC) samples in relation to the presence of KDM2A immunopositivity in intranuclear inclusions (A) and in the cytoplasm (B). Additionally, recurrence-free survival was examined in 47 valid HCC samples in relation to the presence of KDM2A immunopositivity in the cytoplasm of tumor (A) and in adjacent normal tissue (B). We counted inclusions in a standardized area of 23.5 mm² (ten serial sections of each case). Only cases with evaluable material on all ten serial sections were included. Cases containing at least one membrane-bound nuclear inclusion were considered positive; P M 0.05 was defined as statistically significant

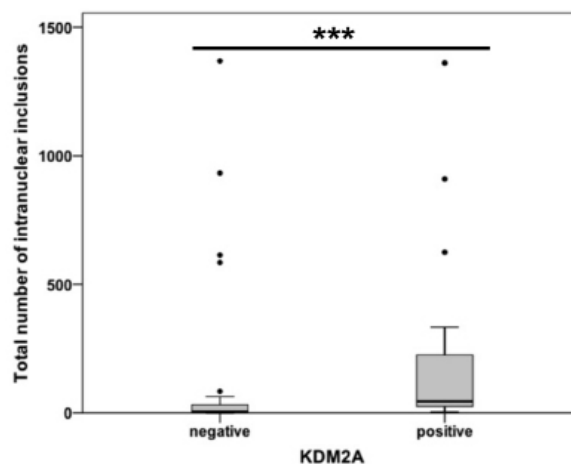


Figure 6. Association between KDM2A immunopositivity and intranuclear inclusions (NI) in hepatocellular carcinoma. The diagram depicts a significant association between positive KDM2A immunoreactivity within NI and the occurrence of NI. *** $P \leq 0.001$

DISCUSSION

In the present study, we have demonstrated accumulation of β -catenin and proteins associated with the Wnt/ beta-Catenin pathway in NI, partly together with autophagy-associated proteins in the same inclusion. The presence of inclusions with KDM2A immunopositivity correlated significantly with overall survival in HCC. We found positive immunostaining for β -catenin, glutamin synthetase and KDM2A within NI [Figure 1]. In addition, other immunohistochemical studies have reported strong immunopositivity for β -catenin within

NI in thyroid carcinomas and pulmonary neuroendocrine tumors^[17,18]. Rezk *et al.*^[18] reported that some cases had β -catenin positive inclusions although they had almost negative cytoplasmic/nuclear staining. This is in line with our results. Though we found in 11 of 19 cases a correlation of β -catenin immunopositivity within NI with immunopositivity in the surrounding cytoplasm, there were 8 cases nearly lacking in cytoplasmic staining. Rezk *et al.*^[18] suggested that β -catenin might play a role in the development of NI as it is involved in organizing actin and microtubule polymerization^[18,19]. Additionally, Gamachi *et al.* also observed NI with immunopositivity for biotin, biotin-binding enzymes and β -catenin in pregnancy-related endometrium and morule-associated neoplastic lesions^[20]; they revealed that though biotin and biotin-binding enzymes were present in neoplastic and non-neoplastic tissues, β -catenin was lacking in non-neoplastic endometrial lesions. Further, NI with immunoreactivity for PAX8 protein were observed in conventional clear cell renal cell carcinoma^[21]. The immunopositivity for PAX8 is interesting as it is a transcription factor that is also involved in the proliferation of tumor cells via the Wnt/ β -catenin pathway; overexpression of PAX8 has been reported in various carcinomas^[21]. These studies did not clarify why NI contained these proteins. In our current study, to clarify the reason for β -catenin immunopositivity in NI, we examined our HCC cases on CTNNB1 exon 3 mutations. We differentiated between immunopositivity within the nucleus and in the intranuclear inclusion and found up to 20 different CTNNB1 mutations, all of which are listed in the COSMIC catalog and most, reported in the NCBI ClinVar database. A positive mutation status for CTNNB1 correlated significantly with nuclear immunopositivity, which is supported by the literature as CTNNB1 mutations are known to be associated with the translocation of β -catenin proteins from the membrane to the nucleus and activation of Wnt/ β -catenin signalling^[22]. However, we found no association between CTNNB1 mutations and positive β -catenin-immunostaining within the inclusions. Recently, we have shown that NI in HCC contained autophagy-associated proteins^[6]. Since we could simultaneously detect degraded cell material and lysosomes in NI by TEM studies, we assumed that biological processes similar to autophagy are taking place in NI^[6]. These autophagy-associated proteins were partly co-localized within the same NI. In the current study, we demonstrate that cases with β -catenin immunopositive NI also harbored NI with immunopositivity for autophagy-associated proteins; this association was significant for p62, cathepsinB/D, ubiquitin and LC3B. We found that p62 immunoreactivity was almost exclusively located in NI whereby cytoplasmic staining was diffuse and very weak. We suggest that high p62 positivity in NI could be caused by several reasons including alterations in p62 nucleocytoplasmic shuttling^[23] as previously reported in our HCC study^[6]. Intriguingly, we also detected co-localization of β -catenin with p62. The latter strongly suggests that biological processes with involvement of β -catenin are taking place within NI. We found in 11 of 19 cases a significant correlation between β -catenin immunopositivity within NI and β -catenin immunoreactivity in the surrounding cytoplasm, and we suppose that some NI with positive β -catenin immunostaining have developed by invagination of the cytoplasm into the NI with further closure of the invagination.

To clarify the factors contributing to β -catenin accumulation in NI, we also studied the immunoreactivity of proteins associated with the Wnt/ β -catenin pathway. We detected immunopositivity within NI for glutamine synthetase, which is a target of β -catenin signalling implicated in the development of HCC^[7,24,25] and for KDM2A having a role in β -catenin degradation^[14,26,27]. Lu *et al.*^[14] reported that lysine demethylase KDM2A demethylates nuclear β -catenin, which then induces the degradation of β -catenin and consequently, the downregulation of Wnt/ β -catenin target genes. In our study, the presence of NI containing KDM2A protein was significantly associated with longer survival in HCC patients; this is in line with our previous work in HCC demonstrating by Kaplan-Meier survival curves that the presence of NI significantly correlated with survival^[6]. Additionally, HCC patients with KDM2A immunopositivity in the cytoplasm showed a significant benefit in both, disease-specific OS and recurrence-free survival. Further, we found that even the presence of immunopositive KDM2A in the cytoplasm of normal tissues adjacent to tumor was associated significantly with recurrence-free survival. It has been documented by immunohistochemical studies that KDM2A positive staining, though predominantly found in the nucleus, can also be detected in the cytoplasm^[28]. Lu *et al.*^[14] reported methylation by KDM2A in both non-phosphorylated and phosphorylated β -catenin,

and in both the cytosol and the nucleus; they suppose that methylation/demethylation plays a role in modulating β -catenin activity. Henderson et al. described that β -catenin can be exported from the nucleus to the cytoplasm where its levels are regulated by degradation^[29]. Our results suggest that methylation of β -catenin by KDM2A and consequently, repressing of Wnt/ β -catenin signaling might play a role in the beneficial survival of patients with immunopositive inclusions and cytoplasm. However, mechanistically driven experiments are necessary to prove this hypothesis. The role of KDM2A in disease is contradictory and seems to be context dependent^[26]. On one hand, KDM2A is upregulated in ovarian^[30], breast and lung cancer^[13,30,31] and associated with a poor prognosis; on the other, it is downregulated in prostate cancer^[32] and in the liver, KDM2A regulates hepatic gluconeogenesis whereby its exogenous expression reduces blood glucose levels^[33]. Reasons for the dual effects of KDM2A may be due to the involvement of KDM2A in various biological signaling pathways, including interacting with the p53-binding protein and NF-kappaB activity by demethylation of the p65 subunit of NF-kappaB^[34]. Further, we found that the increased occurrence of NI was significantly associated with KDM2A immunopositivity in NI. KDM2A is described as a heterochromatin-associated and HP1-interacting protein^[32] and KDM2A is required to sustain centromeric integrity and genomic stability, particularly during mitosis^[32]. A change in chromatin stability has been discussed as a factor contributing to the development of NI^[6,16,35,36]. Thus, we suppose that KDM2A might play a role in the formation of inclusions. The mechanisms that participate in the formation of inclusions are still poorly understood, in spite of several investigations^[35,37]. However, we believe that various factors can induce the formation of inclusions and that KDM2A may be one.

In this study, we demonstrated accumulations of β -catenin and proteins associated with the Wnt/ β -catenin pathway in NI. The simultaneous presence of β -catenin with autophagy-associated proteins, partly co-localized to the same inclusion suggests that biological processes similar to autophagy might take place in NI. Further, we found that the presence of KDM2A immunopositive NI provides a survival benefit to HCC patients. To what extent this is related to possible degradation of β -catenin by KDM2A, and whether NI play a role in these biological processes needs to be analyzed by further experiments.

DECLARATIONS

Acknowledgments

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Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Schwertheim S, Baba HA, Jastrow H, Herold T

Conceptualization: Schwertheim S, Baba HA, Schmid KW

Data acquisition: Herold T, Jastrow H, Baba HA, Schmid KW

Investigation: Schwertheim S, Theurer S, Jastrow H, Herold T, Ting S, Kälsch J, Baba HA, Schmid KW

Methodology: Schwertheim S, Theurer S, Jastrow H, Herold T, Ting S, Kälsch J, Baba HA

Supervision: Schwertheim S, Baba HA, Schmid KW

Writing - original draft preparation: Schwertheim S, Baba HA

Writing - review and editing: Schwertheim S, Theurer S, Jastrow H, Herold T, Ting S, Kälsch J, Baba HA, Schmid KW

Availability of data and materials

The source of the data came from the Institute of Pathology, University Hospital of Essen.

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None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Informed consent was obtained from each patient. The study complied with the Helsinki Declaration of 1975 and the Ethics Committee (Institutional Review Board) of the University Hospital Essen (reference number: 16-6917-BO).

Consent for publication

Not applicable.

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REFERENCES

1. Apte U, Singh S, Zeng G, Cieply B, Virji MA, et al. Beta-catenin activation promotes liver regeneration after acetaminophen-induced injury. *Am J Pathol* 2009;175:1056-65.
2. Hu M, Kurobe M, Jeong YJ, Fuerer C, Ghole S et al. Wnt/beta-catenin signaling in murine hepatic transit amplifying progenitor cells. *Gastroenterology* 2007;133:1579-91.
3. McLin VA, Rankin SA, Zorn AM. Repression of Wnt/beta-catenin signaling in the anterior endoderm is essential for liver and pancreas development. *Development* 2007;134:2207-17.
4. Spee B, Carpino G, Schotanus BA, Katoonizadeh A, Vander Borgh S et al. Characterisation of the liver progenitor cell niche in liver diseases: potential involvement of Wnt and Notch signalling. *Gut* 2010;59:247-57.
5. Van Haele M, Snoeck J, Roskams T. Human liver regeneration: an etiology dependent process. *Int J Mol Sci* 2019;20:2332.
6. Schwertheim S, Westerwick D, Jastrow H, Theurer S, Schaefer CM, et al. Intracellular inclusions in hepatocellular carcinoma contain autophagy-associated proteins and correlate with prolonged survival. *J Pathol Clin Res* 2019;5:164-76.
7. Burke ZD, Tosh D. The Wnt/beta-catenin pathway: master regulator of liver zonation? *BioEssays* 2006;28:1072-7.
8. Long J, Wang H, Lang Z, Wang T, Long M, et al. Expression level of glutamine synthetase is increased in hepatocellular carcinoma and liver tissue with cirrhosis and chronic hepatitis B. *Hepatol Int* 2011;5:698-706.
9. Cadoret A, Ovejero C, Terris B, Souil E, Levy L, et al. New targets of beta-catenin signaling in the liver are involved in the glutamine metabolism. *Oncogene* 2002;21:8293-301.
10. Huang Y, Liu Y, Yu L, Chen J, Hou J, et al. Histone demethylase KDM2A promotes tumor cell growth and migration in gastric cancer. *Tumor Biology* 2015;36:271-8.
11. Dhar SS, Alam H, Li N, Wagner KW, Chung J, et al. Transcriptional repression of histone deacetylase 3 by the histone demethylase KDM2A is coupled to tumorigenicity of lung cancer cells. *J Biol Chem* 2014;289:7483-96.
12. Tanaka Y, Okamoto K, Teye K, Umata T, Yamagiwa N, et al. JmJC enzyme KDM2A is a regulator of rRNA transcription in response to starvation. *EMBO J* 2010;29:1510-22.
13. Wagner KW, Alam H, Dhar SS, Giri U, Li N, et al. KDM2A promotes lung tumorigenesis by epigenetically enhancing ERK1/2 signaling. *J Clin Invest* 2013;123:5231-46.
14. Lu L, Gao Y, Zhang Z, Cao Q, Zhang X, et al. Kdm2a/b lysine demethylases regulate canonical Wnt signaling by modulating the stability of nuclear beta-catenin. *Dev Cell* 2015;33:660-74.
15. Bosman FT, Carneiro F, Hruban RH, Theise ND. WHO classification of tumours of the digestive system (4th edn). Lyon: International Agency for Research on Cancer; 2010.
16. Schwertheim S, Theurer S, Jastrow H, Herold T, Ting S, et al. New insights into intracellular inclusions in thyroid carcinoma: association with autophagy and with BRAFV600E mutation. *PLoS One* 2019;14.
17. Kobayashi S, Tsuta K, Sekine S, Yoshida A, Sasaki N, et al. Pulmonary neuroendocrine tumors with nuclear inclusion. *Pathol Res Pract* 2013;209:574-7.
18. Rezk S, Brynes RK, Nelson V, Thein M, Patwardhan N, et al. Beta-Catenin expression in thyroid follicular lesions: potential role in nuclear envelope changes in papillary carcinomas. *Endocr Pathol* 2004;15:329-37.
19. Ligon LA, Karki S, Tokito M, Holzbaur ELF. Dynein binds to beta-catenin and may tether microtubules at adherens junctions. *Nat Cell Biol* 2001;3:913-7.
20. Gamachi A, Kashima K, Daa T, Nakatani Y, Tsujimoto M, et al. Aberrant intracellular localization of biotin, biotin-binding enzymes, and beta-catenin in pregnancy-related endometrium and morule-associated neoplastic lesions. *Mod Pathol* 2003;16:1124-31.

21. McClain A, Sakowski L, Conti M, Zhang H, Li QK. Intranuclear inclusions in conventional clear cell renal cell carcinoma (ccRCC): diagnosis and differential diagnosis. *Arch Urol Res* 2018;2:5.
22. Kim G, Kurnit KC, Djordjevic B, Singh C, Munsell MF, et al. Nuclear beta-catenin localization and mutation of the CTNNB1 gene: a context-dependent association. *Mod Pathol* 2018;31:1553-9.
23. Pankiv S, Lamark T, Bruun JA, Øvervatn A, Bjørkøy G, et al. Nucleocytoplasmic shuttling of p62/SQSTM1 and its role in recruitment of nuclear polyubiquitinated proteins to promyelocytic leukemia bodies. *J Biol Chem* 2010;285:5941-53.
24. Hartmann F, Tannapfel A. Leberadenome und andere maligne und benigne Lebertumoren. *Der Gastroenterologe* 2016;11:359-67.
25. Sakamoto M. Early HCC: diagnosis and molecular markers. *J Gastroenterol* 2009;44:108-11.
26. Vacik T, Ladinovic D, Raska I. KDM2A/B lysine demethylases and their alternative isoforms in development and disease. *Nucleus* 2018;9:431-41.
27. Yu G, Wang J, Lin X, Diao S, Cao Y, et al. Demethylation of SFRP 2 by histone demethylase KDM 2A regulated osteo-/dentinogenic differentiation of stem cells of the apical papilla. *Cell Prolif* 2016;49:330-40.
28. Rizwani W, Schaal C, Kunigal S, Coppola D, Chellappan S. Mammalian lysine histone demethylase KDM2A regulates E2F1-mediated gene transcription in breast cancer cells. *PLoS One* 2014;9.
29. Henderson BR, Fagotto F. The ins and outs of APC and beta-catenin nuclear transport. *EMBO Rep* 2002;3:834-9.
30. Lu DH, Yang J, Gao LK, Min J, Tang JM, et al. Lysine demethylase 2A promotes the progression of ovarian cancer by regulating the PI3K pathway and reversing epithelial-mesenchymal transition. *Oncol Rep* 2019;41:917-27.
31. Chen JY, Li CF, Chu PY, Lai YS, Chen CH, et al. Lysine demethylase 2A promotes stemness and angiogenesis of breast cancer by upregulating Jagged1. *Oncotarget* 2016;7:27689.
32. Frescas D, Guardavaccaro D, Kuchay SM, Kato H, Poleshko A, et al. KDM2A represses transcription of centromeric satellite repeats and maintains the heterochromatic state. *Cell Cycle* 2008;7:3539-47.
33. Pan D, Mao C, Zou T, Yao AY, Cooper MP, et al. The histone demethylase Jhdm1a regulates hepatic gluconeogenesis. *PLoS Genet* 2012;8.
34. Lu T, Jackson MW, Wang B, Yang M, Chance MR, et al. Regulation of NF-kappaB by NSD1/FBXL11-dependent reversible lysine methylation of p65. *Proc Natl Acad Sci* 2010;107:46-51.
35. Bozler J, Nguyen HQ, Rogers GC, Bosco G. Condensins exert force on chromatin-nuclear envelope tethers to mediate nucleoplasmic reticulum formation in *Drosophila melanogaster*. *G3 (Bethesda)* 2015;5:341-52.
36. Fischer AH. The diagnostic pathology of the nuclear envelope in human cancers. *Adv Exp Med Biol* 2014;773:49-75.
37. Lammerding J, Fong LG, Ji JY, Reue K, Stewart CL, et al. Lamins A and C but Not Lamin B1 Regulate Nuclear Mechanics. *J Biol Chem* 2006;281:25768-80.

Review

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Genetics of alcohol-related hepatocellular carcinoma - its role in risk prediction

Ken Liu^{1,2}, Gontran Verset^{3,4}, Eric Trepo^{3,4}, Devanshi Seth^{1,2,5}

¹Centenary Institute of Cancer Medicine and Cell Biology, The University of Sydney, Sydney, NSW 2006, Australia.

²Faculty of Medicine and Health, The University of Sydney, Sydney, NSW 2006, Australia.

³Department of Gastroenterology, Hepatopancreatology and Digestive Oncology, CUB Hôpital Erasme, Université Libre de Bruxelles, Brussels 1070, Belgium.

⁴Laboratory of Experimental Gastroenterology, Université Libre de Bruxelles, Brussels 1070, Belgium.

⁵Drug Health Services, Royal Prince Alfred Hospital, Missenden Road, Camperdown, NSW 2050, Australia.

Correspondence to: Dr. Devanshi Seth, Centenary Institute, Building 93, Missenden Road, Camperdown, NSW 2050, Australia.
E-mail: d.seth@sydney.edu.au

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy, with increasing incidence worldwide. Alcohol-related cirrhosis (AC) accounts for 30% of the global incidence of HCC and HCC-related deaths. With the decline of hepatitis C virus (HCV) and decreasing HCV-related HCC, AC will soon become the leading cause of HCC. Excess alcohol consumption (> 80 g per day for > 10 years) increases the risk of HCC by 5-fold. However, only up to 35% of excessive drinkers develop cirrhosis and its associated HCC risk. Individual variation in susceptibility to HCC is known, but there is limited information to predict who among the patients is at high risk of progressing to HCC. Clinical risk factors for HCC include male gender, older age, severity of cirrhosis, obesity and presence of type 2 diabetes. In addition to ethnic variability in HCC risk, genetic variants are known to alter the risk of alcohol-related HCC. For example, single nucleotide polymorphisms in *PNPLA3* (rs738409, C>G) and *TM6SF2* (rs58542926, C>T) increase the risk of AC-related HCC, whereas *HSD17B13* (T>A) reduces the risk for HCC. Studies have also confirmed *PNPLA3* and *TM6SF2* to be independent risk factors for AC-related (but not HCV-related) HCC. Combining genetic risk factors with phenotypic/clinical risk factors has been explored for stratification of patients for HCC development. Risk allele rs378409-G in *PNPLA3* when combined with phenotypic/clinical risk factors (BMI, age, sex) has enabled HCC risk stratification of AC patients into low-, intermediate- and high-risk subgroups. Similarly, a combination of the two genetic variants *PNPLA3*-G and *TM6SF2*-T has been independently associated with risk of HCC onset. Using a polygenic risk score approach of incorporating several genetic variants, prognostic performance of polygenic risk score that included *PNPLA3*



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rs378409 and *TM6SF2* rs58542926 improved HCC prediction better than with either variant alone. Incorporating new variants and risk factors has the potential to build better algorithms/models to predict onset, early diagnosis and treatments for AC-related HCC. However, clinical usefulness of these approaches is yet to be determined.

Keywords: Alcohol-related cirrhosis, *PNPLA3*, *HSD17B13*, *TM6SF2*, risk prediction

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy, with increasing incidence worldwide^[1]. Despite screening programs in high-risk populations, long-term outcome is poor with a 5-year survival of 18%, representing the world's third most lethal cancer. More specifically, the World Health Organization estimates that more than a million patients will die from liver cancer in 2030^[2].

In almost 90% of cases, HCC occurs in the context of chronic liver disease, in particular, on the background of cirrhosis^[1,3]. The underlying chronic liver disease promoting liver carcinogenesis varies geographically^[1]. In Asia and sub-Saharan Africa, HCC is mostly caused by hepatitis B virus infection, while in the United States and Europe the current leading etiologies are hepatitis C virus (HCV) infection and alcohol-related cirrhosis (AC) followed by non-alcohol-related fatty liver disease (NAFLD)^[4]. However, the advent of new direct-acting antiviral agents is expected to control HCV-related HCC in upcoming years, and AC will soon become the leading cause of HCC in most high-income countries^[1]. Clinical risk factors for HCC occurrence include male gender, older age, severity of cirrhosis, obesity and presence of type 2 diabetes^[5-7]. Clinical risk models have shown that the individual risk of HCC development is highly variable^[6]. In addition, case-control and cancer database studies have highlighted the impact of ethnic background and a significant familial clustering^[8,9]. For example, individuals of African and Hispanic ancestry are less likely to undergo curative therapies^[10]. Overall, these observations strongly suggest that inherited genetic factors contribute to hepatocarcinogenesis.

Here, we review the current literature on risk factors, with a particular focus on genetic risk variants for alcohol-related HCC occurrence.

EPIDEMIOLOGY AND CHARACTERISTICS OF ALCOHOL-RELATED HCC

Alcohol-related HCC occurs infrequently in patients without pre-existing cirrhosis. Cirrhosis (of any etiology) is the single biggest risk factor for HCC development^[3,11,12]. The annual incidence of HCC in patients with AC is nearly 3%^[13]. The risk of developing AC and HCC parallels the amount of alcohol consumed daily and significantly increases above a threshold of 20 and 30 g for females and males, respectively^[14,15]. Heavy alcohol drinking of more than 80 g per day for longer than 10 years increases the risk of HCC by 5-fold^[16]. More specifically, AC accounts for 30% of the global incidence of HCC and HCC-related deaths, with marked geographical differences^[17]. In Europe, HCC occurrence on the background of alcohol-related liver disease (ALD) varies from 20% in the south (e.g., Italy or Spain), to 63% in eastern countries. In the United States, alcohol accounts for 13% to 23% of HCC cases. Finally, the prevalence of alcohol-related HCC reaches 6% in the Middle East and 14% in North Africa^[17,18]. The influence of alcohol consumption has also been highlighted by the impact of alcohol withdrawal on HCC development. Thus, a meta-analysis reported an annual reduction of HCC risk by 6%-7%^[19].

However, only up to 10%-35% of excessive drinkers develop advanced fibrosis or cirrhosis and its associated HCC risk^[20]. Interestingly, the role of alcohol consumption seems to be milder or even negligible compared to other environmental factors in the setting of HCC occurring in a non-fibrotic liver. For example, a recent case-control study observed that after adjustment of smoking habits and metabolic syndrome features,

alcohol consumption was no longer independently associated with HCC in individuals with F0-F1 fibrosis stage^[21].

HCC is often diagnosed at a later Barcelona Clinic Liver Cancer (BCLC) stage in patients with ALD and with a more severe underlying cirrhosis leading to a worse prognosis compared to other liver diseases^[14,22]. A previous study reported that patients with alcohol-related HCC are often younger and are more frequently diagnosed with a multifocal or infiltrative/massive tumor compared to HCV-related HCC. However, this apparent greater cancer aggressiveness disappeared after adjusting for confounding factors, and prognosis was similar in ALD and HCV patients when stratified by BCLC stages^[23]. Moreover, a recent study did not show significant differences in tumor characteristics between patients with AC- and NAFLD-related HCC^[24]. Overall, the higher proportion of advanced BCLC stages observed in ALD/AC patients might only reflect a lower compliance with surveillance programs, rather than a greater tumor aggressiveness.

LIMITATIONS OF SCREENING STRATEGIES IN PATIENTS WITH AC-RELATED HCC

The American Association for the Study of Liver Diseases and European Association for the Study of the Liver recommend HCC surveillance in all cirrhotic patients using ultrasound, with or without alpha-fetoprotein determination, every 6 months^[25,26]. This surveillance program has been shown to increase overall survival and improve the quality-adjusted life expectancy^[27,28]. However, this periodic surveillance has been shown to be difficult to implement in daily clinical practice, ultimately leading to a significant prevalence of HCC detected at a more advanced stage^[29]. Thus, more than 20% of HCC patients are also diagnosed with an unsuspected cirrhosis^[30]. This phenomenon is even more pronounced in patients with ALD because AC is underdiagnosed due to their poor compliance in cancer surveillance programs^[30]. Therefore, risk factors identified for AC are potential candidates for susceptibility to HCC.

Due to the aforementioned limitations, there is an urgent need for new detection strategies and the development of new highly sensitive, reliable, and easily accessible biomarkers that can either improve the early detection of HCC in high-risk patients with AC or identify individuals at risk of progressive ALD when liver fibrosis is incomplete and potentially reversible^[31].

Individual variation in susceptibility to HCC is known, but there is limited information to predict who among the patients is at high risk of progressing to HCC. A better understanding of the contributing molecular, genetic and epigenetic factors is required to identify drivers of and therapeutic options for hepatocarcinogenesis.

CONTRIBUTION OF GENETIC VARIANTS TO THE PREDICTION OF ALCOHOL-RELATED HCC

The association of genetic variants with the risk of alcohol-related HCC has been reported. Earlier studies targeted genes with known functions, specifically genes known to operate in the pathogenesis of ALD and recently proposed to be part of a “5-hit working model” of disease progression leading to HCC^[32]. These candidate genes, involved in hepatic alcohol metabolism [alcohol dehydrogenase (*ADH*), acetaldehyde dehydrogenase (*ALDH*), ethanol-induced cytochrome P450 (*CYP2E1*), *CYP2E1*-dependent microsomal ethanol oxidizing system (MEOS)] and affecting downstream mechanisms including oxidative stress [*CYP2E1*, glutathione S-transferase (*GST*), manganese superoxide dismutase (*MnSOD*), *N*-acetyltransferase 2, ectonucleotide pyrophosphatase/phosphodiesterase 1, homeostatic iron regulator (*HFE*)], endotoxin release [*CD14*, toll-like receptor 4 (*TLR4*)], immune function (*TNF α* , *IFN γ* , *IL-10*, *IL-1 β* , *CD14*) and fibrogenesis [*TGF β* , *angiotensin*, *leptin*, metalloproteinases (*MMPs*), tissue inhibitors of *MMPs* (*TIMPs*)], have been extensively reviewed for their association to alter risk of ALD/AC and ALD-related HCC^[12,33-35]. But results from most of these earlier studies could not be replicated or confirmed due to limitations in

technology, small sample size, inappropriate study population and not accounting for underlying ethnic variability. The most widely known genetic mutations altering the risk of ALD and ALD-HCC are in the alcohol-metabolizing enzymes. These mutations alter the enzyme kinetics of alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH)^[35,36]. ADH1B rs1229984 induces ADH activity and acetaldehyde formation, whereas ALDH2*2 rs671 reduces ALDH activity, impairing its ability to clear acetaldehyde^[35,37]. Carriage of both mutations results in the accumulation of toxic acetaldehyde levels with intense rise in arterial blood flow to the face, causing the well-known flushing and nausea. In East Asian populations with high prevalence of these mutations, this may result in reduced alcohol intake conferring protection against alcoholism^[38]. Conversely, the risk of developing ALD and ALD-HCC increases in drinkers who carry one or both mutations^[36].

Recent technological advances such as genome-wide association studies (GWAS) and next-generation sequencing have added to the growing field of genetic and epigenetic factors that modulate the risk for ALD/AC-related HCC. In recent years, a few single nucleotide polymorphisms (SNPs) have been discovered that are associated with risk of AC^[39-41]. The single most commonly reproduced association with liver cirrhosis is the rs738409 SNP (p. I148M) in patatin-like phospholipase domain protein 3 (*PNPLA3*), which is also associated with increased HCC risk^[42]. This C>G mutation is accompanied by a change from isoleucine to methionine at a conserved amino acid residue (I148M). Association of rs738409 (C>G) with increased risk of liver diseases has been confirmed in AC^[40,41] and alcohol-related HCC^[43,44]. Dose effect of the G-allele has been shown with ancestry-adjusted odds ratio (OR) increasing by 1.79 per G allele ($P = 1.9 \times 10^{-5}$) for ALD risk^[45] and 1.77 (95%CI: 1.42-2.19, $P = 2.78 \times 10^{-7}$) per G allele for HCC^[46]. The influence of this variant on HCC risk prediction revealed that the rs738409 (GG) genotype was an independent risk factor specifically for alcohol- (but not HCV-) related HCC^[46]. Moreover, OR among the AC patients with HCC increased from 2.87 (95%CI: 1.61-5.10) in carriers of the CG genotype to 12.41 (95%CI: 6.99-22.03) in GG patients^[43].

A study in a Chinese population showed that rs17401966 (A>G) in kinesin-like factor 1 B (*KIF1B*), a tumor suppressor gene, was associated with risk of HCC. The risk of HCC was higher in carriers of the AA genotype, compared to GG or AG, but only in the presence of alcohol (OR 2.36, 95%CI: 1.49-3.74), suggesting an additive gene-environment interaction between rs17401966 and alcohol consumption^[47]. But this association has not been confirmed. Similarly, rs641738 (C>T) in membrane-bound O-acyltransferase 7 (*MBOAT7*), was identified as a risk locus for AC^[40], but has yet to be replicated in other studies as a risk for AC/ALD or HCC.

Another SNP, rs58542926 (*T) in transmembrane 6 superfamily 2 (*TM6SF2*), is strongly associated with the risk for HCC, particularly in patients with AC- and not HCV-related cirrhosis^[44,48]. This variant was independently confirmed to be associated with HCC using a multivariate model adjusted for age, sex, BMI and diabetes (OR 2.5, 95%CI: 1.4-4.3)^[48].

SNPs in hydroxysteroid 17-beta dehydrogenase 13 (*HSD17B13*) are associated with decreased liver transaminases and liver injury^[49]. In particular, recently identified splice variant SNP rs72613567 (T>A), resulting in loss of function and reduced enzyme activity, also showed (1) interactions with *PNPLA3* rs738409 risk allele, with each rs72613567:TA allele lowering the increase in transaminase levels conferred by each *PNPLA3* risk allele (I148M); and (2) allele dose-dependent association of rs72613567:TA with decrease in *PNPLA3* mRNA expression^[39]. Importantly, this rs72613566 (T>A) was associated with lower odds of alcohol- and non-alcohol-related liver diseases/cirrhosis as well as lower risk for HCC. The lower risk conferred by rs72613567 variant was *PNPLA3* allele-dependent for AC and was confirmed in both men and women^[50]. However, the rs72613567-associated lower risk for HCC was *PNPLA3*-dependent only in men^[50].

It is suggested that for risk of AC/ALD-related HCC, *PNPLA3* may be most relevant for the development of steatosis and ALD/AC, and *TM6SF2* and *MBOAT7* contributing towards HCC through inflammation-driven fibrosis^[51]. Intriguingly, SNPs reported so far in *PNPLA3*, *HSD17B13*, *TM6SF2* and *MBOAT7* as being associated with AC/ALD are involved in lipid metabolism and processing, but their role in developing AC-ALD and HCC is yet to be clarified. Further investigations are required into the contribution of genetic factors individually and in combination with other variants, especially those influencing the effect on each other, such as *PNPLA3* and *HSD17B13*. Understanding the roles of SNPs in the biology of liver disease is still in its infancy, and there is limited literature on specific functions of these recently identified SNPs. Although some SNPs are shared among different etiologies of cirrhosis and HCC, the role or interaction of these SNPs remains unclear in complex etiologies that co-exist with AC, such as viral hepatitis and NAFLD-related HCC. Further investigations are required to delineate the contribution of these SNPs to HCC development.

RISK STRATIFICATION FOR AC/ALD-RELATED HCC

Using a combination of genetic risk factors with other phenotypic/clinical risk factors has been explored for the identification and stratification of patients for HCC development^[46,48,52-56]. Using the rs738409 risk variant in *PNPLA3* in combination with other phenotype/clinical factors (BMI, age, sex) enabled HCC risk stratification of low-, intermediate- and high-risk AC patients (hazard ratio (HR) 4.3, 95%CI: 2.7-6.4)^[53]. Similarly, a combination of the two genetic variants *PNPLA3*-G and *TM6SF2*-T was independently associated with risk of HCC onset (HR 2.3, 95%CI: 1.5-3.4)^[48]. Furthermore, the same study also reported that the number of HCC cases with carriage of both *PNPLA3*-G and *TM6SF2*-T risk alleles was significantly higher than carriers of only one risk allele in either SNP.

Most recently, a combination of all reported SNPs in AC, rs738409 (*PNPLA3*), rs6834314 (*HSD17B13*), rs58542926 (*TM6SF2*) and rs626283 (*MBOAT7*), when added to phenotypic factors (BMI, diabetes status, wine and coffee consumption), performed better [area under the curve (AUC) 0.748, 95%CI: 0.721-0.774], compared to only genetic factors (AUC 0.689, 95%CI: 0.660-0.717) or only phenotypic factors (AUC 0.681, 95%CI: 0.651-0.710), in stratifying drinkers with AC from drinkers with no liver disease^[57]. It is encouraging that combining information on genetic variants with other risk factors can improve the identification of patients at risk. Furthermore, these genetic variants have also been shown to modulate severity of NAFLD (and its progression to steatohepatitis, fibrosis and cirrhosis) which commonly co-exists with alcoholic liver disease patients as “dual-etiology fatty liver disease” and accelerates liver injury^[58,59].

Recently, the approach of incorporating several genetic variants in a so-called polygenic risk score (PRS) has been shown to be a successful strategy to improve the prediction of various complex phenotypes^[60]. Thus, this method has been shown to outperform existing clinical models for the prediction of breast cancer with personalized recommendation on screening^[61]. Of note, the addition of other risk factors into a global predictive model improves the overall performances compared to PRS alone^[62]. At the transcriptional level, gene expression signatures gathering several dozens of genes (i.e., Prosigna and MammaPrint) have been included by the European Society for Medical Oncology to its clinical practice guidelines as prognostic and predictive tools to determine the benefit from chemotherapy^[63].

The use of PRS to predict HCC occurrence in AC-related HCC patients is emerging^[52,64]. More specifically, the prognostic performance of PRS including *PNPLA3* rs738409 and *TM6SF2* rs58542926 was higher than when considering *PNPLA3* and *TM6SF2* variants alone^[52,64].

Several other SNPs have been identified as being associated with the risk of HCC, particularly with a viral etiology, but the literature is sparse regarding genetic variants specifically in relation to alcohol-related HCC. Similarly, contributions of molecular markers^[65-67], somatic mutations^[68], chromosomal instability

and tumor microenvironment^[69], and other regulatory components, such as mRNAs^[70], noncoding RNAs^[65,71], epigenetic^[71,72] and mitoepigenetic factors^[73] influencing the risk of alcohol-related HCC are few and overlap with other etiologies^[69,71]. Last but not least, the role of the gut microbiota (fungi, bacteria and viruses), is another emerging factor influencing disease risk in ALD and ALD-HCC^[74,75]. The gut microbiota also engages in alcohol metabolism, thereby altering the risk for ALD pathogenesis. Changes in the gut microbiome significantly correlates with alcohol consumption in human and experimental models, and there is evidence that alcohol and gut metabolites in ALD patients show carcinogenic effects^[74], potentially increasing the risk of HCC.

Genomic studies have revealed several subclasses of HCC. Alcohol-related HCC is associated with *CTNNB1* mutations (WNT- β -catenin signalling pathway); however, direct translation of molecular HCC subclasses into clinical management (i.e., personalized medicine) is yet to be achieved^[76]. The recent success of checkpoint inhibitors in HCC has led to a renewed interest in immunological profiling of HCC and opportunities for personalized medicine. Recently, Sia *et al.*^[77] analyzed the gene expression pattern of inflammatory cells in HCCs of almost 1000 patients. The authors identified a novel molecular class of tumors (in approximately 25% of patients) with an enriched inflammatory response characterized by overexpression of immune-related genes and high expression of PD1 and PD-L1 which may predict response to checkpoint inhibitor immunotherapy. A study by The Cancer Genome Atlas consortium performed multi-platform integrative molecular subtyping on 196 HCCs and found a similar subset of patients with high lymphocyte infiltration (in 22% of patients)^[78]. Of note, the authors showed that the aforementioned *CTNNB1* mutation was associated with a lack of immune infiltrate (so-called cold tumors), which has been observed by others^[78,79]. In a recent first report of prospective genotyping of advanced HCC by next-generation sequencing, *CTNNB1* mutations were associated with primary resistance to immune checkpoint inhibitors^[80]. Patients exhibiting *CTNNB1* mutations all had progressive disease as their best response and a shorter median survival compared to those without mutations (9.1 months *vs.* 15.2 months, respectively). Clearly, the immunological classification of alcohol-related HCCs will become increasingly important as immune-based therapies are added to the limited therapeutic options for patients with advanced disease.

With the discovery of new variants and risk factors, there is the potential to incorporate them for building prediction algorithms/models for AC/ALD-HCC onset, early diagnosis and treatments.

CLINICAL APPLICATIONS OF RISK-STRATIFIED AC/ALD-HCC PATIENTS

In terms of clinical application, patients identified by the above genetic modifiers to be at high risk of developing significant liver fibrosis may be prioritized for early referral to specialist care, with those at low risk remaining in primary care. These select high-risk patients can then be linked with resource-intensive multidisciplinary and evidence-based care involving hepatologists, psychiatrists, and addiction specialists to maximize their chance of obtaining abstinence. Indeed, when prolonged abstinence is achieved, it has been shown to lead to resolution of steatosis and inflammation and even fibrosis regression in some (but not all) patients^[81,82]. Specialist care can also facilitate access to closer monitoring of liver fibrosis using non-invasive tests (e.g., transient elastography, magnetic resonance elastography) and prompt commencement of HCC surveillance (discussed below) when patients are diagnosed with cirrhosis.

Risk stratification for HCC surveillance

Aside from the prediction of patients at risk of advanced fibrosis or cirrhosis, genetic variants (e.g., *PNPLA3*, *TM6SF2* and *HSD17B13*) can also help predict HCC development. As mentioned, these genes predisposing to alcohol-related HCC can be incorporated with other established risk factors for HCC (e.g., male sex, age and obesity) into a validated scoring system to risk-stratify patients for tailored HCC surveillance. Indeed, risk calculators for HCC development already exist for other liver diseases such as

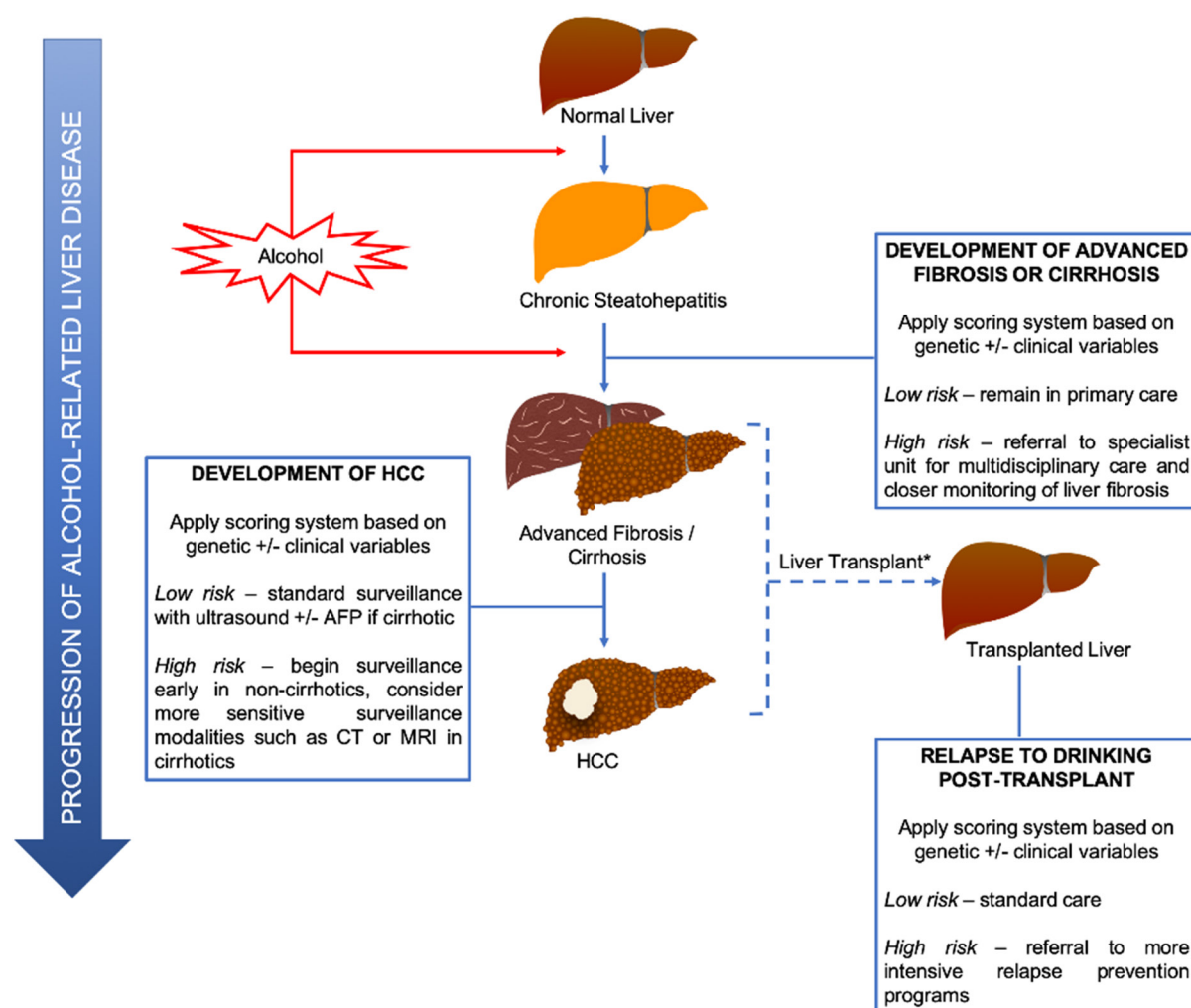


Figure 1. Potential clinical applications of genetic-based tests in alcoholic cirrhosis and related hepatocellular carcinoma. *It would be unethical to use genetic tests in the pre-transplant setting to determine if a patient should be offered liver transplantation. AFP: alpha-fetoprotein; CT: computed tomography; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging

chronic hepatitis B and hepatitis C infection^[83,84]. It is likely that the combination of several genetic variants (rather than any single SNP) with or without clinical variables into a score will be most predictive. Since the surveillance interval of 6 months for patients^[85] was determined on the basis of estimated tumor doubling time (rather than tumor development risk), shortening intervals (e.g., to every 3 months) for ALD patients classified as high-risk may not result in improved outcomes^[86]. However, risk calculators may help identify high-risk patients without cirrhosis, who should undergo surveillance (akin to surveillance of non-cirrhotic chronic hepatitis B patients) or those who should be surveyed with a more sensitive modality (e.g., computed tomography scan or magnetic resonance imaging). Conversely, risk scores may select out a low-risk group of patients with ALD who can safely forego surveillance, especially in resource-poor settings [Figure 1]. The development and application of risk scores using genetics needs to be explored further.

Given the rise of alcohol-related HCC in the post-HCV era, it is imperative that more research be conducted in this area, providing a deeper understanding of the underlying risks and early diagnosis of HCC in patients with ALD. Possibilities exist of repurposing biomarkers and therapeutic agents for alcohol-related HCC identified/used for other etiologies.

CHALLENGES OF CURRENT RISK PREDICTION MODELS FOR ALCOHOL-RELATED HCC

Risk prediction for alcohol-related HCC has been critically missing in the past due to lack of reproducible genetic studies. Recent discoveries on several genetic risk associations with AC have opened the field for using this information for risk prediction, not only for cirrhosis but also for alcohol-related HCC in patients with alcohol use problems.

The contribution of genetic variants, especially *PNPLA3* rs738409, as potential predictors for AC-related HCC has been frequently discussed mainly because ORs often are > 2 , calculated in retrospective cohorts^[46]. However, modest to large ORs and extreme statistical significance do not automatically imply clinical relevance and other statistical metrics such as sensitivity, specificity and negative/positive predictive values might be more relevant^[87]. Although variants in *PNPLA3*, *TM6SF2* and more recently *HSD17B13* modulate the risk of AC-related HCC, the use of these variants in HCC surveillance programs is currently not recommended^[25,26].

Even though predictive models for HCC have generally been successful, they have limited clinical utility currently, especially the use of genetic-based factors identified in one ethnic population but used for prediction in another population. Although recent use of PRS for identification/stratification of at-risk patients is promising, one important limitation of PRS is their applicability in non-European ancestry populations. Indeed, most of the variants used in PRS have been identified in GWAS overwhelmingly conducted in individuals of European descent^[88]. Therefore, the applicability of current PRS is not guaranteed^[89]. Failure to include individuals from diverse ancestry will hamper the use of PRS in the multiethnic population seen in clinical practice^[90]. At the level of gene expression, a 186-gene signature, initially developed to predict HCC recurrence in HCV-infected patients, has shown promising predictive ability for hepatocarcinogenesis in AC patients^[91].

A particular challenge with alcohol-related liver diseases, including HCC, is the complication of alcohol dependence in these patients. Only select patients with alcohol-related HCC undergo liver transplantation (LT). After LT, up to 50% of patients relapse to drinking with 20% returning to harmful drinking with potential recurrence of liver disease^[92]. The heritability of alcohol dependence has previously been estimated to be 25%-50%, and variants in genes encoding alcohol metabolism enzymes (ADH, ALDH) and GABA neurotransmission (GABRA2) have been shown to be associated with alcohol misuse^[93]. Whether these same markers can predict (beyond current clinical markers) recidivism post-LT is currently unknown, so this presents an opportunity for further study. Transplanted patients identified to be at high risk of relapse can be preferentially referred for participation in multidisciplinary relapse prevention programs, which have been shown to be effective^[94]. The prediction of relapsers post-LT will be increasingly important as transplant indications have recently expanded to include select patients with severe alcoholic hepatitis without significant prior abstinence^[95].

Overall, PRS and gene expression signatures in combination with environmental risk factors have the potential to improve the prediction of alcohol-related HCC and pinpoint high-risk individuals. However, to date, evidence of clinical usefulness in this field is lacking. Thus, before genetic variation/expression can impact decision-making and be implemented in daily practice, it will need to be validated in large-scale prospective cohorts evaluating their clinical utility and cost-effectiveness^[89,96]. Moreover, many physicians will require some training to interpret and communicate in a digestible manner the results of genetic testing and its current limitations^[97].

DECLARATIONS

Authors' contributions

Led the overall concept, design, structure, writing, submission and revision of the manuscript in consultation with all co-authors: Seth D

Risk stratification, HCC surveillance and treatment, [Figure 1](#): Liu K
 Introduction, epidemiology of AC-HCC, risk prediction: Trepo E, Verset G
 Introduction, genetic variants, genetic variants risk prediction: Seth D
 All authors critically reviewed the manuscript.

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Consent for publication

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REFERENCES

- Villanueva A. Hepatocellular carcinoma. *Engl J Med* 2019;380:1450-62.
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
- Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018;391:1301-14.
- Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, et al. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019;16:589-604.
- El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004;126:460-8.
- N'Kontchou G, Paries J, Htar MTT, Ganne-Carrie N, Costentin L, et al. Risk factors for hepatocellular carcinoma in patients with alcoholic or viral C cirrhosis. *Clin Gastroenterol Hepatol* 2006;4:1062-8.
- Zaman SN, Melia WM, Johnson RD, Portmann BC, Johnson PJ, et al. Risk factors in development of hepatocellular carcinoma in cirrhosis: prospective study of 613 patients. *Lancet* 1985;1:1357-60.
- Yu MW, Chang HC, Liaw YF, Lin SM, Lee SD, et al. Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. *J Natl Cancer Inst* 2000;92:1159-64.
- Hemminki K, Li X. Familial liver and gall bladder cancer: a nationwide epidemiological study from Sweden. *Gut* 2003;52:592-6.
- Rich NE, Hester C, Odewole M, Murphy CC, Parikh ND, et al. Racial and ethnic differences in presentation and outcomes of hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2019;17:551-9.e1.
- Herbst DA, Reddy KR. Risk factors for hepatocellular carcinoma. *Clin Liver Dis* 2012;1:180-2.
- Stickel F, Schuppan D, Hahn EG, Seitz HK. Cocarcinogenic effects of alcohol in hepatocarcinogenesis. *Gut* 2002;51:132-9.
- Ganne-Carrié N, Chaffaut C, Bourcier V, Archambeaud I, Perarnau JM, et al. Estimate of hepatocellular carcinoma incidence in patients with alcoholic cirrhosis. *J Hepatol* 2018;69:1274-83.
- Morgan TR, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. *Gastroenterology* 2004;127:S87-96.
- Roerecke M, Vafaei A, Hasan OSM, Chrystoja BR, Cruz M, et al. Alcohol consumption and risk of liver cirrhosis: a systematic review and meta-analysis. *Am J Gastroenterol* 2019;114:1574-86.
- Grewal P, Viswanathan VA. Liver cancer and alcohol. *Clin Liver Dis* 2012;16:839-50.
- Ganne-Carrié N, Nahon P. Hepatocellular carcinoma in the setting of alcohol-related liver disease. *J Hepatol* 2019;70:284-93.
- Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. *JAMA Oncol* 2017;3:1683-91.
- Heckley GA, Jarl J, Asamoah BO, G-Gerdtham U. How the risk of liver cancer changes after alcohol cessation: a review and meta-

- analysis of the current literature. *BMC Cancer* 2011;11:446.
20. Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 2011;141:1572-85.
 21. Jean-Frédéric B, Adelaide D, Marie-Quitterie P, Aline M, Caroline B, et al. Diabetes, HBV infection and smoking are independent risk factors for developing hepatocellular carcinoma on non-fibrotic liver in the NoFLIC French multicenter case-control study. *Hepatology* 2015;62:276A.
 22. Costentin CE, Mourad A, Lahmek P, Causse X, Pariente A, et al. Hepatocellular carcinoma is diagnosed at a later stage in alcoholic patients: results of a prospective, nationwide study. *Cancer* 2018;124:1964-72.
 23. Bucci L, Garuti F, Camelli V, Lenzi B, Farinati F, et al.; Italian Liver Cancer (ITA.LI.CA) Group; Italian Liver Cancer ITA LI CA Group. Comparison between alcohol- and hepatitis C virus-related hepatocellular carcinoma: clinical presentation, treatment and outcome. *Aliment Pharmacol Ther* 2016;43:385-99.
 24. Kumar R, Goh BBG, Kam JW, Chang PE, Tan CK. Comparisons between non-alcoholic steatohepatitis and alcohol-related hepatocellular carcinoma. *Clin Mol Hepatol* 2020;26:196-208.
 25. EASL. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236.
 26. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;68:723-50.
 27. Andersson KL, Salomon JA, Goldie SJ, Chung RT. Cost effectiveness of alternative surveillance strategies for hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2008;6:1418-24.
 28. Bolondi L, Sofia S, Siringo S, Gaiani S, Casali A, et al. Surveillance programme of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost effectiveness analysis. *Gut* 2001;48:251-9.
 29. Singal AG, Lampertico P, Nahon P. Epidemiology and surveillance for hepatocellular carcinoma: new trends. *J Hepatol* 2020;72:250-61.
 30. Singal AG, Yopp AC, Gupta S, Skinner CS, Halm EA, et al. Failure rates in the hepatocellular carcinoma surveillance process. *Cancer Prev Res (Phila)* 2012;5:1124-30.
 31. Thursz M, Gual A, Lackner C, Mathurin P, Moreno C, et al. EASL Clinical Practice Guidelines: Management of alcohol-related liver disease. *J Hepatol* 2018;69:154-81.
 32. Teschke R. Alcoholic liver disease: alcohol metabolism, cascade of molecular mechanisms, cellular targets, and clinical aspects. *Biomedicines* 2018;6:106.
 33. Seitz HK, Stickel F. Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. *Biol Chem* 2006;387:349-60.
 34. Stickel F, Hampe J. Genetic determinants of alcoholic liver disease. *Gut* 2012;61:150-9.
 35. Stickel F, Osterreicher CH. The role of genetic polymorphisms in alcoholic liver disease. *Alcohol Alcohol* 2006;41:209-24.
 36. Homann N, Stickel F, König IR, Jacobs A, Junghanns K, et al. Alcohol dehydrogenase 1C*1 allele is a genetic marker for alcohol-associated cancer in heavy drinkers. *Int J Cancer* 2006;118:1998-2002.
 37. Seitz HK, Stickel F. Molecular mechanisms of alcohol mediated carcinogenesis. *Nat Rev* 2007;7:599-612.
 38. Dasgupta A. Genetic polymorphisms of alcohol metabolizing enzymes associated with protection from or increased risk of alcohol abuse. *Alcohol, Drugs, Genes and the Clinical Laboratory* 2017:107-16.
 39. Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. *Engl J Med* 2018;378:1096-106.
 40. Buch S, Stickel F, Trepo E, Way M, Herrmann A, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. *Nat Genet* 2015;47:1443-8.
 41. Tian C, Stokowski RP, Kershenovich D, Ballinger DG, Hinds DA. Variant in PNPLA3 is associated with alcoholic liver disease. *Nat Genet* 2010;42:21-3.
 42. Trepo E, Guyot E, Ganne-Carrie N, Degre D, Gustot T, et al. PNPLA3 (rs738409 C>G) is a common risk variant associated with hepatocellular carcinoma in alcoholic cirrhosis. *Hepatology* 2012;55:1307-8.
 43. Salameh H, Raff E, Erwin A, Seth D, Nischalke HD, et al. PNPLA3 gene polymorphism is associated with predisposition to and severity of alcoholic liver disease. *Am J Gastroenterol* 2015;110:846-56.
 44. Falletti E, Cusigh A, Cmet S, Fabris C, Toniutto P. PNPLA3 rs738409 and TM6SF2 rs58542926 variants increase the risk of hepatocellular carcinoma in alcoholic cirrhosis. *Dig Liver Dis* 2016;48:69-75.
 45. Seth D, Daly AK, Haber PS, Day CP. Patatin-like phospholipase domain containing 3: a case in point linking genetic susceptibility for alcoholic and nonalcoholic liver disease. *Hepatology* 2010;51:1463-5.
 46. Trépo E, Nahon P, Bontempi G, Valenti L, Falletti E, et al. Association between the PNPLA3 (rs738409 C>G) variant and hepatocellular carcinoma: Evidence from a meta-analysis of individual participant data. *Hepatology* 2014;59.
 47. Chen JH, Wang YY, Lv WB, Gan Y, Chang W, et al. Effects of interactions between environmental factors and KIF1B genetic variants on the risk of hepatocellular carcinoma in a Chinese cohort. *World J Gastroenterol* 2016;22:4183-90.
 48. Nahon P, Cao Q, Guyot E, Rufat P, Sutton A, et al. O054 TM6SF2-T and PNPLA3-G genetic variants co-modulate the risk of hepatocellular carcinoma in caucasian patients with alcoholic cirrhosis. Inter-cohort validation in 1068 patients. *J Hepatol* 2015;62:S218-9.
 49. Chambers JC, Zhang W, Sehmi J, Li X, Wass MN, et al. Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. *Nat Genet* 2011;43:1131-8.
 50. Stickel F, Lutz P, Buch S, Nischalke HD, Silva I, et al. Genetic variation in HSD17B13 reduces the risk of developing cirrhosis and hepatocellular carcinoma in alcohol misusers. *Hepatology* 2019; epub ahead of print. doi: 10.1002/hep.30996
 51. Thursz M, Kamath PS, Mathurin P, Szabo G, Shah VH. Alcohol-related liver disease: Areas of consensus, unmet needs and opportunities

- for further study. *J Hepatol* 2019;70:521-30.
52. Yang J, Trépo E, Nahon P, Cao Q, Moreno C, et al. PNPLA3 and TM6SF2 variants as risk factors of hepatocellular carcinoma across various etiologies and severity of underlying liver diseases. *Int J Cancer* 2019;144:533-44.
 53. Guyot E, Sutton A, Rufat P, Laguillier C, Mansouri A, et al. PNPLA3 rs738409, hepatocellular carcinoma occurrence and risk model prediction in patients with cirrhosis. *J Hepatol* 2013;58:312-8.
 54. Kerr TA, Korenblat KM, Davidson NO. MicroRNAs and liver disease. *Transl Res* 2011;157:241-52.
 55. Xu RH, Wei W, Krawczyk M, Wang W, Luo H, et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. *Nat Mater* 2017;16:1155-61.
 56. Yu JH, Suh YJ, Jin YJ, Yeo NY, Jang JW, et al. Prediction model for hepatocellular carcinoma risk in treatment-naïve chronic hepatitis B patients receiving entecavir/tenofovir. *Eur J Gastroenterol Hepatol* 2019;31:865-72.
 57. Whitfield JB, Schwantes-An TH, Cordell HJ, Darlay R, Masson S, et al. Can genotyping predict risk of alcohol-related cirrhosis in high-risk drinkers? *Hepatology* 2019;70:171A-2.
 58. Anstee QM, Day CP. The genetics of nonalcoholic fatty liver disease: spotlight on PNPLA3 and TM6SF2. *Semin Liver Dis* 2015;35:270-90.
 59. Anstee QM, Seth D, Day CP. Genetic factors that affect risk of alcoholic and nonalcoholic fatty liver disease. *Gastroenterology* 2016;150:1728-44.e7.
 60. Torkamani A, Wineinger NE, Topol EJ. The personal and clinical utility of polygenic risk scores. *Nat Rev Genet* 2018;19:581-90.
 61. Maas P, Barrdahl M, Joshi AD, Auer PL, Gaudet MM, et al. Breast cancer risk from modifiable and nonmodifiable risk factors among white women in the United States. *JAMA Oncol* 2016;2:1295-302.
 62. Lee A, Mavaddat N, Wilcox AN, Cunningham AP, Carver T, et al. BOADICEA: a comprehensive breast cancer risk prediction model incorporating genetic and nongenetic risk factors. *Genet Med* 2019;21:1708-18.
 63. Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015;26:v8-30.
 64. Stickel F, Buch S, Nischalke HD, Weiss KH, Gotthardt D, et al. Genetic variants in PNPLA3 and TM6SF2 predispose to the development of hepatocellular carcinoma in individuals with alcohol-related cirrhosis. *Am J Gastroenterol* 2018;113:1475-83.
 65. Scaggiante B, Kazemi M, Pozzato G, Dapas B, Farra R, et al. Novel hepatocellular carcinoma molecules with prognostic and therapeutic potentials. *World J Gastroenterol* 2014;20:1268-88.
 66. Shang S, Plymoth A, Ge S, Feng Z, Rosen HR, et al. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. *Hepatology* 2011;483-90.
 67. Sieghart W, Wang X, Schmid K, Pinter M, König F, et al. Osteopontin expression predicts overall survival after liver transplantation for hepatocellular carcinoma in patients beyond the Milan criteria. *J Hepatol* 2011;54:89-97.
 68. Zhou SL, Zhou ZJ, Hu ZQ, Song CL, Luo YJ, et al. Genomic sequencing identifies WNK2 as a driver in hepatocellular carcinoma and a risk factor for early recurrence. *J Hepatol* 2019;71:1152-63.
 69. Niu ZS, Niu XJ, Wang WH. Genetic alterations in hepatocellular carcinoma: an update. *World J Gastroenterol* 2016;22:9069-95.
 70. Ye QH, Qin LX, Forgues M, He P, Kim JW, et al. Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. *Nat Med* 2003;9:416-23.
 71. Wahid B, Ali A, Rafique S, Idrees M. New insights into the epigenetics of hepatocellular carcinoma. *Biomed Res Int* 2017;2017:1609575.
 72. Toh TB, Lim JJ, Chow EK. Epigenetics of hepatocellular carcinoma. *Clin Trans Med* 2019;8:13.
 73. Lozano-Rosas MG, Chávez E, Aparicio-Cadena AR, Velasco-Loyden G, Sánchez VC. Mitoepigenetics and hepatocellular carcinoma. *Hepatoma Res* 2018;4:19.
 74. Méndez-Sánchez N, Valencia-Rodríguez A, Vera-Barajas A, Abenavoli L, Scarpellini E, et al. The mechanism of dysbiosis in alcoholic liver disease leading to liver cancer. *Hepatoma Res* 2020;6:5.
 75. Neuman MG, Seitz HK, French SW, Malnick S, Tsukamoto H, et al. Alcoholic-hepatitis, links to brain and microbiome: mechanisms, clinical and experimental research. *Biomedicine* 2020;8:63.
 76. Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2016;2:16018.
 77. Sia D, Jiao Y, Martinez-Quetglas I, Kuchuk O, Villacorta-Martin C, et al. Identification of an immune-specific class of hepatocellular carcinoma, based on molecular features. *Gastroenterology* 2017;153:812-26.
 78. Wheeler DA, Roberts LR. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell* 2017;169:1327-41.e23.
 79. Calderaro J, Couchy G, Imbeaud S, Amaddeo G, Letouze E, et al. Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. *J Hepatol* 2017;67:727-38.
 80. Harding JJ, Nandakumar S, Armenia J, Khalil DN, Albano M, et al. Prospective genotyping of hepatocellular carcinoma: clinical implications of next-generation sequencing for matching patients to targeted and immune therapies. *Clin Cancer Res* 2019;25:2116-26.
 81. Theise ND. Histopathology of alcoholic liver disease. *Clin Liver Dis* 2013;2:64-7.
 82. Lackner C, Tiniakos D. Fibrosis and alcohol-related liver disease. *J Hepatol* 2019;70:294-304.
 83. Ganne-Carrie N, Layese R, Bourcier V, Cagnot C, Marcellin P, et al. Nomogram for individualized prediction of hepatocellular carcinoma occurrence in hepatitis C virus cirrhosis (ANRS CO12 CirVir). *Hepatology* 2016;64:1136-47.
 84. Wong VW, Janssen HL. Can we use HCC risk scores to individualize surveillance in chronic hepatitis B infection? *J Hepatol* 2015;63:722-32.
 85. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, et al. AASLD guidelines for the treatment of hepatocellular carcinoma.

- Hepatology 2018;67:358-80.
86. Fujiwara N, Friedman SL, Goossens N, Hoshida Y. Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. *J Hepatol* 2018;68:526-49.
 87. Kraft P, Wacholder S, Cornelis MC, Hu FB, Hayes RB, et al. Beyond odds ratios--communicating disease risk based on genetic profiles. *Nat Rev Genet* 2009;10:264-9.
 88. Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, et al. The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Res* 2019;47:D1005-12.
 89. Sugrue LP, Desikan RS. What are polygenic scores and why are they important? *JAMA* 2019;321:1820.
 90. Wojcik GL, Graff M, Nishimura KK, Tao R, Haessler J, et al. Genetic analyses of diverse populations improves discovery for complex traits. *Nature* 2019;570:514-8.
 91. Nakagawa S, Wei L, Song WM, Higashi T, Ghoshal S, et al. Molecular liver cancer prevention in cirrhosis by organ transcriptome analysis and lysophosphatidic acid pathway inhibition. *Cancer Cell* 2016;30:879-90.
 92. DiMartini A, Day N, Dew MA, Javed L, Fitzgerald MG, et al. Alcohol consumption patterns and predictors of use following liver transplantation for alcoholic liver disease. *Liver Transpl* 2006;12:813-20.
 93. Stickel F, Moreno C, Hampe J, Morgan MY. The genetics of alcohol dependence and alcohol-related liver disease. *J Hepatol* 2017;66:195-211.
 94. Bjornsson E, Olsson J, Rydell A, Fredriksson K, Eriksson C, et al. Long-term follow-up of patients with alcoholic liver disease after liver transplantation in Sweden: impact of structured management on recidivism. *Scand J Gastroenterol* 2005;40:206-16.
 95. Im GY, Cameron AM, Lucey MR. Liver transplantation for alcoholic hepatitis. *J Hepatol* 2019;70:328-34.
 96. Hunter DJ, Drazen JM. Has the genome granted our wish yet? *Engl J Med* 2019;380:2391-3.
 97. Schork AJ, Schork MA, Schork NJ. Genetic risks and clinical rewards. *Nat Genet* 2018;50:1210-1.

Review

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Comprehending the therapeutic effects of stereotactic body radiation therapy for small hepatocellular carcinomas based on imagings

Zhao-Chong Zeng¹, Jia Fan², Jian Zhou², Meng-Su Zeng³, Yi-Xing Chen¹, Zhi-Feng Wu¹, Jing Sun¹, Jian-Ying Zhang¹, Yong Hu¹, Qian-Qian Zhao¹

¹Department of Radiation Oncology, Zhongshan Hospital, Fudan University, Shanghai 200032, China.

²Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai 200032, China.

³Department of Radiology, Zhongshan Hospital, Fudan University, Shanghai 200032, China.

Correspondence to: Dr. Zhao-Chong Zeng, Department of Radiation Oncology, Zhongshan Hospital, Fudan University, Shanghai 200032, China. E-mail: zeng.zhaochong@zs-hospital.sh.cn

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Abstract

Surgical resection or radiofrequency ablation (RFA) is considered first-choice treatment for small hepatocellular carcinomas (HCCs). When a patient has a small HCC that is inoperable or unsuitable for RFA, what are alternative treatments? Some oncologists recommend transarterial chemoembolization (TACE), chemotherapy, molecular-targeted therapy, or immunotherapy. However, these treatments have minimally beneficial effects in small HCCs. Stereotactic body radiation therapy (SBRT) is a liver-directed radical therapy for small HCCs, with treatment outcomes similar to those for surgical resection or RFA, but many oncologists do not comprehend its efficacy or accept this therapy. We herein discuss 11 typical patients who received SBRT for various indications: refusal to undergo resection or RFA; surgical resection or RFA considered difficult or unfeasible; residual cancer after surgical resection or RFA or incomplete iodized oil retention after TACE; or tumor recurrence after resection or RFA. We describe each case, including the radiation field, tumor radiation dose, and response to SBRT in both the tumor and liver parenchyma. These clinical data should help readers understand this new therapeutic technique. We also conducted a literature review and found evidence to support survival benefit with SBRT, including good three- and five-year overall survival rates. The purpose of this article is to encourage readers to accept the concept that SBRT is a low-toxicity and effective therapeutic option for patients with small HCCs, which offers substantial local control and improved overall survival, especially for patients with a tumor that is unresectable or unsuitable for RFA, residual tumor after local therapy, or intrahepatic recurrent tumor.

Keywords: Small hepatocellular carcinomas, stereotactic body radiation therapy, treatment outcomes, toxicity, imaging changes



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INTRODUCTION

Recent technological advances offer precise and safe radiation delivery to tumors in various areas of the body through imaging guidance. External beam radiation therapy (EBRT) has been recommended as a therapeutic option for HCCs that are considered unresectable according to National Comprehensive Cancer Network guidelines because of the tumor's location, inadequate hepatic reserve, or the presence of comorbidities. SBRT is a type of EBRT technique requiring special equipment for patient positioning and the delivery of high-dose radiation to tumors. There is growing evidence supporting the usefulness of SBRT for patients considered unsuitable for hepatectomy or RFA. However, most oncologists or hepatologists do not understand this treatment technique because of limited use of SBRT in their clinical practice. Herein, we discuss 11 typical patients with HCC to clarify the indications, therapeutic outcomes, treatment-related toxicities, and doses of SBRT, as well as the expected post-SBRT imaging features.

DEFINITIONS

SBRT is an advanced technique of hypofractionated EBRT with photons, which delivers large ablative doses of radiation to tumors. This complex technique relies on the following: (1) stringent control of breathing motion for liver cancer, using four-dimensional computed tomography scans to track respiration-induced hepatic movement; (2) extremely precise patient positioning; and (3) image guidance for radiation delivery.

Early-stage HCC is defined as a solitary tumor with a maximum diameter ≤ 5 cm or multiple nodules (≤ 3 total), each with a maximum diameter ≤ 3 cm, without vascular invasion or extrahepatic metastasis and accompanied by Child-Pugh Class A or B hepatic function (Cases 1-3 and 5). Not all small tumors are classified as early-stage because some have decreased in size after treatment (Case 8). If intrahepatic recurrence (Cases 9 and 10) or Child-Pugh Class C (Case 11) function is present, HCC is considered later stage.

CLINICAL EFFECTIVENESS OF SBRT FOR HCC

There is a growing body of evidence indicating the usefulness of SBRT for the management of patients with HCC. We conducted a literature review and identified several retrospective studies involving the use of SBRT for HCC. These studies have primarily included patients in whom surgical resection or RFA was difficult, unfeasible, or rejected, as well as some patients with intermediate- or advanced-stage HCC. The results of these studies are summarized in Table 1. This table is restricted to studies involving the use of ≤ 10 fractions of SBRT.

Overall survival

As shown in Table 1, overall survival (OS) rates have varied between studies. For early-stage HCC, 2-year OS rates of 78%-80%^[1,2], 3-year OS rates of 66%-73%^[2,3], and a 5-year OS rate of 64% after SBRT have been reported^[3].

Local tumor control

As shown in Table 1, local tumor control rates at one and two years were approximately 95% in most studies, especially those reported in more recent years^[1-13].

Bridging before liver transplantation

SBRT is suitable bridging therapy for patients with HCC awaiting liver transplantation. Sapisochin *et al.*^[14] compared the efficacy of SBRT, TACE, and RFA as a bridge to transplantation in a large cohort of patients with HCC and concluded that SBRT can be a safe alternative to the other, more conventional bridging therapies. However, SBRT has been safer than RFA and TACE when ascites or poor coagulation function are present, as often occurs in patients with underlying liver disease.

Table 1. Outcomes of small-sized liver cancers after SBRT

Authors	Year	HCC status	Cases	Dose to tumors	Response(%)				Overall survival(%)				Local control (%)		
					CR	PR	SD	PD	1-Y	2-Y	3-Y	5-Y	1-Y	2-Y	3-Y
Kimura et al. ^[11]	2018	BCLC 0 53.3%; BCLC A 46.7%, Inoperable or unsuitable RFA, or refusal operation or RFA	28	48Gy/4Fx					78.6				95.4	95.4	
			122	TACE + 48Gy/4Fx					80.3						
Takeda et al. ^[12]	2016	BCLC 0-A 84%, C 16%, Salvage SBRT 42%, initial SBRT 36%, intrahepatic recurrence 22%	90	35Gy/5Fx (10%); 40Gy/5Fx (90%)					95.5	80.0	66.7		98.8	96.3	96.3
Su et al. ^[3]	2016	Max. Ø ≤ 5 cm; BCLC stage A 55.3%, B 44.7%, CP A 86.4%, CP B 13.6%	132	42 - 46Gy/3 - 5Fx					94.1		73.5	64.3	90.9		
Wahl et al. ^[4]	2016	Max Ø < 3 cm 73.1%; 3 cm ≤ Ø < 5 cm 23.2%; Ø ≥ 5 cm 3.7%, CP A 68.7%, B 28.9%, C 2.4%	63	30Gy/3Fx - 50Gy/5Fx					74	46			97.4	83.8	
Huertas et al. ^[5]	2015	Ø ≤ 6cm, CP: A5-B8, ECOG ≤ 2, nodules ≤ 3; AJCC stage I 28.6%, II 68.8%, IIIa 1.3%, IIIb 1.3%	77	45Gy/3Fx, 2Fx/W					81.8	56.6			99	99	
Yamashita et al. ^[6]	2014	AJCC stage I 37%, stage II 27%, stage III 8%, recurrence 14%, no stage: 14%	79	BED10 = 96.3Gy (75-106). 40Gy/4Fx - 60Gy/10Fx	45.6	35.4	11.4	5.1		52.9				74.8	
Lo et al. ^[7]	2014	BCLC A 5.7%; B 11.3%; C 83.0%	53	40Gy/4 - 5Fx	32.8	38.8	23.9	4.5	70.1	45.4			73.3	66.8	
Sanuki et al. ^[8]	2014	≤ 5 cm. T1 84.3%; T2 11.4%; T3 4.3%	185	CP A: 40Gy/5Fx CP B: 35Gy/5Fx					95	83	70		99	93	91
Tekeda et al. ^[9]	2014	T1: 68.3%, T2: 15.9%, T3: 15.8%	63	35 - 40Gy/5Fx	80.7	17.7	1.6	0	100	87	73		100	95	92
Yoon et al. ^[10]	2013	Ø < 6 cm; ≤ 3 nodules; CP A or B; normal liver volume > 700 mL; distance between tumor and GI > 2 cm; 92 pts pre-treatment failure	93	30 - 60Gy/3Fx	51.5	21.4	25.2	0	86	53.8			94.8		92.1
Jang et al. ^[11]	2013	BCLC A: 53%; B: 29%; C: 18%. Ø < 7 cm	82	33 - 60Gy/3Fx						63		39		87	
Bibault et al. ^[12]	2013	BCLC A: 62.7%; B: 13.3%; C: 24%. 51% treated with other therapies	75	24 - 45Gy/3Fx (median 45 Gy)					78.5	50.4			89.8	89.8	
Park et al. ^[13]	2013	Ø < 6 cm; nodules ≤ 3; normal liver volume > 700 mL; tumor between GI > 2 cm	26	40 - 50Gy/4-5Fx	25	42.9	32.1	0	88.5	67.2				87.6	

SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; BCLC: Barcelona Clinic Liver Cancer; RFA: radiofrequency ablation; TACE: transarterial chemoembolization; CP: Child-Pugh Classification; Ø: diameter; ECOG: Eastern Cooperative Oncology Group; AJCC: American Joint Committee on Cancer; BED: biologically equivalent dose; GI: gastrointestinal

RADIATION DOSE

The SBRT dose to HCC has been significantly associated with OS ($P = 0.005$) in multivariate analysis. High-dose SBRT may increase local control and improve OS in patients with inoperable HCC^[11]. In most studies described in the literature, a biologically-equivalent dose (BED) 10 of > 80 Gy has been delivered to the tumors [Table 1]. Of the 11 typical patients discussed in this manuscript, all received BED10 of ≥ 78 Gy and are alive and well. Although no evidence has emerged to clearly support a minimum or maximum dose of SBRT for HCC, we recommend BED10 of ≥ 80 Gy.

INDICATIONS FOR SBRT

SBRT may be an effective therapeutic option for early-stage HCC (as defined above). Patients with early-stage HCC usually undergo surgical resection or RFA attempts, unless contraindicated. However, SBRT can

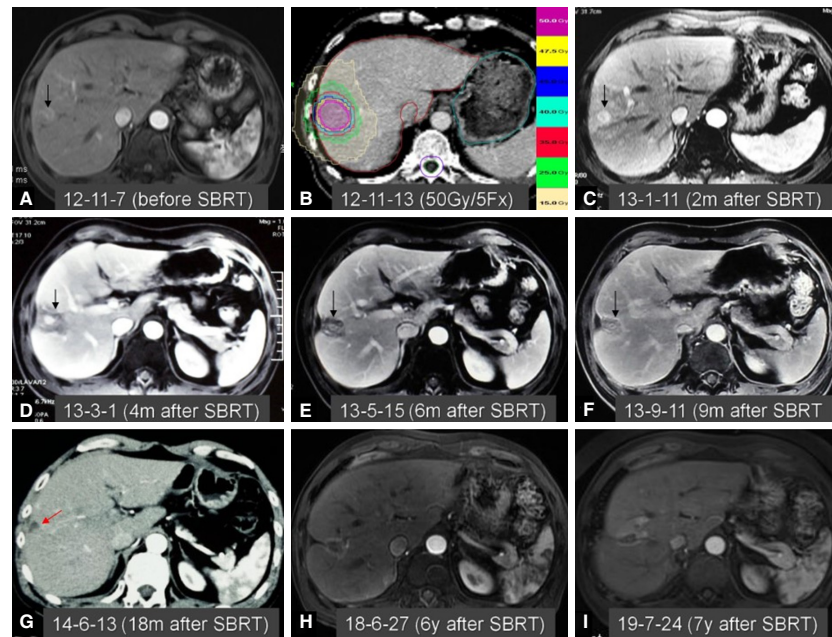


Figure 1. SBRT for HCC in a 59-year-old man who refused surgical resection and RFA. A: Axial arterial phase MRI image showed hyperenhancement of a 1.8-cm liver nodule in the right lobe of the liver; B: the patient received SBRT with a dose of 50 Gy in five fractions; C: axial arterial phase MRI image 2 months after SBRT revealed a stable-size, enhancing lesion (arrowhead). The normal liver parenchyma received 25 Gy and appeared as a low-density area, indicating acute radiation injury; D: axial arterial phase MRI image 4 months after SBRT showed decreased size of the tumor, with atrophy of the perilesional hepatic parenchyma; E: axial arterial phase MRI image 6 months after SBRT showed necrotic changes and a hypovascular target lesion. The perilesional hepatic parenchyma, which received high-dose radiation, also exhibited necrotic changes; F: axial arterial phase MRI image nine months after SBRT demonstrated complete regression of the treated lesion and atrophy of the irradiated hepatic parenchyma; G: axial arterial phase CT image 18 months after SBRT showed a scar in the radiation field. Complete repair of the radiation damage was also observed; H,I: axial arterial phase MRI images 6 years (H) and 7 years (I) after SBRT demonstrated a stable lesion size but the presence of complete necrosis and ring enhancement, indicating a complete radiologic response. Note the SBRT-related changes, which can be differentiated from tumor recurrence. SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; RFA: radiofrequency ablation; MRI: magnetic resonance imaging; CT: computed tomography

be a useful alternative treatment in patients who refuse surgery or RFA. Case 1 [Figure 1] is a typical example of this.

Currently, SBRT is most frequently used when surgical resection or RFA would be difficult or unfeasible, as with tumors located at the center of liver, in the hepatic hilar region, or close to large blood vessels (as in Cases 2 and 3, which are shown in Figures 2 and 3). It is also commonly used in patients who are older [Case 4; Figure 4] or have significant comorbidities, such as poor liver function [Case 5; Figure 5].

SBRT may also be used for residual cancer after surgical resection, as in Case 6 [Figure 6], or after RFA, as in Case 7 [Figure 7]. In these situations, the tumor is often expected to be difficult to treat with surgery or RFA, but these treatments are tried initially. When residual tumor is found during follow-up, SBRT will generally be the most appropriate treatment.

SBRT may serve as adjuvant treatment for intrahepatic tumors with incomplete iodized oil retention, as in Case 8 [Figure 8]. TACE can be used to reduce the size of HCCs; however, it is often considered palliative therapy because of incomplete iodized oil retention by intrahepatic tumors. Adjuvant SBRT can function as consolidation treatment, converting palliative therapy to potentially curative therapy.

SBRT has also been used as salvage treatment for intrahepatic tumor recurrence after RFA or surgical resection [Cases 9 and 10; Figures 9 and 10]. Although metachronous intrahepatic recurrence is sometimes

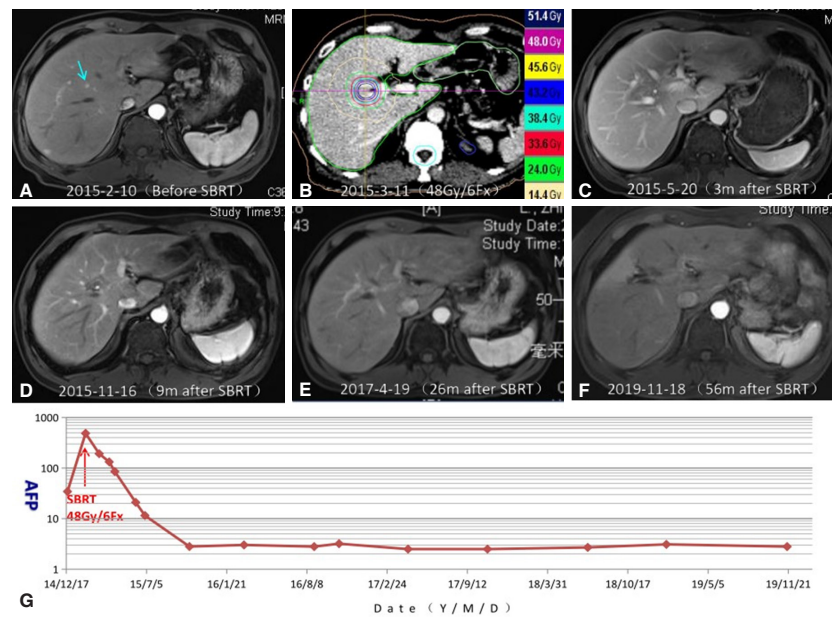


Figure 2. SBRT for very early-stage HCC in a 52-year-old man. Both surgical resection and RFA were considered difficult because the tumor was located in the center of the liver and was too small to be easily detected. A: Axial arterial phase MRI image showed hyper enhancement of an 0.8-cm liver nodule (arrowhead) located in the center of the right lobe of the liver. The patient was clinically diagnosed with very early-stage HCC based on the Barcelona Clinic Liver Cancer staging system; B: the patient received SBRT with a dose of 48 Gy in six fractions; C: axial arterial phase MRI image three months after SBRT demonstrated complete tumor response. Hypodensity in the radiation field (about 30 Gy) indicated the presence of radiation-induced focal liver injury; D: axial arterial phase MRI image 9 months after SBRT showed clear reduction in size of the area of radiation injury; E, F: axial arterial phase MRI images 26 months (E) and 56 months (F) after SBRT revealed complete regression of the tumor lesion; G: serum AFP levels are shown in relation to the treatment timeline. The elevated serum AFP level prior to SBRT dramatically declined to normal ($< 20 \mu\text{g/L}$) after SBRT, and remained within normal limits thereafter. SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; RFA: radiofrequency ablation; MRI: magnetic resonance imaging; AFP: alpha-fetoprotein

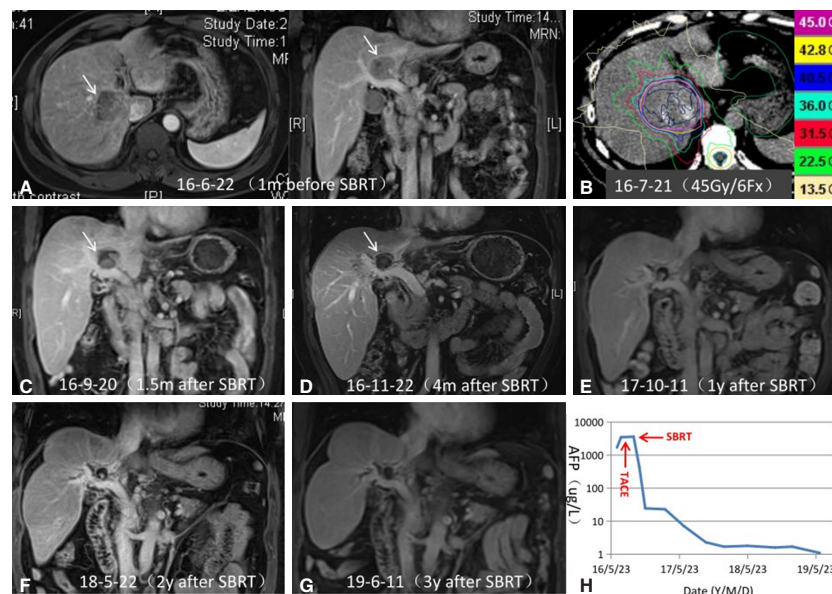


Figure 3. SBRT for unresectable HCC in a 47-year-old man. A: Axial and sagittal MRI images showed a hepatic lesion (arrowhead) near the inferior vena cava and main portal vein. The lesion enhanced in the arterial phase and washed out in the portal venous phase; B: the patient underwent SBRT with a dose of 45 Gy in six fractions; C: arterial phase MRI image 1.5 months after SBRT revealed dramatic regression of the lesion; D: arterial phase MRI image four months after SBRT demonstrated further reduction in size of the lesion, as well as necrosis of the targeted hypovascular lesion, consistent with a nonviable tumor; E-G: MRI images 1 year (E), 2 years (F), and 3 years (G) after SBRT showed progressive reduction in tumor size and complete hypovascularity of the lesion. These findings suggested a good tumor response; H: serum AFP levels are shown in relation to the treatment timeline. The elevated serum AFP level prior to SBRT ($1709 \mu\text{g/L}$) declined to normal ($< 20 \mu\text{g/L}$) after SBRT and remained within normal limits thereafter. SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging; AFP: alpha-fetoprotein

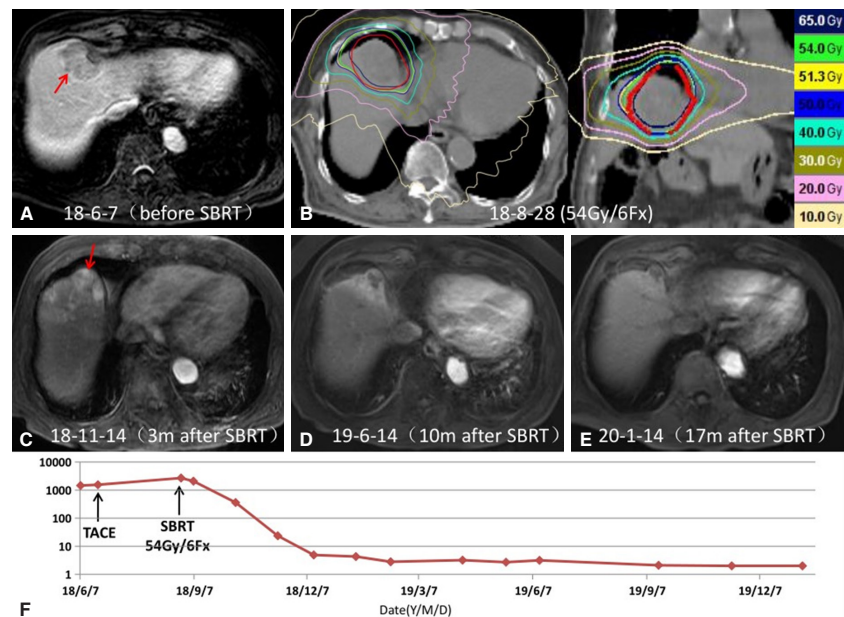


Figure 4. SBRT for HCC in a 99-year-old man for whom SBRT was chosen because of his age and comorbidities. A: Axial venous phase MRI image showed a 6-cm low-density lesion adjacent to the diaphragm (arrow); B: the patient underwent SBRT with a dose of 54 Gy in six fractions; C: axial arterial phase MRI image three months after SBRT demonstrated reduced size of the intrahepatic lesion and vascular enhancement (arrow); D,E: axial arterial phase MRI images 10 months (D) and 17 months (E) after SBRT showed further reduction in size of the tumor, which was hypovascular (arrows). The perilesional liver parenchyma in the enhanced phase appeared as ill-defined areas of enhancement, favoring SBRT-related changes; F: Serum AFP levels are shown in relation to the treatment timeline. AFP levels decreased to normal after SBRT. SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging; AFP: alpha-fetoprotein

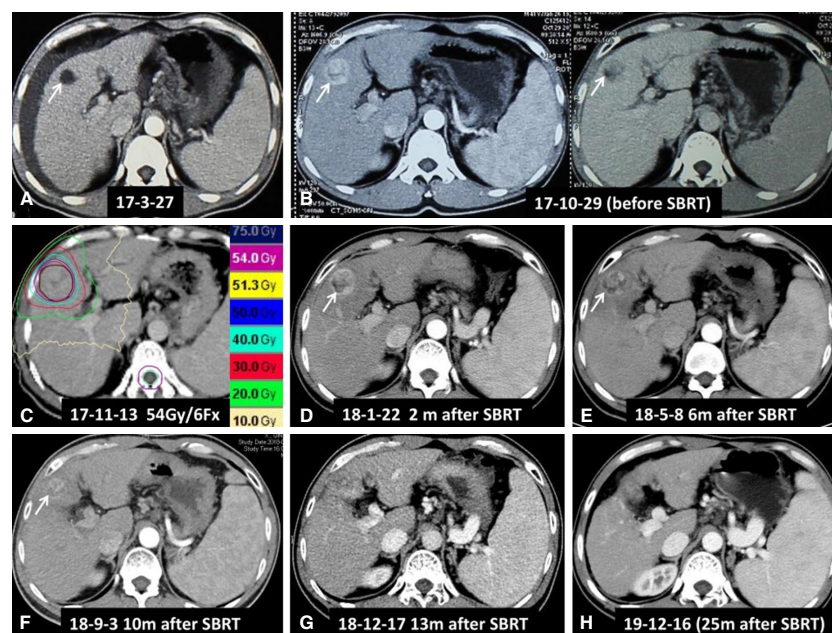


Figure 5. SBRT for HCC consisting of an extensive fat-tissue component in a 41-year-old man with Child-Pugh Class C liver function. A: Axial arterial phase CT image showed a 2-cm nodule containing fat attenuation (arrow). Mild ascites and splenomegaly were also evident; B: multiphasic CT images six months after liver protecting treatment demonstrated enlargement of the lesion to 3 cm (arrow), with enhancement in the arterial phase and washout in the portal venous phase. The patient was diagnosed with HCC with an extensive fat-tissue component. The ascites had disappeared, but splenomegaly was still present; C: the patient underwent SBRT with a dose of 54 Gy in six fractions; D: axial arterial phase CT image two months after SBRT showed the lesion (arrow) with a stable size and partial devascularization; E-G: axial arterial phase CT images 6 months (E), 9 months (F), and 13 months (G) after SBRT demonstrated progressive reduction in tumor size and devascularization of the lesion, consistent with a partial response based on mRECIST criteria; H: axial arterial phase CT image 25 months after SBRT showed complete response of the treated lesion, with marked focal atrophy of the irradiated hepatic parenchyma. SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; CT: computed tomography; mRECIST: modified Response Evaluation Criteria In Solid Tumor

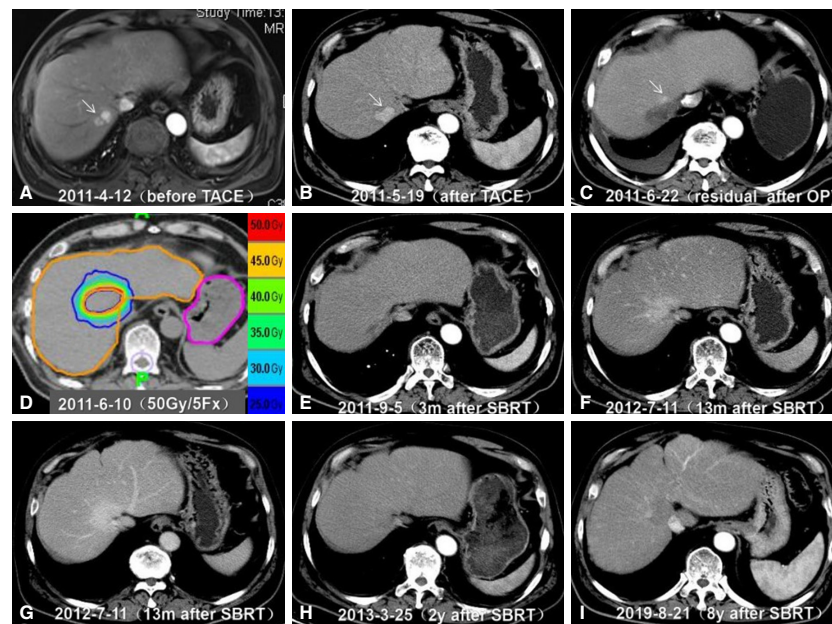


Figure 6. SBRT for residual HCC after surgical resection in a 59-year-old man. A: Axial arterial phase MRI scan 6 years after initial surgery showed a 2.1-cm recurrent hyper-enhancing nodule adjacent to the inferior vein cava, located in the second hepatic hilar segment. The patient underwent TACE for the recurrent lesion; B: axial arterial phase CT image one month after TACE showed a hyper-enhancing lesion (arrow), with no lipiodol deposition. Then, the patient underwent hepatic wedge resection in May 2011; C: axial arterial phase CT image one month after surgery revealed residual tumor with hyper enhancement (arrow); D: the patient underwent SBRT with a dose of 50 Gy in five fractions in June 2011; E: axial arterial phase CT image 3 months after SBRT showed complete response of the target lesion; F,G: Axial arterial (F) and portal venous phases (G) CT images 13 months after SBRT showed persistent enhancement of the radiation field because of post-SBRT changes representing congestion and edema; H,I: Follow-up CT images 2 years (H) and 8 years (I) after SBRT demonstrated a normal liver. SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging; TACE: transarterial chemoembolization; CT: computed tomography

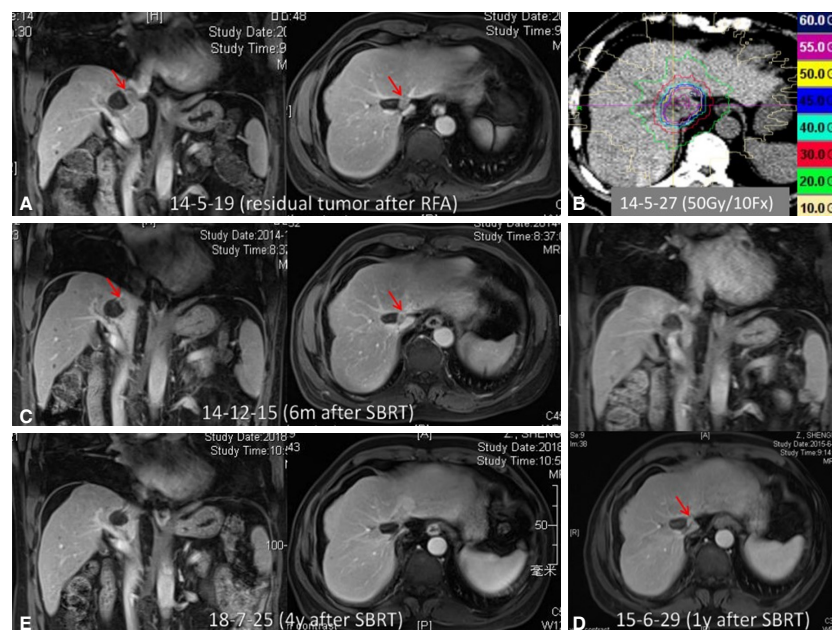


Figure 7. SBRT for residual tumor after RFA in a 66-year-old man. A: Axial and sagittal MRI images approximately 1.5 months after RFA showed a hepatic lesion with a residual cavity and viable tumor (arrowhead) close to the inferior vena cava; B: the patient underwent SBRT with a dose of 50 Gy in 10 fractions for the residual tumor; C-E: MRI images obtained 6 months (C), 1 year (D), and 4 years (E) after SBRT illustrate gradual shrinkage and eventual disappearance of the tumor. SBRT: stereotactic body radiation therapy; RFA: radiofrequency ablation; MRI: magnetic resonance imaging

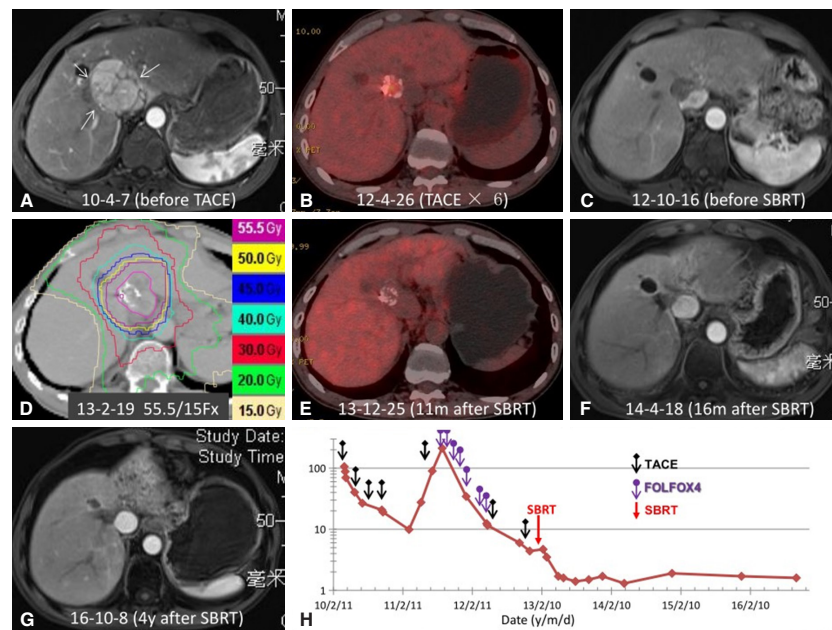


Figure 8. SBRT for residual tumor with incomplete iodized oil retention in a 54-year-old man who had undergone multiple cycles of TACE and chemotherapy. A: Original axial arterial phase MRI image showed a 7.5-cm enhancing mass in the hepatic hilar region, consistent with HCC; B: the lesion decreased substantially in size after six cycles of TACE. FDG PET/CT image demonstrated hypermetabolic activity in the region with incomplete iodized oil retention, consistent with residual viable tumor; C: arterial phase MRI image after multiple TACE cycles and chemotherapy but before SBRT showed no enhancement of the 1.8-cm liver lesion; D: the patient underwent SBRT for residual tumor with a dose of 55.5 Gy in 15 fractions; E: FDG PET/CT image 11 months after SBRT showed no metabolic activity; F: axial arterial phase MRI image 16 months after SBRT demonstrated reduced size and hypovascularity of the treated lesion; G: axial arterial phase MRI image 4 years after SBRT showed stable size and hypovascularity of the treated lesion; H: serum AFP levels are shown in relation to the treatment timeline. AFP further declined to normal after SBRT. SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging; TACE: transarterial chemoembolization; AFP: alpha-fetoprotein; FDG: fludeoxyglucose; PET: positron emission tomography; CT: computed tomography

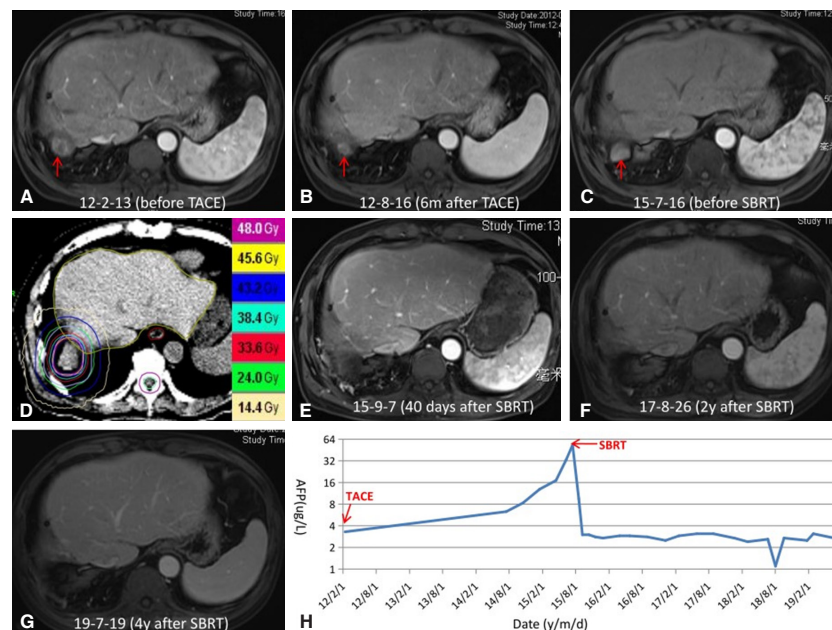


Figure 9. SBRT for recurrent HCC at the surgical margin in a 63-year-old man. A: Axial arterial phase MRI image showed hyper enhancement of a 2.4 cm recurrent focus at the surgical margin (arrowhead). The patient received TACE for this recurrent lesion; B: axial arterial phase MRI image six months after TACE showed reduced size and hypovascularity of the lesion; C: axial arterial phase CT image 3 years after TACE demonstrated enlargement of the treated lesion to 2.9 cm; D: the patient received SBRT with a dose of 48 Gy in six fractions; E-G: CT images 40 days (E), 2 years (F), and 4 years (G) after SBRT showed complete tumor response; H: serum AFP levels are shown in relation to the treatment timeline. The elevated serum AFP prior to SBRT (53 μg/L) declined to normal (2.1 μg/L) after SBRT and remained within normal limits thereafter. SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging; TACE: transarterial chemoembolization; CT: computed tomography; AFP: alpha-fetoprotein

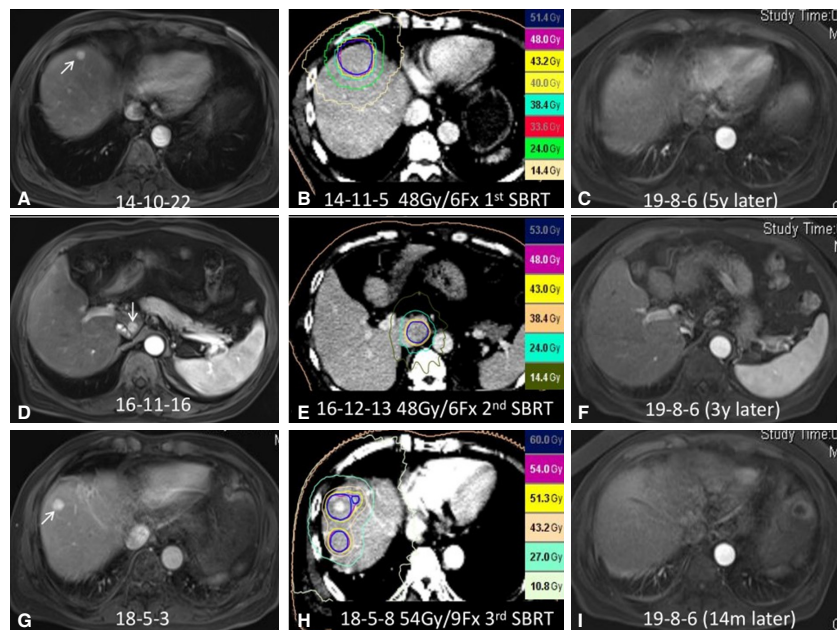


Figure 10. SBRT for repeated de novo HCC in a 63-year-old man with previous surgical resection of HCC. A: Axial arterial phase MRI image showed a 1.2-cm enhancing nodule in the right hepatic lobe, adjacent to the diaphragm; B: the patient received his first course of SBRT with a dose of 48 Gy in six fractions; C: axial arterial phase MRI image 4 years after SBRT demonstrated complete response of this right lobe lesion; D: axial arterial phase MRI image two years after the first SBRT showed a 1-cm enhancing nodule in the caudate lobe of the liver; E: the patient received a second course of SBRT with a dose of 48 Gy in six fractions; F: axial arterial phase MRI image 3 years after the second course of SBRT revealed complete response of the caudate lobe lesion; G: axial arterial phase MRI image 18 months after the second SBRT showed a 1.2-cm enhancing lesion in the right hepatic lobe, as well as an additional lesion at a lower level (not shown), suggesting de novo HCC; H: the patient received a third course of SBRT with a dose of 54 Gy in nine fractions. I: axial arterial phase MRI image one year after this SBRT revealed complete tumor response of the de novo lesion. SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging

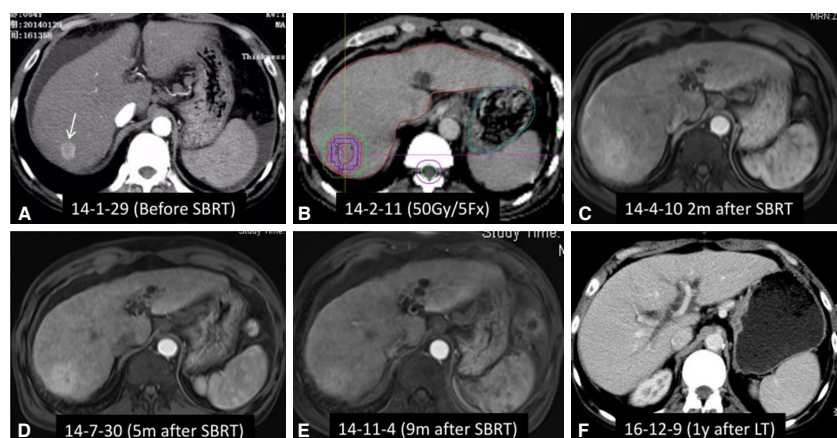


Figure 11. SBRT as a bridge to liver transplantation for HCC in a 55-year-old man with alcohol-related cirrhosis and Child-Pugh Class C liver function. A: Axial arterial phase CT image showed hyper enhancement of a 2 cm mass in the right hepatic lobe (arrowhead), accompanied by moderate ascites. Liver function was scored as Child-Pugh Class C; B: the patient underwent SBRT with a dose of 50 Gy in five fractions; C-E: Axial arterial phase MRI images 1.5 months (C), 5 months (D), and 9 months (E) after SBRT showed complete tumor response. However, focal reaction with enhancement indicated congestion of the hepatic parenchyma in the radiation field. The patient received a liver transplant 22 months after SBRT; F: axial arterial phase CT image one year after liver transplantation demonstrated a normal liver. SBRT: stereotactic body radiation therapy; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging; CT: computed tomography

difficult to distinguish from de novo HCC, a course of SBRT can be considered. In addition, SBRT can be a suitable bridging therapy for patients with HCC awaiting liver transplantation [Case 11; Figure 11].

CONTRAINDICATIONS FOR SBRT IN SMALL HCC

If tumors and luminal structures (esophagus, stomach, duodenum, or intestine) are closely situated at < 1 cm, SBRT is relatively contraindicated for this patient. However, hypofraction imaging-guided radiation therapy could be recommended when HCC is inoperable or unsuitable for RFA.

At least 700 mL of normal liver (Child-Pugh Class A) must receive < 15 Gy. If this condition is not met, we must be careful in choosing such patients.

The safety of liver radiation for HCC in patients with Child-Pugh Class C cirrhosis has not been established, but SBRT could be used as bridging therapy for patients with HCC awaiting liver transplantation.

RESPONSE TO SBRT

HCC

Tumor response rates increase over time after SBRT. Sanuki *et al.*^[15] reported that HCC complete response increased from 24% at three months after SBRT to 67%, 71%, and 93% at 6, 12, and 24 months, respectively, after SBRT^[15]. Using modified Response Evaluation Criteria In Solid Tumor (mRECIST) criteria, complete response occurred within three months after completing SBRT in Cases 9 and 11 and > 9 months after SBRT in Cases 1, 5, and 7. Of note, we prefer to evaluate tumor response to SBRT using European Association for the Study of the Liver (EASL) or mRECIST criteria rather than RECIST criteria^[16]. Case 7, for example, exhibited a complete response according to EASL or mRECIST criteria in the fourth year after completion of SBRT, but only a stable disease and partial response using RECIST criteria, at six months and one year after completing SBRT, respectively. Similarly, Case 5 had a partial response using EASL or mRECIST criteria, but stable disease using RECIST criteria, at six months after completing SBRT.

Liver parenchymal reactions

Early focal liver reaction refers to a surrounding low-intensity area observed on both computed tomography (CT) and magnetic resonance imaging (MRI) scans within six months after completing SBRT. This focal reaction is more visible in patients who undergo initial therapy with SBRT, as shown in Cases 1, 2, and 5.

Delayed focal liver reactions are classified as areas of hyperdensity, isodensity, and hypodensity in all enhanced phases on follow-up MRI or CT > 6 months after SBRT completion^[17,18]. Features of hyperdensity were found in Cases 6 [Figure 6F and G] and 11 [Figure 11D]; features of isodensity were found in Cases 3 [Figure 3E], 7 [Figure 7C and D], and 11 [Figure 11E]; and features of hypodensity were found in Cases 1 [Figure 1E], 2 [Figure 2D], and 5 [Figure 5E].

The incidence of hyperdensity reactions in irradiated hepatic parenchyma may gradually increase after 6 months post-SBRT completion, potentially interfering with accurate assessment of treatment response and being misinterpreted as recurrent tumor. Lack of washout in the delayed phase in hypervascular areas helps distinguish SBRT-related changes from residual or recurrent HCC^[19]. Hyperdensity will typically disappear 2-3 years after treatment, as shown in Case 6 [Figure 6H]. Hypodensity represents the presence of regional liver atrophy within 1-2 years, as shown in Cases 1 [Figure 1F], and 5 [Figure 5H].

The types of focal reaction did not appear to be related to liver function in our cases. The focal liver reaction threshold dose following SBRT for HCC is 30 Gy for livers with Child-Pugh Class A function and 25 Gy for livers with Child-Pugh Class B function, when delivered in five fractions^[20,21]. The doses used in our cases [Figures 1C, 2C, and 5D] were consistent with these thresholds.

TOXICITY

Hepatic damage

A consensus article summarizing the results of 15 previously published studies, including 1063 patients with HCC undergoing SBRT, reported that only eight patients (0.8%) developed Grade 5 liver failure, and most

fatalities from liver failure occurred in patients with Child-Pugh Class B liver function^[22]. However, we have observed no fatal radiation-induced liver disease in our clinical practice.

The safety of SBRT for HCC in patients with Child-Pugh Class C liver function has not been established. Class C function is generally considered a contraindication to all HCC treatment except liver transplantation. No deterioration in liver function occurred after SBRT in Cases 5 and 11, who had Child-Pugh Class C liver function. Furthermore, some patients undergo > 1 course of SBRT, as exemplified by Case 10, who received a total of three courses because of the development of de novo HCCs. Together, these observations suggest that SBRT produces little liver parenchymal damage.

Other toxicities

Gastrointestinal toxicity is another potential concern with SBRT, especially when luminal structures, such as the esophagus, stomach, duodenum, or intestines, are close to the tumors being treated. Grade 3 or higher gastrointestinal toxicities were reported in 1.4% of patients from previously published studies, but fatal gastrointestinal bleeding did not occur^[22]. Other complications, such as rib fractures, chest or abdominal wall pain, biliary stricture, and musculoskeletal discomfort, have been noted occasionally. Overall, most toxicities from SBRT are generally infrequent and mild.

THE LIMITATION OF SBRT

Firstly, there is a lack of randomized clinical trials to compare the treatment outcome among SBRT, resection, and RFA. Thus, SBRT can only be used as an alternative treatment when operation and RFA are impossible. Secondly, vaguely defined SBRT has led to inconsistent radiation doses and fractions globally, with ≤ 5 fractions in the United States and ≤ 10 fractions in the rest of the world. A dose of 55.5 Gy in 15 fractions was delivered to Case 8, and complete response was achieved. This is not strictly part of SBRT; at this point, we are not interested in the definition of SBRT, as we care more about the local control and long-term survival.

CONCLUSION

SBRT is an effective therapeutic option based on proven studies for patients with small HCCs; is complementary to the existing treatment options, as illustrated by the typical cases; and is safe with minimal toxicities.

DECLARATIONS

Authors' contributions

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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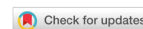
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REFERENCES

- Kimura T, Aikata H, Doi Y, Kimura T, Aikata H, et al. Comparison of stereotactic body radiation therapy combined with or without transcatheter arterial chemoembolization for patients with small hepatocellular carcinoma ineligible for resection or ablation therapies. *Technol Cancer Res Treat* 2018;17:1533033818783450.
- Takeda A, Sanuki N, Tsurugai Y, Iwabuchi S, Matsunaga K, et al. Phase 2 study of stereotactic body radiotherapy and optional transarterial chemoembolization for solitary hepatocellular carcinoma not amenable to resection and radiofrequency ablation. *Cancer* 2016;122:2041-9.
- Su TS, Liang P, Lu HZ, Liang J, Gao YC, et al. Stereotactic body radiation therapy for small primary or recurrent hepatocellular carcinoma in 132 Chinese patients. *J Surg Oncol* 2016;113:181-7.
- Wahl DR, Stenmark MH, Tao Y, Pollom EL, Caoili EM, et al. Outcomes after stereotactic body radiotherapy or radiofrequency ablation for hepatocellular carcinoma. *J Clin Oncol* 2016;34:452-9.
- Huertas A, Baumann AS, Saunier-Kubs F, Salleron J, Oldrini G, et al. Stereotactic body radiation therapy as an ablative treatment for inoperable hepatocellular carcinoma. *Radiother Oncol* 2015;115:211-6.
- Yamashita H, Onishi H, Matsumoto Y, Murakami N, Matsuo Y, et al. Local effect of stereotactic body radiotherapy for primary and metastatic liver tumors in 130 Japanese patients. *Radiat Oncol* 2014;9:112.
- Lo CH, Huang WY, Lee MS, Lin KT, Lin TP, et al. Stereotactic ablative radiotherapy for unresectable hepatocellular carcinoma patients who failed or were unsuitable for transarterial chemoembolization. *Eur J Gastroenterol Hepatol* 2014;26:345-52.
- Sanuki N, Takeda A, Oku Y, Mizuno T, Aoki Y, et al. Stereotactic body radiotherapy for small hepatocellular carcinoma: a retrospective outcome analysis in 185 patients. *Acta Oncol* 2014;53:399-404.
- Takeda A, Sanuki N, Eriguchi T, Kobayashi T, Iwabuchi S, et al. Stereotactic ablative body radiotherapy for previously untreated solitary hepatocellular carcinoma. *J Gastroenterol Hepatol* 2014;29:372-9.
- Yoon SM, Lim YS, Park MJ, Kim SY, Cho B, et al. Stereotactic body radiation therapy as an alternative treatment for small hepatocellular carcinoma. *PLoS One* 2013;8:e79854.
- Jang WI, Kim MS, Bae SH, Cho CK, Yoo HJ, et al. High-dose stereotactic body radiotherapy correlates increased local control and overall survival in patients with inoperable hepatocellular carcinoma. *Radiat Oncol* 2013;8:250.
- Bibault JE, Dewas S, Vautravers-Dewas C, Hollebecque A, Jarraya H, et al. Stereotactic body radiation therapy for hepatocellular carcinoma: prognostic factors of local control, overall survival, and toxicity. *PLoS One* 2013;8:e77472.
- Park JH, Yoon SM, Lim YS, Kim SY, Shim JH, et al. Two-week schedule of hypofractionated radiotherapy as a local salvage treatment for small hepatocellular carcinoma. *J Gastroenterol Hepatol* 2013;28:1638-42.
- Sapisochin G, Barry A, Doherty M, Fischer S, Goldaracena N, et al. Stereotactic body radiotherapy vs. TACE or RFA as a bridge to transplant in patients with hepatocellular carcinoma. An intention-to-treat analysis. *J Hepatol* 2017;67:92-9.
- Sanuki N, Takeda A, Mizuno T, Oku Y, Eriguchi T, et al. Tumor response on CT following hypofractionated stereotactic ablative body radiotherapy for small hypervascular hepatocellular carcinoma with cirrhosis. *AJR Am J Roentgenol* 2013;201:W812-20.
- Oldrini G, Huertas A, Renard-Oldrini S, Taste-George H, Vogin G, et al. Tumor response assessment by MRI following stereotactic body radiation therapy for hepatocellular carcinoma. *PLoS One* 2017;12:e0176118.
- Sanuki-Fujimoto N, Takeda A, Ohashi T, Taste-George H, Vogin G, et al. CT evaluations of focal liver reactions following stereotactic body radiotherapy for small hepatocellular carcinoma with cirrhosis: relationship between imaging appearance and baseline liver function. *Br J Radiol* 2010;83:1063-71.
- Kimura T, Takahashi S, Takahashi I, Nishibuchi I, Doi Y, et al. The time course of dynamic computed tomographic appearance of radiation injury to the cirrhotic liver following stereotactic body radiation therapy for hepatocellular carcinoma. *PLoS One* 2015;10:e0125231.
- Park MJ, Kim SY, Yoon SM, Kim JH, Park SH, et al. Stereotactic body radiotherapy-induced arterial hypervascularity of nontumorous hepatic parenchyma in patients with hepatocellular carcinoma: potential pitfalls in tumor response evaluation on multiphase computed tomography. *PLoS One* 2014;9:e90327.
- Sanuki N, Takeda A, Oku Y, Eriguchi T, Nishimura S, et al. Threshold doses for focal liver reaction after stereotactic ablative body radiation therapy for small hepatocellular carcinoma depend on liver function: evaluation on magnetic resonance imaging with Gd-EOB-DTPA. *Int J Radiat Oncol Biol Phys* 2014;88:306-11.
- Takeda A, Oku Y, Sanuki N, Kunieda E, Koike N, et al. Dose volume histogram analysis of focal liver reaction in follow-up multiphase CT following stereotactic body radiotherapy for small hepatocellular carcinoma. *Radiother Oncol* 2012;104:374-8.
- Zeng ZC, Seong J, Yoon SM, Cheng JC, Lam KO, et al. Consensus on stereotactic body radiation therapy for small-sized hepatocellular carcinoma at the 7th Asia-Pacific Primary Liver Cancer Expert Meeting. *Liver Cancer* 2017;6:264-74.

Review

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Imaging assessment after SBRT for hepatocellular carcinoma

Connie Yip¹, Tiffany Priyanthi Hennedige², Gary J. R. Cook³, Vicky Goh³

¹Radiation Oncology, National Cancer Centre Singapore, Singapore 169610, Singapore.

²Oncologic Imaging, National Cancer Centre Singapore, Singapore 169610, Singapore.

³Cancer Imaging, King's College London, London WC2R 2LS, United Kingdom.

Correspondence to: Dr. Connie Yip, Radiation Oncology, National Cancer Centre Singapore, Singapore 169610, Singapore. E-mail: connie.yip.s.p@singhealth.com.sg

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Abstract

The use of stereotactic body radiotherapy (SBRT) in hepatocellular carcinoma (HCC) has increased over the past few decades. Thus, accurate evaluation of post-SBRT treatment response is essential to avoid over-treatment of responders as well as missing the opportunity to salvage non-responders. There are some intricate imaging differences after liver SBRT compared to those observed after conventional fractionated radiotherapy and other locoregional treatment. We aim to review the imaging changes that occur following SBRT for HCC and their potential clinical implications.

Keywords: Imaging, liver, stereotactic body radiotherapy, hepatocellular carcinoma

INTRODUCTION

Radiation therapy for liver malignancies has evolved over the past few decades. In the past, radiation was predominantly used as a palliative modality due to the limited tolerance of whole liver irradiation. However, with the technological advances achieved with improved resolution of on-board imaging and the ability to deliver highly conformal radiotherapy, we were then able to irradiate liver tumours with high precision and limited bystander damage to normal tissues. Stereotactic body radiotherapy (SBRT) is characterised by high dose per fraction, typically in the range of 5-25 Gy over 1-10 fractions, which is enabled by accurate tumour localisation using daily image guidance. The adoption of SBRT has increased exponentially in primary and secondary liver malignancies with promising local control rates and favourable toxicity profiles^[1-5]. SBRT has also been shown to be a useful local therapy to bridge patients awaiting transplant in primary hepatocellular carcinoma (HCC)^[6].



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Table 1. The different imaging treatment response evaluation criteria for HCC

Treatment response	RECIST 1.1	mRECIST	EASL
Tumour measurements	Uni-dimensional of target lesions	Uni-dimensional of viable tumours (arterial phase enhancement)	Bi-dimensional of viable tumours (arterial phase enhancement)
Number of lesions	2 per organ	2 per organ	Not specified
Complete response (CR)	Disappearance of all target lesions	Disappearance of any intratumoral arterial enhancement in all target lesions	Disappearance of any intratumoral arterial enhancement in all target lesions
Partial response (PR)	≥ 30% reduction in sum of longest diameters of target lesions	≥ 30% reduction in sum of longest diameters of viable target lesions	≥ 50% reduction in sum of the product of bi-dimensional diameters of viable target lesions
Progressive disease (PD)	≥ 20% increase in sum of longest diameters of target lesions	≥ 20% increase in sum of longest diameters of viable target lesions	≥ 25% increase in sum of the product of bi-dimensional diameters of viable target lesions
Stable disease (SD)	Does not meet PR or PD	Does not meet PR or PD	Does not meet PR or PD

HCC: Hepatocellular carcinoma

Nonetheless, treatment response assessment after high ablative doses of radiation can be challenging due to the different radiation dose deposition profiles compared to conventional fractionated radiotherapy. Here, we aim to review the imaging characteristics associated with SBRT in HCC and their implications in our clinical practice.

HISTOPATHOLOGICAL CHANGES AFTER RADIATION

During the acute phase, which is typically defined as less than 3-4 months after radiation, there is deposition of fibrin and subsequently collagen within the centrilobular venules causing obliteration of these small vessels with relative sparing of the larger veins and arterioles. This causes reactive hyperaemia and also hepatic cell loss within the liver^[7,8]. This is collectively known as veno-occlusive disease. In the chronic phase, there is significant reduction of hyperaemia with some degree of hyperplasia and parenchymal fibrosis^[8].

IMAGING RESPONSE ASSESSMENT CRITERIA

Although RECIST 1.1 is a universally accepted set of radiological response evaluation criteria in solid tumours^[9], these criteria do not apply well in HCC as they are based solely on uni-dimensional tumour measurements. Several other response evaluation criteria, which are more sensitive for HCC, have been proposed, including the EASL and modified RECIST (mRECIST) criteria [Table 1]^[10,11]. The EASL criteria use bi-dimensional measurements of viable enhancing tumours, whereas mRECIST uses uni-dimensional measurement of viable tumours. There are a few studies that compared the different evaluation criteria after SBRT treatment for HCC but no definite conclusions could be drawn regarding the most optimal criteria to use in clinical practice^[12,13]. Of interest, one study correlated radiological response to pathological response in 38 patients who underwent orthotopic liver transplants for HCC^[14]. The radiological criteria used included EASL, RECIST and mRECIST. All radiological response criteria compared poorly against the actual pathological response. The positive predictive values and negative predictive values were 73%/29% (EASL), 71%/32% (RECIST) and 74%/40% (mRECIST), respectively. Both computed tomography (CT) agreement (22%-39%) and magnetic resonance imaging (MRI) agreement (31%-39%) with pathologic findings were poor, irrespective of the imaging criteria used. This highlighted the difficulty of using imaging to predict pathological treatment response after SBRT.

EARLY IMAGING CHANGES

Similar to the histopathological changes described above, corresponding imaging changes can be observed during the acute post-SBRT phase. One important point to remember is that the conventional well-defined radiation field edges observed with the use of two- or three-dimensional radiation techniques are no longer seen in SBRT treatment, which uses multiple, often non-coplanar, radiation fields.



Figure 1. Contrast-enhanced computed tomography at 3 months after stereotactic body radiotherapy showing reactive hyperaemia in the surrounding irradiated non-tumorous liver parenchyma

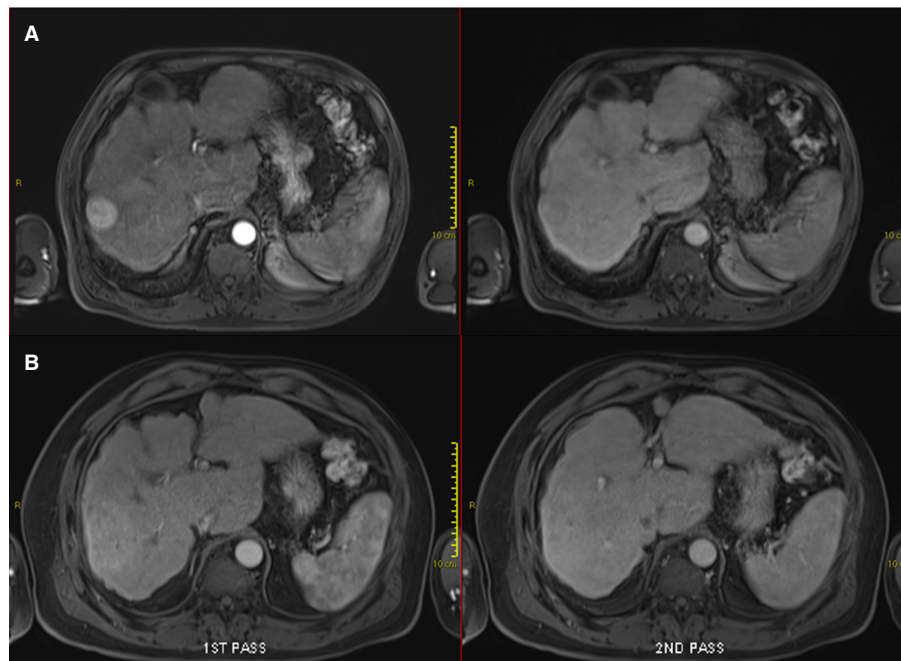


Figure 2. Contrast-enhanced magnetic resonance imaging demonstrating early imaging changes before and after stereotactic body radiotherapy (SBRT): pre-SBRT showing typical arterially enhancing lesion with subsequent wash-out (A); and 3 months after SBRT showing the surrounding arterial enhancement without subsequent wash-out observed, indicative of reactive hyperaemia (B)

During the very early phase, hepatic oedema occurs due to decreased venous outflow secondary to centrilobular obstruction. This is manifested as hypoattenuation on unenhanced CT and hyper-attenuation on contrast-enhanced CT^[15]. On MRI, this is manifested as low signal on T1-weighted images with high signal on T2-weighted images. On diffusion-weighted imaging (DWI), there may be mild restricted diffusion and a slight increase in apparent diffusion coefficient (ADC). Periportal oedema can also occur, although this is short-lived and not specific to radiation therapy. On MRI, this can be observed as widening of periportal space with mild high signal intensity on T2-weighted images^[16].

Following that, reactive hyperaemia is often seen in the surrounding irradiated non-tumorous liver parenchyma as early as 1 month after treatment and peaks at approximately 3 months^[17,18] [Figures 1 and 2]. This focal liver reaction could be difficult to differentiate from residual tumour, a phenomenon also known as “pseudoprogression”. However, reactive arterial hypervascularity is usually followed by no delayed phase washout as opposed to viable tumours^[17]. This is a useful tip during SBRT response assessment to avoid

misdiagnosing this reactive feature as tumour progression. Focal steatosis may also occur, which is typified by decreased attenuation on CT and signal drop on opposed-phase MRI^[16]. In rare circumstances, temporary biliary dilatation is observed, which tends to resolve after a few months^[16].

LATE IMAGING CHANGES

Months after SBRT, the injured hepatocytes are gradually replaced by fibrosis. CT is not usually sensitive in detecting liver fibrosis, although perfusion CT may be more useful for this purpose^[19]. This chronic effect can be better appreciated on MRI with low signal on T1- and T2-weighted images. During the earlier stages of fibrosis, moderate arterial enhancement is observed, which persists in the delayed phase. As this progresses, arterial enhancement diminishes but progressive enhancement in the delayed phase persists^[16].

In most cases, loss of liver volume is observed in the initial 3 months after SBRT. This is followed by subsequent regeneration and increase in liver volume. However, liver atrophy may also occur in some cases with capsular retraction^[16] [Figure 3]. In contrast to the early focal steatosis observed, focal sparing of fatty liver (e.g., in pre-existing fatty liver or post-chemotherapy liver changes) may occur in the months following SBRT due to loss of fat in the hepatocytes^[16].

Finally, tumour response after SBRT can be challenging, as described above. Tumour shrinkage may not happen in the immediate few months after SBRT. Nonetheless, even in the absence of volumetric reduction, progressive reduction in tumour enhancement is consistent with treatment response. This is typically accompanied by a corresponding increase in ADC^[16]. An increment in ADC of 20%-25% was found to be an indicator of SBRT response in a few small retrospective studies^[20,21] [Figure 4]. However, the authors did not use pathological response as the study end-point, and hence are subjected to the same challenges as described with the use of radiological response criteria as an end-point. Furthermore, there is as yet no standardisation of DWI acquisition and interpretation, which hinders its use as a standard response evaluation criterion.

ROLE OF POSITRON EMISSION TOMOGRAPHY

¹⁸F-Fluorodeoxyglucose positron emission tomography (¹⁸F-FDG PET) is not recommended in the staging of early HCC due to its low sensitivity in detecting low-grade, well-differentiated HCC against the background liver activity, precluding an accurate primary tumour assessment. Nonetheless, it is more sensitive in detecting extrahepatic disease compared to local tumour staging^[22]. To our knowledge, there is no study that evaluated the role of ¹⁸F-FDG PET in treatment response assessment after SBRT for primary HCC. However, baseline ¹⁸F-FDG PET maximum SUV value (SUV_{max}) > 3.2 was found to be associated with higher risk of local failure in a cohort of HCC patients treated with SBRT^[23].

Other radionuclides being evaluated in HCC include ¹⁸F-Fluorocholine (¹⁸F-FCH) that is a biomarker for phosphocholine, which is a major metabolite in cancer cells, and ¹¹C-acetate^[24]. ¹⁸F-FCH is reported to have a high sensitivity, approaching 90%, in detecting HCC^[25]. A decrease of ¹⁸F-FCH SUV_{max} > 45% was shown to be associated with longer progression-free survival and improved mRECIST response in patients treated with various locoregional therapies, including SBRT for HCC^[26].

The availability of PET/MR imaging is slowly increasing in some parts of the world. This could be a promising tool in HCC combining the superior MRI soft tissue and primary tumour definition, functional aspects of MRI such as DWI and the superior sensitivity of PET in extrahepatic staging.

PREDICTION OF LIVER TOXICITIES

Although SBRT is relatively well tolerated in most patients, the risk of radiation-induced liver disease (RILD) cannot be underestimated in this group of patients who have pre-existing cirrhosis, particularly those with

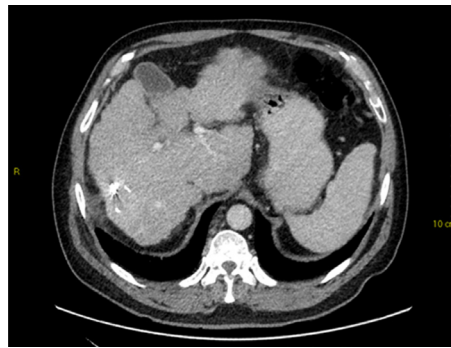


Figure 3. Contrast-enhanced computed tomography at 1 year after stereotactic body radiotherapy showing volume loss with capsular retraction (note fiduciary clips *in-situ*)

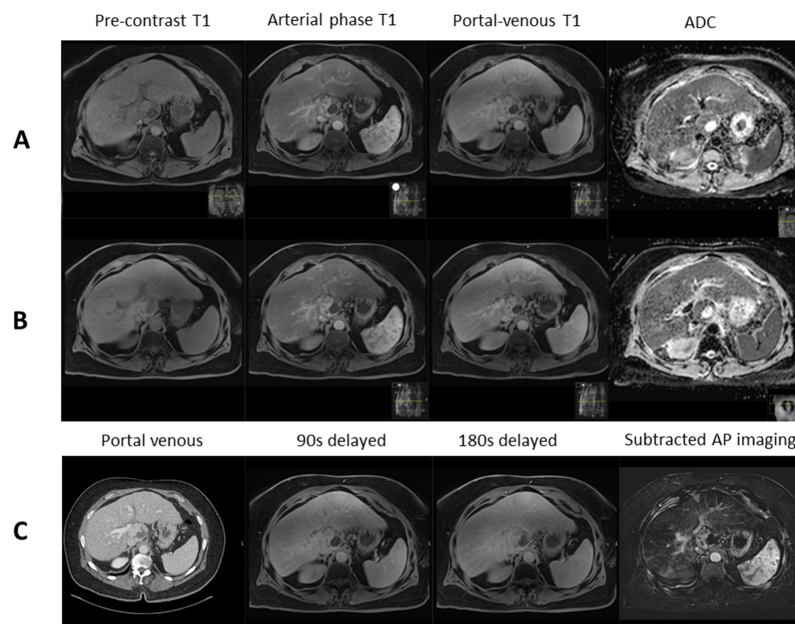


Figure 4. Contrast-enhanced magnetic resonance imaging demonstrating: pre-treatment caudate lobe hepatocellular carcinoma with restricted diffusion ($ADC\ 1101 \times 10^{-6}$) (A); post-stereotactic body radiotherapy scan showing no treatment response with restricted diffusion ($ADC\ 917 \times 10^{-6}$) (B); and corresponding contrast-enhanced computed tomography and delayed imaging with hepatobiliary contrast agent showing perfusional changes around the tumour with no response (C). ADC: apparent diffusion coefficient

Child-Pugh B liver dysfunction. Classical RILD, which is characterised by weight gain, hepatomegaly, anicteric ascites and isolated elevation of alkaline phosphatase (ALP) out of proportion to other liver enzymes, is not usually seen following liver SBRT. In contrast, non-classical RILD, which is characterised by markedly elevated transaminases and jaundice, is more commonly seen after partial liver irradiation such as SBRT^[27].

A combination of clinical and/or biochemical criteria such as Child-Pugh and Model for End-stage Liver Disease (MELD) scores are routinely used as a measure of the severity of cirrhosis in the clinical setting. In addition, serum indocyanine green (ICG) clearance is also widely used to evaluate liver function in clinical practice, although it is a direct measurement of neither the hepatic synthetic nor conjugative function^[28]. ^{99m}Tc-Sulphur Colloid single-photon emission computed tomography (^{99m}Tc-SC SPECT) is considered a liver function imaging tool. ^{99m}Tc-SC is taken by Kupffer cells which have similar perfusion as hepatocytes, thus making it a surrogate of perfused hepatocytes^[29]. Quantitative ^{99m}Tc-SC SPECT uptake was found to have

significant correlations with ICG retention at 15 min (ICG-R15) ($r = -0.84$, $P < 0.0001$)^[29] and clinical Child-Pugh status^[30].

Hepatocyte-specific MRI contrast agents, such as gadoxetate acid (Gd-EOB DTPA), also have the potential to provide information on liver function besides its role as a diagnostic tool in HCC^[31,32]. The rate of T1 relaxation time reduction and T1 map quantifications have been shown to correlate well with ICG clearance^[31-33]. However, its use in this setting requires additional research and validation.

We anticipate that functional liver imaging will play a complementary role to existing cirrhosis severity evaluation criteria, particularly in providing valuable spatial information which can be used to guide HCC treatment. One exciting area of research is the prospect of sparing functional liver as depicted on cross-sectional imaging during radiation planning, which may allow us to reduce the risk of RILD even more^[34].

PROPOSED IMAGING ALGORITHM

We propose that response evaluation should be performed at 3 months after SBRT using multi-phasic CT or MRI. The latter should include DWI sequence and the use of hepatocyte-specific contrast agents should be considered. This should continue 3 monthly in the first year, 3-6 monthly in the second year and 6-12 monthly in the subsequent years depending on the clinical needs. However, the absence of volumetric shrinkage or presence of residual arterial enhancement in the first few months after SBRT should not be deemed tumour relapse unless there are other unequivocal radiological and/or clinical signs of progression. We suggest that early interval imaging should be performed in these cases to confirm disease progression if clinical situation permits.

CONCLUSION

Early post-SBRT imaging features include hepatic and periportal oedema as well as reactive hyperaemia in the surrounding irradiated liver parenchyma. The latter may mimic disease progression, although there is usually no associated delayed washout observed in these cases. In the subsequent months after SBRT, there is hepatic fibrosis which may be followed by atrophy and capsular retraction. PET imaging is currently not routinely used in HCC, although ¹⁸F-FDG PET may have a role for extrahepatic staging, particularly in high-grade HCC. ^{99m}Tc-SC SPECT can also be considered to assess liver function before and after SBRT.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception, design of the study and performed data interpretation: Yip C
Made substantial contributions to the design of the study and data interpretation: Henedige TP, Cook GJR, Goh V

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REFERENCES

1. Méndez Romero A, Wunderink W, Hussain SM, De Pooter JA, Heijmen BJM, et al. Stereotactic body radiation therapy for primary and metastatic liver tumors: a single institution phase I-II study. *Acta Oncol* 2006;45:831-7.
2. Bujold A, Massey CA, Kim JJ, Brierley J, Cho C, et al. Sequential phase I and II trials of stereotactic body radiotherapy for locally advanced hepatocellular carcinoma. *J Clin Oncol* 2013;31:1631-9.
3. Culleton S, Jiang H, Haddad CR, Kim J, Brierley J, et al. Outcomes following definitive stereotactic body radiotherapy for patients with Child-Pugh B or C hepatocellular carcinoma. *Radiother Oncol* 2014;111:412-7.
4. Andratschke N, Alheid H, Allgäuer M, Becker G, Blanck O, et al. The SBRT database initiative of the German Society for Radiation Oncology (DEGRO): patterns of care and outcome analysis of stereotactic body radiotherapy (SBRT) for liver oligometastases in 474 patients with 623 metastases. *BMC Cancer* 2018;18:283.
5. Scorsetti M, Comito T, Clerici E, Franzese C, Tozzi A, et al. Phase II trial on SBRT for unresectable liver metastases: long-term outcome and prognostic factors of survival after 5 years of follow-up. *Radiat Oncol* 2018;13:234.
6. Sapisochin G, Barry A, Doherty M, Fischer S, Goldaracena N, et al. Stereotactic body radiotherapy vs. TACE or RFA as a bridge to transplant in patients with hepatocellular carcinoma. An intention-to-treat analysis. *J Hepatol* 2017;67:92-9.
7. Fajardo LF. The pathology of ionizing radiation as defined by morphologic patterns. *Acta Oncol* 2005;44:13-22.
8. Reed GB, Cox AJ. The human liver after radiation injury. A form of veno-occlusive disease. *Am J Pathol* 1966;48:597-611.
9. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45:228-47.
10. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL Conference. *J Hepatol* 2001;35:421-30.
11. Lencioni R, Llovet J. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010;30:52-60.
12. Vernuccio F, Godfrey D, Meyer M, Williamson H V., Salama JK, et al. Local tumor control and patient outcome using stereotactic body radiation therapy for hepatocellular carcinoma: iRECIST as a potential substitute for traditional criteria. *Am J Roentgenol* 2019;213:1232-9.
13. Price TR, Perkins SM, Sandrasegaran K, Henderson MA, Maluccio MA, et al. Evaluation of response after stereotactic body radiotherapy for hepatocellular carcinoma. *Cancer* 2012;118:3191-8.
14. Mannina EM, Cardenes HR, Lasley FD, Goodman B, Zook J, et al. Role of Stereotactic body radiation therapy before orthotopic liver transplantation: retrospective evaluation of pathologic response and outcomes. *Int J Radiat Oncol* 2017;97:931-8.
15. Takamatsu S, Kozaka K, Kobayashi S, Yoneda N, Yoshida K, et al. Pathology and images of radiation-induced hepatitis: a review article. *Jpn J Radiol* 2018;36:241-56.
16. Lall C, Bhargava P, Sandrasegaran K, Shanbhogue AK, Ramsinghani N, et al. Three-dimensional conformal radiation therapy in the liver: MRI findings along a time continuum. *J Comput Assist Tomogr* 2015;39:356-64.
17. Park MJ, Kim SY, Yoon SM, Kim JH, Park SH, et al. Stereotactic body radiotherapy-induced arterial hypervascularity of non-tumorous hepatic parenchyma in patients with hepatocellular carcinoma: potential pitfalls in tumor response evaluation on multiphase computed tomography. *PLoS One* 2014;9:e90327.
18. Kimura T, Takahashi S, Takahashi I, Nishibuchi I, Doi Y, et al. The time course of dynamic computed tomographic appearance of radiation injury to the cirrhotic liver following stereotactic body radiation therapy for hepatocellular carcinoma. *PLoS One* 2015;10:e0125231.
19. Ronot M, Asselah T, Paradis V, Michoux N, Dorvillius M, et al. Liver fibrosis in chronic hepatitis C virus infection: Differentiating minimal from intermediate fibrosis with perfusion CT. *Radiology* 2010;256:135-42.
20. Yu JJ, Park HC, Lim DH, Choi Y, Jung SH, et al. The role of diffusion-weighted magnetic resonance imaging in the treatment response evaluation of hepatocellular carcinoma patients treated with radiation therapy. *Int J Radiat Oncol* 2014;89:814-21.
21. Lo CH, Huang WY, Hsiang CW, Lee MS, Lin CS, et al. Prognostic significance of apparent diffusion coefficient in hepatocellular carcinoma patients treated with stereotactic ablative radiotherapy. *Sci Rep* 2019;9:14157.
22. Lee SM, Kim HS, Lee S, Lee JW. Emerging role of 18 F-fluorodeoxyglucose positron emission tomography for guiding management of hepatocellular carcinoma. *World J Gastroenterol* 2019;25:1289-306.
23. Huang WY, Kao CH, Huang WS, Chen CM, Chang LP, et al. 18F-FDG PET and combined 18F-FDG-contrast CT parameters as predictors of tumor control for hepatocellular carcinoma after stereotactic ablative radiotherapy. *J Nucl Med* 2013;54:1710-6.
24. Lu RC, She B, Gao WT, Ji YH, Xu DD, et al. Positron-emission tomography for hepatocellular carcinoma: Current status and future prospects. *World J Gastroenterol* 2019;25:4682-95.
25. Talbot JN, Fartoux L, Balogova S, Nataf V, Kerrou K, et al. Detection of hepatocellular carcinoma with PET/CT: A prospective comparison of 18F-Fluorocholine and 18F-FDG in patients with cirrhosis or chronic liver disease. *J Nucl Med* 2010;51:1699-706.
26. Wallace MC, Sek K, Francis RJ, Samuelson S, Ferguson J, et al. Baseline and post-treatment 18F-Fluorocholine PET/CT predicts outcomes in hepatocellular carcinoma following locoregional therapy. *Dig Dis Sci* 2020;65:647-57.

27. Koay EJ, Owen D, Das P. Radiation-induced liver disease and modern radiotherapy. *Semin Radiat Oncol* 2018;28:321-31.
28. Munoz-Schuffenegger P, Ng S, Dawson LA. Radiation-induced liver toxicity. *Semin Radiat Oncol* 2017;27:350-7.
29. Zuckerman E, Slobodin G, Sabo E, Yeshurun D, Naschitz JE, Groshar D. Quantitative liver-spleen scan using single photon emission computerized tomography (SPECT) for assessment of hepatic function in cirrhotic patients. *J Hepatol* 2003;39:326-32.
30. Bowen SR, Chapman TR, Borgman J, Miyaoka RS, Kinahan PE, et al. Measuring total liver function on sulfur colloid SPECT/CT for improved risk stratification and outcome prediction of hepatocellular carcinoma patients. *EJNMMI Res* 2016;6:57.
31. Kamimura K, Fukukura Y, Yoneyama T, Takumi K, Tateyama A, et al. Quantitative evaluation of liver function with T1 relaxation time index on Gd-EOB-DTPA-enhanced MRI: Comparison with signal intensity-based indices. *J Magn Reson Imaging* 2014;40:884-9.
32. Haimerl M, Verloh N, Zeman F, Fellner C, Nickel D, et al. Gd-EOB-DTPA-enhanced MRI for evaluation of liver function: Comparison between signal-intensity-based indices and T1 relaxometry. *Sci Rep* 2017;7:43347.
33. Yoon JH, Lee JM, Kang H, Ahn SJ, Yang H, et al. Quantitative assessment of liver function by using Gadoteric Acid-enhanced MRI: Hepatocyte uptake ratio. *Radiology* 2019;290:125-33.
34. Bowen SR, Saini J, Chapman TR, Miyaoka RS, Kinahan PE, et al. Differential hepatic avoidance radiation therapy: proof of concept in hepatocellular carcinoma patients. *Radiother Oncol* 2015;115:203-10.

Letter to Editor

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For dietary advice in end-stage liver cirrhosis resting metabolic rate should be measured, not estimated

Daphne Bot¹, Anneke Droop¹, Maarten E. Tushuizen², Bart van Hoek²

¹Department of Dietetics, Leiden University Medical Center, Leiden 2300 RC, The Netherlands.

²Department of Gastroenterology and Hepatology, and Transplantation Center, Leiden University Medical Center, Leiden 2300 RC, The Netherlands.

Correspondence to: Daphne Bot, Department of Dietetics, Leiden University Medical Center, Leiden 2300 RC, The Netherlands. E-mail: d.bot@lumc.nl

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Dear Editor,

We read with great interest the article “Frailty and Liver resection: where do we stand?” by Sioutas *et al.*^[1] in *Hepatoma Research*. In this review, the authors summarized the available frailty tools and their impact on postoperative outcomes in patients undergoing liver resection, in particular elderly patients (> 60 years). In addition to this review, we would like to provide extra information regarding this topic based on own research.

One of the variables included in most frailty assessments is nutritional status, e.g., loss of body weight or muscle mass. It is well known that malnutrition has a negative impact on clinical outcome of patients with end-stage liver cirrhosis. Insufficient nutrient intake, impaired digestion or absorption of nutrients, and disturbances in macronutrient metabolism contribute to malnutrition in these patients. The total energy expenditure consists of resting metabolic rate (RMR) and expenditure for physical activity. The Harris and Benedict (HB) equation is widely used in clinical care for estimating RMR^[2]. However, estimating RMR with HB may be unreliable in patients with cirrhosis. These patients can be hyper- or hypometabolic with many individual differences in energy expenditure, especially based on disease severity and body composition. Measuring RMR in a respiratory chamber is reliable but cumbersome^[3]. Cheaper and less complicated devices to perform indirect calorimetry measurements have become available. We compared estimated RMR derived with the HB equation with measured RMR using desktop indirect calorimetry in patients with end-stage liver cirrhosis.



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After obtaining informed consent from 29 consecutive patients with cirrhosis and preparing for liver transplantation, RMR was measured with desktop indirect calorimetry (Fitmate®, Cosmed) and compared to the results estimated by the Harris and Benedict equation. Twenty-nine patients (79.3% male) with liver cirrhosis had a mean (± 1.96 SD) estimated RMR with HB equation of 1771 (± 253) kilocalories, while the mean measured RMR with Fitmate was 1,630 (± 322) kilocalories ($P < 0.05$). The mean (± 1.96 SD) difference in RMR was 140 (± 240) kilocalories, with a minimum of -424 and a maximum of 510 kilocalories difference. The Pearson correlation between measured and estimated RMR was $R = 0.677$ ($P < 0.05$), which is a significant but not strong correlation [Supplementary Figure 1]. Large clinically relevant differences were detected between measured and estimated RMR in patients with liver cirrhosis during screening for liver transplantation. The most likely explanation for the discrepancy is the altered body composition and the frequent presence of ascites in these patients. A limitation of the device used was that it measures VO_2 but calculates VCO_2 . Indirect calorimetry devices that measure both VCO_2 and VO_2 are even more accurate^[4].

In conclusion, for reliable dietary advice in patients with end-stage liver cirrhosis, RMR should be measured with one of these newer easy-to-use devices, and should no longer be estimated with HB and other equations. This can have potential beneficial effects on nutritional status and therefore frailty in patients with liver diseases.

DECLARATIONS

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Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Bot D, Droop A, Tushuizen ME, van Hoek B

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REFERENCES

1. Sioutas GS, Ziogas IA, Tsoulfas G. Frailty and liver resection: where do we stand? *Hepatoma Res* 2020;6:4.
2. Roza AM, Shizgal HM. The harris benedict equation reevaluated: resting energy requirements and the body cell mass. *Am J Clin Nutr* 1984;40:168-82.
3. Verboeket-van der Venne WPHG, Westerterp KR, van Hoek B, Swart GR. Energy expenditure and substrate metabolism in patients with cirrhosis of the liver: effects of the pattern of food intake. *Gut* 1995;36:110-6.
4. Purcell SA, Elliott SA, Ryan AM, Sawyer MB, Prado CM. Accuracy of a portable indirect calorimeter for measuring resting energy expenditure in individuals with cancer. *JPEN J Parenter Enteral Nutr* 2019;43:145-51.

Review

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Contrast-enhanced ultrasonography with Sonazoid in hepatocellular carcinoma diagnosis

Yasunori Minami, Masatoshi Kudo

Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine, Osaka 589-8511, Japan.

Correspondence to: Prof. Yasunori Minami, Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine, 377-2 Ohno-higashi Osaka-sayama, Osaka 589-8511, Japan. E-mail: minkun@med.kindai.ac.jp

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Abstract

With the development of second-generation contrast agents and advancement in contrast harmonic imaging, contrast-enhanced ultrasonography (CEUS) now has the capacity to sensitively and accurately show tumor vascularity. Therefore, marked improvements have been achieved in the diagnosis of focal liver lesions (FLLs), including hepatocellular carcinoma (HCC), by US. In contrast to other agents, Kupffer cells in liver sinusoids take up Sonazoid. Two contrast enhancement phases occur in CEUS with Sonazoid: a vascular phase and Kupffer phase. Images obtained in the Kupffer phase have higher diagnostic sensitivity for hepatic malignancies because the majority of these malignancies do not contain Kupffer cells. Dynamic images obtained in the vascular phase markedly narrow the clinical differential diagnoses of FLLs. The sustainable detection of inconspicuous HCC, adequate guidance of ablation therapy, and accurate assessment of treatment responses in HCC are all facilitated by Sonazoid. The principles, clinical applications, and techniques of CEUS with Sonazoid in the diagnosis of HCC will be reviewed herein.

Keywords: Contrast-enhanced ultrasonography, focal liver lesion, hepatocellular carcinoma, sonazoid

INTRODUCTION

Recent advances in the multi-modality treatment of hepatocellular carcinoma (HCC) have contributed to significant improvement in the prognosis of patients with this type of primary liver cancer. The importance of the early detection of liver nodules, accurate diagnosis of HCC, and tumor staging for treatment planning is increasingly recognized. Guidelines for the utilization of imaging tests in the diagnosis of HCC have been developed by a number of societies, including the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver, the Asian-Pacific Association for the Study of Liver, and the Japan Society of Hepatology^[1-4]. Due to advances in techniques that have contributed to the



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highly sensitive and specific diagnosis of HCC, clinical HCC guidelines now recommend imaging tests. The specificity of single contrast-enhanced imaging is adequate for diagnosing HCC when typical features are observed on dynamic images. Recently, a critical milestone was achieved with integration of Liver Imaging Reporting and Data System (LI-RADS) into the AASLD HCC clinical practice guideline. LI-RADS is a comprehensive algorithm for standardizing the terminology, technique, interpretation and reporting for patients at high risk for HCC^[5].

Contrast-enhanced ultrasonography (CEUS) is now widely used in clinical practice and has markedly expanded the scope of the diagnosis of focal liver lesions (FLLs) by US. In CEUS, second-generation contrast agents, including SonoVue (sulfur hexafluoride), Definity (perflutren lipid), and Sonazoid (perflubutane), are microbubbles composed of a low-solubility gas enveloped by a phospholipid shell^[6]. Kupffer cells in the reticuloendothelial system of the liver take up Sonazoid, which remains in these cells for several hours, resulting in two contrast enhancement phases: a vascular phase and Kupffer phase. Sonazoid is advantageous for the diagnosis of HCC because of the higher diagnostic sensitivity of images obtained in the Kupffer phase for hepatic malignancies; the majority of hepatic neoplasms, particularly malignant tumors, do not contain Kupffer cells^[7]. CEUS LI-RADS is a standardized system for CEUS exams and can allow for accurate categorization of observations in patients with chronic hepatitis B or cirrhosis^[8]. The utility of SonoVue and Definity is supported by CEUS LI-RADS, whereas Sonazoid alone has not been included yet. It is expected that Sonazoid utilization will be incorporated in the next version of CEUS LI-RADS.

Sonazoid was approved for use as an ultrasound contrast agent in Japan in 2007^[9], followed by Norway, Korea, and Singapore. It was subsequently approved in Taiwan in 2018 and China in 2019. CEUS with Sonazoid is now regarded as a valuable diagnostic tool in the management of HCC patients. The principles, clinical applications and techniques of CEUS with Sonazoid in the management of HCC will be reviewed herein.

STRUCTURE AND PHARMACOKINETICS OF SONAZOID

Sonazoid (GE Healthcare, Waukesha, WI, USA) consists of lipid-coated microbubbles containing perfluorocarbon within a well-defined size range (median diameter of approximately 3 μm). These microbubbles are stabilized by a monomolecular membrane of hydrogenated egg phosphatidyl serine that is embedded in an amorphous sucrose structure^[6]. Sonazoid powder is reconstituted with 2 mL of sterile water for administration by injection. The clinical dose of Sonazoid that is generally employed to image liver lesions is 0.015 mL/kg body weight; however, the recommended dose is decreased to 0.0075-0.0010 mL/kg body weight when an US machine with high sensitivity for the detection of contrast agents is used.

Regarding the two contrast enhancement phases in real-time CEUS, the vascular phase (between 10 s and 5-7 min after the injection of Sonazoid) shows tumor vascularity, while the Kupffer phase (from 10 min after the injection) shows hepatic parenchymal findings because Kupffer cells or liver sinusoids take up this contrast agent. The artery- and portal-dominant time zones in the vascular phase are referred to as the arterial and portal phases, respectively [Figure 1]^[10,11]. Contrast enhancement in the Kupffer phase provides important information on FLLs because hypoenhancement indicates HCC, while benign lesions mostly show iso- or hyperenhancement. Imaging patterns, namely, arterial enhancement with Kupffer defects, are an important distinguishing feature of hepatic malignancies.

Dynamic CEUS displays similar, but distinct, vascular patterns to contrast-enhanced computed tomography (CECT); the contrast agents used in US are retained within blood vessels (blood pool contrast agents), whereas those for CT and magnetic resonance imaging (MRI) move into the extracellular space until their concentrations balance between the intravascular and extracellular spaces^[12].

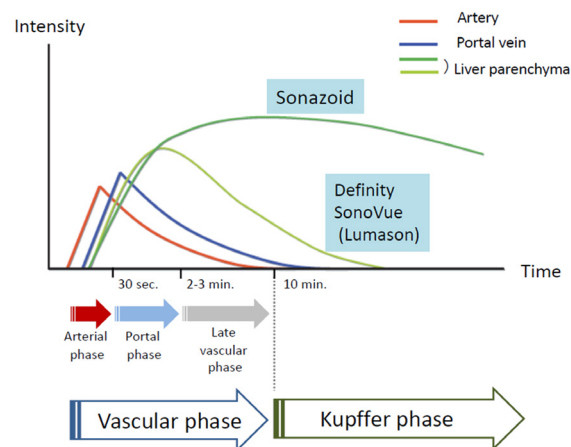


Figure 1. Pharmacokinetic behaviors of US contrast agents. Vascular and Kupffer phase images may be obtained using Sonazoid, but not Definity/SonoVue (Lumason). Sonazoid microbubbles are taken up by Kupffer cells and show homogeneous enhancement in a normally functioning liver parenchyma. Kupffer phase images are generally obtained 10 min after the injection of Sonazoid, the stability of which does not degrade for at least 60 min

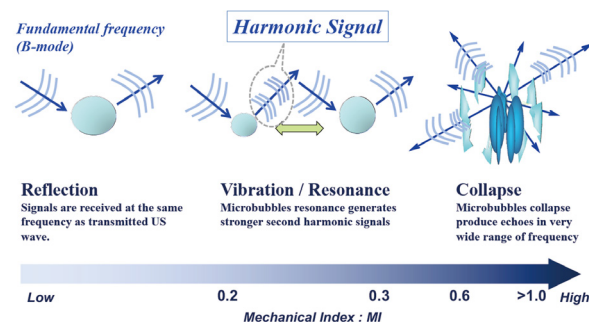


Figure 2. Behavior of microbubbles exposed to ultrasound. Microbubbles oscillate in a symmetrical manner at very low MI (< 0.1) with stable linear scattering. In contrast, asymmetrical oscillations are observed at low/medium MI (0.2-0.6), with microbubbles expanding more than they contract because they are more resistant to compression. This asymmetry causes harmonic emissions. Transient non-linear scattering occurs at high MI (> 0.6), and is followed by microbubble destruction. MI: mechanical index

MEDICAL ULTRASOUND TECHNOLOGIES

The mechanical index (MI) is a measure of the insonation power of microbubbles within an ultrasound field [Figure 2]. Second-generation microbubble contrast agents remain static when MI is very low and scatter the ultrasound beam. As MI increases, microbubbles linearly (MI $<$ approximately 0.2) or non-linearly (approximately $0.2 < \text{MI} < 0.5$) oscillate at their resonance frequency. Real-time scanning with CEUS generally involves low MI of < 0.3 . Microbubbles strongly oscillate at MI of higher than 0.6, expand beyond their limit, and ultimately burst. CEUS images may be generated from signals of the non-linear oscillation of microbubbles or their destruction^[12].

Information obtained on harmonic distortions in echo signals is used in non-linear ultrasonic imaging techniques. The non-linear mechanical behavior of microbubbles in contrast-enhanced imaging or non-linear wave propagation within tissues in tissue harmonic imaging causes harmonic distortions in ultrasound signals. Since echo signals contain both linear and non-linear reflections, the extraction of non-linear echo content is important for ensuring the sufficient performance of harmonic imaging. CEUS-specific modes including pulse inversion, amplitude modulation, and pulse inversion amplitude modulation have been devised to suppress linear tissue signals, thereby enhancing the detection of non-linear microbubble echoes^[13-15] [Figure 3]. The non-CEUS-specific mode, tissue harmonic imaging (THI), which is a non-

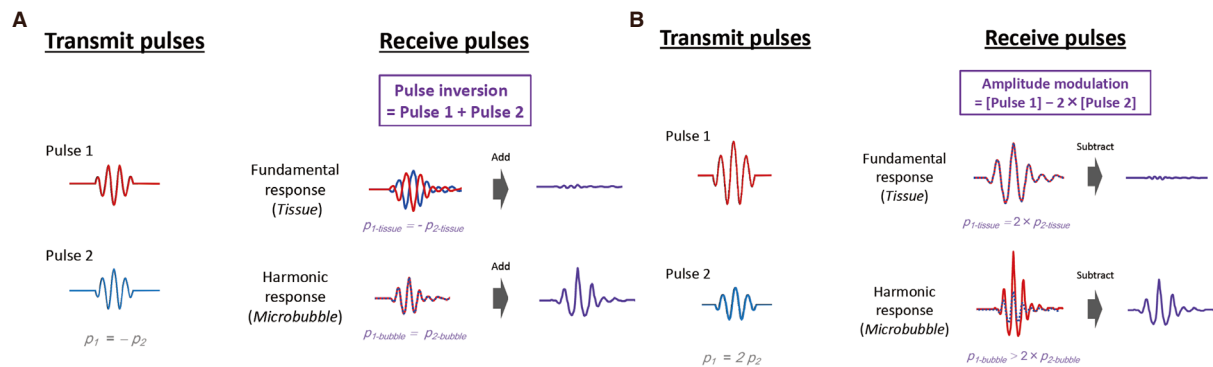


Figure 3. Pulse inversion and amplitude modulation. A: pulse-inversion technique is used in second harmonic imaging. Pulse 1 excites microbubbles, generating a linear fundamental response along with higher harmonic components. The inverted pulse 2 generates the same frequency components, however with different phases. The linear fundamental response from tissue experiences a 180° phase shift relative to the pulse 1 components, whereas the second harmonic response from microbubble experiences a 360° ($= 0^\circ$) phase shift. As a result, the fundamental responses are canceled out and the second harmonic responses are constructively added together; B: an amplitude modulation technique also plays a role in ultrasonic nonlinear imaging. An amplitude pulse is transmitted to eliminate the linear response and to elicit a nonlinear response. Upon reception, the pulse 2 components are rescaled and subtracted. Then, the fundamental response from tissue is canceled, and the second harmonic response from microbubble is leaked out

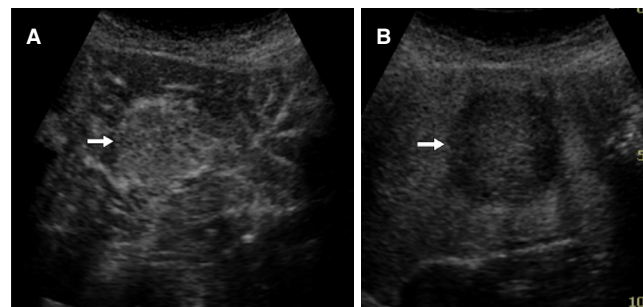


Figure 4. Hepatocellular carcinoma. A: contrast-enhanced ultrasonography shows homogenous strong enhancement (arrow) in the early arterial phase; B: a clear defect (arrow) with an irregular border is observed on a Kupffer phase image

contrast form of native harmonics, is also applied to contrast imaging. THI is advantageous because it provides a better signal-to-noise ratio. Contrast THI with Sonazoid offers a better contrast-to-tissue ratio at the cost of blood flow signals. Therefore, contrast THI provides an overlay view of conventional THI and contrast imaging^[16]. It allows us to observe vessels and inconspicuous lesions in the liver through all phases at high spatial and time resolutions; however, image contrast in the Kupffer phase may be slightly lower than that of the pulse inversion^[17].

CLINICAL APPLICATION OF CEUS

Diagnosis of HCC

Classic HCC generally receive a blood supply from abnormal arteries alone and are diagnosed based on positive enhancement (hypervascularity) in the arterial phase and defects in the Kupffer phase [Figure 4]. Previous studies reported the accurate diagnosis of small HCC (≤ 2 cm) using CEUS at a sensitivity of 81%-95% and specificity of 82%-86%^[18,19]. However, a potential limitation of this imaging modality is that a small subset of atypical HCC does not show hypervascularity in the arterial phase. Tumor hemodynamics change through the process of multistep hepatocarcinogenesis from low grade dysplastic nodule (DN) to moderately differentiated HCC [Figure 5]. The enhancement patterns of HCC are influenced by the degree of cellular differentiation. Arterial and portal blood supplies in pathological early or well-differentiated HCC vary, which increases the challenges associated with reaching an accurate diagnosis. The nodule-in-

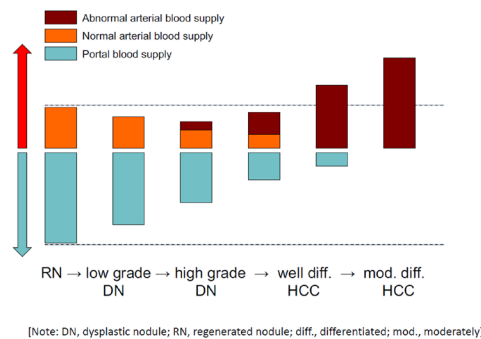


Figure 5. Tumor blood flows and multistep hepatocarcinogenesis. The development of hepatocellular carcinoma (HCC) occurs through a multistep process in the following sequence: large regenerative nodule (RN), low- or high-grade dysplastic nodule (DN), DN with a focus of HCC, well differentiated HCC, and moderately to poorly differentiated HCC. During the dedifferentiation, the intratumoral areas supplied portal blood flow are gradually reduced, whereas the intratumoral areas supplied arterial blood flow are synchronously changed. Normal arterial blood supply is reduced at an early stage, and then abnormal arterial blood supply is finally replaced

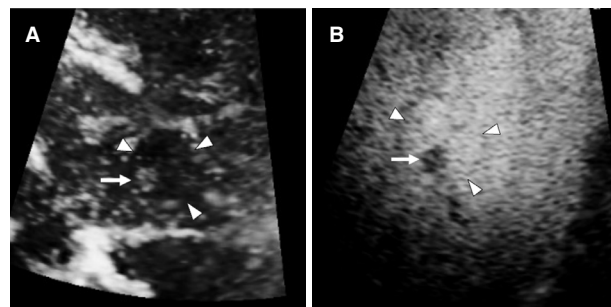


Figure 6. Nodule-in-nodule appearance of hepatocellular carcinoma. A: Arterial phase image shows an inner hypervascular spot (arrow) within the outer hypovascular nodule (arrowheads); B: Kupffer phase image shows better differentiation between a small defect (arrow) and the outer hypointense nodule (arrowheads)

nodule hemodynamic pattern, which is characterized by hyperintense foci in hypointense nodules, is specific for diagnosing early-stage HCC^[20] [Figure 6]. Arterial enhancement is less commonly observed for well-differentiated HCC; nodules are more likely to be isoenhanced or slightly hypoenhanced from the portal to Kupffer phases^[21,22]. Malignant liver lesions including HCC, cholangiocarcinoma, metastasis, *etc.* appear as hypoechoic areas surrounded by hyperechoic background liver in the Kupffer phase due to the depletion of Kupffer cells within them^[11,12]. The Kupffer defect can be easily detected and increasing its diagnostic performance. Moreover, the images of Kupffer defect are also useful to evaluate the macroscopic type of HCC that is a significant prognostic factor of HCC patients^[23].

Repeated contrast injections may be performed when an enhancement defect is identified in the Kupffer phase. This procedure is termed “defect reperfusion imaging” or “the re-injection technique”, and arterial enhancement may be superimposed on Kupffer images of lesions^[24,25]. Defect reperfusion imaging generates a very high detection rate of HCC that is not achievable with conventional B-mode US^[26] [Figure 7]. Kupffer phase image surveillance is also useful for the early detection and confirmation of HCC with the reinjection technique^[27].

Characterization of FLLs

The accurate differential diagnosis of FLLs requires clinical imaging tests, and CEUS plays an important role in the characterization of FLLs. CEUS with Sonazoid can show a high sensitivity of the detection of intranodular blood flow. Most malignant liver lesions are demonstrated as hypoechoic masses from the portal phase to the Kupffer phase, while most benign liver lesions are iso- or hyperechoic during these phases.

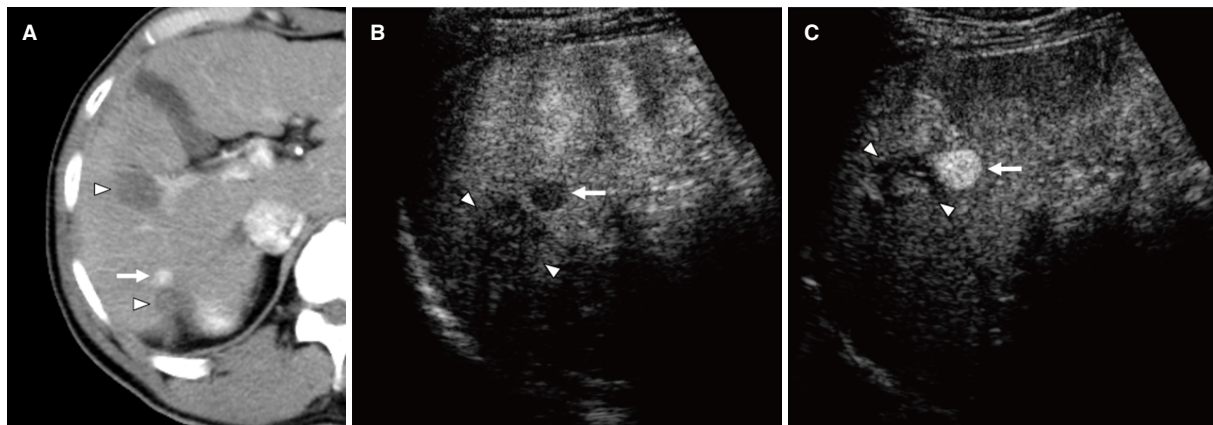


Figure 7. Defect reperfusion imaging for the local progression of hepatocellular carcinoma (HCC) after radiofrequency ablation (RFA). A: a computed tomography image shows focal arterial enhancement of the local progression of HCC (arrow) and RFA-induced coagulation necrosis (arrow heads); B: viable HCC (arrow) in close proximity to the necrotic area (arrow heads) are shown as areas with defects in the Kupffer phase; C: recurrent HCC (arrow) is clearly identified using the Sonazoid reinjection technique, whereas the necrotic area (arrow heads) does not enhance

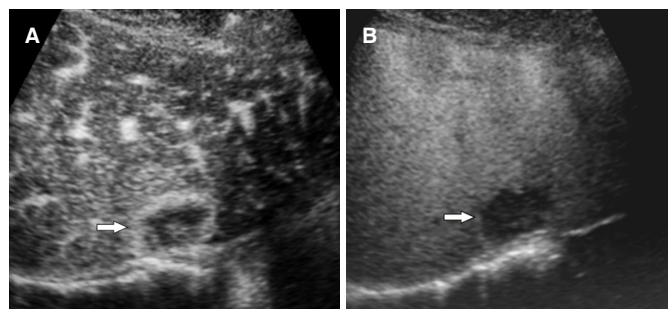


Figure 8. Liver metastasis from colon cancer. A: liver metastasis shows peripheral irregular rim-like enhancement (arrow); B: a clear defect (arrow) is evident in the Kupffer phase

Arterial hypervascularity is generally observed in the tumor periphery of adenocarcinoma liver metastases from many organs including colon, stomach, pancreas, *etc.*, at which tumor cells are abundant, while complete defects are detected in the Kupffer phase^[11] [Figure 8]. Washout is typically more rapid for liver metastases than for HCC, for which it is often slow^[21,28]. However, homogeneous hypervascularity in the arterial phase was previously reported in between 10% and 15% of adenocarcinoma liver metastases^[22]. In addition, renal cell carcinoma or gastrointestinal stromal tumor typically cause hypervascular metastases, and these lesions tend to show homogeneous arterial enhancement that is similar to typical HCC.

Similarities have been identified in the enhancement patterns of intrahepatic cholangiocarcinoma and metastatic liver cancer on CEUS, with rim-like enhancement in the early arterial phase and complete defects in the Kupffer phase commonly being detected^[11]. Nevertheless, it is important to note that approximately 30% of intrahepatic cholangiocarcinomas show hypervascularity and enhancement in the arterial phase^[22], consistent with the typical enhancement pattern of HCC on CEUS. Rapid washout has also been reported for intrahepatic cholangiocarcinoma^[29]. Peripheral rim-like enhancement and quick contrast washout may provide high efficiency in the differentiation of intrahepatic cholangiocarcinoma from HCC.

Hepatocellular adenoma (HCA) often shows arterial hyperenhancement^[28] and approximately 30% of HCAs show mild washout in the late vascular phase of CEUS^[28], making it difficult to distinguish HCA from well-differentiated HCC. In such cases, the individual patient's background such as a history of risk factors for HCC is very important for differentiation, and a biopsy may be necessary for the precise diagnosis.

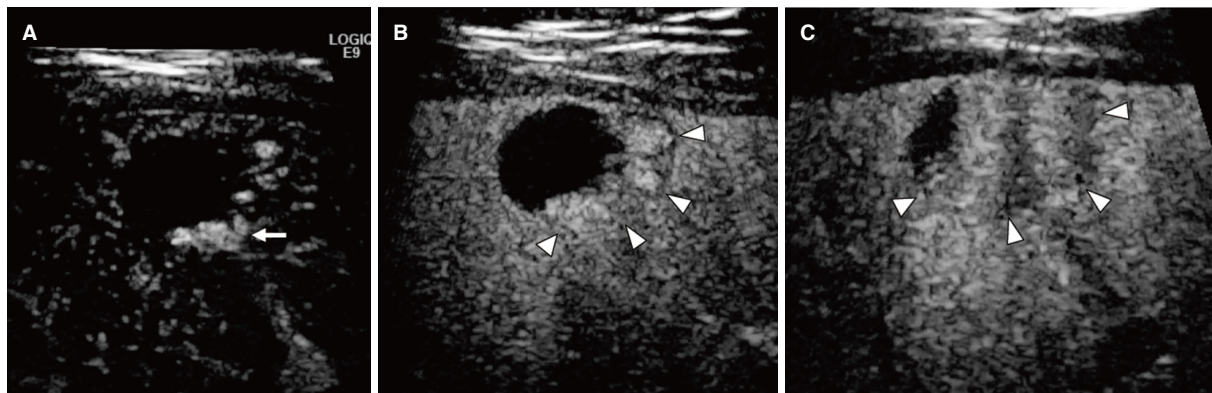


Figure 9. Hepatic hemangioma. A: early arterial phase image shows the typical “peripheral globular enhancement” (arrow) of the lesion; B: the lesion shows “partial centripetal filling” (arrow heads) during the portal phase; C: a progressive filling pattern (arrow heads) is observed in the Kupffer phase

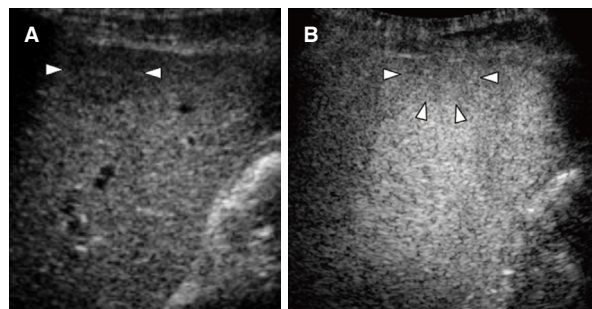


Figure 10. Dysplastic nodule. A: dysplastic nodule (arrowheads) shows hypovascularity in the arterial phase because of the reduction of arterial and portal supplies; B: the Kupffer phase image shows a slightly hypointense lesion with unclear border (arrowheads)

Liver hemangioma shows peripheral nodular enhancement in the arterial phase and centripetal filling through the portal to Kupffer phases of CEUS [Figure 9]^[11]. Although incomplete late filling has been reported in some cases of larger hemangioma, the enhancement pattern of “peripheral nodular arterial enhancement” in combination with “complete filling” resulted in a sensitivity of 98% for the diagnosis of liver hemangioma^[30].

The typical findings of focal nodular hyperplasia on CEUS include hypervascularity in the early arterial phase with a spoke-wheel pattern and an iso-enhanced mass with a hypo-enhanced central scar through the portal to Kupffer phases^[11]. The sensitivity of CEUS for detecting the spoke-wheel pattern is markedly higher than that of color-Doppler imaging.

Hepatic angiomyolipoma (AML) is a relatively rare benign mesenchymal tumor of the liver. Pathologically, it is composed of varying proportions of fat, muscle, and blood vessels. AML is characterized by a hyperechoic nodule on B-mode US due to a fatty component and is generally observed as a hypervascular lesion in the arterial phase and a defective lesion in the Kupffer phase^[4]. However, the diagnosis of hepatic AML is still challenging because imaging characteristics can vary depending on the proportions of its components.

Normal hepatic arteries and portal veins are generally present within low-grade DN which do not show the early uptake of contrast agents in the arterial phase. A previous study indicated that a high-grade DN showed transient hypovascularity in the arterial phase, and this finding was attributed to increased cellularity^[11] [Figure 10].

Inflammatory pseudotumor (IPT) of the liver is a rare benign nodule and can display various enhancement patterns on CEUS due to pathological change during the course of disease progression. When the nodules are abundant in inflammatory cells and granulation tissues, they often appear as an area of diffuse homogeneous hyperenhancement. As more necrosis and fibrosis develop within the nodules, IPT may show heterogeneous or peripheral rim-like enhancement^[31].

Meta-analytic studies reported the ability of CEUS to accurately differentiate between benign and malignant FLLs at a sensitivity of 93% and specificity of 90%, and also demonstrated its similar diagnostic performance to dynamic CECT and MRI^[32,33]. Furthermore, the diagnostic accuracy of CEUS for lesions that were inconclusive on CECT increased from 42%-44% to 89%-92%, and a higher diagnostic confidence level was confirmed^[34,35].

CEUS guidance of biopsy/ablation therapy

The correct placement of the needle into the target tumor for percutaneous biopsy/ablation therapy increases its technical success rate. B-mode US does not accurately detect HCC in the presence of local tumor progression after treatment or true HCC surrounded by large regenerative nodules in cirrhotic livers. The rates of HCC with poor conspicuity on planning B-mode US for ablation therapy ranged from 5.2 to 38.8 in previous reports^[36-39]. CEUS with Sonazoid facilitates needle placement in HCC that is poorly depicted on B-mode US because the defect lesion functions as a target for insertion.

If imaging studies fail to reveal an accurate diagnosis of FLLs, biopsy may be required. The limitations of percutaneous liver biopsy guided with B-mode US include its high rates of false-negative results. However, correct targeting and guiding steps benefit from the use of CEUS with Sonazoid^[40,41]. The diagnosis of benign FLLs may be improved with the utility of CEUS during liver biopsy without surgical intervention.

A previous study reported that the technical success rate of a single radiofrequency ablation (RFA) session was significantly higher with CEUS than with B-mode US (94.7% vs. 65.0%, $P = 0.043$)^[42]. Furthermore, the number of RFA sessions conducted in a historical cohort was smaller with Sonazoid CEUS guidance than with B-mode US guidance^[43,44]. Another study showed that the sustained local control rate was markedly higher for CEUS-guided RFA than for B-mode US-guided RFA (85.3% vs. 66.4% at 2 years)^[45]. In addition, inconspicuity on B-mode US and CEUS represents one of the most difficult conditions for percutaneous RFA. The combination of fusion imaging and CEUS is an effective guidance in ablation therapy for poorly defined HCCs on B-mode US and CEUS/fusion imaging^[46].

CEUS may also help to identify complications immediately after ablation therapy such as active bleeding or segmental infarction of the liver^[47]. Active hemorrhage should be visualized on CEUS as extravasation of microbubbles and infarcted areas can show no enhancement.

Assessment of HCC treatment responses

The complete lack of enhancement in all phases on CEUS was previously demonstrated in patients with complete treatment responses following arterial chemoembolization for HCC; while intratumoral residual or nodular peripheral enhancement was detected in patients with residual or recurrent HCC. CEUS allows for the reliable prediction of the risk of recurrence in patients with HCC within a short period of time (approximately 1 week) after TACE^[48,49].

Important issues to consider in treatment response assessments of RFA are evaluations of the absence of the vascular enhancement of HCC and the ablative margin. Residual HCC shows a focal defect in the Kupffer phase, representing hypervascular enhancement, with reinjections of Sonazoid. However, consistent and accurate assessments of the ablative margin by CEUS is not always possible because the tumor boundary may not be clearly identified on US after RFA^[50].

Sorafenib (Bayer, Leverkusen, Germany) is the first oral multikinase inhibitor developed for advanced unresectable HCC. Analyses of time intensity curves and arrival time parametric imaging facilitated assessments of the early responses of advanced HCC to sorafenib^[51,52]. However, not all lesions of multiple HCC treated with sorafenib exhibit similar behaviors on CEUS; therefore, CEUS may only investigate a few regions of interest and may be limited to treatment response assessments of multiple HCC to systemic chemotherapies.

CONCLUSION

US is a widely used imaging modality for liver diseases because it is minimally invasive, allows for real-time observations, and provides high-resolution images. However, the accuracy of CEUS may be negatively affected by a number of factors, such as acoustic attenuation, various artifacts, and blind areas. Therefore, the favorable and unfavorable characteristics of CEUS need to be carefully considered. This review comprehensively demonstrates the importance of CEUS with Sonazoid for managing patients with HCC. CEUS provides real-time and high-quality images of FLLs, including HCC, in all phases of enhancement, and has markedly increased the accuracy of US-based detection and characterization. CEUS has the ability to differentiate between benign and malignant liver nodules with high accuracy, which is crucial in the management of these patients. Furthermore, CEUS with Sonazoid provides guidance during therapeutic procedures and facilitates assessments of treatment responses. The adequate guidance of ablation and precise monitoring of treatment responses using CEUS with Sonazoid will contribute to further improvement in the prognosis of patients with HCC.

DECLARATIONS

Authors' contributions

Study concepts/study design, data acquisition and manuscript drafting: Minami Y
Manuscript editing: Kudo M

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53:1020-2.
2. European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012;56:908-43.
3. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular

- carcinoma: a 2017 update. *Hepatol Int* 2017;11:317-70.
4. Kudo M, Matsui O, Izumi N, Iijima H, Kadoya M, et al. JSH consensus-based clinical practice guidelines for the management of hepatocellular carcinoma: 2014 update by the liver Cancer study Group of Japan. *Liver Cancer* 2014;3:458-68.
 5. Elsayes KM, Hooker JC, Agrons MM, Kielar AZ, Tang A, et al. 2017 version of LI-RADS for CT and MR imaging: an update. *Radiographics* 2017;37:1994-2017.
 6. de Jong N, Emmer M, van Wamel A, Versluis M. Ultrasonic characterization of ultrasound contrast agents. *Med Biol Eng Comput* 2009;47:861-73.
 7. Lee JY, Minami Y, Choi BI, Lee WJ, Chou YH, et al. The AFSUMB consensus statements and recommendations for the clinical practice of contrast-enhanced ultrasound using sonazoid. *Ultrasonography* 2020;39:191-220.
 8. Wilson SR, Lyshchik A, Piscaglia F, Cosgrove D, Jang HJ, et al. CEUS LI-RADS: algorithm, implementation, and key differences from CT/MRI. *Abdom Radiol (NY)* 2018;43:127-42.
 9. Kudo M. Management of hepatocellular carcinoma in Japan as a world-leading model. *Liver Cancer* 2018;7:134-47.
 10. Minami Y, Kudo M. Review of dynamic contrast-enhanced ultrasound guidance in ablation therapy for hepatocellular carcinoma. *World J Gastroenterol* 2011;17:4952-9.
 11. Terminology and Diagnostic Criteria Committee, Japan Society of Ultrasonics in Medicine. Ultrasound diagnostic criteria for hepatic tumors. *J Med Ultrason* 2014;41:113-23.
 12. Chung YE, Kim KW. Contrast-enhanced ultrasonography: advance and current status in abdominal imaging. *Ultrasonography* 2015;34:3-18.
 13. Eckersley RJ, Chin CT, Burns PN. Optimising phase and amplitude modulation schemes for imaging microbubble contrast agents at low acoustic power. *Ultrasound Med Biol* 2005;31:213-9.
 14. Needles A, Arditi M, Rognin NG, Mehi J, Coulthard T, et al. Nonlinear contrast imaging with an array-based micro-ultrasound system. *Ultrasound Med Biol* 2010;36:2097-106.
 15. Gong P, Song P, Chen S. Improved contrast-enhanced ultrasound imaging with multiplane-wave imaging. *IEEE Trans Ultrason Ferroelectr Freq Control* 2018;65:178-87.
 16. Kono M, Minami Y, Iwanishi M, Minami T, Chishina H, et al. Contrast-enhanced tissue harmonic imaging versus phase inversion harmonic sonographic imaging for the delineation of hepatocellular carcinomas. *Oncology* 2017;92:29-34.
 17. Tanaka H. Current role of ultrasound in the diagnosis of hepatocellular carcinoma. *J Med Ultrason* (2001) 2020;47:239-55.
 18. Niu Y, Huang T, Lian F, Li F. Contrast-enhanced ultrasonography for the diagnosis of small hepatocellular carcinoma: a meta-analysis and meta-regression analysis. *Tumour Biol* 2013;34:3667-74.
 19. Kan M, Hiraoka A, Uehara T, Hidaka S, Ichiryu M, et al. Evaluation of contrast-enhanced ultrasonography using perflubron (Sonazoid®) in patients with small hepatocellular carcinoma: comparison with dynamic computed tomography. *Oncol Lett* 2010;1:485-8.
 20. Zheng RQ, Zhou P, Kudo M. Hepatocellular carcinoma with nodule-in-nodule appearance: demonstration by contrast-enhanced coded phase inversion harmonic imaging. *Intervirology* 2004;47:184-90.
 21. Jang HJ, Kim TK, Burns PN, Wilson SR. Enhancement patterns of hepatocellular carcinoma at contrast-enhanced US: comparison with histologic differentiation. *Radiology* 2007;244:898-906.
 22. Jang JY, Kim MY, Jeong SW, Kim TY, Kim SU, et al. Current consensus and guidelines of contrast enhanced ultrasound for the characterization of focal liver lesions. *Clin Mol Hepatol* 2013;19:1-16.
 23. Hatanaka K, Minami Y, Kudo M, Inoue T, Chung H, et al. The gross classification of hepatocellular carcinoma: usefulness of contrast-enhanced US. *J Clin Ultrason* 2014;42:1-8.
 24. Kudo M, Hatanaka K, Maekawa K. Newly developed novel ultrasound technique, defect reperfusion ultrasound imaging, using sonazoid in the management of hepatocellular carcinoma. *Oncology* 2010;78:40-5.
 25. Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, et al. World Federation for Ultrasound in Medicine; European Federation of Societies for Ultrasound. Guidelines and good clinical practice recommendations for Contrast Enhanced Ultrasound (CEUS) in the liver - update 2012: A WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultrasound Med Biol* 2013;39:187-210.
 26. Kudo M. Diagnostic imaging of hepatocellular carcinoma: recent progress. *Oncology* 2011;81:73-85.
 27. Kudo M, Ueshima K, Osaki Y, Hirooka M, Imai Y, et al. B-Mode ultrasonography versus contrast-enhanced ultrasonography for surveillance of hepatocellular carcinoma: a prospective multicenter randomized controlled trial. *Liver Cancer* 2019;8:271-80.
 28. Yang HK, Burns PN, Jang HJ, Kono Y, Khalili K, et al. Contrast-enhanced ultrasound approach to the diagnosis of focal liver lesions: the importance of washout. *Ultrasonography* 2019;38:289-301.
 29. Liu GJ, Wang W, Lu MD, Xie XY, Xu HX, et al. Contrast-enhanced ultrasound for the characterization of hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *Liver Cancer* 2015;4:241-52.
 30. Dietrich CF, Mertens JC, Braden B, Schuessler G, Ott M, et al. Contrast-enhanced ultrasound of histologically proven liver hemangiomas. *Hepatology* 2007;45:1139-45.
 31. Kong WT, Wang WP, Cai H, Huang BJ, Ding H, et al. The analysis of enhancement pattern of hepatic inflammatory pseudotumor on contrast-enhanced ultrasound. *Abdom Imaging* 2014;39:168-74.
 32. Friedrich-Rust M, Klopffleisch T, Nierhoff J, Herrmann E, Vermehren J, et al. Contrast-enhanced ultrasound for the differentiation of benign and malignant focal liver lesions: a meta-analysis. *Liver Int* 2013;33:739-55.
 33. Xie L, Guang Y, Ding H, Cai A, Huang Y. Diagnostic value of contrast-enhanced ultrasound, computed tomography and magnetic

- resonance imaging for focal liver lesions: a meta-analysis. *Ultrasound Med Biol* 2011;37:854-61.
34. Chung YE, Kim KW. Contrast-enhanced ultrasonography: advance and current status in abdominal imaging. *Ultrasonography* 2015;34:3-18.
 35. Quaiia E, De Paoli L, Angileri R, Cabibbo B, Cova MA. Indeterminate solid hepatic lesions identified on non-diagnostic contrast-enhanced computed tomography: assessment of the additional diagnostic value of contrast-enhanced ultrasound in the noncirrhotic liver. *Eur J Radiol* 2014;83:456-62.
 36. Minami Y, Kudo M, Kawasaki T, Chung H, Ogawa C, et al. Treatment of hepatocellular carcinoma with percutaneous radiofrequency ablation: usefulness of contrast harmonic sonography for lesions poorly defined with B-mode sonography. *AJR Am J Roentgenol* 2004;183:153-6.
 37. Kim PN, Choi D, Rhim H, Rha SE, Hong HP, et al. Planning ultrasound for percutaneous radiofrequency ablation to treat small (≤ 3 Cm) hepatocellular carcinomas detected on computed tomography or magnetic resonance imaging: a multicenter prospective study to assess factors affecting ultrasound visibility. *J Vasc Interv Radiol* 2012;23:627-34.
 38. Kim AY, Lee MW, Rhim H, Cha DI, Choi D, et al. Pretreatment evaluation with contrast-enhanced ultrasonography for percutaneous radiofrequency ablation of hepatocellular carcinomas with poor conspicuity on conventional ultrasonography. *Korean J Radiol* 2013;14:754-63.
 39. Rajesh S, Mukund A, Arora A, Jain D, Sarin SK. Contrast-enhanced US-guided radiofrequency ablation of hepatocellular carcinoma. *J Vasc Interv Radiol* 2013;24:1235-40.
 40. Eso Y, Takai A, Takeda H, Matsumoto T, Lee M, et al. Sonazoid-enhanced ultrasonography guidance improves the quality of pathological diagnosis in the biopsy of focal hepatic lesions. *Eur J Gastroenterol Hepatol* 2016;28:1462-7.
 41. Park HS, Kim YJ, Yu MH, Jung SI, Jeon HJ. Real-time contrast-enhanced sonographically guided biopsy or radiofrequency ablation of focal liver lesions using perflubrobutane microbubbles (sonazoid): value of Kupffer-phase imaging. *J Ultrasound Med* 2015;34:411-21.
 42. Minami Y, Kudo M, Chung H, Kawasaki T, Yagyu Y, et al. Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. *AJR Am J Roentgenol* 2007;188:489-94.
 43. Minami Y, Kudo M, Hatanaka K, Kitai S, Inoue T, et al. Radiofrequency ablation guided by contrast harmonic sonography using perfluorocarbon microbubbles (Sonazoid) for hepatic malignancies: an initial experience. *Liver Int* 2010;30:759-64.
 44. Masuzaki R, Shiina S, Tateishi R, Yoshida H, Goto E, et al. Utility of contrast-enhanced ultrasonography with Sonazoid in radiofrequency ablation for hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011;26:759-64.
 45. Dohmen T, Kataoka E, Yamada I, Miura K, Ohshima S, et al. Efficacy of contrast-enhanced ultrasonography in radiofrequency ablation for hepatocellular carcinoma. *Intern Med* 2012;51:1-7.
 46. Minami T, Minami Y, Chishina H, Arizumi T, Takita M, et al. Combination guidance of contrast-enhanced US and fusion imaging in radiofrequency ablation for hepatocellular carcinoma with poor conspicuity on contrast-enhanced US/fusion imaging. *Oncology* 2014;87:55-62.
 47. Huang DY, Yusuf GT, Daneshi M, Ramnarine R, Deganello A, et al. Contrast-enhanced ultrasound (CEUS) in abdominal intervention. *Abdom Radiol (NY)* 2018;43:960-76.
 48. Kono Y, Lucidarme O, Choi SH, Rose SC, Hassanein TI, et al. Contrast-enhanced ultrasound as a predictor of treatment efficacy within 2 weeks after transarterial chemoembolization of hepatocellular carcinoma. *J Vasc Interv Radiol* 2007;18:57-65.
 49. Xia Y, Kudo M, Minami Y, Hatanaka K, Ueshima K, et al. Response evaluation of transcatheter arterial chemoembolization in hepatocellular carcinomas: the usefulness of sonazoid-enhanced harmonic sonography. *Oncology* 2008;75:99-105.
 50. Zhou P, Kudo M, Minami Y, Chung H, Inoue T, et al. What is the best time to evaluate treatment response after radiofrequency ablation of hepatocellular carcinoma using contrast-enhanced sonography? *Oncology* 2007;72:92-7.
 51. Shiozawa K, Watanabe M, Kikuchi Y, Kudo T, Maruyama K, et al. Evaluation of sorafenib for hepatocellular carcinoma by contrast-enhanced ultrasonography: a pilot study. *World J Gastroenterol* 2012 28;18:5753-8.
 52. Sugimoto K, Moriyasu F, Saito K, Rognin N, Kamiyama N, et al. Hepatocellular carcinoma treated with sorafenib: early detection of treatment response and major adverse events by contrast-enhanced US. *Liver Int* 2013;33:605-15.

Review

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Pathomolecular characterization of HCC in non-cirrhotic livers

Archana Rastogi

Department of Pathology, Institute of Liver & Biliary Sciences, New Delhi-110070, India.

Correspondence to: Prof. Archana Rastogi, Department of Pathology, Institute of Liver & Biliary Sciences, D-1, VASANT KUNJ, New Delhi 110070, India. E-mail: drarchanarastogi@gmail.com

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and usually arises in cirrhotic livers. Increasingly, it is diagnosed in non-cirrhotic livers. A variety of risk factors and etiologies can trigger the development of HCC in non-fibrotic and non-cirrhotic backgrounds. The most important causes are metabolic syndrome and hepatitis B virus infection. Postulated pathogenetic mechanisms are direct carcinogenesis, chronic liver injury and repair cycles, and genetic/epigenetic aberrations. Histopathology has a very important role in the diagnosis of non-cirrhotic HCC. Gross features of non-cirrhotic HCC are quite different from HCC originating in a cirrhotic background. Microscopic characteristics are similar to a classical HCC. However, certain histological variants show a predilection to occur in non-cirrhotic livers. These encompass fibrolamellar, scirrhous, steatohepatic and mixed hepato-cholangiocarcinoma subtypes. Due to the non-cirrhotic background, adenoma, metastasis and most of the other non-neoplastic and neoplastic conditions enter the differential diagnosis. Genomic studies and morpho-molecular classifications of HCC provide further understanding of the molecular pathogenesis of non-cirrhotic HCC. This group however, has rarely been exclusively studied. This review offers an update of etiology, patho-molecular characteristics and differential diagnosis of HCC arising in non-cirrhotic backgrounds.

Keywords: Hepatocellular carcinoma, non-cirrhotic, etiopathogenesis, molecular genetics, pathology, patho-molecular, differential diagnosis



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INTRODUCTION

Hepatocellular carcinoma (HCC) usually arises in a cirrhotic liver and is characterized by tremendous phenotypic and molecular heterogeneity^[1]. Around 20% of HCCs arise in non-cirrhotic livers^[2-5]. The underlying etiologies, risk factors, pathogenetic mechanisms, gross specimen features, histological variants and the differential diagnosis of non-cirrhotic HCCs is quite distinct from HCCs arising in cirrhotic backgrounds. However, these aspects have not been adequately studied and further analysis of the molecular genetics and pathological characteristics in non-cirrhotic HCCs is necessary. In this article, an overview of the clinical, etiological and etiopathogenic, patho-molecular characteristics and differential diagnosis of HCCs arising in non-cirrhotic backgrounds, are presented.

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

HCC is the most common primary malignant liver tumor and usually arises in cirrhotic livers. The occurrence of HCC in non-cirrhotic livers varies across different geographic regions of the world with a prevalence ranging from 7% to 54%^[6]. Most series, however, have reported a prevalence of around 15%-20%^[2-5].

The existence of HCC in non-cirrhotic livers has a bimodal age distribution, with peaks in the second and seventh decades. Non-cirrhotic HCCs have a lower male to female sex ratio (1.3-2.1) in comparison to cirrhotic HCCs where the ratio is 3.2 to 8:1^[6]. HCC in non-cirrhotic patients manifest with non-specific symptoms or a clinically silent course in the initial period. This is due to the lack of surveillance imaging and higher hepatic reserves, leading to a delay in diagnosis until an advanced stage with a larger tumor burden has been reached^[7]. Extrahepatic metastasis is already present in more than 25% of these patients at the time of presentation^[8].

Non-cirrhotic HCCs usually display similar imaging features to cirrhotic HCCs on computed tomography (CT) and MRI, with arterial phase hyper-enhancement followed by washout on portal venous and/or delayed phase imaging^[9,10]. However, due to the non-prototypical background, the development of HCCs in non-cirrhotic livers is one of the most important indications for tissue diagnosis. Imaging features illustrate a solitary mass with or without satellite lesions^[11]. Similar to imaging, serum α -fetoprotein measurements are similar for HCCs arising from both cirrhotic and non-cirrhotic backgrounds^[7].

The prognosis for non-cirrhotic HCCs is usually better than that for cirrhotic HCCs^[7]. Non-cirrhotic HCCs are more amenable to hepatic resection due to the lower risk of liver failure. Patients without cirrhosis have longer survival (postoperative overall survival and recurrence-free survival) than patients with cirrhosis^[7,12]. However, the recurrence rate of HCC in non-cirrhotic livers is very high after surgical resection^[11,13,14].

ETIOLOGIC CONSIDERATIONS

A variety of conditions can be a risk factor for developing non-cirrhotic HCC.

Infections

Hepatitis B virus (HBV) infection is one of the most common underlying etiologies, especially in high incidence areas. Up to 30% of HBV-related HCCs arise in non-cirrhotic livers^[15,16]. HBV infection can directly trigger liver carcinogenesis by integration of the HBV genome into the host hepatocyte DNA. This can cause secondary chromosomal rearrangement and genomic instability, or produce genotoxins such as the HBx protein, resulting in HCC development in non-cirrhotic backgrounds^[7,17]. In addition, the X protein of HBV, through its interaction with p53, interferes with tumor-suppressor activity. This oncogenic impact of HBV can remain even in treated cases or after seroconversion and resolution of HBV^[18,19].

Although relatively less common, chronic HCV-infection is also a risk factor for the development of HCC in non-cirrhotic livers^[8,20,21]. HCV carcinogenesis is likely mediated by viral factors and the host immune response^[7]. Oxidative stress in hepatocytes with sustained necro-inflammatory processes leads to cell injury, repeated cell divisions leading to genetic alterations in stem cells, and cause transformation into dysplastic and malignant phenotypes^[16,22,23]. HCV does not integrate into the host genome, but its direct hepatocarcinogenic potential is attributable to certain HCV gene products (core, NS3, NS4B and NS5A) and has been reported in murine fibroblast culture studies. However, the carcinogenic potential of HCV is much lower than HBV, and HCCs related to HCV mostly develop in cirrhotic livers^[24]. A study reported the annual incidence of HCV-associated HCC to be 0.8% among non-cirrhotic patients, and 2%-8% among cirrhotic patients^[21].

Metabolic syndrome and alcohol related liver disease

Metabolic syndrome is the most frequent cause of HCCs in non-cirrhotic backgrounds^[8]. NAFLD, with or without NASH, is the hepatic manifestation of metabolic syndrome and a common risk factor for HCC development^[25]. 39%-49% of NAFLD-related HCC cases arise in non-cirrhotic livers^[26]. Amongst various etiologic links, patients with NAFLD most frequently develop HCCs in non-cirrhotic backgrounds^[27]. Alcohol related hepatocarcinogenicity is almost exclusively due to the development of cirrhosis^[28,29]. However, excessive alcohol intake in the setting of chronic HCV and diabetes mellitus may potentiate oxidative stress and free radical damage. This can lead to rapid progression to HCC even in non-cirrhotic livers^[8,30]. As shown in one study, 15% of non-cirrhotic HCC patients had alcohol-HCV infection synergism^[8].

The aforementioned cause-effect relationships for HCC development can be due to direct carcinogenic action. Nonetheless, the role of chronic liver inflammation leading to repeated cycles of cell injury and regeneration, and subsequent genetic and epigenetic alterations in hepatocytes, is equally plausible^[31].

Hepatocyte injury resulting from microbial or sterile etiologies activates resident liver immune cells and later, facilitates the recruitment of nonresident immune cells to the liver, thereby mounting a strong inflammatory response. Persistent inflammation as a result of hepatitis virus or microbial attack resulting from breaches of the gut-liver axis lead to the production of proinflammatory cytokines such as IL-6, TNF- α , IL-1 and IL-18 through inflammasome-independent or -dependent pathways. Activated transcription factors make the hepatic milieu a fertile zone for cellular transformation^[32]. Tregs coupled to the activation of Notch and TGF- β are involved in perpetuation of the inflammatory response and HCC development in patients chronically infected with the HBV^[33].

Other liver disorders and tumors

Inherited metabolic and congenital diseases, in particular hereditary hemochromatosis, α -1-antitrypsin deficiency, Wilson disease, type I glycogen storage disease, porphyria, hypercitrullinemia, Alagille syndrome, and congenital hepatic fibrosis have predisposition to developing HCC in non-cirrhotic livers^[6]. Mild iron accumulation is found in background liver parenchyma in non-cirrhotic HCCs, indicating the role of excess iron within hepatocytes as a genotoxic cocarcinogen factor^[34].

Other genotoxic factors such as aflatoxin B1, produced by the fungus *Aspergillus flavus*, can contaminate cereals, legumes, spices and fruits^[35]. It is metabolized by the P450 enzyme in the liver to generate an epoxide, which binds to DNA and leads to the development of non-cirrhotic HCC via p53 mutation^[35,36] and also, amplifies the risk of HCC development among patients with HBV infection through this mutation^[37]. Exposure to microcystins (metabolites of cyanobacterial blooms) through water and aquatic food is also implicated in hepatocarcinogenesis^[38]. Chemical industrial carcinogens such as pesticides, vinyl chloride, arsenic, tobacco combustion derivatives, and radioactive elements such as Thorotrast, can also cause liver cancer^[6].

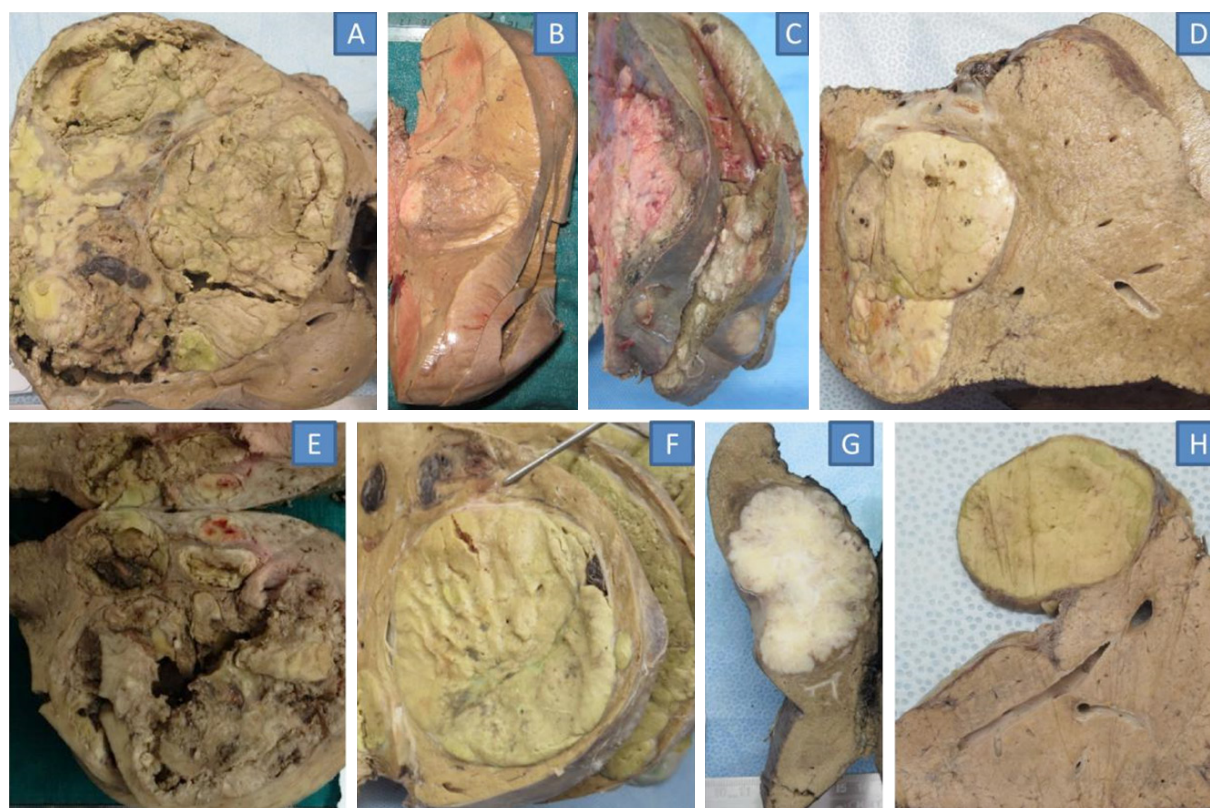


Figure 1. Gross specimens: HCC in a non-cirrhotic background: massive solitary HCC in a patient with NASH (A); single HCC in HBV related liver disease (B); multinodular HCC in a patient infected with HBV (C); steatohepatic variant of HCC in a patient with NASH (D); single large HCC in a patient infected with HCV (E); large HCC with prominent cholestasis and pseudoglandular pattern on microscopy (F); combined HCC-CC in a patient infected with HBV (G); fibrolamellar HCC (H). HCC: hepatocellular carcinoma; HBV: hepatitis B virus

Other liver lesions such as hepatocellular adenoma occur in non-cirrhotic backgrounds and can undergo malignant transformation in around 15% of cases^[39]. Patients taking anabolic C17-alkylated androgenic steroids are also predisposed to HCC development in a non-cirrhotic background^[40].

PATHOLOGICAL FEATURES

Macroscopic evaluation of non-cirrhotic HCC

Gross examination of a non-cirrhotic HCC frequently displays a large solitary mass or a dominant mass with small satellite nodules. This is in contrast to HCCs in cirrhosis, which has either a single nodule or multiple small nodules^[6,24,41]. In a study based on retrospective analysis of the gross specimens of 242 solitary and resected primary HCCs, the absence of cirrhosis was recorded in 45%. Various gross subtypes including expanding nodular, multinodular confluent, nodular with perinodular extension were almost equally prevalent in both cirrhotic and non-cirrhotic HCCs; the infiltrative type however, was far more common in cirrhotic patients^[42] [Figure 1].

Non-cirrhotic HCCs are more likely to develop intratumoral hemorrhage. It shows tumour heterogeneity with variegated appearances due to necrosis and hemorrhage^[24]. Intracellular fat accumulation is more frequently seen in well-differentiated, non-cirrhotic HCCs^[24]. Encapsulated tumors occur significantly more in patients without cirrhosis^[12]. However other studies have reported lack of encapsulation in this group^[43]. Altogether, non-cirrhotic HCCs are markedly different from cirrhotic HCCs in terms of lesion number, dimensions, fat content, intratumoral hemorrhage, and encapsulation^[7].

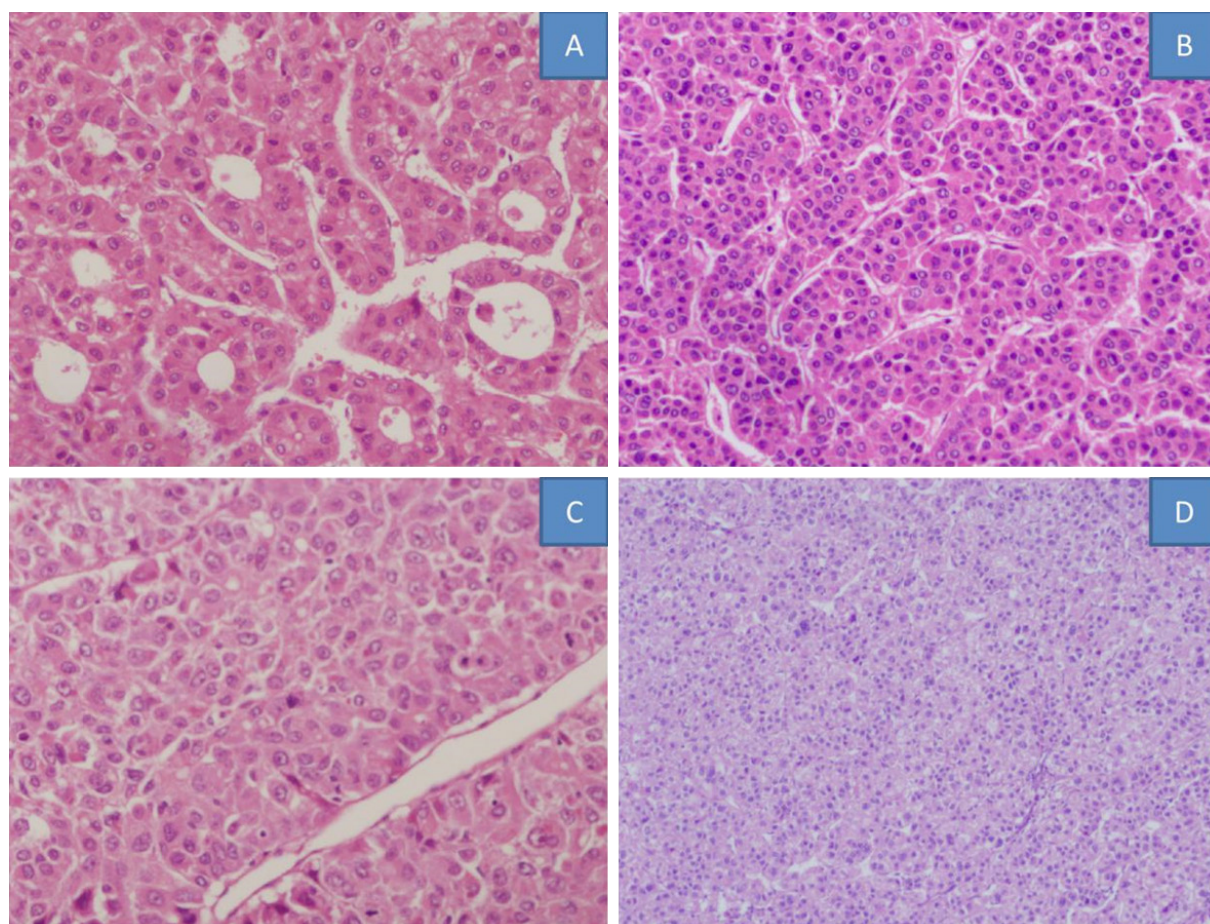


Figure 2. Histological patterns of hepatocellular carcinoma in non-cirrhotic livers: pseudoglandular (A), microtrabecular (B), macrotrabecular (C), compact (D)

The published literature indicate more frequent metastasis, direct invasion of adjacent organs and macroscopic portal vein or hepatic vein invasion in non-cirrhotic HCC, which is probably related to delayed diagnosis or inherent biological aggressiveness^[41].

Fibrolamellar HCCs are a distinct variant of HCCs known to occur in non-cirrhotic livers^[44]. Up to one third of HCCs developing in noncirrhotic backgrounds are of the fibrolamellar type^[2,45]. This variant forms a heterogeneous and well-circumscribed mass^[46]. On cut sections, prominent fibrous septa subdividing the mass into a central zone of scarring and calcifications may be observed^[47].

Histopathology of HCCs arising in non-cirrhotic backgrounds

The histological evaluation of HCC specimens plays a key role in tumor staging and in distinguishing HCC from its precursor lesions or other liver nodules^[48]. Tumor biopsy based diagnosis is recommended for all nodules occurring in non-cirrhotic livers^[1,49,50].

The pathological characteristics are similar to those of an HCC developing in a cirrhotic background. Thickened tumor cell plates, malignant cytology, capillarization of sinusoids and evidence of invasion constitute the principal diagnostic microscopic features. The four major histological patterns in HCC are microtrabecular, compact, macrotrabecular and pseudoglandular. Of these, the trabecular form is the most common histological pattern of HCC, both in cirrhotic and non-cirrhotic livers (41%-76%)^[51] [Figure 2].

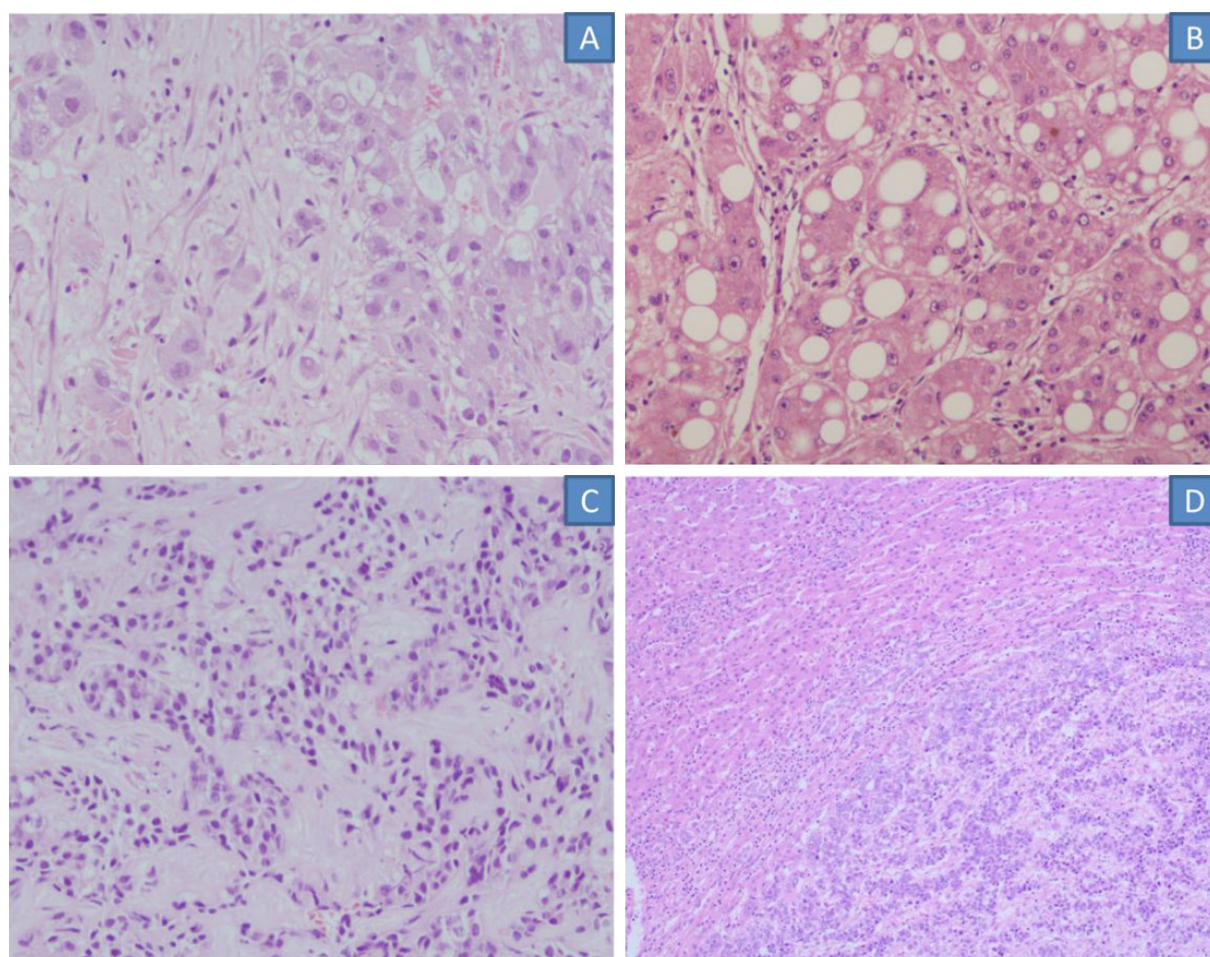


Figure 3. Microscopic sections of histological variants of hepatocellular carcinoma occurring in non-cirrhotic backgrounds: fibrolamellar (A), steatohepatic (B), scirrhous (C), mixed hepatocellular-cholangiocarcinoma (D)

There are several histological subtypes of HCC such as fibrolamellar, steatohepatic, lymphoepithelioma-like carcinoma, combined hepatocholangiocarcinoma, clear cell HCC, sarcomatoid HCC and many others^[52]. These HCC histological subtypes have distinct morphological features, some of which have prognostic importance; recently, several have also been reported to have dysregulation of specific molecular pathways with implications with respect to molecular targeted therapies^[1]. All of the subtypes can be found in cirrhotic and non-cirrhotic livers but fibrolamellar carcinoma is found almost exclusively in non-cirrhotic livers^[52] [Figure 3]. FLC typically occurs as a single tumor in non-cirrhotic livers in younger individuals^[53]. Scirrhous HCCs are often located beneath the liver capsule and are most common in non-cirrhotic livers^[54,55]. Mixed HCC-cholangiocarcinoma subtype also occurs more frequently in non-cirrhotic livers^[6]. The steatohepatic variant of HCC also often occurs in non-cirrhotic backgrounds. Cirrhotomimetic HCC typically arises in cirrhotic livers but in rare cases, can occur in non-cirrhotic livers^[52]. Well-differentiated HCCs are frequent in non-cirrhotic livers and have microscopic fat. Immunohistochemical panel comprising glypican 3 (GPC3), heat shock protein (HSP70) and glutamine synthetase (GS) may assist in diagnosis^[56].

HCC is characterised by cytologic features which have prognostic importance and add heterogeneity to the tumour phenotype^[1]. The occurrence of steatosis, clear cells, cholestasis, giant cells, and other miscellaneous features in HCCs in non-cirrhotic backgrounds is comparable with that in cirrhotic livers. However, a few studies have shown that giant cells, multinucleate cells, local hepatic venous invasion by

tumor, and Mallory bodies were 2.4, 2.0, 1.6 and 1.5 times more likely, respectively, to be found in tumors occurring in patients with cirrhosis than those in patients without cirrhosis^[12].

There is conflicting data on the occurrence of prognostic histological indices in non-cirrhotic HCCs. Nzeako *et al.*^[12] reported that cirrhotic patients, as compared to non-cirrhotic individuals, have a risk of harbouring grade 3-4 tumors and venous invasion of 1.7 and 1.6 times respectively. Whereas extrahepatic extension was reported to be greater in HCC arising in non-cirrhotic liver (20.5% vs. 6.5%)^[41]; others have reported comparable tumor differentiation and portal tree invasion between cirrhotic and non-cirrhotic HCCs^[41,57].

Pathology of background liver

HCC may develop without advanced liver fibrosis or even in normal livers. The non-tumoral liver exhibits features of chronic hepatitis, varying degrees of fibrosis, steatosis, iron overload or other metabolic disorders^[58]. In actuality, very few cases have an absolutely normal liver. However, in a study, 21/87 HCCs occurred in non-cirrhotic livers, and histopathological evaluation showed that nearly a third had no fibrosis in the liver^[8]. Another study of 1,221 patients revealed that 238 (19%) had no cirrhosis yet the grade of fibrosis was \leq F2 in 62% of all non-cirrhotic patients, and F3 in 8%. In another series of surgical resections of HCC, a high rate of non-cirrhotic livers was identified (F0-F1 38%, F2-F3 33%, F4 29%)^[59]. Other features such as liver cell dysplasia, more often the large cell type, was found in 27%-40% of cases^[60,61] and these figures decreased to 6%-20% in the subgroup of non-fibrotic livers^[62,63]. The presence of NAFLD (12% vs. 28%) is more common in patients without cirrhosis than in those with cirrhosis^[64] [Figure 4].

Differential diagnosis of HCCs arising in non-cirrhotic livers

There is a plethora of inflammatory, immunologic, neoplastic and infective lesions that occur in a non-cirrhotic liver background. However, from the point of view of histopathological interpretation, the most important are preneoplastic and neoplastic conditions, HCC variants and tumor metastasis.

Dysplastic nodules are premalignant lesions, which are well-defined and circumscribed. These lesions mostly arise from a background of chronic liver disease and more often, cirrhosis^[65]. These are important for diagnosis as often, they come under the differential diagnosis of well-differentiated HCCs. Immunohistochemical panels comprising glypican 3, HSP-70, glutamine synthetase, CD34 and CK7, are particularly useful in such scenarios^[18] [Figure 5].

The differential diagnosis between hepatocellular adenoma (HCA) and well-differentiated HCC arising in non-cirrhotic livers is another challenging situation, especially the HCA subtype with cytological or architectural atypia^[66]. Clinico-pathological correlation helps to sort this conundrum. Malignant transformation is one of the most important complications of HCA and is reported to occur in 4%-10% of HCA^[67-69]. Awareness about the malignant potential and predisposing risk factors such as male gender, larger tumor size, and β -catenin activated subtype, is crucial^[68].

Focal nodular hyperplasia (FNH) is another important differential consideration for HCCs occurring in non-cirrhotic backgrounds. FNH, a benign lesion resulting from the regenerative response to vascular abnormalities, has a characteristic histomorphology. A central scar with thick-walled vessels and nodular regeneration of hepatocytes, with marked ductular reaction and inflammatory infiltrate at the junction of fibrous bands and hepatocyte nodules, distinguishes FNH from HCC. A “map-like,” geographic pattern of GS staining of FNH is especially useful in diagnostic dilemmas^[70-72].

Awareness of other benign and malignant epithelial, mesenchymal and vascular tumours is crucial. Cholangiocarcinoma is the second most common primary cancer and accounts for 15% of primary liver

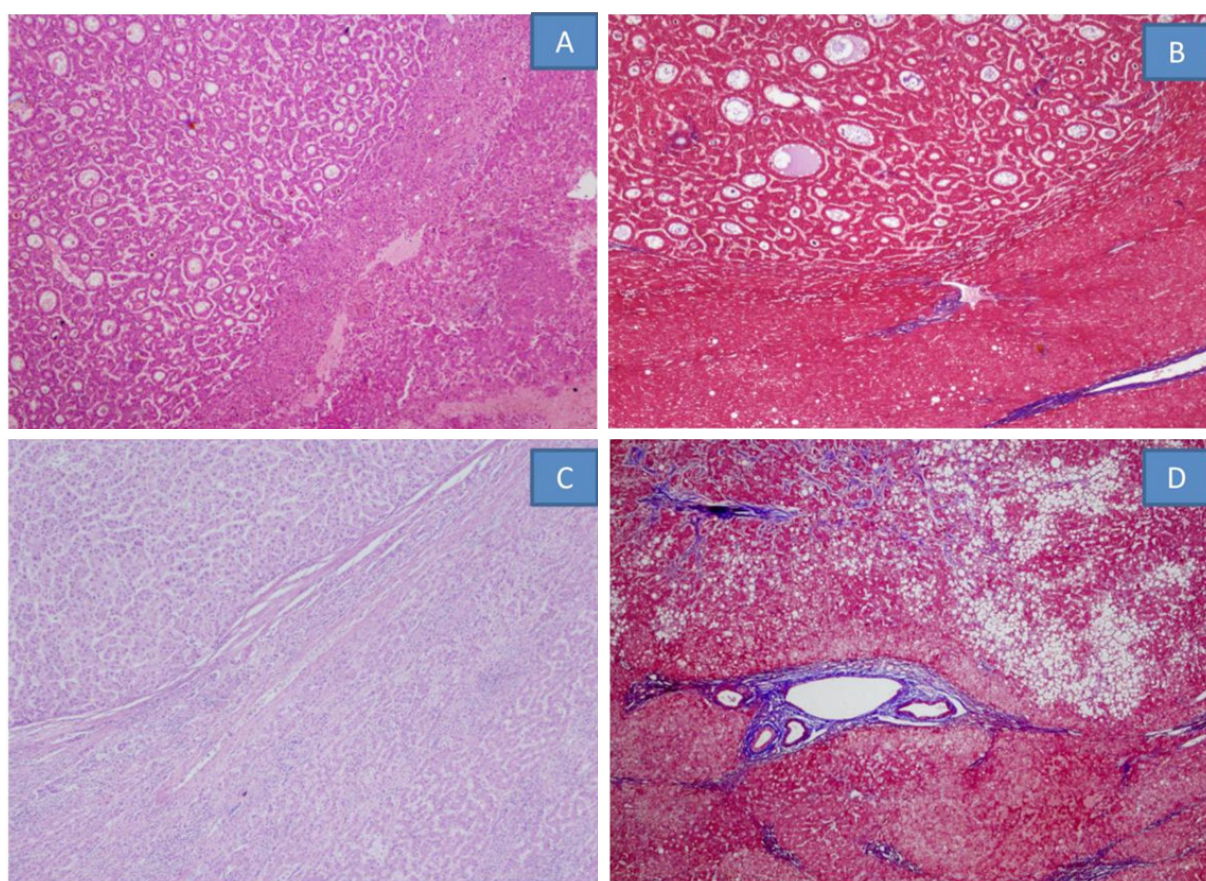


Figure 4. HCC in a non-cirrhotic background: HCC with pseudoglandular pattern in a background with F0-F1 fibrosis (A, HE stain; B Masson Trichrome stain), HCC with microtrabecular pattern in F0 fibrosis (C, HE stain), steatohepatic HCC with F3 fibrosis in background liver (D, MT stain). HCC: hepatocellular carcinoma

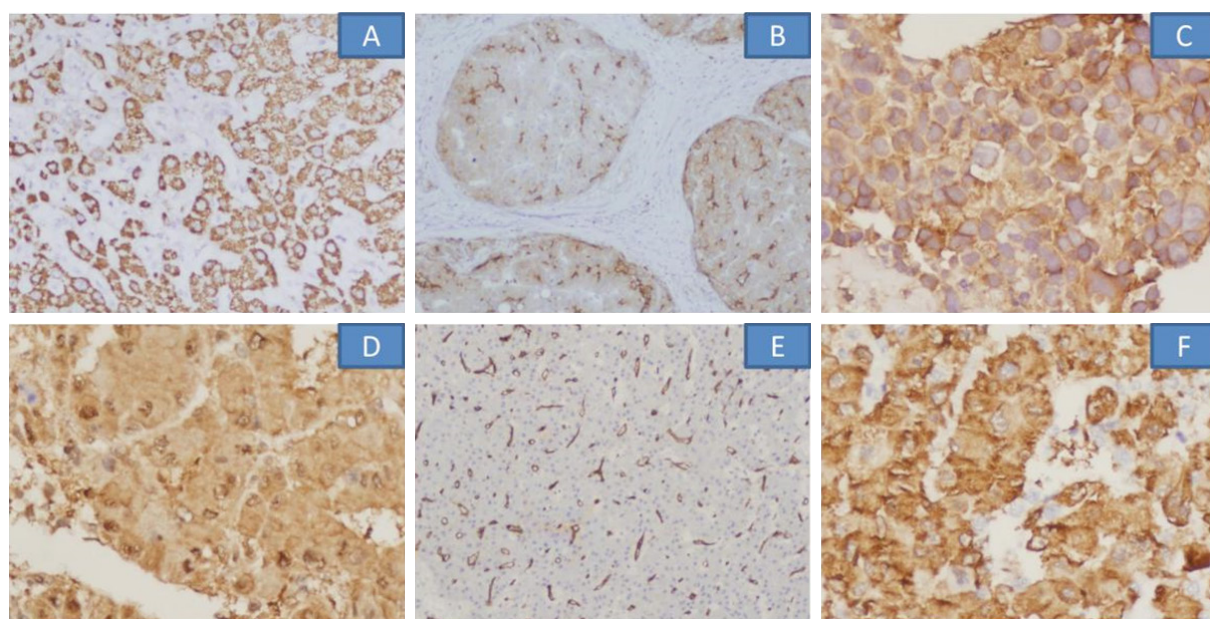


Figure 5. Immunohistochemical markers helpful for confirming hepatocytic origin and in confirming well-differentiated HCC: HepPar1 (A), polyclonal CEA (B), Glypican-3 (C), HSP70 (D), CD34 (E), glutamine synthetase (F). HCC: hepatocellular carcinoma

tumors^[73]. Glands lying in desmoplastic stroma and immunohistochemical expression of mucin1, CK7 and CK19, while negative for hepatocytic origin, aids in the diagnosis^[74]. Bile duct adenoma is often a difficult differential, particularly in intra-operative frozen section examination. A subcapsular location and a well-circumscribed appearance are important clues to the diagnosis. Vascular lesions such as hemangioma, epithelioid hemangioendothelioma, angiosarcoma, and angiomyolipoma are other mimics of HCC on imaging. The histopathology and immunohistochemical markers are quite distinct however, so the diagnosis is straightforward.

Certain HCC variants have a predilection to occur in non-cirrhotic livers. Fibrolamellar, scirrhous, steatohepatic and mixed hepato-cholangiocarcinoma are the more frequent subtypes. Molecular pathways and morpho-molecular features are reviewed in the molecular pathology section.

The fibrolamellar subtype merits special mention. It was first described by Edmondson in 1980, and is a rare entity, accounting for less than 1% of all cases of primary liver cancer^[44]. It is mostly encountered in the young population without any underlying chronic liver disease, or other known predisposing risk factors^[75]. Its key histological features are the presence of lamellar stromal bands surrounding nests of large polygonal eosinophilic tumor cells, which have prominent nucleoli. The tumor cells display the presence of cytoplasmic inclusions - ground-glass pale bodies, eosinophilic cytoplasmic globules, and Mallory-Denk bodies. The immunohistochemical markers CK7 and CD68 are expressed by tumor cells and have a sensitivity of 100% and 96% respectively^[76,77], which are important for confirmation^[47].

Metastasis is the most common hepatic malignancy and occurs in non-cirrhotic livers. In patients without underlying liver disease, HCC accounts for only about 2% of malignant liver neoplasms^[78,79]. The lung, colon, pancreas and breast are the most common primary sites that metastasize to the liver. In some cases, these mimic HCC, particularly clear-cell renal cell carcinoma, clear-cell adenocarcinoma of the female genital organs, adrenal carcinoma and hepatoid adenocarcinoma of the stomach^[78,79].

PATHO-MOLECULAR CHARACTERIZATION OF HCC IN NON-CIRRHOTIC LIVERS

The etiology and etiopathogenesis for a vast number of non-cirrhotic HCCs still remain unknown. Advancements in translational research have made it possible to analyse thousands of molecular targets in HCC using microarray-based technologies as well as next-generation sequencing. However, unlike the cirrhotic HCC and HCA, molecular pathways and classifications have not been exclusively studied in non-cirrhotic HCCs. Genomic studies in this particular group would be able to elucidate the similarities and distinctness of the underlying mechanisms and biology in comparison to the cirrhotic HCC. Despite the scarcity of the literature, recent studies on the molecular pathology of HCC, regardless of the background liver, provides vital information.

The most frequent mutations affect the TERT promoter (60%), which is associated with an increased expression of telomerase. TP53 and CTNNB1 are the next most prevalent mutations. These, combined with low-frequency mutated genes (e.g., AXIN1, ARID2, ARID1A, TSC1/TSC2, RPS6KA3, KEAP1, MLL2), represent the main deregulated pathways in HCC^[80].

Different pathways of genetic alterations point towards different hepatocarcinogenetic mechanisms in HCC with and without cirrhosis. HCCs in non-cirrhotic livers are more often associated with higher β -catenin mutation, p21 expression, p14 inactivation, and global gene methylation, in contrast to higher p53 and Wnt/ β -catenin pathway aberrations seen in cirrhotic HCCs^[81,82]. However, one study showed nuclear p53 labeling in 30% of non-cirrhotic HCCs^[83]. Other studies have also suggested that tumor suppressor genes play an important role in the development of HCC in the absence of cirrhosis^[84] [Figure 6].

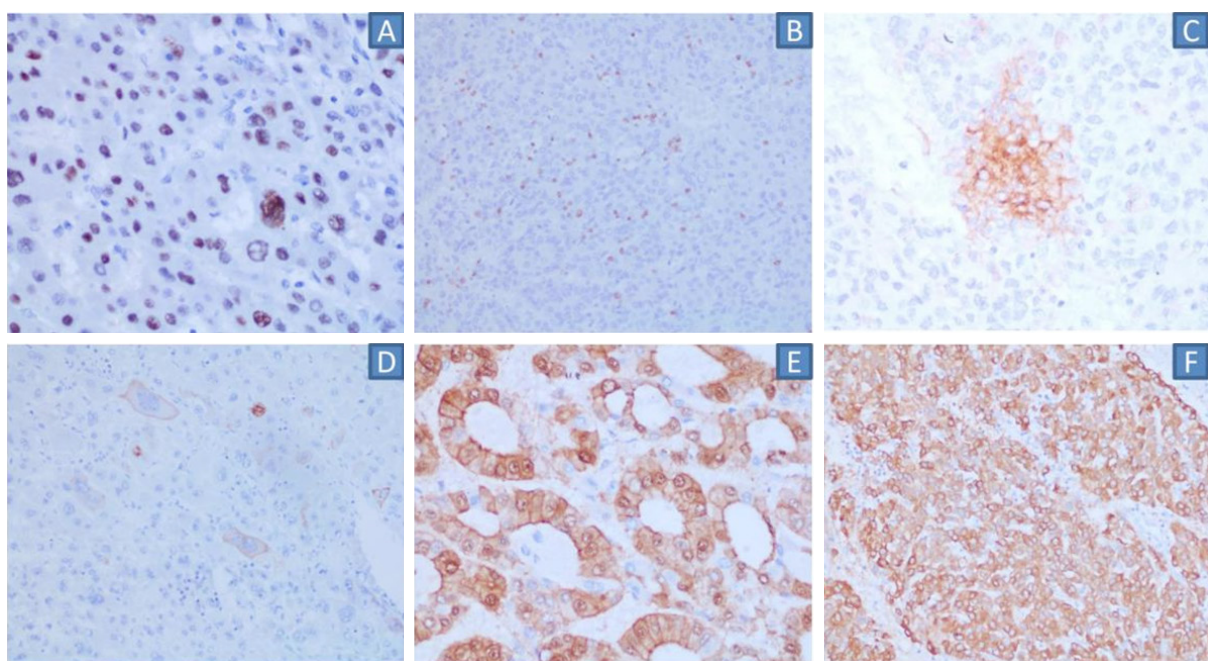


Figure 6. Immunohistochemical markers representative of phenotypic correlates of dysregulated molecular pathways: TP53 (A), PD1 in immune cells (B), PDL1 in tumour cells (C), CK19 in tumour cells (D), β -catenin nuclear positivity (E), glutamine synthetase diffuse expression (F). PD1: Programmed death-1; PD-L1: programmed death-ligand 1

Analysis of miRNAs holds great promise for improving diagnosis and prognosis, along with the therapeutic management of HCCs originating in non-cirrhotic backgrounds. miRNA, particularly hsa-mir-149 expression, is considered an independent risk factor for the poor prognosis of non-cirrhotic HCCs but not for cirrhotic HCCs^[85]. Early and unique changes in circulating miRNA in the serum could allow it to be a biomarker for the early detection of non-cirrhotic HCCs. A similar role of a three mi-RNA panel comprising of miR-92-3p, miR-107, and miR-3126-5p in early HCC was shown by Zhang *et al.*^[86] and Koh *et al.*^[87] reported the difference in expression of 16 miRNAs between non-tumor and HCC tissues in non-cirrhotic livers^[87].

Few studies have pointed towards the role of altered mismatch repair genes in hepatocarcinogenesis. A study to explore the presence of microsatellite instability (MSI) in 37 non-cirrhotic HCC patients with a histologically normal liver, low alcohol intake and absence of HBV and HCV infection^[88], demonstrated that 26 (43%) had MSI (16% of high grade). However, other authors did not find MSI to be significant in non-cirrhotic HCCs^[88,89].

Angiogenesis related characteristics are similar between cirrhotic and non-cirrhotic HCCs^[90]. Favorable outcomes in the fibrolamellar subtype might be partly due to the low number of cytogenetic aberrations in this type of tumor^[91]. Stemness marker expression analysis revealed that 88% of non-cirrhotic HCCs were keratin-19 negative. Expression of K19 in this study demonstrated correlation with less tumor encapsulation and with the presence of p53 mutation^[39].

Certain etiology specific studies have reported important molecular alterations in non-cirrhotic HCCs.

STAT signaling pathways have an important role in HCC-NASH^[92], especially STAT-3 signaling with regard to NASH associated HCC occurring in non-cirrhotic backgrounds^[93]. The literature advocates targeting of the STAT-1 signaling pathway in steatohepatic HCC with cirrhosis or severe fibrosis and NASH,

whereas NASH-HCC without cirrhosis or fibrosis is mediated through the STAT-3 signaling pathway^[92]. Tumor suppressor genes play an important role in the development of steatosis, liver cell damage and HCC development in the absence of cirrhosis in fatty liver disease^[27,84,94]. Patients whose tumors showed heterogeneous staining patterns of glutamine synthetase in non-cirrhotic livers were more commonly overweight or obese^[39].

HBV infection has direct oncogenic potential and the accumulation of mutations in basal core promoters and a high viral load is considered an independent predictor of HCC development. These mutational patterns have the potential to identify those at risk of HCC development. HCC development in HCV infected patients is mainly attributable to sustained necro-inflammatory processes and therefore, occurs on a background of advanced liver fibrosis or frank cirrhosis. Studies have shown that several HCV gene products (core, NS3, NS4B and NS5A) possess transformation potential in murine fibroblast culture, suggesting that HCV also has direct hepatocarcinogenic potential^[16,22,23]. Approximately 46% of HCV-related HCCs exhibit *CTNNB* mutations^[95]. Of these, the majority arise in the absence of underlying cirrhosis.

Overrepresentation of T>C at ApTpX with transcription strand bias, a pattern known to be strongly associated with genotoxic injury, was reported in HCC developing in non-cirrhotic patients with high alcohol and tobacco consumption^[96]. A variety of congenital and acquired conditions also induce the development of HCC without underlying cirrhosis, often through alterations in cell cycle regulation, oxidative stress, and increased levels of tumorigenic growth factors.

Dysregulation of molecular pathways in histological variants of HCC

Macrotrabecular massive HCC (MTM-HCC), a novel distinct subtype of HCC, is characterized by the presence of a macrotrabecular pattern of more than 6 cells thick recorded in > 50% of the tumor as described by Ziol *et al.*^[97]. In another recent study, taking a cut off of > 30% macrotrabecular pattern, MTM-HCC was found to be less often associated with cirrhosis^[98]. This variant shows genetic aberrations, which are related to cell cycle activation, chromosomal instability, the G3 transcriptomic subgroup, FGF19 amplifications and TP53 mutation^[59,99]. MM-HCC is also characterized by the high expression of two key regulators of neoangiogenesis and vascular remodeling, angiopoietin 2 and vascular endothelial growth factor A (VEGFA)^[59,100]. Endothelial-specific molecule 1 (ESM1) was identified as a biomarker for this variant^[59].

The steatohepatic subtype is characterized by prominent steatotic changes in the tumor cells, namely fat accumulation, ballooning degeneration, the presence of Mallory-Denk bodies and peri-cellular fibrosis^[48,101]. SH-HCC also often occurs in non-cirrhotic backgrounds as shown in a recent study that reported SH-HCC in 20% of the 96 HCC cases reviewed^[102]. IL6/JAK/STAT pathway activation, wild type CTNNB1 and TP53 are the molecular pathways implicated in the pathogenesis^[59,103]. However, the literature reports that CTNNB1 mutations (beta catenin pathway alterations) are less frequent in steatohepatic HCCs compared to conventional HCCs^[103].

Lymphocyte-rich HCCs are characterized by an immune rich stroma and have been demonstrated to have cirrhosis in only 46% of cases in a recent comprehensive review^[104]. Molecular studies have revealed that mutations of CTNNB1, AXIN1, APC, NOTCH1 and NOTCH2 were less frequently observed in lymphocyte-rich HCCs than conventional HCCs^[105], suggesting a relation between these pathways and immune exclusion. In lymphoepithelioma-like HCC, oncogenes expressed from chromosome 11q13.3 (CCND1, FGF19, and FGF4) are strongly associated with the immune checkpoint signature (CD274, PDCD1, BTLA, CTLA4, HAVCR2, IDO1, and LAG3). Such differences in genetic aberrations from classical HCCs provide insight into the need for therapeutic strategies that evade immune surveillance seen in classical HCCs^[105,106].

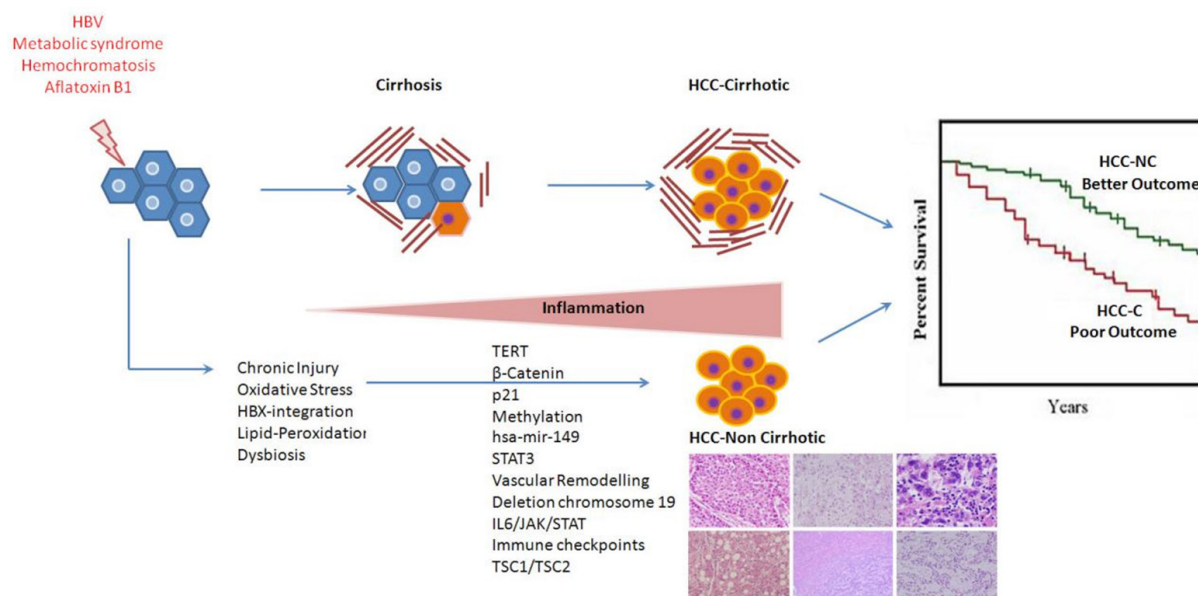


Figure 7. Genetic aberrations in non-cirrhotic hepatocellular carcinomas (HCCs)

Scirrhou HCC is another variant, which is less often associated with liver cirrhosis when compared with conventional HCC^[55]. Genetic studies have highlighted TGF- β signaling, TSC1/TSC2 mutations and the expression of stem cell markers as the main derangements^[59,107].

Fibrolamellar HCC is characterized by a chimeric transcript, found as a result of a deletion in the chromosome 19 - DNAJB1-PRKACA chimeric protein^[108]. This genetic signature is not reported in other tumors, which indicates that the mutation plays a key role in FL-HCC tumorigenesis^[46,109]. Interaction between the fusion kinase and b-catenin^[110] also contribute to the pathogenesis of FLC. Comparative genomic hybridization studies have demonstrated that in contrast to the classical HCC, the TP53, Wnt/ β -catenin, or surviving pathways, are not mutated in FL-HCC^[91,111].

Combined hepatocellular-cholangiocarcinoma (cHCC-CCA) also occurs in the non-cirrhotic liver background. Genomic sequencing shows a profile similar to conventional HCCs^[112]. Studies have shown that the same oncogenic drivers delivered to hepatocytes could generate tumors with either a hepatocellular or biliary phenotype, and such differentiation is mainly dependent upon the microenvironment created by the oncogenic process^[113]. Recurrent alterations in TERT, TP53, cell cycle genes, receptor tyrosine kinase/Ras/PI3K pathway genes, chromatin regulators etc. were identified in cHCC-CCA, while IDH1, IDH2, FGFR2 and BAP1 mutations were absent^[97]. CCND1, MET and ERBB2 amplifications are present at higher frequencies in CHCs compared with HCCs, whereas Wnt pathway alterations are relatively less frequent^[114]. The literature shows that in comparison to HCCs, TP53 mutations occur twice as frequently in cHCC-CCAs. TP53 mutations in HCC are usually associated with a worse prognosis and poorly differentiated histomorphology^[59,115]. This suggests that cHCC-CCA is more similar to poorly differentiated HCCs and explains its worse prognosis [Figure 7].

CONCLUSION

HCCs arising in non-cirrhotic livers are distinct from cirrhotic HCCs in many ways. The risk factors, etiologies, pathogenesis, histopathology and the differential diagnosis, along with genomic pathways, differ from the typical cirrhotic HCC. Current knowledge of phenotypic-molecular alterations are based on studies on HCC irrespective of the liver background. Studies devoted exclusively to non-cirrhotic HCCs

are scarce. In the era of molecular targeted therapies, there is an urgent need to analyze the carcinogenetic mechanisms and pathology based phenotypic correlates of specific molecular aberrations in HCCs originating in non-cirrhotic livers.

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REFERENCES

1. Rastogi A. Changing role of histopathology in the diagnosis and management of hepatocellular carcinoma. *World J Gastroenterol* 2018;24:4000-13.
2. Gaddikeri S, McNeeley MF, Wang CL, Bhargava P, Dighe MK, et al. Hepatocellular carcinoma in the noncirrhotic liver. *Am J Roentgenol* 2014;203:W34-47.
3. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011;365:1118-27.
4. Wörns MA, Bosslet T, Victor A, Koch S, Hoppe-Lotichius M, et al. Prognostic factors and outcomes of patients with hepatocellular carcinoma in non-cirrhotic liver. *Scand J Gastroenterol* 2012;47:718-28.
5. Schütte K, Kipper M, Kahl S, Bornschein J, Götze T, et al. Clinical characteristics and time trends in etiology of hepatocellular cancer in Germany. *Digestion* 2013;87:147-59.
6. Trevisani F, Frigerio M, Santi V, Grignaschi A, Bernardi M. Hepatocellular carcinoma in non-cirrhotic liver: a reappraisal. *Dig Liver Dis* 2010;42:341-7.
7. Zhang Y, Wang C, Xu H, Xiao P, Gao Y. Hepatocellular carcinoma in the noncirrhotic liver: a literature review. *Eur J Gastroenterol Hepatol* 2019;31:743-8.
8. Schütte K, Schulz C, Poranzke J, Antweiler K, Bornschein J, et al. Characterization and prognosis of patients with hepatocellular carcinoma (HCC) in the non-cirrhotic liver. *BMC Gastroenterol* 2014;14:117.
9. Lafitte M, Laurent V, Soyer P, Ayav A, Balaj C, et al. MDCT features of hepatocellular carcinoma (HCC) in non-cirrhotic liver. *Diagn Interv Imaging* 2016;97:355-60.
10. Di Martino M, Saba L, Bosco S, Rossi M, Miles KA, et al. Hepatocellular carcinoma (HCC) in non-cirrhotic liver: clinical, radiological and pathological findings. *Eur Radiol* 2014;24:1446-54.
11. Desai A, Sandhu S, Lai JP, Sandhu DS. Hepatocellular carcinoma in non-cirrhotic liver: a comprehensive review. *World J Hepatol*

- 2019;11:1-18.
12. Nzeako UC, Goodman ZD, Ishak KG. Hepatocellular carcinoma in cirrhotic and noncirrhotic livers. A clinico-histopathologic study of 804 North American patients. *Am J Clin Pathol* 1996;105:65-75.
13. Adam R, Bismuth H, Castaing D, Waechter F, Navarro F, et al. Repeat hepatectomy for colorectal liver metastases. *Ann Surg* 1997;225:51-60; discussion 60-2.
14. Hadjittofi C, Athanasopoulos PG, Koti RS, Konstantinidou SK, Davidson BR. Long-term survival with repeated resections of recurrent hepatocellular carcinoma in a non-cirrhotic liver: case report and brief review of the literature. *Ann Transl Med* 2016;4:112.
15. el-Refaie A, Savage K, Bhattacharya S, Khakoo S, Harrison TJ, et al. HCV-associated hepatocellular carcinoma without cirrhosis. *J Hepatol* 1996;24:277-85.
16. Ray RB, Meyer K, Ray R. Hepatitis C virus core protein promotes immortalization of primary human hepatocytes. *Virology* 2000;271:197-204.
17. Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat Rev Cancer* 2006;6:674-87.
18. Sherman M. Hepatocellular carcinoma: epidemiology, risk factors, and screening. *Semin Liver Dis* 2005;25:143-54.
19. Wong GL, Chan HL, Chan HY, Tse PC, Tse YK, et al. Accuracy of risk scores for patients with chronic hepatitis B receiving entecavir treatment. *Gastroenterology* 2013;144:933-44.
20. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016;65:727-33.
21. Bruix J, Sherman M. American association for the study of liver diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53:1020-2.
22. Park JS, Yang JM, Min MK. Hepatitis C virus nonstructural protein NS4B transforms NIH3T3 cells in cooperation with the Ha-ras oncogene. *Biochem Biophys Res Commun* 2000;267:581-7.
23. Sakamuro D, Furukawa T, Takegami T. Hepatitis C virus nonstructural protein NS3 transforms NIH 3T3 cells. *J Virol* 1995;69:3893-6.
24. Lee DH, Lee JM. Primary malignant tumours in the non-cirrhotic liver. *Eur J Radiol* 2017;95:349-61.
25. Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. *J Hepatol* 2012;56:1384-91.
26. Thompson SM, Garg I, Ehman EC, Sheedy SP, Bookwalter CA, et al. Non-alcoholic fatty liver disease-associated hepatocellular carcinoma: effect of hepatic steatosis on major hepatocellular carcinoma features at MRI. *Br J Radiol* 2018;91:20180345.
27. Ertle J, Dechêne A, Sowa J, Penndorf V, Herzer K, et al. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *Int J Cancer* 2011;128:2436-43.
28. Adami HO, Hsing AW, McLaughlin JK, Trichopoulos D, Hacker D, et al. Alcoholism and liver cirrhosis in the etiology of primary liver cancer. *Int J Cancer* 1992;51:898-902.
29. London WT, McGlynn KA. Liver cancer. In: Schottenfeld D, Fraumeni Jr JF, editors. *Cancer epidemiology and prevention*. New York: Oxford University Press; 1996. pp. 772-93.
30. Burra P, Zanetto A, Germani G. Liver transplantation for alcoholic liver disease and hepatocellular carcinoma. *Cancers (Basel)* 2018;10:46.
31. Jiang HY, Chen J, Xia CC, Cao LK, Duan T, et al. Noninvasive imaging of hepatocellular carcinoma: from diagnosis to prognosis. *World J Gastroenterol* 2018;24:2348-62.
32. Ramakrishna G, Rastogi A, Trehanpati N, Sen B, Khosla R, et al. From cirrhosis to hepatocellular carcinoma: new molecular insights on inflammation and cellular senescence. *Liver Cancer* 2013;2:367-83.
33. Trehanpati N, Shrivastav S, Shivakumar B, Khosla R, Bhardwaj S, et al. Analysis of notch and TGF- β signaling expression in different stages of disease progression during hepatitis B virus infection. *Clin Transl Gastroenterol* 2012;3:e23.
34. Turlin B, Juguet F, Moirand R, Le Quilleuc D, Loréal O, et al. Increased liver iron stores in patients with hepatocellular carcinoma developed on a noncirrhotic liver. *Hepatology* 1995;22:446-50.
35. Ozakyol A. Global epidemiology of hepatocellular carcinoma (HCC epidemiology). *J Gastrointest Canc* 2017; 48:238-40.
36. Liu CJ, Chen BF, Chen PJ, Lai MY, Huang WL, et al. Role of hepatitis B virus precore/core promoter mutations and serum viral load on noncirrhotic hepatocellular carcinoma: a case-control study. *J Infect Dis* 2006;194:594-9.
37. Villanueva A. Hepatocellular carcinoma. *N Engl J Med* 2019;380:1450-62.
38. Zheng C, Zeng H, Lin H, Wang J, Feng X, et al. Serum microcystin levels positively linked with risk of hepatocellular carcinoma: a case-control study in southwest China. *Hepatology* 2017;66:1519-28.
39. Liu TC, Vachharajani N, Chapman WC, Brunt EM. Noncirrhotic hepatocellular carcinoma: derivation from hepatocellular adenoma? Clinicopathologic analysis. *Mod Pathol* 2014;27:420-32.
40. Farrell GC, Joshua DE, Uren RF, Baird PJ, Perkins KW, et al. Androgen-induced hepatoma. *Lancet* 1975;22:430-2.
41. Trevisani F, D'Intino PE, Caraceni P, Pizzo M, Stefanini GF, et al. Etiologic factors and clinical presentation of hepatocellular carcinoma. Differences between cirrhotic and noncirrhotic Italian patients. *Cancer* 1995;75:2220-32.
42. Lee Y, Park H, Lee H, Cho JY, Yoon YS, et al. The clinicopathological and prognostic significance of the gross classification of hepatocellular carcinoma. *J Pathol Transl Med* 2018;52:85-92.
43. Kojiro M. *Pathology of hepatocellular carcinoma*. Hoboken: Wiley; 2009. pp. 1-184.
44. Craig JR, Peters RL, Edmondson HA, Omata M. Fibrolamellar carcinoma of the liver: a tumor of adolescents and young adults with distinctive clinico-pathologic features. *Cancer* 1980;46:372-9.
45. Houben KW, McCall JL. Liver transplantation for hepatocellular carcinoma in patients without underlying liver disease: a systematic

- review. *Liver Transpl Surg* 1999;5:91-5.
46. Lin CC, Yang HM. Fibrolamellar carcinoma: a concise review. *Arch Pathol Lab Med* 2018;142:1141-5.
 47. Van Eyken P, Sciot R, Brock P, Casteels-Van Daele M, Ramaekers FC, et al. Abundant expression of cytokeratin 7 in fibrolamellar carcinoma of the liver. *Histopathology* 1990;17:101-7.
 48. Cheuk-Lam Lo R. An update on the histological subtypes of hepatocellular carcinoma. *Hepatoma Res* 2019;5:41.
 49. International consensus group for hepatocellular neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 2009;49:658-64.
 50. Agni RM. Diagnostic histopathology of hepatocellular carcinoma: a case-based review. *Semin Diagn Pathol* 2017;34:126-37.
 51. Bosman FTCE, Hruban RH, Theise ND. World Health Organization classification of tumours: pathology and genetics of tumours of the digestive system. Lyon, France: IARC Press; 2010.
 52. Torbenson MS. Morphologic Subtypes of hepatocellular carcinoma. *Gastroenterol Clin North Am* 2017;46:365-91.
 53. Ang CS, Kelley RL, Choti MA, Cosgrove DP, Chou JF, et al. Clinicopathologic characteristics and survival outcomes of patients with fibrolamellar carcinoma: data from the fibrolamellar carcinoma consortium. *Gastrointest Cancer Res* 2013;6:3-9.
 54. Fujii T, Zen Y, Harada K, Harada K, Niwa HN, Masuda S, et al. Participation of liver cancer stem/progenitor cells in tumorigenesis of scirrhous hepatocellular carcinoma-human and cell culture study. *Hum Pathol* 2008;39:1185-96.
 55. Lee JH, Choi MS, Gwak GY, Gwak GY, Lee JH, et al. Clinicopathologic characteristics and long-term prognosis of scirrhous hepatocellular carcinoma. *Dig Dis Sci* 2012;57:1698-707.
 56. Trojan J, Zangos S, Schnitzbauer AA. Diagnostics and treatment of hepatocellular carcinoma in 2016: standards and developments. *Visc Med* 2016;32:116-120.
 57. Chang CH, Chau GY, Lui WY, Tsay SH, King KL, et al. Long-term results of hepatic resection for hepatocellular carcinoma originating from the noncirrhotic liver. *Arch Surg* 2004;139:320-5.
 58. Paradis V, Zalinski S, Chelbi E, Guedj N, Degos F, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. *Hepatology* 2009;49:851-9.
 59. Calderaro J, Couchy G, Imbeaud S, Amadeo G, Letouzée E, et al. Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. *J Hepatol* 2017;67:727-38.
 60. Okuda K, Nakashima T, Kojiro M, Kondo Y, Wada K. Hepatocellular carcinoma without cirrhosis in Japanese patients. *Gastroenterology* 1989;97:140-6.
 61. Shimada M, Rikimaru T, Sugimachi K, Hamatsu T, Yamashita Y, et al. The importance of hepatic resection for hepatocellular carcinoma originating from nonfibrotic liver. *J Am Coll Surg* 2000;191:531-7.
 62. Grando-Lemaire V, Guettier C, Chevret S, M Beaugrand, Trinchet JC. Hepatocellular carcinoma without cirrhosis in the West: epidemiological factors and histopathology of the non-tumorous liver. *Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire. J Hepatol* 1999;31:508-13.
 63. Bralet MP, Régimbeau JM, Pineau P, Dubois S, Loas G, et al. Hepatocellular carcinoma occurring in nonfibrotic liver: epidemiologic and histopathologic analysis of 80 French cases. *Hepatology* 2000;32:200-4.
 64. van Meer S, van Erpecum KJ, Sprengers D, Coenraad MJ, Klümpen HJ, et al. Hepatocellular carcinoma in cirrhotic versus noncirrhotic livers: results from a large cohort in the Netherlands. *Eur J Gastroenterol Hepatol* 2016;28:352-9.
 65. Pittman ME, Brunt EM. Anatomic pathology of hepatocellular carcinoma: histopathology using classic and new diagnostic tools. *Clin Liver Dis* 2015;19:239-59.
 66. Evason KJ, Grenet JP, Ferrell LD, Kakar S. Atypical hepatocellular adenoma-like neoplasms with β -catenin activation show cytogenetic alterations similar to well-differentiated hepatocellular carcinomas. *Hum Pathol* 2013;44: 750-8.
 67. Dokmak S, Paradis V, Vilgrain V, Sauvanet A, Farges O, et al. A single-center surgical experience of 122 patients with single and multiple hepatocellular adenomas. *Gastroenterology* 2009;137:1698-705.
 68. Bioulac-Sage P, Laumonier H, Couchy G, Bail BL, Cunha A, et al. Hepatocellular adenoma management and phenotypic classification: the Bordeaux experience. *Hepatology* 2009;50:481-9.
 69. Deneve JL, Pawlik TM, Cunningham S, Clary B, Reddy S, et al. Liver cell adenoma: a multicenter analysis of risk factors for rupture and malignancy. *Ann Surg Oncol* 2009;16:640-8.
 70. Roncalli M, Sciarra A, Tommaso LD. Benign hepatocellular nodules of healthy liver: Focal nodular hyperplasia and hepatocellular adenoma. *Clin Mol Hepatol* 2016;22:199-211.
 71. Libbrecht L, Cassiman D, Verslype C, Maleux G, Van Hees D, et al. Clinicopathological features of focal nodular hyperplasia-like nodules in 130 cirrhotic explant livers. *Am J Gastroenterol* 2006;101:2341-6.
 72. Bioulac-Sage P, Laumonier H, Rullier A, Cubel G, Laurent C, et al. Over-expression of glutamine synthetase in focal nodular hyperplasia: a novel easy diagnostic tool in surgical pathology. *Liver Int* 2009;29:459-65.
 73. Nakanuma Y, Sripa B, Vatanasapt B, Leong A, Ponchon T, et al. Intrahepatic cholangiocarcinoma. In: Hamilton SR, Aaltonen LA, editors. *WHO Classification of Tumors, Pathology and Genetics Tumors of the Digestive System*. Lyon: IARC Press; 2000. pp. 173-80.
 74. Suriawinata AA, Thung SN. Malignant liver tumors. *Clin Liver Dis* 2002;6:527-54, ix.
 75. El-Serag HB, Davila JA. Is fibrolamellar carcinoma different from hepatocellular carcinoma? A US population-based study. *Hepatology* 2004;39:798-803.
 76. Ward SC, Huang J, Tickoo SK, Thung SN, Ladanyi M, et al. Fibrolamellar carcinoma of the liver exhibits immunohistochemical evidence of both hepatocyte and bile duct differentiation. *Mod Pathol* 2010;23:1180-90.
 77. Ross HM, Daniel HD, Vivekanandan P, Kannangai R, Yeh MM, et al. Fibrolamellar carcinomas are positive for CD68. *Mod Pathol*

- 2011;24:390-5.
78. Schlageter M, Terracciano LM, D'Angelo S, Sorrentino P. Histopathology of hepatocellular carcinoma. *World J Gastroenterol* 2014;20:15955-64.
79. Melato M, Laurino L, Mucli E, Valente M, Okuda K. Relationship between cirrhosis, liver cancer, and hepatic metastases. An autopsy study. *Cancer* 1989;64:455-9.
80. Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM. Genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology* 2015;149:1226-39.e4.
81. Tretiakova MS, Shabani-Rad MT, Guggisberg K, Hart J, Andera RA, et al. Genomic and immunophenotypical differences between hepatocellular carcinoma with and without cirrhosis. *Histopathology* 2010;56:683-93.
82. Tannapfel A, Wittekind C. Genes involved in hepatocellular carcinoma: deregulation in cell cycling and apoptosis. *Virchows Arch* 2002;440:345-52.
83. Sanyal AJ, Yoon SK, Lencioni R. The etiology of hepatocellular carcinoma and consequences for treatment. *Oncologist* 2010;15 Suppl 4:14-22.
84. Herzer K, Hofmann TG, Teufel A, Schimanski CC, Moehler M, et al. IFN-alpha-induced apoptosis in hepatocellular carcinoma involves promyelocytic leukemia protein and TRAIL independently of p53. *Cancer Res* 2009;69:855-62.
85. Mei Y, You Y, Xia J, Gong JP, Wang YB. Identifying differentially expressed microRNAs between cirrhotic and non-cirrhotic hepatocellular carcinoma and exploring their functions using bioinformatic analysis. *Cell Physiol Biochem* 2018;48:1443-56.
86. Zhang Y, Li T, Qiu Y, Zhang T, Guo P, et al. Serum microRNA panel for early diagnosis of the onset of hepatocellular carcinoma. *Medicine (Baltimore)* 2017;96:e5642.
87. Koh YS, Kim JH, Cai H, Li LH, Kim HS, et al. Dysregulated microRNAs in non-cirrhotic hepatocellular carcinoma. *Genes Genom* 2013;35:759-65.
88. Chiappini F, Gross-Goupil M, Saffroy R, Azoulay D, Emile JF, et al. Microsatellite instability mutator phenotype in hepatocellular carcinoma in non-alcoholic and non-virally infected normal livers. *Carcinogenesis* 2004;25:541-7.
89. Togni R, Bagla N, Muesan P, Miquel R, O'Grady J, et al. Microsatellite instability in hepatocellular carcinoma in non-cirrhotic liver in patients older than 60 years. *Hepatol Res* 2009;39:266-73.
90. Zeng W, Gouw AS, van den Heuvel MC, Molema G, Poppema S, et al. Hepatocellular carcinomas in cirrhotic and noncirrhotic human livers share angiogenic characteristics. *Ann Surg Oncol* 2010;17:1564-71.
91. Kakar S, Chen X, Ho C, Burgart LJ, Sahai V, et al. Chromosomal changes in fibrolamellar hepatocellular carcinoma detected by array comparative genomic hybridization. *Mod Pathol* 2009;22:134-41.
92. Grohmann M, Wiede F, Dodd GT, Gurzov EN, Ooi GJ, et al. Obesity drives STAT-1-dependent NASH and STAT-3-dependent HCC. *Cell* 2018;175:1289-306.e20.
93. Takakura K, Oikawa T, Nakano M, Saeki C, Torisu Y, et al. Recent insights into the multiple pathways driving non-alcoholic steatohepatitis-derived hepatocellular carcinoma. *Front Oncol* 2019;9:762.
94. Cazanave SC, Mott JL, Elmi NA, Bronk SF, Werneburg NW, et al. JNK1-dependent PUMA expression contributes to hepatocyte lipopoptosis. *J Biol Chem* 2009;284:26591-602.
95. Shanbhogue AK, Prasad SR, Takahashi N, Vikram R, Sahani DV. Recent advances in cytogenetics and molecular biology of adult hepatocellular tumors: implications for imaging and management. *Radiology* 2011;258:673-93.
96. Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, et al. Exome sequencing of hepatocellular carcinoma identifies new mutations signatures and potential therapeutic targets. *Nat Genet* 2015;47:505-11.
97. Ziol M, Pote N, Amadeo G, Laurent A, Nault JC, et al. Macrotrabecular-massive hepatocellular carcinoma: a distinctive histological subtype with clinical relevance. *Hepatology* 2018;68:103-12.
98. Jeon Y, Benedict M, Taddei T, Jain D, Zhang X. Macrotrabecular hepatocellular carcinoma: an aggressive subtype of hepatocellular carcinoma. *Am J Surg Pathol* 2019;43:943-8.
99. Boyault S, Rickman DS, de Reynies A, Balabaud C, Rebouissou S, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 2007;45:42-52.
100. Zhang L, Yang N, Park JW, Katsaros D, Fracchioli S, et al. Tumor-derived vascular endothelial growth factor up-regulates angiopoietin-2 in host endothelium and destabilizes host vasculature, supporting angiogenesis in ovarian cancer. *Cancer Res* 2003;63:3403-12.
101. Salomao M, Yu WM, Brown RS Jr, Emond JC, Lefkowitz JH. Steatohepatic hepatocellular carcinoma (SH-HCC): a distinctive histological variant of HCC in hepatitis C virus-related cirrhosis with associated NAFLD/NASH. *Am J Surg Pathol* 2010;34:1630-6.
102. Tan PS, Nakagawa S, Goossens N, Venkatesh A, Huang T, et al. Clinicopathological indices to predict hepatocellular carcinoma molecular classification. *Liver Int* 2016;36:108-18.
103. Ando S, Shibahara J, Hayashi A, Fukayama M. β -catenin alteration is rare in hepatocellular carcinoma with steatohepatic features: immunohistochemical and mutational study. *Virchows Arch* 2015;467:535-42.
104. Labgaa I, Stueck A, Ward SC. Lymphoepithelioma-like carcinoma in liver. *Am J Pathol* 2017;187:1438-44.
105. Chan AW, Zhang Z, Chong CC, Tin EK, Chow C, et al. Genomic landscape of lymphoepithelioma-like hepatocellular carcinoma. *J Pathol* 2019;249:166-72.
106. Han Y, Wu Y, Yang C, Huang J, Guo Y, et al. Dynamic and specific immune responses against multiple tumor antigens were elicited in patients with hepatocellular carcinoma after cell-based immunotherapy. *J Transl Med* 2017;15:64.
107. Seok JY, Na DC, Woo HG, Roncalli M, Kwon SM, et al. A fibrous stromal component in hepatocellular carcinoma reveals a cholangiocarcinoma-like gene expression trait and epithelial-mesenchymal transition. *Hepatology* 2012;55:1776-86.

108. Honeyman JN, Simon EP, Robine N, Chiaroni-Clarke R, Darcy DG, et al. Detection of a recurrent DNAJB1-PRKACA chimeric transcript in fibrolamellar hepatocellular carcinoma. *Science* 2014;343:1010-4.
109. Riggle KM, Turnham R, Scott JD, Yeung RS, Riehle KJ. Fibrolamellar hepatocellular carcinoma: mechanistic distinction from adult hepatocellular carcinoma. *Pediatr Blood Cancer* 2016;63:1163-7.
110. Kastenhuber ER, Lalazar G, Houlihan SL, Tschaharganeh DF, Baslan T, et al. DNAJB1-PRKACA fusion kinase interacts with beta-catenin and the liver regenerative response to drive fibrolamellar hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 2017;114:13076-84.
111. Wilkens L, Bredt M, Flemming P, Kubicka S, Klempnauer J, et al. Cytogenetic aberrations in primary and recurrent fibrolamellar hepatocellular carcinoma detected by comparative genomic hybridization. *Am J Clin Pathol* 2000;114:867-74.
112. Joseph NM, Tsokos CG, Umetsu SE, Shain AH, Kelley RK, et al. Genomic profiling of combined hepatocellular-cholangiocarcinoma reveals similar genetics to hepatocellular carcinoma. *J Pathol* 2019;248:164-78.
113. Seehawer M, Heinzmann F, D'Artista L, Harbig J, Roux PF, et al. Necroptosis microenvironment directs lineage commitment in liver cancer. *Nature* 2018;562:69-75.
114. Cancer Genome Atlas Research Network. Comprehensive and inte-grative genomic characterization of hepatocellular carcinoma. *Cell* 2017;169:1327-41.
115. Zhan P, Ji YN. Prognostic significance of TP53 expression for patients with hepatocellular carcinoma: a meta-analysis. *Hepatobiliary Surg Nutr* 2014;3:11-7.

Case Report

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Long-term survival of occult hepatitis B associated hepatocellular carcinoma following surgery and antiviral therapy

Tina Boortalary¹, Ernest Rosato², Christopher Roth³, Xiangdong David Ren⁴, Selena Y. Lin⁵, Hie-Won Hann⁶

¹Department of Medicine, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA.

²Department of Surgery, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA.

³Department of Radiology, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA.

⁴Reniguard Life Sciences Inc., Malvern, PA 19355, USA.

⁵JBS Science, Inc., Doylestown, PA 18902, USA.

⁶Division of Liver Disease Prevention Center, Division of Gastroenterology and Hepatology, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA.

Correspondence to: Hie-Won Hann, MD, FAASLD, Department of Medicine, Division of Gastroenterology and Hepatology, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA. E-mail: hie-won.hann@jefferson.edu

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Abstract

Occult hepatitis B infection (OBI) is characterized by absent hepatitis B surface antigen (HBsAg), low or undetectable serum hepatitis B viral DNA (HBV-DNA), and detectable DNA in the liver. There is debate over whether OBI increases the risk of hepatocellular carcinoma (HCC). We present a patient with negative HBsAg and a large HCC tumor who underwent a large right hepatic lobectomy. Initially, the etiology of HCC was unknown, but through more sensitive molecular testing, it was believed to be due to OBI. In this case report, we discuss the patient's clinical course, the effect of antiviral therapy, mechanism of carcinogenesis in OBI, and the need for more rigorous HBV DNA assay testing for the detection of OBI.

Keywords: Occult hepatitis B infection, OBI-associated HCC, HBsAg negative HCC

INTRODUCTION

Hepatitis B virus (HBV) has caused over 50 percent of hepatocellular carcinoma (HCC) cases worldwide^[1]. The prognosis for HCC is generally poor especially when patients present with multifocal disease. Radical liver resection is usually ineffective as new tumors can present in the remnant liver. High levels of HBV-DNA are believed to increase the risk for HCC and sensitive molecular testing has identified OBI as a risk factor in



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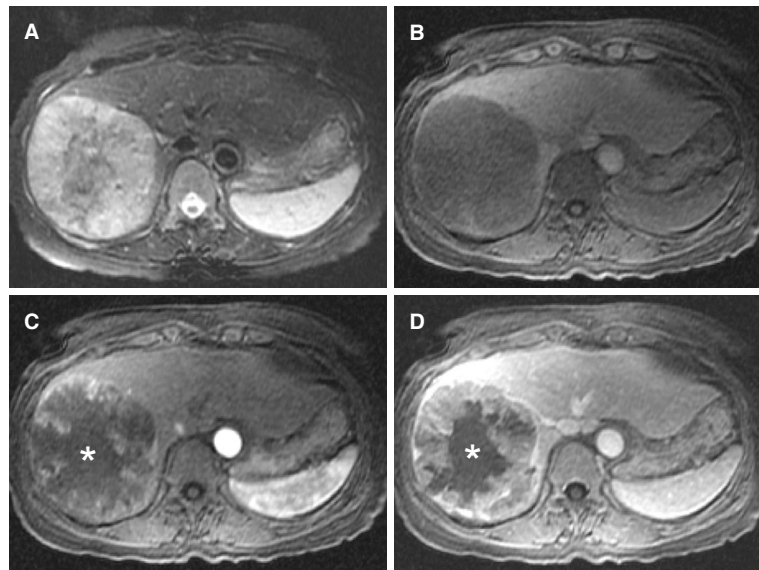


Figure 1. Contrast-enhanced magnetic resonance imaging showing a large liver mass. The axial T2-weighted fat-suppressed image (A) shows a large hyperintense mass replacing most of the right hepatic lobe. The corresponding T1-weighted fat-suppressed pre-contrast (B), arterial phase post-contrast (C) and delayed post-contrast (D) images demonstrate hypointensity with patchy arterial hyperenhancement and washout with capsule appearance of the periphery and central necrosis (asterisk)

the progression of cancer. OBI has been recognized as a possible phase of chronic hepatitis B infection and is characterized by absent HBsAg, low or undetectable serum HBV-DNA, and detectable DNA in the liver^[2]. While the clinical significance of OBI remains unknown, the frequency varies across populations, and with the specificity and sensitivity of routine laboratory assays. The prevalence is as high as 41%-90% in those with prior HBV exposure in high-prevalence areas, and 5%-20% in low-prevalence areas^[3,4]. Except for cases of replication-defective variants or S escape mutants that produce undetectable modified HBsAg, most OBI are capable of replicating but are suppressed in their activity by host defense mechanisms^[5].

We present the case of a patient with negative HBsAg and a large, 10 cm hepatocellular carcinoma who underwent a right hepatic lobectomy. The patient subsequently required lung resection for metastasis 4 years later. After tumor recurrence, additional testing revealed the presence of OBI. The patient was started on anti-HBV therapy and has remained disease free for the past 16 years. The patient's earlier course was published previously^[6]. This is a follow up of the patient's 16-year course to date. In this paper, we describe the patient's initial hepatic surgical resection, the subsequent lung resection complicated by postoperative infection, and the patient's long-term management, functional outcome, and survival.

CASE REPORT

A 64-year-old woman presented with right shoulder pain for one month and was found to have a 9 cm mass on magnetic resonance imaging (MRI) scan in the right lobe of the liver [Figure 1]. Physical exam revealed a hard, non-tender mass in the right upper quadrant (RUQ) extending to the pelvis. Ultrasound-guided core biopsy of the mass was compatible with HCC. Family history was negative for known HBV or liver cancer. Her mother died from injuries sustained during a bomb explosion when the patient was 14. Her father died of pulmonary disease. Her three younger siblings (60F, 56F, 52M) were well without liver disease. The patient has three children. Her 43-year-old daughter was found to be HBsAg positive whilst her 2nd daughter, aged 37-year-old, was negative for HBsAg but positive for anti-HBc total, suggestive of past exposure. Data was unavailable on her youngest daughter. The patient has a history of depressive disorder and has been on Prozac for the past 12 years.

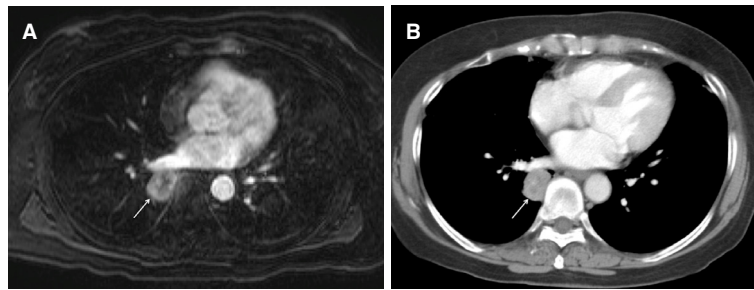


Figure 2. Magnetic resonance imaging and computed tomography (CT) showing a right lower lobe mass. The axial T1-weighted fat-suppressed postcontrast (A) and enhanced CT (B) images show an enhancing mass in the right lower lobe (arrow)

On initial presentation, the patient was not in acute distress. Blood results revealed an AFP of 7,981 ng/mL, HBsAg (-), Anti-HBs (+), Anti-HBc total (+), Anti-HCV (-), HBV DNA (-), serum albumin 4.0 g/dL, total bilirubin 0.7 mg/dL, ALT 17, AST 95, alkaline phosphatase 98 U/L, WBC 5.4 K/ μ L, platelets 229 K/ μ L, serum creatinine 0.7 mg/dL and normal coagulation studies. HBsAg was determined with a quantitative HBsAg assay (AxSYM, Abbott Laboratories, IL, USA).

The patient was evaluated for potential curative resection. computed tomography (CT) and MRI staging studies did not reveal intrahepatic or distant metastatic disease. No regional adenopathy was identified. She was assessed to be medically fit for resection and her calculated remnant liver volumes were acceptable. In the operating room, a staging laparoscopy revealed no evidence of peritoneal metastases. There was no evidence of macro-nodular cirrhosis or portal hypertension. Intraoperative ultrasound of the liver confirmed a single large right hepatic lobe HCC without evidence of satellite lesions or additional tumors. Through abdominal exploration, there was suspicion of invasion of the right diaphragm at the bare area of the liver. A portion of the right diaphragm was resected with the right hepatic lobe to achieve grossly clean margins and the diaphragm was repaired primarily. The patient had an uneventful recovery and was discharged home. Final pathology revealed moderately differentiated HCC.

Follow up AFP decreased to 2.4 mg/mL at 4 months post-surgery. Four years later, the patient's AFP increased to 25.5 ng/mL and peaked at 79.8 ng/mL 3 months later. Abdominal MRI showed a 3.2 cm mass behind the heart [Figure 2]. Chest CT confirmed a mass behind the right pulmonary vein and she underwent video-assisted thoracoscopic surgery (VATS) to remove the right lower lobe lung mass. Histologically, the lung mass was confirmed to be HCC. Her postoperative course was complicated by continued pleural effusions and empyema for which she underwent a right lower lobectomy and decortication via VATS [Figure 3A-C].

At this juncture, questions of whether the patient's HCC could be attributed to HBV infection were raised. While she had remained HBV DNA negative by the commercial assay, her daughters' positive HBV markers prompted consultation with a laboratory where more sensitive HBV DNA testing had been developed^[6,7]. The analytical sensitivity was 15-20 copies/mL, while at the time of assay development, the sensitivity of the Roche Cobas HBV DNA assay was at ~150 copies/mL. Since HBV DNA tends to mutate, it is possible that our assay detected a HBV strain that the commercial assay could not due to rare mutation(s).

HCC tissue from the original liver tumor, the lung tumor, and the serum specimens collected both at the time of her HCC diagnosis and at the time of her lung metastasis were sent to the laboratory. DNA was extracted from formalin-fixed liver or lung tissue blocks using the DNeasy Blood & Tissue Kit from Qiagen. The extracted DNA was then subject to real time PCR^[7].

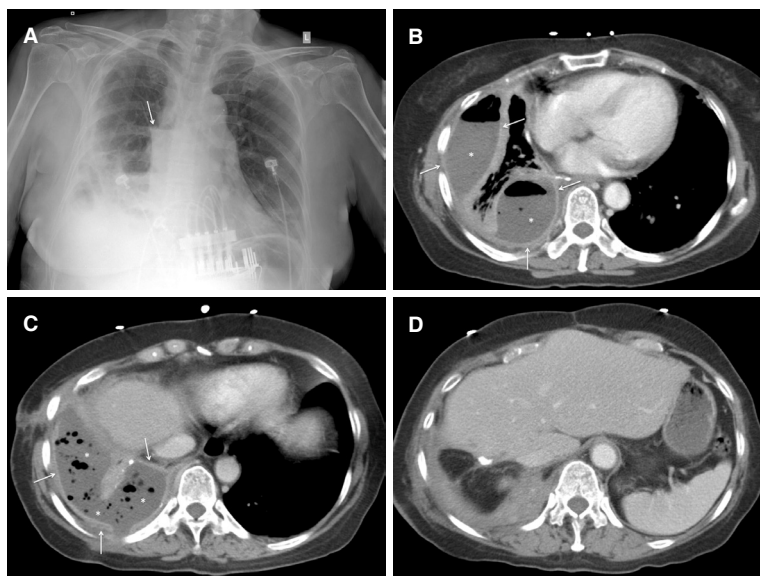


Figure 3. Chest radiograph and computed tomography (CT) showing empyema. The frontal chest radiograph (A) shows layering fluid in the right pleural space with an air-fluid level more medially, overlapping the right paramediastinal shadow (arrow) corresponding to the empyema. Enhanced axial CT images (B and C) show loculated fluid (asterisks) in the right pleural space with pockets of gas surrounded by a thick, enhancing rim (arrows) typical of an empyema. More caudally in the upper abdomen (D), hepatectomy changes are demonstrated post-resection of the previously noted large hepatic mass with regeneration of the left lobe

The liver tumor was positive for HBV DNA while the metastatic lung mass was negative for HBV DNA. Serum samples from the time of her diagnosis of HCC and subsequent lung metastasis (both negative by commercial assay) showed HBV DNA levels of 3,271 copies/mL and 52 copies/mL respectively.

Based on these findings, the patient was started on lamivudine 150 mg daily, 1 year after lung metastasis resection. At that time, her HBV profile by commercial assay showed HBsAg (-), Anti-HBs (+), Anti-HBc total (+), anti-HAV (+), and AFP 2.8 ng/mL. Seven years after lamivudine therapy, her anti-HBc total became negative, which was suggestive of possible decrease or elimination of the HBV covalently closed circular DNA (cccDNA) in her liver.

For the past 12 years, after resection of lung metastasis and antiviral therapy, she has had no evidence of recurrence of the HCC and has maintained undetectable HBV DNA levels. Imaging shows that her left hepatic lobe has hypertrophied [Figure 3D].

DISCUSSION

Well-established risk factors for the development of HCC in patients with chronic HBV infection are viral load and the presence of HBeAg and HBsAg^[8-10]. However, studies have demonstrated a high rate of OBI in patients with HCC who are immunocompromised during chemotherapy for malignancy^[11,12], as well as in patients with hepatitis C^[13]. The 38%-73% of patients from endemic areas with cryptogenic HCC actually have underlying OBI^[13-15]. Despite such evidence, the direct correlation between OBI and carcinogenesis remains controversial. While some studies have linked OBI to hepatocellular carcinoma^[16,17], other studies have failed to show direct causality^[15,18].

Occult HBV can persist in hepatocytes as both integrated DNA or as a free episome known as covalently closed circular DNA (cccDNA), while maintaining transcription activity and synthesizing proteins at low levels^[15]. HBV can promote carcinogenesis through the integration of HBV sequences into the host genome, as well as through mild continuous micro-inflammation, contributing to chronic liver disease and cirrhosis^[19].

With our patient, the etiology of the HCC was unclear given her negative HBsAg and undetectable serum viral load. Suspicion for HBV as the possible cause for her HCC was prompted by HBV positivity in her daughters. It is likely that she was infected with HBV during her reproductive period and vertically transmitted the virus to her daughters. Additionally, the presence of HBV DNA in the liver tumor and the more sensitive HBV serum DNA assay suggested a case of OBI. Indeed, the failure of commercial HBV assays to detect HBV DNA has been reported, particularly in patients harboring treatment-resistant mutations^[20]. This highlights the need for a more rigorous HBV DNA assay test and reinforces the need to target at least three different locations in the HBV genome^[2]. Furthermore, HBV DNA assays used in diagnosing OBI should be able to distinguish between the detection of integrated HBV DNA and replication competent HBV DNA, which encompasses both cccDNA and/or relaxed circular DNA (rcDNA), the direct product of transcriptionally active cccDNA.

The presence of integrated HBV in OBI-associated HCC has recently been reported at a high frequency (76%)^[21] and in cccDNA-negative patients (88%)^[22]. The presence of integrated HBV DNA in OBI-HCC can further complicate disease management and antiviral regimens. However, its detection can play a critical role in patients' HCC management as new HBV-directed T cell immunotherapies emerge. Recently, the potential application of HBV-specific T cells in targeting HBV antigens derived from integrated HBV DNA has been shown to have antiviral and anti-tumor effects^[23].

In this report, we present a rare case of a patient with OBI with HCC who survived multiple resections, including resection of a pulmonary metastasis, and had no recurrence of disease or tumor burden after beginning antiviral therapy. This case is interesting for many reasons and offers several educational points. HBV DNA was negative in the commercial assay suggesting that the patient had reached a "functional cure" in the setting of negative HBsAg and positive anti-HBs. Numerous studies have shown that seroconversion of HBsAg is associated with improved clinical outcomes^[24,25]. Unfortunately, the association between her HCC and HBV profile was unclear at presentation. It was not clarified until later in her clinical course when a more sensitive assay found a viral load of 3,271 copies/mL in her serum. Therefore, it is important to realize that the diagnosis of OBI can be challenging given different serological presentations and the limitations of routine assays. Further, it has been reported that spontaneous HBsAg seroconversion does not mean complete elimination of HBV and patients can still have risk of developing HBV associated HCC^[26].

Even if the diagnosis of OBI is made, management of such patients can be difficult as there are no guidelines regarding the initiation of antivirals or screening for HCC. Additionally, the prognosis of OBI-associated HCC is unclear and outcome studies are limited. A prior study investigated surgical outcomes in patients with OBI and HCC. They found that patients with OBI were younger at the time of surgery but did not differ in disease free survival or overall survival compared to those with HCC attributed to other carcinogenetic factors such as alcohol abuse, NASH, and diabetes^[27]. Regarding the management of OBI related HCC, studies have shown that after the development of HCC, anti-HBV therapy can prevent recurrence or new HCC in the majority of cases^[28-30].

In summary, this case suggests a strong role for OBI in HCC development and indicates that OBI can be cured with anti-HBV treatment and complete surgical removal of HBV infected hepatocytes and other cells. Further studies are required to better define the role of OBI in carcinogenesis, and to determine the mechanisms by which it exerts pro-oncogenic activity.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception, design of the study and writing of the paper: Boortalary T, Hann HW

Made contributions to surgical aspects of the paper: Rosato E

Made contributions to radiological imaging: Roth C

Made contributions on providing details on HBV DNA assay: Ren XD

Made contributions to concept of OBI-HCC: Lin SY

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Dr. Hie-Won Hann has received research grants from Gilead Sciences, Assembly Biosciences, Trio-Health and has served on the National Advisory Board of Gilead.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Maucort-Boulch D, de Martel C, Franceschi S, Plummer M. Fraction and incidence of liver cancer attributable to hepatitis B and c viruses worldwide. *Int J Cancer* 2018;142:2471-7.
2. Raimondo G, Locarnini S, Pollicino T, Levrero M, Zoulim F, Lok AS, et al. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. *J Hepatol* 2019;71:397-408.
3. Conjeevaram HS, Lok AS. Occult hepatitis B virus infection: a hidden menace? *Hepatology* 2001;34:204-6.
4. Kim YS, Jang JY, Eun SH, Cheon YK, Kim YS, et al. Detection of intrahepatic HBV DNA in hbsag-negative liver diseases. *Korean J Hepatol* 2006;12:201-8.
5. Pollicino T, Raimondo G. Occult hepatitis B infection. *J Hepatol* 2014;61:688-9.
6. Wong SY, Ren XD, Hann HW. Development of hepatocellular carcinoma in patients with chronic hepatitis B long after achieving hbsag seroconversion: a need for an improved hepatitis B virus DNA assay. *Clin Microbiol* 2013;2:127.
7. Loeb KR, Jerome KR, Goddard J, Huang ML, Cent A, et al. High-throughput quantitative analysis of hepatitis B virus DNA in serum using the taqman fluorogenic detection system. *Hepatology* 2000;32:626-9.
8. Chen CJ, Yang HI, Su J, Jen CL, You SL, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006;295:65-73.
9. Chen CF, Lee WC, Yang HI, Chang HC, Jen CL, et al. Changes in serum levels of HBV DNA and alanine aminotransferase determine risk for hepatocellular carcinoma. *Gastroenterology* 2011;141:1240-8, 1248.e1-2.
10. Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, et al. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. *Gastroenterology* 2012;142:1140-9.e3; quiz e13-4.
11. Pitini V, Rizzo M, Arrigo C, Mondello P, Altavilla G. Fatal hepatocellular carcinoma in a patient with occult hepatitis B virus infection following the administration of R-chop for diffuse large B-cell lymphoma. *Case Rep Hematol* 2012;2012:803298.
12. Evens AM, Jovanovic BD, Su YC, Raisch DW, Ganger D, et al. Rituximab-associated hepatitis B virus (HBV) reactivation in lymphoproliferative diseases: Meta-analysis and examination of fda safety reports. *Ann Oncol* 2011;22:1170-80.
13. Wong DK, Huang FY, Lai CL, Poon RT, Seto WK, Fung J, et al. Occult hepatitis B infection and HBV replicative activity in patients with cryptogenic cause of hepatocellular carcinoma. *Hepatology* 2011;54:829-36.
14. Shim CW, Park JW, Kim SH, Kim JS, Kim BH, et al. Noncirrhotic hepatocellular carcinoma: Etiology and occult hepatitis B virus infection in a hepatitis B virus-endemic area. *Therap Adv Gastroenterol* 2017;10:529-36.
15. Muto J, Sugiyama M, Shirabe K, Mukaide M, Kirikae-Muto I, et al. Frequency and characteristics of occult hepatitis B infection among hepatocellular carcinoma patients in japan. *Ann Hepatol* 2018;17:596-603.
16. Pollicino T, Squadrito G, Cerenzia G, Cacciola I, Raffa G, et al. Hepatitis B virus maintains its pro-oncogenic properties in the case of occult HBV infection. *Gastroenterology* 2004;126:102-10.

17. Squadrito G, Cacciola I, Alibrandi A, Pollicino T, Raimondo G. Impact of occult hepatitis B virus infection on the outcome of chronic hepatitis C. *J Hepatol* 2013;59:696-700.
18. Lok AS, Everhart JE, Di Bisceglie AM, Kim HY, Hussain M, et al. Occult and previous hepatitis B virus infection are not associated with hepatocellular carcinoma in united states patients with chronic hepatitis C. *Hepatology* 2011;54:434-42.
19. Torbenson M, Thomas DL. Occult hepatitis B. *Lancet Infect Dis* 2002;2:479-86.
20. Lindh M, Hannoun C, Malmstrom S, Lindberg J, Norkrans G. Lamivudine resistance of hepatitis B virus masked by coemergence of mutations in probe region of the cobas amplicor assay. *J Clin Microbiol* 2006;44:2587-9.
21. Saitta C, Tripodi G, Barbera A, Bertuccio A, Smedile A, et al. Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma. *Liver Int* 2015;35:2311-7.
22. Wong DK, Cheng SCY, Mak LL, To EW, Lo RC, et al. Among patients with undetectable hepatitis B surface antigen and hepatocellular carcinoma, a high proportion has integration of HBV DNA into hepatocyte DNA and no cirrhosis. *Clin Gastroenterol Hepatol* 2020;18:449-56.
23. Tan AT, Yang N, Lee Krishnamoorthy T, Oei V, Chua A, et al. Use of expression profiles of HBV-DNA integrated into genomes of hepatocellular carcinoma cells to select t cells for immunotherapy. *Gastroenterology* 2019;156:1862-76.e9.
24. Yip TC, Chan HL, Wong VW, Tse YK, Lam KL, et al. Impact of age and gender on risk of hepatocellular carcinoma after hepatitis B surface antigen seroclearance. *J Hepatol* 2017;67:902-8.
25. Ahn SH, Park YN, Park JY, Chang HY, Lee JM, et al. Long-term clinical and histological outcomes in patients with spontaneous hepatitis B surface antigen seroclearance. *J Hepatol* 2005;42:188-94.
26. Yuen MF, Wong DK, Fung J, Ip P, But D, et al. Hbsag seroclearance in chronic hepatitis B in asian patients: Replicative level and risk of hepatocellular carcinoma. *Gastroenterology* 2008;135:1192-9.
27. Koga H, Kai K, Aishima S, Kawaguchi A, Yamaji K, et al. Occult hepatitis B virus infection and surgical outcomes in non-B, non-C patients with curative resection for hepatocellular carcinoma. *World J Hepatol* 2017;9:1286-95.
28. Hann HW, Bergin D, Coben R, DiMarino AJ. Prevention of new hepatocellular carcinoma with concomitant antiviral therapy in chronic hepatitis B patients whose initial tumor was successfully ablated. *Int J Cancer* 2011;128:739-42.
29. Hann HW, Coben R, Brown D, Needleman L, Rosato E, et al. A long-term study of the effects of antiviral therapy on survival of patients with HBV-associated hepatocellular carcinoma (HCC) following local tumor ablation. *Cancer Med* 2014;3:390-6.
30. Yuan P, Chen P, Qian Y. Evaluation of antiviral therapy performed after curative therapy in patients with HBV-related hepatocellular carcinoma: an updated meta-analysis. *Can J Gastroenterol Hepatol* 2016;2016:5234969.

Case Report

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Combined transarterial chemoembolization and stereotactic body radiation therapy as a bridge therapy to liver transplant for hepatocellular carcinoma

Bui Quang Bieu¹, Nguyen Dinh Chau¹, Nguyen Xuan Kien¹, Le Van Thanh², Vu Van Quang², Thai Doan Ky³, Nguyen Tien Thinh³, Mai Hong Bang³

¹Department of Radiation Oncology and Radiosurgery, Military Central Hospital 108, Hanoi 100000, Vietnam.

²Department of Hepatobiliary and Pancreatic Surgery, Military Central Hospital 108, Hanoi 100000, Vietnam.

³Department of Gastroenterology and Hepatobiliary, Military Central Hospital 108, Hanoi 100000, Vietnam.

Correspondence to: Dr. Bui Quang Bieu, Department of Radiation Oncology and Radiosurgery, Military Central Hospital 108, Hanoi 100000, Vietnam. E-mail: buiquangbieu@gmail.com

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Abstract

Liver transplant (LT) is the curative treatment for patients with hepatocellular carcinoma (HCC). Bridge therapies are local treatments given to patients on the LT waitlist, to prevent tumor progression and to reduce the dropout rate. Case presentation: We reported a 40-year-old man diagnosed with Barcelona-Clinic Liver Cancer BCLC intermediate stage HCC and Child-Pugh A5 hepatitis B virus cirrhosis who underwent combined bridge therapies to LT. Firstly, the patient received transarterial chemoembolization (TACE) for two times and showed a partial response. Then he underwent stereotactic body radiation therapy (SBRT) with a total dose of 45 Gy in 3 fractions. Three months later, the tumor size and serum protein induced by Vitamin K absence or antagonists-II, alpha fetoprotein levels decreased gradually. In June 2019 a suitable donor was found and his LT was successfully performed. Conclusion: We propose that a combination of TACE and SBRT was feasible as bridge therapy for HCC patients on the LT waitlist.

Keywords: Transarterial chemoembolization, stereotactic body radiation therapy, bridge therapy, hepatocellular carcinoma, liver transplant



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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the 5 most common cancers worldwide, and its incidence is increasing in Vietnam and Southeast Asian countries^[1]. According to Milan criteria, the liver transplant (LT) is the treatment of choice for HCC patients with tumor less than 5 cm and up to 3 tumors ≤ 3 cm. The 5 years survival rate in these patients are 70% with less than 20% recurrence rate^[2]. However, not all HCC patients can undergo transplantation due to a lack of liver donors, resulting in an extended time on the waiting list (WL) and a high dropout rate^[3].

Bridging treatments are locoregional therapies given to HCC patients on the WL to reduce the disease progression as well as the dropout rate. These treatments act as a temporary “bridge” until a suitable donor is identified. Liver resection, radiofrequency ablation (RFA), transarterial chemoembolization (TACE), and stereotactic body radiation therapy (SBRT) are main bridge modalities to LT in patients with HCC^[2]. The rates of drop out at 6 months and 1 year were estimated as high as 12% and 15%-30%, respectively, if HCC is left untreated^[4,5]. The strongest risk factors for dropout from WL are tumor size ≥ 3 cm or multiple tumors, waiting time ≥ 6 months, alpha fetoprotein (AFP) ≥ 200 ng/mL, and poor response to bridging therapies^[6]. In patients with HCC within Milan criteria, bridging therapy is estimated to decrease the dropout rate to 0-10%. To reduce the dropout rate from the WL, a consensus statement recommends that bridging therapies should be considered for HCC patients with one nodule size 2 cm-5 cm or up to 3 nodules each ≤ 3 cm, expected to wait longer than 6 months^[2].

Another aim of bridge therapy is to treat patients initially outside criteria for LT to fulfill Milan criteria which allows entry to the WL for LT after an adequate period of follow-up. In this case, bridge therapy is used as a downstaging procedure^[3].

There are several studies of bridge therapy to LT for HCC with TACE^[7-9] or SBRT^[10-12] alone. Some trials have shown that adjuvant SBRT post-TACE was safe and effective for patients with HCC^[13-15]. In this paper, we report an HCC patient in the BCLC intermediate stage who we successfully treated with a combination of TACE and SBRT as a bridge therapy to LT in our center.

CASE REPORT

A 39-year-old male with a history chronic hepatitis B virus infection was diagnosed with intermediate BCLC HCC in October 2018. His liver function test (LFT) showed that he had Child-Pugh A5 cirrhosis. The tumor was in VI-VII segments and the patient characteristics were shown in [Table 1](#). He had one tumor with a size bigger than 5 cm and very high serum AFP levels.

Because the patient was young with a good performance status of ECOG 0, the LT team decided that he was an optimal candidate for LT and bridge therapy was needed to downstage the tumor while he was on the WL. Two DC beads TACE treatments were carried out in October and November 2018. The DC beads TACE technique was done in the same manner as conventional TACE. We used doxorubicin (Ebewe, Austria) loaded with DC-beads (Bicompatibles, UK) at least 90-120 min before the intervention. The dose of doxorubicin was 100 mg per each treatment. Two sizes of DC-beads were used (100-300 μm and 300-500 μm). These treatments resulted in partial response according to the modified Response Evaluation Criteria in Solid Tumors and Response Evaluation Criteria in Cancer of the Liver^[16].

Due to serum AFP level still too high post-TACE, as well as there was no suitable donor, we decided that he needs further bridge therapy with SBRT. The patient underwent SBRT with a total dose of 45 Gy in 3 fractions, one fraction delivered in every other day in January 2019.

Table 1. Patient characteristics pre-treatment and post-TACE and SBRT

Lab test and imaging	Pre-treatment	Post-TACE 1 months	Post-SBRT 3 months
AFP (IU/mL)	2479	1128	143
PIVKA-II (mAU/mL)	272.6	29.4	0.8
Child Pugh	A5	A5	A5
AST	28	43	47
ALT	33	65	70
Tumor size (mm)	71 × 60 × 53	72 × 63 × 43	60 × 43 × 38

PIVKA: a protein induced by vitamin K absence; AST: aspartate transaminase; ALT: alanine transaminase; TACE: transarterial chemoembolization; SBRT: stereotactic body radiation therapy; AFP: alpha fetoprotein

SBRT procedure for the patient

CT simulation in GE CT 580 RT (USA)

The patient lied supine, arm up, and was immobilized in a vacuum bag (Qfix - USA). A non-contrast 4D CT performed with 2.5 mm slice thickness (used for treatment planning). A contrast-enhanced 4D CT with 2.5 mm slice thickness, intravenous injection of Omnipaque (GE Healthcare) 2 mL/kg, and 2.5 mL/second was performed (used for target volume contouring). Both 4D CT data sets were then transferred to the treatment planning system (TPS).

Target volume and organs at risk contouring using Eclipse 13.6 (Varian, USA)

The average CT images were created for both non-contrast and contrast-enhanced 4D CT data sets. Non-contrast average CT was co-registered and fused with the contrast-enhanced one. Then 10 gross tumor volumes (GTVs) were contoured in 10 contrast-enhanced 4D CT data sets: only delineated tumor with contrast enhancement. The 10 GTVs were copied to contrast-enhanced average CT data set and combined to create internal target volume (ITV). The ITV was propagated from contrast-enhanced average CT data set to the non-contrast one. The planning target volume (PTV) was created in non-contrast average CT from ITV: PTV = ITV + 5 mm. The organs at risk were contoured in non-contrast average CT including liver, lung, heart, stomach, duodenum, small and large bowel, spinal cord, chest wall, kidneys, and gall bladder.

Dose prescription

The prescription dose was 45 Gy in 3 fractions, based on normal tissue constraints (the report of AAPM Task Group 101^[17]).

Treatment planning

Treatment technique was VMAT with 2 coplanar arcs. Plan optimization was done in Eclipse 13.6 (Varian, USA). Based on normal tissue constraints, we selected a treatment plan with a total dose of 45 Gy in 3 fractions for the patient [Figure 1].

SBRT treatment plan parameters were shown in Table 2, with plan normalization 100% prescription dose covered 95% PTV and doses of OARs were within tolerance of AAPM Task Group 101^[16]. Normal liver volume received less than 17 Gy (V17) was 812.7 mL.

Quality assurance of treatment plan

The treatment plan was verified by portal dose dosimetry (Varian, USA) with a 2%/1mm gamma passing rate of 99.5%.

Treatment delivery

The patient was treated in TrueBeam STx (Varian, USA) with position and immobilization just like in CT simulation. We used the Optical Surface Monitoring System (OSMS - Vision RT, UK) to help set up and

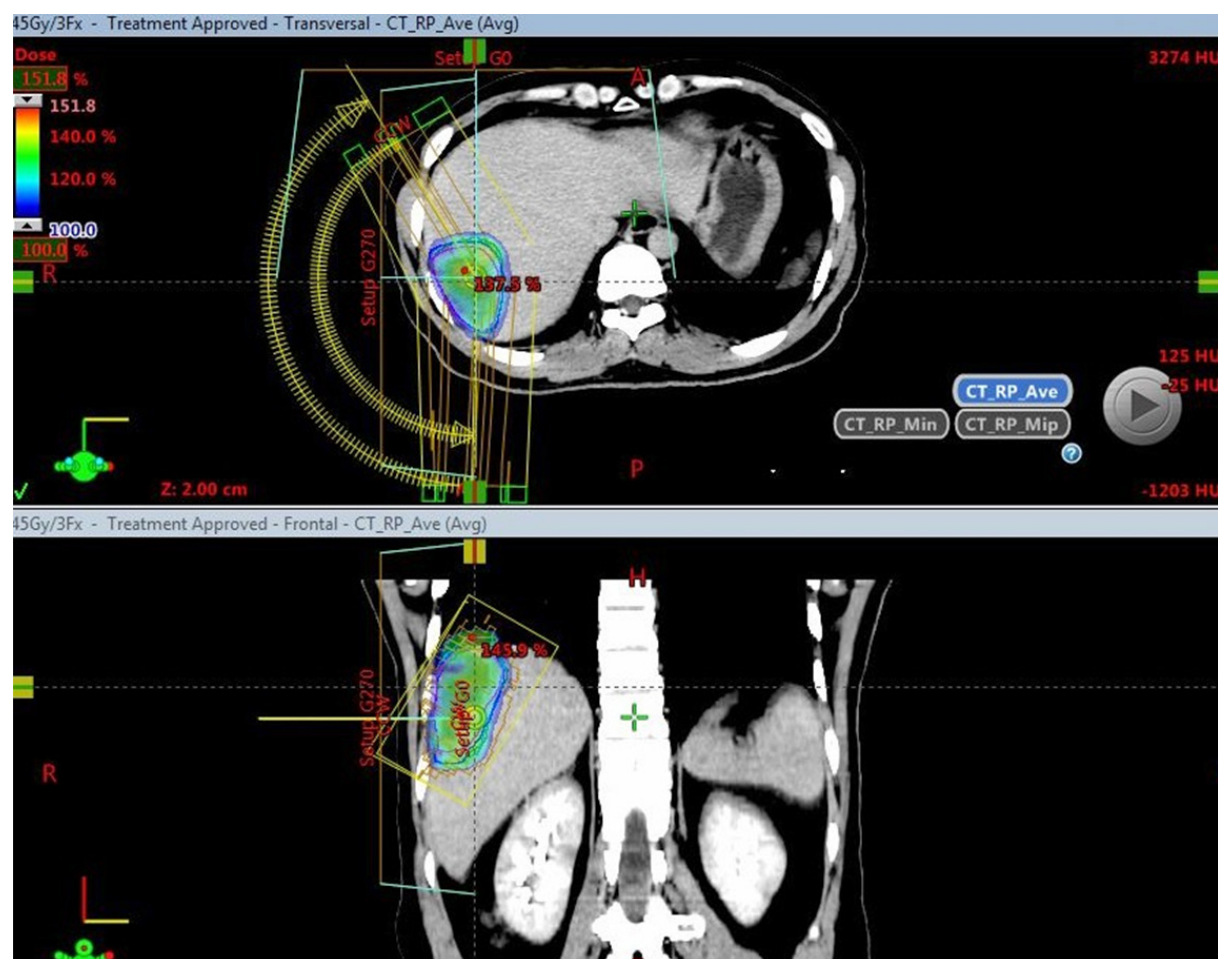


Figure 1. SBRT treatment plan and 100% dose color wash (with courtesy of Department of Radiation Oncology and Radiosurgery, Military Central Hospital 108). SBRT: stereotactic body radiation therapy

Table 2. SBRT treatment plan parameters

Structures	Volume (cm ³)	Min dose (Gy)	Max dose (Gy)	Mean dose (Gy)
ITV	130	43.97	68.31	55.62
PTV	201.3	34.68	68.31	53.44
Liver	1235.1	0.26	66.46	17.11
Normal liver	1112.5	0.26	65.93	12.87
Gallbladder	28.9	3.03	24.42	12.43
Duodenum	40	0.26	8.08	2.3
Stomach	265.5	0.26	5.4	2.3
Jejunum	327.8	0.08	2.8	0.5
Ileum	769.3	0.07	14.62	1.14
Esophagus	12	3.43	8.54	5.65
Lung	888.9	0.13	65.98	3.81
Heart	359.3	0.19	8.9	2.16
Spinal cord	35.9	0.03	7.48	2.08
Right kidney	129.2	0.14	2.02	0.53
Left kidney	134.3	0.05	0.48	0.17

SBRT: stereotactic body radiation therapy; ITV: internal target volume; PTV: planning target volume

monitor patient movement during treatment. The patient's position was verified by cone-beam CT before each fraction. After the verification was done, the linac was beamed on to treat the patient. Total treatment

time for each fraction was about 30 min: patient set up and verification 20 min, beam on 10 min. The total SBRT course lasted for two weeks.

Follow-up

The patient was treated with tenofovir (Savi Tenofovir) 300 mg one tablet per day and scheduled for checkup one month post-SBRT and then every 3 months. Clinical examination, lab tests, and imaging were done during checkup including cell blood count, LFT, serum AFP, PIVKA II, abdominal ultrasound, chest Xray and abdominal CT.

The patient was well tolerated with SBRT and showed only minimum adverse effects such as mild fever and increased AST, ALT. Three months post SBRT, his LFT was still Child Pugh A5, serum PIVKA II and AFP levels were decreased, the tumor size was also decreased with central necrosis [Table 1, Figure 2]. In July 2019, we found a suitable donor for him, and his LT was successfully performed in August 2019. His post LT histopathology report showed that the tumor was mostly necrotized. He went on with tenofovir (Savi Tenofovir) 300 mg one tablet per day, tacrolimus (Prograf) 1 mg six capsules per day to treat HBV, and prevent rejection and routine follow-up. Until now, seven months post LT, he has no evidence of HCC, his LFT is normal with a mild increase of AST, ALT.

DISCUSSION

Several locoregional therapies have been used as bridging treatments for HCC patients awaiting LT. The most common treatments include TACE, RFA, and recently SBRT. Nowadays, TACE is still the most widely used as bridging therapy. In the procedure, a chemotherapeutic drug (commonly doxorubicin, cisplatin or mitomycin C), emulsified in lipiodol with embolizing material, is injected into the hepatic artery branch that feeding the tumor, to induce hypoxemia and tumor necrosis. The technique has been enhanced by drug-eluting beads (DEB-TACE), which allows a higher dose and uptake of chemotherapeutic drugs into the tumor and less systemic toxicity. In the histological examination, TACE achieves a complete pathological response in less than 30% of cases. Some studies focused on the efficacy of TACE as bridging treatment to LT on dropout rates in WL, survival, and recurrence after LT. The reported results of bridge therapy with TACE are controversial and no prospective randomized control trials have confirmed its efficacy in reducing dropout rates^[2]. Several authors demonstrated that a good response to TACE (necrosis > 60%) is significantly related to improved long-term survival after LT and a lower recurrence rate^[18]. Others did not find any significant advantage of bridge therapy with TACE in overall and recurrence-free survival after LT in HCC patients^[19,20].

SBRT uses stereotactic conformal RT with 1-5 fractions of large fraction sizes (8-20 Gy/fraction) to the tumor while reducing the dose to adjacent normal tissues. The precise treatment and steep dose gradient within the target volume lead to excellent conformity with steep dose fall-off and high dose delivery to the target volume^[21]. These advantages of SBRT over conventional radiotherapy allows a high chance of tumor control and minimizing treatment toxicities. SBRT for liver tumors was first introduced in the 1990s^[22]; however, it has not frequently been performed because of the concern of radiation-induced liver disease (RILD). Recently, with the development of medical linear accelerators and motion management solutions as well as supported data, SBRT has been recommended as a local treatment for HCC by NCCN guidelines. Now it is considered as an option for HCC patients who are not candidates to other bridging therapies^[23].

Data regarding the use of SBRT as a bridging treatment are emerging. In a paper by Sandroussi *et al.*^[24], ten HCC patients awaiting LT with tumor diameters ranging from 2.5 to 10.8 cm received conformal radiation therapy in 5-6 fractions. The treatment was done in nine patients with acceptable toxicities. Five patients underwent LT, and their explant pathology report showed that tumor necrosis ranging from 40%-90%. At a median follow-up of 6 months, no patients had tumor recurrence after LT. The author suggested that

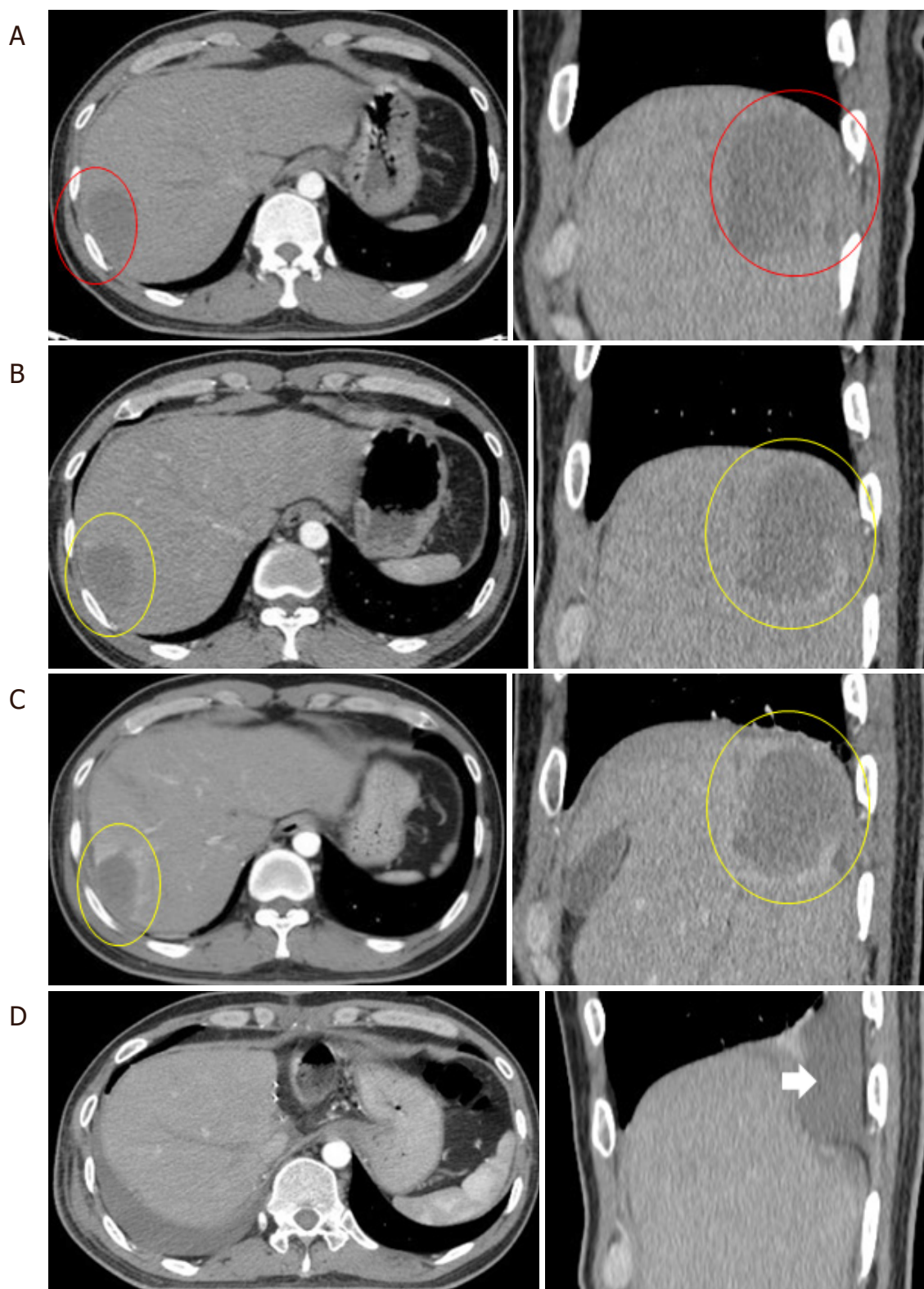


Figure 2. The patient's CT images. A: non-contrast enhancing primary tumor (red rings) in arterial phase CT images pre SBRT; B, C: mild contrast-enhancing of normal liver tissue surrounding the primary tumor (yellow rings) in arterial phase CT images one month and three months post-SBRT; D: new transplanted liver and local pleural effusion in the right lung (white arrow). With courtesy of Department of Radiation Oncology and Radiosurgery and Department of Hepatobiliary and Pancreatic Surgery, Military Central Hospital 108. SBRT: stereotactic body radiation therapy

conformal radiation therapy is a safe and efficacious local bridging therapy for patients with HCC on the WL for LT^[24]. In another article, Sapisochin *et al.*^[12] reported an intention to treat analysis about SBRT used as bridge therapy in HCC patients not eligible for other locoregional treatments and observed similar drop-out rate with SBRT and TACE or RFA. SBRT is proven to be safe and effective for tumors with a diameter < 6 cm, even in lesions near the central biliary system, where surgery or RFA is impossible^[25]. In a recent study by Moore *et al.*^[26], 23 early-stage HCC patients who were not candidates for resection or local therapy treated with SBRT as bridge therapy to LT. The median prescribed doses to the tumor and the normal livers were 54 Gy (range 30-54 Gy) and 6.0 Gy (range 1.6-12.6 Gy), respectively. 22 patients had no significant changes in lab tests in 12 weeks follow-up but one patient developed RILD. 16 patients were on WL post SBRT and 11 were successfully transplanted. The median overall survival (OS) and progression-free survival (PFS) for the transplanted patients were not reached (range, 2.0-53.7 months, and 54 months, respectively) and were 23 and 14 months, respectively for the non-transplanted patients. Pathology report of liver explant post LT revealed 3 tumors (27.3%) with complete response (CR), 6 tumors (54.5%) with partial response (PR), and stable disease in 2 tumors (18.2%). The authors concluded that SBRT was effective and safe to be used as a bridge therapy to LT without compromising the surgical procedure^[26]. Furthermore, in a retrospective study, Gresswell *et al.*^[27] found that SBRT with functional treatment planning can be used safely as a bridge to LT in select patients with CP \geq 8 cirrhosis.

Until now, there are no guidelines available to define which bridging therapy is the preferred treatment for specific patients. The choice of suitable bridging therapies has to be tailored to the patient's status, the tumor characteristics, and more important the center experience. RFA is the treatment of choice in patients with a single tumor size < 5 cm. The benefit of RFA as bridging therapy is best seen in patients with small tumors < 3 cm and < 1-year waiting time^[28]. TACE should be considered for patients with HCC between 3-5 cm, because nodules with 3 cm of diameter or more are better vascularized, with a large feeding artery, therefore the effectiveness of TACE appears to be better; whereas smaller HCC has not yet a completely developed arterial neoangiogenesis^[29,30]. SBRT has the advantage to treat the tumors adjacent to the central biliary system, in the liver dome or subcapsular HCC, these lesions are not suitable for RFA. However, SBRT is not suitable for tumors close to the duodenum, stomach, or bowel, for high risk of ulcer, hemorrhage, and perforation^[25]. Up to now, there are several ongoing prospective phases 2 and 3 randomized trials to compare the safety and effectiveness of SBRT and TACE as a bridge therapy to LT^[31,32].

Experiences with combined therapies such as SBRT and TACE have been published in recent years, mostly in the scenario of unresectable HCC with diameter > 3 cm^[33,34]. The rationale for treatment combination is to achieve a higher local control rate due to higher rates of complete tumor necrosis. The potential advantages when combining TACE followed by SBRT are: (1) TACE is most effective at the center of the HCC and failures are most commonly seen at the periphery of the tumor, where the ischaemic effects of TACE are least potent because the surrounding normal liver parenchyma is well oxygenated; (2) on the contrary, SBRT is most effective in the well-oxygenated periphery of the HCC and failures often occur in the more hypoxic zone at the tumor center; (3) large tumors that are not suitable for SBRT alone become more amenable to this therapy following TACE due to the effect of TACE in the hypoxic area at the tumor center; and (4) a theoretical radio-sensitization by the cytotoxic agents used in TACE may result in the improvement of tumor response^[34].

The approach of SBRT and TACE combination may be applied even as bridging therapy to LT. A retrospective study combined TACE followed by SBRT in patients with HCC size \geq 3 cm by Jacob *et al.*^[33] showed that local recurrence was significantly decreased in the TACE plus SBRT group (10.8%) when compared with the TACE-only group (25.8%) ($P = 0.04$). The TACE plus SBRT group also had significantly longer OS than the TACE-only one (33 months and 20 months, respectively; $P = 0.02$). The author supposed that combined TACE and SBRT resulted in a survival advantage over treatment with TACE

alone in HCC patients with tumor size ≥ 3 cm and a prospective randomized clinical trial is required to confirm the result^[33]. In another pilot phase II trial, Buckstein *et al.*^[34] combined drug-eluting bead (DEB) TACE followed by SBRT in 25 HCC patients with single tumor size 4-7 cm. 92% of target lesions showed objective response including 64% CR ($n = 16$) and 28% PR ($n = 7$). 2-year OS and PFS were 67% and 52%, respectively. Cause-specific survival (CSS) was 91% at 1 year and 83% at 2 years. He suggested that the results show very promising response rates when combining TACE and SBRT in large, unresectable HCC with the excellent OS, PFS, and CSS^[34]. However, up to date, we do not found any paper address the combination of TACE and SBRT as a bridge therapy to LT for patients with HCC.

In our case, the patient had a big tumor size > 5 cm with very high serum PIVKA II and AFP levels so we combined both TACE and SBRT to increase the chance of local control as well as downstage the lesion to LT criteria. TACE was performed first and adjuvant SBRT was followed to exploit the advantage of combination therapies. It took 6 months for both therapies to downstage the tumor and 10 months on WL for the patient to find a suitable donor. After two TACE treatments, the tumor showed partial response but the serum AFP was still higher than 200 ng/mL. The patient had a high risk of drop out from the WL for LT post TACE because there was still no suitable donor for him. Adding SBRT had kept the patient in the WL and finally, his LT was successfully done.

However, SBRT for HCC is a highly specialized procedure requiring both clinical and technical expertise. Considerations such as accurate target localization, rigid patient immobilisation, strict motion management, and proximity to adjacent viscera such as bowel and biliary structures must be considered prior to, and throughout treatment. As such, this technique can only be delivered safely and effectively in experienced centers with synchronized equipment, coupled with a well-oiled multi-disciplinary team comprising radiation oncologists, medical physicists and radiation therapy technologists.

At our center, we use 4D CT, gating, breath-hold techniques, or abdominal compression to manage the respiratory movement of the tumor, so that we can treat the tumor precisely with a 3-5 mm margin from ITV to PTV. Moreover, when combining SBRT with TACE as a bridge therapy to LT, it is necessary to have close teamwork between surgeons, gastroenterologists, and radiation oncologists. Additionally, adjuvant SBRT to TACE can do more harm to liver function and cause gastrointestinal toxicities such as bleeding or ulcer. So patient selection is very important to safely combine TACE and SBRT. Our patient had a good liver function with CP A5 and normal liver volume > 700 mL. His tumor was close to the chest wall and liver dome so it is difficult for RFA and more suitable for adjuvant SBRT post-TACE. That is why he had only grade hepatic toxicity and no gastrointestinal toxicities post-TACE and SBRT. It is also important that after bridge therapy with TACE and SBRT, patients must continue the treatment of chronic liver disease, in our case was HBV, to prevent tumors from progression.

Finally, we should be cautious when evaluating treatment response after TACE combined with SBRT for HCC. With this patient, we found that the tumor size did not change one month after SBRT and only decreased slightly 3-month post-SBRT with central necrosis. Interestingly, contrast enhancement was seen in normal liver tissue surrounding the primary tumor one month post SBRT and it seemed increased with time [Figure 2B and C]. It was a normal liver reaction after SBRT and can be misinterpreted with tumor progression. In a series of 26 HCC patients treated with SBRT, Price *et al.*^[35] found that this phenomenon can even last 6 months post-SBRT. He suggested that nonenhancement on imaging, a surrogate for ablation, maybe a more useful indicator than size reduction in evaluating HCC response to SBRT in the first 6 to 12 months^[36]. Sanuki-Fujimoto *et al.*^[36] also noted in their study that the CT appearances of the normal liver seen in reaction to the treatment of an HCC by SBRT were therefore related to background liver function and should not be misread as recurrence of HCC.

In conclusion, Via this case report and review of literature, we suppose that bridging therapy combining SBRT and TACE may be most beneficial over TACE alone in HCC patients with a high risk of drop-out from WL for LT. It is necessary to perform a randomized trial to provide more evidence to guide the treatment options for these patients.

DECLARATIONS

Acknowledgments

We acknowledge our patient for providing informed consent for this case report.

Authors' contributions

Made substantial contributions to conception and design of the case report and performed data analysis and interpretation: Bieu BQ

Performed data acquisition, as well as providing administrative, technical, and material support: Chau ND, Kien NX, Thanh LV, Quang VV, Ky TD, Thinh NT, Bang MH

Read and approved the final manuscript: Bieu BQ, Chau ND, Kien NX, Thanh LV, Quang VV, Ky TD, Thinh NT, Bang MH

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Obtain the informed consent from patient.

Consent for publication

Not applicable.

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REFERENCES

1. IARC. Cancer Today. Available from: <http://gco.iarc.fr/today/>. [Last accessed on 24 Jul 2020]
2. Coletta M, Nicolini D, Benedetti Cacciaguerra A, Mazzocato S, Rossi R, et al. Bridging patients with hepatocellular cancer waiting for liver transplant: all the patients are the same? *Transl Gastroenterol Hepatol* 2017;2:78.
3. Cescon M, Cucchetti A, Ravaioli M, Pinna AD. Hepatocellular carcinoma locoregional therapies for patients in the waiting list. Impact on transplantability and recurrence rate. *J Hepatol* 2013;58:609-18.
4. Washburn K, Edwards E, Harper A, Freeman R. Hepatocellular carcinoma patients are advantaged in the current liver transplant allocation system. *Am J Transplant* 2010;10:1643-8.
5. Yao FY, Bass NM, Nikolai B, Davern TJ, Kerlan R, et al. Liver transplantation for hepatocellular carcinoma: analysis of survival according to the intention-to-treat principle and dropout from the waiting list. *Liver Transpl* 2002;8:873-83.
6. Yao FY, Bass NM, Nikolai B, Merriman R, Davern TJ, et al. A follow-up analysis of the pattern and predictors of dropout from the waiting list for liver transplantation in patients with hepatocellular carcinoma: implications for the current organ allocation policy. *Liver Transpl* 2003;9:684-92.
7. Millonig G, Graziadei IW, Freund MC, Jaschke W, Stadlmann S, et al. Response to preoperative chemoembolization correlates with outcome after liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl* 2007;13:272-9.
8. Otto G, Herber S, Heise M, Lohse AW, Mönch C, et al. Response to transarterial chemoembolization as a biological selection criterion for

- liver transplantation in hepatocellular carcinoma. *Liver Transpl* 2006;12:1260-7.
9. De Luna W, Sze DY, Ahmed A, Ha BY, Ayoub W, et al. Transarterial chemoembolization for hepatocellular carcinoma as downstaging therapy and a bridge toward liver transplantation. *Am J Transplant* 2009;9:1158-68.
 10. Katz AW, Chawla S, Qu Z, Kashyap R, Milano MT, et al. Stereotactic hypofractionated radiation therapy as a bridge to transplantation for hepatocellular carcinoma: clinical outcome and pathologic correlation. *Int J Radiat Oncol Biol Phys* 2012;83:895-900.
 11. O'Connor JK, Trotter J, Davis GL, Dempster J, Klintmalm GB, et al. Long-term outcomes of stereotactic body radiation therapy in the treatment of hepatocellular cancer as a bridge to transplantation. *Liver Transpl* 2012;18:949-54.
 12. Sapisochin G, Barry A, Doherty M, Fischer S, Goldaracena N, et al. Stereotactic body radiotherapy vs. TACE or RFA as a bridge to transplant in patients with hepatocellular carcinoma. An intention-to-treat analysis. *J Hepatol* 2017;67:92-9.
 13. Paik EK, Kim MS, Jang WI, Seo YS, Cho CK, et al. Benefits of stereotactic ablative radiotherapy combined with incomplete transcatheter arterial chemoembolization in hepatocellular carcinoma. *Radiat Oncol* 2016;11:22.
 14. Honda Y, Kimura T, Aikata H, Kobayashi T, Fukuhara T, et al. Stereotactic body radiation therapy combined with transcatheter arterial chemoembolization for small hepatocellular carcinoma. *J Gastroenterol Hepatol* 2013;28:530-6.
 15. Cho JY, Paik YH, Park HC, Yu JI, Sohn W, et al. The feasibility of combined transcatheter arterial chemoembolization and radiotherapy for advanced hepatocellular carcinoma. *Liver Int* 2014;34:795-801.
 16. Kudo M, Ikeda M, Ueshima K, Sakamoto M, Shiina S, et al. Response evaluation criteria in cancer of the liver version 5 (RECICL 2019 revised version). *Hepatol Res* 2019;49:981-9.
 17. Benedict SH, Yenice KM, Followill D, Galvin JM, Hinson W, et al. Stereotactic body radiation therapy: the report of AAPM Task Group 101. *Med Phys* 2010;37:4078-101.
 18. Allard MA, Sebahg M, Ruiz A, Guettier C, Paule B, et al. Does pathological response after transarterial chemoembolization for hepatocellular carcinoma in cirrhotic patients with cirrhosis predict outcome after liver resection or transplantation? *J Hepatol* 2015;63:83-92.
 19. Decaens T, Roudot-Thoraval F, Bresson-Hadni S, Meyer C, Gugenheim J, et al. Impact of pretransplantation transarterial chemoembolization on survival and recurrence after liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2005;11:767-75.
 20. She WH, Cheung TT. Bridging and downstaging therapy in patients suffering from hepatocellular carcinoma waiting on the list of liver transplantation. *Transl Gastroenterol Hepatol* 2016;1:34.
 21. Choi SH, Seong J. Stereotactic body radiotherapy: does it have a role in management of hepatocellular carcinoma? *Yonsei Med J* 2018;59:912-22.
 22. Blomgren H, Lax I, Näslund I, Svanström R. Stereotactic high dose fraction radiation therapy of extracranial tumors using an accelerator. Clinical experience of the first thirty-one patients. *Acta Oncol* 1995;34:861-70.
 23. Kollmann D, Selzner N, Selzner M. Bridging to liver transplantation in HCC patients. *Langenbecks Arch Surg* 2017;402:863-71.
 24. Sandroussi C, Dawson LA, Lee M, Guindi M, Fischer S, et al. Radiotherapy as a bridge to liver transplantation for hepatocellular carcinoma. *Transpl Int* 2010;23:299-306.
 25. Eriguchi T, Takeda A, Sanuki N, Oku Y, Aoki Y, et al. Acceptable toxicity after stereotactic body radiation therapy for liver tumors adjacent to the central biliary system. *Int J Radiat Oncol Biol Phys* 2013;85:1006-11.
 26. Moore A, Cohen-Naftaly M, Tobar A, Kundel Y, Benjaminov O, et al. Stereotactic body radiation therapy (SBRT) for definitive treatment and as a bridge to liver transplantation in early stage inoperable Hepatocellular carcinoma. *Radiat Oncol* 2017;12:163.
 27. Gresswell S, Tobillo R, Hasan S, Uemura T, Machado L, et al. Stereotactic body radiotherapy used as a bridge to liver transplant in patients with hepatocellular carcinoma and Child-Pugh score ≥ 8 cirrhosis. *J Radiosurg SBRT*. 2018;5:261-7.
 28. Belghiti J, Carr BI, Greig PD, Lencioni R, Poon RT. Treatment before liver transplantation for HCC. *Ann Surg Oncol* 2008;15:993-1000.
 29. European Association For The Study Of The Liver., European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012;56:908-43.
 30. Golfieri R, Cappelli A, Cucchetti A, Piscaglia F, Carpenzano M, et al. Efficacy of selective transarterial chemoembolization in inducing tumor necrosis in small (<5 cm) hepatocellular carcinomas. *Hepatology* 2011;53:1580-9.
 31. ClinicalTrials.gov. Stereotactic Body Radiation Therapy (SBRT) Versus Trans-Arterial Chemoembolization (TACE) as Bridge to Liver Transplant (SBRTvsTACE). Available from: <https://clinicaltrials.gov/ct2/show/NCT02182687>. [Last accessed on 24 Jul 2020].
 32. ClinicalTrials.gov. Stereotactic Body Radiation Therapy (SBRT) vs Trans-Arterial Chemoembolization (TACE) as Bridge to Transplant (SBRTvsTACE). Available from: <https://clinicaltrials.gov/ct2/show/NCT03960008>. [Last accessed on 24 Jul 2020].
 33. Jacob R, Turley F, Redden DT, Saddekni S, Aal AK, et al. Adjuvant stereotactic body radiotherapy following transarterial chemoembolization in patients with non-resectable hepatocellular carcinoma tumours of ≥ 3 cm. *HPB (Oxford)* 2015;17:140-9.
 34. Buckstein M, Kim E, Facciuto M, Sung M, Taouli B, et al. Phase II trial using combination of TACE and SBRT for unresectable single large HCC: interim report. *Int J Radiat Oncol Biol Phys* 2019;105:E214-5.
 35. Price TR, Perkins SM, Sandrasegaran K, Henderson MA, Maluccio MA, et al. Evaluation of response after stereotactic body radiotherapy for hepatocellular carcinoma. *Cancer* 2012;118:3191-8.
 36. Sanuki-Fujimoto N, Takeda A, Ohashi T, Kunieda E, Iwabuchi S, et al. CT evaluations of focal liver reactions following stereotactic body radiotherapy for small hepatocellular carcinoma with cirrhosis: relationship between imaging appearance and baseline liver function. *Br J Radiol* 2010;83:1063-71.

Review

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Risk factors for hepatocellular carcinoma recurrence after liver transplantation

Pierluigi Toniutto, Ezio Fornasiere, Elisa Fumolo, Davide Bitetto

Hepatology and Liver Transplantation Unit, Azienda Sanitaria Universitaria Integrata, Udine 33100, Italy.

Correspondence to: Prof. Pierluigi Toniutto, Hepatology and Liver Transplantation Unit, Azienda Sanitaria Universitaria Integrata, P.zale S.M. della Misericordia 1, Udine 33100, Italy. E-mail: pierluigi.toniutto@uniud.it

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Abstract

Liver transplantation (LT) provides an excellent option for the long-term survival of patients with unresectable hepatocellular carcinoma (HCC) based on the Milan criteria. Despite careful selection of patients, HCC may still recur after LT, which represents the most important negative predictor of post-transplant survival. The growing demand for LT in HCC has led to the expansion of patient selection criteria, with a resultant increase in the risk of post-transplant HCC recurrence. Numerous tumor and host factors predict HCC recurrence. The morphological, histological, and serological characteristics of tumors in predicting HCC recurrence have been extensively studied. Furthermore, the type and duration of anticancer response before LT has also been considered a surrogate marker of tumor aggressiveness and is associated with the risk of recurrence. The demographic and clinical characteristics of recipients, as well as the type and duration of exposure to immunosuppressive therapy, represent the main host-related risk factors. Many studies have attempted to describe predictive models for the risk of HCC recurrence, considering evaluable parameters both before and after LT. Although many models have been proposed, relatively few have been externally validated on different patient populations. This paper aims to comprehensively summarize the available data on the predictive factors of HCC recurrence after LT, and to examine and discuss those that have been externally validated.

Keywords: Liver transplantation, hepatocellular carcinoma, tumor recurrence, risk predictive model



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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer and is ranked as the sixth most common neoplasm and the third leading cause of cancer death^[1]. In Western countries, the vast majority of HCCs develop in the presence of liver cirrhosis caused by chronic viral hepatitis, alcohol abuse, and - more recently - following the complications of non-alcoholic steatohepatitis^[2,3].

In 1963, liver transplantation (LT) was originally introduced in clinical practice with the concept of treating unresectable liver tumors^[4]. Early LT experiences were unsatisfactory since it became clear that post-transplant survival was reduced due to high rates of primary tumor recurrence^[5]. The major revolution in improving the survival of HCC transplanted patients has been the introduction of more stringent selection criteria. The Milan criteria, published in 1996, highlighted that the selection of patients to be transplanted should be based on both the number (up to three) and diameter (up to 5 cm) of HCC nodules^[6]. In the absence of macrovascular invasion and extrahepatic spread, LT allowed patients a 4-year survival rate of 75%^[6]. Despite these excellent results, HCC recurred in 8% of patients and was the main cause of death^[6]. In subsequent studies focused on LT patients fulfilling the Milan criteria, HCC recurrence were found in 10%-16% of cases^[7-9]. These data demonstrated that HCC recurrence occurs despite the application of stringent selection criteria for LT, and is likely due to HCC dissemination from circulating cancer cells and micrometastases before or during total hepatectomy^[10]. A recent debate on the need to expand the Milan criteria for LT poses the question of whether the price to pay for adopting this policy is increased HCC recurrence^[11].

The purpose of this paper is to present a review of the scientific data available on the risk factors for HCC recurrence after LT. Risk factors related mainly to cancer and the host, as well as the impact of immunosuppressive therapy regimens adopted after transplantation, have been considered. Since many of the risk factors have been incorporated into predictive HCC recurrence risk models, this review focuses only on models that have been validated in different cohorts of LT patients.

FACTORS ASSOCIATED WITH HCC RECURRENCE AFTER LIVER TRANSPLANTATION

The development of post-LT HCC recurrence appears to be multifactorial [Table 1]. Risk factors involved in HCC recurrence may be divided into factors related to the tumor and those unrelated to the tumor. Risk factors related to the tumor are those pertaining to the morphological, histological, and serological characteristics of HCC, as well as those from the response of HCC to anticancer treatments. Risk factors unrelated to the tumor are those referring to the demographic and clinical characteristics of the recipient (age, gender, severity of underlying liver disease), of the liver graft (percentage of steatosis, cold ischemia time, living versus cadaveric donor), and the impact of immunosuppression after LT. All of these have been studied extensively to develop risk prediction models of HCC recurrence after LT.

Risk prediction models can be classified into three categories: preoperative, postoperative, and general models. Preoperative models consider the morphological, serological, and histological characteristics of HCC [Table 2]. Thus, these models may be adopted to select a candidate for LT by estimating the future risk of developing HCC recurrence. Postoperative models [Table 3] are developed from histological risk factors based on the evaluation of HCC characteristics in the explanted liver, such as tumor grade and the presence of microvascular invasion (MVI). General risk models are derived from a combination of pre- and postoperative risk factors; for this reason, they cannot be used to select candidates with HCC for LT. Conversely, general risk models can be adopted to determine optimal screening intervals for HCC recurrence in high-risk patients or to design clinical trials on neo-adjuvant therapies^[12].

Table 1. Classification of risk factors implicated in hepatocellular carcinoma recurrence after liver transplantation

Risk factors	
Tumor-related	
Morphology	<ul style="list-style-type: none"> Number and size of the nodules Macrovascular invasion Extrahepatic spread
Histology	<ul style="list-style-type: none"> Grading Microvascular invasion Genetic signature
Expression of serum markers	<ul style="list-style-type: none"> AFP DCP CRP NLR PLR
Molecular markers	<ul style="list-style-type: none"> TP53 mutations Gene expression signatures FAI HDACs and MMPs miRNAs CTC
Response to anticancer treatments	<ul style="list-style-type: none"> Bridge treatments Downstaging treatments
Tumor unrelated	
Recipient characteristics	<ul style="list-style-type: none"> Age, gender Severity of underlying liver disease Immunological status
Donor characteristics	<ul style="list-style-type: none"> Age, gender LDLT vs. DCD Percentage of graft steatosis Cold and warm ischemia times
Immunosuppressive regimen after liver transplantation	<ul style="list-style-type: none"> CNI mTOR inhibitors

AFP: alpha-fetoprotein; DCP: des-gamma-carboxyprothrombin; CRP: C-reactive protein; NLR: neutrophil/lymphocyte ratio; PLR: platelet/lymphocyte ratio; TP53: tumor protein p53; FAI: fractional allelic imbalance; HDACs: histone deacetylases; MMPs: matrix metalloproteinases; miRNAs: micro-RNAs; CTC: circulating tumor cells; LDLT: living donor liver transplantation; DCD: deceased donor; CNI: calcineurin inhibitors; mTOR: mammalian target of rapamycin

PRE-LIVER TRANSPLANTATION TUMOR-RELATED RISK FACTORS ASSOCIATED WITH HCC RECURRENCE

Morphological factors

The simplest morphological characteristics of HCC, such as the number of nodules and their size, were adopted to develop the Milan criteria^[6]. The reason why these criteria, based exclusively on morphological features, made it possible to obtain an accurate selection of patients with HCC for LT, is linked to the size and number of HCC nodules which are considered as a surrogate marker for the presence of MVI and/or poor differentiation of the tumor^[13,14]. It has been demonstrated that the presence of MVI and/or poor HCC differentiation are independent predictors for HCC recurrence^[15]. Since the Milan criteria were published, several studies conducted in Western countries have reported similar survival rates of HCC liver transplanted patients using less stringent morphologic selection criteria. These results suggest that the Milan criteria might exclude some patients with HCC who may benefit from LT^[16]. Thus, several studies have since explored the possibility of expanding the Milan criteria by considering only the morphologic characteristics of the tumor(s), which are assessed in the pre-LT period using radiologic techniques^[17-20]. Among these studies, two were validated in different patient cohorts. The first was conducted in China

Table 2. The pre-operative models assessing the risk of hepatocellular carcinoma recurrence after liver transplantation. Only risk models that have been externally validated for use before liver transplantation are presented

Authors - model name	Factors included in the model	Outcomes after liver transplantation
Mazzaferro <i>et al.</i> ^[6] Milan criteria	Number (up to three identified as < 3 cm in diameter) and size (up to 5 cm if single) of nodules	4-year post-LT survival 75% 4-year RFS 83%
Fan <i>et al.</i> ^[21] Fudan-Shanghai criteria	Number and size of nodules (≤ 9 cm if single, no more than 3 lesions with the largest ≤ 5 cm), total tumor diameter ≤ 9 cm	3-years post-LT survival 80% 3-years RFS 88%
Yao <i>et al.</i> ^[23] San Francisco (UCSF) criteria	Number and size of nodules (≤ 6.5 cm if single or 2-3 lesions ≤ 4.5 cm), total tumor diameter ≤ 8 cm	5-year RFS 80.7%
DuBay <i>et al.</i> ^[51] Toronto criteria	Tumor confined to the liver, no poor histologic differentiation on biopsy, AFP serum levels < 400 ng/mL	5-year post-LT survival 70% 5-year RFS 66%
Toso <i>et al.</i> ^[55] Toso criteria	Total tumor volume ≤ 115 cm ³ and AFP serum levels ≤ 400 ng/mL	4-year post-LT survival 74.6% 4-year RFS 68%
Duvoux <i>et al.</i> ^[43] French model	Size and number of nodules (≤ 3 cm, between 3-6 cm or ≥ 6 cm) and AFP serum levels ≤ 100 , between 100-1000, or > 1000 ng/mL	5-year post-LT survival 69.9% 5-year RFS 66.6%
Mazzaferro <i>et al.</i> ^[59] Metroticket 2.0	Number and size of nodules (up-to-seven criteria) and AFP serum levels	5-year post-LT survival 74.9% 5-year RFS 77.9%
Zheng <i>et al.</i> ^[53] Hangzhou criteria	HCC ≤ 8 cm or > 8 cm associated with AFP < 400 ng/mL and tumor histological grade I or II	5-year post LT survival 70.7% 5-years RFS 62.4%
Kaido <i>et al.</i> ^[68] Kyoto criteria (LDLT)	Up to 10 nodules ≤ 5 cm in diameter and DCP serum levels ≤ 400 mAU/mL	5-year post-LT survival 82% 5-year HCC recurrence rate 4.4%
Lee <i>et al.</i> ^[71] MoRAL model (LDLT)	DCP and AFP serum levels	5-year post-LT survival 86% 5-year RFS 66.3%

LDLT: living donor liver transplantation; AFP: alpha-fetoprotein; DCP: des-gamma-carboxyprothrombin; RFS: recurrence-free survival

Table 3. The post-operative models to assess the risk of hepatocellular carcinoma recurrence after liver transplantation. In the table are presented the only risk models that have been externally validated for use after liver transplantation

Authors - model name	Factors included in the model	Outcomes after liver transplantation
Onaca <i>et al.</i> ^[113]	Single lesion ≤ 6 cm or 2-4 lesions ≤ 5 cm each	5-year post-LT survival 67.8% 5-year RFS 63.9%
Mazzaferro <i>et al.</i> ^[11] Up-to-seven Metroticket criteria	Sum of the size of the largest tumor (in cm) and the number of tumors not exceeding 7 in the absence of MVI	5-year post-LT survival 71.2% 5-year recurrence rate 9.1%
Decaens <i>et al.</i> ^[114]	Number of nodules, largest tumor diameter, and tumor differentiation	5-year RFS 60.2% 5-year recurrence rate 20.8%
Halazun <i>et al.</i> ^[115] Combo-MoRAL score	Pre LT largest HCC nodule < 3 cm, NLR < 5 and AFP < 200 ng/mL plus post LT HCC number < 3, largest nodule < 3 cm, HCC histological grade < 4 and no MVI	5-years RFS 80%
Mehta <i>et al.</i> ^[116] RETREAT criteria	AFP serum levels at LT, the sum of the largest viable tumor diameter, and number of viable tumors	5-year recurrence rate 12.8%

MVI: microvascular invasion; NLR: neutrophil/lymphocyte ratio; AFP: alpha-fetoprotein; LT: liver transplantation; RFS: recurrence-free survival

in patients transplanted for HCC due mainly to chronic hepatitis B virus (HBV) infection. The authors demonstrated that expanding the indications for LT in patients with solitary HCC ≤ 9 cm in diameter, or with no more than 3 lesions (the largest ≤ 5 cm) with a total tumor diameter of ≤ 9 cm, there was no significant difference in 1- and 3-year survival and in recurrence-free survival as compared to the Milan criteria^[21]. The aforementioned Fudan-Shanghai criteria were subsequently validated in seven Shanghai liver transplant centers, which included 1,078 patients^[22]. The second study was conducted in the US and gave rise to the University of California San Francisco (UCSF) criteria^[23]. These criteria suggested that having a single lesion ≤ 6.5 cm or 2-3 lesions ≤ 4.5 cm each, with a total tumor diameter ≤ 8 cm, resulted in 5-years post-LT recurrence-free survival in 80.7% of cases, which was not worse compared to that observed when applying the Milan criteria.

The most important limitation of morphologic criteria based exclusively on radiological imaging -performed by contrasted computed tomography (CT) scan or magnetic resonance imaging (MRI) - is the accuracy in detecting any single lesion in the liver, and, more importantly, to properly characterize it.

The American College of Radiology developed the Liver Imaging Reporting and Data System (LI-RADS) to standardize the acquisition, interpretation, reporting, and data collection of liver imaging^[24]. LI-RADS is being increasingly adopted in clinical practice for patients at high risk of HCC, thereby enabling the categorization of observations from LR-1 (definitely benign) to LR-5 (definitely HCC) based on the level of suspicion for HCC. However, a recent meta-analysis^[25] derived from 14 studies showed that the performance of LI-RADS for diagnosing HCC has a sensitivity of 67% and specificity of 92%. These data confirm previous reports indicating that radiologic imaging alone inaccurately stages as many as 20%-25% of patients undergoing LT for HCC^[26-28].

Histological factors

To increase the prognostic accuracy of the predictive models of HCC recurrence based exclusively on morphological data, some authors explored using the histological characteristics of HCC obtained by nodule biopsy performed before LT. Cillo *et al.*^[26] selected 33 patients with HCC for LT based on tumor grading obtained by liver biopsy. Patients with moderately to well-differentiated HCC had a 5-year post-LT survival of 75% despite approximately one-third of them failing to meet the Milan criteria at explanted liver examination. With respect to MVI, Shah *et al.*^[28] evaluated 155 patients with confirmed HCC after LT that satisfied the Milan criteria, then assessed the presence of MVI via pathological analysis. The presence of MVI was significantly associated with both the number and size of the nodules and, more importantly, 68% of patients who developed HCC recurrence were positive for MVI. Despite the undoubted diagnostic value of pre-LT pathological assessment of tumor grading and MVI, routine tumor biopsy is often unfeasible either due to the presence of multiple nodules or the risk of cancer cells seeding^[29].

To overcome these limitations, a recent approach to non-invasively detect the presence of MVI in HCC is the application of 18F-FDG PET/CT imaging^[30]. In HCC, the growth rate and activity of glycolytic enzymes are related^[31]. Thus, contrary to what occurs in well-differentiated HCC, poorly differentiated HCC cells exhibit low glucose-6 phosphatase activity and high 18F-FDG uptake^[32]. Recent studies appear to confirm that maximum standardized uptake values of 18F-FDG PET/CT imaging are strongly correlated with the histological characteristics of HCC, such as MVI and tumor grade^[33-35]. The optimal cutoff values for the SUVmax of HCC (SUVmax T) and SUVmax of the normal liver (SUVmax L) in predicting MVI have been identified as 3.80 and 1.49, respectively^[36]. Moreover, in a study that enrolled 34 HCC liver transplanted patients, none of the patients with a SUVmax L/T ratio > 1.5 had well-differentiated HCC^[37]. A further improvement in the radiological detection of MVI in patients with HCC was derived from the application of gadoteric acid-enhanced MRI and 18F-FDG PET/CT examinations. With the application of these radiological techniques, the presence of peritumoral enhancement and the ratio of SUVmax T/SUVmean L ≥ 1.2 had a statistically significant association with MVI, with an odds ratios of 10.6 and 14.2, respectively^[38]. When both MRI and PET/CT imaging techniques have been applied in combination, the sensitivity and specificity for the prediction of MVI were 78.6 and 80% respectively^[30]. Recent experiences have demonstrated how the combination of 18F-FDG PET/CT can represent valid help in selecting LT patients with HCC who exceed the Milan criteria. A Japanese study^[39] that enrolled 182 living donor liver transplanted (LDLT) patients with HCC demonstrated that, in patients exceeding the Milan criteria with negative 18F-FDG PET/CT and alpha-fetoprotein (AFP) serum levels < 115 ng/mL, the 5-year HCC recurrence rate was not statistically different from those fulfilling the Milan criteria (19% compared to 7%, $P = 0.1$). Similar results have been obtained in a Korean study involving LDLT, wherein patients exceeding either the Milan or UCSF criteria, but with negative 18F-FDG PET/CT, had 5-year HCC-free survival rates after LT of 73.3 and 72.8%, respectively^[40]. However, these encouraging results require extensive validation in Western populations, in patients with different etiologies of liver disease, and in LT performed using cadaveric donors. Undoubtedly, the combined use of 18F-FDG PET/CT could represent a new and more accurate system to non-invasively assess the morphological and histological features of HCC in the near future, which could guide the selection of patients for LT.

Biological markers

Biological markers can be divided into three categories: (1) serum markers directly related to HCC biology, such as AFP and des-gamma-carboxyprothrombin (DCP); (2) systemic inflammation markers, such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR)^[41]; and (3) molecular biomarkers in tumor tissue and in serum, such as DNA alterations/mutations, enzymes, and micro-RNAs (miRNAs)^[42].

Serum markers

AFP

AFP is a surrogate marker of HCC differentiation and vascular invasion^[43,44]; thus, the measurement of AFP serum levels before LT has been proposed as a useful tool to identify patients with a high risk of HCC recurrence^[45]. Although AFP has proven to be a valid and simple tool to discriminate HCC recurrence risk, opinions regarding what the discriminating plasma values of the protein should be are not unanimous. Several authors have suggested that serial measurements of AFP levels might be more accurate than a single measurement. Increasing AFP levels to more than 15 ng/mL^[46,47], more than 50 ng/mL/month^[48], or 0.1 ng/mL/day^[49] during the LT waiting period have been proposed as strong predictors for HCC recurrence.

The most effective method of using AFP serum levels to predict HCC recurrence after LT is to associate it with tumor morphological criteria^[50]. Four selection criteria for LT in HCC patients, including AFP serum levels and the morphologic characteristics of the tumor - both evaluated pre-LT - have been extensively validated. The “Toronto criteria”^[51] were derived from a general assumption that acceptable survival rates in LT for HCC can be achieved for any size or number of HCC, provided that: imaging studies ruled out vascular invasion, the HCC was confined to the liver, and the HCC was not poorly differentiated on biopsy. The authors demonstrated that by applying these criteria, the only pre-transplant variable associated with 5-year disease-free survival was an AFP serum level value > 400 IU/mL at the time of transplant. In the validation cohort of the study^[52], it was confirmed that AFP was the only independent predictive variable associated with post-LT survival and HCC recurrence, albeit with a cutoff value of 500 IU/mL.

The association of morphologic and histologic characteristics of HCC and AFP serum levels has also been utilized in China to develop the Hangzhou criteria^[53]. These criteria were defined as no portal vein tumor invasion, HCC diameter ≤ 8 cm, or patients who have HCC larger than 8 cm would still be eligible for LT if their AFP serum levels were < 400 ng/mL, and their HCC histological grade was I or II. The 1- and 3-year survival rates of patients transplanted for HCC within the Hangzhou criteria were not significantly different from those transplanted within the Milan criteria.

The criteria proposed by Toso *et al.*^[54] were derived from a large study including over 6000 patients. The authors demonstrated that a total tumor volume (TTV) of ≤ 115 cm³ and AFP serum levels ≤ 400 ng/mL identified patients at low risk of HCC recurrence after LT more effectively than both the Milan and UCSF criteria. The TTV-AFP criteria were validated in a prospective study with patients from different European countries and Canada^[55].

The Liver Transplantation French Group developed and validated a prognostic model for predicting HCC recurrence after LT, known as the AFP model^[43]. This model considered the predictive variables of AFP serum level, and size and the number of nodules, with different cutoffs for each variable. The following points were assigned for tumor size: 0, 1, or 4 points when the largest tumor size was ≤ 3 cm, 3-6 cm, or > 6 cm, respectively. Concerning the number of nodules, 0 or 2 points were assigned for the presence of ≤ 3 or ≥ 4 nodules. Moreover, 0, 2, or 3 points were assigned to AFP serum levels ≤ 100, 100-1000, or > 1000 ng/mL, respectively. The maximum score obtainable from the AFP model was 9. Patients with a final score of up to

2 points were classified as having a low risk of HCC recurrence; on the contrary, patients with a final score ≥ 3 were classified as high risk. Interestingly, patients with AFP serum levels > 1000 ng/mL reached 3 points irrespective of the number or size of nodules; therefore, they could immediately be classified as patients at high risk of HCC recurrence. The validation of the AFP model in several countries^[44,56-58] and LDLT confirms that this model better discriminated the risk of HCC recurrence compared to the Milan criteria. It is evident that patients within the Milan criteria but with AFP serum levels > 1000 ng/mL experienced HCC recurrence in 37.7% of cases, compared to patients exceeding the Milan criteria but with AFP < 100 ng/mL, with HCC recurrence in 14.4% of cases^[41]. Due to the aforementioned characteristics, the AFP model was adopted for the liver allocation policy of France in 2013.

More recently, Mazzaferro *et al.*^[59] developed a new predictive model of HCC recurrence based on AFP serum levels and the morphological characteristics of HCC in Italy, and validated this model in Asian patient cohorts. This model, known as the Metroticket 2.0 model, identified the sum of tumor number and size, and the \log_{10} of AFP level as being significantly associated with HCC-specific death. For patients with HCC to have a 70% chance of HCC-specific survival 5 years after transplantation, their AFP level should be < 200 ng/mL and the sum of the number and size of tumors (in centimeters) should not exceed 7 cm; if the AFP level was 200-400 ng/mL, the sum of the number and size of tumors should be ≤ 5 cm; if their AFP level was 400-1000 ng/mL, the sum of the number and size of tumors should be ≤ 4 cm. This model, based on serum AFP level and HCC number and size, outperformed the Milan, UCSF, and AFP French model to predict which patients will survive for 5 years after LT.

The availability of recent models that combine the morphological and biological elements of the tumor has made it possible for patients with HCC, who would have been excluded by applying selection models based exclusively on morphological characteristics of the tumor, to undergo LT. More importantly, this has been achieved by keeping post-transplant survival unchanged. The relative simplicity of calculating the size and number of nodules in addition to AFP serum levels as surrogate biological markers of the tumor, make these models suitable for extensive and standardized use. An important innovation of these models is their possibility of being used “dynamically”, to evaluate the evolution of the tumor in the patient before the transplant. This implies that these models could be used in addition with the response to neo-adjuvant treatments as the reference criteria for defining transplant feasibility in patients with HCC.

DCP

Increased serum DCP levels have been detected in patients with HCC^[60] and they correlate with the degree of HCC malignancy, including the presence of intrahepatic metastases, capsule infiltration, and portal vein invasion^[61,62]. Moreover, HCC expressing normal levels of AFP and increased levels of DCP showed a lower grade of differentiation and more frequent MVI^[63,64]. For these reasons, DCP has been proposed as a stronger predictor of HCC recurrence after LT than AFP^[61,65]. Two Japanese groups have developed and validated the selection criteria for LDLT by considering the size and number of nodules as well as DCP serum levels. The Kyoto criteria^[66-68] were considered for LT patients with up to 10 HCC ≤ 5 cm in diameter and DCP serum levels ≤ 400 mAU/mL. Patients that exceeded the Milan criteria but met the Kyoto criteria had similar HCC recurrence rates to patients within the Milan criteria^[66]. Similar results were obtained by adopting the Kyushu criteria^[69], which selects for LT patients with any number of HCC < 5 cm in diameter and DCP serum levels < 300 mAU/ml. These criteria were more sensitive than the UCSF and Kyoto criteria in predicting HCC recurrence^[61,70].

A further attractive strategy to construct a model to predict HCC recurrence involves combining AFP and DCP levels. The MoRAL model^[71] was developed from this hypothesis in patients exceeding the Milan criteria who underwent LDLT. The authors included a total of 566 consecutive patients who underwent LDLT in Korea, 410 of which exceeded the Milan criteria. Serum levels of AFP and DCP provided good

discriminatory function with respect to time to HCC recurrence. A low MoRAL score (cutoff ≤ 314.8) was associated with significantly longer recurrence-free (vs. > 314.8) and overall survival in the cohort exceeding the Milan criteria. The 5-year recurrence-free and overall survival rates of patients exceeding the Milan criteria with a low MoRAL score were as high as 66.3% and 82.6%, respectively. Moreover, patients within the Milan criteria with a high MoRAL score showed a higher risk of recurrence than patients exceeding the Milan criteria with a low MoRAL score.

Only one retrospective study^[72] combining AFP and DCP serum levels to predict HCC recurrence was performed outside of Asia, in the US. The authors demonstrated that AFP ≥ 250 ng/mL and DCP ≥ 7.5 ng/mL were associated with a higher risk of HCC recurrence. When AFP and DCP were combined with the Milan criteria, the hazard ratio increased from 2.6 for outside the Milan criteria to 8.6 for outside the Milan criteria and AFP ≥ 250 ng/mL, and to 7.2 for outside the Milan criteria and DCP ≥ 7.5 ng/mL.

Systemic inflammation biomarkers

The option of considering inflammatory markers as elements associated with greater HCC invasiveness or with more frequent recurrence after LT arises from certain studies conducted on C-reactive protein (CRP). CRP is a protein synthesized by hepatocytes in response to systemic inflammation and has been implicated in the prognosis of HCC^[73]. Some Asian studies have demonstrated that in patients outside the Milan criteria, high serum CRP levels were significantly associated with a higher risk of HCC recurrence^[74,75].

Besides CRP, recent studies have identified NLR and PLR as two inflammation markers involved in the prognosis of HCC^[41]. Halazun *et al.*^[76] reported that in patients within the Milan criteria, an NLR ≥ 5 was associated with worse recurrence-free survival after LT than in patients with an NLR < 5 (25% vs. 75%). These observations led the authors to propose a risk model for predicting HCC recurrence that includes NLR and tumor size > 3 cm in diameter. Further studies have been conducted to assess the real impact of NLR on the independent risk of HCC recurrence along with MVI and the size and number of nodules. Recent meta-analyses have highlighted that the cutoff value of NLR is rather heterogeneous among different studies, which is justified since the results obtained were not comparable. By applying an NLR cutoff value of 4, the majority of studies have indicated that a high NLR value is associated with MVI and a lower HCC recurrence-free survival after LT^[77,78].

Regarding PLR, high PLR has been associated with a significant increase in HCC recurrence after LT^[79]. Notably, the prognostic value of PLR in determining the risk of HCC recurrence should be evaluated with caution. Moreover, some reports indicate that the absolute platelet count seems to be as important as PLR. In patients with a platelet count $\geq 75 \times 10^9/L$ on the day before transplant, the HCC recurrence rate was significantly higher than in patients with a platelet count $< 75 \times 10^9/L$ (28.2% vs. 13.2%)^[80]; interestingly the former group of patients presented more frequently with poorly differentiated HCC, MVI, and bile duct invasion compared to the latter group.

Many hypotheses have been proposed in an attempt to explain the pathophysiological mechanisms involved in determining the influence of NLR and PLR in HCC recurrence after LT. Both neutrophils and platelets are implicated in promoting vascular invasion and the development of metastasis of tumors by increasing the production of vascular endothelial growth factor (VEGF)^[81,82]. Furthermore, platelets may promote the establishment of HCC metastases by blocking tumor cell removal^[83,84]. Since high NLR and PLR are determined by low lymphocyte numbers, it may be hypothesized that the reduction in lymphocyte numbers could result in impaired immune surveillance against HCC^[41]. While existing studies conducted on inflammatory markers have provided interesting results, they also have several limitations. Specifically, the retrospective nature of the studies and the small number of cases represent the major limitations. Moreover, the different cutoffs applied to NLR and PLR make adoption of these models for predicting the risk of HCC recurrence in the clinical practice unvalidated.

Molecular biomarkers

Several studies investigated the usefulness of molecular biomarkers to predict HCC recurrence in liver transplanted patients^[85]. Regarding DNA alterations/mutations evaluable in liver tissue, the presence of TP53 mutations, high fractional allelic loss, significant hypo-methylation of 8 tumor suppression genes, and the absence of CTNNB1 mutations, identified a molecular subclass of aggressive HCC. These features were predictive of reduced recurrence-free survival in a small group of 25 liver transplanted patients^[86]. A further interesting approach is to evaluate the impact of gene expression signatures in liver tissue, both inside and outside of the tumor, in predicting HCC recurrence. Applying this approach in a large cohort of 132 liver transplanted patients for HCC outside Milan criteria, Miltiadous *et al.*^[87] demonstrated that the S2 molecular subclass^[88] and progenitor cell markers (CK19 signature^[89]) were independent predictors of overall survival and of HCC recurrence after LT, respectively.

The fractional allelic imbalance rate index (FAI), determined from tissue samples, is used to compare the acquired mutational load between different tumors^[90]. FAI was evaluated in a cohort of 71 liver transplanted patients with HCC, 18 of whom experienced tumor recurrence. Among the 19 microsatellites evaluated, 3 loci (D3S2303, D9S251, and D9S254) were found to be predictive of recurrence after LT^[91]. If confirmed in other prospective studies, and in patients outside the Milan criteria, FAI could represent an interesting tool to identify recipients at higher risk of tumor recurrence.

Enzymes such as histone deacetylases (HDACs) and matrix metalloproteinases (MMPs) have also been studied in liver transplanted patients. HDACs regulate genes and are involved in tumor cell proliferation, differentiation, invasion, and metastasis. Liver transplanted patients carrying T alleles in HDAC1 rs1741981 and HDAC3 rs 2547547 single nucleotide polymorphisms have been found to have a low risk of HCC recurrence^[92]. On the contrary, high expression of HDAC3 was related to high risk of HCC recurrence in HBV positive recipients^[93].

MMPs are extracellular matrix-degrading enzymes that can be secreted by tumor cells to enhance tumor invasiveness and metastasis. Although results among different studies are conflicting^[42], MMP-9 and MMP-2 positive staining in stromal tissue adjacent to tumors seems to be associated with HCC recurrence^[94]. While there is a rationale for investigating the role of enzymes in predicting HCC recurrence, the results of the studies are still too heterogeneous to draw solid conclusions for use in clinical practice.

Besides DNA changes and aberrant gene expression, miRNAs have been evaluated as potential prognostic biomarkers in HCC. An interesting report proposed a prognostic score incorporating the expression in liver tissue of miR-214, miR-3187, and the Milan criteria, which improved the accuracy of predicting HCC recurrence compared with the Milan criteria alone^[95]. Despite several studies reporting a potential role of miRNAs in predicting HCC recurrence^[85], none of them were really prospective and adequately powered. The major limitation in introducing miRNAs in clinical practice is probably the need for a liver tissue biopsy to evaluate their expression. To overcome this important limitation, liquid biopsy has emerged as a minimally invasive alternative approach to analyze HCC components without the need of tissue samples. This method allows the analysis of DNA, RNA in extracellular vesicles such as the exosomes, or circulating tumor cells released by the tumor in the bloodstream^[85]. Among the few studies that have applied this technology, Nakano *et al.*^[96] demonstrated that circulating exosomal miR-92b could have clinical value for predicting HCC recurrence post-LT. The main limitation of liquid biopsy is the low sensitivity and lack of reproducibility when different technologies were applied^[97].

Among the serological markers that can be measured before transplantation, AFP remains the most clinically useful to date, albeit with the major limitation that it can only be used in protein secreting HCC.

HCC response to anticancer treatments as a surrogate biological marker of tumor aggressiveness

Alongside the morphological, histological, and serological criteria used to predict the risk of HCC recurrence after LT, an innovative and more flexible approach involves considering the response of HCC to anticancer loco-regional treatments as a surrogate marker of the biological aggressiveness of the tumor - and thus, of the risk of post-LT recurrence. This approach shifts the concept of selecting HCC patients for LT from the characteristics of disease presentation to the final characteristics of the tumor after it has undergone available treatment. Regarding this point, it is important to differentiate “bridge treatments” from “downstaging treatments”. Bridge treatments refer to patients already on the waiting list for LT that undergo HCC loco-regional treatments to reduce dropout rates from the waiting list. Downstaging treatments refer to those initially applied to patients beyond the accepted criteria for LT (e.g., Milan, UCSF, TTV-AFP, and others) to bring the tumor back within the accepted criteria for LT^[98]. The increasing rate of applying the approach of merging HCC tumor stage and response to anticancer treatments in European countries^[99] is justified by observations that post-LT survival outcomes in patients with HCC exceeding the Milan criteria with objective and sustained response to pre-LT therapies are not significantly different compared to those patients who meet conventional criteria at presentation^[100]. In the US, the UCSF downstaging protocol has recently been adopted as a national policy for granting priority listing for LT^[101]. This protocol implies that the initial selection criteria are single lesions ≤ 8 cm, or 2-3 lesions < 5 cm with total tumor diameter < 8 cm, or 4-5 nodules all < 3 cm with total tumor diameter < 8 cm. In a retrospective analysis of the UNOS database of 3819 patients who underwent LT from 2012 to 2015, and were classified as always within the Milan criteria or achieving UNOS downstaging criteria (UNOS-DS), 3-year post-LT survival was 83.2% for the Milan criteria and 79.1% for the UNOS-DS. The 3-year HCC recurrence probability was 6.9% for Milan and 12.8% for UNOS-DS. Interestingly, AFP ≥ 100 ng/mL was the only independent predictor of HCC recurrence in downstaging groups^[102]. The application of downstaging protocols involves a minimum observation period of 3 months of disease stability from successful downstaging to LT^[101,103]. This implies that liver transplant centers should adopt dynamic graft allocation protocols to assure “on time” LT in those patients who can maximize the benefit of successful downstaging^[99]. Thus, both AASLD and EASL guidelines recommended loco-regional treatments in patients with HCC exceeding the Milan criteria and to consider those who underwent successful downstaging as candidates for LT, when this status is maintained for at least 3-6 months^[104-106].

Although the rationale for supporting the application of downstaging strategies exists, it is much more complex to evaluate the ways in which to obtain it. There are in fact multiple loco-regional therapies applicable for the treatment of patients with HCC with both bridging and downstaging purposes. These therapies include transarterial chemoembolization (TACE) and radioembolization, percutaneous ethanol injection, radiofrequency ablation, and stereotactic body radiation. Liver resection may be a further part of a multimodal downstaging strategy^[107,108]. The long list of therapeutic strategies highlights how indications to perform them can be varied between different transplant centers, thus results obtained are not always comparable^[107]. Since every treatment can negatively impact upon residual liver function, it has been proposed that only patients with adequate liver function (Child Pugh class A/B) and with serum bilirubin ≤ 3 mg/dL can be candidates for downstaging procedures^[102,103]. TACE is the most commonly used treatment, so it is recommended as first-line for downstaging^[108].

A further critical element relates to the method used in various studies to judge the response to loco-regional treatments. In more recent studies, this has been done by applying the modified Response Evaluation Criteria in Solid Tumors (mRECIST), which assesses both the change in tumor volume and in arterial enhancement by means of contrast CT or MRI scan of the liver^[109]. A very recent report demonstrated that the addition of the mRECIST criteria into the Metroticket 2.0 framework improved its predictive ability^[110]. However, although sufficiently detailed, the mRECIST criteria may have reproducibility limits between different transplant centers, such that the results obtained by loco-regional therapies are not always reproducible.

Despite these important limitations, it has recently been accepted that patients with HCC listed for LT and receiving loco-regional treatments associated with objective response, improved waitlist and post-transplant outcomes. More importantly, the degree of tumor response to loco-regional treatments may help in defining LT priority in candidates with HCC^[108].

POST-LIVER TRANSPLANTATION TUMOR-RELATED RISK FACTORS ASSOCIATED WITH HCC RECURRENCE

In all prognostic risk models, the number and size of tumors, as well as the presence of MVI, were found to be statistically associated with the risk of HCC recurrence after LT. As previously mentioned, it is known that pre-transplant staging methods based on radiologic imaging fail to predict the exact number and size of HCC at pathology in approximately 25%-35% of patients due to over- or understaging^[28,111,112]. These observations justified the development of HCC recurrence risk models based on the accurate assessment of tumor burden in the explanted liver. In a large US database of HCC liver transplanted patients, Onaca *et al.*^[113] evaluated the number and size of HCC in all explanted livers and correlated them with HCC-free survival after LT. The authors demonstrated that patients with 2-4 tumors < 5 cm or with a single lesion < 6 cm had recurrence-free survival equivalent to patients with a single tumor of 3.1-5.0 cm or 2-3 lesions all < 3 cm in diameter, which represents the Milan criteria.

As previously reported, the assessment of MVI before LT is difficult and often inaccurate^[29]; on the contrary, evaluation of the presence of MVI appears very accurate on histological examination obtained in the explanted liver. On this basis, Mazzaferro *et al.*^[111] developed a predictive model of the risk of mortality and recurrence of HCC after LT based on histopathological analysis performed on the explanted liver. The histological characteristics evaluated include the number and size of the nodules, the presence of MVI, and the grading of the tumor. The authors collected a sample of 1,556 patients transplanted for HCC from several US, European, and Asian centers, of which only 444 had tumor characteristics under the Milan criteria at explant. The combination of HCC characteristics exceeding the Milan criteria but resulting in an estimated 5-year overall survival of at least 70% generated a subgroup of patients that, in the absence of MVI, fulfilled the so-called “up-to-seven criteria”, which involves seven being the result of the sum of the size (in cm) and the number of tumors, for any given HCC. The overall survival reported in this subgroup of patients was 71.2%, which was similar (73.3%) to that obtained in the subgroup of patients fulfilling the Milan criteria, irrespective of the presence of MVI. On the contrary, patients exceeding the up-to-seven criteria, plus patients with MVI who were beyond the Milan criteria and within the up-to-seven criteria, had a 5-year survival rate after LT of 48.1%. The presence of MVI at any size and number category of tumors was paralleled by a significant worsening of survival and the cumulative incidence of HCC recurrence.

A similar model was developed and validated in France^[114], which considered the pathological characteristics of HCC assessed in the explanted liver, including the number, size, and grading of tumors. To obtain a final numeric risk score, the authors attributed point values for any of the following tumor characteristics: the number of nodules, the diameter of the largest nodule, and tumor differentiation (well, moderate and poor). For Cox regression analysis, the number of nodules, maximal diameter of the largest nodule, and tumor differentiation were independent predictors of HCC-free survival. Interestingly, in patients with a score < 4, there was no significant difference in 5-year tumor-free survival between those within and exceeding the Milan criteria. A very similar approach was followed in one of the largest single institution dataset of HCC patients undergoing LT in the US^[115], to update the original MoRAL score. The evaluation of histology in the explanted liver showed that grade IV HCC, the presence of more than 3 lesions, the largest tumor size > 3 cm, and MVI were independently associated with HCC recurrence. This model, called the post-MoRAL score, was therefore combined with the original pre-MoRAL score to

develop a new model, called the combo-MoRAL score. In this new model, patients were stratified according to an increased HCC recurrence risk score ranging from 0 to 26. Those patients presenting a combo-MoRAL score of up to 6 were identified as low-intermediate risk, while those exceeding 6 points were considered to have a high-very high risk of HCC recurrence. Patients outside the Milan criteria who had a combo-MoRAL score of up to 6 experienced a risk of recurrence similar to those within the Milan criteria. A recent and particularly relevant Risk Estimation of Tumor Recurrence After Transplant (RETREAT) score^[116] was developed and validated for patients with HCC that met the Milan criteria based on imaging. A total of 1061 patients were enrolled in the study, which was developed in the US and validated in Canada. In the development cohort, 9.4% of patients had MVI and 22.1% exceeded the Milan criteria on explant. Cumulative probabilities of HCC recurrence at 1 and 5 years were 5.7% and 12.8%, respectively. For multivariable Cox proportional hazards regression, three variables were independently associated with HCC recurrence: MVI, AFP value at time of LT, and the sum of the largest viable tumor diameter and the number of viable tumors on explant. The RETREAT score was created using these three variables, with scores ranging from 0 to 5 or higher being highly predictive of HCC recurrence. The RETREAT model was able to stratify 5-year post-LT recurrence risk ranging from less than 3% with a score of 0 to greater than 75% with a score of 5 or higher.

A critical point that can be applied to the majority of models that aim to predict HCC recurrence is that the time of recurrence after transplant is rarely mentioned. Although this time is variable, several studies have identified a peak of HCC recurrence within 2-3 years after transplant while after 5 years, recurrence is very infrequent^[117]. Since HCC recurrence after LT impacts negatively on overall survival, the time to recurrence represents an important prognostic factor. Early (within 12 months after LT) HCC recurrence is associated with a more severe prognosis^[117]. The reasons why it occurs may be related to the presence of non-detected extra-hepatic HCC metastases at the time of transplant, or as a consequence of circulating neoplastic clones of HCC able to engraft and growing in the transplanted liver or in other organs. Recurrences occurring more than 12 months following LT, particularly if associated with AFP serum levels < 100 ng/mL at the time of recurrence, are associated with a better prognosis and with 5-year survival nearing 50%^[118,119]. These data strongly suggest maintaining a very stringent surveillance of the recurrence of HCC in the first 3 years after LT, prolonging up to the fifth year, since very late recurrence of the tumor is also associated, if promptly discovered and treated, with a better prognosis^[10]. Regarding the best way to perform surveillance, in the absence of specific evidence-based risk stratification criteria, the majority of LT centers suggest to perform a total body contrast CT or MRI scan every 6 months for at least 3 years, that can then be extended to 5 years after LT, in addition to AFP serum measurements^[117].

Taking into account the aforementioned considerations HCC recurrence risk models based on the evaluation of tumor characteristics on the explanted liver should improve post-LT HCC surveillance strategies and to help identify patients who may benefit from future adjuvant therapies. On the contrary, all of these models have the major limitation in that they cannot be used to select patients with HCC for LT.

TUMOR UNRELATED RISK FACTORS ASSOCIATED WITH HCC RECURRENCE

Transplant type

Conflicting results exist regarding the potential impact of influencing HCC recurrence following LT performed using cadaveric donors versus LDLT^[120-126]. In addition to more advanced clinical characteristics of the tumor that are often recognized in LDLT^[120], there are other potential explanations to support the hypothesis that HCC recurrence may be more frequent in LDLT when compared to using cadaveric donors. A potential mechanism to promote tumor progression and recurrence may be related to the release of growth factors in the course of liver regeneration involving a partial graft. Moreover, it has been demonstrated that small-sized grafts are more likely to cause acute phase graft injury, promoting cell adhesion, increased angiogenesis, and cell migration. All of these factors may contribute to increased

HCC recurrence^[127-129]. A further mechanism could be linked to the short waiting time on LDLT waiting lists^[130,131]. This short waiting time may not be able to highlight tumors with greater biological aggressiveness, expressed as having rapid growth over time. Finally, the LDLT technique associated with sparing of the inferior vena cava and with more extensive manipulation of the liver during transplant operations may contribute towards increasing the risk of HCC recurrence^[132]. The same considerations may be applied to LT performed by the piggyback technique with cadaveric donors since it theoretically carries a higher risk of positive vena cava margins and requires greater manipulation of the diseased liver, thus leading to an increased risk of HCC spread^[133]. Nevertheless, it remains important to highlight that the piggyback technique is the preferred approach in many liver transplant centers since it provides several advantages compared to conventional techniques, such as shorter operation times, anhepatic phases, warm ischemia times, and stays in intensive care units^[132,134].

Graft and donor-related factors

Besides the use of partial grafts for LT, several reports have indicated that prolonged cold and warm ischemia times could be associated with an increased risk of HCC recurrence^[135]. Multiple biological mechanisms have been proffered to explain how ischemia-reperfusion injury can affect the risk of HCC recurrence, based on *in vivo* and *in vitro* experiments^[136-138]. One of these mechanisms hypothesizes that the exposure of micrometastases to prolonged hypoxia could lead to the abnormal expression of genes and cytokines that increase angiogenesis, cell proliferation, and growth^[136]. Notably, it has been hypothesized that female grafts may be more susceptible to ischemia-reperfusion injury and may have increased sensitivity to reoxygenation damage following prolonged cold storage^[139,140]. Furthermore, hypoxia stabilizes and activates the transcription factor for hypoxia - inducible factor, which represents a key oxygen response regulator able to activate the transcription of genes stimulating angiogenesis such VEGF-A^[141,142]. However, the relationship between prolonged graft ischemia time and the risk of HCC recurrence was most convincing in recipients who presented additional risk factors for recurrence at baseline, such as HCC beyond the Milan criteria, the presence of MVI, or high AFP serum levels^[126].

The increased susceptibility to ischemia-reperfusion injury of older grafts support some observations, thus indicating that recipients transplanted with older donors experienced HCC recurrence more frequently than those transplanted with younger donors^[143]. Although these observations are of interest, previous studies have not confirmed them in subsequent studies^[144,145].

Given the growing number of donors with metabolic syndrome, one aspect that appears to be of particular interest is the potential impact of graft steatosis on the risk of HCC recurrence. It has been accepted that grafts with moderate to severe steatosis present low tolerability to ischemia-reperfusion injury^[146]. This injury may be able to promote a release of lipid peroxides and downregulate the secretion of adipokines that can protect the steatotic grafts^[147]. Thus, the induced inflammatory cascade within the graft could be responsible for the increase in angiogenesis, which is considered the key factor in promoting tumor recurrence. All of these observations have not been validated in prospective studies and a large number of patients; thus, no current evidence exists to modify the allocation criteria for steatotic grafts based on the presence of HCC in recipients.

There is emerging evidence that persistent HBV infection contributes to cancer development within the liver, increasing genetic instability of and promoting hepatocyte destruction and regeneration. Several reports indicate that in patients transplanted for HCC related to chronic HBV infection, the reappearance of active HBV replication in the graft was associated with an increased risk of HCC recurrence and with shorter overall survival^[126]. These reports were more frequent in the past when prophylaxis against HBV recurrence was given only with immunoglobulins against HBV or with Lamivudine.

Recipient characteristics

Overweight or obese recipients transplanted for HCC seem to be more exposed to HCC recurrence compared to lean recipients^[148]. The mechanisms proposed to support this observation are very similar to those discussed for steatotic grafts. The altered production of adipokines could occur in obese patients, and are known to be responsible for increased cell proliferation and the reduction of apoptosis in neoplastic cell lines. In certain cases, adipokines can also be responsible for the increased expression of VEGF and other mediators capable of increasing angiogenesis and tumor recurrence^[149,150].

Clinical studies have identified a body mass index cutoff value of $> 30 \text{ kg/m}^2$ or the presence of obesity as independent predictors of more frequent HCC recurrence^[148,151]. Regarding recipient age, several studies have reported that elderly patients who underwent LT experienced lower survival and higher rates of HCC recurrence^[126]; however, the mechanisms involved in explaining these observations are not entirely clear. The most probable hypothesis is that advanced age represents a factor associated with reduced efficiency of the immune system in reducing the development of neoplastic cell clones, which would be more evident during prolonged immunosuppressive therapy^[152].

Impact of immunosuppression schemes adopted after LT

The impact of immunosuppressive therapy after LT has been extensively studied with regard to the development of metabolic complications such as diabetes, arterial hypertension, hyperlipidemia, and renal failure. This was motivated by the observation that the main cause of mortality in the medium to long term after LT is not linked to allograft dysfunction, but rather to the development of metabolic complications and *de novo* tumors^[153].

Much less studied has been the impact of immunosuppressive therapy in modifying the risk of HCC recurrence after LT. The role of immunosuppression in promoting oncologic cell transformations has been extensively proven in both *in vitro* and *in vivo* studies^[154]. Furthermore, research conducted in cell lines and observational clinical studies indicate that not only the type and the schemes of immunosuppressive treatments, but also the total immunosuppressive load, are likely determinants in promoting cancer recurrence^[154].

Among the very few studies designed to evaluate the impact of immunosuppressive treatment on HCC recurrence after LT, a high level of heterogeneity among patients and HCC selection criteria are present. Furthermore, many immunosuppressive schemes have adopted the simultaneous use of different classes of drugs, with potential pro- or anti-oncogenic effects that may not always be synergistic. Thus, the inherent limitations in the design of these studies make it very difficult to draw solid conclusions.

A recent literature review^[154] identified 21 studies, of which only one was prospective and randomized, while two were meta-analyses and evaluated the potential anticancer effect of sirolimus^[155]. Other studies evaluated the impact of the type and load of calcineurin inhibitors (CNIs), as well as the impact of corticosteroids or anti-thymoglobulin antibodies. By summarizing the results of these studies, which highlighted HCC recurrence rates ranging from 12% to 54%, two key messages may be reported: (1) an increased rate of HCC recurrence risk was associated with higher exposure to both CNIs (cyclosporine or tacrolimus); and (2) the use of a mammalian target of rapamycin (mTOR) inhibitors was associated with a lower risk of HCC recurrence^[156]. To confirm the potential beneficial role of the mTOR inhibitor sirolimus in decreasing the risk of HCC recurrence after LT, a prospective multicenter and randomized study was performed^[157]. The results of this study were disappointing since they only showed a lower recurrence rate 3 years after LT in patients with early HCC, though this advantage was lost at 5 years. Moreover, in patients with HCC within the Milan criteria, the rate of recurrence was not statistically different from that obtained by adopting immunosuppressive schemes without mTOR inhibitors^[154]. The main limitations of this study

were likely related to the high heterogeneity of the immunosuppressive drugs added to sirolimus and to having left the LT centers completely free to manage therapy with steroids.

Studies performed with the other mTOR inhibitor, everolimus^[158], suggested that patients treated with this inhibitor had significantly lower HCC recurrence rates when compared to those treated with sirolimus or with CNI. However, it is important to note that everolimus-treated recipients had a shorter follow-up period and were more frequently transplanted for HCC within the Milan criteria. Overall, based on the available data from retrospective studies, meta-analyses, and post-hoc assessments of randomized trials, it seems advisable to consider mTOR inhibition-based immunosuppression after transplantation for HCC, particularly in patients who exceed the Milan criteria; however, prospective data are required to verify this claim.

If immunosuppression load appears to serve a determinant role in cancer recurrence^[159,160], it seems justified to explore immunosuppressive protocols aimed at weaning exposure to immunosuppressive drugs. The liver is a solid organ that - more than other organs - is suitable for tolerating transplantation with total withdrawal of immunosuppression without developing rejection. This phenomenon is known as operational tolerance^[161]. A large European prospective study on immunosuppressive treatment withdrawal, which enrolled 102 adult LT recipients, showed that 40% of them reached the status of operational tolerance for at least 1 year^[162]. The clinical predictors for achieving operational tolerance were a minimal time of 3 years from LT to immunosuppression weaning, the absence of autoimmune liver disease, and recipient age > 60 years^[163]. Regarding the impact of operational tolerance on HCC recurrence, 17 patients transplanted for HCC were enrolled in a protocol of immunosuppression weaning in Italy^[163]. The results demonstrated that no HCC recurrence was detected in those patients achieving operational tolerance, while one patient who presented only transient tolerance experienced HCC recurrence after 3 years from LT and died within 4 years. Despite these promising results, the identification criteria for precisely selecting patients who may develop operational tolerance remain lacking. Furthermore, if the purpose of utilizing the development of operational tolerance is to reduce the risk of HCC recurrence, it would be essential to obtain the suspension of immunosuppression within the first 2 to 3 years after LT, since this is the time interval during which the recurrence of HCC is more likely^[117].

CONCLUSION

LT remains an effective treatment in patients with unresectable HCC within the Milan criteria or in those who can be downstaged to being within the Milan criteria; however, HCC recurs despite careful selection of recipients. The growing demand for LT for HCC is expected, and the probable modest increase in the availability of organs to allocate for this indication - in the face of a clear decrease in transplantation for hepatitis C virus-related liver disease - makes the intention to expand the Milan criteria justifiable. The key question will now be: how can we balance the expansion of the Milan criteria with the risk of an exponential increase in HCC recurrence after LT? From a mathematical perspective, squaring the circle is a geometry problem that consists of constructing a square with the same compass and ruler with the same area as a circle. The study of the relationship between circumference and diameter of the rim has accompanied the history of humanity since the invention of the wheel. In 212 B.C., Archimedes was the first to attempt a calculation based on geometry through the method of exhaustion, which consisted of attempting to trap the circumference between a registered and a circumscribed polygon. Using 96-sided polygons, he calculated the value of π at 3.14163. Trapping that number in a finite series of digits is a feat that almost all mathematicians do, and when the mathematician Johan Lambert showed that it is irrational in 1761, he took the real challenge to calculate the highest number of decimal places to make it as precise as possible. Lambert's proof marks a turning point since it shows, that our number cannot be trapped in the relationship between two numbers in practice (one in the numerator and one in the denominator); thus, it cannot be "rationalized".

From a clinical perspective, the search for the perfect solution between expanding the Milan criteria and maintaining a sufficiently low risk of tumor recurrence to allow a patient survival rate of at least 60% at 5 years represents a major challenge for the coming years. The models used to predict the risk of HCC recurrence based on the pre-transplant evaluation of all tumor characteristics (including its response to treatments) currently represent those that combine both rigorous aspects of patient selection and organ allocation for transplant. These two factors work synergistically to increase the benefit, effectiveness, and justice of transplantation for HCC. The evaluation of the results obtained by adopting these criteria on large patient series in different geographical areas will be crucial for defining their validity and applicability.

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Authors' contributions

Wrote the paper: Toniutto P

Selected bibliography and collaborate in writing the paper: Fornasiere E, Fumolo E, Bitetto D

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REFERENCES

1. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018;391:1301-14.
2. Simonetti RG, Camma C, Fiorello F, Politi F, D'Amico G, et al. Hepatocellular carcinoma. A worldwide problem and the major risk factors. *Dig Dis Sci* 1991;36:962-72.
3. Toniutto P, Zanetto A, Ferrarese A, Burra P. Current challenges and future directions for liver transplantation. *Liver Int* 2017;37:317-27.
4. Starzl TE, Marchioro TL, Vonkaulla KN, Hermann G, Brittain RS, et al. Homotransplantation of the Liver in Humans. *Surg Gynecol Obstet* 1963;117:659-76.
5. Starzl TE, Groth CG, Bretschneider L, Penn I, Fulginiti VA, et al. Orthotopic homotransplantation of the human liver. *Ann Surg* 1968;168:392-415.
6. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996;334:693-9.
7. Plessier A, Codes L, Consigny Y, Sommacale D, Dondero F, et al. Underestimation of the influence of satellite nodules as a risk factor for post-transplantation recurrence in patients with small hepatocellular carcinoma. *Liver Transpl* 2004;10:S86-90.
8. Escartin A, Sapisochin G, Bilbao I, Vilallonga R, Bueno J, et al. Recurrence of hepatocellular carcinoma after liver transplantation. *Transplant Proc* 2007;39:2308-10.
9. Valdivieso A, Bustamante J, Gastaca M, Uriarte JG, Ventoso A, et al. Management of hepatocellular carcinoma recurrence after liver transplantation. *Transplant Proc* 2010;42:660-2.
10. Roayaie S, Schwartz JD, Sung MW, Emre SH, Miller CM, et al. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl* 2004;10:534-40.
11. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, et al. Predicting survival after liver transplantation in patients with

- hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009;10:35-43.
12. Al-Ameri AAM, Wei X, Wen X, Wei Q, Guo H, et al. Systematic review: risk prediction models for recurrence of hepatocellular carcinoma after liver transplantation. *Transpl Int* 2020;33:697-712.
 13. Shah SA, Tan JC, McGilvray ID, Cattral MS, Levy GA, et al. Does microvascular invasion affect outcomes after liver transplantation for HCC? A histopathological analysis of 155 consecutive explants. *J Gastrointest Surg* 2007;11:464-71.
 14. Zhang X, Li J, Shen F, Lau WY. Significance of presence of microvascular invasion in specimens obtained after surgical treatment of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2018;33:347-54.
 15. Jonas S, Bechstein WO, Steinmuller T, Herrmann M, Radke C, et al. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. *Hepatology* 2001;33:1080-6.
 16. Costentin CE, Bababekov YJ, Zhu AX, Yeh H. Is it time to reconsider the Milan Criteria for selecting patients with hepatocellular carcinoma for deceased-donor liver transplantation? *Hepatology* 2019;69:1324-36.
 17. Herrero JI, Sangro B, Quiroga J, Pardo F, Herraiz M, et al. Influence of tumor characteristics on the outcome of liver transplantation among patients with liver cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2001;7:631-6.
 18. Roayaie S, Frischer JS, Emre SH, Fishbein TM, Sheiner PA, et al. Long-term results with multimodal adjuvant therapy and liver transplantation for the treatment of hepatocellular carcinomas larger than 5 centimeters. *Ann Surg* 2002;235:533-9.
 19. Kneteman NM, Oberholzer J, Al Saghier M, Meeberg GA, Blitz M, et al. Sirolimus-based immunosuppression for liver transplantation in the presence of extended criteria for hepatocellular carcinoma. *Liver Transpl* 2004;10:1301-11.
 20. Silva M, Moya A, Berenguer M, Sanjuan F, Lopez-Andujar R, et al. Expanded criteria for liver transplantation in patients with cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2008;14:1449-60.
 21. Fan J, Zhou J, Xu Y, Qiu SJ, Wu ZQ, et al. Indication of liver transplantation for hepatocellular carcinoma: Shanghai Fudan Criteria. *Zhonghua Yi Xue Za Zhi* 2006;86:1227-31.
 22. Fan J, Yang GS, Fu ZR, Peng ZH, Xia Q, et al. Liver transplantation outcomes in 1,078 hepatocellular carcinoma patients: a multi-center experience in Shanghai, China. *J Cancer Res Clin Oncol* 2009;135:1403-12.
 23. Yao FY, Xiao L, Bass NM, Kerlan R, Ascher NL, et al. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. *Am J Transplant* 2007;7:2587-96.
 24. American College of Radiology. Liver Imaging Reporting and Data System (LI-RADS). Available from: <https://www.acr.org/Clinical-Resources/Reporting-and-Data-Systems/LI-RADS/CT-MRI-LI-RADS-v2017>. [Last accessed on 21 Jul 2020]
 25. Lee S, Kim SS, Roh YH, Choi JY, Park MS, et al. Diagnostic performance of CT/MRI Liver imaging reporting and data system v2017 for hepatocellular carcinoma: a systematic review and meta-analysis. *Liver Int* 2020;40:1488-97.
 26. Cillo U, Vitale A, Bassanello M, Boccagni P, Brolese A, et al. Liver transplantation for the treatment of moderately or well-differentiated hepatocellular carcinoma. *Ann Surg* 2004;239:150-9.
 27. Sotiropoulos GC, Malago M, Molmenti E, Paul A, Nadalin S, et al. Liver transplantation for hepatocellular carcinoma in cirrhosis: is clinical tumor classification before transplantation realistic? *Transplantation* 2005;79:483-7.
 28. Shah SA, Tan JC, McGilvray ID, Cattral MS, Cleary SP, et al. Accuracy of staging as a predictor for recurrence after liver transplantation for hepatocellular carcinoma. *Transplantation* 2006;81:1633-9.
 29. Cuccurullo V, Di Stasio GD, Mazzarella G, Cascini GL. Microvascular Invasion in HCC: the molecular imaging perspective. *Contrast Media Mol Imaging* 2018;2018:9487938.
 30. Yaprak O, Acar S, Ertugrul G, Dayangac M. Role of pre-transplant 18F-FDG PET/CT in predicting hepatocellular carcinoma recurrence after liver transplantation. *World J Gastrointest Oncol* 2018;10:336-43.
 31. Sweeney MJ, Ashmore J, Morris HP, Weber G. Comparative biochemistry hepatomas. Iv. isotope studies of glucose and fructose metabolism in liver tumors of different growth rates. *Cancer Res* 1963;23:995-1002.
 32. Torizuka T, Tamaki N, Inokuma T, Magata Y, Sasayama S, et al. In vivo assessment of glucose metabolism in hepatocellular carcinoma with FDG-PET. *J Nucl Med* 1995;36:1811-7.
 33. Yang SH, Suh KS, Lee HW, Cho EH, Cho JY, et al. The role of (18)F-FDG-PET imaging for the selection of liver transplantation candidates among hepatocellular carcinoma patients. *Liver Transpl* 2006;12:1655-60.
 34. Lee SD, Kim SH. Role of positron emission tomography/computed tomography in living donor liver transplantation for hepatocellular carcinoma. *Hepatobiliary Surg Nutr* 2016;5:408-14.
 35. Kornberg A, Kupper B, Tannapfel A, Buchler P, Krause B, et al. Patients with non-[18 F]fluorodeoxyglucose-avid advanced hepatocellular carcinoma on clinical staging may achieve long-term recurrence-free survival after liver transplantation. *Liver Transpl* 2012;18:53-61.
 36. Lin CY, Liao CW, Chu LY, Yen KY, Jeng LB, et al. Predictive value of 18F-FDG PET/CT for vascular invasion in patients with hepatocellular carcinoma before liver transplantation. *Clin Nucl Med* 2017;42:e183-e87.
 37. Bailly M, Venel Y, Orain I, Salame E, Ribeiro MJ. 18F-FDG PET in liver transplantation setting of hepatocellular carcinoma: predicting histology? *Clin Nucl Med* 2016;41:e126-9.
 38. Ahn SY, Lee JM, Joo I, Lee ES, Lee SJ, et al. Prediction of microvascular invasion of hepatocellular carcinoma using gadoteric acid-enhanced MR and (18)F-FDG PET/CT. *Abdom Imaging* 2015;40:843-51.
 39. Takada Y, Kaido T, Shirabe K, Nagano H, Egawa H, et al. Significance of preoperative fluorodeoxyglucose-positron emission tomography in prediction of tumor recurrence after liver transplantation for hepatocellular carcinoma patients: a Japanese multicenter study. *J Hepatobiliary Pancreat Sci* 2017;24:49-57.
 40. Lee SD, Kim SH, Kim SK, Kim YK, Park SJ. Clinical Impact of 18F-Fluorodeoxyglucose positron emission tomography/computed tomography in living donor liver transplantation for advanced hepatocellular carcinoma. *Transplantation* 2015;99:2142-9.

41. Citores MJ, Lucena JL, de la Fuente S, Cuervas-Mons V. Serum biomarkers and risk of hepatocellular carcinoma recurrence after liver transplantation. *World J Hepatol* 2019;11:50-64.
42. Pommergaard HC, Burcharth J, Rosenberg J, Rasmussen A. Serologic and molecular biomarkers for recurrence of hepatocellular carcinoma after liver transplantation: a systematic review and meta-analysis. *Transplant Rev (Orlando)* 2016;30:171-7.
43. Duvoux C, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, et al. Liver transplantation for hepatocellular carcinoma: a model including alpha-fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012;143:986-94 e3; quiz e14-5.
44. Notarpaolo A, Layese R, Magistri P, Gambato M, Colledan M, et al. Validation of the AFP model as a predictor of HCC recurrence in patients with viral hepatitis-related cirrhosis who had received a liver transplant for HCC. *J Hepatol* 2017;66:552-9.
45. Hakeem AR, Young RS, Marangoni G, Lodge JP, Prasad KR. Systematic review: the prognostic role of alpha-fetoprotein following liver transplantation for hepatocellular carcinoma. *Aliment Pharmacol Ther* 2012;35:987-99.
46. Vibert E, Azoulay D, Hoti E, Iacopinelli S, Samuel D, et al. Progression of alpha-fetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. *Am J Transplant* 2010;10:129-37.
47. Lai Q, Avolio AW, Graziadei I, Otto G, Rossi M, et al. Alpha-fetoprotein and modified response evaluation criteria in solid tumors progression after locoregional therapy as predictors of hepatocellular cancer recurrence and death after transplantation. *Liver Transpl* 2013;19:1108-18.
48. Han K, Tzimas GN, Barkun JS, Metrakos P, Tchervenkov JL, et al. Preoperative alpha-fetoprotein slope is predictive of hepatocellular carcinoma recurrence after liver transplantation. *Can J Gastroenterol* 2007;21:39-45.
49. Dumitra TC, Dumitra S, Metrakos PP, Barkun JS, Chaudhury P, et al. Pretransplantation alpha-fetoprotein slope and milan criteria: strong predictors of hepatocellular carcinoma recurrence after transplantation. *Transplantation* 2013;95:228-33.
50. Burra P, Giannini EG, Caraceni P, Ginanni Corradini S, Rendina M, et al. Specific issues concerning the management of patients on the waiting list and after liver transplantation. *Liver Int* 2018;38:1338-62.
51. DuBay D, Sandroussi C, Sandhu L, Cleary S, Guba M, et al. Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. *Ann Surg* 2011;253:166-72.
52. Sapisochin G, Goldaracena N, Laurence JM, Dib M, Barbas A, et al. The extended Toronto criteria for liver transplantation in patients with hepatocellular carcinoma: a prospective validation study. *Hepatology* 2016;64:2077-88.
53. Zheng SS, Xu X, Wu J, Chen J, Wang WL, et al. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 2008;85:1726-32.
54. Toso C, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. *Hepatology* 2009;49:832-8.
55. Toso C, Meeberg G, Hernandez-Alejandro R, Dufour JF, Marotta P, et al. Total tumor volume and alpha-fetoprotein for selection of transplant candidates with hepatocellular carcinoma: a prospective validation. *Hepatology* 2015;62:158-65.
56. Varona MA, Soriano A, Aguirre-Jaime A, Garrido S, Oton E, et al. Risk factors of hepatocellular carcinoma recurrence after liver transplantation: accuracy of the alpha-fetoprotein model in a single-center experience. *Transplant Proc* 2015;47:84-9.
57. Pinero F, Tisi Bana M, de Ataide EC, Hoyos Duque S, Marciano S, et al. Liver transplantation for hepatocellular carcinoma: evaluation of the alpha-fetoprotein model in a multicenter cohort from Latin America. *Liver Int* 2016;36:1657-67.
58. Rhu J, Kim JM, Choi GS, Kwon CHD, Joh JW. Validation of the alpha-fetoprotein model for hepatocellular carcinoma recurrence after transplantation in an Asian population. *Transplantation* 2018;102:1316-22.
59. Mazzaferro V, Sposito C, Zhou J, Pinna AD, De Carlis L, et al. Metroticket 2.0 Model for analysis of competing risks of death after liver transplantation for hepatocellular carcinoma. *Gastroenterology* 2018;154:128-39.
60. Fujiyama S, Morishita T, Hashiguchi O, Sato T. Plasma abnormal prothrombin (des-gamma-carboxy prothrombin) as a marker of hepatocellular carcinoma. *Cancer* 1988;61:1621-8.
61. Shirabe K, Itoh S, Yoshizumi T, Soejima Y, Taketomi A, et al. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma-with special reference to the serum levels of des-gamma-carboxy prothrombin. *J Surg Oncol* 2007;95:235-40.
62. Pote N, Cauchy F, Albuquerque M, Voitot H, Belghiti J, et al. Performance of PIVKA-II for early hepatocellular carcinoma diagnosis and prediction of microvascular invasion. *J Hepatol* 2015;62:848-54.
63. Okuda H, Nakanishi T, Takatsu K, Saito A, Hayashi N, et al. Comparison of clinicopathological features of patients with hepatocellular carcinoma seropositive for alpha-fetoprotein alone and those seropositive for des-gamma-carboxy prothrombin alone. *J Gastroenterol Hepatol* 2001;16:1290-6.
64. Hong YM, Cho M, Yoon KT, Chu CW, Yang KH, et al. Risk factors of early recurrence after curative hepatectomy in hepatocellular carcinoma. *Tumour Biol* 2017;39:1010428317720863.
65. Taketomi A, Sanefuji K, Soejima Y, Yoshizumi T, Uchiyama H, et al. Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. *Transplantation* 2009;87:531-7.
66. Takada Y, Ito T, Ueda M, Sakamoto S, Haga H, et al. Living donor liver transplantation for patients with HCC exceeding the Milan criteria: a proposal of expanded criteria. *Dig Dis* 2007;25:299-302.
67. Fujiki M, Takada Y, Ogura Y, Oike F, Kaido T, et al. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. *Am J Transplant* 2009;9:2362-71.
68. Kaido T, Ogawa K, Mori A, Fujimoto Y, Ito T, et al. Usefulness of the Kyoto criteria as expanded selection criteria for liver transplantation for hepatocellular carcinoma. *Surgery* 2013;154:1053-60.
69. Soejima Y, Taketomi A, Yoshizumi T, Uchiyama H, Aishima S, et al. Extended indication for living donor liver transplantation in patients

- with hepatocellular carcinoma. *Transplantation* 2007;83:893-9.
70. Shirabe K, Taketomi A, Morita K, Soejima Y, Uchiyama H, et al. Comparative evaluation of expanded criteria for patients with hepatocellular carcinoma beyond the Milan criteria undergoing living-related donor liver transplantation. *Clin Transplant* 2011;25:E491-8.
 71. Lee JH, Cho Y, Kim HY, Cho EJ, Lee DH, et al. Serum tumor markers provide refined prognostication in selecting liver transplantation candidate for hepatocellular carcinoma patients beyond the Milan criteria. *Ann Surg* 2016;263:842-50.
 72. Chaiteerakij R, Zhang X, Addissie BD, Mohamed EA, Harmsen WS, et al. Combinations of biomarkers and Milan criteria for predicting hepatocellular carcinoma recurrence after liver transplantation. *Liver Transpl* 2015;21:599-606.
 73. Zheng Z, Zhou L, Gao S, Yang Z, Yao J, et al. Prognostic role of C-reactive protein in hepatocellular carcinoma: a systematic review and meta-analysis. *Int J Med Sci* 2013;10:653-64.
 74. An HJ, Jang JW, Bae SH, Choi JY, Yoon SK, et al. Serum C-reactive protein is a useful biomarker for predicting outcomes after liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl* 2012;18:1406-14.
 75. Kim YK, Kim SH, Lee SD, Hong SK, Park SJ. Pretransplant serum levels of C-reactive protein predict prognoses in patients undergoing liver transplantation for hepatocellular carcinoma. *Transplant Proc* 2015;47:686-93.
 76. Halazun KJ, Hardy MA, Rana AA, Woodland DC, Luyten EJ, et al. Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma. *Ann Surg* 2009;250:141-51.
 77. Najjar M, Agrawal S, Emond JC, Halazun KJ. Pretreatment neutrophil-lymphocyte ratio: useful prognostic biomarker in hepatocellular carcinoma. *J Hepatocell Carcinoma* 2018;5:17-28.
 78. Xu ZG, Ye CJ, Liu LX, Wu G, Zhao ZX, et al. The pretransplant neutrophil-lymphocyte ratio as a new prognostic predictor after liver transplantation for hepatocellular cancer: a systematic review and meta-analysis. *Biomark Med* 2018;12:189-99.
 79. Lai Q, Melandro F, Larghi Laureiro Z, Giovanardi F, Ginanni Corradini S, et al. Platelet-to-lymphocyte ratio in the setting of liver transplantation for hepatocellular cancer: a systematic review and meta-analysis. *World J Gastroenterol* 2018;24:1658-65.
 80. Han S, Lee S, Yang JD, Leise MD, Ahn JH, et al. Risk of posttransplant hepatocellular carcinoma recurrence is greater in recipients with higher platelet counts in living donor liver transplantation. *Liver Transpl* 2018;24:44-55.
 81. Kusumanto YH, Dam WA, Hospers GA, Meijer C, Mulder NH. Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. *Angiogenesis* 2003;6:283-7.
 82. Bambace NM, Holmes CE. The platelet contribution to cancer progression. *J Thromb Haemost* 2011;9:237-49.
 83. Gay LJ, Felding-Habermann B. Contribution of platelets to tumour metastasis. *Nat Rev Cancer* 2011;11:123-34.
 84. Bihari C, Rastogi A, Shasthry SM, Bajpai M, Bhadoria AS, et al. Platelets contribute to growth and metastasis in hepatocellular carcinoma. *APMIS* 2016;124:776-86.
 85. von Felden J, Villanueva A. Role of molecular biomarkers in liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2020;26:823-31.
 86. Nishida N, Nishimura T, Kaido T, Minaga K, Yamao K, et al. Molecular scoring of hepatocellular carcinoma for predicting metastatic recurrence and requirements of systemic chemotherapy. *Cancers (Basel)* 2018;10.
 87. Miltiados O, Sia D, Hoshida Y, Fiel MI, Harrington AN, et al. Progenitor cell markers predict outcome of patients with hepatocellular carcinoma beyond Milan criteria undergoing liver transplantation. *J Hepatol* 2015;63:1368-77.
 88. Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009;69:7385-92.
 89. Villanueva A, Hoshida Y, Battiston C, Tovar V, Sia D, et al. Combining clinical, pathology, and gene expression data to predict recurrence of hepatocellular carcinoma. *Gastroenterology* 2011;140:1501-12.e2.
 90. Dvorchik I, Schwartz M, Fiel MI, Finkelstein SD, Marsh JW. Fractional allelic imbalance could allow for the development of an equitable transplant selection policy for patients with hepatocellular carcinoma. *Liver Transpl* 2008;14:443-50.
 91. Pagano D, Barbera F, Conaldi PG, Seidita A, Di Francesco F, et al. Role of allelic imbalance in predicting hepatocellular carcinoma (HCC) recurrence risk after liver transplant. *Ann Transplant* 2019;24:223-33.
 92. Yang Z, Zhou L, Wu LM, Xie HY, Zhang F, et al. Combination of polymorphisms within the HDAC1 and HDAC3 gene predict tumor recurrence in hepatocellular carcinoma patients that have undergone transplant therapy. *Clin Chem Lab Med* 2010;48:1785-91.
 93. Wu LM, Yang Z, Zhou L, Zhang F, Xie HY, et al. Identification of histone deacetylase 3 as a biomarker for tumor recurrence following liver transplantation in HBV-associated hepatocellular carcinoma. *PLoS One* 2010;5:e14460.
 94. Zhang Q, Chen X, Zhou J, Zhang L, Zhao Q, et al. CD147, MMP-2, MMP-9 and MVD-CD34 are significant predictors of recurrence after liver transplantation in hepatocellular carcinoma patients. *Cancer Biol Ther* 2006;5:808-14.
 95. Liese J, Peveling-Oberhag J, Doering C, Schnitzbauer AA, Herrmann E, et al. A possible role of microRNAs as predictive markers for the recurrence of hepatocellular carcinoma after liver transplantation. *Transpl Int* 2016;29:369-80.
 96. Nakano T, Chen IH, Wang CC, Chen PJ, Tseng HP, et al. Circulating exosomal miR-92b: Its role for cancer immunoediting and clinical value for prediction of posttransplant hepatocellular carcinoma recurrence. *Am J Transplant* 2019;19:3250-62.
 97. Sanchez-Lorencio MI, Ramirez P, Saenz L, Martinez Sanchez MV, De La Orden V, et al. Comparison of Two types of liquid biopsies in patients with hepatocellular carcinoma awaiting orthotopic liver transplantation. *Transplant Proc* 2015;47:2639-42.
 98. Pavel MC, Fuster J. Expansion of the hepatocellular carcinoma Milan criteria in liver transplantation: future directions. *World J Gastroenterol* 2018;24:3626-36.
 99. Cillo U, Burra P, Mazzaferro V, Belli L, Pinna AD, et al. A multistep, consensus-based approach to organ allocation in liver transplantation: toward a "Blended Principle Model". *Am J Transplant* 2015;15:2552-61.
 100. Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, et al. Recommendations for liver transplantation for hepatocellular carcinoma:

- an international consensus conference report. *Lancet Oncol* 2012;13:e11-22.
101. Yao FY, Mehta N, Flemming J, Dodge J, Hameed B, et al. Downstaging of hepatocellular cancer before liver transplant: long-term outcome compared to tumors within Milan criteria. *Hepatology* 2015;61:1968-77.
 102. Mehta N, Dodge JL, Grab JD, Yao FY. National experience on down-staging of hepatocellular carcinoma before liver transplant: influence of tumor burden, alpha-fetoprotein, and Wait Time. *Hepatology* 2020;71:943-54.
 103. Yao FY, Fidelman N. Reassessing the boundaries of liver transplantation for hepatocellular carcinoma: where do we stand with tumor down-staging? *Hepatology* 2016;63:1014-25.
 104. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358-80.
 105. Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. *Gastroenterology* 2016;150:835-53.
 106. European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236.
 107. Bryce K, Tsochatzis EA. Downstaging for hepatocellular cancer: harm or benefit? *Transl Gastroenterol Hepatol* 2017;2:106.
 108. Mehta N, Bhangui P, Yao FY, Mazzaferro V, Toso C, et al. Liver transplantation for hepatocellular carcinoma. Working Group Report from the ILTS Transplant Oncology Consensus Conference. *Transplantation* 2020;104:1136-42.
 109. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010;30:52-60.
 110. Cucchetti A, Serenari M, Sposito C, Di Sandro S, Mosconi C, et al. Including mRECIST in the Metroticket 2.0 criteria improves prediction of hepatocellular carcinoma-related death after liver transplant. *J Hepatol* 2020;73:342-8.
 111. O'Malley ME, Takayama Y, Sherman M. Outcome of small (10-20 mm) arterial phase-enhancing nodules seen on triphasic liver CT in patients with cirrhosis or chronic liver disease. *Am J Gastroenterol* 2005;100:1523-8.
 112. Grasso A, Stigliano R, Morisco F, Martinez H, Quaglia A, et al. Liver transplantation and recurrent hepatocellular carcinoma: predictive value of nodule size in a retrospective and explant study. *Transplantation* 2006;81:1532-41.
 113. Onaca N, Davis GL, Jennings LW, Goldstein RM, Klintmalm GB. Improved results of transplantation for hepatocellular carcinoma: a report from the international registry of hepatic tumors in liver transplantation. *Liver Transpl* 2009;15:574-80.
 114. Decaens T, Roudot-Thoraval F, Badran H, Wolf P, Durand F, et al. Impact of tumour differentiation to select patients before liver transplantation for hepatocellular carcinoma. *Liver Int* 2011;31:792-801.
 115. Halazun KJ, Najjar M, Abdelmessih RM, Samstein B, Griesemer AD, et al. Recurrence after liver transplantation for hepatocellular carcinoma: a new MORAL to the story. *Ann Surg* 2017;265:557-64.
 116. Mehta N, Heimbach J, Harnois DM, Sapisochin G, Dodge JL, et al. Validation of a risk estimation of tumor recurrence after transplant (RETREAT) score for hepatocellular carcinoma recurrence after liver transplant. *JAMA Oncol* 2017;3:493-500.
 117. Verna EC, Patel YA, Aggarwal A, Desai AP, Frenette C, et al. Liver transplantation for hepatocellular carcinoma: Management after the transplant. *Am J Transplant* 2020;20:333-47.
 118. Goldaracena N, Mehta N, Scalera I, Sposito C, Atenafu EG, et al. Multicenter validation of a score to predict prognosis after the development of HCC recurrence following liver transplantation. *HPB (Oxford)* 2019;21:731-8.
 119. Sapisochin G, Goldaracena N, Astete S, Laurence JM, Davidson D, et al. Benefit of treating hepatocellular carcinoma recurrence after liver transplantation and analysis of prognostic factors for survival in a large euro-american series. *Ann Surg Oncol* 2015;22:2286-94.
 120. Lo CM, Fan ST, Liu CL, Chan SC, Ng IO, et al. Living donor versus deceased donor liver transplantation for early irresectable hepatocellular carcinoma. *Br J Surg* 2007;94:78-86.
 121. Vakili K, Pomposelli JJ, Cheah YL, Akoad M, Lewis WD, et al. Living donor liver transplantation for hepatocellular carcinoma: Increased recurrence but improved survival. *Liver Transpl* 2009;15:1861-6.
 122. Bhangui P, Vibert E, Majno P, Salloum C, Andreani P, et al. Intention-to-treat analysis of liver transplantation for hepatocellular carcinoma: living versus deceased donor transplantation. *Hepatology* 2011;53:1570-9.
 123. Sandhu L, Sandroussi C, Guba M, Selzner M, Ghanekar A, et al. Living donor liver transplantation versus deceased donor liver transplantation for hepatocellular carcinoma: comparable survival and recurrence. *Liver Transpl* 2012;18:315-22.
 124. Xiao GQ, Song JL, Shen S, Yang JY, Yan LN. Living donor liver transplantation does not increase tumor recurrence of hepatocellular carcinoma compared to deceased donor transplantation. *World J Gastroenterol* 2014;20:10953-9.
 125. Park MS, Lee KW, Suh SW, You T, Choi Y, et al. Living-donor liver transplantation associated with higher incidence of hepatocellular carcinoma recurrence than deceased-donor liver transplantation. *Transplantation* 2014;97:71-7.
 126. Gu XQ, Zheng WP, Teng DH, Sun JS, Zheng H. Impact of non-oncological factors on tumor recurrence after liver transplantation in hepatocellular carcinoma patients. *World J Gastroenterol* 2016;22:2749-59.
 127. Yang ZF, Poon RT, Luo Y, Cheung CK, Ho DW, et al. Up-regulation of vascular endothelial growth factor (VEGF) in small-for-size liver grafts enhances macrophage activities through VEGF receptor 2-dependent pathway. *J Immunol* 2004;173:2507-15.
 128. Man K, Lo CM, Xiao JW, Ng KT, Sun BS, et al. The significance of acute phase small-for-size graft injury on tumor growth and invasiveness after liver transplantation. *Ann Surg* 2008;247:1049-57.
 129. Shi JH, Huitfeldt HS, Suo ZH, Line PD. Growth of hepatocellular carcinoma in the regenerating liver. *Liver Transpl* 2011;17:866-74.
 130. Kulik L, Abecassis M. Living donor liver transplantation for hepatocellular carcinoma. *Gastroenterology* 2004;127:S277-82.
 131. Fisher RA, Kulik LM, Freise CE, Lok AS, Shearon TH, et al. Hepatocellular carcinoma recurrence and death following living and deceased donor liver transplantation. *Am J Transplant* 2007;7:1601-8.
 132. Grat M, Kornasiewicz O, Lewandowski Z, Skalski M, Zieniewicz K, et al. The impact of surgical technique on the results of liver

- transplantation in patients with hepatocellular carcinoma. *Ann Transplant* 2013;18:448-59.
133. Mangus RS, Fridell JA, Vianna RM, Cooper AB, Jones DT, et al. Use of the piggyback hepatectomy technique in liver transplant recipients with hepatocellular carcinoma. *Transplantation* 2008;85:1496-9.
134. Khan S, Silva MA, Tan YM, John A, Gunson B, et al. Conventional versus piggyback technique of caval implantation; without extra-corporeal veno-venous bypass. A comparative study. *Transpl Int* 2006;19:795-801.
135. Nagai S, Yoshida A, Facciuto M, Moonka D, Abouljoud MS, et al. Ischemia time impacts recurrence of hepatocellular carcinoma after liver transplantation. *Hepatology* 2015;61:895-904.
136. van der Bilt JD, Kranenburg O, Nijkamp MW, Smakman N, Veenendaal LM, et al. Ischemia/reperfusion accelerates the outgrowth of hepatic micrometastases in a highly standardized murine model. *Hepatology* 2005;42:165-75.
137. Man K, Ng KT, Lo CM, Ho JW, Sun BS, et al. Ischemia-reperfusion of small liver remnant promotes liver tumor growth and metastases--activation of cell invasion and migration pathways. *Liver Transpl* 2007;13:1669-77.
138. Ku Y, Kusunoki N, Shiotani M, Maeda I, Iwasaki T, et al. Stimulation of haematogenous liver metastases by ischaemia-reperfusion in rats. *Eur J Surg* 1999;165:801-7.
139. Gasbarrini A, Addolorato G, Di Campli C, Simoncini M, Montemagno S, et al. Gender affects reperfusion injury in rat liver. *Dig Dis Sci* 2001;46:1305-12.
140. Burra P, De Martin E, Gitto S, Villa E. Influence of age and gender before and after liver transplantation. *Liver Transpl* 2013;19:122-34.
141. Brahimi-Horn MC, Pouyssegur J. Harnessing the hypoxia-inducible factor in cancer and ischemic disease. *Biochem Pharmacol* 2007;73:450-7.
142. Axelson H, Fredlund E, Ovenberger M, Landberg G, Pahlman S. Hypoxia-induced dedifferentiation of tumor cells--a mechanism behind heterogeneity and aggressiveness of solid tumors. *Semin Cell Dev Biol* 2005;16:554-63.
143. Sharma P, Welch K, Hussain H, Pelletier SJ, Fontana RJ, et al. Incidence and risk factors of hepatocellular carcinoma recurrence after liver transplantation in the MELD era. *Dig Dis Sci* 2012;57:806-12.
144. Cameron AM, Ghobrial RM, Yersiz H, Farmer DG, Lipshutz GS, et al. Optimal utilization of donor grafts with extended criteria: a single-center experience in over 1000 liver transplants. *Ann Surg* 2006;243:748-53; discussion 53-5.
145. Segev DL, Maley WR, Simpkins CE, Locke JE, Nguyen GC, et al. Minimizing risk associated with elderly liver donors by matching to preferred recipients. *Hepatology* 2007;46:1907-18.
146. Tashiro H, Kuroda S, Mikuriya Y, Ohdan H. Ischemia-reperfusion injury in patients with fatty liver and the clinical impact of steatotic liver on hepatic surgery. *Surg Today* 2014;44:1611-25.
147. Jimenez-Castro MB, Casillas-Ramirez A, Mendes-Braz M, Massip-Salcedo M, Gracia-Sancho J, et al. Adiponectin and resistin protect steatotic livers undergoing transplantation. *J Hepatol* 2013;59:1208-14.
148. Mathur A, Franco ES, Leone JP, Osman-Mohamed H, Rojas H, et al. Obesity portends increased morbidity and earlier recurrence following liver transplantation for hepatocellular carcinoma. *HPB (Oxford)* 2013;15:504-10.
149. Saxena NK, Sharma D, Ding X, Lin S, Marra F, et al. Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells. *Cancer Res* 2007;67:2497-507.
150. Rega G, Kaun C, Demyanets S, Pfaffenberger S, Rychli K, et al. Vascular endothelial growth factor is induced by the inflammatory cytokines interleukin-6 and oncostatin m in human adipose tissue in vitro and in murine adipose tissue in vivo. *Arterioscler Thromb Vasc Biol* 2007;27:1587-95.
151. Siegel AB, Lim EA, Wang S, Brubaker W, Rodriguez RD, et al. Diabetes, body mass index, and outcomes in hepatocellular carcinoma patients undergoing liver transplantation. *Transplantation* 2012;94:539-43.
152. Martins PN, Pratschke J, Pascher A, Fritsche L, Frei U, et al. Age and immune response in organ transplantation. *Transplantation* 2005;79:127-32.
153. Watt KD. Keys to long-term care of the liver transplant recipient. *Nat Rev Gastroenterol Hepatol* 2015;12:639-48.
154. Lerut J, Iesari S, Foguene M, Lai Q. Hepatocellular cancer and recurrence after liver transplantation: what about the impact of immunosuppression? *Transl Gastroenterol Hepatol* 2017;2:80.
155. Schnitzbauer AA, Schlitt HJ, Geissler EK. Influence of immunosuppressive drugs on the recurrence of hepatocellular carcinoma after liver transplantation: a gap between basic science and clinical evidence. *Transplantation* 2011;91:1173-6.
156. Liang W, Wang D, Ling X, Kao AA, Kong Y, et al. Sirolimus-based immunosuppression in liver transplantation for hepatocellular carcinoma: a meta-analysis. *Liver Transpl* 2012;18:62-9.
157. Geissler EK, Schnitzbauer AA, Zulke C, Lamby PE, Proneth A, et al. Sirolimus use in liver transplant recipients with hepatocellular carcinoma: a randomized, multicenter, open-label phase 3 trial. *Transplantation* 2016;100:116-25.
158. Cholongitas E, Mamou C, Rodriguez-Castro KI, Burra P. Mammalian target of rapamycin inhibitors are associated with lower rates of hepatocellular carcinoma recurrence after liver transplantation: a systematic review. *Transpl Int* 2014;27:1039-49.
159. Vivarelli M, Cucchetti A, La Barba G, Ravaioli M, Del Gaudio M, et al. Liver transplantation for hepatocellular carcinoma under calcineurin inhibitors: reassessment of risk factors for tumor recurrence. *Ann Surg* 2008;248:857-62.
160. Rodriguez-Peralvarez M, Tsochatzis E, Naveas MC, Pieri G, Garcia-Caparras C, et al. Reduced exposure to calcineurin inhibitors early after liver transplantation prevents recurrence of hepatocellular carcinoma. *J Hepatol* 2013;59:1193-9.
161. Lerut J, Sanchez-Fueyo A. An appraisal of tolerance in liver transplantation. *Am J Transplant* 2006;6:1774-80.
162. Benitez C, Londono MC, Miquel R, Manzia TM, Abraldes JG, et al. Prospective multicenter clinical trial of immunosuppressive drug withdrawal in stable adult liver transplant recipients. *Hepatology* 2013;58:1824-35.
163. Angelico R, Parente A, Manzia TM. Using a weaning immunosuppression protocol in liver transplantation recipients with hepatocellular carcinoma: a compromise between the risk of recurrence and the risk of rejection? *Transl Gastroenterol Hepatol* 2017;2:74.

Review

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Liver imaging reporting and data system and CT/MRI diagnosis of hepatocellular carcinoma

Devaraju Kanmaniraja, Victoria Chernyak

Radiology, Montefiore Medical Center, The Bronx, New York, NY 10467, USA.

Correspondence to: Prof. Victoria Chernyak, Radiology, Montefiore Medical Center, The Bronx, 111 East 210th St Bronx, New York, NY 10467, USA. E-mail: vichka17@hotmail.com

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Abstract

The Liver Imaging Reporting and Data System (LI-RADS) is a comprehensive and robust system which provides an algorithmic approach to stratify the probability of hepatocellular carcinoma (HCC) for each observation found in patients at risk for HCC. LI-RADS uses a standardized terminology and approach to improve communication between the radiologist and clinicians. LI-RADS version 2018 is noteworthy for its adoption by the American Association for the Study of Liver Disease into its HCC practice guidance. This manuscript provides an overview of the history of LI-RADS, reviews the Computed tomography/magnetic resonance imaging diagnostic algorithm, highlights the key diagnostic criteria for each category, and discusses the advantage of incorporating LI-RADS in clinical practice.

Keywords: Liver Imaging Reporting and Data System, hepatocellular carcinoma, cirrhosis, hepatocellular carcinoma diagnosis

INTRODUCTION

Hepatocellular cancer (HCC) is the most common primary malignancy of the liver^[1]. It is the fifth most common cancer in the world and fourth leading cause of cancer mortality^[1,2]. The incidence of HCC in the United States has been rapidly rising over the last 20 years and predicted to increase until 2030^[3,4]. Imaging plays a critical role in the management of HCC, as the diagnosis of HCC is usually made non-invasively in the appropriate at-risk patient population, based on imaging features alone without the need of pathologic



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confirmation^[5]. When biopsy is required, it is often done with image guidance; furthermore, imaging plays an integral role in patients undergoing surveillance or following loco-regional therapy^[6]. The Liver Imaging Reporting and Data System (LI-RADS) is a comprehensive and dynamic system which provides a standardized means of assessing and communicating the entire spectrum of lesions and pseudolesions in studies performed in patients who are at risk for developing HCC^[7,8]. LI-RADS is developed by an international multidisciplinary consortium of radiologists, hepatologists, hepatobiliary surgeons, and hepatopathologists^[7,8].

OVERVIEW

Multiple societies and organizations have proposed image-based systems for the diagnosis of HCC. In 2008, the American College of Radiology proposed the LI-RADS with the primary goal of standardizing the lexicon, interpretation, and reporting of imaging studies specifically in patients who are at risk for developing HCC. The aim was to establish a comprehensive algorithm that would accurately stratify the probability of HCC and malignancy in each observation, while still maintaining a high specificity for HCC^[7]. The first version was released in 2011, followed by major updates in 2013, 2014, 2017, and 2018. With the most recent changes in 2018, LI-RADS was integrated into the American Association for the Study of Liver Disease (AASLD) practice guidance for HCC^[6].

Currently, LI-RADS has four individual algorithms that are used in different clinical contexts. Ultrasound LI-RADS is used for surveillance of at-risk patients. Contrast-enhanced ultrasound LI-RADS is used for the diagnosis of HCC. Computed tomography/magnetic resonance imaging (CT/MRI) LI-RADS algorithm is used for diagnosis and staging of HCC. Treatment response LI-RADS algorithm is used following local-regional therapy. This manuscript focuses on LI-RADS CT/MRI diagnostic algorithm.

CT/MRI DIAGNOSTIC ALGORITHM

LI-RADS CT/MRI Diagnostic Algorithm version 2018 (v2018) includes eight diagnostic categories which are applied to individual observations with increasing probability of HCC and malignancy with higher categories. The various categories do not have an exact correlation to the histologic categories but instead reflect the probability of individual observations being benign, HCC, or non-HCC malignancy. For instance, although all observations in LR-1 are benign and all observations in LR-5 are HCC, the opposite is not true, as many benign lesions may not be categorized LR-1 and not all HCC meet criteria for the LR-5 category^[9].

A multiphase contrast-enhanced study is required for appropriate assessment of liver observations. Multiphase studies include late arterial phase, portal venous phase, and delayed phase. Extracellular contrast agents are used for CT exams, while either an extracellular or hepatobiliary agent may be used for MRI^[7]. Extracellular MRI contrast agents provide lesion characteristics based primarily on blood flow, while hepatobiliary agents utilize information from both blood flow and the hepatocellular function^[6]. Although recent meta-analyses studies have shown multiphase contrast-enhanced MRI to have a higher sensitivity than CT for the diagnosis of HCC with similar specificity, there is currently insufficient evidence to recommend one modality or contrast agent over the other^[6,7,10]. Practitioners and institutions are encouraged to make decisions based on their best judgement, as well as to develop their own approach based on a multidisciplinary consensus that would be best suited both for the individual patient and the institution as a whole^[6]. In treatment-naïve patients, the unenhanced phase on CT is optional, while it is required in patients following loco-regional therapy^[7]. Late arterial phase (AP) imaging is strongly preferred over the early AP to improve detection of the arterial phase hyperenhancement, an imaging feature that is required for imaging diagnosis of HCC^[7].

LI-RADS CT/MRI diagnostic algorithm is equally applicable to CT and MRI. It is important to note, however, that the assigned categories may be discordant between the two modalities. Several studies have demonstrated that the LI-RADS categories are discordant between CT and MRI in about 35%-70% of cases^[9]. MRI categorizes more benign lesions as LR-1 compared to CT (25%-30% of those lesions were categorized as LR-3 on CT)^[11,12]. When excluding the LR-1 category, MRI-assigned categories are higher compared to the CT-assigned categories (12%-31% of LR-5 observations on MRI were categorized as LR-4, 12% as LR-3, and 15%-29% were not seen on CT)^[9,11,12]. The discrepancies in category assignment is likely multifactorial, and they are related to inherent differences between the modalities, inter-reader disagreements, and the fact that some of the imaging features are only assessable by MRI.

To maintain high specificity of the HCC diagnosis, the LI-RADS algorithm must be applied to a well-defined at-risk target population with a high pretest probability of HCC^[7,13]. It is essential that the patients meet the inclusion criteria for LI-RADS patient population, and have none of the exclusion criteria. The LI-RADS patient population includes patients with cirrhosis, chronic hepatitis B with or without cirrhosis, and a personal history of HCC^[7,13]. The exclusion criteria include patients < 18 years of age, those with vascular causes of cirrhosis, or those with congenital hepatic fibrosis^[8].

The LI-RADS CT/MRI algorithm is applied in a stepwise fashion beginning with LR-NC^[7]. This is followed by LR-TIV, LR-1, LR-2, and LR-M. Once the above categories are excluded, the CT/MRI diagnostic table is then used to assign an observation LR-3, LR-4, or LR-5 category. Observations with a pathological diagnosis are reported as such and not assigned a LI-RADS category to avoid confusion and uncertainty (i.e., pathology proven intrahepatic cholangiocarcinoma or pathology proven hemangioma). The exception to this is in pathological proven benign or premalignant hepatocellular lesions such as regenerative or dysplastic nodules, which are assigned LI-RADS categories^[9]. The rationale is that such lesions may evolve over time and progress to frank malignancy.

The CT/MRI diagnostic table uses a combination of the major features [Figure 1] to assign LR-3, LR-4, and LR-5 categories, with the option of applying ancillary features (AF) to adjust the final category. The LI-RADS category can be upgraded or downgraded by one, if there are more than one AF favoring malignancy or benignity, respectively^[14]. It is important to note that an LR-4 observation cannot be upgraded to LR-5 based on AF^[14]. The category is also not adjusted where there are ancillary features favoring both benignity and malignancy^[14].

CT/MRI diagnostic categories

LR-NC: not categorizable

Observations are included in this category when they cannot be meaningfully categorized because of image omission and/or degradation, preventing assessment of one or more of its major features resulting in the inability to differentiate categories in which cancer is unlikely (LR-1 or LR-2) from categories in which cancer is likely (LR-4, LR-5, and LR-M)^[7,15]. LR-NC should be applied only to observations that are specifically affected by the limitation and not applied to the entire liver^[15]. Management in this category includes a repeat diagnostic imaging usually within three months with the option of using an alternative modality or contrast agent to improve diagnostic quality^[16].

LR-TIV: definite tumor in vein

Observations in this category demonstrate definite enhancing soft tissue in vein, regardless of the presence of parenchymal mass [Figure 2]^[7,15]. Approximately 92% of observations are malignant^[17]. Approximately 80% of LR-TIV is attributed to HCC, and some intrahepatic cholangiocarcinoma (iCCA) and combined HCC-cholangiocarcinoma (cHCC-CCA) may also cause tumor in vein^[17]. When a LR-5 parenchymal observation is associated with TIV, it is reported as "LR-TIV, definitely due to HCC", and, in the absence of

CT/MRI Diagnostic Table

Arterial phase hyperenhancement (APHE)		No APHE		Nonrim APHE		
Observation size (mm)		< 20	≥ 20	< 10	10-19	≥ 20
Count additional major features: •Enhancing “capsule” •Nonperipheral “washout” •Threshold growth	None	LR-3	LR-3	LR-3	LR-3	LR-4
	One	LR-3	LR-4	LR-4	LR-4	LR-5
	≥ Two	LR-4	LR-4	LR-4	LR-5	LR-5



Observations in this cell are categorized based on one additional major feature:

- LR-4 – if enhancing “capsule”
- LR-5 – if nonperipheral “washout” **OR** threshold growth

If unsure about the presence of any major feature: characterize that feature as absent

Figure 1. Liver Imaging Reporting and Data System computed tomography/magnetic resonance imaging diagnostic table, used to assign LR-3, LR-4, and LR-5 categories. Reprinted with permission from Ref.^[8]

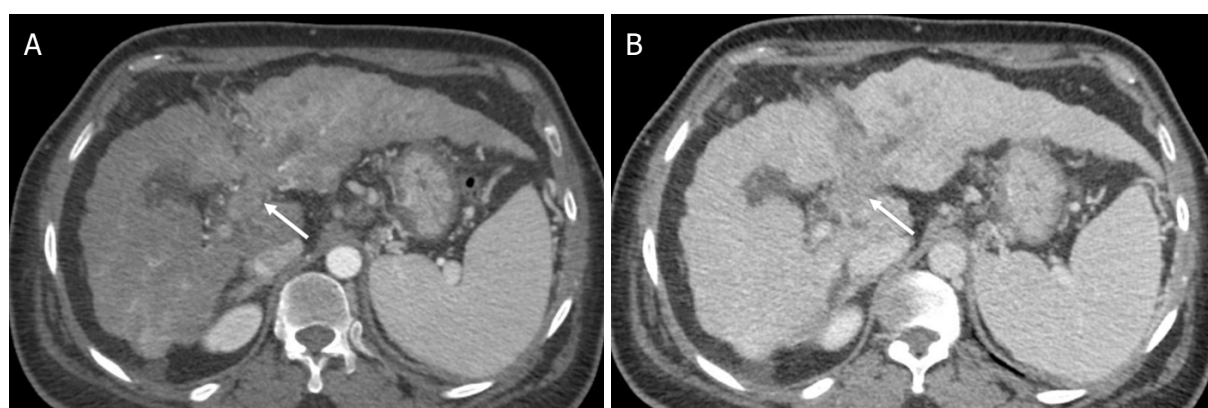


Figure 2. LR-TIV (Definite tumor in vein). Axial computed tomography in a 63-year-old woman with hepatitis C cirrhosis. Arterial phase (A); and portal venous phase (B) demonstrate definite enhancing soft tissue extending to the left portal vein (arrow). The observation is categorized LR-TIV. Note that the presence of parenchymal mass is not required for this category

a discernable parenchymal mass, it is reported as “LR-TIV, probably due to HCC”^[9]. Occasionally, TIV may be associated with a targetoid parenchymal mass, in which case the category is reported as “LR-TIV, may be due to non-HCC malignancy”^[9]. Management in this category requires multidisciplinary discussion and may require a biopsy if the tumor in vein is not definitely due to HCC^[16,18].

LR-1: definitely benign

Observations in this category have a 100% certainty of being benign [Figure 3]^[7,15]. Observations in this category are usually benign non-hepatocellular lesions or vascular pseudolesions and include cysts, hemangiomas, focal fatty deposition, or sparing and perfusion related changes^[9]. Benign lesions when evaluated by MRI are more often categorized as LR-1, compared to CT^[11,12]. Management of observations in this category involves routine surveillance in six months^[16,18].

LR-2: probably benign

Observations in this category have a high probability but lack 100% certainty of being benign [Figure 4]^[7,15]. A recent systematic review showed about 13% of observations in this category to be HCC and 14% of the

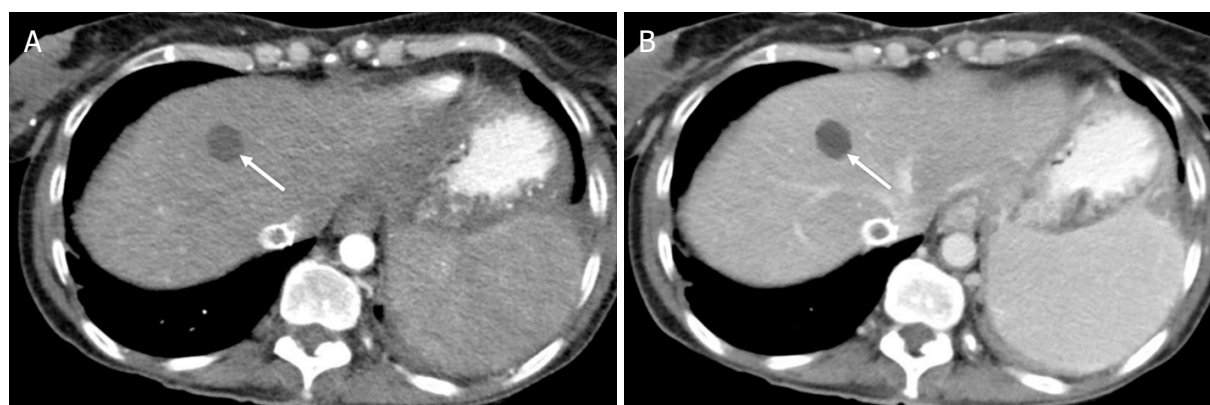


Figure 3. LR-1 (Definitely benign). Axial computed tomography in a 53-year-old woman with hepatitis C cirrhosis. Arterial phase (A); and portal venous phase (B) demonstrate a 17-mm well-defined round observation (arrow) with attenuation values of simple fluid, consistent with a definite simple cyst



Figure 4. LR-2 (Probably benign). Axial computed tomography in a 46-year-old woman with alcoholic cirrhosis. Arterial phase (A); portal venous phase (B); and delayed phases (C) demonstrate an 8-mm well-defined hypodense observation. The observation is too small to definitively characterize, but probably represents a small cyst

observations to be malignant^[17]. The majority of the observations in this category are the same as in LR-1 but display atypical imaging features, which result in less than 100% certainty of making the diagnosis. Additionally, distinctive nodules less than 20 mm without major imaging features of HCC, features of LR-M, and ancillary features favoring malignancy are categorized LR-2^[9]. LR-2 distinctive nodules include siderotic nodules, T1-hyperintense nodules, T2-hypointense nodules, and nodules hyperintense on hepatobiliary phase. Some LR-3 observation can be down-categorized to LR-2, if there are ancillary

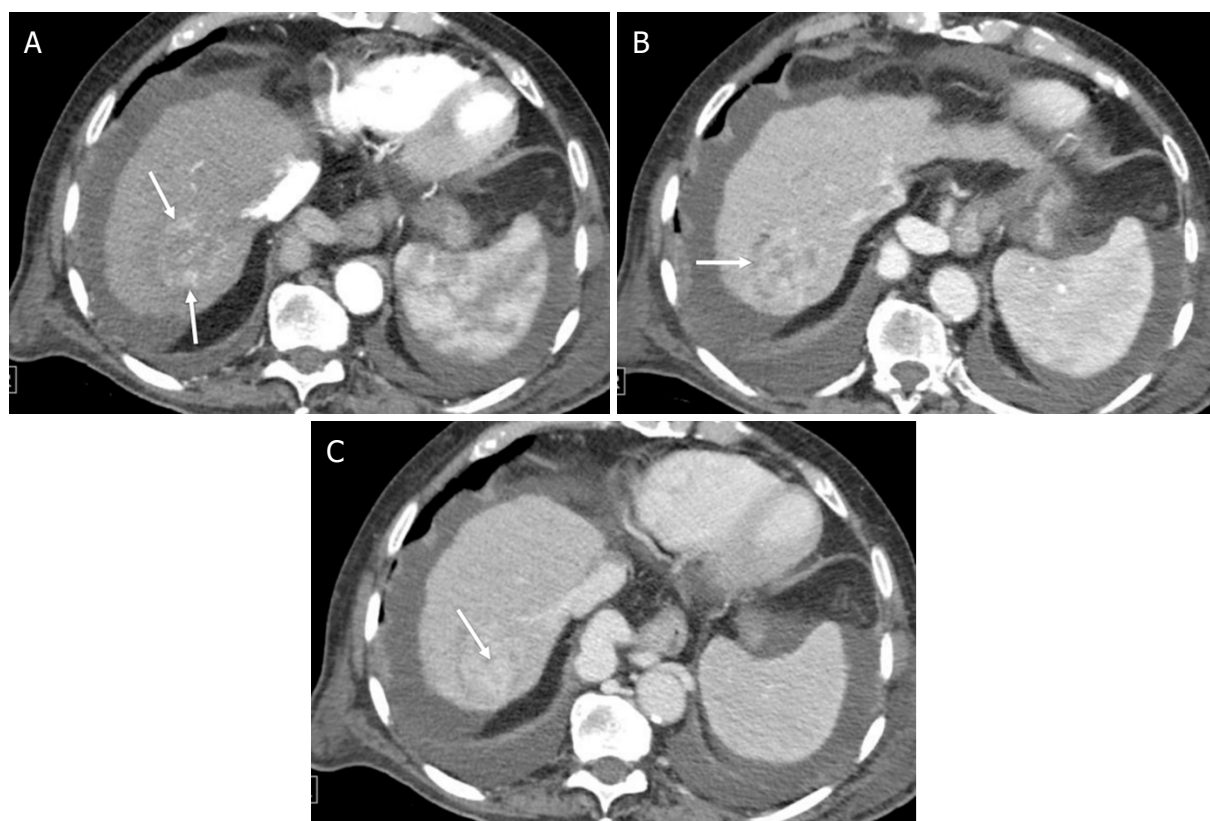


Figure 5. LR-M (Probably or definitely malignant, not hepatocellular carcinoma specific). Axial Computed Tomography in an 80-year-old man with hepatitis C cirrhosis. Arterial phase (A); portal venous phase (B); and delayed phases (C) demonstrate a 40-mm observation with rim arterial phase hyperenhancement [arrows (A)], peripheral washout appearance [arrow (B)], and delayed central enhancement [arrow (C)] observation. Subsequent biopsy confirmed an intrahepatic cholangiocarcinoma

features favoring benignity and no ancillary features of malignancy^[9]. Management in LR-2 category usually includes routine surveillance in six months; however, occasionally repeat diagnostic imaging with a different modality in six months and/or multimodality discussion may be warranted^[16,18].

LR-M: probably or definitely malignant, not HCC specific

Observations in this category have a high probability or 100% certainty of being malignant but the features are not specific for HCC [Figure 5]^[7,15]. Approximately 93% of observations in this group are malignant, with non-HCC malignancies such as iCCA, cHCC-CCA, and metastases comprising the majority of the LR-M category and HCC accounting for 36% of the observations in this group^[17]. On rare occasions, benign lesions such as sclerosed hemangiomas may have features that meet the criteria for LR-M. Both targetoid and non-targetoid lesions are included in this category. Targetoid lesions include observations with a targetoid morphology, such as targetoid dynamic enhancement pattern, targetoid diffusion restriction, and targetoid transitional or hepatobiliary phase hypointensity^[19]. Non-targetoid lesions in the LR-M group include observations that do not meet the criteria for LR-5 or LR-TIV and with at least one of the following features: infiltrative appearance, marked restricted diffusion, necrosis or severe ischemia, or other features suggesting non-HCC malignancy^[19]. Management requires multidisciplinary discussion and often a biopsy is needed for diagnosis, staging, and management^[16,18].

LR-3: intermediate probability of malignancy

Observations in this category include malignant and nonmalignant lesions, resulting in a moderate probability of being malignant [Figure 6]^[7,15]. Forty percent of observations in this category are malignant

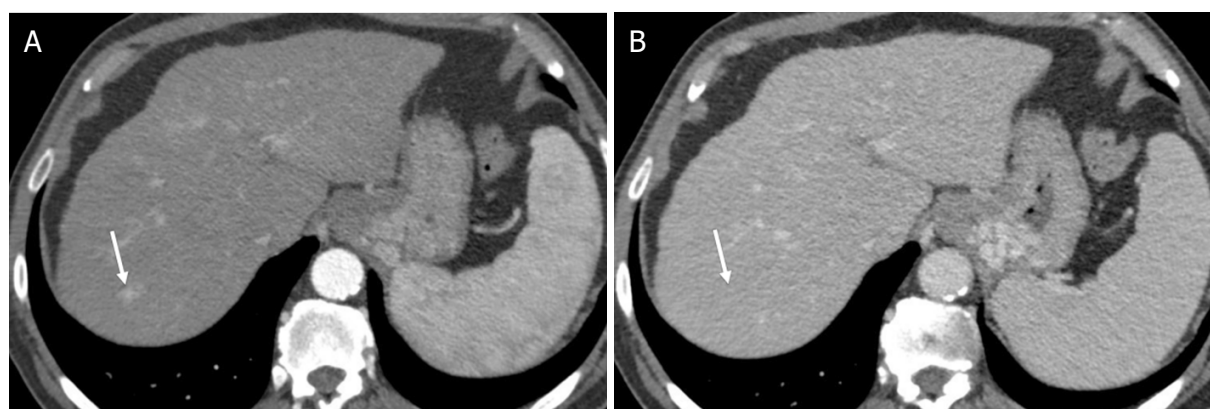


Figure 6. LR-3 (Intermediate probability hepatocellular carcinoma). Axial computed tomography in a 68-year-old man with hepatitis C cirrhosis. Arterial phase (A); and portal venous phase (B) demonstrate a 12-mm observation with nonrim arterial phase hyperenhancement [arrow (A)] and no washout appearance or enhancing capsule appearance [arrow (B)]

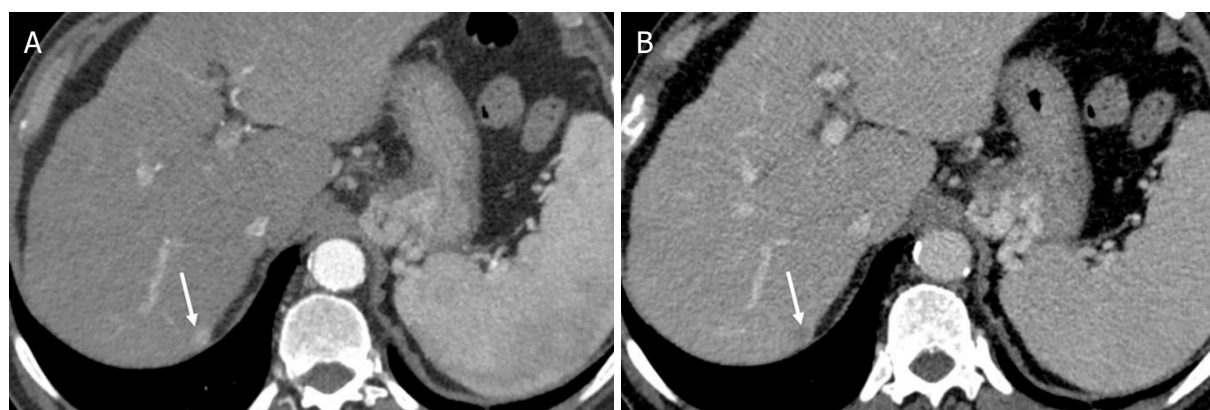


Figure 7. LR-4 (Probable hepatocellular carcinoma). Axial computed tomography in a 68-year-old man with hepatitis C cirrhosis. Arterial phase (A); and portal venous phase (B) demonstrate a 9-mm observation with nonrim arterial phase hyperenhancement [arrow (A)], nonperipheral washout appearance [arrow (B)], and no enhancing capsule appearance

with 38% being HCC^[17]. Observations < 20 mm demonstrating nonrim arterial phase hyperenhancement (APHE) alone are categorized in this category^[7,15]. Observations without APHE can also be categorized in this group if they are < 20 mm with ≤ 1 additional major feature, or if the observations are ≥ 20 mm with no additional major features^[7,15]. LR-4 observations can be down-categorized to LR-3 if there are ≥ 1 AF of benignity^[7,15]. Management options include repeat diagnostic imaging in 3-6 months with or without the use of an alternative modality or contrast agent and occasionally a multidisciplinary discussion may be warranted^[16,18].

LR-4: probably HCC

Observations in this category have a high probability but not 100% certainty of being HCC [Figure 7]^[7,15]. The probability of observations being HCC in this category approaches 75%-80%^[17,20]. Observations in this category include observations < 10 mm with nonrim APHE and ≥ 1 additional major feature, observations that are 10-19 mm with nonrim APHE and enhancing “capsule” as the only major feature, and observations ≥ 20 mm with nonrim APHE and no additional major feature. Observations without nonrim APHE can be categorized LR-4 with the size < 20 mm and ≥ 2 additional major features or with size ≥ 20 mm and ≥ 1 additional major feature^[7,15]. LR-3 observations with ≥ 1 AF favoring malignancy can be upgraded to LR-4, while LR-5 observations with ≥ 1 AF favoring benignity can be downgraded to LR-4^[9]. Management often

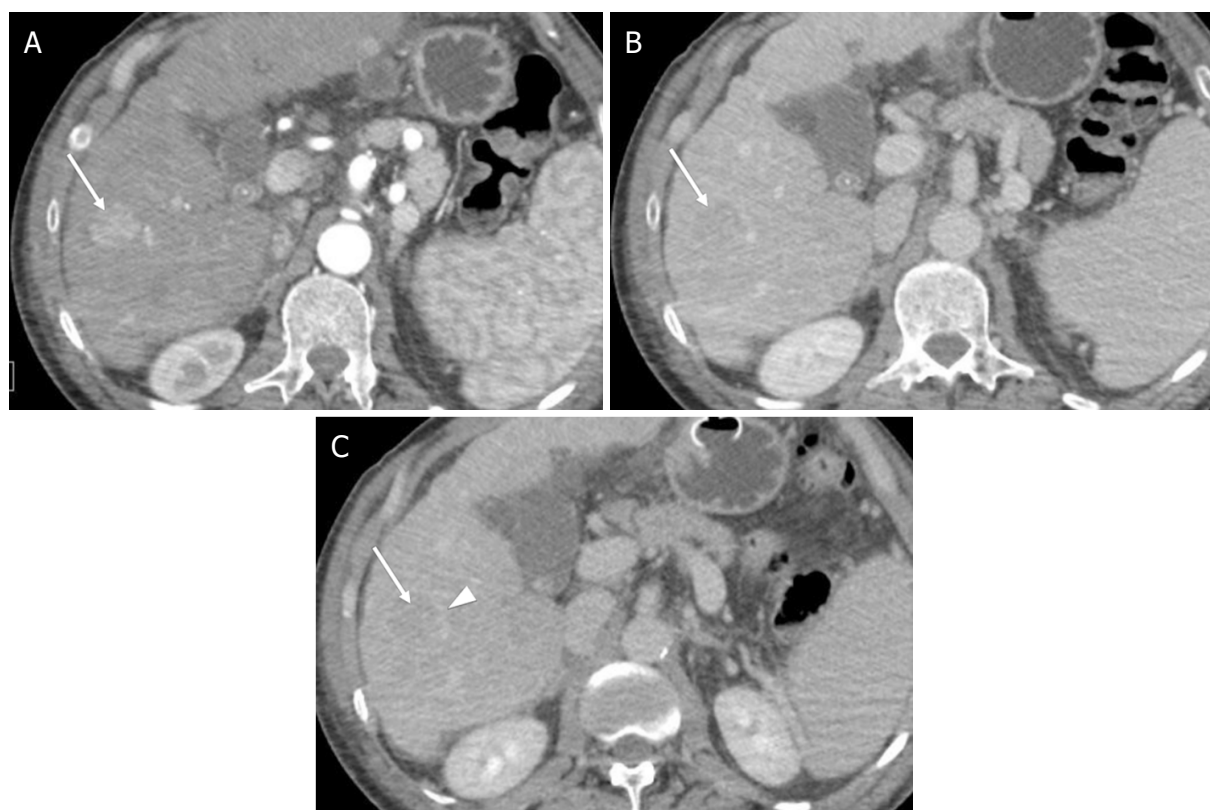


Figure 8. LR-5 (Definite hepatocellular carcinoma). Axial computed tomography in a 60-year-old man with hepatitis C cirrhosis. Arterial phase (A); portal venous phase (B); and delayed phases (C) demonstrate a 21-mm observation with nonrim arterial phase hyperenhancement [arrow (a)], nonperipheral washout appearance [arrow (B,C)], and enhancing capsule appearance [arrowhead (c)]

involves multidisciplinary discussion and may include repeat or alternate imaging within three months, biopsy, or, in some patients, definitive treatment without confirmatory biopsy^[16,18].

LR-5: definitely HCC

Observations in this category have 100% certainty of being HCC [Figure 8]^[7,15]. Ninety-four percent of observations in this category are HCC and 97% are malignant^[17]. Since HCC can be diagnosed based on imaging features alone and treated without the need for pathologic confirmation, observations in this category have specificity close to 100% with resulting modest sensitivity in the range of 50%-80%^[21-23]. Size ≥ 10 mm and nonrim APHE are absolute requirements to LR-5 categorization^[7,15]. Observations measuring > 20 mm with ≥ 1 additional major feature and observations measuring 10-19 mm with ≥ 2 additional major features are included in this category^[7,15]. Observations measuring 10-19 mm with either nonperipheral “washout” or threshold growth as the only additional major feature are also categorized LR-5.^[7,15] Ancillary features cannot be used to upgrade LR-4 observations to LR-5 in order to maintain the required high specificity in this category^[7,15]. Management includes multidisciplinary discussion for staging and treatment without the need for a biopsy^[16,18]. In some patients, a tissue sample may be required for histologic grading, molecular characterization, or enrollment in clinical trials^[16,18].

INTEGRATION INTO AASLD

LI-RADS has undergone several major updates since its initial release in 2011. The most recent update (LI-RADS v2018) has revised its criteria for LR-5 (definite HCC), and as a result LI-RADS has been integrated into the AASLD HCC practice guidelines and simplified^[6]. In v2018, 10-19-mm observations with nonrim APHE and nonperipheral “washout” are categorized LR-5, regardless of visualization on the

antecedent surveillance ultrasound^[7,8]. Additionally, v2018 updated the definition for threshold growth to be congruent with that of the Organ Procurement and Transplantation Network: threshold growth is defined as $\geq 50\%$ increase in size of a mass in ≤ 6 months^[8]. Any other size increase, including new observations and observations with $\geq 100\%$ increase in size on studies > 6 months apart, are characterized subthreshold growth^[8].

LI-RADS: ADVANTAGES FOR CLINICIANS

As the diagnosis of HCC is often made based on imaging findings alone in patients at risk without the need for a biopsy confirmation, it is imperative to maintain a high level of accuracy among radiologists in both the interpretation and the reporting of liver observations^[24-26]. LI-RADS uses a strict diagnostic criterion and an algorithmic approach to determine the likelihood of focal liver observations being HCC, while maintaining substantial inter-reader reliability and a high specificity for HCC^[17,27,28]. In fact, LI-RADS inter-reader agreement on MRI compares favorably to other commonly used RAD systems, such as breast and prostate^[29].

The use of non-standardized lexicon in liver imaging reports may result in confusion, especially when communicating results to the referring clinicians^[30]. The same phrase may be used differently by different radiologists and may be interpreted differently by the referring clinicians^[31]. Corwin *et al.*^[31] reported that the use of radiology phrases such as “consistent with HCC” and “suspicious for HCC” were associated with a high variability in the LI-RADS categories. Conversely, use of LI-RADS terminology and reporting results in a more comprehensive, clearer, and ultimately more actionable report^[32].

DISCUSSION

While LI-RADS offers numerous advantages for radiologists, clinicians, and patients, the system is not perfect. Unlike many other imaging-based systems for HCC diagnosis which have binary approach for diagnosis (i.e., HCC *vs.* all other lesions), LI-RADS utilizes ordinal categories which reflect increasing probability of HCC. Furthermore, LI-RADS is unique in that it recognizes the increased risk of non-HCC malignancies in patients with cirrhosis and provides diagnostic criteria for LR-M category. This level of detail requires greater complexity of the diagnostic criteria, and as a result LI-RADS may be intimidating for novice users or may be perceived as too cumbersome for clinical practice. However, a recent survey of radiologists and clinicians found that nearly 90% preferred the use of LI-RADS compared to other standardized reporting systems^[33].

LI-RADS has undergone several major updates since its original release in 2011. As scientific evidence accumulates, and user feedback is accrued, the criteria are refined and updated^[7]. The goal of each release is to improve accuracy and ease of use^[7]. The need for the system to be in congruence with the latest scientific knowledge is balanced against the need for a stable system^[7]. As a result, major updates to the system are planned for every 3-5 years.

CONCLUSION

This manuscript reviews CT/MRI LI-RADS. CT/MRI LI-RADS is a comprehensive and dynamic system with standardized terminology and an algorithmic diagnostic approach for the diagnosis and subsequent management of HCC, enabling clear communication between radiologist and clinicians. The most recent version has revised its criteria for LR-5 and simplified its definition for threshold growth, and it is now incorporated into the AASLD HCC practice guidelines.

DECLARATIONS

Authors' contributions

Made substantial contributions to drafting, editing and finalizing of the draft: Kanmaniraja D, Chernyak V

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Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Chernyak V is the consultant for Bayer; Kanmaniraja D declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. GLOBOCAN International Agency for Research on Cancer (IARC). Available from: <https://gco.iarc.fr/>. [Last accessed on 7 July 2020]
2. World Health Organization Mortality Database. WHO Statistical Information System. 2008. Available from: <https://www.who.int/news-room/fact-sheets/detail/cancer>. [Last accessed on 7 July 2020].
3. Petrick JL, Kelly SP, Altekruse SF, McGlynn KA, Rosenberg PS. Future of hepatocellular carcinoma incidence in the United States forecast through 2030. *J Clin Oncol* 2016;34:1787-94.
4. Petrick JL, Braunlin M, Laversanne M, Valery PC, Bray F, et al. International trends in liver cancer incidence, overall and by histologic subtype, 1978-2007. *Int J Cancer* 2016;139:1534-45.
5. Tang A, Bashir MR, Corwin MT, Cruite I, Dietrich CF, et al. Evidence supporting LI-RADS major features for CT- and MR imaging-based diagnosis of hepatocellular carcinoma: a systematic review. *Radiology* 2018;286:29-48.
6. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;68:723-50.
7. Chernyak V, Fowler KJ, Kamaya A, Kielar AZ, Elsayes KM, et al. Liver imaging reporting and data system (LI-RADS) Version 2018: imaging of hepatocellular carcinoma in at-risk patients. *Radiology* 2018;289:816-30.
8. The American College of Radiology LI-RADS v2018 Core. Available from: <https://www.acr.org/-/media/ACR/Files/RADS/LI-RADS/LI-RADS-2018-Core.pdf?la=en>. [Last accessed on 29 Jun 2020]
9. Chapter-8-LIRADS-Categories. ACR. Available from: <https://www.acr.org/-/media/ACR/Files/Clinical-Resources/LIRADS/Chapter-8-LIRADS-Categories.pdf?la=en>. [Last accessed on 29 Jun 2020]
10. Roberts LR, Sirlin CB, Zaiem F, Almasri J, Prokop LJ, et al. Imaging for the diagnosis of hepatocellular carcinoma: a systematic review and meta-analysis. *Hepatology* 2018;67:401-21.
11. Basha MAA, AlAzzazy MZ, Ahmed AF, Yousef HY, Shehata SM, et al. Does a combined CT and MRI protocol enhance the diagnostic efficacy of LI-RADS in the categorization of hepatic observations? A prospective comparative study. *Eur Radiol* 2018;28:2592-603.
12. Corwin MT, Fananapazir G, Jin M, Lamba R, Bashir MR. Differences in liver imaging and reporting data system categorization between MRI and CT. *AJR Am J Roentgenol* 2016;206:307-12.
13. Tang A, Hallouch O, Chernyak V, Kamaya A, Sirlin CB. Epidemiology of hepatocellular carcinoma: target population for surveillance and diagnosis. *Abdom Radiol (NY)* 2018;43:13-25.
14. Chernyak V, Tang A, Flusberg M, Papadatos D, Bijan B, et al. LI-RADS((R)) ancillary features on CT and MRI. *Abdom Radiol (NY)* 2018;43:82-100.
15. Santillan C, Chernyak V, Sirlin C. LI-RADS categories: concepts, definitions, and criteria. *Abdom Radiol (NY)* 2018;43:101-10.
16. Chapter-11-Management. ACR. Available from: <https://www.acr.org/-/media/ACR/Files/Clinical-Resources/LIRADS/Chapter-11-Management.pdf?la=en>. [Last accessed on 29 Jun 2020]
17. van der Pol CB, Lim CS, Sirlin CB, McGrath TA, Salameh JP, et al. Accuracy of the liver imaging reporting and data system in computed tomography and magnetic resonance image analysis of hepatocellular carcinoma or overall malignancy-a systematic review. *Gastroenterology* 2019;156:976-86.
18. Mitchell DG, Bruix J, Sherman M, Sirlin CB. LI-RADS (Liver Imaging Reporting and Data System): summary, discussion, and consensus of the LI-RADS Management Working Group and future directions. *Hepatology* 2015;61:1056-65.
19. Fowler KJ, Potretzke TA, Hope TA, Costa EA, Wilson SR. LI-RADS M (LR-M): definite or probable malignancy, not specific for hepatocellular carcinoma. *Abdom Radiol (NY)* 2018;43:149-57.

20. Choi SH, Byun JH, Kim SY, Lee SJ, Won HJ, et al. Liver Imaging Reporting and Data System v2014 with gadoxetate disodium-enhanced magnetic resonance imaging: validation of LI-RADS Category 4 and 5 Criteria. *Invest Radiol* 2016;51:483-90.
21. Chernyak V, Flusberg M, Berman J, Fruitman KC, Kobi M, et al. Liver Imaging Reporting and Data System (LI-RADS) v2018: impact on categorization and hepatocellular carcinoma staging. *Liver Transpl* 2019;25:1488-502.
22. Lee SM, Lee JM, Ahn SJ, Kang HJ, Yang HK, et al. LI-RADS Version 2017 versus Version 2018: diagnosis of hepatocellular carcinoma on gadoxetate disodium-enhanced MRI. *Radiology* 2019;292:655-63.
23. Ren AH, Zhao PF, Yang DW, Du JB, Wang ZC, et al. Diagnostic performance of MR for hepatocellular carcinoma based on LI-RADS v2018, compared with v2017. *J Magn Reson Imaging* 2019;50:746-55.
24. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358-80.
25. Chernyak V, Santillan CS, Papadatos D, Sirlin CB. LI-RADS((R)) algorithm: CT and MRI. *Abdom Radiol (NY)* 2018;43:111-26.
26. Tang A, Valasek MA, Sirlin CB. Update on the liver imaging reporting and data system: what the pathologist needs to know. *Adv Anat Pathol* 2015;22:314-22.
27. Fowler KJ, Tang A, Santillan C, Bhargavan-Chatfield M, Heiken J, et al. Interreader Reliability of LI-RADS Version 2014 algorithm and imaging features for diagnosis of hepatocellular carcinoma: a large international multireader study. *Radiology* 2018;286:173-85.
28. Kang JH, Choi SH, Lee JS, Park SH, Kim KW, et al. Interreader agreement of liver imaging reporting and data system on MRI: a systematic review and meta-analysis. *J Magn Reson Imaging* 2020; doi: 10.1002/jmri.27065.
29. Chernyak V, Sirlin CB. Editorial for “Interreader Agreement of Liver Imaging Reporting and Data System on MRI: a systematic review and meta analysis”. *J Magn Reson Imaging* 2020; doi: 10.1002/jmri.27133.
30. Goldberg-Stein S, Chernyak V. Adding value in radiology reporting. *J Am Coll Radiol* 2019;16:1292-8.
31. Corwin MT, Lee AY, Fananapazir G, Loehfelm TW, Sarkar S, et al. Nonstandardized terminology to describe focal liver lesions in patients at risk for hepatocellular carcinoma: implications regarding clinical communication. *AJR Am J Roentgenol* 2018;210:85-90.
32. Flusberg M, Ganeles J, Ekinici T, Goldberg-Stein S, Paroder V, et al. Impact of a structured report template on the quality of CT and MRI reports for hepatocellular carcinoma diagnosis. *J Am Coll Radiol* 2017;14:1206-11.
33. Alenazi AO, Elsayes KM, Marks RM, Yacoub JH, Hecht EM, et al. Clinicians and surgeon survey regarding current and future versions of CT/MRI LI-RADS. *Abdom Radiol (NY)* 2020;45:2603-11.

Systematic Review

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Global pattern and trend of liver cancer survival: a systematic review of population-based studies

Yu-Fei Jiang^{1,2,3}, Zhuo-Ying Li^{1,2}, Xiao-Wei Ji^{1,2}, Qiu-Ming Shen^{1,2}, Jia-Yi Tuo^{1,2}, Hui-Yun Yuan³, Yong-Bing Xiang^{1,2,3}

¹School of Public Health, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China.

²State Key Laboratory of Oncogene and Related Genes & Department of Epidemiology, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200032, China.

³Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200032, China.

Correspondence to: Prof. Yong-Bing Xiang, State Key Laboratory of Oncogenes and Related Genes & Department of Epidemiology, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, No. 25, Lane 2200, Xie Tu Road, Shanghai 200032, China. E-mail: ybxiaang@shsci.org

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Abstract

Aim: To describe the global pattern and trend of liver cancer survival, using data from the population-based studies or cancer registration.

Methods: By searching CNKI, Wanfang Data, PubMed, Web of Science, EMBASE and SEER. All population-based survival studies of liver cancer from 1 January 2000 to 30 April 2020 were collected and evaluated by patient gender, time period, and country. The overall or age-standardized five-year relative survival rate was used to describe the pattern and changes in liver cancer survival over the past decades.

Results: Globally, the highest age-standardized five-year relative survival rate was observed in Italy (18.0%, 2005-2007) and the highest overall five-year relative survival rate was observed in Korea (34.6%, 2012-2016), when compared to other countries. The most remarkable increase in overall five-year relative survival rate can be seen in Korea (from 10.7% during 1993-1995 to 34.6% during 2012-2016). In general, worldwide, the five-year relative survival rate of younger patients with liver cancer was higher than old people. For most countries, the five-year relative survival rate of liver cancer was slightly higher in women than in men. In China, the overall five-year relative survival rate of liver cancer in Taiwan was higher than that in other areas, while Cixian of Hebei and Qidong of Jiangsu were lower.



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Conclusion: Over the past decades, the survival rates of liver cancer have gradually improved, but great variations are also observed globally. Worldwide, younger patients with liver cancer have experienced a better prognosis. Gender disparity in liver cancer survival was not obvious.

Keywords: Primary liver cancer, relative survival rate, prognosis, population-based study, cancer registration

INTRODUCTION

Primary liver cancer (PLC) is the sixth most common cancer and the fourth most common cause of cancer death worldwide^[1]. The top five countries with the highest incidence of liver cancer are Mongolia (71.8/100,000), Thailand (33.7/100,000), North Korea (32.3/100,000), Japan (27.9/100,000), and China (27.6/100,000)^[2]. In the United States, the incidence has increased rapidly by 2% to 3% per year from 2007 to 2016, although the change was smaller than that in previous years^[3]. According to Global cancer statistics 2018^[1], an estimated 841,080 incident cases of liver cancer occurred worldwide, with 392,868 in China, accounting for 46.71%.

Certainly, incidence, mortality, and prevalence are commonly applied to describe the burden of disease. However, it is also crucial to comprehend and employ survival rate, which is another important descriptive indicator of disease burden and widely used in the evaluation of cancer prognosis. Survival data are available from three sources: clinical studies, hospital-based follow-up data, and population-based follow-up data^[4]. Interpretations of the outcomes of each source are different. The population-based follow-up data include the survival information of all patients in the population, which can reflect the cancer survival status of the entire population. Population-based survival data usually exclude death certificate only (DCO) and autopsy cases during analysis because evidence of diagnosis is weak.

Cancer registries are the premise and foundation of cancer prevention and control. They help obtain comprehensive, accurate, and timely information on the incidence, mortality, survival, and other factors related to cancer in the population^[4]. Survival analysis can be conducted with these data, and provide valuable indicators such as population-based relative survival rate (RSR) for the effectiveness of cancer control and reflect the prospects of cure in a country or region^[5]. To describe the global pattern, chronological changes, and enable comparisons between different populations or regions, this review collected all available population-based survival rates of primary liver cancer in different populations.

METHODS

Data source

A literature search of related studies from 1 January 2000 to 30 April January 2020 was conducted using the databases of CNKI, Wanfang Data, PubMed, Web of Science, EMBASE and SEER, with the following keywords: “liver cancer”, “hepatocellular carcinoma”, “HCC”, “population-based survival studies”, “relative survival”, “observed survival” “cancer registry”. Two researchers collected the data independently according to the search criteria, and 129 articles were retrieved by titles and abstracts. After screening with the following criteria: (1) provided RSR or observed survival rate (OSR) of patients with primary liver cancer; and (2) data were population-based or from cancer registries, and excluding duplicate, incomplete or unavailable articles. The final analysis included 53 studies, 9 of which were in Chinese and the remaining 44 were in English [Figure 1].

Statistical analysis

Estimates of one to five-year RSRs from the published studies were extracted. We used overall and age-standardized 5-year RSR mainly to describe and compare different countries or regions, age groups, and

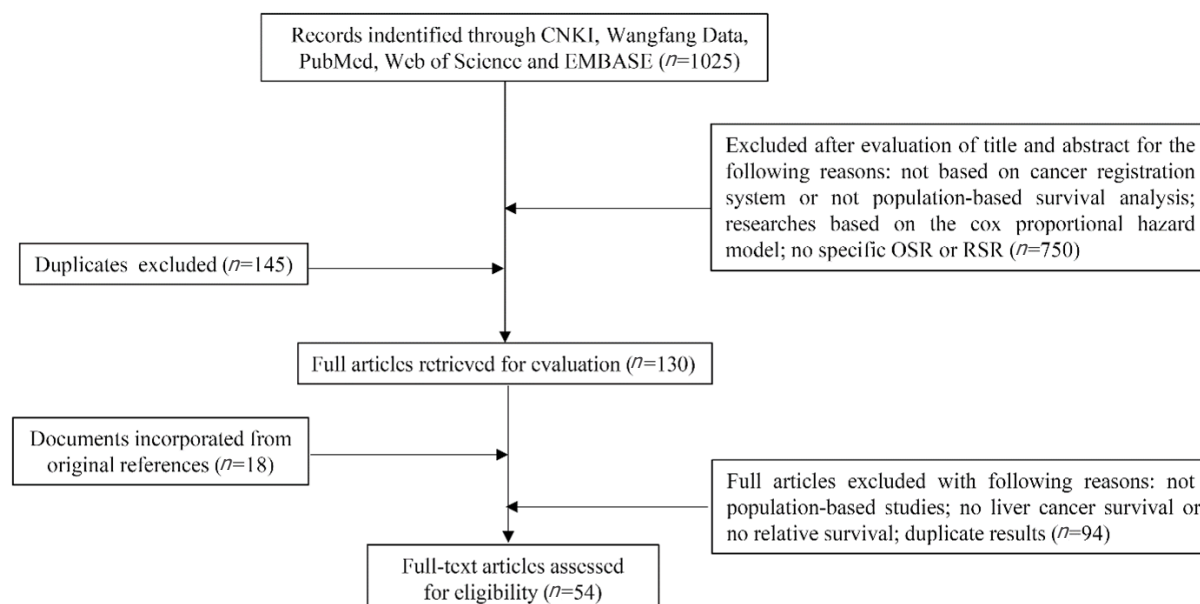


Figure 1. Study selection process. OSR: observed survival rate; RSR: relative survival rate

gender. Most of the included publications provided the age-standardized relative survival rates which were extracted. SPSS 22.0 and Excel 2016 were used for data management and analysis.

RESULTS

Global pattern and trend

Table 1 shows the sex-specific overall 5-year RSRs from Korea^[6-11], Japan^[12], Singapore^[13], USA^[14,15], and Europe^[14,16-22]. Table 2 shows the sex-specific age-standardized 5-year RSRs mainly from Singapore^[13] and Europe^[16,19,23,24]. In addition, the overall 10-year RSRs in Japan during 2002-2006 were 9.6% for men and 9.1% for women. It can be inferred from Tables 1 and 2 that although the 5-year RSRs of PLC in women is higher than that in men in most countries or regions, the difference is not obvious. The greatest difference in overall 5-year RSRs between male and female was observed in Scotland during 2005-2007 (4.4% for male and 10.6% for female)^[18], followed by the USA during 1986-1988 (3.5% for male and 8.5% for female)^[14]. In age-standardized 5-year RSRs, the greatest difference was observed in Norway during 1990-1994 and 1994-1998 (both 5.0% for male and 11.0% for female)^[24]. When comparing Tables 1 and 2 to determine whether age-standardization of 5-year RSRs has an effect on outcomes, the dissimilarity is not striking. In Europe (1990-1994)^[16], after age standardization, the 5-year RSRs in both men and women decreased; in France (1989-1997)^[19], the indicator in men increased, but remained the same in women. Besides, some studies have provided one to five-year RSRs or one and five-year age-standardized RSRs, as the details displayed in Supplementary Tables 1 and 2.

Figure 2A and B demonstrate age-standardized and overall 5-year RSRs since 1974 of PLC respectively, in selected countries and regions from Asia^[6-12,25-28], North America^[11,12,14,15,29-32], Europe^[14,16,17,19,21,22,33-36], and Africa^[37,38]. Figure 2A revealed that the highest age-standardized 5-year RSR was 18.0% and observed in Italy during 2005-2007^[36], followed by Canada during 2004-2006^[30], which was 17.0%. The lowest age-standardized 5-year RSR was 2.3% observed in Iceland during 1995-1999^[17]. Figure 2B showed that in the 1980s and before, the overall 5-year RSRs in all regions was lower than 5%, with the lowest being the USA (1977-1981)^[32] and Vaud of Switzerland (1984-1988)^[22], both of which were 2.0%. However, after entering the 21st century, the 5-year RSRs in most regions have improved greatly. In general, the survival rates have

Table 1. Population-based sex-specific overall 5-year relative survival rates of primary liver cancer in selected countries

Region	Year	5-year RSR (%)	
		Male	Female
Korea ^[6-11]	1993-1995	9.9	13.6
	1996-2000	12.9	14.2
	2001-2005	20.1	20.3
	2007-2011	28.5	28.7
	2005-2009	25.1	25.1
	2008-2012	30.4	29.3
	2010-2014	33.1	31.9
	2012-2016	35.2	32.7
Japan ^[12]	2006-2010	26.6	26.7
	1993-1996	21.0	21.8
	1997-1999	23.7	21.8
USA ^[14,15]	1983-1985	2.7	6.2
	1986-1988	3.5	8.5
	1989-1991	3.4	7.0
	1992-1994	4.7	5.3
	1996-1998	8.3	9.1
	1999-2001	10.9	12.1
	2002-2004	14.9	14.7
	2005-2009	17.7	17.3
	2010-2016	20.8	20.9
Europe ^[14,16,17]	1983-1985	2.8	5.3
	1986-1988	3.7	5.2
	1989-1991	5.3	6.0
	1990-1994	7.0	7.0
	1992-1994	7.2	7.0
	1995-1999	8.9	8.4
	1985-1989	0.4	0.5
Scotland ^[18]	1990-1994	2.2	5.4
	1995-1999	5.5	7.1
	2000-2004	8.7	8.7
	2005-2007	4.4	10.6
	1989-1997	7.0	9.0
France	Total ^[19]		
	Côte-d'Or, Burgundy ^[20]	1.1	2.0
		4.6	2.6
Spain ^[21]	1996-2005	10.3	10.3
	2000-2007	13.8	10.6
Switzerland ^[22]	Vaud		
		-	22.0
		3.0	-
	1989-1993	8.0	7.0

-: No report or non-available in the original articles; RSR: relative survival rate

gradually increased in all regions with time. Korea's growth was the most obvious, rising from 10.7% in the early 1990s to 34.6% during 2012-2016^[6-11].

Time changes in survival rates for liver cancer were also reviewed in our study. Figure 3 shows the age-standardized 5-year RSRs of PLC in specific regions in Europe^[36] at a specific calendar period. It was confirmed again in Figure 3 that the RSRs of PLC have been increasing over time. During three identical periods, we found that the rates were almost higher in Southern Europe (12.0% during 1999-2001, 14.0% during 2002-2004, 17.0% during 2005-2007), but consistently poorest in Eastern Europe (6.0% during 1999-2001 and 2002-2004, 7.0% in 2005-2007).

Figure 4 shows a comparison of age-specific relative survival rates of PLC. Because some reports did not provide age-specific survival data, and some reports adopted different age groups, Figure 4 demonstrated

Table 2. Population-based sex-specific age-standardised 5-year relative survival rates of primary liver cancer in selected countries

Region	Year	Age-standardised 5-year RSR (%)	
		Male	Female
Singapore ^[13]	1968-1972	5.0	3.0
	1973-1977	1.0	6.0
	1978-1982	3.0	8.0
	1983-1987	2.0	3.0
	1988-1992	3.0	2.0
Europe ^[16]	1990-1994	6.2	6.7
Austria ^[16]	1990-1994	7.0	-
Czech Republic ^[16]	1990-1994	1.1	3.4
Denmark ^[16,23,24]	1989-1993	3.0	3.0
	1990-1994	-	2.3
	1994-1998	4.0	4.0
England ^[16]	1999-2003	3.0	5.0
	1990-1994	6.1	7.2
	1990-1994	5.5	-
Estonia ^[16]	1989-1993	4.0	5.0
	1990-1994	3.9	4.4
	1994-1998	7.0	7.0
Finland ^[16,23,24]	1999-2003	8.0	8.0
	1989-1993	14.0	-
	1994-1998	7.0	-
Iceland ^[23,24]	1999-2003	7.0	-
	1989-1993	8.0	9.0
	1990-1994	6.9	-
France ^[16,19]	1989-1997	8.0	9.0
	1990-1994	6.9	-
	1990-1994	-	3.8
Germany ^[16]	1990-1994	6.2	8.6
Italy ^[16]	1990-1994	6.2	5.8
The Netherlands ^[16]	1990-1994	6.0	8.0
Norway ^[16,23,24]	1989-1993	6.0	8.0
	1990-1994	2.1	3.2
	1994-1998	5.0	11.0
Poland ^[16]	1999-2003	5.0	11.0
	1990-1994	-	1.3
Scotland ^[16]	1990-1994	-	4.8
Slovakia ^[16]	1990-1994	-	1.8
Slovenia ^[16]	1990-1994	-	4.9
Spain ^[16]	1990-1994	10.4	11.6
Sweden ^[16,23,24]	1989-1993	5.0	3.0
	1990-1994	2.9	3.1
	1994-1998	6.0	7.0
Switzerland ^[16]	1999-2003	7.0	8.0
	1990-1994	5.9	-
Wales ^[16]	1990-1994	5.1	6.3

-: No reports or non-available in the original articles; RSR: relative survival rate

the age-specific 5-year RSRs from Europe^[16,17,36], USA^[15], Japan^[26], Canada^[30,31], Korea^[11], and China^[39-41] in the period between 1990-2010. The 5-year RSRs decreased with age. The rates of patients aged 15-44, followed by those aged 45-54, were higher than other age groups, while the prognosis of patients aged 75 or older was poor. In the age groups of 35-44 and 45-54, the rate in Canada (2004-2006)^[30] was markedly higher than that in other regions. In the age 65-74 group, the rate of Korea (2006-2010)^[11] was markedly higher than that in other regions, and that in those age 75 or older, was highest in the Zhejiang Province (2005-2010)^[41] of China compared to other regions or countries. Of note however, some reports estimate RSRs after excluding DCO and autopsy cases^[11-14,18,21,22,24,26,28,30,31,35,36,39,40,42-45].

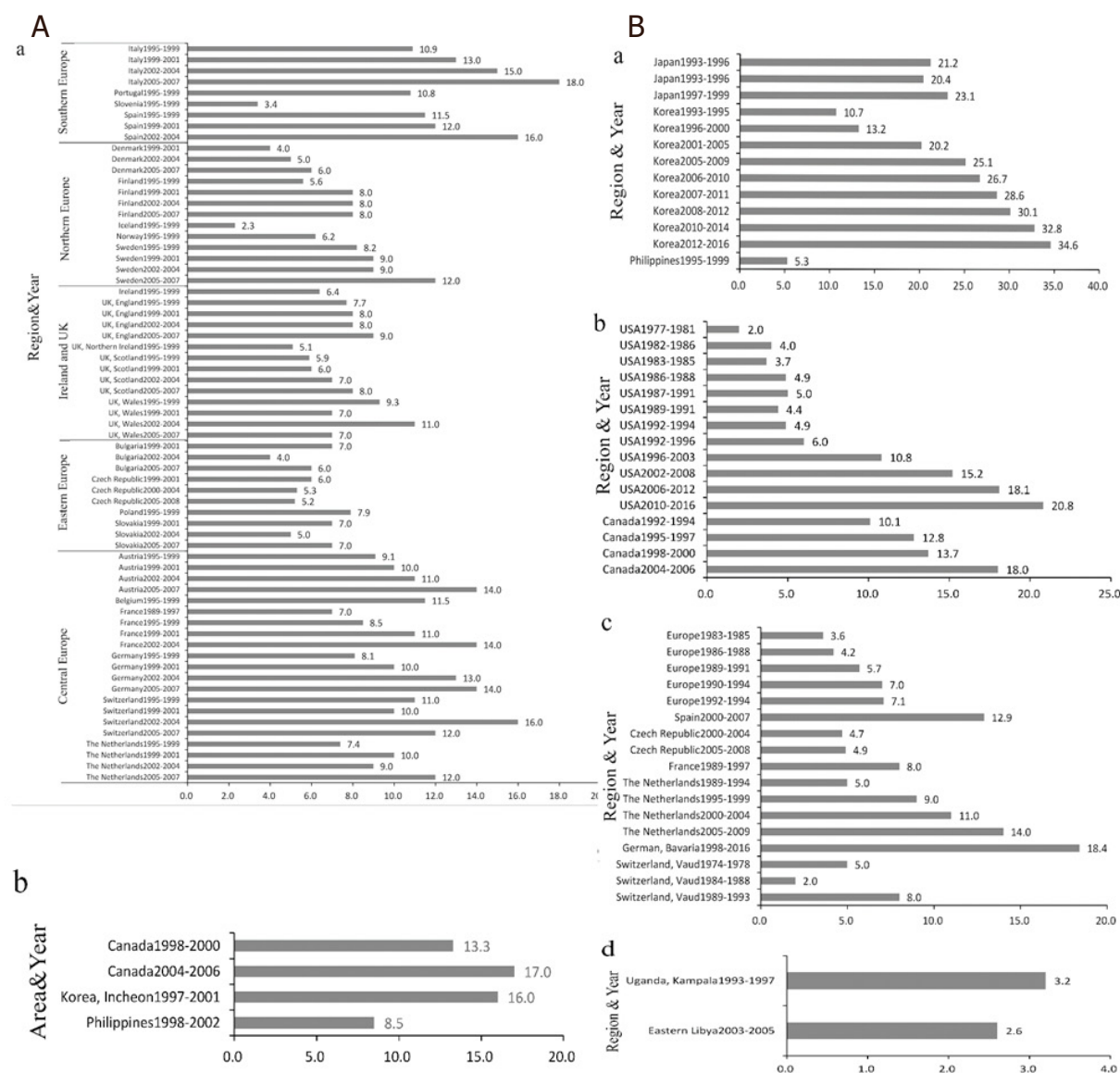


Figure 2. Age-standardised and overall 5-year relative survival rates of primary liver cancer in some selected countries during 1974-2016. A: Age-standardised 5-year relative survival rates (a: Europe [14,16,19,21,22,33-35]; b: other regions [17,19,35,36]; c: North America [11,12,14,15,29-32]; d: Africa [37,38]). *The divisions of Europe refer to the EUROCARE report

Liver cancer survival in China

Table 3 shows the details of the population-based overall and age-standardized 5-year RSRs of PLC in some areas in China. It mainly includes the survival of PLC in the nation [46], Beijing [45], Shanghai [39], Zhejiang Province [41], Liaoning Province [28], Taiwan [47], Hong Kong [28], Haining and Jiashan (Zhejiang Province) [48], Cixian (Hebei Province) [49,50], Huaian (Jiangsu Province) [51], Qidong (Jiangsu Province) [40,52], and Jintan district (Changzhou, Jiangsu Province) [53]. Among them, cases of DCO were explicitly excluded in the reports of Shanghai [39], Qidong of Jiangsu [40], Haining, and Jiashan of Zhejiang [48], and Liaoning [28].

As shown in Table 3, the 5-year RSRs of PLC improved gradually over time. The age-standardized 5-year RSRs of liver cancer patients in China (2003-2015) are lower than that of Korea and Japan for a similar time period. For the overall 5-year RSRs, gender difference was not found in our review as the survival rates of liver cancer in women were not consistently higher than that in men. The highest overall 5-year RSR of

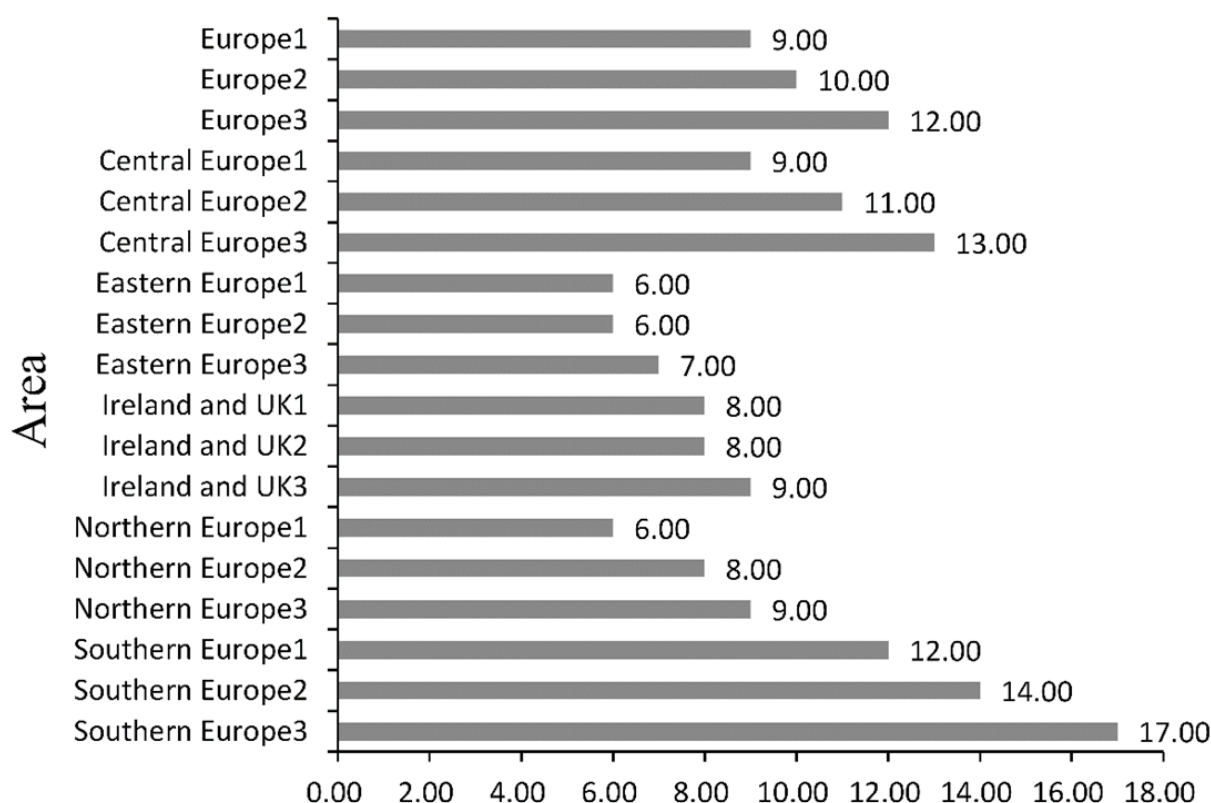


Figure 3. The age-standardised 5-year relative survival rates of primary liver cancer in different areas of Europe*, 1999-2007^[36]. 1: 1999-2001; 2:2002-2004; 3: 2005-2007. *The divisions of Europe refer to the EURO CARE report

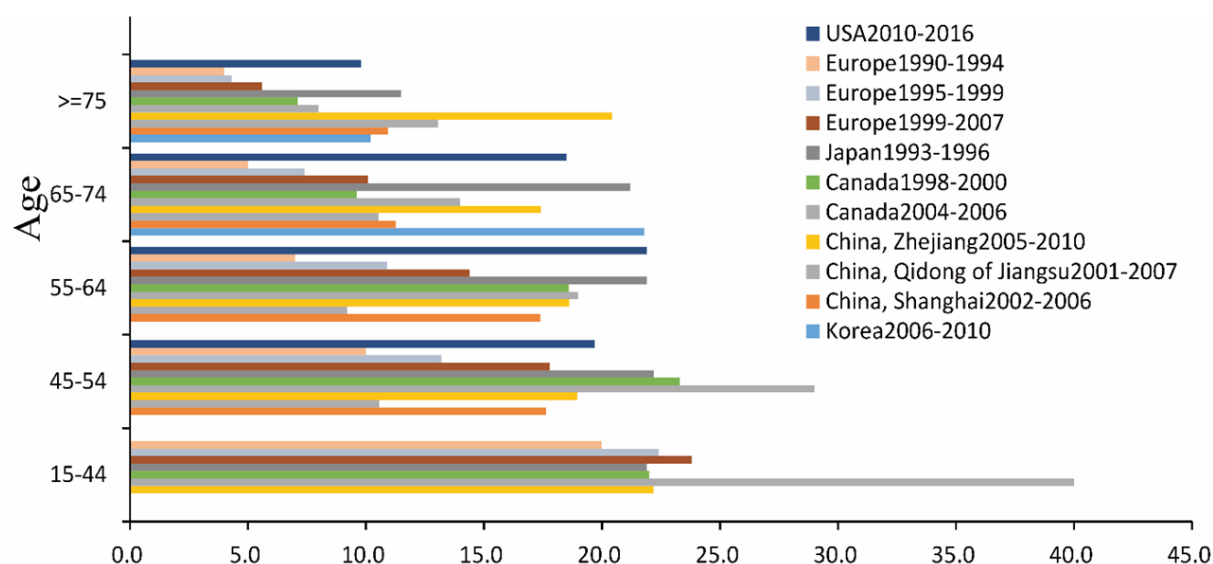


Figure 4. Age-specific 5-year relative survival rates of primary liver cancer in different years in some selected countries and regions^[11,15-17,26,30,36,39-41]

28.9% was in Taiwan during 2004-2008, which was 28.1% and 31.7% in men and women respectively^[47]. Since 2000, the overall 5-year RSR of Cixian (Hebei Province) was the lowest, at only 4.2% in 2000-2002^[49].

Table 3. Population-based overall and age-standardised 5-year relative survival rates of PLC in some areas of China

Area			Year	5-year RSR (%)			Age-standardised 5-year RSR (%)			
				Total	Male	Female	Total	Male	Female	
China ^[46]			2003-2005	-	-	-	10.1	10.2	10.3	
			2006-2008	-	-	-	10.1	10.0	11.0	
			2009-2011	-	-	-	9.8	9.8	10.7	
			2012-2015	-	-	-	12.1	12.2	13.1	
East China	Shanghai ^[39]	Total ^[41]	2002-2006	15.5	16.0	14.8	-	-	-	
			2005-2010	19.1	19.5	18.0	-	-	-	
	Zhejiang	Haining and Jiashan ^[48]	2003-2006	10.3	9.8	11.4	10.2	-	-	
			2007-2010	8.9	9.5	7.9	9.0	-	-	
			2011-2014	10.6	11.3	8.9	10.2	-	-	
	Jiangsu	Huaian ^[51]	2010	8.4	8.9	6.9	-	-	-	
			Jintan District of Changzhou ^[53]	2012-2013	11.6	-	-	-	-	-
		Qidong ^[40,52]	1972-2011	4.7	4.5	5.4	-	-	-	
			1973-1977	2.8	-	-	-	-	-	
			1978-1982	1.4	-	-	-	-	-	
	1983-1987		2.6	-	-	-	-	-		
	North China	Taiwan ^[47]	Beijing ^[45]	1988-1992	4.7	-	-	-	-	-
				1993-1997	4.7	-	-	-	-	-
				1998-2002	5.1	-	-	-	-	-
				2001-2007	10.0	9.8	10.6	-	-	-
				2003-2007	7.1	-	-	-	-	-
Hebei ^[49,50]		Cixian	2004-2008	28.9	28.1	31.7	27.6	27.0	31.5	
			1982-1983	-	2.2	2.4	-	-	-	
			1987-1988	-	3.4	5.3	-	-	-	
			2000-2002	4.2	-	-	-	-	-	
			2003-2013	7.6	7.1	8.7	-	-	-	
South China	Hong Kong ^[28]		1996-2001	-	-	-	22.4	-	-	
Northeast China	Liaoning ^[28]		2000-2002	-	-	-	10.7	8.8	15.2	

-: No reports or non-available in the original articles; RSR: relative survival rate

DISCUSSION

Survival data based on clinical trials, hospital-based follow-up studies, and population-based cancer registration are disparate in their aims, methods of survival estimation, and application. This study collected overall or age-standardized RSRs of liver cancer worldwide so that we can describe the prognosis of liver cancer in the general population, and make comparisons between different countries and regions. All publications in the study were from the cancer registries or population-based survival analysis, which aimed to provide valuable information for epidemiologists, basic scientists, oncologists, and clinical physicians in liver cancer research.

The aim of clinical trials and hospital-based follow-up studies are quite different from that of population-based survival studies. The survival obtained from clinical trials or studies comes from the evaluation of certain therapeutics, and generally adopts overall survival (defined as the date from randomization to death from any cause) and progression-free survival (defined as the date from randomization until progression or death from any cause) as endpoints. For instance, a randomized, phase 3 clinical trial published in the *New England Journal of Medicine* evaluated cabozantinib as compared with placebo in previously treated patients with advanced hepatocellular carcinoma, and demonstrated that cabozantinib treatment significantly prolonged survival in patients with longer overall survival and progression-free survival (median overall survival and median progression-free survival were 10.2 months and 5.2 months, respectively) compared to placebo (8.0 months and 1.9 months, respectively)^[54]. Hospital-based survival or follow-up studies rely on hospital-based cancer follow-up or registries that collect survival information

of patients who have been hospitalized, which reflects the service's capacity and treatment effects in a particular department. For example, using follow-up data in a hospital's registry, a study in Qidong of Jiangsu Province from China^[55] calculated the 5-year observed survival rate of patients with liver cancer from 2002 to 2016 to be 14.6% [Supplementary Table 3], which was higher than the population-based OSR of 8.9% reported in the general population of Qidong during 2001-2007. For hospital-based survival, the follow-up time starts from the first hospitalization date, while the population-based cancer registry starts from the date of diagnosis of cancer. However, according to the data in China, the survival rates of PLC in the more developed areas such as Shanghai and Zhejiang were higher than that of other areas and the national average during the similar period, and studies have additionally supported the observation that the survival rates of PLC in urban areas were higher than that in the rural areas for the same period.

A common reason to study population-based cancer survival is to estimate the net survival, a measure of patient survival following primary cancer in the absence of other causes of death^[56], which can be obtained by calculating disease-specific survival. Since the estimation of net survival must rely on complete and accurate information on the cause of death, which is often difficult to obtain, an alternative indicator - RSR^[5] - can be used. RSR is defined as the ratio of the observed survival rate (where all causes of deaths are considered as events) to the expected survival rate (which is estimated from national population life tables stratified by sex, age, and calendar period) in the general population with the same distribution of key demographic factors (sex, age, calendar, period, and country). It provides a measure of the excess mortality hazard experienced by cancer patients, irrespective of whether the excess mortality is directly or indirectly attributable to the cancer^[56] and enables direct comparison of survival rates between different populations or regions by eliminating the effects of age, gender, ethnicity, and calendar period on cancer survival to some extent. In addition, to further eliminate the effect of age structure, international comparisons of RSRs ought to use age-standardized relative survival^[57].

It is apparent from our review then that the prognosis of PLC has shown continuous improvement overtime, whether in China or around the world. Over the past decades, numerous changes in clinical practice, public health, and social economy may affect the survival of PLC. For instance, advances in imaging diagnosis, clinical treatment such as chemoembolization, ablation, and surgical resection techniques, increased surveillance and screening for early-stage disease and anti-cancer health education, the improvement of socio-economic status (SES) and the transformation of peoples' health consciousness and lifestyles^[39,58,59]. However, it is these factors that can improve survival rates that may lead to regional disparity in survival rates of PLC as well, because of their varying degrees of development between different regions. Globally, the 5-year RSRs in Africa such as Eastern Libya and Uganda Kampala are much poorer than in the countries of Europe and North America during the same time period, and 5-year RSRs of PLC were also varied across regions in Europe. Studies have shown that people from the highest SES have better survival outcomes compared to those in the lowest SES^[60-62]. The low SES and the attendant delayed diagnosis and treatment, unfair distribution of medical resources, incomplete medical insurance systems, lack of health education, and other factors will all affect cancer survival.

The data we have summarized from the literature implied that there are gender and age disparities in liver cancer survival. For example, in the majority of countries and regions, the prognosis of liver cancer in women was better than men, although the situation was not systematic. Therefore, it is inappropriate to draw the conclusion that the prognosis of women with PLC is better than men. Gender-specific distinctions in the survival rate of PLC require more population-based follow-up studies. In terms of the age at diagnosis, survival was highest among patients in the 35-44 age group, followed by the 45-54 age group, and lowest for the 75 or older age group. This might have been due to the presence of comorbidities and various chronic diseases in the aged patients that reduced their tolerance of cancer treatments or affected physicians' decisions for treatment options, as compared to younger patients^[63-65]. In addition, studies have

suggested that the proportion of late-diagnosis of PLC in the older age group was higher, resulting in poor long-term survival^[39].

When comparing survival rates in different countries, times, or populations, *etc.*, the following points need to be considered. Firstly, the relative survival rate was the only indicator analyzed in this review. However, descriptive indicators of cancer survival also include observed survival rate (all the OSRs collected were showed in [Supplementary Table 3](#)), cause-specific survival, *etc.* The estimation methods and their interpretations are completely different and cannot be substituted for each other. Next, close attention to additional comorbidities or variables (such as age, gender, ethnicity, *etc.*) used in survival rate estimation in the study is required. As in this review, some studies excluded patients aged under 15 or 20 during analysis^[11,18,21,30,34,35,44,47]. Thirdly, it should be noted whether DCO cases or autopsy cases were excluded from the analysis, which would affect outcomes.

In conclusion, we summarized one to five-years RSRs of liver cancer, which were markedly distinct between different regions or periods in the same region. This implied that the region, period, and age might affect the survival rate of PLC; however, whether gender is a relevant factor remains to be studied. Therefore, more attention should be drawn to PLC prevention and screening, in particular, must be developed and implemented. Epidemiological, basic, and clinical studies of PLC have a long way to go still.

DECLARATIONS

Authors' contributions

Conducted the study and collected publications and abstract data and wrote the first draft: Jiang YF

Double check the collected publications and abstract data: Li ZY

Reviewed and approved the final version of the paper: Jiang YF, Li ZY, Ji XW, Shen QM, Tuo JY, Yuan HY, Xiang YB

Primary responsibility for final content, designed the research study and obtained funding: Xiang YB

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
2. Ying Q, Wang Y. Global prevalence and trend of liver cancer. *China Cancer* 2020;29:185-91.
3. Howlader N, Noone A, Krapcho M, Miller D, Brest A, et al. SEER cancer statistics review, 1975-2016, National Cancer Institute. In: Bethesda MD, editor. Based on November 2018 SEER data submission posted to the SEER web site, April 2019. Available from: https://seer.cancer.gov/csr/1975_2016/. [Last accessed on 3 Jul 2020]
4. Cancer Hospital Chinese Academy of Medical Science. Chinese Guideline for Cancer Registration (2016)ed. Beijing: People's Medical Publishing House(PMPH) Pub; 2016. pp. 33-75.
5. Hakulinen T. Cancer survival corrected for heterogeneity in patient withdrawal. *Biometrics* 1982;38: 933-42.
6. Jung KW, Park S, Kong HJ, Won YJ, Lee JY, et al. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2009. *Cancer Res Treat* 2012;44:11-24.
7. Jung KW, Won YJ, Kong HJ, Oh CM, Lee DH, et al. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2011. *Cancer Res Treat* 2014;46:109-23.
8. Jung KW, Won YJ, Kong HJ, Oh CM, Cho H, et al. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2012. *Cancer Res Treat* 2015;47:127-41.
9. Jung KW, Won YJ, Oh CM, Kong HJ, Lee DH, et al. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2014. *Cancer Res Treat* 2017;49:292-305.
10. Jung KW, Won YJ, Kong HJ, Lee ES. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2016. *Cancer Res Treat* 2019;51:417-30.
11. Jung KW, Won YJ, Kong HJ, Oh CM, Shin A, et al. Survival of korean adult cancer patients by stage at diagnosis, 2006-2010: national cancer registry study. *Cancer Res Treat* 2013;45:162-71.
12. Matsuda T, Ajiki W, Marugame T, Ioka A, Tsukuma H, et al. Population-based survival of cancer patients diagnosed between 1993 and 1999 in Japan: a chronological and international comparative study. *Jpn J Clin Oncol* 2011;41:40-51.
13. Chia KS, Du WB, Sankaranarayanan R, Sankila R, Seow A, et al. Population-based cancer survival in Singapore, 1968 to 1992: an overview. *Int J Cancer* 2001;93:142-7.
14. Capocaccia R, Sant M, Berrino F, Simonetti A, Santi V, et al. Hepatocellular carcinoma: trends of incidence and survival in Europe and the United States at the end of the 20th century. *Am J Gastroenterol* 2007;102:1661-70.
15. Howlader N, Noone AM, Krapcho M, Miller D, Brest A, et al. SEER Cancer Statistics Review, 1975-2017, National Cancer Institute. Available from: https://seer.cancer.gov/csr/1975_2017/. [Last accessed on 30 Jun 2020].
16. Sant M, Aareleid T, Berrino F, Bielska Lasota M, et al. EUROCORE-3: survival of cancer patients diagnosed 1990-94--results and commentary. *Ann Oncol* 2003;14:v61-118.
17. Sant M, Allemani C, Santaquilani M, Knijn A, Marchesi F, et al. EUROCORE-4. Survival of cancer patients diagnosed in 1995-1999. Results and commentary. *Eur J Cancer* 2009;45:931-91.
18. Dunbar JK, Dillon J, Garden OJ, Brewster DH. Increasing survival of hepatocellular carcinoma patients in Scotland: a review of national cancer registry data. *HPB* 2013;15:279-85.
19. Bossard N, Velten M, Remontet L, Belot A, Maarouf N, et al. Survival of cancer patients in France: a population-based study from The Association of the French Cancer Registries (FRANCIM). *Eur J Cancer* 2007;43:149-60.
20. Guin B, Minello A, Cottet V, Lepage C, Hillon P, et al. A 30-year, population-based study shows improved management and prognosis of hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2010;8:986-91.
21. Chirlaque MD, Salmeron D, Galceran J, Ameijide A, Mateos A, et al. Cancer survival in adult patients in Spain. Results from nine population-based cancer registries. *Clin Transl Oncol* 2018;20:201-11.
22. Levi F, Randimbison L, Te VC, Franceschi S, La Vecchia C. Trends in survival for patients diagnosed with cancer in Vaud, Switzerland, between 1974 and 1993. *Ann Oncol* 2000;11:957-63.
23. Storm HH, Engholm G, Hakulinen T, Tryggvadottir L, Klint A, et al. Survival of patients diagnosed with cancer in the Nordic countries up to 1999-2003 followed to the end of 2006. A critical overview of the results. *Acta Oncol* 2010;49:532-44.
24. Klint A, Engholm G, Storm HH, Tryggvadottir L, Gislum M, et al. Trends in survival of patients diagnosed with cancer of the digestive organs in the Nordic countries 1964-2003 followed up to the end of 2006. *Acta Oncol* 2010;49:578-607.
25. Redaniel MT, Laudico A, Mirasol-Lumague MR, Gondos A, Brenner H. Cancer survival differences between European countries and an urban population from the Philippines. *Eur J Public Health* 2011;21:221-8.
26. Tsukuma H, Ajiki W, Ioka A, Oshima A. Survival of cancer patients diagnosed between 1993 and 1996: a collaborative study of population-based cancer registries in Japan. *Jpn J Clin Oncol* 2006;36:602-7.
27. Laudico AV, Mirasol-Lumague MR, Mapua CA, Uy GB, Toral JA, et al. Cancer incidence and survival in Metro Manila and Rizal Province, Philippines. *Jpn J Clin Oncol* 2010;40:603-12.
28. Li Y, Yu L, Na J, Li S, Liu L, et al. Survival of cancer patients in northeast China: analysis of sampled cancers from population-based cancer registries. *Cancer Res Treat* 2017;49:1106-13.
29. Jemal A, Ward EM, Johnson CJ, Cronin KA, Ma J, et al. Annual report to the nation on the status of cancer, 1975-2014, featuring survival. *J Natl Cancer Inst* 2017;109:djx030.
30. Ellison LF, Wilkins K. An update on cancer survival. *Health Rep* 2010;21:55-60.
31. Ellison LF. Measuring the effect of including multiple cancers in survival analyses using data from the Canadian Cancer Registry. *Cancer*

- Epidemiol 2010;34:550-5.
32. El-Serag HB, Mason AC, Key C. Trends in survival of patients with hepatocellular carcinoma between 1977 and 1996 in the United States. *Hepatology* 2001;33:62-5.
33. De Toni EN, Schlesinger-Raab A, Fuchs M, Schepp W, Ehmer U, et al. Age independent survival benefit for patients with hepatocellular carcinoma (HCC) without metastases at diagnosis: a population-based study. *Gut* 2020;69:168-76.
34. Witjes CD, Karim-Kos HE, Visser O, van den Akker SA, de Vries E, et al. Hepatocellular carcinoma in a low-endemic area: rising incidence and improved survival. *Eur J Gastroenterol Hepatol* 2012;24:450-7.
35. Pavlik T, Majek O, Buchler T, Vyzula R, Petera J, et al. Trends in stage-specific population-based survival of cancer patients in the Czech Republic in the period 2000-2008. *Cancer Epidemiol* 2014;38:28-34.
36. Lepage C, Capocaccia R, Hackl M, Lemmens V, Molina E, et al. Survival in patients with primary liver cancer, gallbladder and extrahepatic biliary tract cancer and pancreatic cancer in Europe 1999-2007: results of EUROCARE-5. *Eur J Cancer* 2015;51:2169-78.
37. El Mistiri M, Salati M, Marcheselli L, Attia A, Habil S, et al. Cancer incidence, mortality, and survival in Eastern Libya: updated report from the Benghazi Cancer Registry. *Ann Epidemiol* 2015;25:564-8.
38. Gondos A, Brenner H, Wabinga H, Parkin DM. Cancer survival in Kampala, Uganda. *Br J Cancer* 2005;92:1808-12.
39. Peng H, Zheng Y, Wang CF, Gong YM, Wu CX, et al. Survival analysis of liver cancer between 2002 and 2006 in Shanghai. *Chin Oncol* 2016;561-8.
40. Chen JG, Zhu J, Zhang YH, Chen YS, Ding LL. Survival of liver cancer during 2001-2007 in Qidong city. *Chin J Cancer Prevent Treat* 2011;18:568-70.
41. Fei FR, Hu RY, Gong WW, Pan J, Wang M. Analysis of mortality and survival rate of liver cancer in Zhejiang Province in China: a general population-based study. *Can J Gastroenterol Hepatol* 2019;2019:1074286.
42. Lim GH, Wong CS, Chow KY, Bhalla V, Chia KS. Trends in long-term cancer survival in Singapore: 1968-2002. *Ann Acad Med Singapore* 2009;38:99-105.
43. Yang YJ, Bae JM. Survival rates of the 5 major cancers in Jeju Island residents, 2000-2001. *J Prev Med Public Health* 2007;40:213-7.
44. Ito Y, Miyashiro I, Ito H, Hosono S, Chihara D, et al. Long-term survival and conditional survival of cancer patients in Japan using population-based cancer registry data. *Cancer Sci* 2014;105:1480-6.
45. Wang QJ, Zhu WX, Li L, Xing XM. Cancer survival in urban Beijing. *China Cancer* 2001;05:17-8.
46. Zeng HM, Chen WQ, Zheng RS, Zhang SW, Ji JS, et al. Changing cancer survival in China during 2003-15: a pooled analysis of 17 population-based cancer registries. *Lancet Global Health* 2018;6:e555-67.
47. Chiang CJ, Lo WC, Yang YW, You SL, Chen CJ, et al. Incidence and survival of adult cancer patients in Taiwan, 2002-2012. *J Formos Med Assoc* 2016;115:1076-88.
48. Li H, Yang J, Du L, Li Q, Zhu C, et al. Cancer survival in Haining and Jiashan cancer registry areas of Zhejiang Province. *China Cancer* 2019;29:14-21.
49. He YT, Zeng Y, Xu H, Song G, Tian G, et al. Survival rate among cancer patients in Cixian county, 2000-2002. *Chin J Publ Heal* 2011;27:1107-10.
50. Li D, Li D, Song G, Liang D, Chen C, et al. Cancer survival in Cixian of China, 2003-2013: a population-based study. *Cancer Med* 2018;7:1537-45.
51. Luo S, Pan E, He Y, Zhang Q, Wang C, et al. Analysis of survival rates among residents newly diagnosed with malignant cancers during 2010 in Huai'an City. *Chin J Cancer Prevent Treat* 2016;23:1049-52.
52. Yao HR, Chen H, Chen JG, Zhu J, Zhang YH. Long-term survival trends for liver cancer in Qidong: 1972 to 2011. *Chin J Hepatol* 2014;22:921-5.
53. He Y, Zhou X, Lv YL. Analysis of the malignant tumor incidence and survival in Jintan district of Changzhou from 2012 to 2013. *Jiangsu J Prevent Med* 2019;30:518-21. Available form: <http://jsyf.cbpt.cnki.net/WKB2/WebPublication/paperDigest.aspx?paperID=beb308e0-c2ea-4038-850f-dd73acc4cb9a>. [Last accessed on 3 Jul 2020]
54. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N Engl J Med* 2018;379:54-63.
55. Chen JG, Chen HZ, Zhu J, Yang YL, Zhang YH, et al. Cancer survival in patients from a hospital-based cancer registry, China. *J Cancer* 2018;9:851-60.
56. Engholm G, Gislum M, Bray F, Hakulinen T. Trends in the survival of patients diagnosed with cancer in the Nordic countries 1964-2003 followed up to the end of 2006. Material and methods. *Acta Oncologica* 2010;49:545-60.
57. Corazziari I, Quin M, Capocaccia R. Standard cancer patient population for age standardising survival ratios. *Eur J Cancer* 2004;40:2307-16.
58. Jarnagin W, Chapman WC, Curley S, D'Angelica M, Rosen C, et al. Surgical treatment of hepatocellular carcinoma: expert consensus statement. *HPB (Oxford)* 2010;12:302-10.
59. Sangiovanni A, Del Ninno E, Fasani P, De Fazio C, Ronchi G, et al. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. *Gastroenterology* 2004;126:1005-14.
60. Yun EH, Lim MK, Oh JK, Park JH, Shin A, et al. Combined effect of socioeconomic status, viral hepatitis, and lifestyles on hepatocellular carcinoma risk in Korea. *Br J Cancer* 2010;103:741-6.
61. Artinyan A, Mailey B, Sanchez-Luege N, Khalili J, Sun CL, et al. Race, ethnicity, and socioeconomic status influence the survival of patients with hepatocellular carcinoma in the United States. *Cancer* 2010;116:1367-77.
62. Jembere N, Campitelli MA, Sherman M, Feld JJ, Lou W, et al. Influence of socioeconomic status on survival of hepatocellular carcinoma

- in the Ontario population; a population-based study, 1990-2009. *PLoS One* 2012;7:e40917.
63. Brenner H, Arndt V. Recent increase in cancer survival according to age: higher survival in all age groups, but widening age gradient. *Cancer Causes Control* 2004;15:903-10.
 64. Thein HH, Khoo E, Campitelli MA, Zaheen A, Yi Q, et al. Trends in relative survival in patients with a diagnosis of hepatocellular carcinoma in Ontario: a population-based retrospective cohort study. *CMAJ Open* 2015;3:E208-16.
 65. De Angelis R, Sant M, Coleman MP, Francisci S, Baili P, et al. Cancer survival in Europe 1999-2007 by country and age: results of EURO-CARE-5-a population-based study. *Lancet Oncol* 2014;15:23-34.

Review

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Contrast-enhanced ultrasound liver reporting and data system for hepatocellular carcinoma diagnosis

Alexander M. Vezeridis¹, Yuko Kono²

¹Interventional Radiology, Stanford University Medical Center, Stanford, CA 94305, USA.

²Department of Radiology, Department of Medicine, University of California, San Diego, CA 92103, USA.

Correspondence to: Prof. Yuko Kono, Department of Radiology, Department of Medicine, University of California, 200 W Arbor Dr. MC 8413, San Diego, CA 92103, USA. E-mail: ykono@health.ucsd.edu

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Abstract

Contrast-enhanced ultrasound (CEUS) is a powerful imaging modality for the diagnosis of focal liver lesions, including hepatocellular carcinoma (HCC). The American College of Radiology Contrast-Enhanced Ultrasound Liver Reporting and Data System (CEUS LI-RADS[®]) was created as a standardized reporting system to facilitate consistent and high-quality technique, interpretation, reporting, and data collection for CEUS diagnosis of HCC. This article describes the history and background of CEUS LI-RADS[®], its major concepts and algorithm, and the differences between CEUS LI-RADS[®] and CT/MRI LI-RADS[®].

Keywords: Contrast-enhanced ultrasound, LI-RADS, hepatocellular carcinoma, diagnosis

INTRODUCTION

Hepatocellular carcinoma (HCC) can be diagnosed non-invasively by imaging without histological confirmation as endorsed by major liver society guidelines^[1,2]. Contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) have been used for HCC diagnosis, with contrast-enhanced ultrasound (CEUS) recognized as an alternative imaging modality^[3]. Indeed, focal liver lesion characterization is the most widely recognized and used application for CEUS^[4].

CEUS is an advanced form of ultrasound imaging utilizing tiny microbubble contrast agents, with diameters similar to that of red blood cells^[5]. After intravenous injection, the microbubbles stay in the



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bloodstream for minutes, allowing ultrasound imaging machines with specialized contrast-specific modes to visualize differences in the vascularity of tissues of interest^[6]. The microbubbles resonate and exhibit harmonic signals under very weak ultrasound exposure while the background tissues exhibit only a linear response to ultrasound signals^[7]. Contrast-specific imaging utilizes this unique property of microbubble contrast agents, and cancels the linear tissue signal, resulting in a contrast-enhanced microbubble-only image^[8]. CEUS is highly sensitive owing to the intrinsically high contrast of microbubbles^[7].

Microbubble contrast agents demonstrate multiple contrast phases in the liver after they are injected intravenously, progressing from arterial phase (~10-20 s to 30-45 s after injection) to portal venous phase (~30-45 s to 2 min after injection) to late phase (~2 min to 4-6 min after injection)^[4,9]. Most malignancies within the liver derive their blood supply from the hepatic arteries and not the portal vein. As a result, malignancy typically manifests on CEUS as hyperenhancing compared to the surrounding liver on the arterial phase and as hypoenhancing in the portal venous and late phases. This temporal reduction in the enhancement of a lesion from earlier to later phases is also known as washout^[9]. It is important to note that the washout of CEUS is different from that of CT or MRI, as microbubble contrast agents of CEUS are purely intravascular, whereas CT and MR contrast agents are both intravascular as well as interstitial. As a result, fibrous lesions such as intrahepatic cholangiocarcinoma (ICC) will appear to have delayed enhancement by CT or MRI, while they will demonstrate washout on CEUS^[10].

ADVANTAGES AND LIMITATIONS OF CEUS

CEUS has a number of advantages as an imaging modality. CEUS is a real-time imaging technique with much higher temporal resolution than CT or MRI, and therefore it does not suffer from mistiming or acquiring contrast phases that are too early or too late, as can be seen with CT or MRI^[10]. CEUS is highly sensitive to the microbubble contrast agents^[7]. Therefore, CEUS is a good modality to confirm subtle enhancement when it cannot be seen on CT/MRI^[5,11].

CEUS does not use ionizing radiation as does CT. CEUS is also less costly than CT or MRI^[12]. The microbubble contrast agent for CEUS is safe in patients with severe renal impairment, unlike those used in CT or MRI^[13,14]. CEUS is approved for the pediatric population and is a very safe alternative that does not require sedation or anesthesia^[15].

CEUS has some potential limitations that the practitioner must recognize to tailor its use appropriately. CEUS is usually not suitable for staging of hepatocellular carcinoma, as it is difficult to survey the entire liver effectively with CEUS^[16]. In general, a few lesions can be characterized in one session of CEUS.

CEUS may require more planning and time on the part of the interpreting physician as lesions of interest must be identified on the precontrast portion of the examination and correlated to the prior imaging^[5,17]. As a result, the interpreting physician is often involved in the acquisition of CEUS. As CEUS is acquired by a sonographer and/or interpreting physician, it is typically more operator-dependent than CT or MRI. The microbubble imaging agent Lumason® (Bracco Diagnostics, Monroe Township, NJ) became the first agent approved by the US Food and Drug Administration (FDA) for contrast-enhanced abdominal ultrasound imaging in adults and children in April 2016. While this agent has been used worldwide under the name SonoVue® for well over a decade before this^[13], given the relatively short history of FDA-approved microbubble contrast agents, practitioner knowledge of CEUS acquisition techniques and interpretation is generally less than conventional ultrasound, CT, and MRI.

HISTORY AND BACKGROUND OF CEUS LI-RADS

The American College of Radiology (ACR) Liver Reporting and Data System (LI-RADS®) was created as a standardized reporting system to facilitate consistent and high-quality technique, interpretation, reporting,

and data collection for diagnosis of HCC in patients at risk of HCC, initially for CT and MRI in 2011^[18]. Recognizing the value and worldwide utilization of CEUS to diagnose HCC, ACR convened a Working Group of international experts in CEUS to develop CEUS LI-RADS® starting in April 2014. The first official version of CEUS LI-RADS® (version 2016) was published online in September 2016^[19].

Similar to CT/MRI LI-RADS®, CEUS LI-RADS® is updated at regular intervals. As of the time of writing, the Working Group is finalizing the CEUS LI-RADS® v2019 manual. The most updated versions of LI-RADS®, including CEUS LI-RADS® version 2017, can be found on the ACR website (<https://www.acr.org/Clinical-Resources/Reporting-and-Data-Systems/LI-RADS/>).

CEUS is recognized as one of the imaging modalities for HCC diagnosis by many societies worldwide including European Association for the Study of the Liver (EASL)^[2], the Asian Pacific Association for the Study of the Liver (APASL)^[20], Japanese Society of Hepatology^[21], Korean Liver Cancer Study Group-National Cancer Center^[22], Canadian Association for the Study of the Liver (CASL)^[23], Italian Association for the Study of the Liver (AISF)^[24], and the World Federation for Ultrasound in Medicine and Biology-European Federation of Society for Ultrasound in Medicine and Biology (WFUMB-EFSUMB)^[17]. Historically it was removed in the prior American Association for the Study of Liver Diseases (AASLD) guidelines^[25] and EASL 2012 guidelines^[26] due to the concern of misdiagnosis of ICC as HCC based on a small retrospective study^[27]. The authors reviewed 21 ICC cases retrospectively and reported 10/21 (47.6%) of ICC showed homogeneous hyperenhancement followed by a washout on CEUS, therefore they could have been misdiagnosed as HCC. However, subsequent experience and publications do not support the interpretation of this small retrospective study^[28-30]. While current EASL guidelines now endorse CEUS^[2], AASLD has not included CEUS in its new guidelines^[3]. More data and experience in the United States may help recognition of CEUS for HCC diagnosis in the United States.

MAJOR FEATURES OF CEUS LI-RADS®

The characteristic appearance of HCC by CEUS is due to the purely intravascular nature of the microbubble contrast agent as well as the biology of HCC, which is a vascular tumor that derives its blood supply from the hepatic artery (and not the portal vein). As a result, HCC has a typical CEUS appearance of arterial phase hyperenhancement (APHE) and relative hypoenhancement (washout) in the portal venous or late phase compared to the surrounding liver^[9]. The washout of HCC is most commonly late (defined as > 60 s) and mild. As a result of these observations regarding the appearance of HCC by CEUS, the “major features” that are used to define HCC by CEUS LI-RADS® are arterial phase hyperenhancement and late and mild washout^[19].

DESCRIPTION OF THE CEUS LI-RADS ALGORITHM

The algorithm of CEUS LI-RADS® integrates the major features of HCC by CEUS (enhancement and washout) as well as size to stratify the likelihood of HCC by imaging appearance^[19]. The stratification is performed according to the same Likert scale of CT/MRI LI-RADS®, from LI-RADS 1 (LR-1) meaning definitely benign to LI-RADS 5 (LR-5) meaning definitely HCC [Table 1]. Figure 1 is the CEUS LI-RADS® algorithm and its diagnostic table for LR-3 to LR-5.

If one or more major features cannot be assessed due to image omission or degradation, the nodule/observation should be designated as LR-NC (non-categorizable).

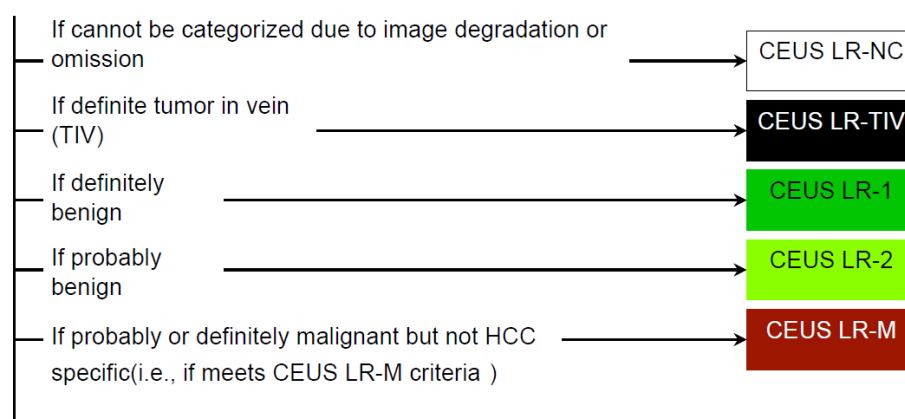
Tumor in a vein (LR-TIV) is categorized when the classic arterial hyperenhancement and late mild washout of HCC are seen in soft tissue mass within a vein. A review and subsequent meta-analysis of the diagnostic accuracy of CEUS for diagnosis of a tumor in vein support high sensitivity and specificity from several

Table 1. The CEUS LI-RADS categories

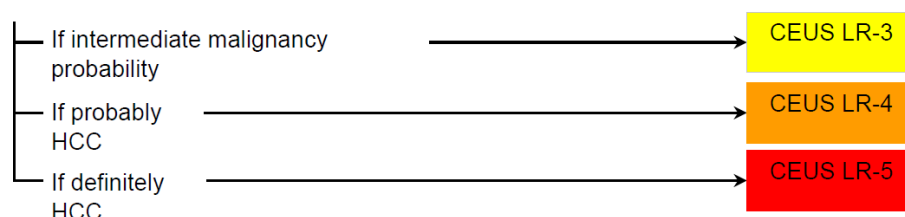
CEUS LR-NC	Not Categorizable: The observation that cannot be meaningfully categorized because image omission or degradation prevents the assessment of one or more major features
CEUS LR-1	Definitely Benign: 100% certainty that the observation is not malignant
CEUS LR-2	Probably Benign: High probability but not 100% certainty that the observation is not malignant
CEUS LR-3	Intermediate probability of malignancy: Non-malignant and malignant entities each have a moderate probability
CEUS LR-4	Probably HCC: High probability but not 100% certainty that the observation is HCC
CEUS LR-5	Definitely HCC: 100% certainty that the observation is HCC
CEUS LR-M	Probably or definitely malignant, not HCC specific: High probability or 100% certainty observation is malignant but features are not HCC specific
CEUS LR-TIV	Tumor in vein: 100% certainty there is malignancy with tumor in vein

CEUS LI-RADS: Contrast-Enhanced Ultrasound Liver Reporting and Data System; HCC: hepatocellular carcinoma

Untreated observation visible on precontrast US and without pathologic proof



Otherwise, use CEUS diagnostic table below



Arterial phase hyperenhancement (APHE)	No APHE		APHE (not rim, not peripheral discontinuous globular)	
Nodule size (mm)	< 20	≥ 20	< 10	≥ 10
No washout of any type	CEUS LR-3	CEUS LR-3	CEUS LR-3	CEUS LR-4
Late and mild washout	CEUS LR-3	CEUS LR-4	CEUS LR-4	CEUS LR-5

Figure 1. The CEUS LI-RADS Algorithm and Diagnostic Table (reproduced with permission from the ACR). CEUS LI-RADS: Contrast-Enhanced Ultrasound Liver Reporting and Data System; HCC: hepatocellular carcinoma; ACR: American College of Radiology

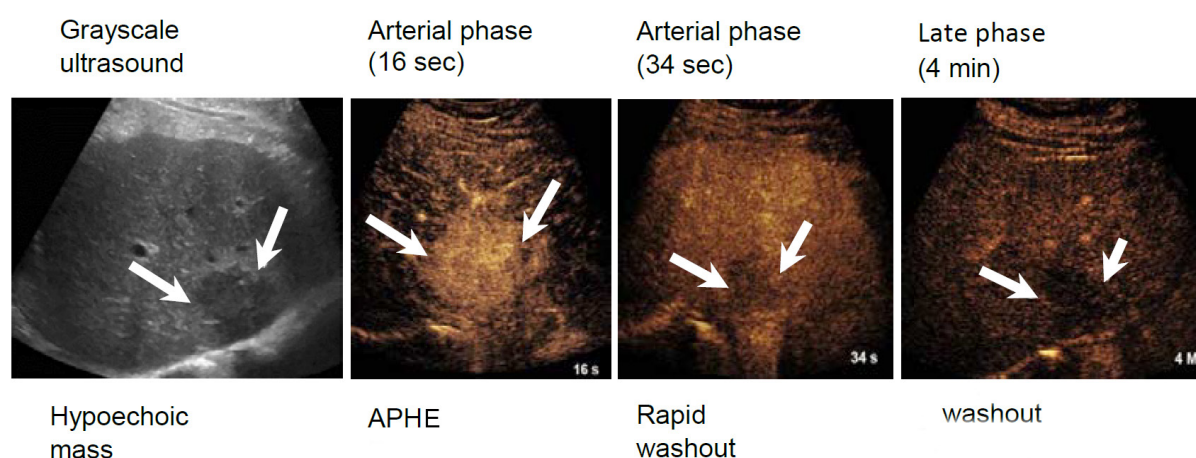


Figure 2. Example case of a LR-M nodule. 78-year-old male with HBV and a growing nodule on MRI, approximately 3 cm, previously diagnosed as a hemangioma on MRI. CEUS imaging demonstrated arterial phase hyperenhancement at 16 s followed by almost immediate washout by 34 s. Biopsy showed the mass to be intrahepatic cholangiocarcinoma (image reproduced with permission from the ACR). MRI: magnetic resonance imaging; HBV: hepatitis B virus; CEUS: contrast-enhanced ultrasound; ACR: American College of Radiology

published studies^[31,32], with a pooled sensitivity of 94% and a pooled specificity of 99%. On the other hand, diagnosis using CT or MRI can be more challenging with a poorer reported performance for more widely available CT/MRI techniques. The reported sensitivity and specificity for contrast-enhanced CT is 43% and 100%, respectively^[33]. More recent reports of conventional MRI findings suggest that sensitivity and specificity up to 100% and 90%, respectively, can be achieved with a “careful” evaluation of findings^[34]. Arguably, diagnosis is simpler and more direct using CEUS as indirect signs (such as primary mass size and distance from a vein) are not used.

LR-1 (definitely benign) observations include cysts, hemangiomas, focal hepatic fat deposition, focal hepatic fat sparing, and hypertrophic pseudo mass. The reader is referred to several excellent reviews for a thorough description of the imaging appearance of these entities^[35-39]. An examination performed as a followup of observation could also be categorized as LR-1 if the observation spontaneously disappeared on followup.

LR-2 (probably benign) observations include several classes of nodules. First, a distinct nodule was seen on non-contrast grayscale ultrasound < 10 mm without APHE is categorized at LR-2 (if ≥ 10 mm, the nodule would be categorized as LR-3). Nonmasslike (i.e., regional or geographic) isoenhancement of any size would also be categorized as LR-2. Second, an LR-3 nodule that has been stable for ≥ 2 years could be downgraded to an LR-2. Third, observations that are *probably* LR-1 - cysts, hemangiomas, focal hepatic fat deposition, focal hepatic fat sparing, and hypertrophic pseudo mass - but are not *definite* by imaging criteria would be categorized as LR-2.

LR-M (Probably or malignant, not HCC specific) is an important category. Major criteria include rim arterial phase hyperenhancement with any washout, OR early (< 1 min) OR marked (becomes black, punched out appearance within 2 min). An example LR-M case is shown in Figure 2. The existence of the LR-M category is crucial to maintain the high specificity of LR-5. All LR-5 should be HCC, while not all HCC are categorized in LR-5. LR-M are typically non-hepatocellular malignancy such as ICC, and metastasis, however, some HCC especially poorly differentiated HCC is most likely categorized as LR-M. LR-M are recommended to have a biopsy for diagnosis, therefore, the diagnosis will be accurately achieved not by imaging but by histology.

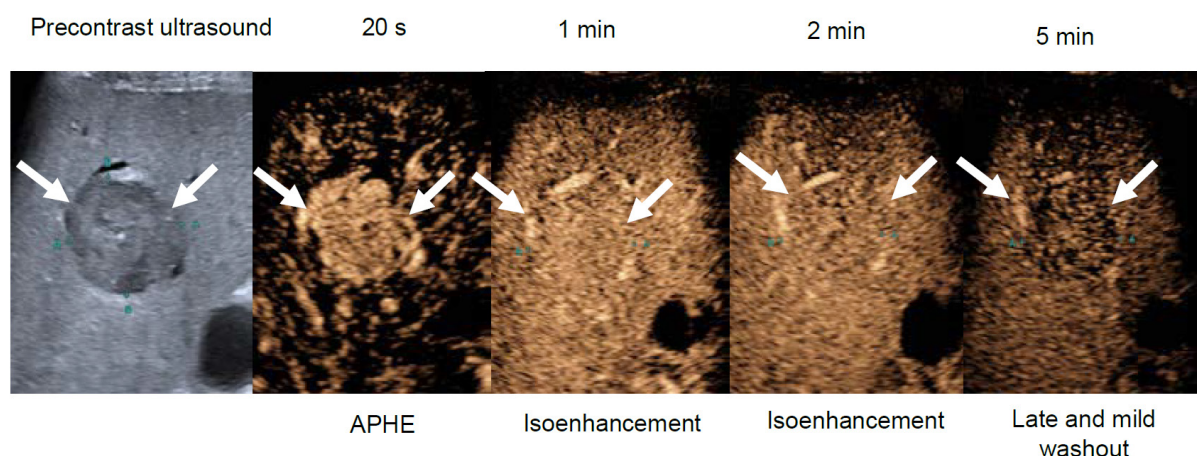


Figure 3. Example case of a LR-5 nodule. A 34 mm observation in a 66 year-old man with HCV cirrhosis, demonstrating arterial phase hyperenhancement (APHE), isoenhancement through the portal venous and late phase, with late mild washout observed at 5 min after contrast injection. (Image reproduced with permission from the ACR). HCV: hepatitis C virus; ACR: American College of Radiology

After the above lesions are categorized appropriately and therefore excluded from consideration as possible or definite HCC, the CEUS LI-RADS[®] diagnostic table is applied to categorize LR-3, LR-4, and LR-5. The table integrates the presence of arterial hyperenhancement, presence of late mild washout, and nodule size [Figure 1]. It is important to distinguish that the arterial hyperenhancement used to categorize nodules as LR-3, LR-4, or LR-5 should not be “rim” or peripherally continuous, as this is not characteristic of HCC. Rather, such arterial phase hyperenhancement would categorize a nodule as LR-M (probably or definitely malignant but not HCC specific). Also, if a nodule shows washout and it is not the late mild washout typical of HCC, then such a nodule would be categorized as LR-M. Examples of washout atypical of HCC include early washout (within 60 s) and marked washout that results in a “punched out” appearance within 2 min. Figure 3 is a typical HCC (LR-5) case.

After the above algorithm is applied, ancillary imaging features may be used to upgrade or downgrade a nodule between CEUS LI-RADS[®] categories. These ancillary imaging features include nodule-in-nodule/mosaic architecture (favoring HCC in particular), definite growth (favoring malignancy, not HCC in particular), size stability more than 2 years and size reduction (favoring benignity). Of note, a nodule cannot be upgraded to LR-5 in keeping with stringent criteria to maintain high specificity of an LR-5 categorization, and imaging features can only upgrade/downgrade by a maximum of one category (i.e., LR-3 to LR-4). Additionally, CEUS LI-RADS[®] has fewer ancillary imaging features than CT/MR LI-RADS[®].

INDICATIONS FOR CEUS

Taking into account the advantages and potential drawbacks of CEUS, as well as the major features and algorithm of CEUS LI-RADS[®], the following are common indications for CEUS in patients at risk for HCC: (1) assessment of nodules ≥ 10 mm detected on surveillance ultrasound; (2) assessment of observations that are indeterminate on prior CT or MRI (i.e., LR-3, LR-4, or LR-M); (3) detection of arterial phase hyperenhancement when it is suspected that contrast mistiming is suspected as a cause of lack of arterial enhancement on prior CT or MRI; (4) detection of CEUS washout when CT or MRI washout is indeterminate but shows APHE; (5) further evaluation of biopsied observation with inconclusive histology; (6) guiding biopsy or percutaneous ablation of observations difficult to visualize with precontrast US; (7) guiding biopsy of heterogeneous observations; (8) monitor changes in enhancement pattern over time for selected CEUS LR-3 or CEUS LR-4 observations; and (9) differentiating tumor in vein (‘tumor thrombus’) from bland thrombus. With regards to the first indication listed above, studies and experience have shown

that having CEUS available at the time of surveillance ultrasound reduces time to diagnosis, healthcare expenditures, and patient anxiety^[12,40].

Similar to CT/MRI LI-RADS®, CEUS LI-RADS® should only be applied in populations at risk for HCC by AASLD guidelines, including patients with cirrhosis of any etiology or noncirrhotic patients with chronic hepatitis B.

DIFFERENCES BETWEEN CEUS LI-RADS AND CT/MRI LI-RADS

As the reader may be more acquainted with CT/MRI LI-RADS®, we will highlight some differences between CEUS LI-RADS® and CT/MRI LI-RADS® to reinforce the utility and some unique aspects of CEUS.

CEUS practitioners have long used the term “nodule” to describe possible lesions of interest, so in CEUS LI-RADS® “nodule” is often used interchangeably with the term “observation”. In CT/MRI LI-RADS®, the term “observation” is favored as APHE on CT/MRI may be due to arteriportal shunting or other pseudo-lesions. By contrast, CEUS does not show these AP shunts and is a very important tool to differentiate them from HCC without washout^[10]. Conversely, a suspected arteriportal shunt on CT/MRI associated with a distinct nodule on US will raise suspicion for HCC^[41].

CEUS and CT/MRI exhibit differences in enhancement and washout due to differences in the contrast agents employed for each. CEUS agents are several microns in diameter, which confines them to be purely intravascular contrast agents. CT/MRI contrast agents are much smaller in size and therefore have an extracellular interstitial phase of contrast. As a result of these differences, a “capsule” appearance is typically seen in HCC when assessed by CT/MRI and is therefore a major criterion for categorization as LR-5 by CT/MRI LI-RADS®. Capsule appearance is not seen by CEUS and is therefore not one of the major features used for categorization by CEUS LI-RADS®^[10].

DISCUSSION

Structured reporting systems such as BI-RADS® and LI-RADS® emerged due to the need for increased consistency of imaging diagnostics^[18]. These systems typically are created based on existing data and expertise, but the validation of real-world use is critical. An international multi-center prospective clinical trial has been organized by members of the CEUS LI-RADS® working group to assess the diagnostic accuracy of CEUS for HCC diagnosis in patients at risk for HCC, assess the interreader reliability of diagnosis, and to validate CEUS LI-RADS®^[42]. Recruitment is still ongoing as of the time of writing.

There are existing retrospective data to support the use of CEUS LI-RADS® and provide the prevalence of HCC within each of the categories from a large international case series out of Europe and Canada. Terzi *et al.*^[43] showed that in 848 patients with 1006 lesions of median size 2 cm, the specificity of LR-5 for HCC diagnosis was 98.5%. Concern has been raised historically that CEUS may misdiagnose ICC, however, this was not found to be of concern in this large case series. None of the 519 LR-5 nodules were pure ICC, although one nodule was found to represent mixed HCC-ICC. Regarding the LR-M category (8% of lesions), 38% were ICC, 10% were mixed HCC-ICC, 48% were HCC, and 2% were metastases. A large study of 2020 patients with hepatitis B virus (HBV) infection from China validated CEUS LI-RADS® for patient populations in whom HBV is endemic - with LR-5 showing high specificity of 96% and positive predictive value of 98% for the diagnosis of HCC^[44]. More recently, similar diagnostic accuracy was observed in another large retrospective study of patients from China who were predominantly (92%) HBV positive - with LR-5 showing a sensitivity and specificity of 73% and 97%, respectively for nodules 20 mm or less^[45].

Retrospective data regarding the interreader reliability of CEUS LI-RADS® is encouraging. The largest

retrospective study of 1,366 patients showed excellent specificity for HCC of 90.2% with an interreader agreement of 0.61-0.73 for LI-RADS categories. Another retrospective study of 258 patients comparing inexperienced and experienced radiologists showed that not only was interreader agreement high between the two groups, but diagnostic accuracy was also excellent when using CEUS LI-RADS® (sensitivity 84.2%-87.5%, sensitivity 90.6%-97%)^[46]. A smaller case series of 50 patients found that the interreader agreement of CEUS LI-RADS® categories was only fair, with a kappa of 0.309, and that agreement of APHE was higher than that of washout^[47].

CONCLUSION

In summary, CEUS LI-RADS® is a recent addition to the constellation of LI-RADS® structured reporting systems for HCC. Retrospective data supports a high specificity for the diagnosis of HCC by the LR-5 category, and a large multi-center prospective validation study is ongoing. Additional future areas of expansion of CEUS LI-RADS® include treatment assessment.

DECLARATIONS

Author contributions

Conception and writing of this work: Vezeridis AM, Kono Y

Availability of data and materials

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Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358-80.
2. European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236.
3. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;68:723-50.
4. Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, et al. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver - update 2012: a WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultrasound Med Biol* 2013;39:187-210.
5. Jo PC, Jang HJ, Burns PN, Burak KW, Kim TK, et al. Integration of contrast-enhanced US into a multimodality approach to imaging of nodules in a cirrhotic liver: how i do it. *Radiology* 2017;282:317-31.
6. Quaia E. Microbubble ultrasound contrast agents: an update. *Eur Radiol* 2007;17:1995-2008.
7. Ophir J, Parker KJ. Contrast agents in diagnostic ultrasound. *Ultrasound Med Biol* 1989;15:319-33.
8. Wilson SR, Burns PN. An algorithm for the diagnosis of focal liver masses using microbubble contrast-enhanced pulse-inversion

- sonography. *AJR Am J Roentgenol* 2006;186:1401-12.
9. Yang HK, Burns PN, Jang HJ, Kono Y, Khalili K, et al. Contrast-enhanced ultrasound approach to the diagnosis of focal liver lesions: the importance of washout. *Ultrasonography* 2019;38:289-301.
10. Kim TK, Noh SY, Wilson SR, Kono Y, Piscaglia F, et al. Contrast-enhanced ultrasound (CEUS) liver imaging reporting and data system (LI-RADS) 2017 - a review of important differences compared to the CT/MRI system. *Clin Mol Hepatol* 2017;23:280-9.
11. Sugimoto K, Moriyasu F, Shiraishi J, Saito K, Taira J, et al. Assessment of arterial hypervascularity of hepatocellular carcinoma: comparison of contrast-enhanced US and gadoxetate disodium-enhanced MR imaging. *Eur Radiol* 2012;22:1205-13.
12. Streb JW, Tchelepi H, Malhi H, Deurdulian C, Grant EG. Retrospective analysis of contrast-enhanced ultrasonography effectiveness in reducing time to diagnosis and imaging-related expenditures at a single large United States County hospital. *Ultrasound Q* 2019;35:99-102.
13. Piscaglia F, Bolondi L, Italian Society for Ultrasound in M, Biology Study Group on Ultrasound Contrast A. The safety of Sonovue in abdominal applications: retrospective analysis of 23188 investigations. *Ultrasound Med Biol* 2006;32:1369-75.
14. Yusuf GT, Sellars ME, Deganello A, Cosgrove DO, Sidhu PS. Retrospective analysis of the safety and cost implications of pediatric contrast-enhanced ultrasound at a single center. *AJR Am J Roentgenol* 2017;208:446-52.
15. Ntoulia A, Anupindi SA, Darge K, Back SJ. Applications of contrast-enhanced ultrasound in the pediatric abdomen. *Abdom Radiol (NY)* 2018;43:948-59.
16. Lyschchik A, Kono Y, Dietrich CF, Jang HJ, Kim TK, et al. Contrast-enhanced ultrasound of the liver: technical and lexicon recommendations from the ACR CEUS LI-RADS working group. *Abdom Radiol (NY)* 2018;43:861-79.
17. Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, et al. Guidelines and good clinical practice recommendations for Contrast Enhanced Ultrasound (CEUS) in the liver - update 2012: a WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultrasound Med Biol* 2013;39:187-210.
18. Tang A, Singal AG, Mitchell DG, Hecht EM, Fowler KJ, et al. Introduction to the Liver Imaging Reporting and Data System for Hepatocellular Carcinoma. *Clin Gastroenterol Hepatol* 2019;17:1228-38.
19. Kono Y, Lyschchik A, Cosgrove D, Dietrich CF, Jang HJ, et al. Contrast Enhanced Ultrasound (CEUS) Liver Imaging Reporting and Data System (LI-RADS®): the official version by the American College of Radiology (ACR). *Ultraschall Med* 2017;38:85-6.
20. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017;11:317-70.
21. Kudo M, Matsui O, Izumi N, Iijima H, Kadoya M, et al. JSH Consensus-Based Clinical Practice Guidelines for the Management of Hepatocellular Carcinoma: 2014 Update by the Liver Cancer Study Group of Japan. *Liver Cancer* 2014;3:458-68.
22. Korean Liver Cancer Study G, National Cancer Center K. 2014 Korean Liver Cancer Study Group-National Cancer Center Korea practice guideline for the management of hepatocellular carcinoma. *Korean J Radiol* 2015;16:465-522.
23. Burak KW, Sherman M. Hepatocellular carcinoma: consensus, controversies and future directions. A report from the Canadian Association for the Study of the Liver Hepatocellular Carcinoma Meeting. *Can J Gastroenterol Hepatol* 2015;29:178-84.
24. Italian Association for the Study of the L, Panel AE, Committee AC, Bolondi L, Cillo U, Colombo M, et al. Position paper of the Italian Association for the Study of the Liver (AISF): the multidisciplinary clinical approach to hepatocellular carcinoma. *Dig Liver Dis* 2013;45:712-23.
25. Bruix J, Sherman M, American Association for the Study of Liver D. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53:1020-2.
26. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012;56:908-43.
27. Vilana R, Forner A, Bianchi L, Garcia-Criado A, Rimola J, et al. Intrahepatic peripheral cholangiocarcinoma in cirrhosis patients may display a vascular pattern similar to hepatocellular carcinoma on contrast-enhanced ultrasound. *Hepatology* 2010;51:2020-9.
28. Han J, Liu Y, Han F, Li Q, Yan C, et al. The degree of contrast washout on contrast-enhanced ultrasound in distinguishing intrahepatic cholangiocarcinoma from hepatocellular carcinoma. *Ultrasound Med Biol* 2015;41:3088-95.
29. Li R, Yuan MX, Ma KS, Li XW, Tang CL, et al. Detailed analysis of temporal features on contrast enhanced ultrasound may help differentiate intrahepatic cholangiocarcinoma from hepatocellular carcinoma in cirrhosis. *PLoS One* 2014;9:e98612.
30. Wildner D, Bernatik T, Greis C, Seitz K, Neurath MF, et al. CEUS in hepatocellular carcinoma and intrahepatic cholangiocellular carcinoma in 320 patients - early or late washout matters: a subanalysis of the DEGUM multicenter trial. *Ultraschall Med* 2015;36:132-9.
31. Danila M, Sporea I, Popescu A, Șirli R. Portal vein thrombosis in liver cirrhosis - the added value of contrast enhanced ultrasonography. *Med Ultrason* 2016;18:218-33.
32. Chen J, Zhu J, Zhang C, Song Y, Huang P. Contrast-enhanced ultrasound for the characterization of portal vein thrombosis vs tumor-in-vein in HCC patients: a systematic review and meta-analysis. *Eur Radiol* 2020;30:2871-80.
33. Tublin ME, Dodd GD, Baron RL. Benign and malignant portal vein thrombosis: differentiation by CT characteristics. *AJR Am J Roentgenol* 1997;168:719-23.
34. Sandrasegaran K, Tahir B, Nutakki K, Akisik FM, Bodanapally U, et al. Usefulness of conventional MRI sequences and diffusion-weighted imaging in differentiating malignant from benign portal vein thrombus in cirrhotic patients. *AJR Am J Roentgenol* 2013;201:1211-9.
35. Burrowes DP, Medellin A, Harris AC, Milot L, Wilson SR. Contrast-enhanced US approach to the diagnosis of focal liver masses. *Radiographics* 2017;37:1388-400.
36. Burrowes DP, Kono Y, Medellin A, Wilson SR. Update: contrast-enhanced US approach to the diagnosis of focal liver masses. *Radiographics* 2020;40:E16-20.
37. Dietrich CF, Tana C, Caraianni C, Dong Y. Contrast enhanced ultrasound (CEUS) imaging of solid benign focal liver lesions. *Expert Rev*

- Gastroenterol Hepatol 2018;12:479-89.
38. Dietrich CF. Contrast-enhanced ultrasound of benign focal liver lesions. *Ultraschall Med* 2019;40:12-29.
 39. Kim TK, Lee E, Jang HJ. Imaging findings of mimickers of hepatocellular carcinoma. *Clin Mol Hepatol* 2015;21:326-43.
 40. Lanka B, Jang HJ, Kim TK, Burns PN, Wilson SR. Impact of contrast-enhanced ultrasonography in a tertiary clinical practice. *J Ultrasound Med* 2007;26:1703-14.
 41. Hu J, Bhayana D, Burak KW, Wilson SR. Resolution of indeterminate MRI with CEUS in patients at high risk for hepatocellular carcinoma. *Abdom Radiol (NY)* 2020;45:123-33.
 42. Kono Y, Sirlin CB, Fetzer DT, Kim TK, Rodgers SK, et al. Time to clarify common misconceptions about the liver imaging reporting and data system for contrast-enhanced US. *Radiology* 2020;295:245-7.
 43. Terzi E, Iavarone M, Pompili M, Veronese L, Cabibbo G, et al. Contrast ultrasound LI-RADS LR-5 identifies hepatocellular carcinoma in cirrhosis in a multicenter retrospective study of 1,006 nodules. *J Hepatol* 2018;68:485-92.
 44. Zheng W, Li Q, Zou XB, Wang JW, Han F, et al. Evaluation of contrast-enhanced US LI-RADS version 2017: application on 2020 liver nodules in patients with hepatitis B infection. *Radiology* 2020;294:299-307.
 45. Huang JY, Li JW, Lu Q, Luo Y, Lin L, et al. Diagnostic accuracy of CEUS LI-RADS for the characterization of liver nodules 20 mm or smaller in patients at risk for hepatocellular carcinoma. *Radiology* 2020;294:329-39.
 46. Wang JY, Feng SY, Xu JW, Li J, Chu L, et al. Usefulness of the contrast-enhanced ultrasound liver imaging reporting and data system in diagnosing focal liver lesions by inexperienced radiologists. *J Ultrasound Med* 2020; doi: 10.1002/jum.15242.
 47. Schellhaas B, Hammon M, Strobel D, Pfeifer L, Kielisch C, et al. Interobserver and intermodality agreement of standardized algorithms for non-invasive diagnosis of hepatocellular carcinoma in high-risk patients: CEUS-LI-RADS versus MRI-LI-RADS. *Eur Radiol* 2018;28:4254-64.

Original Article

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Validation of novel Japanese indication criteria and biomarkers among living donor liver transplantation recipients with hepatocellular carcinoma - a single center retrospective study

Akihiko Ichida, Nobuhisa Akamatsu, Kiyoshi Hasegawa

Artificial Organ and Transplantation Surgery Division, Department of Surgery, University of Tokyo, Tokyo 113-8655, Japan.

Correspondence to: Dr. Nobuhisa Akamatsu, Artificial Organ and Transplantation Surgery Division, Department of Surgery, Graduate school of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: nakamats-tyk@umin.ac.jp

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Abstract

Aim: To validate a novel Japanese indication criteria for liver transplantation (LT) for hepatocellular carcinoma (HCC), i.e., the 5-5-500 criteria (nodule size ≤ 5 cm in diameter, nodule number ≤ 5 , and alfa-fetoprotein (AFP) value ≤ 500 ng/mL) and the Japanese double eligibility criteria (DEC) (patients meeting the Milan or the 5-5-500 criteria) in the University of Tokyo cohort. The usefulness of biomarkers in predicting the recurrence of HCC was also verified.

Methods: The overall survival and recurrence rates of patients meeting the Milan, 5-5-500, and the Japanese DEC were compared among 153 patients who underwent living donor LT (LDLT) between 1996 and 2019. A receiver-operating characteristics curve analysis was conducted to evaluate the usefulness of AFP, lens culinaris agglutinin-reactive fraction of AFP, des-gamma-carboxy prothrombin, neutrophil-lymphocyte ratio, and the platelet-lymphocyte ratio to detect recurrence.

Results: The 5-year recurrence rate for all patients, those meeting the Japanese DEC, 5-5-500 criteria, and the Milan criteria was 10.9%, 9.2%, 7.4%, and 7.6%, respectively. Compared with the conventional Milan criteria, the 5-5-500 criteria and the Japanese DEC could increase the number of eligible LDLT candidates by 6.1% and 11.4%. Among five biomarkers, the area under the curve value of AFP was the highest (0.852).



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Conclusion: The results suggest that the 5-5-500 criteria and the Japanese DEC are the appropriate selection criteria for patients with HCC in LDLT. Among five biomarkers investigated, AFP was most reliable to predict HCC recurrence, which justified the utilization of AFP in the 5-5-500 criteria and the Japanese DEC.

Keywords: Indication criteria of liver transplantation for hepatocellular carcinoma, the 5-5-500 criteria, the Japanese double eligibility criteria, alfa-fetoprotein, the lens culinaris agglutinin-reactive fraction of alfa-fetoprotein, the des-gamma-carboxy prothrombin, the neutrophil-lymphocyte ratio, the platelet-lymphocyte ratio

INTRODUCTION

Expansion of the conventional Milan criteria^[1] has been debated over the last two decades^[2-11]. Though recurrence of hepatocellular carcinoma (HCC) after liver transplantation (LT) has decreased since the proposal of the Milan criteria in 1996, it has been criticized as being too restrictive^[11]. Initially, several extended criteria focused on expanding the upper limit of tumor size and number were reported^[2-5]. In recent years, however, many authors have reported that an extended criteria should include the parameters reflecting the biologic behavior of the tumor^[6-10]. With regard to the biomarkers incorporated into the expanded criteria, tumor markers such as alfa-fetoprotein (AFP)^[7,10,12-15] and des-gamma-carboxy prothrombin (DCP)^[6,8,15] have been investigated by many researchers and recently, other markers such as the neutrophil-lymphocyte ratio (NLR)^[16,17], the platelet-lymphocyte ratio (PLR)^[17], and fluorine-18-fluorodeoxyglucose positron emission tomography (FDG-PET)^[18,19] were reported to be useful for selection of the high risk group and prediction for recurrence. Another biomarker, the lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), is widely used for the prediction of malignant biological behavior and poor prognosis of HCC patients in Japan, and may be a possible biomarker for recurrent HCC after LT^[20-23].

In Japan, living-donor liver transplantation (LDLT) has been the mainstay for end-stage liver disease patients with HCC due to the severe scarcity of deceased donors. While the gold standard has long been the Milan criteria, several center-oriented expanded criteria have been reported^[3,6,8]. We proposed the Tokyo criteria in 2007, the detail of which was as follows; the number of tumors should be five or less, and the maximum diameter should be 5 cm or less, without distant metastasis nor vascular invasion^[3]. Similarly, Kyoto and Kyushu advocated their own expanded criteria and included DCP as a biological marker^[6,8]. These expanded criteria, however, had not been approved by the government, and those beyond the Milan criteria but still within each expanded criterion had to undergo LDLT as private practice, which led us to establish the government-approved expanded criterion. Most recently, the 5-5-500 criteria (nodule size \leq 5 cm in diameter, nodule number \leq 5, and AFP value \leq 500 ng/mL) was established based on retrospective data analysis of the Japanese Liver Transplant Registry by our colleagues^[24]. This expanded criteria was approved as the new national selection criteria for liver transplant candidates with HCC and started in August 2019. Now, the double eligibility criteria (DEC), Milan + 5-5-500, has been adopted as the new indication criteria for Japanese patients with HCC.

The aim of the present study was to validate the Japanese DEC and the 5-5-500 criteria in our single-center cohort. In addition, the usefulness of biological markers (AFP, AFP-L3, DCP, NLR, and PLR) in predicting the recurrence of HCC after LT was also verified.

METHODS

Patients

From January 1996 until the end of 2019, a total of 563 adult patients underwent LDLT at the University of Tokyo Hospital. Among them, 153 patients were treated for HCC and were the subjects of the present study. Preoperative diagnosis of HCC was based on dynamic multi-detector computed tomography (MDCT)

performed within a month before LT in all cases. Lesions presenting with typical radiological characteristics of classical HCC, that is, lesions with arterial phase enhancement and low density during the portal phase, were diagnosed as HCC to be counted and measured. In cases that underwent pretransplant locoregional treatments, only the size of the viable lesion was measured on the basis of MDCT before LDLT, and on the basis of pathological findings after LDLT. Essentially, we used the Milan criteria as a standard indication for LT for HCC; however, we allowed the expanded criteria, i.e., Tokyo criteria, in a private practice setting as mentioned above. Six cases exceeding the Tokyo criteria exceptionally, underwent LDLT in the early period. We did not use biomarkers such as AFP and DCP in patient selection.

Donor selection and postoperative management

Until 2015, the estimated graft volume to the recipient standard liver volume (SLV) ratio must be over 40% for LT at our institution. Since 2016, we have changed the threshold of the graft volume criteria to 35% of the recipient SLV. The left liver was the first choice for the graft if it satisfied the lower limit. Otherwise, right liver procurement was indicated if the estimated right liver graft volume was less than 70% of the donor's total liver volume, and a right lateral sector graft was used in selected cases. Details of donor evaluation and graft selection are described elsewhere^[25]. The basic immunosuppression regimen comprised tacrolimus and steroid for all recipients, and the doses of each drug were gradually tapered over 6 months after LDLT. Our detailed postoperative recipient management including the immunosuppression protocol has been described elsewhere^[26]. We do not modify immunosuppression for HCC recipients and do not use m-TOR inhibitors nor adjuvant chemotherapies. All patients were followed up at our department after LT according to the following protocol: monthly measurements of AFP and DCP, abdominal ultrasound performed every 3 months, and contrast-enhanced dynamic MDCT every 6 months. Recurrence was defined as the emergence of radiological findings in MDCT or magnetic resonance imaging compatible with typical HCC.

Statistical analysis

Categorical variables were expressed as number (%) and continuous variables were expressed as median with range. NLR and PLR were calculated by dividing the number of neutrophils or platelets, respectively, by the number of lymphocytes. Patient overall survival and recurrence rates were calculated using Kaplan-Meier with Log rank test. A receiver-operating characteristics (ROC) curve analysis and Youden index were used to define the ideal cut-off values for AFP, AFP-L3, DCP, NLR, and PLR to detect recurrence. Univariate and multivariate analysis was performed using a Cox proportional hazards model to identify the predictors of recurrence. Factors with a P value less than 0.05 in a Cox proportional-hazard model as a univariate analysis were considered potential risk factors and further analyzed in a multivariate Cox model. The hazard ratio (HR) and 95% confidence interval (CI) were calculated for each variable. Although seventeen variables listed in the table were examined as potential risk factors, AFP-L3 was excluded from multivariate analysis because of the quantity of missing data (AFP-L3 was not checked in 19 patients). Beyond the Milan, 5-5-500, and Japanese DEC were also excluded from multivariate analysis because they were not considered to be independent factors but composite factors having a strong relation to tumor number, size, and AFP value. All statistical calculations were performed using JMP Pro 15 (SAS Institute Inc., Cary, NC, USA). P values less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

Recipient demographics are summarized in Table 1. There were 116 males and 37 females, with a median age of 56 (range, 37-67) years. The median model for end-stage liver disease score was 12 (range, 2-34). Hepatitis C virus positive cases comprised 57% of the cohort. Sixty percent (92/153) had undergone locoregional treatments for HCC before LT, including 69 cases with transcatheter arterial chemoembolization, 31 with radiofrequency ablation, 23 with percutaneous ethanol injection therapy, and 14 with surgical

Table 1. Characteristics of recipients with hepatocellular carcinoma

	Recipients with hepatocellular carcinoma (n = 153)
Pretransplant factors	
Age (years)	56 (37-67)
Gender (male/female)	116/37
Model for end-stage liver disease score	12 (2-34)
Child-Pugh score	9 (5-14)
Child-Pugh classification	
Child A	11 (7%)
Child B	70 (46%)
Child C	72 (47%)
Etiology	
HBV	44 (29%)
HCV	85 (56%)
HBV, HCV, co-infection	2 (1%)
Alcohol	6 (4%)
Primary Biliary Cholangitis	5 (3%)
Nonalcoholic Steatohepatitis	4 (3%)
Other	7 (5%)
Pretransplant treatments	
Transcatheter arterial chemoembolization	69 (45%)
Radiofrequency ablation	31 (20%)
Percutaneous ethanol injection therapy	23 (15%)
Liver resection	14 (9%)
Proton therapy	1 (1%)
Biomarker	
AFP (ng/mL)	16 (1-11999)
AFP-L3 (%)	5 (undetectable-88)
DCP (mAU/mL)	33 (6-13248)
NLR	2.4 (0.5-27.7)
PLR	74 (18-578)
Tumor number (pretransplant)	1 (0-14)
Tumor size (pretransplant) (cm)	2 (0-8)
Tumor number (explants)	2 (1-19)
Tumor size (explants) (cm)	2 (0.5-11)
Differentiation	
Well differentiated	67 (44%)
Moderately differentiated	67 (44%)
Poorly differentiated	8 (5%)
Necrosis	11 (7%)
Intraoperative factors	
Operation time (min)	860 (601-1890)
Total blood loss (mL)	5390 (568-53835)
Graft volume (g)	554 (300-880)
Graft volume ratio to standard liver volume (%)	45 (28-68)

HBV: hepatitis B virus; HCV: hepatitis C virus; AFP: alpha-fetoprotein; AFP-L3: lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; DCP: des-gamma-carboxy prothrombin; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio

resections. There were no cases of intentional downstaging or bridging therapy in this cohort. The median number and size of the tumor were 1 (range, 0-14) and 2.0 (range, 0-8.0) cm in the preoperative radiologic evaluation, while these were 2 (range, 1-19) and 2.0 (range, 0.5-11.0) cm in pathological examination of the explants. In histologic examination of explants, vascular invasion was confirmed in 21/153 (13.7%) cases, and 67 (44%), 67 (44%), 8 (5%), and 11 (7%) patients had well-, moderately-, poorly-differentiated HCC, and necrosis, respectively. Two patients were diagnosed as having combined HCC-cholangiocarcinoma histologically. The median follow-up period after LDLT was 139 (range, 1-231) months for all patients.

Validation of the 5-5-500 criteria and the Japanese DEC

The relationship of the Milan criteria, the 5-5-500 criteria, and the Japanese DEC is presented in the Venn diagram [Supplementary Figure 1]. The number of patients and patients with recurrence meeting each indication criteria was summarized in Supplementary Table 1. The recurrence rate was the lowest in patients meeting the 5-5-500 criteria (6.5%) followed by the Milan criteria (6.9%) and then the Japanese DEC (8.2%). All criteria achieved the target of a recurrence rate below 10%. When focusing on each area of the Venn diagram, the recurrence rate was the highest (42.9%) in patients within the Milan but beyond the 5-5-500 criteria and in patients beyond the Japanese DEC. Meanwhile, the recurrence rate in patients within the 5-5-500 but beyond the Milan criteria was lower (20%) than these patients. As for the comparison of the number of patients, the number of patients included in the 5-5-500 criteria was larger than that included in the conventional Milan criteria by eight (6.1% increase). In the Japanese DEC, 15 additional patients were included (11.5% increase). The overall survival and recurrence rate curves in patients meeting each indication criteria are presented in Figure 1. The 5-year overall survival and the 5-year recurrence rate of all the patients, patients meeting the Japanese DEC, 5-5-500 criteria, and Milan criteria was 76.9%, 77.9%, 79.0%, and 76.2%, and 10.9%, 9.2%, 7.4%, and 7.6%, respectively. There was no significant difference both in the 5-year recurrence and 5-year survival rates amongst each criterion.

Usefulness of biomarkers in predicting the recurrence of HCC

The results of the ROC curve analysis for biomarkers is presented in Figure 2. Among the five biomarkers, the area under the curve (AUC) value of AFP was the highest (0.852). The sensitivity of AFP was also the highest (86.7%). Meanwhile, the false-positive rate (1-specificity) of AFP-L3 was the lowest (8.3%). Patient recurrence rate curves stratified by each biomarker using the cutoff value obtained from the ROC curve analysis are presented in Supplementary Figure 2. Though recurrence rate curves were well stratified with AFP, AFP-L3, and DCP ($P < 0.0001$), significant results were not obtained with NLR and PLR ($P = 0.076$ and $= 0.263$ respectively).

Factors associated with HCC recurrence

Risk factors associated with HCC recurrence were evaluated with univariate and multivariate analyses. Univariate analysis revealed that beyond the Milan, 5-5-500, and Japanese DEC were all significant predictors [Table 2]. Among these three criteria, the hazard ratio and P value beyond the 5-5-500 criteria was the highest (7.99) and the smallest (0.0005), respectively. Except for factors associated with these three criteria, the high AFP value ≥ 60 ng/mL, high AFP-L3 value ($\geq 35\%$), high DCP value (≥ 130 mAU/mL), and large tumor size (≥ 2.0 cm) were all identified as significant predictors by univariate analysis. Among the five biomarkers evaluated, the hazard ratio and P value of a high AFP value was the highest (11.50) and the smallest (< 0.0001), respectively. Multivariate analysis revealed that high AFP and DCP values were the independent significant predictors.

DISCUSSION

The results of the present study suggest that the 5-5-500 criteria and the Japanese DEC are appropriate and acceptable since the 5-year recurrence rate in patients meeting these criteria were both below 10% in our cohort. Compared with the conventional Milan criteria, the 5-5-500 criteria and the Japanese DEC could increase the number of eligible LDLT candidates by 6.1% and 11.4%, respectively. As for the usefulness of biomarkers in predicting the recurrence of HCC, AFP seems to be the most reliable. Though there were some missing data, AFP-L3 also seems promising.

In Japan, the national insurance system had restricted LDLT to those falling within the Milan criteria until recently, although some centers have been performing LDLT in private practice with a center-oriented expanded criteria that has achieved a 5-year patient survival over 80% and a 5-year recurrence rate of 10%^[27,28]. Consequently, a few patients had given up the chance for LDLT because of financial reasons,

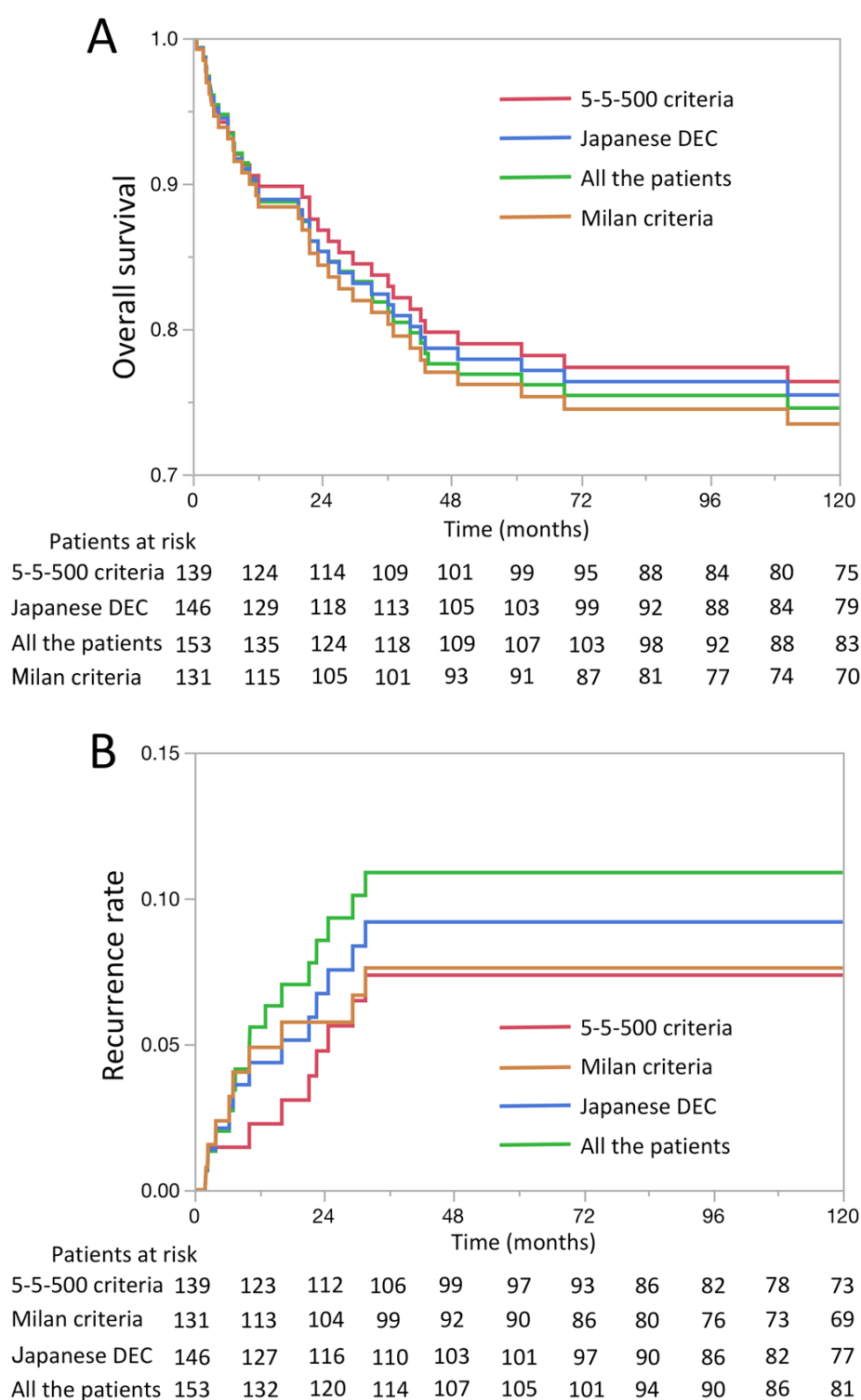
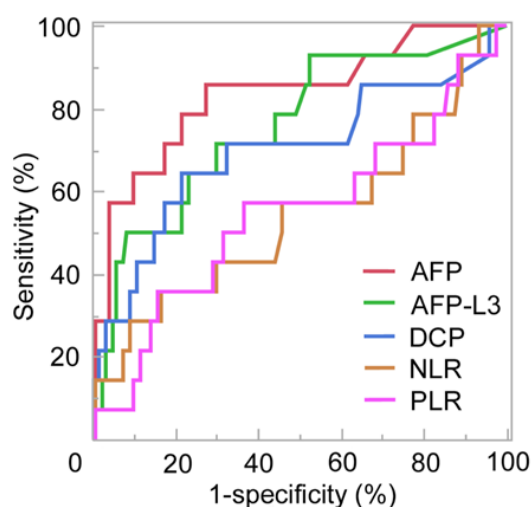


Figure 1. Overall survival (A) and recurrence rate (B) of patients meeting each indication criteria. DEC: double eligibility criteria

despite the potential of a live donor, and there has been strong demands to expand insurance coverage for those beyond the Milan criteria. When establishing government-approved expanded criteria, achieving a 5-year recurrence rate of less than 10% and a 5-year survival rate of over 70% seems reasonable and socially acceptable in the setting of LDLT for HCC^[29], which was achieved in the benchmark study by



	Sensitivity (%)	1-specificity (%)	AUC	Cutoff value
AFP	86.7	23.9	0.852	55ng/mL
AFP-L3	50.0	8.3	0.754	35%
DCP	66.7	20.3	0.719	132mAU/mL
NLR	40.0	15.9	0.564	5.4
PLR	33.3	15.2	0.533	138

Figure 2. Receiver operating characteristic curves of true-positive (sensitivity) vs. false-positive (1 - specificity) rates with respect to recurrence plotted for AFP, AFP-L3, DCP, NLR, and PLR. AFP: alpha-fetoprotein; AFP-L3: lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; DCP: des-gamma-carboxy prothrombin; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; AUC: area under the curve

Table 2. Univariate and multivariate analysis for recurrence

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Age \geq 60 years	1.02 (0.28-2.99)	0.970		
Gender (male)	1.16 (0.37-5.09)	0.816		
MELD score \geq 12	1.22 (0.44-3.64)	0.703		
Child-pugh score \geq 9	1.11 (0.40-3.57)	0.844		
HBV infection	2.00 (0.70-5.58)	0.187		
HCV infection	0.51 (0.17-1.41)	0.194		
Pre-treatment therapies	1.78 (0.61-6.43)	0.303		
AFP \geq 60 ng/mL	11.50 (3.64-50.51)	< 0.0001	6.16 (1.84-28.20)	0.002
AFP-L3 \geq 35%*	8.72 (2.97-25.57)	0.0002		
DCP \geq 130 mAU/mL	7.22 (2.56-23.26)	0.0002	4.42 (1.52-14.60)	0.006
NLR \geq 5	2.47 (0.83-6.85)	0.101		
PLR \geq 140	1.90 (0.53-5.56)	0.298		
Tumor number \geq 2 (pretransplant)	2.46 (0.87-7.90)	0.089		
Tumor size \geq 2.0 cm (pretransplant)	9.06 (1.82-164)	0.004	4.74 (0.90-87.59)	0.071
Beyond Milan criteria*	3.92 (1.31-10.89)	0.016		
Beyond 5-5-500 criteria*	7.99 (2.67-22.22)	0.0005		
Beyond Japanese DEC*	5.80 (1.32-18.33)	0.024		

*These variables were excluded from multivariate analysis. HR: hazard ratio; CI: confidence interval; MELD score: model for end-stage liver disease score; HBV: hepatitis B virus; HCV: hepatitis C virus; AFP: alpha-fetoprotein; AFP-L3: lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; DCP: des-gamma-carboxy prothrombin; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; DEC: double eligibility criteria

Mazzaferro *et al.*^[1]. In line with this recommendation, the 5-5-500 criteria was established with the intent to enable the maximal enrollment of candidates while securing a 5-year recurrence rate below 10% and a 5-year survival rate over 70% based on a retrospective data analysis of the Japanese nationwide survey^[24]. Because the exclusion of patients within the Milan but beyond the 5-5-500 criteria seems not socially acceptable nor rationale, and considering the worldwide prevalence and acceptance of the Milan criteria, the Japanese DEC, Milan + 5-5-500, was adopted as the new indication criteria now in Japan.

In the present study, the 5-year recurrence and survival rate in patients meeting the 5-5-500 criteria and the Japanese DEC were superior to those socially accepted as mentioned above [Figure 1]. In addition, the number of LDLT candidates increased considerably using these criteria [Supplementary Figure 1 and Supplementary Table 1]. The outcomes of survival and recurrence were similar to our previous national report^[24] though the increase of LDLT candidates was a bit modest in the present study. Univariate analysis revealed that both beyond the 5-5-500 criteria and beyond the Japanese DEC were significant predictors of recurrence [Table 2]. Meanwhile, the recurrence rate was higher in patients beyond the Japanese DEC [Supplementary Figure 1 and Supplementary Table 1]. On the basis of these findings, we consider that the Japanese DEC are the appropriate selection criteria to maximize the number of LDLT candidates while securing acceptable outcomes. The major concern is that the recurrence rate was considerably high (42.9%) in patients within the Milan but beyond the 5-5-500 criteria in the present study [Supplementary Table 1]. The exclusion of patients within the Milan criteria, however, seems not socially acceptable at present. In addition, when the Japanese DEC was adopted, the 5-year recurrence and survival rate still fell within the target as a whole.

Amongst five biomarkers, AFP seems to be the most reliable marker with the highest AUC value [Figure 2]. The usefulness of AFP in predicting recurrence after LT has been investigated by many researchers^[12-14,30-32], and AFP is incorporated in some selection^[7,10,12,33] and prognostic models^[12-14]. The AFP model, developed by the Liver Transplantation French Study Group, combines serum AFP level, tumor size, and tumor number^[12]. Another famous prognostic model is the RETREAT score, which incorporated microvascular invasion, tumor diameter, and tumor number other than the AFP value as prognostic variables^[13]. Another prognostic model, the TRAIN score, incorporated the AFP slope, which was defined as [(final-AFP)-(initial-AFP)]/time^[14]. The cut-off value of AFP differs from study to study, ranging from 15 ng/mL to 1000 ng/mL^[7,10,12,13,30-33]. The AFP cut-off value of 60 ng/mL, used in the present study, is relatively low compared with those used in other studies, however, the cut-off value was shown to be useful in predicting recurrence [Supplementary Figure 2]. The present results as well as the previous reports justify the use of pretransplant AFP values in the expanded indication criteria of LT for HCC patients.

AFP-L3, a reliable marker for the diagnosis of HCC^[20], proved to be a promising marker for recurrence after LT since the specificity of AFP-L3 was the highest [Figure 2] and patient recurrence rate curves were well stratified using AFP-L3 [Supplementary Figure 2]. However, there has been little study^[34] investigating the usefulness of AFP-L3 in predicting HCC recurrence after LT. Highly sensitive AFP-L3 became available around 2010 in Japan, which enabled the measurement of AFP-L3 even in patients with total AFP levels below 20 ng/mL^[20,35-37]. Highly sensitive AFP-L3 is reported to be 5-10 times more sensitive than conventional AFP-L3^[37]. Along with these studies in non-transplant HCC patients, the present results warrant further investigation and validation for the usefulness and efficacy of AFP-L3 in predicting HCC recurrence after LT.

The AUC value of DCP was the 3rd highest [Figure 2] and multivariate analysis revealed that DCP is one of the independent risk factors for recurrence [Table 2] in this cohort. Though DCP has not been commonly used in the west^[38], some argued that DCP is more predictive than AFP^[8,39], and indeed, DCP is incorporated in the extended indication criteria of LT at two major centers in Japan^[6,8]. A new prognostic

model was developed in Korea, i.e., the MoRAL score, using only serum levels of AFP and DCP^[15], which was shown to be more effective than the Milan criteria in predicting recurrence after LT. While DCP is criticized for not being a routine laboratory test in the West and for its dependence on vitamin K status and warfarin administration in clinical settings, reports from Asia as well as the present study warrant further study on the DCP in predicting HCC recurrence after LT.

NLR and PLR are indicators of inflammatory status previously reported as prognostic markers for the recurrence of various cancers, including HCC^[16,17]. As the usefulness of NLR has been presented in both DDLT and LDLT settings^[40,41], NLR is incorporated in some prognostic models^[14,42]. Although the usefulness of PLR has also been reported since 2012, supporting evidence is still limited^[40,41]. NLR and PLR were not as useful as AFP, AFP-L3, and DCP in predicting HCC recurrence after LT in the present study [Figure 2, Table 2, Supplementary Figure 2]. One of the drawbacks of these inflammatory markers may be the inconstant nature of neutrophil, platelet, and lymphocyte counts. This is more so in cirrhotic patients who suffer from portal hypertension, splenomegaly, and consequently, pancytopenia. As for other biomarkers, we could not evaluate the usefulness of FDG-PET, one of the promising biomarkers reported previously^[18,19], because FDG-PET was not routinely performed at our institute.

Our analysis has several weaknesses related to its retrospective design and the limited number of patients included. Both the present and the national cohorts used in the establishment of the 5-5-500 criteria were based on the long time-course with a considerable number of cases from nearly 20 years ago. As the developments and advances in imaging modalities, anti-viral treatments, and immunosuppression regimens might have changed practice in the management of LT considerably over the last two decades, it seems mandatory to validate the criteria in the recent cohort or in the prospective study. Although the usefulness of tumor downstaging before LT has been reported recently^[43], unfortunately there was no case of intentional downstaging in the present cohort. In Japan, where the indication of LT for HCC is restricted to those with decompensated cirrhosis by the national insurance system, HCC patients with compensated cirrhosis are usually recommended for locoregional treatments and will be referred for LT when they develop decompensated cirrhosis not amenable to locoregional treatments. The downstaging strategy for those beyond the selection criteria and the expansion of the indication criteria are two opposite ways to expand the indication of LT for candidates, which should be compared and discussed in future studies.

In conclusion, the present study suggests that both the 5-5-500 criteria and the Japanese DEC are appropriate for patients with HCC in LDLT. AFP, including AFP-L3, was demonstrated to be a reliable biomarker and could reasonably be incorporated into the expanded selection criteria. Further validation with more recent cases and a prospective study is warranted.

DECLARATIONS

Authors' contributions

Conception and design: Ichida A, Akamatsu N

Provision of study materials or patients: Ichida A

Collection and assembly of data: Ichida A, Akamatsu N

Data analysis and interpretation: Ichida A, Akamatsu N

Manuscript writing: Ichida A, Akamatsu N, Hasegawa K

Final approval of manuscript: Ichida A, Akamatsu N, Hasegawa K

Availability of data and materials

The data used in the present study were submitted to the journal.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The current study was approved as project number 2158-(6) by Graduate School of Medicine and Faculty of Medicine, the University of Tokyo Research Ethics Committee/Institutional Review Board. Informed consent was obtained in the form of opt-out on the web-site (<http://www.u-tokyo-hbp-transplant-surgery.jp/>).

Consent for publication

Not applicable.

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REFERENCES

1. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996;334:693-9.
2. Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001;33:1394-403.
3. Sugawara Y, Tamura S, Makuuchi M. Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. *Dig Dis* 2007;25:310-12.
4. Lee SG, Hwang S, Moon DB, Ahn CS, Kim KH, et al. Expanded indication criteria of living donor liver transplantation for hepatocellular carcinoma at one large-volume center. *Liver Transpl* 2008;14:935-45.
5. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009;10:35-43.
6. Ito T, Takada Y, Ueda M, Haga H, Maetani Y, et al. Expansion of selection criteria for patients with hepatocellular carcinoma in living donor liver transplantation. *Liver Transpl* 2007;13:1637-44.
7. Toso C, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the scientific registry of transplant recipients database. *Hepatology* 2008;49:832-8.
8. Taketomi A, Sanefuji K, Soejima Y, Yoshizumi T, Uchiyama H, et al. Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. *Transplantation* 2009;87:531-7.
9. DuBay D, Sandroussi C, Sandhu L, Cleary S, Guba M, et al. Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. *Ann Surg* 2011;253:166-72.
10. Kim JM, Kwon CH, Joh JW, Park JB, Lee JH, et al. Expanded criteria for liver transplantation in patients with hepatocellular carcinoma. *Transplant Proc* 2014;46:726-9.
11. Llovet JM. Expanding HCC criteria for liver transplant: the urgent need for prospective, robust data. *Liver Transpl* 2006;12:1741-3.
12. Duvoux C, Roudot Thoraval F, Decaens T, Pessione F, et al. Liver transplantation for hepatocellular carcinoma: a model including α -fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012;143:986-94.
13. Mehta N, Heimbach J, Harnois DM, Sapisochin G, Dodge JL, et al. Validation of a risk estimation of tumor recurrence after transplant (RETREAT) score for hepatocellular carcinoma recurrence after liver transplant. *JAMA Oncol* 2017;3:493-500.
14. Lai Q, Nicolini D, Inostroza Nunez M, Iesari S, Goffette P, et al. A novel prognostic index in patients with hepatocellular cancer waiting for liver transplantation: Time-Radiological-response-Alpha-fetoprotein-INflammation (TRAIN) score. *Ann Surg* 2016;264:787-96.
15. Lee JH, Cho Y, Kim HY, Cho EJ, Lee DH, et al. Serum tumor markers provide refined prognostication in selecting liver transplantation candidate for hepatocellular carcinoma patients beyond the Milan criteria. *Ann Surg* 2016;263:842-50.
16. Halazun KJ, Hardy MA, Rana AA, Woodland DC 4th, Luyten EJ, et al. Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma. *Ann Surg* 2009;250:141-51.
17. Zheng J, Cai J, Li H, Zeng K, He L, et al. Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio as prognostic predictors for hepatocellular carcinoma patients with various treatments: a meta-analysis and systematic review. *Cell Physiol Biochem* 2017;44:967-81.
18. Hong G, Suh KS, Suh SW, Yoo T, Kim H, et al. Alpha-fetoprotein and (18)F-FDG positron emission tomography predict tumor recurrence better than Milan criteria in living donor liver transplantation. *J Hepatol* 2016;64:852-9.
19. Takada Y, Kaido T, Shirabe K, Nagano H, Egawa H, et al. Significance of preoperative fluorodeoxyglucose-positron emission tomography in prediction of tumor recurrence after liver transplantation for hepatocellular carcinoma patients: a Japanese multicenter study. *J*

- Hepatobiliary Pancreat Sci 2017;24:49-57.
20. Nakao K, Ichikawa T. Recent topics on alpha-fetoprotein. *Hepatol Res* 2013;43:820-5.
 21. Cillo U, Giuliani T, Polacco M, Herrero Manley LM, Crivellari G, et al. Prediction of hepatocellular carcinoma biological behavior in patient selection for liver transplantation. *World J Gastroenterol* 2016;22:232-52.
 22. Kobayashi T, Aikata H, Honda F, Nakano N, Nakamura Y, et al. Preoperative fluorine 18 fluorodeoxyglucose positron emission tomography/computed tomography for prediction of microvascular invasion in small hepatocellular carcinoma. *J Comput Assist Tomogr* 2016;40:524-30.
 23. Imura S, Teraoku H, Yoshikawa M, Ishikawa D, Yamada S, et al. Potential predictive factors for microvascular invasion in hepatocellular carcinoma classified within the Milan criteria. *Int J Clin Oncol* 2018;23:98-103.
 24. Shimamura T, Akamatsu N, Fujiyoshi M, Kawaguchi A, Morita S, et al. Expanded living-donor liver transplantation criteria for patients with hepatocellular carcinoma based on the Japanese nationwide survey: the 5-5-500 rule - a retrospective study. *Transpl Int* 2019;32:356-68.
 25. Kokudo N, Sugawara Y, Imamura H, Sano K, Makuuchi M. Tailoring the type of donor hepatectomy for adult living donor liver transplantation. *Am J Transplant* 2005;5:1694-703.
 26. Akamatsu N, Sugawara Y, Kaneko J, Arita J, Sakamoto Y, et al. Low platelet counts and prolonged prothrombin time early after operation predict the 90 days morbidity and mortality in living-donor liver transplantation. *Ann Surg* 2017;265:166-72.
 27. Furukawa H, Shimamura T, Suzuki T, Taniguchi M, Nakanishi K, et al. Liver transplantation for hepatocellular carcinoma: the Japanese experience. *J Hepatobiliary Pancreat Sci* 2010;17:533-8.
 28. Tamura S, Sugawara Y, Kokudo N. Living donor liver transplantation for hepatocellular carcinoma: the Japanese experience. *Oncology* 2011;81:111-5.
 29. Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, et al. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012;13:e11-22.
 30. Todo S, Furukawa H, Tada M. Extending indication: role of living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2007;13:S48-54.
 31. Mailey B, Artinyan A, Khalili J, Denitz J, Sanchez-Luege N, et al. Evaluation of absolute serum α -fetoprotein levels in liver transplant for hepatocellular cancer. *Arch Surg* 2011;146:26-33.
 32. Berry K, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. *Liver Transpl* 2013;19:634-45.
 33. Zheng SS, Xu X, Wu J, Chen J, Wang WL, et al. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 2008;85:1726-32.
 34. Wongjarupong N, Negron-Ocasio GM, Chaiteerakij R, Addissie BD, Mohamed EA, et al. Model combining pre-transplant tumor biomarkers and tumor size shows more utility in predicting hepatocellular carcinoma recurrence and survival than the BALAD models. *World J Gastroenterol* 2018;24:1321-31.
 35. Tamura Y, Igarashi M, Kawai H, Suda T, Satomura S, et al. Clinical advantage of highly sensitive on-chip immunoassay for fucosylated fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. *Dig Dis Sci* 2010;55:3576-83.
 36. Hanaoka T, Sato S, Tobita H, Miyake T, Ishihara S, et al. Clinical significance of the highly sensitive fucosylated fraction of alpha-fetoprotein in patients with chronic liver disease. *J Gastroenterol Hepatol* 2011;26:739-44.
 37. Toyoda H, Kumada T, Tada T. Highly sensitive Lens clinaris agglutinin-reactive alpha-fetoprotein: a new tool for the management of hepatocellular carcinoma. *Oncology* 2011;81:61-5.
 38. Olthoff KM, Goldberg DS. The MoRAL path beyond Milan. *Ann Surg* 2016;263:851-2.
 39. Shirabe K, Itoh S, Yoshizumi T, Soejima Y, Taketomi A, et al. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma-with special reference to the serum levels of des-gamma-carboxy prothrombin. *J Surg Oncol* 2007;95:235-40.
 40. Rosenblatt RE, Tafesh ZH, Halazun KJ. Role of inflammatory markers as hepatocellular cancer selection tool in the setting of liver transplantation. *Transl Gastroenterol Hepatol* 2017;2:95.
 41. Citores MJ, Lucena JL, de la Fuente S, Cuervas-Mons V. Serum biomarkers and risk of hepatocellular carcinoma recurrence after liver transplantation. *World J Hepatol* 2019;11:50-64.
 42. Halazun KJ, Najjar M, Abdelmessih RM, Samstein B, Griesemer AD, et al. Recurrence after liver transplantation for hepatocellular carcinoma: a new MORAL to the story. *Ann Surg* 2017;265:557-64.
 43. Mazzaferro V, Citterio D, Bhoori S, Bongini M, Miceli R, et al. Liver transplantation in hepatocellular carcinoma after tumour downstaging (XXL): a randomised, controlled, phase 2b/3 trial. *Lancet Oncol* 2020;21:947-56.

Review

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Role of imaging in management of hepatocellular carcinoma: surveillance, diagnosis, and treatment response

Azeez Osho¹, Nicole E. Rich^{1,*}, Amit G. Singal^{2,*}

¹Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX 75390-8887, USA.

²Division of Digestive and Liver Diseases, UT Southwestern Medical Center, Dallas, TX 75390-8887, USA.

*These two authors are co-senior authors.

Correspondence to: Dr. Amit G. Singal, Division of Digestive and Liver Diseases, UT Southwestern Medical Center, 5959 Harry Hines Blvd, POB 1, Suite 420, Dallas, TX 75390-8887, USA. E-mail: amit.singal@utsouthwestern.edu

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Abstract

Imaging plays a notable role in hepatocellular carcinoma (HCC) surveillance, diagnosis, and treatment response assessment. Whereas HCC surveillance among at-risk patients, including those with cirrhosis, has traditionally been ultrasound-based, there are increasing data showing that this strategy is operator-dependent and has insufficient sensitivity when used alone. Several novel blood-based and imaging modalities are currently being evaluated to increase sensitivity for early HCC detection. Multi-phase computed tomography (CT) or contrast-enhanced magnetic resonance imaging (MRI) should be performed in patients with positive surveillance tests to confirm a diagnosis of HCC and perform cancer staging, as needed. HCC is a unique cancer in that most cases can be diagnosed radiographically without histological confirmation when demonstrating characteristic features such as arterial phase hyperenhancement and delayed phase washout. The Liver Imaging Reporting and Data System offers a standardized nomenclature for reporting CT or MRI liver findings among at-risk patients. Finally, cross-sectional imaging plays a critical role for assessing response to any HCC therapy as well as monitoring for HCC recurrence in those who achieve complete response.

Keywords: Liver cancer, ultrasound, screening, computed tomography, magnetic resonance imaging, contrast-enhanced ultrasound, Liver Imaging Reporting and Data System



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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the fourth leading cause of cancer-related death worldwide^[1]. Common risk factors for the development of HCC include alcohol use, chronic hepatitis B (HBV) or hepatitis C infection, and nonalcoholic fatty liver disease (NAFLD). Prognosis for HCC depends on tumor stage at diagnosis; curative treatment options for early stage tumors provide 5-year survival exceeding 70%, whereas late stage HCC is only amenable to palliative therapies with a median survival of 2-3 years. Imaging plays a central role in the management of patients with HCC, including surveillance, diagnosis, and assessing treatment response. The aim of this review is to discuss best practices for imaging along the care spectrum of HCC.

ROLE OF IMAGING FOR HCC SURVEILLANCE

Given the strong association between early detection and improved survival, the American Association for the Study of Liver Diseases (AASLD) and European Association for the Study of Liver (EASL) recommend HCC surveillance in at-risk patients, including subgroups with chronic HBV and those with cirrhosis from any etiology^[2,3]. HCC surveillance is supported by a large randomized controlled trial (RCT) in patients with HBV that showed a 37% reduction in mortality^[4]. Although there is no similar RCT among patients with cirrhosis, several cohort studies have highlighted an association between surveillance and improved early detection, curative treatment receipt, and overall survival^[5].

Ultrasound for HCC surveillance

The preferred imaging modality for HCC surveillance across all major professional liver organizations worldwide has been, and remains, abdominal ultrasound^[2,3,6,7]. Ultrasound has many advantages including being readily available, inexpensive, and non-invasive with a favorable safety profile. A systematic review of test modalities for HCC surveillance found that ultrasound has a high sensitivity of 94% to detect HCC at any stage; however, its sensitivity to detect early stage HCC is significantly lower at only 63%^[8]. Furthermore, the wide variation in ultrasound sensitivity between studies highlights the operator-dependent nature of the examination. High ultrasound quality relies heavily on the experience of the individual performing the ultrasound examination as well as the radiologist interpreting the examination^[9,10]. These challenges have been observed in breast cancer screening, with ultrasound being more useful than mammography in women with dense breast tissue, but one of its limitations being variable quality based on inherent operator dependence^[11]. Standardization of examination technique and establishment of minimum reporting requirements, as has been done for breast ultrasonography^[12], can improve the quality of ultrasound-based screenings^[13]. For HCC, regional differences have been observed in ultrasound sensitivity and align with differences in technique^[8]. In the U.S., ultrasound is typically performed by technicians with select frozen images interpreted by a radiologist at a later time, whereas physicians in other regions of the world often perform and interpret ultrasound in real time^[14]. Recent data have also highlighted the impact of patient characteristics on ultrasound effectiveness. In a retrospective cohort study of 941 patients undergoing surveillance ultrasound, 191 (20.3%) were deemed to be of inadequate quality for exclusion of HCC lesions^[15]. In multivariable analysis, inadequate ultrasound quality was associated with obesity and alcohol- or nonalcoholic steatohepatitis (NASH)-related cirrhosis, suggesting that inadequate ultrasound quality and poor sensitivity may be more common as the prevalence of obesity and NASH continue to rise globally^[16,17]. Since this study, the Liver Imaging Reporting and Data System (LI-RADS) has proposed that ultrasound assessment and reporting include an ultrasound visualization score, including score A (no or minimal limitation), score B (moderate limitations that may obscure small masses), and score C (severe limitations that significantly lower sensitivity for focal liver lesions). The visualization score is based on liver heterogeneity, beam attenuation or shadowing, proportion of liver visualized, and proportion of diaphragm visualized. Routine reporting of visualization is an important step that helps clinicians interpret ultrasound results; however, further data are needed to verify

that poor visualization is in fact associated with lower HCC detection as well as determining the optimal surveillance strategies in patients with limited visualization. A recent pilot study suggests that repeat ultrasound in patients with limited visualization (scores B or C) could have sufficient visualization (score A) in approximately half of cases; however, validation of these results are needed in larger cohorts^[18].

Various surveillance intervals have been proposed^[19]. The AASLD and EASL recommend semi-annual surveillance, which appears reasonable on the basis of the median doubling time of HCC tumors^[20]. A retrospective multicenter study among 649 HCC patients from Italy found patients detected by semi-annual surveillance had smaller tumor burden and improved survival compared to patients submitted to annual surveillance (40.3 months *vs.* 30 months, respectively, $P = 0.03$)^[21]. An RCT evaluated if shorter intervals would further improve early detection and survival but found that a 6-month surveillance interval provided similar early HCC detection compared to a 3-month surveillance interval (79% *vs.* 70%, $P = 0.30$)^[22].

Role of biomarkers for HCC surveillance

Professional societies offer differing guidance regarding the additional value of serum biomarkers over ultrasound alone for HCC surveillance. The best studied biomarker for HCC surveillance is alpha fetoprotein (AFP), which has been validated in all five phases of biomarker development^[23]. The AASLD and Asian Pacific Association for the Study of the Liver (APASL) both recommend ultrasound with or without AFP^[2], whereas the EASL recommends ultrasound alone, citing the poor performance characteristics of AFP^[2,3,6,7]. The AASLD and APASL guidelines cite the improvement in sensitivity when adding AFP to ultrasound in clinical practice^[8]. Various AFP cutoffs have been proposed, with the AASLD and APASL recommending AFP cutoffs of 20 and 200 ng/mL, respectively^[2]. Notably, AFP levels can be increased in patients with active hepatitis, and thus, AFP is less accurate in patients with active HCV infection, whereas AFP has higher sensitivity in other subgroups (e.g., patients with cirrhosis and HIV infection)^[24,25]. In contrast, AFP has better sensitivity in individuals with HBV and HCV, who are either receiving or have completed antiviral treatments, and therefore, lower threshold values can be established in using AFP for surveillance in this patient population^[26,27].

Although AFP alone has poor sensitivity for early stage HCC and poor specificity in patients with viral hepatitis, several studies have suggested a potential benefit of using AFP as an adjunct surveillance test with ultrasound. A meta-analysis of cohort studies on this topic demonstrated that the combination of ultrasound and AFP had a significantly higher sensitivity for early stage HCC compared to ultrasound alone (63% *vs.* 45%, respectively)^[8]. Although this was associated with a drop in specificity (92% for ultrasound alone *vs.* 84% for ultrasound plus AFP), this was not felt to be clinically significant and the diagnostic odds ratio of the combination remained higher using the two tests together 7(95%CI: 3-15) *vs.* 8(95%CI: 3-23, respectively). A study by Atiq and colleagues quantified physical harms related to ultrasound with or without AFP, as not all false positive lesions may prompt diagnostic evaluation^[28]. They found 1 in 4 patients with cirrhosis experience physical harms for false positive or indeterminate surveillance tests, which are more often related to ultrasound than AFP monitoring - in part related to some patients having diagnostic evaluation for indeterminate ultrasound results and providers not ordering diagnostic evaluation in many patients with false positive AFP levels.

Other biomarkers, such as lens culinaris agglutinin-reactive AFP (AFP-L3) and des-gamma-carboxy-prothrombin (DCP), have been evaluated in phase 2 (case-control) biomarker studies but appear to have insufficient performance if used alone^[23]. Therefore, there has been increasing interest in biomarker panels, such as GALAD, which combines AFP, AFP-L3, and DCP with patient age and gender, and has been shown to have promising performance in large case-control studies^[29,30]. In a large multi-national case-control study with 6,834 patients (2,430 HCC and 4,404 controls), GALAD demonstrated a sensitivity of 60%-80% for early HCC detection. A recent study by Yang *et al.*^[31] compared GALAD to ultrasound for HCC

detection and found GALAD to be superior to ultrasound, with an area under the curve (AUC) of 0.95 (95%CI: 0.93-0.97) vs. 0.82 (95%CI: 0.77-0.87), respectively. When the GALAD model was combined with ultrasound, the GALADUS score had significantly better performance compared to ultrasound alone with a sensitivity of 95%, specificity of 91% and an AUC of 0.98 (95%CI: 0.96-0.99). In addition, there has also been increased interest in methylated DNA markers and circulating tumor cells (CTCs) for early detection of HCC^[32,33]. However, these biomarkers still require evaluation in large phase II and phase III studies before adoption in clinical practice^[34].

CT/MRI for HCC surveillance

Given the limitations of ultrasound-based surveillance, there has been increasing interest in alternative imaging modalities, such as computed tomography (CT) or magnetic resonance imaging (MRI), but neither is recommended by current practice guidelines^[2,3,6,7]. Although both CT and MRI have been shown to be superior in sensitivity and specificity for HCC diagnosis and staging compared to ultrasound (discussed below), there are limited data evaluating these tests in a surveillance manner. A small randomized trial comparing semi-annual ultrasound to annual multiphase CT found that ultrasound was similar in sensitivity but less costly than CT^[35]. Further, CT is associated with screening harms including radiation exposure and potential contrast injury^[36,37]. Therefore, there has been increasing interest in MRI surveillance, which obviates some of these concerns. A prospective cohort study from South Korea (PRIUS study) comparing ultrasound and MRI surveillance in patients with cirrhosis found that MRI had significantly higher sensitivity for early stage HCC (86% vs. 27.9%) as well as higher specificity (97% vs. 94.4%)^[38]. The authors of this study subsequently performed a cost-effectiveness analysis, suggesting that MRI may be cost-effective; however, these data still require validation in non-HBV Western populations^[39]. Furthermore, there would be concerns about radiologic capacity and patient acceptability if an MRI-based strategy were adopted in larger populations. There have been increasing data on alternative MRI strategies, including abbreviated MRI and non-contrast MRI. Abbreviated MRI protocols use selected sequences from a full diagnostic protocol and can shorten the examination from ~45 min to ~15 min, which may address some concerns about radiologic capacity and improve cost-effectiveness^[40]. Abbreviated MRI protocols have been studied for HCC diagnosis and characterization of lesions^[40,41], but no trials or studies have been done specifically for surveillance. There is an ongoing clinical trial at Seoul National University Hospital comparing annual abbreviated MRI to ultrasound for early HCC detection (NCT03731923). Two recent studies have also evaluated non-contrast MRI as a possible surveillance strategy. A *post-hoc* analysis of the PRIUS study suggested that non-contrast MRI is superior to ultrasound for HCC detection, with per-lesion and per-examination sensitivity of 77.1% and 79.1% for non-enhanced MRI compared to just 25.0% and 27.9% for ultrasound, respectively^[42]. Specificity of non-contrast MRI was also higher than that of ultrasound 97.9% vs. 94.5%, $P < 0.001$. In addition, the estimated scan time was < 6 min with a total room occupancy time of only 25-35 min. Two ongoing prospective trials, MIRACLE-HCC (NCT02514434) and MAGNUS-HCC (NCT02551250), are comparing non-contrast MRI and ultrasound for surveillance of HCC^[43,44].

Most analyses for HCC surveillance have tried to implement a “one-size-fits-all” strategy for all at-risk patients, despite known variation in HCC risk between patients with cirrhosis. For example, a validated tissue-based signature has been shown to accurately risk stratify patients with cirrhosis into high, intermediate, and low risk of HCC, with annual HCC incidences of 5.8, 2.2, and 1.5%, respectively^[45]. Similarly, other risk stratification markers can accurately distinguish patients with high risk and low risk of developing HCC^[46]. Accurate risk stratification could allow more intensive and costly surveillance strategies to be applied to those at highest risk, while using lower intensity and inexpensive surveillance strategies in lower risk patients. A modeling study suggested that a risk-stratified approach was cost-effective compared to ultrasound and AFP in all patients^[47]. Currently, the Japan Society of Hepatology (JSH) is the only professional society that recommends a differential HCC surveillance strategy by individual patient risk, i.e.,

ultrasound and serum biomarkers for most patients, with multiphase CT or MRI considered in the highest risk patients^[7].

Unfortunately, a systematic review found that less than 20% of patients with cirrhosis in the U.S. undergo HCC surveillance, with even lower rates of guideline-concordant semi-annual surveillance^[48,49]. Patients and providers have reported potential barriers to surveillance including knowledge deficits, time constraints, and financial costs of tests that need to be addressed to increase surveillance utilization^[50,51]. Studies have demonstrated promise for inreach efforts such as electronic medical record reminders or outreach strategies including mailed invitations to complete ultrasound surveillance^[52-54].

While awaiting ongoing trial data for both novel biomarkers and cross-sectional imaging techniques, ultrasound with or without AFP remains the gold standard surveillance strategy.

ROLE OF IMAGING IN HCC DIAGNOSIS

For surveillance to be effective, recall procedures must be followed for patients with abnormal surveillance tests^[55]. In patients with an ultrasound nodule < 1 cm in maximum diameter, the risk of HCC is low and professional society guidelines recommend repeat short-interval ultrasound in ~3 months. If the lesion is stable in size, it can be followed on ultrasound; however, diagnostic evaluation with cross-sectional imaging (i.e., contrast-enhanced MRI or 4-phase CT) is recommended once a lesion is ≥ 1 cm in size^[2] [Figure 1].

HCC is unique compared to other cancers, in that the diagnosis can often be made radiographically without histological confirmation. Historically, HCC diagnosis has been made on the basis of the presence of “arterial enhancement and delayed washout”, i.e., hypervascularity during the arterial phase and hypointensity on the portal venous or delayed phases of imaging. This classic appearance is related to the liver’s dual blood supply, where the background liver receives most of its blood supply from the portal vein and HCC lesions obtain most of their blood supply from hepatic artery branches. In the setting of cirrhosis, this appearance was demonstrated to have a specificity of 95% for the diagnosis of HCC^[56,57].

LI-RADS criteria

More recently, the American Association for Cancer Research (AACR) and AASLD have adopted the LI-RADS criteria for HCC diagnosis and classification, and have chosen specific populations for which these criteria should be applied, namely patients with cirrhosis and chronic hepatitis B infection^[57]. The LI-RADS criteria do not apply to pediatric patients or patients with cirrhosis secondary to vascular disorders (e.g., Budd-Chiari syndrome, sinusoidal obstruction syndrome)^[58]. The LI-RADS criteria include a combination of major and minor imaging criteria, and classifies lesions on a scale ranging from LR-1 (definitely benign) to LR-5 (definitely HCC) or LR-M (malignant but not definite for HCC) [Table 1]. Major LI-RADS criteria include arterial phase hyperenhancement (APHE), delayed washout, enhancing capsule, and threshold growth. Patients with LR-1 and LR-2 lesions are definitely and likely benign, respectively, so most of these patients can return to ultrasound-based surveillance. Patients with LR-3 and LR-4 lesions have an intermediate risk of HCC, so these patients can be considered for continued observation versus biopsy after multidisciplinary discussion. A recent systematic review found 38 and 74% of LR-3 and LR-4 lesions were HCC, respectively, highlighting that these lesions should not be ignored and must be followed clinically^[59]. In this systematic review, LR-5 lesions had a positive predictive value of 94% for being HCC, and therefore do not warrant biopsy for histological confirmation prior to treatment. The LR-M classification is reserved for lesions that are suspicious for malignancy but have features that are not definite for HCC, e.g., peripheral enhancement, and can be seen in other malignancies such as intrahepatic cholangiocarcinoma. Therefore, biopsy is typically recommended in these cases to make a definitive diagnosis. It should also be noted that the LI-RADS criteria do not apply to patients without cirrhosis and/or chronic HBV infection, as the positive predictive value of the aforementioned classic imaging

Diagnostic Algorithm for HCC

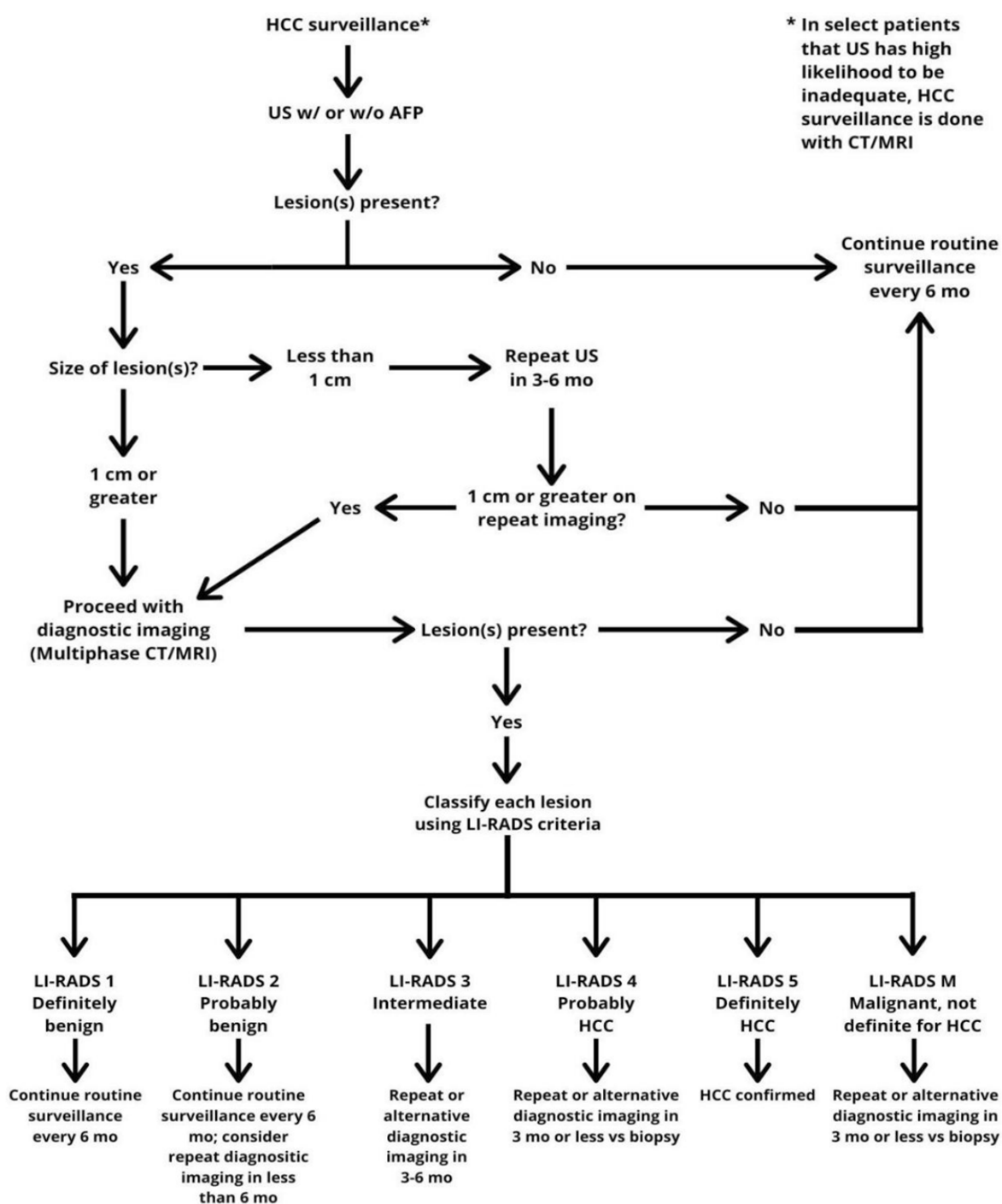


Figure 1. Diagnostic algorithm for HCC. *In select patients in whom US has high likelihood to be inadequate, HCC surveillance may be performed using contrast-enhanced CT or MRI. HCC: hepatocellular carcinoma; CT: computed tomography; MRI: magnetic resonance imaging; US: ultrasound

findings is substantially lower^[60]. Therefore, these patients should typically undergo biopsy for histological confirmation prior to treatment^[2].

Table 1. LI-RADS classification for liver lesions

Non-rim arterial phase enhancement		Absent		Present		
Observed size of lesion (mm)		< 20	≥ 20	< 10	10-19	≥ 20
Presence of additional major features	None	LR-3	LR-3	LR-3	LR-3	LR-4
Enhancing "capsule"	One	LR-3	LR-4	LR-4	LR-4/LR-5*	LR-5
Nonperipheral washout	≥ Two	LR-4	LR-4	LR-4	LR-5	LR-5
Threshold growth						

*If a lesion is classified in this category and has enhancing capsule, it is categorized as LR-4. However, if a lesion is classified in this category and has either nonperipheral washout OR threshold growth, it is classified as LR-5. LI-RADS: Liver Imaging Reporting and Data System

CT/MRI for HCC diagnosis

Several studies have compared the accuracy of CT and MRI for the diagnosis of HCC. In a meta-analysis by Roberts *et al.*^[61], contrast-enhanced CT was compared to both extracellular contrast-enhanced MRI and Eovist MRI for HCC diagnosis. Compared to CT, MRI had a significantly higher sensitivity (82% *vs.* 66%) with similar specificity (92% *vs.* 91%). In addition, MRI was more sensitive for diagnosis of HCC in lesions < 1 cm compared to CT (69% *vs.* 49%), although specificity was lower (46% *vs.* 69%, respectively). Conversely, MRI was associated with higher cost, greater technical complexity (including longer scan time), and less consistent imaging quality (e.g., difficulty with breath holding, difficulty holding still, large volume ascites). Thus, although MRI was noted to have marginally higher sensitivity compared to CT in this meta-analysis, one imaging modality could not be definitively recommended over the other, and the choice of modality should be individualized considering both the risks of either imaging test and the patient's clinical status^[61].

Although contrast-enhanced MRI and 4-phase CT are the primary modalities used for HCC diagnosis in the Western world, APASL guidelines recommend the use of hepatobiliary agents (e.g., gadoxetic acid or Eovist), which can provide information on hepatocellular function in addition to blood flow. This recommendation is largely based on data suggesting increased sensitivity to detect HCC lesions compared to dynamic CT and MRI. A 2015 meta-analysis compared the diagnostic performance of dynamic CT, MRI using conventional extracellular contrast agents, and MRI using hepatobiliary contrast agents. Overall, on a per-lesion basis, MRI was more sensitive than CT for HCC diagnosis (80% *vs.* 68%, $P = 0.02$). In subgroup analyses, the per-lesion sensitivity of gadoxetic acid-enhanced MRI was significantly higher compared to MRI using other contrast agents (87% *vs.* 74%, $P = 0.03$)^[62]. However, gadoxetic acid-based MRI has some limitations in the diagnosis of HCC. While HCC are usually hypointense on the hepatobiliary phase (due to lack of contrast uptake by the tumor compared to the background liver showing peak enhancement at this time), up to 20% of HCCs will have uptake of the contrast and instead appear hyperintense^[63]. Additionally, the classic feature of "pseudocapsule" may not be apparent due to lack of a delayed phase, leading to misdiagnosis^[6]. Furthermore, in patients with more advanced cirrhosis, decreased contrast uptake in the background liver may lead to lower sensitivity for HCC detection^[64].

PET for HCC diagnosis

The use of positron-emission tomography (PET) has been evaluated for diagnosis of HCC but has not produced favorable results for detection of primary tumors. The most widely used radiotracer is ¹⁸F-fluoro-deoxy-6-glucose phosphate (FDG), which has added utility in assessing metabolic cellular function, but ¹⁸F-FDG uptake in PET/CT for primary HCC has only been seen in 40% of cases^[65]. This is due to high ¹⁸F-FDG uptake by both normal hepatocytes and malignant neoplastic cells associated with HCC, resulting in difficulty in identifying HCC lesions^[66,67]. However, PET may be beneficial for diagnosis of extrahepatic or metastatic HCC. One of the most common sites for extrahepatic HCC metastasis is the retroperitoneal lymph nodes, and the sensitivity of FDG PET/CT to detect lymph node metastasis is greater than in other areas of the body^[68]. Other forms of PET imaging that have been studied for HCC diagnosis include PET

MRI and immuno-PET/CT. PET MRI has the benefit of improved soft tissue contrast and a lack of ionizing radiation. However, its availability is limited and requires a technologist experienced in both nuclear medicine and MRI for accurate interpretation^[69]. Immuno-PET/CT uses ⁸⁹Zr-tagged monoclonal antibodies to target glypican-3, a cell surface protein that is highly expressed in HCC, and has shown improvement in differentiating primary HCC cells from normal hepatocytes and identifying small HCC lesions compared to PET alone^[65]. However, studies evaluating immuno-PET have been limited to animal models, and further studies are needed before its routine use in clinical practice^[70].

Contrast-enhanced ultrasound for HCC diagnosis

There has also been increasing interest in the role of contrast-enhanced ultrasound (CEUS) for HCC diagnosis. This imaging modality uses the intravenous administration of microbubble contrast agents to evaluate the hyperenhancement of a liver nodule in “real-time”. These contrast agents have a short half-life of only a few minutes and are eliminated through respiration, eliminating concerns for potential renal toxicity seen with most contrast agents used for CT and MRI^[71]. The LI-RADS criteria have been modified for using CEUS for characterization of liver nodules, similar to the LI-RADS criteria for CT/MRI^[72]. A meta-analysis showed that the pooled sensitivity and specificity of CEUS to detect HCC was 85 and 91%, respectively; however, the authors noted the findings were limited by publication bias^[73]. There are several notable limitations of CEUS that are similar to conventional ultrasound in HCC diagnosis. First, ultrasound is operator-dependent, which may lead to inconsistencies in diagnosis outside of expert centers^[74]. Second, CEUS can also be limited by patient-level factors, including large body habitus, overlying bowel gas, poor acoustic windows, and movement artifact^[72,74]. A limitation of CEUS in HCC diagnosis that differs from conventional ultrasound involves the nuances of contrast administration to properly characterize suspicious lesions. Multiple injections of contrast may be needed to properly classify lesions, thereby limiting its role for staging, and the administration of contrast must be done in a medically controlled setting to ensure safety^[74]. Lastly, CEUS has lower detection rate for washout than CT/MRI^[75], and its ability to distinguish HCC from intrahepatic cholangiocarcinoma (ICC) has been controversial^[76,77]. However, some studies have suggested that dynamic, timed administration of contrast can be used in CEUS to help distinguish the two malignancies, as the rapid loss of signal intensity in the early portal phase is more characteristic of ICC than HCC^[78]. Additional criteria have been proposed to distinguish ICC and HCC using CEUS with reported improved performance but require further validation^[79]. Based on current practice guidelines, CEUS is reserved as a second-line diagnostic imaging modality when multiphase CT or MRI are indeterminate in HCC diagnosis, although data continue to evolve regarding its potential role^[7].

ROLE OF IMAGING FOR POST-TREATMENT RESPONSE AND SURVEILLANCE

Patients with early stage HCC are typically eligible for curative therapies including local ablation, surgical resection, or liver transplantation. Although resection and local ablation are considered curative, they are associated with a high risk of recurrence, approaching up to 70% at 5 years^[80]. Therefore, close observation is critical, with most centers performing CT or MRI every 3 months for the first 1-2 years and then semi-annual surveillance with CT or MRI thereafter. Some centers return to ultrasound-based surveillance after a period of 4-5 years, although there is substantial center-to-center variation. Liu and colleagues used clinical and tumor features to risk stratify patients into 3 categories (low, intermediate, and high risk of recurrence) following surgical resection to determine the optimal time interval for post-hepatectomy surveillance imaging^[81]. They calculated recurrence detection rates between consecutive CT for each surveillance schedule for each risk group, and found surveillance schedules could be tailored on the basis of risk; for example, low-risk patients could undergo surveillance CT every four months for the first two years and yearly over the next three years without compromising surveillance benefits while reducing examination costs and radiation burden.

Surveillance after liver transplantation

Liver transplantation has the advantage of curing not only HCC but also the underlying cirrhosis, and is thus associated with significantly lower recurrence rates (~10% at 5 years) when restricted to patients within the Milan criteria on imaging (one lesion < 5 cm or 2-3 lesions each < 3 cm, without vascular invasion or extrahepatic spread)^[82]. More recently, liver transplant criteria have been expanded to include patients who are “downstaged”, i.e., patients with larger tumor burden who are treated with locoregional therapy (LRT) and brought to within Milan criteria. Radiographic response is used as a prognostic biomarker and serves as a surrogate for tumor biology, with those who exhibit response likely having favorable tumor biology. Several single and multicenter studies have shown similar survival and rates of post-transplant recurrence among extended-criteria patients who were successfully downstaged with LRT compared to those who initially presented within Milan criteria^[83,84]. In the largest multicenter study to date including patients with HCC from 10 of 11 UNOS regions that underwent liver transplantation, Kardashian *et al.*^[85] found 5-year overall survival to be acceptable in patients downstaged to within Milan criteria compared to those initially within Milan criteria (64% vs. 71%). In addition, the authors noted that AFP response to LRT provided a useful adjunct to radiographic response in assessing likelihood of successful downstaging^[85]. Post-transplant, HCC surveillance is evolving from a one-size-fits-all strategy to a tailored one based on an individual's risk of recurrence. The Risk Estimation of Tumor Recurrence After Transplant (RETREAT) score developed and validated in a multicenter study by Mehta and colleagues includes 3 variables - AFP at time of transplantation, presence of microvascular invasion, and the largest viable tumor diameter (cm) plus the number of viable tumors on explant pathology^[86]. Patients are assigned a risk score of 0-8 based on the presence or absence of these features. The RETREAT score accounts for the effect of pre-transplant LRT (as only viable tumor on explant is counted) and stratifies 5-year HCC recurrence risk - noted to be < 3% in patients with a score of 0 to > 75% in patients with a score ≥ 5. Post-operative imaging surveillance intervals can then be tailored on the basis of an individual patient's RETREAT score. For instance, a patient with a RETREAT score of five might undergo surveillance with CT chest and abdomen every three to four months for the first two years followed by every six months through year five, while patients with a RETREAT score of zero might not require post-transplant imaging surveillance at all.

Surveillance after locoregional therapies

LRTs, including ablation, transarterial chemoembolization, transarterial radioembolization, and radiation therapy, are a standard treatment for patients with early to intermediate stage HCC who are not candidates for surgical resection or liver transplantation. Furthermore, as previously mentioned, LRT also has a role in downstaging and “bridging” patients to surgical treatments including transplantation. Response to LRT is typically assessed radiographically using CT or MRI, with serum biomarkers used as adjuncts. One of these such systems is known as the Response Evaluation Criteria in Solid Tumors (RECIST), which uses tumor size and characteristics, involvement of lymph nodes, maximum number of target lesions, and disease progression to qualify treatment response for malignancy^[87]. The use of RECIST has several limitations for HCC response assessment, as it does not consider tumor necrosis nor decrease in tumor size in HCC treated with LRT, and antitumor activity may be poorly correlated. To overcome these limitations, EASL recommended measuring change in area of tumor enhancement to assess treatment response, and in 2008, the AASLD proposed modified RECIST (mRECIST) criteria to include change in lesion size, lesion characteristics, and viable portions of the lesions determined by arterial phase enhancement to determine the response to treatment^[88,89]. Both RECIST and mRECIST criteria classify treatment response for HCC lesions as complete, partial, stable disease, progressive disease, or development of new lesion(s). Overall response by both the EASL and mRECIST criteria have been associated with survival and are thus preferable to RECIST for HCC response assessment^[90]. Still, mRECIST has some notable limitations. First, the response assessment requires radiologic expertise as ascertainment of viable tumor may not be straightforward. Second, patients with underlying renal disease or impairment may be unable to tolerate a contrast-enhanced examination and, therefore, mRECIST evaluation^[91]. In addition, timing of contrast

administration and imaging acquisition must be precise to prevent misinterpretation. LI-RADS has also proposed a nomenclature for reporting response, categorizing patients as having residual disease (LR-TR viable), having complete response with no viable tumor (LR-TR non-viable), or situations in which it is unclear if there is viable tumor remaining (LR-TR equivocal). There are few, if any, data comparing LI-RADS response assessment to other response assessments such as mRECIST.

Surveillance after systemic therapy

Systemic therapy is the mainstay of treatment for patients with advanced HCC, and valid radiologic response criteria are critical for the assessment of treatment response in clinical trials. Sorafenib, an oral multikinase inhibitor, was the first chemotherapy agent approved for first-line treatment of HCC in the U.S. on the basis of data from the multicenter, randomized, double-blind placebo-controlled Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) trial^[92,93]. Since 2017, several additional tyrosine kinase inhibitors (TKIs) for HCC have been approved for first- and second-line indications, including lenvatinib, regorafenib and cabozantinib^[94-96]. A study by Edeline *et al.*^[97] compared RECIST to mRECIST in patients receiving sorafenib for treatment of advanced HCC and found mRECIST objective response correlated with an increased overall survival. Similarly, Kudo *et al.*^[98] demonstrated that objective response per mRECIST was associated with improved survival in a post-hoc analysis of the REFLECT Trial including patients treated with sorafenib or lenvatinib. Median overall survival for patients with an objective response was 22.4 months, compared to 11.4 months for non-responders^[98]. Most recently, Llovet and colleagues evaluated 21 clinical trials in HCC and found a moderate correlation between progression-free survival or time to progression and overall survival; however, a hazard ratio of ≤ 0.6 appeared to be a potential surrogate for improved survival^[99]. Overall, these data highlight the importance of monitoring for both response and progression.

With the introduction of checkpoint inhibitors (e.g., nivolumab, pembrolizumab, atezolizumab/bevacizumab), durable response rates in approximately 15%-30% of patients have been observed^[100]. In an exploratory analysis of data from Checkmate 040, patients with durable objective responses appeared to have prolonged survival compared to those with stable disease or progressive disease^[101]. However, it is possible some patients treated with immunotherapy may display “pseudoprogression”, a distinct radiologic pattern in which deep and durable responses occur after initial progression^[102,103]. Although this is well described for other tumor types, e.g., melanoma, it is currently unclear how commonly this occurs in patients with HCC. This phenomenon has resulted in the development of specific imaging response assessment guidelines (irRECIST and iRECIST) for this population, in which radiographic progression must be confirmed with repeat imaging 4-8 weeks after the first response assessment^[104,105]. These various response assessment classifications are being used in ongoing HCC clinical trials and there has yet to emerge a standard across all trials^[106]. Despite this potential uncertainty regarding optimal ways to assess response, monitoring for treatment response or disease progression can identify patients who are benefiting from therapy and those who may benefit from transition to an alternative treatment. Our institutional practice is to monitor patients with cross-sectional imaging every 2 months while on systemic therapy.

FUTURE DIRECTIONS FOR IMAGING IN HCC

Radiomics, the automated high-throughput extraction and analysis of quantitative and phenotypic features from radiographic images^[107], has emerged as a non-invasive tool for diagnosis and prognostication in several cancers, including HCC^[108]. Qualitative and quantitative radiomics features may predict HCC recurrence and treatment response^[109], and are promising as novel biomarkers that may be complementary to existing serum biomarkers for HCC surveillance and treatment response assessment. However, lack of reproducibility is a major challenge and further validation studies are needed prior to the adoption of radiomics in routine clinical practice.

SUMMARY

Imaging has played a significant role in the advancements of surveillance, diagnosis, and treatment of HCC. Across all professional societies, ultrasound is the most recognized imaging modality for HCC screening among at-risk patients. CT and MRI are currently not recommended for surveillance given similar sensitivities as ultrasound and cost-effectiveness, but recent trials are studying abbreviated MRI protocols for surveillance. Non-invasive diagnosis of HCC relies heavily on CT and MRI with application of the LI-RADS in classifying suspicious lesions for HCC. PET imaging is best utilized to identify extrahepatic metastases but has poor performance for diagnosis of primary HCC. CEUS has also been studied for its role in HCC diagnosis and is currently accepted as a second line imaging modality in most professional societies. Imaging with CT and MRI has also been shown to be effective in monitoring treatment response, with most centers using RECIST or mRECIST for trial analysis.

DECLARATIONS

Authors' contributions

Conception of manuscript: Singal AG

Drafting of manuscript: Osho A, Rich NE

Critical revisions of manuscript: Rich NE, Singal AG

Administrative support: Singal AG

Availability of data and materials

Not applicable.

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Conflicts of interest

Dr. Singal AG has served on advisory boards or as consultant for Genentech, Bayer, Eisai, Exelixis, BMS, Merck, Wako Diagnostics, Glycotest, Exact Sciences, Roche, and TARGET Pharmsolutions. Other authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCE

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
2. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;68:723-50.
3. European Association for the Study of the Liver. European association for the study of the liver. EASL Clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236.
4. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004;130:417-22.

5. Singal AG, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. *PLoS Med* 2014;11:e1001624.
6. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017;11:317-70.
7. Kokudo N, Takemura N, Hasegawa K, Takayama T, Kubo S, et al. Clinical practice guidelines for hepatocellular carcinoma: the Japan Society of Hepatology 2017 (4th JSH-HCC guidelines) 2019 update. *Hepatol Res* 2019;49:1109-13.
8. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, et al. Surveillance imaging and alpha fetoprotein for early detection of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. *Gastroenterology* 2018;154:1706-18.e1.
9. Jeong WK. Surveillance of hepatocellular carcinoma: is only ultrasound enough? *Clin Mol Hepatol* 2017;23:222-3.
10. Lee CI, Chen LE, Elmore JG. Risk-based breast cancer screening: implications of breast density. *Med Clin North Am* 2017;101:725-41.
11. Berg WA, Blume JD, Cormack JB, Mendelson EB. Operator dependence of physician-performed whole-breast US: lesion detection and characterization. *Radiology* 2006;241:355-65.
12. Madjar H, Rickard M, Jellins J, Otto R. IBUS guidelines for the ultrasonic examination of the breast. *Eur J Ultrasound* 1999;9:99-102.
13. Choi EJ, Lee EH, Kim YM, Chang YW, Lee JH, et al; on the behalf of the Alliance for Breast Cancer Screening in Korea (ABCS-K). Interobserver agreement in breast ultrasound categorization in the Mammography and Ultrasonography Study for Breast Cancer Screening Effectiveness (MUST-BE) trial: results of a preliminary study. *Ultrasonography* 2019;38:172-80.
14. Finberg HJ. Whither (with?) the ultrasound specialist? *J Ultrasound Med* 2004;23:1543-7.
15. Simmons O, Fetzer DT, Yokoo T, Marrero JA, Yopp A, et al. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. *Aliment Pharmacol Ther* 2017;45:169-77.
16. Mellinger JL, Shedden K, Winder GS, Tapper E, Adams M, et al. The high burden of alcoholic cirrhosis in privately insured persons in the United States. *Hepatology* 2018;68:872-82.
17. Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, et al. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World J Gastroenterol* 2017;23:8263-76.
18. Schoenberger H, Fetzer D, Yokoo T, Marrero J, Singal AG. Correlates of ultrasound quality for HCC surveillance in patients with cirrhosis. *Gastroenterology* 2020;158:S-91.
19. Santagostino E, Colombo M, Rivi M, Rumi MG, Rocino A, et al; Study Group of the Association of Italian Hemophilia Centers. A 6-month versus a 12-month surveillance for hepatocellular carcinoma in 559 hemophiliacs infected with the hepatitis C virus. *Blood* 2003;102:78-82.
20. Rich NE, John BV, Parikh ND, Rowe I, Mehta N, et al. Hepatocellular carcinoma demonstrates heterogeneous growth patterns in a multi-center cohort of patients with cirrhosis. *Hepatology* 2020; doi: 10.1002/hep.31159.
21. Santi V, Trevisani F, Gramenzi A, Grignaschi A, Mirici-Cappa F, et al; Italian Liver Cancer (ITA.LI.CA) Group. Semiannual surveillance is superior to annual surveillance for the detection of early hepatocellular carcinoma and patient survival. *J Hepatol* 2010;53:291-7.
22. Trinchet JC, Chaffaut C, Bourcier V, Degos F, Henrion J, et al; Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire (GRETCH). Ultrasonographic surveillance of hepatocellular carcinoma in cirrhosis: a randomized trial comparing 3- and 6-month periodicities. *Hepatology* 2011;54:1987-97.
23. Rich N, Singal AG. Hepatocellular carcinoma tumour markers: current role and expectations. *Best Pract Res Clin Gastroenterol* 2014;28:843-53.
24. Gopal P, Yopp AC, Waljee AK, Chiang J, Nehra M, et al. Factors that affect accuracy of α -fetoprotein test in detection of hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2014;12:870-7.
25. Yang JD, Dai J, Singal AG, Gopal P, Addissie BD, et al. Improved performance of serum alpha-fetoprotein for hepatocellular carcinoma diagnosis in HCV cirrhosis with normal alanine transaminase. *Cancer Epidemiol Biomarkers Prev* 2017;26:1085-92.
26. Minami T, Tateishi R, Kondo M, Nakagomi R, Fujiwara N, et al. Serum alpha-fetoprotein has high specificity for the early detection of hepatocellular carcinoma after hepatitis C virus eradication in patients. *Medicine (Baltimore)* 2015;94:e901.
27. Wong GL, Chan HL, Tse YK, Chan HY, Tse CH, et al. On-treatment alpha-fetoprotein is a specific tumor marker for hepatocellular carcinoma in patients with chronic hepatitis B receiving entecavir. *Hepatology* 2014;59:986-95.
28. Atiq O, Tiro J, Yopp AC, Muffler A, Marrero JA, et al. An assessment of benefits and harms of hepatocellular carcinoma surveillance in patients with cirrhosis. *Hepatology* 2017;65:1196-205.
29. Berhane S, Toyoda H, Tada T, Kumada T, Kagebayashi C, et al. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. *Clin Gastroenterol Hepatol* 2016;14:875-86.e6.
30. Best J, Bechmann LP, Sowa JP, Sydor S, Dechêne A, et al. GALAD score detects early hepatocellular carcinoma in an international cohort of patients with nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2020;18:728-35.e4.
31. Yang JD, Addissie BD, Mara KC, Harmsen WS, Dai J, et al. GALAD score for hepatocellular carcinoma detection in comparison with liver ultrasound and proposal of GALADUS score. *Cancer Epidemiol Biomarkers Prev* 2019;28:531-8.
32. Ye Q, Ling S, Zheng S, Xu X. Liquid biopsy in hepatocellular carcinoma: circulating tumor cells and circulating tumor DNA. *Mol Cancer* 2019;18:114.
33. Ahn JC, Teng PC, Chen PJ, Posadas E, Tseng HR, et al. Detection of circulating tumor cells and their implications as a novel biomarker for diagnosis, prognostication, and therapeutic monitoring in hepatocellular carcinoma. *Hepatology* 2020; doi: 10.1002/hep.31165.
34. Chalasani NP, Ramasubramanian T, Bruinsma JJ, et al. Combined methylated DNA and protein markers: An accurate blood-based test for early stage detection of hepatocellular carcinoma, In American Association for the Study of Liver Diseases: *Hepatology* 2019; 70(S1): 72A-73A. Available from <https://aasldpubs.onlinelibrary.wiley.com/doi/10.1002/hep.30940> [Last accessed on 26 Aug 2020]
35. Pocha C, Dieperink E, McMaken KA, Knott A, Thuras P, et al. Surveillance for hepatocellular cancer with ultrasonography vs. computed

- tomography -- a randomised study. *Aliment Pharmacol Ther* 2013;38:303-12.
36. Harris PS, Hansen RM, Gray ME, Massoud OI, McGuire BM, et al. Hepatocellular carcinoma surveillance: an evidence-based approach. *World J Gastroenterol* 2019;25:1550-9.
 37. Andersson KL, Salomon JA, Goldie SJ, Chung RT. Cost effectiveness of alternative surveillance strategies for hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2008;6:1418-24.
 38. Kim SY, An J, Lim YS, Han S, Lee JY, et al. MRI with liver-specific contrast for surveillance of patients with cirrhosis at high risk of hepatocellular carcinoma. *JAMA Oncol* 2017;3:456-63.
 39. Kim HL, An J, Park JA, Park SH, Lim YS, et al. Magnetic resonance imaging is cost-effective for hepatocellular carcinoma surveillance in high-risk patients with cirrhosis. *Hepatology* 2019;69:1599-613.
 40. Lee JY, Huo EJ, Weinstein S, Santos C, Monto A, et al. Evaluation of an abbreviated screening MRI protocol for patients at risk for hepatocellular carcinoma. *Abdom Radiol (NY)* 2018;43:1627-33.
 41. Khatri G, Pedrosa I, Ananthakrishnan L, de Leon AD, Fetzer DT, et al. Abbreviated-protocol screening MRI vs. complete-protocol diagnostic MRI for detection of hepatocellular carcinoma in patients with cirrhosis: An equivalence study using LI-RADS v2018. *J Magn Reson Imaging* 2020;51:415-25.
 42. Park HJ, Jang HY, Kim SY, Lee SJ, Won HJ, et al. Non-enhanced magnetic resonance imaging as a surveillance tool for hepatocellular carcinoma: comparison with ultrasound. *J Hepatol* 2020;72:718-24.
 43. An C, Kim DY, Choi JY, Han KH, Roh YH, et al. Noncontrast magnetic resonance imaging versus ultrasonography for hepatocellular carcinoma surveillance (MIRACLE-HCC): study protocol for a prospective randomized trial. *BMC Cancer* 2018;18:915.
 44. Kim HA, Kim KA, Choi JI, Lee JM, Lee CH, et al. Comparison of biannual ultrasonography and annual non-contrast liver magnetic resonance imaging as surveillance tools for hepatocellular carcinoma in patients with liver cirrhosis (MAGNUS-HCC): a study protocol. *BMC Cancer* 2017;17:877.
 45. Hoshida Y, Villanueva A, Sangiovanni A, Sole M, Hur C, et al. Prognostic gene expression signature for patients with hepatitis C-related early-stage cirrhosis. *Gastroenterology* 2013;144:1024-30.
 46. Ioannou GN, Green P, Kerr KF, Berry K. Models estimating risk of hepatocellular carcinoma in patients with alcohol or NAFLD-related cirrhosis for risk stratification. *J Hepatol* 2019;71:523-33.
 47. Goossens N, Bian CB, Hoshida Y. Tailored algorithms for hepatocellular carcinoma surveillance: Is one-size-fits-all strategy outdated? *Curr Hepatol Rep* 2017;16:64-71.
 48. Singal AG, Yopp A, S Skinner C, Packer M, Lee WM, et al. Utilization of hepatocellular carcinoma surveillance among American patients: a systematic review. *J Gen Intern Med* 2012;27:861-7.
 49. Singal AG, Li X, Tiro J, Kandunoori P, Adams-Huet B, et al. Racial, social, and clinical determinants of hepatocellular carcinoma surveillance. *Am J Med* 2015;128:90.e1-7.
 50. Farvardin S, Patel J, Khambaty M, Yerokun OA, Mok H, et al. Patient-reported barriers are associated with lower hepatocellular carcinoma surveillance rates in patients with cirrhosis. *Hepatology* 2017;65:875-84.
 51. Simmons OL, Feng Y, Parikh ND, Singal AG. Primary care provider practice patterns and barriers to hepatocellular carcinoma surveillance. *Clin Gastroenterol Hepatol* 2019;17:766-73.
 52. Beste LA, Ioannou GN, Yang Y, Chang MF, Ross D, et al. Improved surveillance for hepatocellular carcinoma with a primary care-oriented clinical reminder. *Clin Gastroenterol Hepatol* 2015;13:172-9.
 53. Singal AG, Tiro JA, Murphy CC, Marrero JA, McCallister K, et al. Mailed outreach invitations significantly improve HCC surveillance rates in patients with cirrhosis: a randomized clinical trial. *Hepatology* 2019;69:121-30.
 54. Singal AG, Tiro JA, Marrero JA, McCallister K, Mejias C, et al. Mailed outreach program increases ultrasound screening of patients with cirrhosis for hepatocellular carcinoma. *Gastroenterology* 2017;152:608-15.e4.
 55. Fetzer DT, Rodgers SK, Harris AC, Kono Y, Wasnik AP, et al. Screening and surveillance of hepatocellular carcinoma: an introduction to ultrasound liver imaging reporting and data system. *Radiol Clin North Am* 2017;55:1197-209.
 56. Marrero JA, Hussain HK, Nghiem HV, Umar R, Fontana RJ, et al. Improving the prediction of hepatocellular carcinoma in cirrhotic patients with an arterially-enhancing liver mass. *Liver Transpl* 2005;11:281-9.
 57. Tang A, Bashir MR, Corwin MT, Cruite I, Dietrich CF, et al; LI-RADS Evidence Working Group. Evidence supporting LI-RADS major features for CT- and MR imaging-based diagnosis of hepatocellular carcinoma: a systematic review. *Radiology* 2018;286:29-48.
 58. Elsayes KM, Kielar AZ, Chernyak V, Morshid A, Furlan A, et al. LI-RADS: a conceptual and historical review from its beginning to its recent integration into AASLD clinical practice guidance. *J Hepatocell Carcinoma* 2019;6:49-69.
 59. van der Pol CB, Lim CS, Sirlin CB, McGrath TA, Salameh JP, et al. Accuracy of the liver imaging reporting and data system in computed tomography and magnetic resonance image analysis of hepatocellular carcinoma or overall malignancy-a systematic review. *Gastroenterology* 2019;156:976-86.
 60. Shao S, Liang Y, Kuang S, Chen J, Shan Q, et al. Diagnostic performance of LI-RADS version 2018 in differentiating hepatocellular carcinoma from other hepatic malignancies in patients with hepatitis B virus infection. *Bosn J Basic Med Sci* 2020;20:401-10.
 61. Roberts LR, Sirlin CB, Zaiem F, Almasri J, Prokop LJ, et al. Imaging for the diagnosis of hepatocellular carcinoma: a systematic review and meta-analysis. *Hepatology* 2018;67:401-21.
 62. Lee YJ, Lee JM, Lee JS, Lee HY, Park BH, et al. Hepatocellular carcinoma: diagnostic performance of multidetector CT and MR imaging-a systematic review and meta-analysis. *Radiology* 2015;275:97-109.
 63. Kim JY, Kim MJ, Kim KA, Jeong HT, Park YN. Hyperintense HCC on hepatobiliary phase images of gadoxetic acid-enhanced MRI: correlation with clinical and pathological features. *Eur J Radiol* 2012;81:3877-82.

64. Sirlin CB, Hussain HK, Jonas E, Kanematsu M, Min Lee J, et al. Consensus report from the 6th International forum for liver MRI using gadoxetic acid. *J Magn Reson Imaging* 2014;40:516-29.
65. Lu RC, She B, Gao WT, Ji YH, Xu DD, et al. Positron-emission tomography for hepatocellular carcinoma: Current status and future prospects. *World J Gastroenterol* 2019;25:4682-95.
66. Brix G, Ziegler SI, Bellemann ME, Doll J, Schosser R, et al. Quantification of [(18F)]FDG uptake in the normal liver using dynamic PET: impact and modeling of the dual hepatic blood supply. *J Nucl Med* 2001;42:1265-73.
67. Wudel LJ Jr., Delbeke D, Morris D, Rice M, Washington MK, et al. The role of [18F]fluorodeoxyglucose positron emission tomography imaging in the evaluation of hepatocellular carcinoma. *Am Surg* 2003;69:117-24; discussion 124-6.
68. Jadvar H. Hepatocellular carcinoma and gastroenteropancreatic neuroendocrine tumors: potential role of other positron emission tomography radiotracers. *Semin Nucl Med* 2012;42:247-54.
69. Ehman EC, Johnson GB, Villanueva-Meyer JE, Cha S, Leynes AP, et al. PET/MRI: Where might it replace PET/CT? *J Magn Reson Imaging* 2017;46:1247-62.
70. Hernandez R, Sun H, England CG, Valdovinos HF, Ehlerding EB, et al. CD146-targeted immunoPET and NIRF imaging of hepatocellular carcinoma with a dual-labeled monoclonal antibody. *Theranostics* 2016;6:1918-33.
71. Jo PC, Jang HJ, Burns PN, Burak KW, Kim TK, et al. Integration of contrast-enhanced US into a multimodality approach to imaging of nodules in a cirrhotic liver: how I do it. *Radiology* 2017;282:317-31.
72. Kim TK, Noh SY, Wilson SR, Kono Y, Piscaglia F, et al. Contrast-enhanced ultrasound (CEUS) liver imaging reporting and data system (LI-RADS) 2017 - a review of important differences compared to the CT/MRI system. *Clin Mol Hepatol* 2017;23:280-9.
73. Zhang J, Yu Y, Li Y, Wei L. Diagnostic value of contrast-enhanced ultrasound in hepatocellular carcinoma: a meta-analysis with evidence from 1998 to 2016. *Oncotarget* 2017;8:75418-26.
74. Bartolotta TV, Taibbi A, Midiri M, Lagalla R. Contrast-enhanced ultrasound of hepatocellular carcinoma: where do we stand? *Ultrasonography* 2019;38:200-14.
75. Aubé C, Oberti F, Lonjon J, Pageaux G, Seror O, et al; CHIC Group. EASL and AASLD recommendations for the diagnosis of HCC to the test of daily practice. *Liver Int* 2017;37:1515-25.
76. Vilana R, Forner A, Bianchi L, Garcia-Criado A, Rimola J, et al. Intrahepatic peripheral cholangiocarcinoma in cirrhosis patients may display a vascular pattern similar to hepatocellular carcinoma on contrast-enhanced ultrasound. *Hepatology* 2010;51:2020-9.
77. Chen LD, Xu HX, Xie XY, Xie XH, Xu ZF, et al. Intrahepatic cholangiocarcinoma and hepatocellular carcinoma: differential diagnosis with contrast-enhanced ultrasound. *Eur Radiol* 2010;20:743-53.
78. Wildner D, Pfeifer L, Goertz RS, Bernatik T, Sturm J, et al. Dynamic contrast-enhanced ultrasound (DCE-US) for the characterization of hepatocellular carcinoma and cholangiocellular carcinoma. *Ultraschall Med* 2014;35:522-7.
79. Chen LD, Ruan SM, Liang JY, Yang Z, Shen SL, et al. Differentiation of intrahepatic cholangiocarcinoma from hepatocellular carcinoma in high-risk patients: a predictive model using contrast-enhanced ultrasound. *World J Gastroenterol* 2018;24:3786-98.
80. Shiina S, Teratani T, Obi S, Sato S, Tateishi R, et al. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005;129:122-30.
81. Liu D, Fong DY, Chan AC, Poon RT, Khong PL. Hepatocellular carcinoma: surveillance CT schedule after hepatectomy based on risk stratification. *Radiology* 2015;274:133-40.
82. Pavel MC, Fuster J. Expansion of the hepatocellular carcinoma Milan criteria in liver transplantation: Future directions. *World J Gastroenterol* 2018;24:3626-36.
83. Yao FY, Mehta N, Flemming J, Dodge J, Hameed B, et al. Downstaging of hepatocellular cancer before liver transplant: long-term outcome compared to tumors within Milan criteria. *Hepatology* 2015;61:1968-77.
84. Yao FY, Xiao L, Bass NM, Kerlan R, Ascher NL, et al. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. *Am J Transplant* 2007;7:2587-96.
85. Kardashian A, Florman SS, Haydel B, Ruiz RM, Klintmalm GB, et al. Liver transplantation outcomes in a U.S. multicenter cohort of 789 patients with hepatocellular carcinoma presenting beyond milan criteria. *Hepatology* 2020; doi: 10.1002/hep.31210.
86. Mehta N, Heimbach J, Harnois DM, Sapisochin G, Dodge JL, et al. Validation of a risk estimation of tumor recurrence after transplant (RETREAT) score for hepatocellular carcinoma recurrence after liver transplant. *JAMA Oncol* 2017;3:493-500.
87. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45:228-47.
88. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, et al. Clinical management of hepatocellular carcinoma. conclusions of the barcelona-2000 EASL conference. *J Hepatol* 2001;35:421-30.
89. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010;30:52-60.
90. Gillmore R, Stuart S, Kirkwood A, Hameeduddin A, Woodward N, et al. EASL and mRECIST responses are independent prognostic factors for survival in hepatocellular cancer patients treated with transarterial embolization. *J Hepatol* 2011;55:1309-16.
91. Fournier L, Ammari S, Thiam R, Cuénod CA. Imaging criteria for assessing tumour response: RECIST, mRECIST, Cheson. *Diagn Interv Imaging* 2014;95:689-703.
92. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-90.
93. Cheng A, Kang Y, Chen Z, Tsao C, Qin S, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009;10:25-34.
94. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, et al. Cabozantinib in patients with advanced and progressing

- hepatocellular carcinoma. *N Engl J Med* 2018;379:54-63.
95. Bruix J, Qin S, Merle P, Granito A, Huang Y, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;389:56-66.
 96. Kudo M, Finn RS, Qin S, Han K, Ikeda K, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018;391:1163-73.
 97. Edeline J, Boucher E, Rolland Y, Vauléon E, Pracht M, et al. Comparison of tumor response by response evaluation criteria in solid tumors (RECIST) and modified RECIST in patients treated with sorafenib for hepatocellular carcinoma. *Cancer* 2012;118:147-56.
 98. Kudo M, Finn RS, Qin S, Han K, Ikeda K, et al. Analysis of survival and objective response (OR) in patients with hepatocellular carcinoma in a phase III study of lenvatinib (REFLECT). *JCO* 2019;37:186.
 99. Llovet JM, Montal R, Villanueva A. Randomized trials and endpoints in advanced HCC: role of PFS as a surrogate of survival. *J Hepatol* 2019;70:1262-77.
 100. Bangaru S, Marrero JA, Singal AG. Review article: new therapeutic interventions for advanced hepatocellular carcinoma. *Aliment Pharmacol Ther* 2020;51:78-89.
 101. El-khoueiry AB, Melero I, Yau TC, Crocenzi TS, Kudo M, et al. Impact of antitumor activity on survival outcomes, and nonconventional benefit, with nivolumab (NIVO) in patients with advanced hepatocellular carcinoma (aHCC): Subanalyses of CheckMate-040. *JCO* 2018;36:475.
 102. Grierson P, Crites D, Ruzinova MB, Yano M, Lim KH. Distinct clinical and magnetic resonance features of metastatic hepatocellular carcinoma treated with pembrolizumab: a case report of late response after pseudoprogression. *Hepatol Commun* 2018;2:148-51.
 103. Wong DJ, Lee J, Choo SP, Thng CH, Hennesdige T. Hyperprogressive disease in hepatocellular carcinoma with immune checkpoint inhibitor use: a case series. *Immunotherapy* 2019;11:167-75.
 104. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol* 2017;18:e143-52.
 105. Henze J, Maintz D, Persigehl T. RECIST 1.1, irRECIST 1.1, and mRECIST: how to do. *Curr Radiol Rep* 2016;4.
 106. Edeline J, Karwal M, Zhu AX, Finn RS, Cattani S, et al. RECIST v1.1 and irRECIST outcomes in advanced HCC treated with pembrolizumab (pembro). *JCO* 2020;38:528.
 107. Zwanenburg A, Vallières M, Abdalah MA, Aerts HJWL, Andrearczyk V, et al. The image biomarker standardization initiative: standardized quantitative radiomics for high-throughput image-based phenotyping. *Radiology* 2020;295:328-38.
 108. Kim Y, Furlan A, Borhani AA, Bae KT. Computer-aided diagnosis program for classifying the risk of hepatocellular carcinoma on MR images following liver imaging reporting and data system (LI-RADS). *J Magn Reson Imaging* 2018;47:710-22.
 109. Hectors SJ, Lewis S, Besa C, King MJ, Said D, et al. MRI radiomics features predict immuno-oncological characteristics of hepatocellular carcinoma. *Eur Radiol* 2020;30:3759-69.

Review

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Can radiotherapy finally “go live” in the management of liver metastases?

Reggie G. John¹, Francis Ho¹, Gokula K. Appalanaido², Desiree Chen¹, Jeremy Tey¹, Yu Yang Soon¹, Balamurugan A. Vellayappan¹

¹Department of Radiation Oncology, National University Cancer Institute Singapore, National University Hospital, Singapore 119228, Singapore.

²Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, Jln Tun Hamdan Sheikh Tahir, Pulau Pinang 13200, Malaysia.

Correspondence to: Dr. Balamurugan A. Vellayappan, Department of Radiation Oncology, National University Cancer Institute Singapore, National University Hospital, 1E Kent Ridge Road, Level 7 Tower Block, Singapore 119228, Singapore.

E-mail: bala_vellayappan@nuhs.edu.sg

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Abstract

Liver metastases can present synchronously or at different time points. While systemic therapy continues to be the mainstay of treatment for patients with liver metastases, it is unlikely to completely eradicate the disease. Surgical “metastectomy” for patients with limited metastatic burden, particularly from colorectal cancers, has been shown to improve survival. However, owing to medical co-morbidities or tumour location, not all patients are eligible for surgical resection. In recent years, there has been an increase in the use of non-surgical techniques, including high dose radiation using stereotactic body radiotherapy, or brachytherapy, to ablate liver metastases. The purpose of this narrative review is to describe the role of radiotherapy in the management of liver metastases, both for local ablation and symptom palliation. We will elaborate on the techniques used, patient selection process, expected outcomes and toxicities based on the current literature.

Keywords: Radiotherapy, stereotactic body radiotherapy, liver metastases, brachytherapy, palliation

INTRODUCTION

The liver is one of the most common sites for metastases from primary cancers of the colon, pancreas, breast, and lung. Liver metastases are associated with considerable morbidity and shortened survival.



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While systemic therapy is still the mainstay of treatment, most tumour responses are short-lived. Moreover, the response to systemic therapy can be mixed, with some tumours regressing and others remaining stable or progressing. Aggressive local therapy (such as surgical resection) can be considered for patients with oligometastatic disease. For example, surgical resection is recommended for patients with isolated liver metastases from colorectal primaries, with the potential of long-term disease control^[1]. Early studies demonstrated a 30% 5-year survival in patients who underwent “metastectomy” for one to three liver metastases^[2]. Factors that determine patient eligibility for resection include the size, number, and location of lesions, and hepatic reserve. While surgical techniques have improved, not all patients are good surgical candidates because of surgical factors and patient co-morbidities. Thus, such patients may be considered for non-surgical liver-directed therapies. These include invasive techniques such as radio-frequency ablation (RFA) and non-invasive techniques such as stereotactic body radiotherapy (SBRT).

Traditionally, the role of radiation therapy in liver metastases has been purely for palliation, as the tolerance of whole liver to radiation is limited to 30 Gy (in 2 Gy fractions)^[3], and sustained tumor control is very unlikely at such doses. Technological advances with improvements in target localization, patient immobilization, motion management, and delivery of conformal radiation have allowed the use of high doses of radiation to ablate liver metastases. Moreover, mounting evidence shows that high doses of radiation can be delivered to small targets within the liver without causing toxicity^[3]. In the context of SBRT, doses ranging from 45 to 60 Gy, over three to five fractions (given over 1-2 weeks), is delivered conformally to the target while sparing normal liver parenchyma.

The purpose of this narrative review is to describe the role of radiotherapy in liver metastases - both in the setting of ablative treatment (including SBRT and brachytherapy) for patients with oligometastatic disease, and in the setting of symptom palliation in patients with uncontrolled liver metastases. We will elaborate on the treatment technique, patient selection, expected outcomes and treatment-related toxicities.

USE OF RADIOTHERAPY IN PATIENTS WITH OLIGOMETASTATIC LIVER DISEASE

Hellman and Weichselbaum were the first to introduce the concept of oligometastatic disease, which represented an intermediate state in the spectrum between locally confined and widely metastatic cancer^[4]. They proposed that the process of metastatic disease occurs in a step-wise manner, and patients with limited disease should be managed aggressively. In more recent years, advances in systemic and targeted therapy have rendered a greater number of patients with upfront widely metastatic disease to a state of limited volume metastatic disease. In these patients, aggressive management of drug-resistant clones may improve cancer outcomes. However, to date, there is no universally accepted definition of oligometastasis with regards to the number of lesions involved. The most accepted number of metastatic lesions is considered to be 5 or less (with up to 3 metastases in any one organ).

Although surgery and RFA have a longer history of being used in management of oligometastatic disease involving the liver, there are no trials directly comparing these to SBRT. However, the use of SBRT has been reinvigorated by a recently published randomized phase II trial (SABR-COMET) which investigated the use of SBRT in patients with oligometastatic disease (including liver metastases). They compared SBRT to standard of care palliative treatment, and showed an overall survival benefit with SBRT^[5].

The role of SBRT in oligometastatic liver disease

Technique

Stereotactic radiosurgery was first applied for intracranial targets, and similar concepts have been adapted to treat extracranial targets. SBRT involves the use of high doses of radiation delivered to a well-defined target whilst minimizing radiation to surrounding healthy tissue. The American College of Radiology and American Society for Radiation Oncology defines SBRT as the use of very large doses of radiation, defined

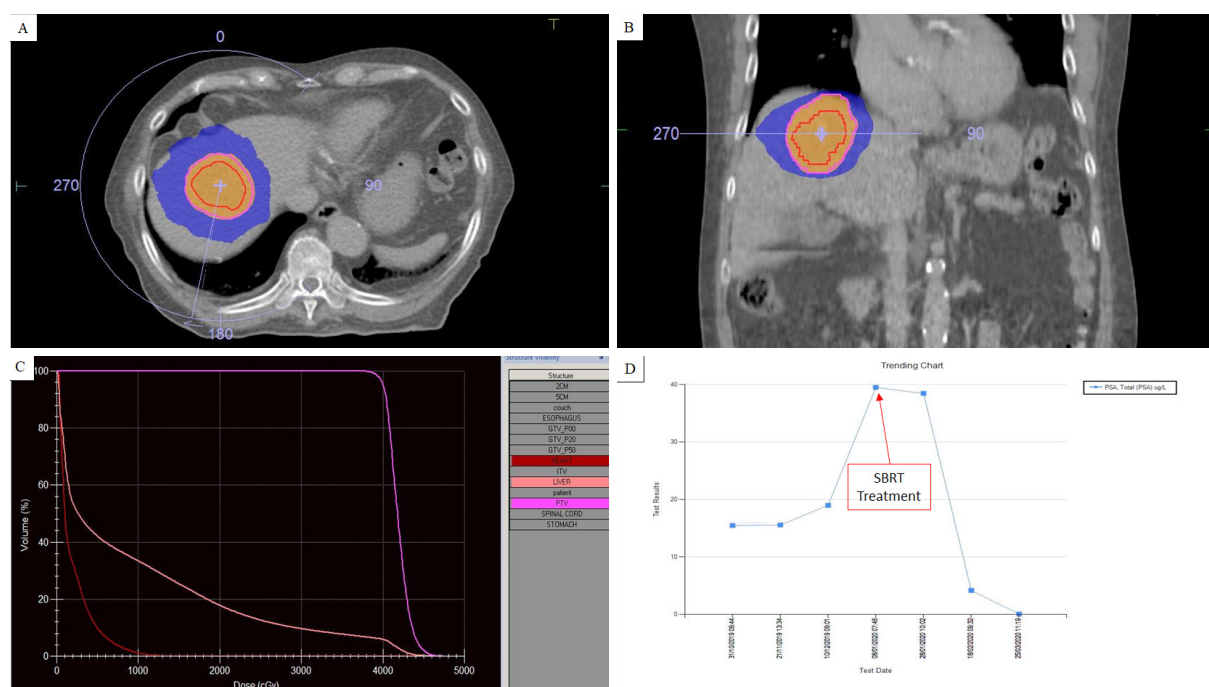


Figure 1. Example of an SBRT plan (40 Gy in 5 fractions) for a single hepatic metastasis in a patient with a castration-resistant prostate primary who had progressed after abiraterone. Axial image; red line showing gross tumour volume, peach line showing planning target volume, orange colourwash showing the 95% isodose line, and blue colourwash showing the 50% isodose line (A) coronal image; showing the 50% isodose line away from the heart (B) dose-volume histogram of the SBRT plan (C) dramatic PSA response following SBRT treatment (D). SBRT: stereotactic body radiotherapy

as more than 6 Gy per fraction, given over few (up to 5) fractions^[6]. This is in contrast to conventional external beam radiotherapy which is usually given in 1.8-2 Gy fractions, leading to protracted treatments.

SBRT is administered via a linear accelerator (LINAC) delivering ionizing radiation in the form of megavoltage photons. The radiation dose is highly conformal to the target, leading to a rapid dose fall off outside the target. This is achieved using multi-directional beams or arc therapy and modulating the intensity of each beam. Patient immobilisation is essential, and generally whole-body vacuum bags are utilised. As each treatment session can last up to 30 min, patient comfort and reproducibility are important for the accurate delivery of SBRT. The liver moves as much as 2-3 cm in the cranio-caudal direction. Motion management strategies are therefore critical in SBRT. Several methods exist such as 4D CT scanning with abdominal compression, breath holding in the form of active breathing control or voluntary breath holding, respiratory gating (synchronizing delivery of RT with specified respiratory phases) and real-time tumour tracking systems using radio-opaque fiducial markers. On-board imaging must be incorporated prior the delivery of SBRT. This allows for the online correction of patient position. Several solutions exist in modern linear accelerators, such as integrated cone-beam computed tomography or magnetic resonance imaging (MR-LINAC). An example of an SBRT plan is shown in Figure 1.

Patient selection

Patient selection for SBRT is critical and should take into account factors such as age, performance status, disease burden and patient preferences. In addition, tumour factors such as the volume of hepatic disease, location of metastases (particularly proximity to critical structures such as bowel, biliary tract, and heart), number of lesions (less than 3), size (preferably each less than 6 cm, and combined less than 15 cm) should be considered. In addition, sufficient hepatic reserve (ideally total liver volume more than 1000 mL, with at least 700 mL spared from doses more than 15 Gy) is essential, to mitigate toxicities, as will be explained below.

Table 1. Outcomes of SBRT to liver metastases from selected recent studies

Authors	Study design	n	Primary tumor	Dose/fractionation (#)	Median Followup in months	2 year LC (%)	2 year OS (%)
Scorsetti <i>et al.</i> ^[7] 2015	Prospective (Phase 2)	42	CRC	75 Gy/3 #	24	91	65
Goodman <i>et al.</i> ^[8] 2016	Retrospective	81	CRC 66.6% Breast 7.4% Lung 3.7% Ovarian 3.7% GI 13.6% Others 4.9%	32-60 Gy/3-5 #	33	90.5	68.6
McPartlin <i>et al.</i> ^[9] 2017	Prospective (Phase 1 & 2)	60	CRC	22.7-62.1 Gy/6 #	28.1	32	26
Joo <i>et al.</i> ^[10] 2017	Retrospective	70	CRC	45-60 Gy/3-4 #	34.2	73	75
Mahadevan <i>et al.</i> ^[11] 2018	Retrospective	427	CRC 44.3% Lung 12.2% Breast 9.8% GI 7.7% Gynae 5.9% Pancreas 4.9% Other 15.2%	Median 45 (12-60) Gy/median 3 (1-5) #	14	72	49

SBRT: stereotactic body radiotherapy; LC: local control; OS: overall survival; CRC: colorectal cancer; GI: gastrointestinal

Outcomes

Local control of hepatic metastases with SBRT are generally encouraging with most studies achieving approximately 80% at 2 years (range 32% to 91%) [Table 1]. This is mostly influenced by size of tumour, prior treatment, and biologically equivalent dose delivered. Median overall survival after SBRT can vary from 26% to 75% at 2 years [Table 1]. However, it is recognised that the patient's overall prognosis may be related to extra-hepatic metastases, thus reinforcing the need for multimodal treatment with effective systemic therapy as opposed to monotherapy with either alone. In most studies concerning the outcomes of SBRT for liver metastases, patients would have received systemic therapy (e.g., chemotherapy, targeted therapy) before and/or after SBRT. This reinforces the need for both effective local and systemic therapy.

Toxicity

Radiation-induced liver disease (RILD) is a feared complication which can be hard to manage^[12]. RILD typically presents 4-8 weeks after completion of radiotherapy (RT). The occurrence of RILD is related to the volume of liver irradiated, pre-existing hepatic functional reserve, and patient co-morbidities. Classic RILD symptoms include fatigue, abdominal pain, anicteric ascites and hepatomegaly. RILD however is more common in whole liver RT (WLRT), although it can occur with SBRT^[13]. Collateral damage to nearby structures is known to occur, including biliary obstruction and stricture formation (for lesions near the porta hepatis), and gastro-intestinal injury (resulting in bleeding, perforation or strictures). With adherence to known dose limits, the risks of these complications can be reduced to below 5%.

Comparison of SBRT with RFA

The most common technique of thermal ablation is radiofrequency ablation (RFA). RFA uses a high frequency alternating electric current which produces ionic agitation and frictional heating, thereby heating tumour tissue to over 60 degrees Celsius. Tumour heating causes extracellular and intracellular dehydration, resulting in tissue destruction by coagulative necrosis. RFA can be performed percutaneously, laparoscopically, or during open surgery^[14]. RFA is usually limited, however, by proximity to the biliary tree as well as to blood vessels because of the "heat sink" effect. Stang *et al.*^[15] reported that local recurrence rates were 5% to 42% after RFA and that the dominant factor affecting local failure rates were the size of the lesion, particularly those larger than 3 cm. Jackson *et al.*^[16] also reported similar efficacy of SBRT and RFA for lesions smaller than 2 cm, however SBRT achieved better local control comparatively for lesions larger than 2 cm^[16]. As such, RFA and SBRT are complementary modalities. SBRT is preferred for lesions near blood vessels or the dome of the liver, and for larger lesions.

The role of interstitial brachytherapy for oligometastatic liver disease

Technique

Although brachytherapy has a long history in oncology, it was not until the early 1980s when it was used for liver tumours. Dritschilo *et al.*^[17] described the percutaneous implantation of interstitial brachytherapy applicators under sonographic guidance in 1986. Subsequently, intraoperative catheter placement (under direct visualisation and/or sonography-assisted) has also been described^[18]. Later, in 2004, Rieke *et al.*^[19] published a phase II trial using CT-guidance for interstitial high dose-rate brachytherapy (HDRBT) of liver tumours which were unsuitable for thermal ablation.

The choice of image guidance for interstitial liver HDRBT application is operator-specific. In general, the use of CT, or a hybrid method combining ultrasound and CT, is the preferred choice. CT guidance allows better visualization of gastrointestinal structures, major vessels and intrahepatic bile ducts. The use of ultrasound, even when combined with CT, can reduce radiation exposure of the healthcare personnel involved.

The procedure is performed under sterile conditions using local anaesthesia and mild to moderate sedation. The skin puncture site is identified based on CT images. Under CT-guidance, the applicator is advanced past the liver capsule, into the target, in the same phase of breathing. The applicators are advanced at least 5 mm beyond the target, to account for breathing motion. After insertion of the applicators, CT or MRI with contrast is usually performed with thin axial cuts (2 mm) for applicator reconstruction. Doses in the range of 15-25 Gy in a single fraction are usually prescribed depending on the histology and organs-at-risk tolerance. Colorectal and sarcoma metastases tend to be radioresistant, and are usually treated with 25 Gy. In view of the clear dose-response reported by Rieke *et al.*^[20], higher doses should be used when possible.

Applicators are removed immediately after completing the treatment. An example of a CT HDRBT plan is shown in [Figure 2](#).

Patient selection

Most centres adopt the selection criteria used by Mohnike *et al.*^[21], with some variation. These include Child-Pugh score of B8 or less, platelet counts > 50,000, prothrombin time < 1.5X. Generally, chemo- and radio-sensitive primaries (such as lymphomas and germ-cell tumours) are excluded. Chemotherapy should be withheld one week pre- and post-HDRBT. The time-interval from prior therapies need to be considered: for RFA at least 1 month, Yttrium-90 at least 6 months, and brachytherapy to the same site at least 3 months. Lesion size and number have no specific cut-off, provided that not more than one third of normal liver parenchyma receives more than 5 Gy. The requirement is more stringent for patients with cirrhotic-appearing livers, with an aim for not more than half of non-target liver tissue to receive more than 5 Gy. Proximity of the target to major vessels or the target adjacent to the hilum is also not a contraindication^[22,23].

Outcomes

Local control rates are highly dependent on the isodose lines covering the target's periphery. In a prospective trial of three single fraction HDRBT dose levels, Rieke *et al.*^[20] (2010) reported a recurrence in only 1 out of 33 lesions (3%) in the 25 Gy group, in contrast to 34 out of 98 lesions (35%) recurring in the 15 Gy group.

Overall, local control rates with HDRBT appear favourable, as shown in [Table 2](#).

Toxicity

Interstitial HDRBT in the liver is a well-tolerated procedure. Post-procedural fever is very common and is related to cytokine-release. Nausea and vomiting may also occur, which is usually related to the volume

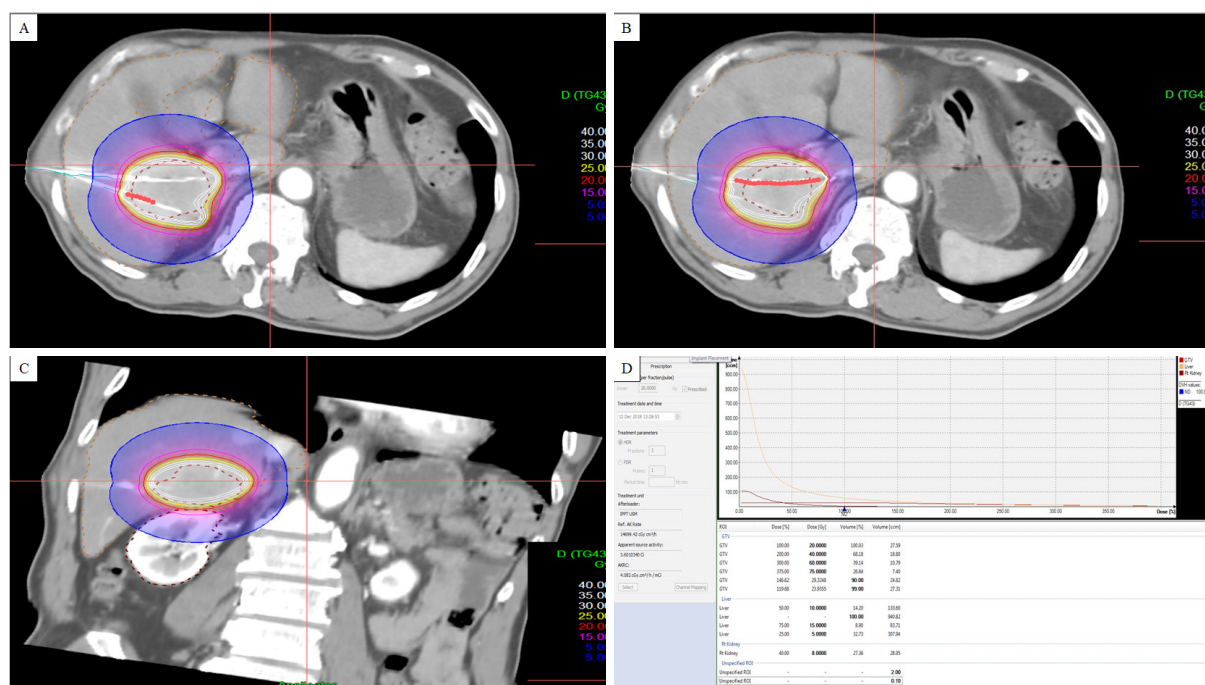


Figure 2. Example of a CT HDRBT plan for a single hepatic metastasis. axial images; the patient has 2 after-loading catheters advanced into the lesion. Dose distribution is adjusted by 3D treatment planning. The planned minimal enclosing dose was 20 Gy (red line) (A, B), coronal image (C), dose-volume histogram (D). HDRBT: high dose-rate brachytherapy

Table 2. Outcomes of CT-HDRBT to liver metastases from selected recent studies

Authors	Study design	n	Primary tumor	Dose/fractionation (#)	Median follow up in months	1 year LC (%)	1 year OS (%)
Ricke <i>et al.</i> ^[20] 2010	Prospective (Phase III)	73	Colorectal	15-25 Gy/1 #	15.2	74.9	NR
Wieners <i>et al.</i> ^[24] 2011	Prospective (Phase II)	41	Breast	15-25 Gy/1 #	18	93.5	79
Colletini <i>et al.</i> ^[25] 2012	Prospective	37	Breast	15-20 Gy/1-4 #	11.6	97.4	80
Sharma <i>et al.</i> ^[26] 2013	Prospective	10	Breast 30% CRC 20% GB 20% Stomach 20% Others 10%	20 Gy/1 #	9	75%	NR
Kieszko <i>et al.</i> ^[27] 2018	Retrospective	61	GI 75.4% Breast 11.5% Lung 8.2% Others 4.9%	15-25 Gy/1 #	11	70.7	79.6
Omari <i>et al.</i> ^[28] 2019	Retrospective	14	Renal	16 (6.5-27.4) Gy/1-5 #	10	92.6 (at median 10.2 months)	NR

HDRBT: high dose-rate brachytherapy; LC: local control; OS: overall survival; GI: gastrointestinal; GB: gallbladder; NR: not reported

of treatment. Prophylactic anti-emetics may be used to counteract these effects. Pain is also a common complaint which may be treated with appropriate analgesia.

Procedure-related toxicity, such as bleeding, is usually limited to the subcapsular space and rarely requires transfusion. Potentially serious, but rare, complications include intra-hepatic biliary occlusion, liver abscess, gastrointestinal ulceration, and non-classic RILD, occurring in less than 1% of cases^[21].

Table 3. Outcomes of recent palliative whole or partial liver irradiation

Authors	Primary tumor	n	Treatment	Dose/fractionation (#)	Outcome	Toxicities
Bydder <i>et al.</i> ^[33] 2003	CRC 39% NSCLC 4% Esophageal 11% SCLC 7% Other 29%	28	WLRT/PLRT	10 Gy/2 #	54% partial or complete symptomatic response	2 patients with Grade 3 vomiting and diarrhoea
Yin <i>et al.</i> ^[35] 2014	CRC	19	WLRT + tumor boost + concurrent chemotherapy	53.4 Gy (including boost)/# NR	52.6% overall response	2 patients with Grade 3 elevated bilirubin
Edyta <i>et al.</i> ^[34] 2015	Colon 59% Stomach 26% Pancreas 15%	27	WLRT	Mean 17 Gy/5-12 #	40% partial or complete symptomatic response	1 patient with Grade 3 vomiting and diarrhoea

CRC: colorectal cancer; NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; NR: not reported

Comparison between Brachytherapy, SBRT and RFA

Unlike SBRT, interstitial HDRBT to the liver delivers highly lethal doses of radiation from inside to out. As such, the physical property of HDRBT plays to its advantage, as the central part of the tumour is often radio-resistant due to tumour hypoxia. Hass *et al.*^[29] performed a dosimetric comparison and demonstrated HDRBT to be superior to SBRT in terms of tumour coverage, whilst reducing the dose received by the unaffected liver parenchyma. However, SBRT has better dose manoeuvring options as compared to HDRBT, especially in non-oval shaped targets. SBRT has the advantage of being a non-invasive procedure which reduces the procedure-associated risks, such as bleeding and infection.

Compared to RFA, the outcomes of interstitial HDRBT liver are not affected by the proximity to the great vessels or vascularized tumour (“heat sink effect”). Lesions near the main intra-hepatic biliary ducts are better treated with HDRBT compared to RFA^[22]. Similarly, lesions near segment VII/VIII, and those near the diaphragm, are technically difficult to treat with RFA. RFA is limited by lesion size, as discussed earlier, whilst in HDRBT, large lesions can be treated with the use of multiple applicators. RFA does have the advantage of real-time manoeuvrability, whereby under ultrasound guidance the operator can keep the RFA probe away from non-static structures such as the stomach and small intestine.

THE USE OF RADIOTHERAPY FOR PALLIATION OF SYMPTOMS

In cases of uncontrolled hepatic metastases, patients may consequently experience abdominal pain (from capsular stretch), nausea and vomiting, jaundice, and constitutional symptoms such as weight loss or night sweats. In general, systemic therapy for palliation can be used for such patients, although a large number will eventually be refractory in end-stage disease. WLRT or partial liver irradiation (PLRT) has been shown to effectively palliate such patients, thereby improving quality of life^[30-32]. Examples of regimens include 8Gy/1 fraction, 21Gy/7 fractions, and 30Gy/15 fractions. Bydder *et al.*^[33] reported prospectively between 53% to 66% improvement in symptoms at 2 weeks. Edyta *et al.*^[34] reported a 100% improvement in symptoms at 1 month in a retrospective study. The results of these studies are shown in further detail in Table 3.

Palliative liver radiotherapy is delivered using a simple method of conventional radiotherapy. Patients are positioned supine and treated with 2 to 3 portals, including most of the liver. Treatment is generally well-tolerated and serious adverse events are rare. Most patients may experience grade 1 to 2 anorexia, and nausea and vomiting following treatment with radiotherapy, and these can be managed symptomatically. Dexamethasone and anti-emetics are useful to counter radiotherapy-induced nausea.

CONCLUSION

Alongside surgical resection of hepatic metastases, local ablative therapies in the form of SBRT and CT-HDRBT have a role in the management of oligometastatic disease. Prospective randomized trials comparing

the various modalities are needed to elucidate comparative long-term outcomes of RT specifically. Dose selection is currently arbitrary, based on lesion size, location and liver function. However, we acknowledge that the spectrum of primary tumours may have varying radio-sensitivity, and an attempt to tailor the dose (biologically-guided treatment), according to the different primaries, should be investigated^[36]. In addition, the patient and disease should be considered holistically. As such, multidisciplinary discussion and collaboration between surgeons, interventional radiologists and oncologists is crucial. Treatment options should be personalized, with the pros and cons of each therapy balanced against the risk of disease progression. On the other end of the palliative spectrum, low-dose whole or partial liver radiotherapy may be used for patients with high disease burden and severe symptoms.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and design of the work: John RG, Vellayappan BA

Drafted the manuscript: John RG, Appalanaido GK, Vellayappan BA

Reviewed the manuscript and provided approval for publication of the content: John RG, Ho F, Appalanaido GK, Chen D, Tey J, Soon YY, Vellayappan BA

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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Not applicable.

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REFERENCES

1. Van Cutsem E, Nordlinger B, Adam R, Köhne CH, Pozzo C, et al. Towards a pan-European consensus on the treatment of patients with colorectal liver metastases. *Eur J Cancer* 2006;42:2212-21.
2. Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. *Ann Surg* 1999;230:309-18.
3. Pan CC, Kavanagh BD, Dawson LA, Li XA, Das SK, et al. Radiation-associated liver injury. *Int J Radiat Oncol Biol Phys* 2010;76:S94-100.
4. Hellman S, Weichselbaum RR. Oligometastases. *J Clin Oncol* 1995;13:8-10.
5. Palma DA, Olson R, Harrow S, Gaede S, Louie AV, et al. Stereotactic ablative radiotherapy versus standard of care palliative treatment in patients with oligometastatic cancers (SABR-COMET): a randomised, phase 2, open-label trial. *Lancet* 2019;393:2051-8.
6. Potters L, Kavanagh B, Galvin JM, Hevezi JM, Janjan NA, et al. American society for therapeutic radiology and oncology (ASTRO) and American college of radiology (ACR) practice guideline for the performance of stereotactic body radiation therapy. *Int J Radiat Oncol Biol Phys* 2010;76:326-32.
7. Scorsetti M, Comito T, Tozzi A, Navarria P, Fogliata A, et al. Final results of a phase II trial for stereotactic body radiation therapy for patients with inoperable liver metastases from colorectal cancer. *J Cancer Res Clin Oncol* 2015;14:543-53.
8. Goodman BD, Mannina EM, Althouse SK, Maluccio MA, Cárdenes HR. Long-term safety and efficacy of stereotactic body radiation

- therapy for hepatic oligometastases. *Pract Radiat Oncol* 2016;6:86-95.
9. McPartlin A, Swaminath A, Wang R, Pintilie M, Brierley J, et al. Long-term outcomes of phase 1 and 2 studies of SBRT for hepatic colorectal metastases. *Int J Radiat Oncol Biol Phys* 2017;99:388-95.
 10. Joo JH, Park JH, Kim JC, Yu CS, Lim SB, et al. Local control outcomes using stereotactic body radiation therapy for liver metastases from colorectal cancer. *Int J Radiat Oncol Biol Phys* 2017;99:876-83.
 11. Mahadevan A, Blanck O, Lanciano R, Peddada A, Sundararaman S, et al. Stereotactic body radiotherapy (SBRT) for liver metastasis - clinical outcomes from the international multi-institutional RSSearch(R) patient registry. *Radiat Oncol* 2018;13:26.
 12. Ingold JA, Reed GB, Kaplan HS, Bagshaw MA. Radiation hepatitis. *Am J Roentgenol Radium Ther Nucl Med* 1965;93:200-8.
 13. Hoyer M, Roed H, Traberg Hansen A, Ohlhuis L, Petersen J, et al. Phase II study on stereotactic body radiotherapy of colorectal metastases. *Acta Oncol* 2006;45:823-30.
 14. Decadt B, Siriwardena AK. Radiofrequency ablation of liver tumours: systematic review. *Lancet Oncol* 2004;5:550-60.
 15. Stang A, Fischbach R, Teichmann W, Bokemeyer C, Braumann D. A systematic review on the clinical benefit and role of radiofrequency ablation as treatment of colorectal liver metastases. *Eur J Cancer* 2009;45:1748-56.
 16. Jackson WC, Tao Y, Mendiratta-Lala M, Bazzi L, Wahl DR. Comparison of stereotactic body radiation therapy and radiofrequency ablation in the treatment of intrahepatic metastases. *Int J Radiat Oncol Biol Phys* 2018;100:950-8.
 17. Dritschilo A, Grant EG, Harter KW, Holt RW, Rustgi SN, et al. Interstitial radiation therapy for hepatic metastases: sonographic guidance for applicator placement. *AJR Am J Roentgenol* 1986;147:275-8.
 18. Thomas DS, Nauta RJ, Rodgers JE, Popescu GF, Nguyen H, et al. Intraoperative high-dose rate interstitial irradiation of hepatic metastases from colorectal carcinoma. Results of a phase I-II trial. *Cancer* 1993;71:1977-81.
 19. Ricke J, Wust P, Wieners G, Beck A, Cho CH, et al. Liver malignancies: CT-guided interstitial brachytherapy in patients with unfavorable lesions for thermal ablation. *J Vasc Interv Radiol*, 2004 15:1279-86.
 20. Ricke J, Mohnike K, Pech M, Seidensticker M, Rühl R, et al. Local response and impact on survival after local ablation of liver metastases from colorectal carcinoma by computed tomography-guided high-dose-rate brachytherapy. *Int J Radiat Oncol Biol Phys* 2010;78:479-85.
 21. Mohnike K, Wolf S, Damm R, Seidensticker M, Seidensticker R, et al. Radioablation of liver malignancies with interstitial high-dose-rate brachytherapy: complications and risk factors. *Strahlenther Onkol* 2016;192:288-96.
 22. Tselis N, Chatzikonstantinou G, Kolotas C, Milickovic N, Baltas D, et al. Computed tomography-guided interstitial high dose rate brachytherapy for centrally located liver tumours: a single institution study. *Eur Radiol* 2013 23:2264-70.
 23. Colletini F, Singh A, Schnapauff D, Powerski MJ, Denecke T, et al. Computed-tomography-guided high-dose-rate brachytherapy (CT-HDRBT) ablation of metastases adjacent to the liver hilum. *Eur J Radiol* 2013;82:e509-14.
 24. Wieners G, Mohnike K, Peters N, Bischoff J, Kleine-Tebbe A, et al. Treatment of hepatic metastases of breast cancer with CT-guided interstitial brachytherapy - a phase II-study. *Radiother Oncol* 2011;100:314-9.
 25. Colletini F, Golenia M, Schnapauff D, Poellinger A, Denecke T, et al. Percutaneous computed tomography-guided high-dose-rate brachytherapy ablation of breast cancer liver metastases: initial experience with 80 lesions. *J Vasc Interv Radiol* 2012;23:618-26.
 26. Sharma DN, Thulkar S, Sharma S, Gandhi AK, Haresh KP, et al. High-dose-rate interstitial brachytherapy for liver metastases: first study from India. *J Contemp Brachytherapy* 2013;5:70-5.
 27. Kieszko D, Cisek P, Kordzińska-Cisek I, Grzybowska-Szatowska L. Treatment of hepatic metastases with computed tomography-guided interstitial brachytherapy. *Oncol Lett* 2018;15:8717-22.
 28. Omari J, Heinze C, Damm R, Hass P, Janitzky A, et al. Radioablation of hepatic metastases from renal cell carcinoma with image-guided interstitial brachytherapy. *Anticancer Res* 2019;39:2501-8.
 29. Hass P, Mohnike K, Kropf S, Brunner TB, Walke M, et al. Comparative analysis between interstitial brachytherapy and stereotactic body irradiation for local ablation in liver malignancies. *Brachytherapy* 2019;18:823-8.
 30. Yeo SG, Kim DY, Kim TH, Kim SY, Hong YS, et al. Whole-liver radiotherapy for end-stage colorectal cancer patients with massive liver metastases and advanced hepatic dysfunction. *Radiat Oncol* 2010;5:97.
 31. Cunningham SC, Choti MA, Bellavance EC, Pawlik TM. Palliation of hepatic tumors. *Surg Oncol* 2007;16:277-91.
 32. Greco C, Catalano G, Di Grazia A, Orecchia R. Radiotherapy of liver malignancies. From whole liver irradiation to stereotactic hypofractionated radiotherapy. *Tumori* 2004;90:73-9.
 33. Bydder S, Spry NA, Christie DR, Roos D, Burmeister BH, et al. A prospective trial of short-fractionation radiotherapy for the palliation of liver metastases. *Australas Radiol* 2003;47:284-8.
 34. Edyta WR, Jakub L, Jerzy W. Whole liver palliative radiotherapy for patients with massive liver metastases. *Asian Pac J Cancer Prev* 2015;16:6381-4.
 35. Yin H, Lu K, Qiao WB, Zhang HY, Sun D, et al. Whole-liver radiotherapy concurrent with chemotherapy as a palliative treatment for colorectal patients with massive and multiple liver metastases: a retrospective study. *Asian Pac J Cancer Prev* 2014;15:1597-602.
 36. Torres-Roca JF. A molecular assay of tumor radiosensitivity: a roadmap towards biology-based personalized radiation therapy. *Per Med* 2012;9:547-57.

Review

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Contrast-enhanced ultrasound of focal liver masses

Stephanie R. Wilson¹, Christina Merrill²

¹Department of Radiology, University of Calgary, Calgary T2N 2T9, Canada.

²Department of Radiology, Foothills Medical Centre, Calgary T2N 2T9, Canada.

Correspondence to: Prof. Stephanie R. Wilson, Department of Radiology, University of Calgary, 1403 29 Street NW, Calgary T2N 2T9, Canada. E-mail: stephanie.wilson@ahs.ca

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Abstract

Non-invasive imaging is the current method of choice for the characterization of frequently discovered focal liver disease. Although historically, contrast-enhanced computed tomography (CT) and magnetic resonance (MR) scans have been selected for this purpose, contrast-enhanced ultrasound (CEUS) now offers a less expensive and safer method to acquire the same information. Performed with the intravenous injection of a microbubble contrast agent, CEUS provides some unique advantages that make it a valuable addition to an imaging toolbox. CEUS is performed in dynamic real-time, providing superior temporal resolution compared to other modalities and allowing detection of enhancement regardless of its timing or duration. CEUS is performed with a purely intravascular contrast agent, providing accurate depiction of the presence of microbubbles in the circulation in all phases of imaging. This compares with CT and MR contrast agents, which have a well-recognized interstitial phase. Resulting discordant imaging may occur especially in the portal venous phase, when CT and MR may show pseudoenhancement from interstitial contrast, while CEUS will accurately show washout in malignant tumors. Lastly, the contrast specific software used to perform CEUS has an excellent subtraction technique, which produces a contrast only image with high sensitivity to enhancement in thin septations and small nodules. CEUS makes a positive contribution to liver mass characterization in any situation.

Keywords: Contrast-enhanced ultrasound, liver, cancer, diagnosis

INTRODUCTION

Focal liver masses are a frequent occurrence and reflect a wide spectrum of histology ranging from benign and totally insignificant tumors to life-threatening malignancies. Incidental discovery on imaging,



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performed for an unrelated reason, is common, especially for benign tumors. Alternately, tumors may be found as a result of a directed search in those with symptoms, with primary malignancy elsewhere, or in those identified to be at risk for hepatocellular carcinoma (HCC).

Regardless of the nature or method of discovery of a focal liver mass, contrast-enhanced imaging is now a mainstay for its noninvasive characterization. Originally, computed tomography (CT) and magnetic resonance (MR) scans comprised the recommended modalities for this purpose. However, since the introduction of microbubble contrast agents for ultrasound (US) two decades ago, CEUS has become an additional lower cost and safer option with unique benefits^[1].

The late approval of a contrast agent for CEUS in the United States in 2016 makes this an expanding but still introductory modality in North America today. However in other parts of the world, such as Europe, the role of CEUS has been well established by international and national guidelines^[2-4]. Here, we describe the CEUS technique, emphasizing its unique benefits for characterization of focal liver disease.

Ultrasound contrast agents: what are they and how do they work?

Ultrasound contrast agents are comprised of tiny encapsulated bubbles of a perfluoropropane gas within a variable, usually lipid, shell. They are on the same order of size as a red blood cell, and their distribution in the circulation is sparse. They are purely intravascular as their size does not allow them to pass into the interstitial tissues. After their venous injection, they are quickly exhaled by the lungs. They are very safe in clinical practise for adults and for children, they have no nephrotoxicity, and their imaging requires no ionizing radiation^[5-7].

Specialized imaging techniques

The successful imaging of microbubble contrast agents is facilitated by their oscillation when they are viewed in a low-power US field. To detect the signal from the oscillating microbubbles in the circulation, CEUS is performed with contrast specific software^[8]. This generally includes pulse inversion imaging, whereby two pulses are sent down each scan line with subtraction of the returning echoes^[9]. The background tissue, a linear reflector, sends back a wave which is a reflection of the insonating wave. Their subtraction, therefore, equals zero, totally removing the background signal. The result is a relatively black image at the beginning of an imaging sequence for CEUS. The returning echoes from the oscillating microbubble contrast agents, by comparison, will produce additive signals so that there is great augmentation of the Doppler signal from blood. The result of these sequences is the creation of an incredibly sensitive microbubble only image, with removal of the signals from the background tissues. The intensity of the signal on the image occurs in direct proportion to the volume of microbubbles within the region of view. Today, all high-end US systems offer a contrast specific software package with low mechanical index (MI) imaging and also a bubble tracking technique. This bubble tracking may involve a transient brief high MI insonation which destroys bubbles within the field of view, following which the refilling of the vessels may be tracked, improving their visualization.

Contrast-enhanced imaging diagnosis of all liver masses on all modalities focuses on their enhancement following contrast injection. Although CEUS follows the very same principles as CT and MR scan for diagnoses, with similar result, CEUS shows, in addition, unique capabilities that give it a special place for this application^[10].

Unique capabilities of CEUS

Dynamic real-time imaging

Microbubble contrast agents allow US imaging of organ and lesion perfusion in real time. In comparison to CT or MR which creates single snapshots in time, CEUS shows all images as dynamic real-time studies.

Therefore, CEUS has superior temporal resolution compared to CT and MR scan, and it has, consequently, the ability to show enhancement changes regardless of their timing or intensity.

Subtraction technique

CEUS uses a subtraction technique which effectively removes all of the background echogenicity such that all imaging reflects microbubbles only. This microbubble only image provides exceptional sensitivity for CEUS to show enhancement even in very small nodules and thin septations.

Purely intravascular contrast agent

CEUS is performed with purely intravascular contrast agents which will therefore always reflect the presence of contrast agent in a tumor or the liver vasculature. Contrast agents for CT and MR, by comparison, have a well-recognized interstitial phase whereby contrast may leak out of the vasculature into the interstitium of a tumor. The resultant discordant imaging between CEUS and CT/MRI scan makes a very positive contribution to the diagnosis of focal liver masses^[11].

These unique features of CEUS imaging require that CEUS have its own algorithms.

CEUS IMAGING OF FOCAL LIVER MASSES

CEUS of a focal liver mass is based on the enhancement changes that occur over time in a nodule, which is preidentified on greyscale US and maintained within the field of view for the entire scan. These changes are described for the nodule relative to the adjacent liver parenchyma. Initial imaging of the pre-identified nodule should be performed continuously, from contrast injection until peak arterial phase enhancement. Subsequent imaging is intermittent (5-10 s every 30-60 s) to detect any washout and assess its degree, until the end of useful enhancement, generally around 5 to 6 min.

This described technique, with only a brief initial acquisition of multiframe continuous images, audio video interleave (avi), will minimize microbubble destruction. This improves the ability to detect late washout and assess its degree.

Additional valuable scanning techniques include sweeping the entire liver in the portal/late phase with suspended inspiration to identify additional areas of abnormal enhancement, especially washout. Furthermore, injections can be repeated as needed including “on top” of a prior injection to assess vascularity of an identified area of washout.

ALGORITHMS FOR DIAGNOSIS OF FOCAL LIVER MASSES ON CEUS

Experience with the use of microbubble contrast agents for liver mass characterization has led to the recognition that multiple constant features lead to reliable differentiation of benign and malignant masses and also to their specific accurate diagnoses. Resultant algorithms for diagnosis of focal liver tumors on contrast imaging are all based on interpretation of enhancement features of an identified nodule relative to the adjacent liver from the injection of the contrast agent to the end of useful enhancement, generally around 5 or 6 min following injection^[12]. Time zero corresponds to the beginning of the saline flush following the contrast injection^[13]. On CEUS, all timing is given in seconds and minutes rather than in phases. Nonetheless, we include both specific timing and phases for completeness and modality comparisons.

Arterial phase (AP) (from 10 to 20 - 30 to 45 s)^[13] observations include:

Enhancement (yes or no)

(1) **Intensity** (hyper, iso, hypo, or nonenhancing)

(2) **Pattern of AP hyperenhancement (APHE)** (Diffuse, nodule-in-nodule, rim, peripheral discontinuous globular, or stellate)

(3) Direction of vascular filling (centrifugal or centripetal)

Portal venous phase (PVP) (45 s - 2 min) and **Late phase (LP)** (from 2 min to 5 or 6 min) observations include:

Washout (yes or no)

(1) **Timing** (rapid, < 1 min; or late, > 1 min)

(2) **Intensity** (weak, with some bubbles remaining in a nodule which is less enhanced than the liver; or marked, whereby the nodule appears black or punched out by 2 min)

Importance of the clinical scenario

Additionally, for all contrast-enhanced liver imaging, interpretations must be made with knowledge of demographic and clinical information. Age and sex are helpful as are relevant health events with the greatest importance given to a history of cancer and also risk factors for development of HCC. The clinical indication for a scan, whether it be for surveillance for metastatic tumor or HCC or related to symptoms may also be directive. The algorithmic interpretation of CEUS should always be guided by what is a reasonable answer for the patient under study.

FUNDAMENTALS FOR INTERPRETATION OF CEUS OF FOCAL LIVER DISEASE

Principle 1. The intensity in a CEUS image reflects the number of microbubbles in the field of view

As these are exclusively in blood vessels, echo-enhancement is therefore indicative of the volume of blood and its change with time indicative of the rate of perfusion in the region of interest. All interpretations compare nodule enhancement levels with the adjacent and enhancing liver parenchyma.

Principle 2. Determination of malignancy: importance of washout

Most malignant masses are identified by washout of the mass in the portal venous or late phase^[14,15]. Washout refers to the decline in the enhancement of a mass relative to that of the adjacent liver parenchyma, following initial AP enhancement. Therefore, if washout is present, malignancy should be considered likely [Figures 1 and 2]. Conversely, if washout is not present and if the mass shows sustained enhancement, there is a high likelihood that it is benign^[16]. These rules are guidelines and there are, of course, exceptions. These exceptions to this important rule are very important, since occasionally, benign tumors, focal nodular hyperplasia (FNH), hemangioma, and most often adenoma, may show washout, and some malignant tumors, especially precursor nodules for HCC and well-differentiated HCC, may show no washout. Nonetheless, the fundamental rule is invaluable.

Principle 3. Differentiation of malignancy: importance of timing and intensity of washout

Although CT and MR scan also determine the presence of washout, CEUS additionally evaluates the intensity and timing of washout, further differentiating hepatocellular from nonhepatocellular malignancy^[17]. HCC tends to show late (later than 1 min) and weak washout (so that some bubbles remain in the washout zone) [Figure 1, Video 1], whereas all nonhepatocellular malignancies, including intrahepatic cholangiocarcinoma (ICC), lymphoma and metastasis are characterized by rapid (earlier than 1 min) and marked washout, so that all bubbles are absent from the nodule, making it appear as a black, punched out hole [Figure 2, Video 2]^[18,19]. This rapid washout may even occur so early as to be within the traditionally defined AP, prior to 30 s.

Principle 4. Detection of malignancy

A natural consequence of the two previous principles is that the portal venous phase is the optimal time to detect metastases, when their conspicuity will be increased relative to the enhanced background parenchyma^[20]. This increased conspicuity makes sweeping of the entire liver at this time the ideal

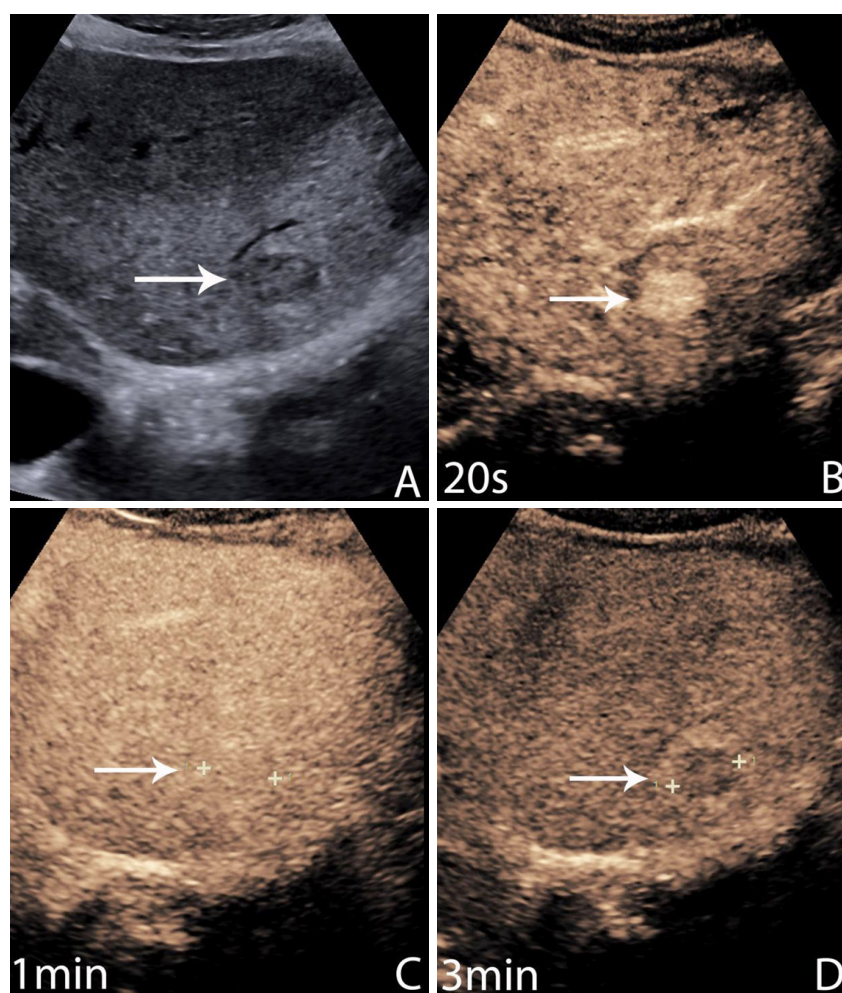


Figure 1. Hepatocellular carcinoma (HCC). 64-year-old male with a history of cirrhosis related to alcohol and fatty liver. Mass found on regular US surveillance. A greyscale image shows a small hypoechoic liver nodule in a very heterogeneous liver (A); at the peak of arterial phase (AP) enhancement, 20 s, the mass is brightly enhanced (B); at one minute, there is isoenhancement. The nodule is now invisible but marked by the arrow (C); there is late and weak washout at 3 min. Weak washout shows less enhancement than the immediately adjacent liver parenchyma but retains bubble signals within the nodule as here. AP hyperenhancement and late weak washout comprise the diagnostic features of HCC (D)

technique and timing for improved detection of metastatic liver masses on CEUS. Although CEUS is not generally involved in the protocolled search for metastases which are developed for follow-up of common tumors of the colon, lung and breast, in particular, it has been shown that CEUS is comparable to CT scan in tumor detection and exceeds CT in some instances^[21]. Additionally, CEUS plays an important role in the oncology patient, resolving many indeterminate CT and MR scans and generally problem-solving in this population. In our department, CEUS is invaluable to resolve the low-attenuation small indeterminate masses shown regularly on portal venous phase CT scans, easily differentiating small cysts from solid tumors with CEUS features of metastatic disease^[22].

Principle 5. Diagnosis of benign tumors

Benign tumors commonly encountered on CEUS examinations include hemangioma, focal nodular hyperplasia and, much less often, hepatic adenoma. It is recognized that CEUS has a unique capability for their diagnosis in that all may demonstrate highly suggestive enhancement patterns in the AP^[23]. Optimally shown by the real-time dynamic scanning afforded by CEUS. Recognizing the significance of washout as an indicator of malignancy, it follows that sustained enhancement, such that the lesion will

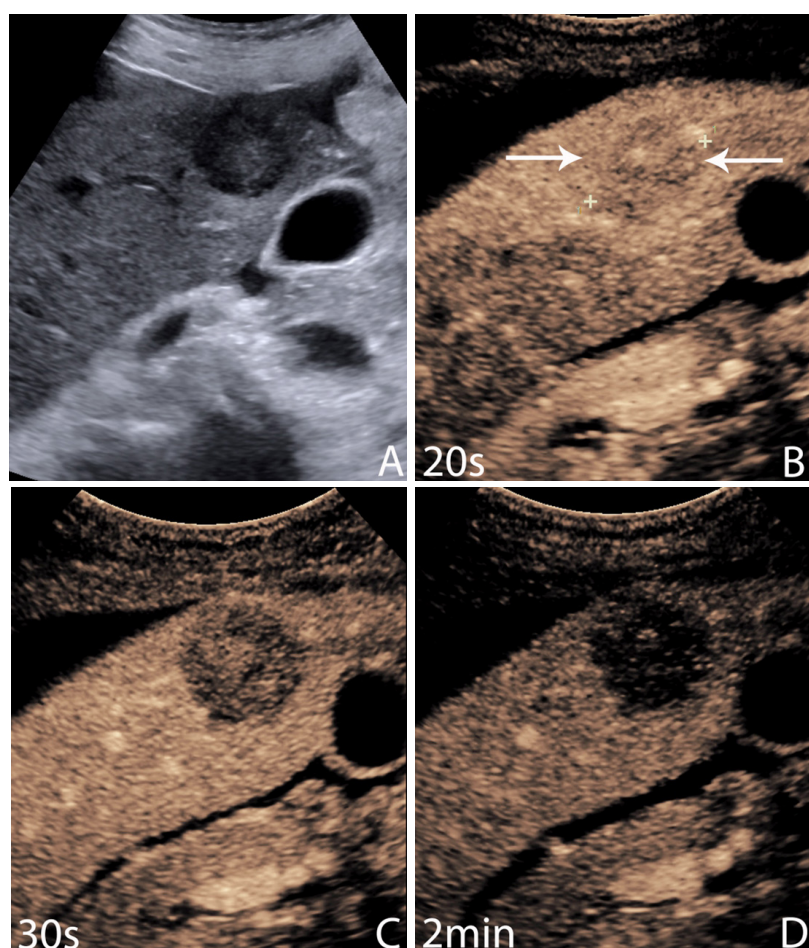


Figure 2. Metastasis. 70-year-old female with abdominal ultrasound performed for right upper quadrant pain, query gallstones. Greyscale ultrasound shows a focal hypoechoic mass in the right lobe of the liver (A); at 20 s, the mass is hypoenhanced, appearing very slightly less bright than the liver (B); at 30 s, there is already washout of the mass, so that it is now much less enhanced than the adjacent liver. It is also less enhanced than it was at 20 s (C); at 2 min, the mass appears as a black hole due to marked washout. This rapid transition from enhancement to washout is typical of metastatic disease. Biopsy showed adenocarcinoma, of unknown origin (D)

remain as enhanced as the adjacent liver to 4 or even 5 min following injection, is highly associated with benign outcome. **Hemangiomas** are characterized by peripheral nodular enhancement with centripetal progression of this enhancement over time [Figure 3, Video 3]. If these enhancement patterns are rapidly changing, they may not be appreciated on CT and MR scans, both of which obtain snapshots in time. This is especially important for the recognition of rapid, or “flash”, filling hemangiomas on CEUS which may show only as a zone of APHE on CT/MRI scan.

FNH is a highly vascular tumor and is characterized by stellate vessel morphology with filling of the lesion from its center to the periphery^[24] [Figure 4, Video 4].

Hepatic adenoma, a fairly rare liver tumor, is an exception to these general rules and may show diffuse enhancement or a more specific filling from the periphery to the center of the lesion [Figure 5, Video 5]. Additionally, adenoma is shown to have the possibility for washout, occurring in up to 50% of cases^[25].

The above described benign tumors, FNH and adenoma, are most often detected incidentally, often on imaging performed for a totally unrelated reason. Adenoma has the additional association of long history of use of oral contraceptives. Both of these tumors have a propensity to occur in asymptomatic young

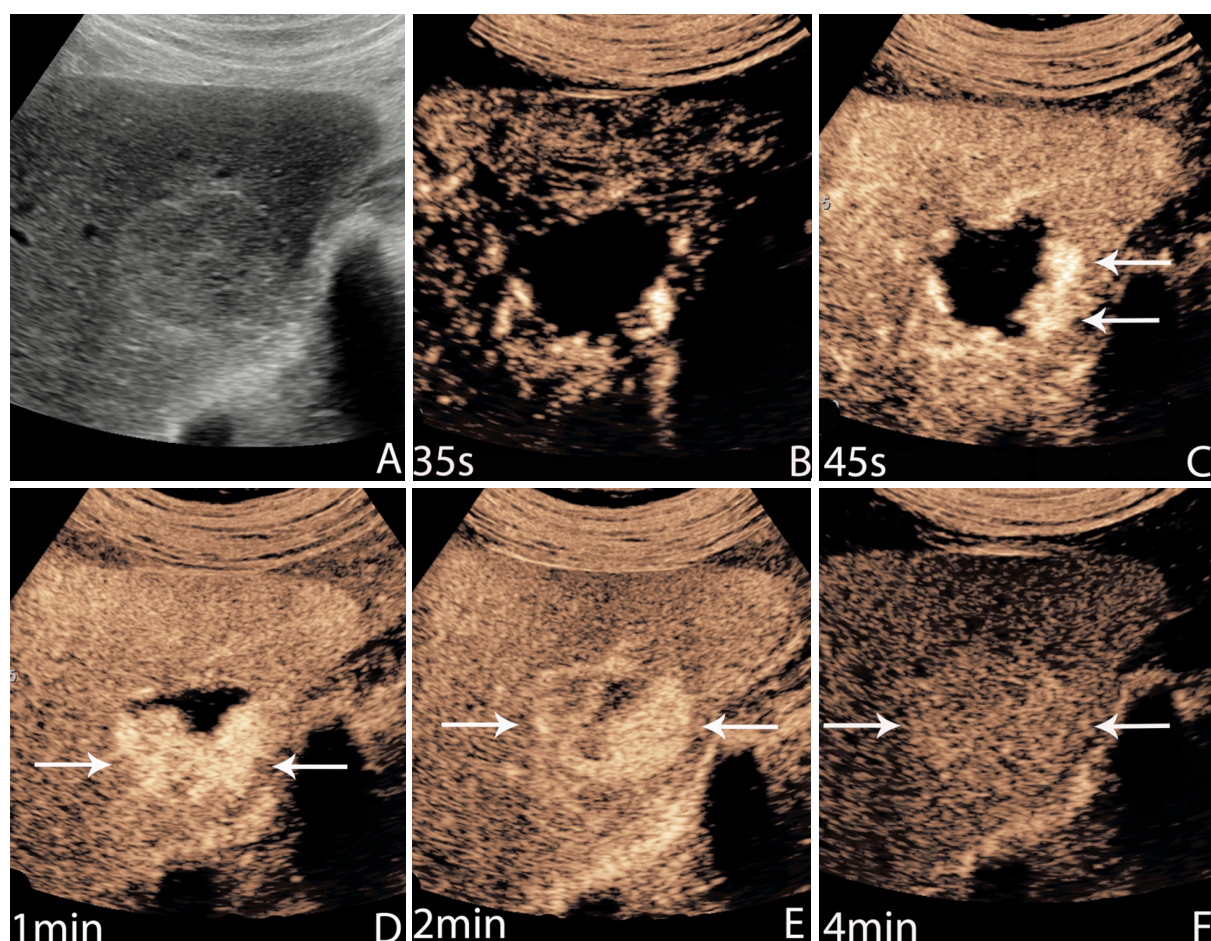


Figure 3. Hemangioma. 39-year-old asymptomatic female with an incidentally discovered liver mass during an US examination for unrelated reasons. A greyscale image shows a focal slightly hypoechoic mass with a thin echogenic rim. It is suggestive of an atypical hemangioma (A); an early AP image, at 35 s, shows a rim of peripheral enhancement with tiny peripheral puddles (B); there is centripetal progression by 45 s with no linear vascularity (C); by 1 min, there is further centripetal progression with eccentric growth of the peripheral puddles of enhancement (D); by 2 min, there is virtually complete fill in with non-uniform enhancement (E); there is sustained enhancement to 4 min. Peripheral nodular enhancement with sustained enhancement is the classic description of an insignificant hemangioma. Hemangiomas may fill very rapidly, within seconds, or very slowly as here, over several minutes (F)

females, and CEUS ranks with MRI performed with a liver specific agent with a dual elimination pathway for accurate differentiation of the two.

Principle 6. Precise diagnosis of malignant tumors

Unlike benign lesions, malignant tumors are not generally characterized as reliably by their AP enhancement pattern, which may be highly variable, but instead by the identification of washout in the portal venous and late phases^[26]. Nonetheless, HCC most often shows a globular pattern of APHE [Figure 1, Video 1]. Nonhepatocellular malignancies may show a similar pattern but also frequently show highly suggestive rim enhancement in the AP^[27].

Principle 7. Discordance of imaging between CEUS and CT/MRI scan

Discordance of imaging between CEUS and CT/MRI scan in the portal venous phase is often related to differences in the mechanism of action of the contrast agents for each modality^[11]. Microbubble contrast agents for ultrasound are purely intravascular agents and they do not have any interstitial phase. This is unlike contrast agents for CT and MR scanning where there is a well-recognized interstitial phase,

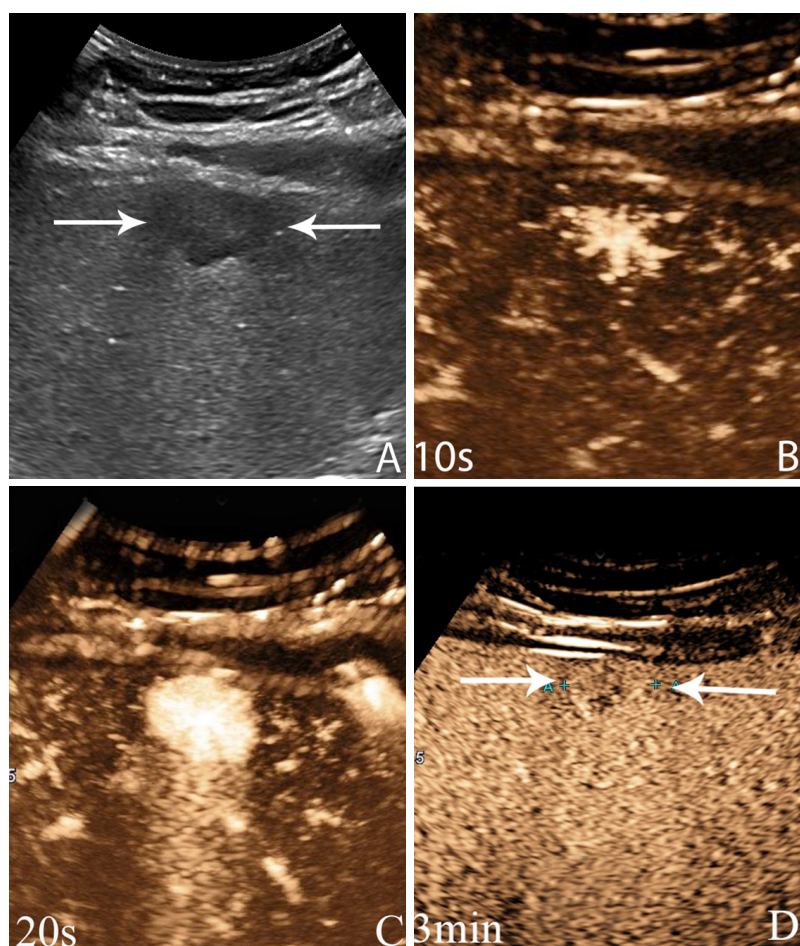


Figure 4. Focal nodular hyperplasia. 25-year-old woman with an incidentally discovered liver mass found during ultrasound performed for examination of her bowel. An incidentally discovered superficial focal hypoechoic liver mass (A); an early arterial phase (AP) image shows a stellate pattern of linear vessels in the center of the mass (B); at the peak of AP enhancement, the mass is brightly and uniformly enhanced (C); at 3 min, the mass continues to show sustained enhancement greater than the adjacent liver (D)

especially evident in malignant tumors with permeable vascular endothelium and a fibrous stroma, such as ICC. Here, the contrast agent leaks into the interstitium, creating a type of pseudoenhancement in the late phase. Thereby, CEUS will show APHE and rapid washout for ICC whereas CT and MR scan may show instead sustained enhancement. This sustained enhancement is in fact pseudoenhancement related to the presence of interstitial contrast agent. This produces a valuable discordance with MR scan of these tumors where washout is appropriately recognized in association with malignant tumors on CEUS only^[28]. In our own unpublished data, this is most important for diagnosis of cholangiocarcinoma in any liver, either cirrhotic or normal.

ACCURACY OF CEUS

CEUS shows comparable performance with CT and MR for liver mass characterization and for the prediction of malignancy^[26,29]. In a prospective multicenter trial with 1,349 patients, there was no statistically significant difference between CEUS and spiral CT in liver tumor differentiation (malignant or benign) and tumor specification^[30]. In a meta-analysis of 21 studies, CEUS showed a sensitivity of 88% (95%CI: 87%-90%) and specificity of 81% (95%CI: 79%-84%), and diagnostic odds ratio (DOR) was 38.62 (95%CI: 13.64%-109.35%), while all parameters were similar with no statistical difference from CECT or CEMRI^[31]. Furthermore, although of secondary importance to characterization, CEUS is shown to

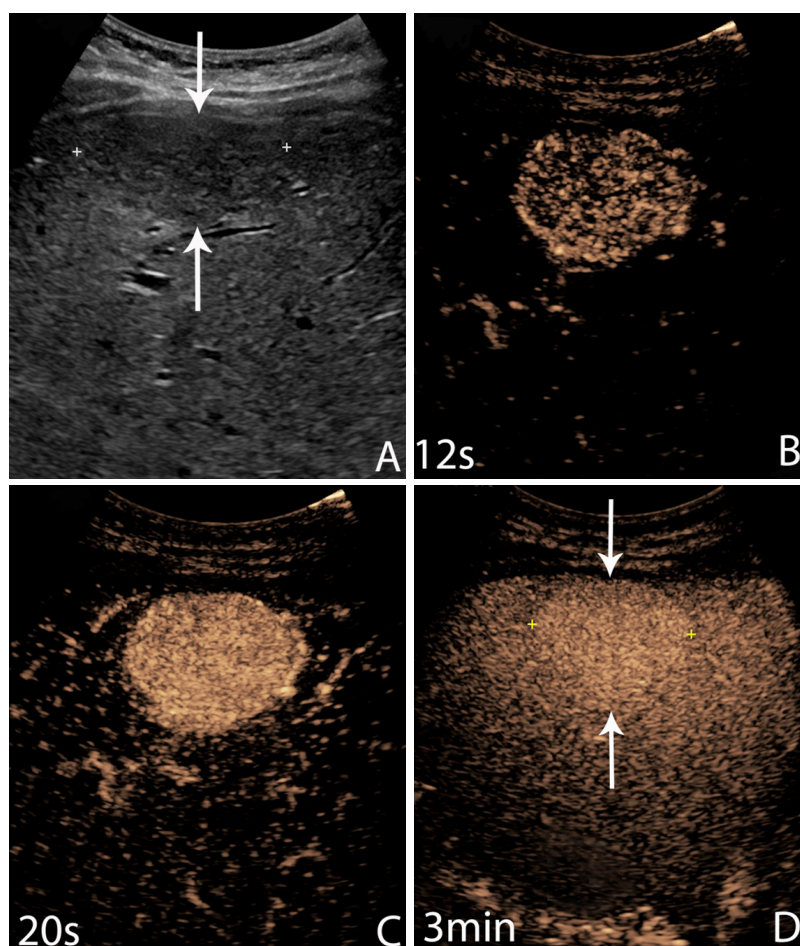


Figure 5. Adenoma. 34-year-old woman with a long continuous history of oral contraceptive use. a greyscale image shows a superficial focal hypoechoic mass (arrows) in a fatty liver (A); an early arterial phase (AP) image at 12 s shows peripheral irregular vessels (B); at the peak of AP enhancement, 20 s, the mass is uniformly hyperenhanced (C); at 3 min, the mass shows sustained enhancement such that it is still slightly brighter than the adjacent enhanced liver parenchyma (D)

have equivalent, and in some cases superior, performance to CECT and CEMR scan in the detection of metastatic disease.

In conclusion, CEUS provides for the determination of malignancy and allows excellent differential diagnosis of a focal liver mass. CEUS regularly resolves an indeterminate result on CT/MRI and is exceptional as a problem-solving tool in liver imaging. CEUS has many similarities to contrast-enhanced CT/MRI but also unique and valuable differences, most showing the additional benefit of inclusion of CEUS with CT and MR for liver imaging.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and data collection: Wilson SR, Merrill C

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Wilson SR reports equipment support from Siemens, Samsung and Philips. Wilson SR reports a research grant from Samsung. Merrill C declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Romanini L, Passamonti M, Aiani L, Cabassa P, Raieli G, et al. Economic assessment of contrast-enhanced ultrasonography for evaluation of focal liver lesions: a multicentre Italian experience. *Eur Radiol* 2007;17 Suppl 6:F99-106.
2. Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, et al. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver--update 2012: a WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultraschall Med* 2013;34:11-29.
3. Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, et al; World Federation for Ultrasound in Medicine. European Federation of Societies for Ultrasound. Guidelines and good clinical practice recommendations for Contrast Enhanced Ultrasound (CEUS) in the liver - update 2012: a WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultrasound Med Biol* 2013;39:187-210.
4. Piscaglia F, Nolsøe C, Dietrich CF, Cosgrove DO, Gilja OH, et al. The EFSUMB Guidelines and Recommendations on the Clinical Practice of Contrast Enhanced Ultrasound (CEUS): update 2011 on non-hepatic applications. *Ultraschall Med* 2012;33:33-59.
5. Piscaglia F, Bolondi L; Italian Society for Ultrasound in Medicine and Biology (SIUMB) Study Group on Ultrasound Contrast Agents. The safety of Sonovue in abdominal applications: retrospective analysis of 23188 investigations. *Ultrasound Med Biol* 2006;32:1369-75.
6. Darge K, Papadopoulou F, Ntoulia A, Bulas DI, Coley BD, et al. Safety of contrast-enhanced ultrasound in children for non-cardiac applications: a review by the Society for Pediatric Radiology (SPR) and the International Contrast Ultrasound Society (ICUS). *Pediatr Radiol* 2013;43:1063-73.
7. Schwarze V, Marschner C, Negrão de Figueiredo G, Rübenthaler J, Clevert DA. Single-center study: evaluating the diagnostic performance and safety of contrast-enhanced ultrasound (CEUS) in pregnant women to assess hepatic lesions. *Ultraschall Med* 2020;41:29-35.
8. Averkiou M. Nonlinear pulsing schemes for the detection of ultrasound contrast agents. Available from: https://www.academia.edu/25447720/Nonlinear_pulsing_schemes_for_the_detection_of_ultrasound_contrast_agents. [Last accessed on 13 Aug 2020].
9. Burns PN, Wilson SR, Simpson DH. Pulse inversion imaging of liver blood flow: improved method for characterizing focal masses with microbubble contrast. *Invest Radiol* 2000;35:58-71.
10. Burns PN, Wilson SR. Focal liver masses: enhancement patterns on contrast-enhanced images--concordance of US scans with CT scans and MR images. *Radiology* 2007;242:162-74.
11. Wilson SR, Kim TK, Jang HJ, Burns PN. Enhancement patterns of focal liver masses: discordance between contrast-enhanced sonography and contrast-enhanced CT and MRI. *AJR Am J Roentgenol* 2007;189:W7-12.
12. Wilson SR, Burns PN. An algorithm for the diagnosis of focal liver masses using microbubble contrast-enhanced pulse-inversion sonography. *AJR Am J Roentgenol* 2006;186:1401-12.
13. Lyshchik A, Kono Y, Dietrich CF, Jang HJ, Kim TK, et al. Contrast-enhanced ultrasound of the liver: technical and lexicon recommendations from the ACR CEUS LI-RADS working group. *Abdom Radiol (NY)* 2018;43:861-79.
14. Burns PN, Wilson SR. Microbubble contrast for radiological imaging: 1. Principles. *Ultrasound Q* 2006;22:15-8.
15. Stobel D, Seitz K, Blank W, Schuler A, Dietrich C, et al. Contrast-enhanced ultrasound for the characterization of focal liver lesions--diagnostic accuracy in clinical practice (DEGUM multicenter trial). *Ultraschall Med* 2008;29:499-505.
16. Friedrich-Rust M, Klopffleisch T, Nierhoff J, Herrmann E, Vermehren J, et al. Contrast-Enhanced Ultrasound for the differentiation of benign and malignant focal liver lesions: a meta-analysis. *Liver Int* 2013;33:739-55.
17. Bhayana D, Kim TK, Jang HJ, Burns PN, Wilson SR. Hypervascular liver masses on contrast-enhanced ultrasound: the importance of washout. *AJR Am J Roentgenol* 2010;194:977-83.
18. Sporea I, Martie A, Bota S, Sirli R, Popescu A, et al. Characterization of focal liver lesions using contrast enhanced ultrasound as a first

- line method: a large monocentric experience. *J Gastrointest Liver Dis* 2014;23:57-63.
19. Kong WT, Wang WP, Huang BJ, Ding H, Mao F. Value of wash-in and wash-out time in the diagnosis between hepatocellular carcinoma and other hepatic nodules with similar vascular pattern on contrast-enhanced ultrasound. *J Gastroenterol Hepatol* 2014;29:576-80.
 20. Murphy-Lavalley J, Jang HJ, Kim TK, Burns PN, Wilson SR. Are metastases really hypovascular in the arterial phase? The perspective based on contrast-enhanced ultrasonography. *J Ultrasound Med* 2007;26:1545-56.
 21. Albrecht T, Blomley MJ, Burns PN, Wilson S, Harvey CJ, et al. Improved detection of hepatic metastases with pulse-inversion US during the liver-specific phase of SHU 508A: multicenter study. *Radiology* 2003;227:361-70.
 22. Weskott HP. Detection and characterization of liver metastases. *Radiologe* 2011;51:469-74.
 23. Laumonier H, Cailliez H, Balabaud C, Possenti L, Zucman-Rossi J, et al. Role of contrast-enhanced sonography in differentiation of subtypes of hepatocellular adenoma: correlation with MRI findings. *AJR Am J Roentgenol* 2012;199:341-8.
 24. Kim TK, Jang HJ, Burns PN, Murphy-Lavalley J, Wilson SR. Focal nodular hyperplasia and hepatic adenoma: differentiation with low-mechanical-index contrast-enhanced sonography. *AJR Am J Roentgenol* 2008;190:58-66.
 25. Dietrich CF, Mertens JC, Braden B, Schuessler G, Ott M, et al. Contrast-enhanced ultrasound of histologically proven liver hemangiomas. *Hepatology* 2007;45:1139-45.
 26. Durot I, Wilson SR, Willmann JK. Contrast-enhanced ultrasound of malignant liver lesions. *Abdom Radiol (NY)* 2018;43:819-47.
 27. Lee HS, Kim MJ, An C. How to utilize LR-M features of the LI-RADS to improve the diagnosis of combined hepatocellular-cholangiocarcinoma on gadoxetate-enhanced MRI? *Eur Radiol* 2019;29:2408-16.
 28. Fowler KJ, Potretzke TA, Hope TA, Costa EA, Wilson SR. LI-RADS M (LR-M): definite or probable malignancy, not specific for hepatocellular carcinoma. *Abdom Radiol (NY)* 2018;43:149-57.
 29. Trillaud H, Bruel JM, Valette PJ, Vilgrain V, Schmutz G, et al. Characterization of focal liver lesions with SonoVue-enhanced sonography: international multicenter-study in comparison to CT and MRI. *World J Gastroenterol* 2009;15:3748-56.
 30. Seitz K, Bernatik T, Strobel D, Blank W, Friedrich-Rust M, et al. Contrast-enhanced ultrasound (CEUS) for the characterization of focal liver lesions in clinical practice (DEGUM Multicenter Trial): CEUS vs. MRI--a prospective comparison in 269 patients. *Ultraschall Med* 2010;31:492-9.
 31. Guang Y, Xie L, Ding H, Cai A, Huang Y. Diagnosis value of focal liver lesions with SonoVue®-enhanced ultrasound compared with contrast-enhanced computed tomography and contrast-enhanced MRI: a meta-analysis. *J Cancer Res Clin Oncol* 2011;137:1595-605.

Review

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Viral hepatitis as a risk factor for the development of hepatocellular carcinoma

Saleh A. Alqahtani^{1,2}, Massimo Colombo³

¹Liver Transplantation Unit, King Faisal Specialist Hospital & Research Centre, Riyadh 12713, Saudi Arabia.

²Division of Gastroenterology and Hepatology, Johns Hopkins University, Baltimore, MD 21287, USA.

³Center of Translational Research in Hepatology, Humanitas Hospital, Rozzano 20089, Italy.

Correspondence to: Dr. Massimo Colombo, Center of Translational Research in Hepatology, Humanitas Hospital, Manzoni 56, Rozzano 20089, Italy. E-mail: mcolombo46@yahoo.it

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Abstract

Hepatocellular carcinoma (HCC) is the fourth leading global cause of tumor-related mortality. HCC has a high prevalence in patients with chronic liver diseases, and it mostly results from cirrhosis caused by infection with blood-borne viruses. Despite the implementation of various diagnostic and prevention strategies, the rates of new HCC cases and mortality are increasing globally due to the aging and growth of the world population as well as their increased exposure to dominant risk factors like alcohol, hepatitis B and C, and clinical correlates of metabolic syndrome. Modeling studies indicate that sanitation practices, implementation of vaccination programs against hepatitis B, and expanded recognition and treatment of patients with chronic hepatitis B and C could greatly contribute to the eradication of viral hepatitis B and C. While the availability of generic antiviral drugs could partially overcome the bottleneck represented by the lack of resources in low and middle-income countries, where viral hepatitis is the leading cause of liver cancer, the enthusiasm for the prevention of liver cancer through antiviral therapy is mitigated by the risk of cancer in many patients who are treated late in the hepatitis course. The present work aimed to review in detail the various types, epidemiology, and carcinogenesis mechanisms of viral infections that are associated with a significantly increased risk for the development of HCC.

Keywords: Antiviral agents, hepatitis viruses, hepatocellular carcinoma, blood-borne hepatitis, cirrhosis, hepatitis B vaccine



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INTRODUCTION

Chronic infections with blood-borne hepatitis B (HBV), hepatitis C (HCV), and hepatitis D (HDV) viruses are the dominant causes of hepatocellular carcinoma (HCC) worldwide. In 2018, the death toll of HCC was 810,000 persons, and the attributable fractions of HCC due to HBV and HCV were 33% and 21%, respectively^[1,2]. In selected regions of Eurasia, the Far East, and Africa, HDV stands as a significant risk factor for HCC and liver-related mortality^[3]. While this cancer is on the rise globally, reflecting the continuing growth of the world population, the threat is not annulled by the lifestyle changes of people at risk, and many hopes are posed on the delivery of effective sanitation interventions. Mirroring the frequency and geographical distribution of blood-borne viral hepatitis, the prevalence of HCC has long been lower in developed regions than in developing regions. Yet, more recently, some peculiar changes in disease trends have emerged. Based on the 2012 data of the World Health Organization (WHO), HCC mortality was on the rise in northern Europe, North America, and some parts of Asia (China, India, and Korea), mainly as a consequence of epidemics of blood-borne viral hepatitis due to such parenteral risk behaviors, such as drug injections, tattoos, and unsafe sex. Conversely, HCC is declining in traditionally high-risk countries, including the Mediterranean European nations, Japan, and Hong Kong, as a consequence of improved sanitation, screening of blood donors, and mass vaccination of newborns against HBV. Of note, the latter also prevents the spread of HDV, another important player in the arena of HCC known to enhance cancer risk in HBV carriers^[4-6]. While projections have predicted a decline of HCC mortality following massive access of infected patients to antiviral therapy against HBV and HCV, currently, only a minority of individuals with chronic hepatitis B (CHB) or C have been diagnosed, and an even smaller percentage of them has received effective antiviral therapy. The global goal of eliminating viral hepatitis as a public health threat by 2030 is expected to prevent HCC-related mortality by 65%. It would require a 7% annual decline of the global burden of viral hepatitis, a goal that has been reached by only a dozen countries to date^[7].

HEPATITIS B

HBV is a small, partially double-stranded DNA virus and a major contributor to chronic liver disease. The virus has a specific predilection for the liver, where it persists in hepatocyte nuclei in the form of chromosomal insertions of HBV DNA sequences and episomal covalently-closed circular DNA (cccDNA)^[8]. Approximately 15%-40% of HBV carriers develop CHB^[9]. The 5-year cumulative incidence of cirrhosis in untreated CHB is 8%-20%, with an annual risk of HCC in cirrhotic patients of 2%-5%^[10]. At the beginning of the 1980s, a highly effective HBV vaccine was developed, and it proved to be very successful in reducing the disease burden. Nevertheless, the global number of HBV infections remains to be high, in part due to ineffective vaccination implementation programs in many less-developed countries and a high rate of perinatal transmission in certain parts of the world^[11].

HBV is generally considered to be the strongest epidemiologic factor associated with HCC. Worldwide, CHB is responsible for almost half of all HCC cases, but the importance of this risk factor varies significantly between regions (e.g., critical in East Asia, but less so in Europe)^[12]. Many studies have revealed that HBV-infected patients have a 15- to 20-fold increased risk for the development of HCC compared to non-infected individuals^[13,14]. However, several effective antiviral therapies (e.g., nucleoside/nucleotide analogs (NAs) have been developed for patients with HBV over the last decade, and these agents were shown to reduce the rate of HCC occurrence in cirrhotic HBV patients^[15].

Risk factors for HCC in HBV patients

A long list of risk factors for disease progression to HCC in CHB patients have been described. Firstly, several host-related factors have been shown to influence the HCC risk, with a higher risk in older patients and HBV carriers of African American origin^[16-19]. Additionally, HCC is known to have a male

preponderance, and several single-nucleotide polymorphisms have been identified to be associated with a higher genetic susceptibility for HCC^[16,18-22]. Also, the lifestyle of HBV carriers can have a profound influence on HCC risk. For instance, heavy alcohol use was found to accelerate the development of cirrhosis in HBV patients, ultimately resulting in a 1.3- to 8.4-fold increase in HCC risk^[23]. Similarly, tobacco smoking in HBV carriers was described to be directly correlated with the development of liver cancer. In this respect, a meta-analysis from 2010 reported a synergistic effect in HCC risk for individuals who smoke and have HBV infection. Compared to HBV-negative nonsmokers, the risk of HCC was 1.87 times greater for HBV-negative smokers, 15.8 for HBV-positive nonsmokers, and 21.6 for HBV-positive smokers^[24]. More recently, this finding was confirmed in a large Chinese population-based case-control study^[25]. There is an increasing body of evidence indicating an important role of metabolic risk factors in the disease process of CHB. For example, a high body mass index (BMI) has been shown to worsen the disease outcomes of HBV carriers. In a large Korean population-based cohort study, a strong association was revealed between high BMI and a higher risk for HCC among patients with CHB infection^[26]. Also, diabetes mellitus (DM) was shown to have a synergistic impact on the HBV disease course, as amply illustrated by a large meta-analysis, including almost 22,000 patients with CHB^[27]. In this analysis, HBV patients with type 2 DM were found to have a significantly increased risk of HCC (pooled HR = 1.77, 95%CI: 1.28-2.47) and worse overall mortality (pooled RR = 1.93, 95%CI: 1.64-2.27) compared to CHB patients without DM^[27]. That being said, the relationship between nonalcoholic fatty liver disease (NAFLD) and hepatitis is complex and requires further clarification. Interestingly, HBV infection seems to protect patients from the development of steatosis, metabolic syndrome, and insulin resistance^[28], whereas the presence of NAFLD-related steatosis impacts on the replication of HBV. The results of a large case-control study revealed that treatment-naïve CHB patients with NAFLD had significantly lower levels of serum HBV DNA compared to CHB without steatosis^[29]. This, however, does not protect against liver damage, as the presence of steatosis was found to be associated with a higher rate of liver fibrosis and subsequent progression to HCC in HBV-infected patients, independent of antiviral therapy^[30,31].

Also, specific virus-related features have been shown to impact the HCC risk, including HBV DNA levels, viral genotype, hepatitis B e-antigen/surface antigen (HBeAg/HBsAg) levels, mutations in the HBV genome, and coinfections with other hepatitis viruses or human immunodeficiency virus (HIV)^[19,20,32-34]. With respect to viral factors, a high viral load has proved to be a strong predictor of the HCC risk, independent of whether or not the patient has cirrhosis, or displays high levels of serum HBsAg levels^[18,20,32]. In relation to the HBV genotype, a large meta-analysis, which included more than 14,500 patients, demonstrated that genotype C was associated with a higher risk of HCC compared to the other major genotypes^[35]. In the past, several large-scale studies have established baseline HBV DNA levels as a prognostic indicator in CHB patients. However, given the fact that the new effective antiviral therapies can induce a complete viral response in the majority of patients, the prognostic significance of serum HBV DNA levels has substantially diminished^[36]. Finally, studies have also identified double mutations in the basal core promoter of the HBV genome as an independent predictor for an increased risk of HCC^[37].

Accumulating evidence indicates that an occult hepatitis B infection (OBI) may be a risk factor for HCC. OBI refers to a condition in which HBV DNA persists in the liver tissue (and the serum in some cases) in the absence of circulating HBsAg^[38]. A long list of studies has demonstrated the persistence of HBV infection in a large proportion of HBsAg-negative HCC patients^[39]. While the exact relationship between OBI and HCC remains to be elucidated^[40], the available data suggest that OBI is not carcinogenic *per se*, but that the minimal lesions produced by the presence of the occult virus might induce a worse liver disease course in the presence of co-existing causative agents of liver injury (e.g., HCV and alcohol abuse)^[39]. This hypothesis is supported by studies indicating a higher prevalence of OBI in HCV-infected patients with HCC compared to HCV carriers who do not develop HCC^[41-43]. Other studies, however, failed to show a correlation between serum anti-HBV detectability and HCC risk in HCV-infected patients^[44,45].

Table 1. HCC prediction models for HBV-infected patients

	CU-HCC ^[18]	GAG-HCC ^[49]	REACH-B ^[16]	mREACH-B ^[52]	LSM-HCC ^[50]	PAGE-B ^[51]
Components	Age Albumin Bilirubin Cirrhosis HBV DNA	Age Gender BCP mutation Cirrhosis HBV DNA	Age Gender ALT level HBeAG status HBV DNA	Age Gender ALT level HBeAG status LS value	Age Albumin HBV DNA Liver stiffness	Age Gender Platelet level
Risk score	Low: < 5 Medium: 5-20 High: > 20	Low: < 100 High: ≥ 100	Low: ≤ 5 Medium: 6-11 High: ≥ 12	Low: < 10 High: ≥ 10	Low: < 11 High: ≥ 11	Low: ≤ 9 Medium: 10-17 High: ≥ 18
Negative predictive value	97% at 10 years	99% at 10 years	98% at 10 years	96.8% at 5 years	99.4% at 10 years	100% at 5 years

HBV: hepatitis B virus; HCC: hepatocellular carcinoma; BCP: basal core promoter; CU: Chinese University; GAG: Guide with age, gender, HBV DNA, core promoter mutations and cirrhosis; LSM: liver stiffness measurement; PAGE-B: score based on age, gender, and platelets count for HCC in CHB; REACH-B: risk estimation for HCC in CHB; mREACH-B: modified REACH-B

Therefore, the most recent report of the Taormina occult HBV expert panel concluded that further studies on molecular epidemiology and carcinogenesis are required to confirm the role of OBI in HCC development^[40].

As such, both viral- and host-related features were shown to profoundly impact HCC development in patients with HBV. However, the most important variables with respect to the HCC risk relate to the stage of liver disease. Historically, assessing the fibrosis status of the liver required a liver biopsy. However, due to the invasive nature of liver biopsy and its potential complications, this cannot be performed routinely in all CHB patients. To address this, several noninvasive methods have been validated to assess fibrosis in patients with chronic liver disease, of which transient elastography using the FibroScan® device is the most popular^[46-48].

In order to help clinicians predict the risk of HCC in patients with CHB, several risk scores have been designed that incorporate host, viral, and liver characteristics. An overview of the most frequently used HCC risk prediction models is depicted in Table 1^[16,18,49-51]. Of note, most of these scoring systems were validated before the availability of effective direct-acting antiviral (DAA) therapies. To assess the performance of the different risk scores in a contemporary setting, these conventional HCC prediction models were compared to the “modified risk estimation for HCC in CHB” (mREACH-B) score^[52]. After a median follow-up of 75.3 months, 125 of the 1,308 subjects (9.6%) enrolled in this study developed HCC. Interestingly, the mREACH-B score proved to be associated with a significantly higher area under the receiver operating characteristic curve (AUROC) for the prediction of HCC development at 3 and 5 years (AUROC: 0.828 and 0.806, respectively), compared to the “liver stiffness measurement-HCC” (LSM-HCC) (AUROC: 0.777 and 0.759, respectively), “guide with age, gender, HBV DNA, core promoter mutations and cirrhosis-HCC” (GAG-HCC) (AUROC: 0.751 and 0.757, respectively), REACH-B (AUROC: 0.717 and 0.699, respectively), and “Chinese university-HCC” (CU-HCC) (AUROC: 0.698 and 0.700, respectively) scores ($P < 0.05$)^[52]. As such, the prognostic performance of the mREACH-B score seems to be superior to that of the more conventional risk models.

Carcinogenic mechanisms

A potential carcinogenic mechanism that is mediated through HBV consists of HBV genome integration. In the vast majority of HCC cases (80%-90%), HBV DNA was found to be integrated into the host hepatocyte genome^[53]. Cancer-related DNA integrations do not occur randomly; interestingly, they seem to be an early event that occurs before the development of HCC. Large-scale sequencing studies revealed recurrent HBV DNA integration sites at genetic loci that encode for proteins with a potential role in the initiation of hepatocellular carcinogenesis (e.g., *CCNE1*, *TERT*, and *MLL4*)^[53,54]. Immune-mediated processes ultimately

play a dominant role in the development of HCC in HBV-infected patients. In this light, elevated serum levels of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin (IL)-1 β are typical hallmarks of HBV-infected patients^[55]. The long-lasting hepatic inflammation caused by the host's immune defense in response to CHB infection accelerates hepatocyte turnover, leading to increased mutations. As such, the proinflammatory environment in the liver of HBV patients indirectly contributes to the development of liver fibrosis, cirrhosis, and HCC progression^[56].

In recent years, several studies have revealed an important role for micro-RNAs (miRNAs) in the HBV-related tumorigenesis. The miRNAs are small noncoding RNAs of 20-25 nucleotides in length that regulate the expression of certain target genes. For instance, HBeAg induces the expression of macrophage miRNA-155, which leads to an accelerated liver injury through the increased production of inflammatory cytokines (mediated by the targeting of *BCL-6*, *SHIP-1*, and *SOCS-1*)^[57]. A deregulated miRNA expression (e.g., downregulation of miRNA-145 and upregulation of miRNA-224) occurs early and accumulates overtime in the stages of HBV-associated multistep hepatocarcinogenesis^[58]. Apart from the deregulated miRNA expression, HBV was also shown to cause other epigenetic changes and regulate the expression of cellular oncogenes and tumor suppressor genes through a process of promotor hypo- or hypermethylation^[59].

HBV-encoded proteins also play a role in the inflammation processes that lead to the development of HCC. In this respect, the HBV core protein and its splice variant HBeAg stimulate an immune response in the host, resulting in an increased production of proinflammatory cytokines^[60]. A special role in hepatocarcinogenesis has also been described for the HBx protein, per the model of transgenic mice expressing HBx protein published in 1991^[61]. Further research into the role of HBx revealed that this protein transactivates binding sites for the transcription factors AP-1 and NF- κ B, and it activates the p53 and β -catenin signaling pathways involved in chromatin remodeling. All these signaling cascades were shown to be involved in the development of HCC (recently reviewed by Kanda *et al.*^[62]). As such, HBx seems to play an essential role in the transcriptional modulation that contributes to hepatocarcinogenesis^[62].

Prevention of HBV-related HCC

In 2016, the WHO, along with other health authorities, launched a campaign for expanding the recognition and treatment of HBV with the goal of eliminating viral hepatitis by 2030^[63]. A package of high impact interventions of high impact was designed, following modeling studies of hepatitis epidemiology, with the anticipation of benefits conferred by articulated interventions like sanitation and even mass vaccination against HBV of newborns. Indeed, early-in-life infection with HBV is the major risk factor fueling the global reservoir of CHB, and it predisposes those individuals to HCC development. In WHO's vision, vaccination of all newborns is linked with the implementation of screening of blood donors and harm reduction policies for people who inject drugs (i.e., exchange of sterile syringes and needles coupled with opioid substitution therapies)^[63]. Strategies to interrupt vertical transmission of HBV through vaccination are in place in almost all WHO member countries, yet only 39% of newborns received the HBV vaccine globally^[64]. By 2015, however, 84% of all infants globally received the vaccine (WHO target for 2020 was set at 90%). Over the last few years, the accumulated evidence showed that vaccination had substantially contributed to shrinking the burden of HBV by over 30%. In China, campaigns of mass vaccination led to a decline of HBsAg carrier state from approximately 90% in the eighties to 1% nowadays. In turn, this averted 2.8-3.5 million future HBV-related deaths, of which the majority is associated with HCC^[64]. The immune prophylaxis against HBV is long-lasting, although multiple long-term studies have demonstrated a decline of protective serum anti-HBs levels (10 IU/mL) over time. While 60% of vaccinated people are anti-HBs serum positive at year 20, more than 95% retain the ability to mount an anamnestic response after a challenge dose at that time point^[65]. However, to achieve optimal rates of immunization (95%), all neonates need to receive their first dose of the HBV vaccine as soon as possible after birth, preferably within 24 h.

In contrast, neonates born to HBV-infected mothers should receive the vaccine along with hepatitis B immunoglobulins within 12 h^[66]. Suboptimal immunoprophylaxis in neonates can occur and is related to mothers with positive serum HBeAg or high viral load. Suboptimal immunoprophylaxis can also be due to the delivery of less than the recommended three doses of vaccine, an event which occurs in 60% of HBV vaccine recipients. In adulthood, failure to achieve 95% immunization rates is mostly seen in persons older than 60 years of age and patients with morbidities like cancer, immunosuppression, renal failure, HIV, and organ transplantation. Mammalian cell-derived recombinant vaccines incorporating preS1 and preS2 antigens have shown enhanced immunogenicity and might be used to overcome non-response to second-generation recombinant vaccines.

While HBV vaccination stands as the only pragmatic approach to prevent mortality from the HDV, a recent report from WHO sheds a dark light on the other pillar of the WHO campaign of viral hepatitis elimination, being the prolonged treatment of the HBV infected population with NAs. Currently, less than 10% of all patients chronically infected with HBV have been identified and successfully linked to care with anti-HBV antivirals. This constraint might have important consequences as chemoprevention of HCC is more likely to be successful when antiviral therapy is started before the development of cirrhosis^[67]. The advent of a safe, effective, and user-friendly third-generation NAs, such as tenofovir disoproxil fumarate and entecavir, overrode the constraints represented by first- and second-generation anti-HBV NAs that caused studies to be flawed by referral biases, high rates of treatment failures, and ultimately by suboptimal percent suppression of HBV. Collectively, both population and cohort studies that have been carried out in both hemispheres of the globe showed that the incidence and mortality of HCC could have been prevented in a majority of patients if they received NAs for more than five years^[68,69]. Given the differences in patient access (entecavir is contraindicated in lamivudine-experienced patients), market distribution, and genetic sequences of HBV polymerase targeted by the two NAs, non-randomized studies comparing the HCC risk reduction following HBV suppression were unable to conclusively demonstrate the superiority of one regimen over the other^[70]. Both NAs fail to clear the nuclei of infected hepatocytes from HBV DNA sequences integrated into chromosomes and from free viral cccDNA, two events that are known to play a role in the neoplastic transformation of the liver in HBV carriers^[67]. More recently, both cohort and population studies provided some evidence that statins and aspirin may confer protection against HCC in HBV carriers. This effect was described to result in their ability to interfere with liver cell metabolism and inflammatory processes engaged in cell carcinogenesis. In a cohort of more than 7000 patients with CHB, statins were associated with a cumulative dose-response reduction of HCC risk of 74% over an observation period of 7 years, after adjustment for important confounders like age, sex, cirrhosis, antiviral therapy, and correlates of metabolic syndrome^[71]. These observations confirm previous observations and aligned with other studies of statins showing potential anticancer activity in other cancer types (i.e., breast, colon, and prostate cancer) through inhibition of the downstream products of the mevalonate pathway, which are crucial for malignant cell proliferation while inhibiting hepatic fibrogenesis, another significant risk factor of HCC^[72-74]. Last but not least, statins may also counteract HBV by slowing down cholesterol synthesis and HBV replication^[75]. In Taiwan, a nationwide cohort study of more than 10,000 patients with CHB showed a statistically significant risk reduction of HCC in patients who received daily aspirin compared with 1:4 matched controls^[76]. Prevention of HCC by aspirin is biologically plausible, considering that this drug may prevent the progression of liver disease and liver carcinogenesis through different mechanisms involving blockade of platelets, modulation of bioactive lipids, and inhibition of the proinflammatory cyclooxygenase-2 enzyme^[77-79].

HEPATITIS C

While HBV is the most common underlying HCC etiology worldwide, HCV is responsible for most cases in Western countries^[1]. In patients with a chronic HCV infection, the risk of HCC gradually increases as liver fibrosis progresses. Once cirrhosis is established, the annual incidence of HCC is high, at 1% to 7%

per year^[17]. Overall, it has been established that HCV infected patients have a 15-20 fold increased risk of developing HCC compared with HCV negative patients^[14,80,81].

Risk factors for HCC in HCV patients

The rate of HCC progression varies greatly among patients with chronic HCV infection, and this is due to the existence of a complex interplay between host, viral, and environmental factors. Similar to what was described for HBV, the most important risk factor for the development of HCC in patients with a chronic HCV infection is the underlying liver disease^[82]. Apart from that, several other concurrent risk factors that impact the HCC risk in patients with HCV have been identified. To begin, male sex and older age have universally been described as independent risk factors for the development of HCC in patients with a chronic HCV infection^[82-84]. Also, a coinfection with HBV or HIV seems to influence the course of an HCV infection. Several studies have demonstrated that coinfection with HIV promotes the progression of fibrosis and cirrhosis in patients with HCV, resulting in a significantly increased risk for severe liver disease^[85-88]. As a result, it is widely accepted that an HIV coinfection in HCV patients also increases the risk of HCC compared to HCV mono-infected patients^[89]. However, recent data from two prospective French cohorts demonstrate that this is no longer the case in the current context of more effective combination antiretroviral therapies and increased access to HCV therapy. In this analysis, the 5-year cumulative incidence of HCC and liver decompensation did not differ significantly between HIV/HCV coinfecting and HCV mono-infected patients (8.5% vs. 13.2%, $P = 0.12$ and 12.8% vs. 15.6%, $P = 0.40$, respectively)^[90]. Also, patients with a dual HBV/HCV infection have a higher risk of progression to cirrhosis and decompensated liver disease compared to patients with an HCV mono-infection^[91,92]. Already in 1998, a meta-analysis of more than 30 case-control studies demonstrated a synergistic effect of HCV and HBV on the incidence of HCC^[14]. This observation was later confirmed by a second, Chinese meta-analysis indicating that a dual infection by HBV and HCV was associated with a higher risk of HCC than each infection alone [odds ratio (OR) for the development of HCC for coinfecting patients: 35.7]^[13]. Similarly, an Italian study reported a yearly HCC incidence of 6.4% in HBV/HCV coinfecting patients as compared to 2.0% and 3.7% in HBV and HCV mono-infected patients, respectively. At 10-years, this translated into a cumulative HCC rate of 45%, 16%, and 28%, respectively^[93]. For HBV/HCV dual infection, the research data suggest that the HBV replication status is the crucial factor affecting the risk for HCC. HCV patients with active HBV replication have twice the risk of HCC compared to those with latent HBV and HCV, while the risk in coinfecting patients with undetectable HBV DNA levels is similar to that of mono-infected HCV patients^[83]. As discussed in the section on HBV, more research is needed to shed light on the potential effect of OBI on the risk for HCC in HCV-infected patients^[40]. Intriguingly, certain HCV genotypes seem to be associated with a higher risk of HCC, particularly genotype 3 which is associated with an 80% higher risk of HCC compared to genotype 1^[94], contradicting a previous meta-analysis that associated genotype 1 with a 78% increased risk of HCC relative to all other genotypes and a 60% increased risk among patients with cirrhosis^[95].

Similar to what was described for HBV, there are significant associations between lifestyle factors and the HCC risk in HCV patients. A meta-analysis has shown a significant increase in the relative risk of HCC in smokers (relative risk 23) compared to non-smokers (relative risk 7.9) with HCV^[24]. Also, alcohol consumption was shown to accelerate liver fibrosis in HCV-infected patients, resulting in an increased risk for progression to cirrhosis and HCC. In a study evaluating the natural history of liver fibrosis progression in 2,235 HCV patients, daily alcohol consumption of at least 50 g resulted in a 34% increase in the rate of fibrosis progression^[96]. In line with this, a meta-analysis involving more than 15,000 HCV patients demonstrated that heavy alcohol intake (210-560 g per week) was associated with a 3.54 relative risk for the development of decompensated cirrhosis^[97]. A study that specifically looked into the effect of alcohol on the development of HCC in HCV patients revealed a 2-fold increase in HCC risk for drinkers of more than 60 g per day^[98]. Similarly, a case-control study revealed an OR for HCC development of 26.1 in HCV-carriers with an alcohol intake of 0-40 g/day, rising to 62.6 and 126 among patients drinking a daily dose of

41–80 g and more than 80 g per day, respectively^[99]. Interestingly, studies have indicated that the synergistic effect of alcohol on the HCC risk in HCV patients is not restricted to heavy drinkers and that even light to modest alcohol use can promote the development of cirrhosis (and subsequent HCC) in HCV patients^[100]. In line with this, a study including 192 HCV patients with compensated cirrhosis, reported a 5-year cumulative HCC rate of 10.6% among abstainers as compared to 23.8% among HCV patients with a light to moderate alcohol consumption (median intake: 15 g/day)^[101].

HCV also comes with an increased risk of HCC in patients with DM. A meta-analysis evaluating the association between DM and HCC in chronic HCV patients indicated a 2- to 3-fold increased risk^[102]. Similarly, a population-based cohort study from Taiwan indicated that DM increases the risk of HCC in HCV-infected patients (HR = 1.36, 95%CI: 1.16–1.68;)^[103]. Finally, NAFLD is a prominent characteristic of a chronic HCV infection. In patients with genotype 3 HCV, the presence of NAFLD is directly linked to the viral load, and in this setting, the NAFLD is considered to be of viral origin^[104]. In contrast, in patients with other HCV genotypes, the NAFLD is linked to host factors such as obesity. Studies have consistently identified (NAFLD-related) steatosis as an independent factor associated with fibrosis progression in HCV patients^[105–108]. As such, it is not surprising to see that several studies (both retrospective and prospective) have demonstrated that steatosis in HCV patients is also strictly associated with the development of HCC^[109–113]. An interesting study in this respect demonstrated that American chronic HCV patients were found to progress more rapidly to HCC than their counterparts in China, with underlying fatty liver disease as the prominent factor fueling this difference^[114].

Several prediction models for HCC have been developed for patients with an HCV infection (e.g., HALT-C, REVEAL-HCV, and SCORE_{HCC}), but the performances of these scores are suboptimal, and their use and validity in clinical practice are limited^[115–117].

Carcinogenic mechanisms

In contrast to HBV, HCV is a single-stranded RNA virus with little potential to integrate its genetic material into the host. Therefore, HCV must exhibit its tumorigenic potential less directly. The mechanisms involved in this process mainly involve two parts: induction of chronic inflammation and the expression of viral proteins. HCV-induced HCC development is a multistep process that involves the establishment of chronic HCV infection, persistent chronic hepatic inflammation, progressive liver fibrogenesis, initiation of neoplastic clones accompanied by irreversible somatic genetic/epigenetic alterations, and progression of the malignant clones in a carcinogenic tissue microenvironment^[118].

Several proinflammatory cytokines, including TNF- α , IL-1, IL-23, IL-6, and lymphotoxins α and β have been implicated in chronic liver inflammation and the development of HCC^[119]. Specifically, for HCV-mediated hepatocarcinogenesis, a high ratio of TNF- α /IL-10 levels has been observed in the sera of patients with severe liver damage and HCC^[120]. Apart from immunological disturbances, epigenetic processes were found to be involved in the development of HCV-related HCC. Like HBV-related HCC, HCV also seems to profoundly impact the expression of certain miRNAs^[121]. Similar to HBV, miRNA-155 also plays a role in the development of HCC in HCV-infected patients. miRNA-155 levels are markedly increased in patients infected with HCV, and this overexpression was found to stimulate hepatocyte proliferation and tumorigenesis by activating Wnt signaling^[122]. Other epigenetic studies have suggested a prominent role for noncoding RNAs in the development of HCV-related HCC^[123,124].

With respect to the role of HCV-related proteins in the development of liver cancer, the HCV core protein and NS5A seem to be of interest^[62]. In 1996, the HCV core protein was found to induce a carcinogenic phenotype in primary rat embryo through a RAS-mediated mechanism^[125]. The HCV core protein was also shown to activate the MAPK signaling pathway, upregulate Wnt/ β -catenin signaling, suppress apoptosis

pathways, and activate TGF- β , PI3K/Akt/mTOR, NF- κ B, p53, IL-6/Stat3, and the androgen receptor pathways. Through these pathways, the HCV core protein could regulate cell growth, differentiation, apoptosis, transcription, and angiogenesis^[62,126]. Similarly, NS5A interacts with multiple pro-oncogenic pathways, including β -catenin, PI3K/Akt/mTOR, NF- κ B, and p53^[62,126].

Finally, insulin resistance, commonly observed in patients with HCV, plays a crucial role in the development of HCC in HCV patients. Intensive research identified that cross-talk between the HCV core protein and molecules regulating insulin signaling might affect HCV-related hepatocarcinogenesis^[62].

Prevention of HCV-related HCC

Every year, *de novo* HCV affects 1.75 million persons, and more than 350,000 people die of HCV-related cirrhosis or liver cancer^[66,127]. Since new HCV infections outnumber the sum of people who die of end-stage hepatitis C and those pharmacologically cured, HCV elimination is also a priority of the WHO^[63]. This goal is achievable because the virus lacks a non-human reservoir, cannot amplify in the environment and can be identified with simple and accurate diagnostic tests. At the same time, practical interventions can be delivered to interrupt transmission and cure both acute and chronic infections^[128]. With an articulated package of interventions similar to the one in place for HBV elimination, except for vaccine prophylaxis, which is not available, the strategy designed by WHO is expected to provide treatment to 90% of all infected individuals by 2030 worldwide^[63]. The clinical benefits of HCV elimination are undisputed and are already well-established since the ages of interferon therapy for HCV. The treatment strategy is also well acknowledged by all international liver societies, which strongly recommend antiviral treatment of all HCV infected patients, independent of the severity of the underlying liver disease^[129,130]. A sustained virologic response (SVR) heralds improvement of portal hypertension and fibrosis progression in patients with chronic hepatitis and may reduce HCC incidence and all-cause mortality^[130]. Significant HCC chemoprevention already surfaced in the interferon studies, where an SVR was found to be associated with a relative risk reduction of 74% compared to non-responders^[131]. This HCC chemoprevention by SVR was further amplified by the advent of safe and effective DAA against HCV. These agents extended the range and clinical benefits of antiviral therapy. Also, DAAs can be indicated in patients with decompensated liver disease who were either ineligible for treatment with interferon or did not respond to the therapy. HCC chemoprevention by DAA was firmly established through the retrospective scrutiny of large cohorts of patients in the US, Europe, and Asia. Moreover, this finding was further confirmed by prospective population studies. In a Veterans Health Administration cohort, which included more than 20,000 patients with advanced liver disease and multiple comorbidities, DAAs reduced the risk of developing HCC by 72% compared to non-responders^[132]. More recently, an analysis of the Veterans Affairs cohorts including more than 15,000 HCV patients demonstrated that patients who achieved an SVR after DAA treatment had a significantly lower all-cause mortality (78.9% reduction) and a lower HCC incidence (83.5% reduction) than those who did not achieve an SVR [Figure 1]^[133,134].

In a prospective population study in Sicily, Italy, the incidence rate of HCC at one year was 2.6% in SVR patients compared to 8% in non-responders with a clear cut association between HCC risk and liver disease severity^[134]. Importantly, these studies had the additional merit of wiping out any doubt about the safety of DAA therapy in patients with advanced hepatitis C as they counteracted the initial observations of high rates of clinically aggressive *de novo* HCC after an SVR to DAA that were identified in small groups of cirrhotic patients in Spain and Italy^[135-137]. Nowadays, it is clear that early occurrence of *de novo* HCC is confined to patients who harbor magnetic resonance imaging (MRI)-undefined liver nodules at the onset of DAA therapy and is promoted by the imbalance of field immunity caused by the swift eradication of HCV^[136]. Early, aggressive recurrence was also a complication of HCV eradication with DAAs in patients with a history of HCC^[137,138], where antiviral therapy aims to halt the progression of hepatitis C towards liver failure and prevent the onset of second primary tumors which result from both direct and indirect

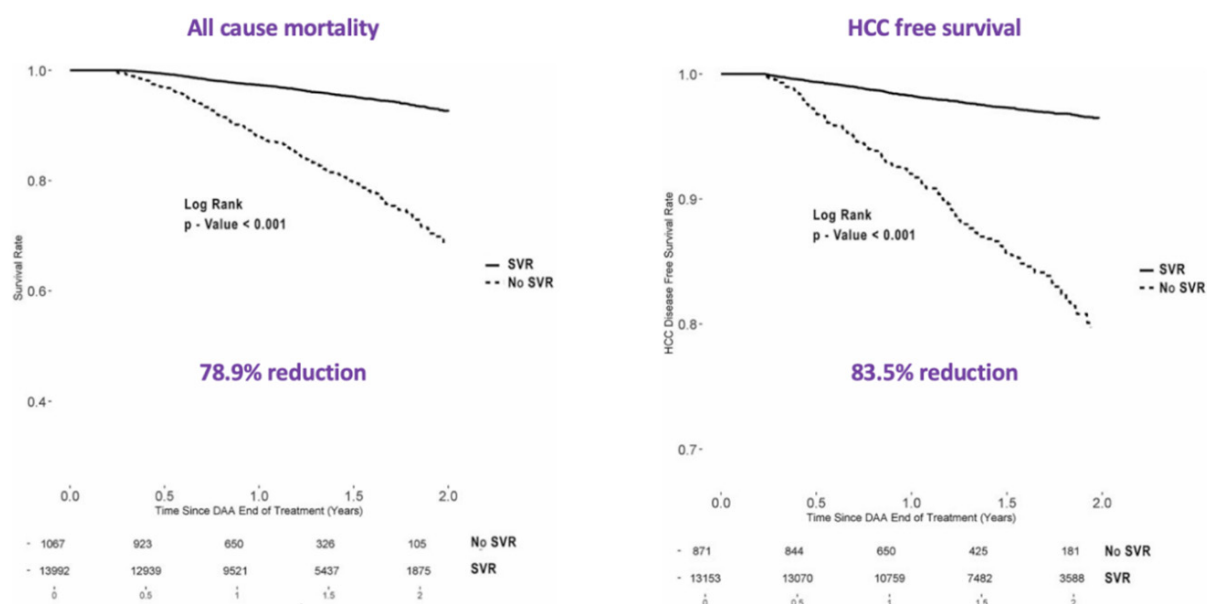


Figure 1. Reduction in HCC incidence and all-cause mortality in patients with advanced hepatitis C who achieve an SVR to DAA. Data from the Veteran Health Administration cohort^[133]. HCC: Hepatocellular carcinoma; SVR: sustained virologic response; DAA: direct-acting antiviral

carcinogenic damage of hepatocellular DNA fueled by unrested HCV replication^[139]. The latter usually takes place 1 to 2 years after the cure of a primary HCC (late recurrence), at variance with earlier recurrence that is caused by the proliferation of pre-existing cancer cells surviving the removal of the primary tumor, for which no effective adjuvant therapy exists. The early recurrence depends on tumor size and cell grading, reflecting the cancer cells that invade the tumor vessels and/or to tumor satellites emerging far from parental HCC^[140], the evidence is mounting that early recurrence of HCC in DAA-treated patients is often bound to pre-existing liver nodules with undefined vascular patterns at MRI^[138].

In a final note on anti-HCV therapy, it is important to underscore that achieving an SVR to antiviral therapy does not tell the whole story. In fact, data from Lens *et al.*^[141] reported in 2017 indicate that obtaining an SVR to all-oral anti-HCV therapy in patients with HCV-associated cirrhosis indeed leads to decrease in a hepatic venous pressure gradient, but that clinically significant hypertension did persist in 78% of patients. These patients have a continued risk for liver decompensation, and subsequently, maintain a higher risk of HCC^[141].

A recent study based on Swedish nationwide registries has demonstrated a significant reduction of HCC risk in a population including more than 50,000 HCV infected and 10,000 HBV infected adults. The latter had been chronically exposed to low doses of aspirin (HR = 0.69, 95%CI: 0.62-0.76). After adjusting for relevant confounding morbidities, chemoprevention of liver cancer was confirmed and found to be associated with a similar risk reduction of liver-related mortality^[142]. These findings align with studies done in HBV patients showing chemoprevention of HBV-related liver cancer following long-term exposure to aspirin.

HEPATITIS D

HDV is a small replication-defective RNA virus that relies on HBV to replicate and propagate. Due to a lack of dedicated studies assessing the prevalence of HDV, the global disease burden of HDV is likely underestimated^[143]. In a recently published systematic review and meta-analysis, 650-700 million people

have a chronic HBV infection globally, of whom 60-70 million have an HDV coinfection, which is almost twice as much as the previous estimate^[144]. HDV does not integrate into the genome of hepatocytes, making a direct oncogenic mechanism unlikely. However, preliminary data have indicated the potential indirect oncogenic effects of this virus. HDV can modify several key signaling pathways with a known role in cirrhosis and hepatocarcinogenesis, including the activation of the TGF- β , NF- κ B, and JAK-STAT signaling pathways^[145-147].

Studies comparing HCC incidence between HBV/HDV coinfecting and HBV mono-infected patients provide better insights on the oncogenic impact of HDV. A critical Eurohep study demonstrated that HBV/HDV-positive cirrhotic patients followed for a median of 6.6 years had a twofold increase in mortality risk compared to patients with HBV-related cirrhosis^[148]. Moreover, the estimated risk for HCC was 13% among cirrhotic HBV/HDV patients compared to 2%-4% for cirrhotic patients with an HBV mono-infection, corresponding to a threefold increase in HCC risk for coinfecting patients^[148]. Also, a large Swedish retrospective cohort study demonstrated a significantly higher risk of HCC for patients with acute (RR = 6.1, 95%CI: 2.8-11.7) or chronic (RR = 3.9, 95%CI: 1.6-7.2) HDV^[149]. Similarly, an American study, including 2,175 HBV patients, found a 2.9-fold increase in the incidence of HCC in individuals with an HBV/HDV coinfection (OR = 2.1, 95%CI: 1.1-3.9)^[150].

Cohort studies evaluating the HCC incidence among patients with HDV yielded variable results. Among 299 HDV infected patients who were included in a single-center Italian study (diagnosed between 1987 and 2006), 46 HCC cases were reported, accounting for an annual rate of 2.8%^[151]. Interestingly, the authors of this analysis later identified high serum levels of HDV RNA as a predictor of cirrhosis and liver cancer in HDV patients^[152]. In a large cohort study which included 1,576 HDV patients, the annual HCC incidence was slightly lower at 1.9%^[153]. The results obtained in other cohort studies with a fewer patients were inconsistent and reported an annual HCC incidence among HDV patients of 3%-13%^[154-156]. Finally, a recent study addressed the HDV-associated mortality in HIV/HBV coinfecting patients. HDV infection appeared to be strongly associated with overall death (HR = 2.33, 95%CI: 1.41-3.84), liver-related death (HR = 7.71, 95%CI: 3.13-18.97), and HCC occurrence (HR = 9.3, 95%CI: 3.03-28.61)^[157].

Very recently, a meta-analysis pooling data from 93 studies compared the HCC risk in patients with HBV/HDV and HBV alone (68 case-control studies with 22,862 patients and 25 cohort studies with 75,427 patients) was published^[5]. Patients with HBV/HDV had a significantly higher HCC risk than patients with an HBV mono-infection (pooled OR = 1.28, 95%CI: 1.05-1.57). Of note, this association was particularly pronounced in studies with HIV-infected patients (pooled OR = 7.13, 95%CI: 2.83-17.92)^[5].

In summary, though most studies evaluating the incidence of HCC in HDV-infected patients have provided low-level evidence, data support an association between HDV and the development of HCC. Similar to what was described for HBV and HCV, the objective of antiviral treatment in patients with a chronic HDV infection is to eliminate HDV and HBV and, as such, prevent the long-term detrimental effects of hepatitis D on the liver. Unfortunately, the treatment options for patients with HDV are limited, and HDV elimination is not commonly achieved. In fact, 1-year of interferon- α (IFN- α) only induces a sustained HDV clearance in 10%-20% of patients^[158]. More recently, several studies have evaluated the use of pegylated IFN- α in HDV patients. While response rates in these trials were higher than what was seen with classical IFN- α , sustained clearance of HDV RNA proved feasible in only about a quarter of patients^[159-161]. Data on the impact of IFN- α treatment on the natural history of hepatitis D are scarce. In an extended follow-up of 36 chronic HDV patients treated with one year of IFN- α found that some patients experienced regression of their advanced stage fibrosis, indicating a positive effect on the natural HDV disease course^[162]. Less favorable data come from the HIDIT trial in which pegylated IFN- α was used to treat chronic HDV. In this trial, 58% of patients who were shown to be HDV RNA negative 6 months

after therapy experienced a late HDV relapse^[163]. Interestingly, these late relapses were not associated with clinical complications. This might indicate that a prolonged virologic response to pegylated IFN- α , even if not sustained, can be clinically relevant in patients with chronic hepatitis D^[163].

CONCLUSION

Blood-borne viral hepatitis is a dominant cause of HCC worldwide, and the positive effect of their eradication on the risk of developing HCC has been extensively demonstrated even in patients carrying significant metabolic comorbidities that predispose them to the neoplastic transformation of the liver. Along this line, intriguing data have emerged, suggesting a significant chemoprophylactic activity of liver metabolism modifiers such as statins and aspirin. All in all, the implementation of articulated interventions of sanitation make reaching the WHO goal of viral elimination more realistic in some small countries like Georgia or Iceland, where elimination programs can easily be run, than in large countries where it is difficult even to identify hepatitis carriers. Further mitigating our belief of being on the right track for global elimination of viral hepatitis was the increasing hesitance against hepatitis B vaccination. In recent years, it has amounted to the level of reaching an alarming proportion of 30% in many high-income countries. While this could be counteracted by the recent pandemic of 2019 coronavirus disease (shortly, COVID-19) that has restored confidence in public health policies based on vaccination, another critical point in the fight against viral HCC is the existence of awareness campaigns and screening programs, since the first essential step in the viral hepatitis cure pathway is to be aware of the infection. Identification of defined risk cohorts, including baby-boomers, people who inject drugs, prisoners, and men who have sex with men, improves the cost-effectiveness of screening programs aimed to target infected populations; however, determining the infectious status is irrelevant if effective linkage-to-care programs are not in place. The availability of generic antivirals can partially overcome the bottleneck represented by the lack of resources in low and middle-income countries where HBV and HCV prevail as risk factors for HCC.

DECLARATIONS

Authors' contributions

Equally made substantial contributions to the conception and design of the study and performed data analysis and interpretation: Alqahtani SA, Colombo M

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Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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REFERENCES

1. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al; Global Burden of Disease Liver Cancer Collaboration. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. *JAMA Oncol* 2017;3:1683-91.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
3. Caviglia GP, Rizzetto M. Treatment of hepatitis D: an unmet medical need. *Clin Microbiol Infect* 2020;26:824-7.
4. Liu KSH, Seto WK, Lau EHY, Wong DK, Lam YF, et al. A territorywide prevalence study on blood-borne and enteric viral hepatitis in Hong Kong. *J Infect Dis* 2019;219:1924-33.
5. Alfaiaite D, Clément S, Gomes D, Goossens N, Negro F. Chronic hepatitis D and hepatocellular carcinoma: a systematic review and meta-analysis of observational studies. *J Hepatol* 2020; doi: 10.1016/j.jhep.2020.02.030.
6. Liu Z, Jiang Y, Yuan H, Fang Q, Cai N, et al. The trends in incidence of primary liver cancer caused by specific etiologies: results from the Global Burden of Disease Study 2016 and implications for liver cancer prevention. *J Hepatol* 2019;70:674-83.
7. Maticic M, Mondelli MU. Elimination of viral hepatitis: where do we stand in the year 2020? *Clin Microbiol Infect* 2020;26:816-7.
8. Lucifora J, Protzer U. Attacking hepatitis B virus cccDNA--The holy grail to hepatitis B cure. *J Hepatol* 2016;64:S41-8.
9. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009;50:661-2.
10. Raffetti E, Fattovich G, Donato F. Incidence of hepatocellular carcinoma in untreated subjects with chronic hepatitis B: a systematic review and meta-analysis. *Liver Int* 2016;36:1239-51.
11. Trépo C, Chan HLY, Lok A. Hepatitis B virus infection. *Lancet* 2014;384:2053-63.
12. Baecker A, Liu X, La Vecchia C, Zhang ZF. Worldwide incidence of hepatocellular carcinoma cases attributable to major risk factors. *Eur J Cancer Prev* 2018;27:205-12.
13. Shi J, Zhu L, Liu S, Xie WF. A meta-analysis of case-control studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma in China. *Br J Cancer* 2005;92:607-12.
14. Donato F, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. *Int J Cancer* 1998;75:347-54.
15. Tawada A, Kanda T, Imazeki F, Yokosuka O. Prevention of hepatitis B virus-associated liver diseases by antiviral therapy. *Hepatol Int* 2016;10:574-93.
16. Yang H, Yuen M, Chan HL, Han K, Chen P, et al. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. *Lancet Oncol* 2011;12:568-74.
17. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, et al. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019;16:589-604.
18. Wong VW, Chan SL, Mo F, Chan TC, Loong HH, et al. Clinical scoring system to predict hepatocellular carcinoma in chronic hepatitis B carriers. *J Clin Oncol* 2010;28:1660-5.
19. Wong VW, Janssen HL. Can we use HCC risk scores to individualize surveillance in chronic hepatitis B infection? *J Hepatol* 2015;63:722-32.
20. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, et al; Taiwan Community-Based Cancer Screening Project Group. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002;347:168-74.
21. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004;130:417-22.
22. Zhang H, Zhai Y, Hu Z, Wu C, Qian J, et al. Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. *Nat Genet* 2010;42:755-8.
23. Iida-Ueno A, Enomoto M, Tamori A, Kawada N. Hepatitis B virus infection and alcohol consumption. *World J Gastroenterol* 2017;23:2651-9.
24. Chuang SC, Lee YC, Hashibe M, Dai M, Zheng T, et al. Interaction between cigarette smoking and hepatitis B and C virus infection on the risk of liver cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2010;19:1261-8.
25. Liu X, Baecker A, Wu M, Zhou JY, Yang J, et al. Interaction between tobacco smoking and hepatitis B virus infection on the risk of liver cancer in a Chinese population. *Int J Cancer* 2018;142:1560-7.
26. Kim K, Choi S, Park SM. Association of high body mass index and hepatocellular carcinoma in patients with chronic hepatitis B virus infection: a Korean population-based cohort study. *JAMA Oncol* 2018;4:737-9.
27. Tan Y, Wei S, Zhang W, Yang J, Yang J, et al. Type 2 diabetes mellitus increases the risk of hepatocellular carcinoma in subjects with chronic hepatitis B virus infection: a meta-analysis and systematic review. *Cancer Manag Res* 2019;11:705-13.
28. Joo EJ, Chang Y, Yeom JS, Ryu S. Hepatitis B virus infection and decreased risk of nonalcoholic fatty liver disease: a cohort study. *Hepatology* 2017;65:828-35.
29. Hui RWH, Seto WK, Cheung KS, Mak LY, Liu KSH, et al. Inverse relationship between hepatic steatosis and hepatitis B viremia: results of a large case-control study. *J Viral Hepat* 2018;25:97-104.
30. Seto WK, Hui RWH, Mak LY, Fung J, Cheung KS, et al. Association between hepatic steatosis, measured by controlled attenuation parameter, and fibrosis burden in chronic hepatitis B. *Clin Gastroenterol Hepatol* 2018;16:575-83.e2.
31. Wong GL, Chan HL, Yu Z, Chan AW, Choi PC, et al. Coincidental metabolic syndrome increases the risk of liver fibrosis progression in patients with chronic hepatitis B--a prospective cohort study with paired transient elastography examinations. *Aliment Pharmacol Ther* 2014;39:883-93.

32. Chen CJ, Yang HI, Su J, Jen CL, You SL, et al; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006;295:65-73.
33. Wong VW, Sung JJ. Diagnosis and personalized management of hepatitis B including significance of genotypes. *Curr Opin Infect Dis* 2012;25:570-7.
34. Kao JH, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. *Gastroenterology* 2003;124:327-34.
35. Wong GL, Chan HL, Yiu KK, Lai JW, Chan VK, et al. Meta-analysis: the association of hepatitis B virus genotypes and hepatocellular carcinoma. *Aliment Pharmacol Ther* 2013;37:517-26.
36. Lee HW, Yoo EJ, Kim BK, Kim SU, Park JY, et al. Prediction of development of liver-related events by transient elastography in hepatitis B patients with complete virological response on antiviral therapy. *Am J Gastroenterol* 2014;109:1241-9.
37. Sumi H, Yokosuka O, Seki N, Arai M, Imazeki F, et al. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* 2003;37:19-26.
38. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 2008;49:652-7.
39. Pollicino T, Saitta C. Occult hepatitis B virus and hepatocellular carcinoma. *World J Gastroenterol* 2014;20:5951-61.
40. Raimondo G, Locarnini S, Pollicino T, Levrero M, Zoulim F, et al; Taormina Workshop on Occult HBV Infection Faculty Members. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. *J Hepatol* 2019;71:397-408.
41. Wang H, Swann R, Thomas E, Innes HA, Valerio H, et al. Impact of previous hepatitis B infection on the clinical outcomes from chronic hepatitis C? A population-level analysis. *J Viral Hepat* 2018;25:930-8.
42. Ikeda K, Marusawa H, Osaki Y, Nakamura T, Kitajima N, et al. Antibody to hepatitis B core antigen and risk for hepatitis C-related hepatocellular carcinoma: a prospective study. *Ann Intern Med* 2007;146:649-56.
43. Coppola N, Onorato L, Sagnelli C, Sagnelli E, Angelillo IF. Association between anti-HBc positivity and hepatocellular carcinoma in HBsAg-negative subjects with chronic liver disease: a meta-analysis. *Medicine (Baltimore)* 2016;95:e4311.
44. Stroffolini T, Almasio PL, Persico M, Bollani S, Benvenuto L, et al; Italian Association of the Study of the Liver Disease (AISF). Lack of correlation between serum anti-HBc detectability and hepatocellular carcinoma in patients with HCV-related cirrhosis. *Am J Gastroenterol* 2008;103:1966-72.
45. Kitab B, Ezzikouri S, Alaoui R, Nadir S, Badre W, et al. Occult HBV infection in Morocco: from chronic hepatitis to hepatocellular carcinoma. *Liver Int* 2014;34:e144-50.
46. Kim JH, Kim MN, Han KH, Kim SU. Clinical application of transient elastography in patients with chronic viral hepatitis receiving antiviral treatment. *Liver Int* 2015;35:1103-15.
47. Jung KS, Kim SU, Ahn SH, Park YN, Kim DY, et al. Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). *Hepatology* 2011;53:885-94.
48. Huang R, Jiang N, Yang R, Geng X, Lin J, et al. Fibroscan improves the diagnosis sensitivity of liver fibrosis in patients with chronic hepatitis B. *Exp Ther Med* 2016;11:1673-7.
49. Yuen MF, Tanaka Y, Fong DY, Fung J, Wong DK, et al. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. *J Hepatol* 2009;50:80-8.
50. Wong GL, Chan HL, Wong CK, Leung C, Chan CY, et al. Liver stiffness-based optimization of hepatocellular carcinoma risk score in patients with chronic hepatitis B. *J Hepatol* 2014;60:339-45.
51. Papatheodoridis G, Dalekos G, Sypsa V, Yurdaydin C, Buti M, et al. PAGE-B predicts the risk of developing hepatocellular carcinoma in Caucasians with chronic hepatitis B on 5-year antiviral therapy. *J Hepatol* 2016;64:800-6.
52. Jung KS, Kim SU, Song K, Park JY, Kim DY, et al. Validation of hepatitis B virus-related hepatocellular carcinoma prediction models in the era of antiviral therapy. *Hepatology* 2015;62:1757-66.
53. Sung WK, Zheng H, Li S, Chen R, Liu X, et al. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat Genet* 2012;44:765-9.
54. Wang M, Xi D, Ning Q. Virus-induced hepatocellular carcinoma with special emphasis on HBV. *Hepatol Int* 2017;11:171-80.
55. Wu S, Kanda T, Nakamoto S, Jiang X, Nakamura M, et al. Cooperative effects of hepatitis B virus and TNF may play important roles in the activation of metabolic pathways through the activation of NF- κ B. *Int J Mol Med* 2016;38:475-81.
56. Xie Y. Hepatitis B virus-associated hepatocellular carcinoma. In: Cai Q, Yuan Z, Lan K, editors. *Infectious Agents Associated Cancers: Epidemiology and Molecular Biology*. Singapore: Springer; 2017. pp. 11-21.
57. Wang W, Bian H, Li F, Li X, Zhang D, et al. HBeAg induces the expression of macrophage miR-155 to accelerate liver injury via promoting production of inflammatory cytokines. *Cell Mol Life Sci* 2018;75:2627-41.
58. Gao P, Wong CC, Tung EK, Lee JM, Wong CM, et al. Deregulation of microRNA expression occurs early and accumulates in early stages of HBV-associated multistep hepatocarcinogenesis. *J Hepatol* 2011;54:1177-84.
59. Tian Y, Ou JH. Genetic and epigenetic alterations in hepatitis B virus-associated hepatocellular carcinoma. *Virol Sin* 2015;30:85-91.
60. Kanda T, Wu S, Sasaki R, Nakamura M, Haga Y, et al. HBV core protein enhances cytokine production. *Diseases* 2015;3:213-20.
61. Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 1991;351:317-20.
62. Kanda T, Goto T, Hirotsu Y, Moriyama M, Omata M. Molecular mechanisms driving progression of liver cirrhosis towards hepatocellular carcinoma in chronic hepatitis B and C infections: a review. *Int J Mol Sci* 2019;20:1358.
63. WHO. Global health sector strategy on viral hepatitis 2016-2021. Geneva, Switzerland: WHO; 2016.

64. Ward JW, Hinman AR. What is needed to eliminate hepatitis b virus and hepatitis C virus as global health threats. *Gastroenterology* 2019;156:297-310.
65. Poovorawan Y, Chongsrisawat V, Theamboonlers A, Bock HL, Leyssen M, et al. Persistence of antibodies and immune memory to hepatitis B vaccine 20 years after infant vaccination in Thailand. *Vaccine* 2010;28:730-6.
66. WHO. Global hepatitis report, 2017. Geneva, Switzerland: WHO; 2017.
67. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu., European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017;67:370-98.
68. Chiang CJ, Yang YW, Chen JD, You SL, Yang HI, et al. Significant reduction in end-stage liver diseases burden through the national viral hepatitis therapy program in Taiwan. *Hepatology* 2015;61:1154-62.
69. Papatheodoridis GV, Idilman R, Dalekos GN, Buti M, Chi H, et al. The risk of hepatocellular carcinoma decreases after the first 5 years of entecavir or tenofovir in Caucasians with chronic hepatitis B. *Hepatology* 2017;66:1444-53.
70. Li M, Lv T, Wu S, Wei W, Wu X, et al. Tenofovir versus entecavir in lowering the risk of hepatocellular carcinoma development in patients with chronic hepatitis B: a critical systematic review and meta-analysis. *Hepatol Int* 2020;14:105-14.
71. Goh MJ, Sinn DH, Kim S, Woo SY, Cho H, et al. Statin use and the risk of hepatocellular carcinoma in patients with chronic hepatitis B. *Hepatology* 2020;71:2023-32.
72. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Statins are associated with a reduced risk of hepatocellular cancer: a systematic review and meta-analysis. *Gastroenterology* 2013;144:323-32.
73. Jiang P, Mukthavaram R, Chao Y, Nomura N, Bharati IS, et al. In vitro and in vivo anticancer effects of mevalonate pathway modulation on human cancer cells. *Br J Cancer* 2014;111:1562-71.
74. Eberlein M, Heusinger-Ribeiro J, Goppelt-Strube M. Rho-dependent inhibition of the induction of connective tissue growth factor (CTGF) by HMG CoA reductase inhibitors (statins). *Br J Pharmacol* 2001;133:1172-80.
75. Tsan YT, Lee CH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis B virus infection. *J Clin Oncol* 2012;30:623-30.
76. Lee TY, Hsu YC, Tseng HC, Yu SH, Lin JT, et al. Association of daily aspirin therapy with risk of hepatocellular carcinoma in patients with chronic hepatitis B. *JAMA Intern Med* 2019;179:633-40.
77. Kern MA, Schubert D, Sahi D, Schöneweiss MM, Moll I, et al. Proapoptotic and antiproliferative potential of selective cyclooxygenase-2 inhibitors in human liver tumor cells. *Hepatology* 2002;36:885-94.
78. Sitia G, Aiolfi R, Di Lucia P, Mainetti M, Fiocchi A, et al. Antiplatelet therapy prevents hepatocellular carcinoma and improves survival in a mouse model of chronic hepatitis B. *Proc Natl Acad Sci U S A* 2012;109:E2165-72.
79. Calvisi DF. Inhibition of hepatitis B virus-associated liver cancer by antiplatelet therapy: a revolution in hepatocellular carcinoma prevention? *Hepatology* 2013;57:848-50.
80. Mancebo A, González-Diéguez ML, Cadahía V, Varela M, Pérez R, et al. Annual incidence of hepatocellular carcinoma among patients with alcoholic cirrhosis and identification of risk groups. *Clin Gastroenterol Hepatol* 2013;11:95-101.
81. Mahale P, Torres HA, Kramer JR, Hwang LY, Li R, et al. Hepatitis C virus infection and the risk of cancer among elderly US adults: a registry-based case-control study. *Cancer* 2017;123:1202-11.
82. Chang KC, Wu YY, Hung CH, Lu SN, Lee CM, et al. Clinical-guide risk prediction of hepatocellular carcinoma development in chronic hepatitis C patients after interferon-based therapy. *Br J Cancer* 2013;109:2481-8.
83. Kruse RL, Kramer JR, Tyson GL, Duan Z, Chen L, et al. Clinical outcomes of hepatitis B virus coinfection in a United States cohort of hepatitis C virus-infected patients. *Hepatology* 2014;60:1871-8.
84. Huang CF, Yeh ML, Huang CI, Lin YJ, Tsai PC, et al. Risk of hepatitis C virus related hepatocellular carcinoma between subjects with spontaneous and treatment-induced viral clearance. *Oncotarget* 2017;8:43925-33.
85. Graham CS, Baden LR, Yu E, Mrus JM, Carnie J, et al. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. *Clin Infect Dis* 2001;33:562-9.
86. Giordano TP, Kramer JR, Soucek J, Richardson P, El-Serag HB. Cirrhosis and hepatocellular carcinoma in HIV-infected veterans with and without the hepatitis C virus: a cohort study, 1992-2001. *Arch Intern Med* 2004;164:2349-54.
87. López-Diéguez M, Montes ML, Pascual-Pareja JF, Quereda C, Von Wichmann MA, et al; GESIDA 37/03-FIPSE 36465/03-NEAT IG5 Study Group. The natural history of liver cirrhosis in HIV-hepatitis C virus-coinfected patients. *AIDS* 2011;25:899-904.
88. Tovo CV, Becker SC, Almeida PR, Galperim B, Chaves S. Progression of liver fibrosis in monoinfected patients by hepatitis C virus and coinfecting by HCV and human immunodeficiency virus. *Arq Gastroenterol* 2013;50:19-22.
89. Puoti M, Prestini K, Putzolu V, Zanini B, Baiguera C, et al. HIV/HCV co-infection: natural history. *J Biol Regul Homeost Agents* 2003;17:144-6.
90. Salmon-Ceron D, Nahon P, Layese R, Bourcier V, Sogni P, et al; ANRS CO12 CirVir and ANRS CO13 HEPACVIH study groups. Human Immunodeficiency Virus/Hepatitis C Virus (HCV) Co-infected Patients With Cirrhosis Are No Longer at Higher Risk for Hepatocellular Carcinoma or End-Stage Liver Disease as Compared to HCV Mono-infected Patients. *Hepatology* 2019;70:939-54.
91. Konstantinou D, Deutsch M. The spectrum of HBV/HCV coinfection: epidemiology, clinical characteristics, viral interactions and management. *Ann Gastroenterol* 2015;28:221-8.
92. Lee LP, Dai CY, Chuang WL, Chang WY, Hou NJ, et al. Comparison of liver histopathology between chronic hepatitis C patients and chronic hepatitis B and C-coinfected patients. *J Gastroenterol Hepatol* 2007;22:515-7.
93. Chiaramonte M, Stroffolini T, Vian A, Stazi MA, Floreani A, et al. Rate of incidence of hepatocellular carcinoma in patients with compensated viral cirrhosis. *Cancer* 1999;85:2132-7.

94. Kanwal F, Kramer JR, Ilyas J, Duan Z, El-Serag HB. HCV genotype 3 is associated with an increased risk of cirrhosis and hepatocellular cancer in a national sample of U.S. Veterans with HCV. *Hepatology* 2014;60:98-105.
95. Raimondi S, Bruno S, Mondelli MU, Maisonneuve P. Hepatitis C virus genotype 1b as a risk factor for hepatocellular carcinoma development: a meta-analysis. *J Hepatol* 2009;50:1142-54.
96. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997;349:825-32.
97. Hutchinson SJ, Bird SM, Goldberg DJ. Influence of alcohol on the progression of hepatitis C virus infection: a meta-analysis. *Clin Gastroenterol Hepatol* 2005;3:1150-9.
98. Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. *Am J Epidemiol* 2002;155:323-31.
99. Tagger A, Donato F, Ribero ML, Chiesa R, Portera G, et al; Brescia HCC Study. Case-control study on hepatitis C virus (HCV) as a risk factor for hepatocellular carcinoma: the role of HCV genotypes and the synergism with hepatitis B virus and alcohol. *Int J Cancer* 1999;81:695-9.
100. Monto A, Patel K, Bostrom A, Pianko S, Pockros P, et al. Risks of a range of alcohol intake on hepatitis C-related fibrosis. *Hepatology* 2004;39:826-34.
101. Vandenbulcke H, Moreno C, Colle I, Knebel JF, Francque S, et al. Alcohol intake increases the risk of HCC in hepatitis C virus-related compensated cirrhosis: a prospective study. *J Hepatol* 2016;65:543-51.
102. Dyal HK, Aguilar M, Bartos G, Holt EW, Bhuket T, et al. Diabetes mellitus increases risk of hepatocellular carcinoma in chronic hepatitis C virus patients: a systematic review. *Dig Dis Sci* 2016;61:636-45.
103. Huang TS, Lin CL, Lu MJ, Yeh CT, Liang KH, et al. Diabetes, hepatocellular carcinoma, and mortality in hepatitis C-infected patients: a population-based cohort study. *J Gastroenterol Hepatol* 2017;32:1355-62.
104. Adinolfi LE, Rinaldi L, Guerrero B, Restivo L, Marrone A, et al. NAFLD and NASH in HCV infection: prevalence and significance in hepatic and extrahepatic manifestations. *Int J Mol Sci* 2016;17:803.
105. Castéra L, Hézode C, Roudot-Thoraval F, Bastie A, Zafrani ES, et al. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. *Gut* 2003;52:288-92.
106. Monto A, Alonzo J, Watson JJ, Grunfeld C, Wright TL. Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus, and alcohol. *Hepatology* 2002;36:729-36.
107. Cross TJ, Quaglia A, Hughes S, Joshi D, Harrison PM. The impact of hepatic steatosis on the natural history of chronic hepatitis C infection. *J Viral Hepat* 2009;16:492-9.
108. Lonardo A, Adinolfi LE, Restivo L, Ballestri S, Romagnoli D, et al. Pathogenesis and significance of hepatitis C virus steatosis: an update on survival strategy of a successful pathogen. *World J Gastroenterol* 2014;20:7089-103.
109. Salomao M, Yu WM, Brown RS Jr, Emond JC, Lefkowitz JH. Steatohepatic hepatocellular carcinoma (SH-HCC): a distinctive histological variant of HCC in hepatitis C virus-related cirrhosis with associated NAFLD/NASH. *Am J Surg Pathol* 2010;34:1630-6.
110. Tanaka A, Uegaki S, Kurihara H, Aida K, Mikami M, et al. Hepatic steatosis as a possible risk factor for the development of hepatocellular carcinoma after eradication of hepatitis C virus with antiviral therapy in patients with chronic hepatitis C. *World J Gastroenterol* 2007;13:5180-7.
111. Pekow JR, Bhan AK, Zheng H, Chung RT. Hepatic steatosis is associated with increased frequency of hepatocellular carcinoma in patients with hepatitis C-related cirrhosis. *Cancer* 2007;109:2490-6.
112. Kumar D, Farrell GC, Kench J, George J. Hepatic steatosis and the risk of hepatocellular carcinoma in chronic hepatitis C. *J Gastroenterol Hepatol* 2005;20:1395-400.
113. Ohata K, Hamasaki K, Toriyama K, Matsumoto K, Saeki A, et al. Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Cancer* 2003;97:3036-43.
114. Rao H, Wu E, Fu S, Yang M, Feng B, et al. The higher prevalence of truncal obesity and diabetes in American than Chinese patients with chronic hepatitis C might contribute to more rapid progression to advanced liver disease. *Aliment Pharmacol Ther* 2017;46:731-40.
115. Singal AG, Mukherjee A, Elmunzer BJ, Higgins PD, Lok AS, et al. Machine learning algorithms outperform conventional regression models in predicting development of hepatocellular carcinoma. *Am J Gastroenterol* 2013;108:1723-30.
116. El-Serag HB, Kanwal F, Davila JA, Kramer J, Richardson P. A new laboratory-based algorithm to predict development of hepatocellular carcinoma in patients with hepatitis C and cirrhosis. *Gastroenterology* 2014;146:1249-55.e1.
117. Ikeda M, Fujiyama S, Tanaka M, Sata M, Ide T, et al. Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon. *J Gastroenterol* 2005;40:148-56.
118. Hoshida Y, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. *J Hepatol* 2014;61:S79-90.
119. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010;140:883-99.
120. Aroucha DC, do Carmo RF, Moura P, Silva JL, Vasconcelos LR, et al. High tumor necrosis factor- α /interleukin-10 ratio is associated with hepatocellular carcinoma in patients with chronic hepatitis C. *Cytokine* 2013;62:421-5.
121. Braconi C, Valeri N, Gasparini P, Huang N, Taccioli C, et al. Hepatitis C virus proteins modulate microRNA expression and chemosensitivity in malignant hepatocytes. *Clin Cancer Res* 2010;16:957-66.
122. Zhang Y, Wei W, Cheng N, Wang K, Li B, et al. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. *Hepatology* 2012;56:1631-40.
123. Hou W, Bonkovsky HL. Non-coding RNAs in hepatitis C-induced hepatocellular carcinoma: dysregulation and implications for early detection, diagnosis and therapy. *World J Gastroenterol* 2013;19:7836-45.

124. Plissonnier ML, Herzog K, Levrero M, Zeisel MB. Non-coding RNAs and hepatitis C virus-induced hepatocellular carcinoma. *Viruses* 2018;10:591.
125. Ray RB, Lagging LM, Meyer K, Ray R. Hepatitis C virus core protein cooperates with ras and transforms primary rat embryo fibroblasts to tumorigenic phenotype. *J Virol* 1996;70:4438-43.
126. Kanda T, Yokosuka O, Omata M. Hepatitis C virus and hepatocellular carcinoma. *Biology (Basel)* 2013;2:304-16.
127. WHO. Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016.2018; Available from: http://origin.who.int/healthinfo/global_burden_disease/estimates/en/. [Last accessed on 22 Jul 2017]
128. Edlin BR, Winkelstein ER. Can hepatitis C be eradicated in the United States? *Antiviral Res* 2014;110:79-93.
129. European Association for the Study of the Liver, European Association for the Study of the Liver. EASL Recommendations on Treatment of Hepatitis C 2018. *J Hepatol* 2018;69:461-511.
130. Cooke GS, Andrieux-meyer I, Applegate TL, Atun R, Burry JR, et al. Accelerating the elimination of viral hepatitis: a lancet gastroenterology & hepatology commission. *Lancet Gastroenterol Hepatol* 2019;4:135-84.
131. Morgan RL, Baack B, Smith BD, Yartel A, Pitasi M, et al. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann Intern Med* 2013;158:329-37.
132. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, et al. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. *Gastroenterology* 2017;153:996-1005.e1.
133. Backus LI, Belperio PS, Shahoumian TA, Mole LA. Impact of sustained virologic response with direct-acting antiviral treatment on mortality in patients with advanced liver disease. *Hepatology* 2019;69:487-97.
134. Calvaruso V, Cabibbo G, Cacciola I, Petta S, Madonia S, et al; Rete Sicilia Selezione Terapia-HCV (RESIST-HCV). Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. *Gastroenterology* 2018;155:411-21.e4.
135. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016;65:727-33.
136. Colombo M, Boccaccio V. HCV therapy and risk of liver cancer recurrence: who to treat? *Nat Rev Gastroenterol Hepatol* 2018;15:392-3.
137. Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016;65:719-26.
138. Sangiovanni A, Alimenti E, Gattai R, Filomia R, Parente E, et al. Undefined/non-malignant hepatic nodules are associated with early occurrence of HCC in DAA-treated patients with HCV-related cirrhosis. *J Hepatol* 2020; doi: 10.1016/j.jhep.2020.03.030.
139. Lemon SM, McGivern DR. Is hepatitis C virus carcinogenic? *Gastroenterology* 2012;142:1274-8.
140. Sasaki A, Kai S, Iwashita Y, Hirano S, Ohta M, et al. Microsatellite distribution and indication for locoregional therapy in small hepatocellular carcinoma. *Cancer* 2005;103:299-306.
141. Lens S, Alvarado-Tapias E, Mariño Z, Londoño MC, Llop E, et al. Effects of all-oral anti-viral therapy on HVPg and systemic hemodynamics in patients with hepatitis C Virus-associated cirrhosis. *Gastroenterology* 2017;153:1273-83.e1.
142. Simon TG, Ma Y, Ludvigsson JF, Chong DQ, Giovannucci EL, et al. Association between aspirin use and risk of hepatocellular carcinoma. *JAMA Oncol* 2018;4:1683-90.
143. Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. *Lancet* 2011;378:73-85.
144. Chen HY, Shen DT, Ji DZ, Han PC, Zhang WM, et al. Prevalence and burden of hepatitis D virus infection in the global population: a systematic review and meta-analysis. *Gut* 2019;68:512-21.
145. Choi SH, Jeong SH, Hwang SB. Large hepatitis delta antigen modulates transforming growth factor-beta signaling cascades: implication of hepatitis delta virus-induced liver fibrosis. *Gastroenterology* 2007;132:343-57.
146. Park CY, Oh SH, Kang SM, Lim YS, Hwang SB. Hepatitis delta virus large antigen sensitizes to TNF-alpha-induced NF-kappaB signaling. *Mol Cells* 2009;28:49-55.
147. Williams V, Brichler S, Khan E, Chami M, Dény P, et al. Large hepatitis delta antigen activates STAT-3 and NF-κB via oxidative stress. *J Viral Hepat* 2012;19:744-53.
148. Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep). *Gut* 2000;46:420-6.
149. Ji J, Sundquist K, Sundquist J. A population-based study of hepatitis D virus as potential risk factor for hepatocellular carcinoma. *J Natl Cancer Inst* 2012;104:790-2.
150. Kushner T, Serper M, Kaplan DE. Delta hepatitis within the Veterans Affairs medical system in the United States: prevalence, risk factors, and outcomes. *J Hepatol* 2015;63:586-92.
151. Romeo R, Del Ninno E, Rumi M, Russo A, Sangiovanni A, et al. A 28-year study of the course of hepatitis Delta infection: a risk factor for cirrhosis and hepatocellular carcinoma. *Gastroenterology* 2009;136:1629-38.
152. Romeo R, Foglieni B, Casazza G, Spreafico M, Colombo M, et al. High serum levels of HDV RNA are predictors of cirrhosis and liver cancer in patients with chronic hepatitis delta. *PLoS One* 2014;9:e92062.
153. Wranke A, Pinheiro Borzacov LM, Parana R, Lobato C, Hamid S, et al; Hepatitis Delta International Network. Clinical and virological heterogeneity of hepatitis delta in different regions world-wide: The Hepatitis Delta International Network (HDIN). *Liver Int* 2018;38:842-50.
154. Niro GA, Smedile A, Ippolito AM, Ciancio A, Fontana R, et al. Outcome of chronic delta hepatitis in Italy: a long-term cohort study. *J Hepatol* 2010;53:834-40.
155. Gheorghe L, Iacob S, Simionov I, Vadan R, Gheorghe C, et al. Natural history of compensated viral B and D cirrhosis. *Rom J*

- Gastroenterol 2005;14:329-35.
156. Buti M, Homs M, Rodriguez-Frias F, Funalleras G, Jardí R, et al. Clinical outcome of acute and chronic hepatitis delta over time: a long-term follow-up study. *J Viral Hepat* 2011;18:434-42.
 157. Béguelin C, Moradpour D, Sahli R, Suter-Riniker F, Lüthi A, et al; Swiss HIV Cohort Study. Hepatitis delta-associated mortality in HIV/HBV-coinfected patients. *J Hepatol* 2017;66:297-303.
 158. Yurdaydin C. Treatment of chronic delta hepatitis. *Semin Liver Dis* 2012;32:237-44.
 159. Wedemeyer H, Yurdaydin C, Dalekos GN, Erhardt A, Çakaloğlu Y, et al; HIDIT Study Group. Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med* 2011;364:322-31.
 160. Kabaçam G, Dalekos GN, Çakaloğlu Y, Zachou K, Bock T, et al. Pegylated interferon-based treatment in patients with advanced liver disease due to chronic delta hepatitis. *Turk J Gastroenterol* 2012;23:560-8.
 161. Heller T, Rotman Y, Koh C, Clark S, Haynes-Williams V, et al. Long-term therapy of chronic delta hepatitis with peginterferon alfa. *Aliment Pharmacol Ther* 2014;40:93-104.
 162. Farci P, Roskams T, Chessa L, Peddis G, Mazzoleni AP, et al. Long-term benefit of interferon alpha therapy of chronic hepatitis D: regression of advanced hepatic fibrosis. *Gastroenterology* 2004;126:1740-9.
 163. Heidrich B, Yurdaydin C, Kabaçam G, Ratsch BA, Zachou K, et al; HIDIT-1 Study Group. Late HDV RNA relapse after peginterferon alpha-based therapy of chronic hepatitis delta. *Hepatology* 2014;60:87-97.

Review

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Alternative approach of hepatocellular carcinoma surveillance: abbreviated MRI

Ryan L. Brunsing¹, Kathryn J. Fowler², Takeshi Yokoo³, Guilherme Moura Cunha², Claude B. Sirlin², Robert M. Marks^{4,5}

¹Department of Radiology, Stanford University, Stanford, CA 94305, USA.

²Liver Imaging Group, Department of Radiology, University of California San Diego, San Diego, CA 92093, USA.

³Department of Radiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.

⁴Department of Radiology, Naval Medical Center San Diego, San Diego, CA 92134, USA.

⁵Department of Radiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20892, USA.

Correspondence to: Dr. Robert M. Marks, Department of Radiology, Naval Medical Center San Diego, San Diego, CA 92134, USA. E-mail: robert.m.marks.mil@mail.mil

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Abstract

This review focuses on emerging abbreviated magnetic resonance imaging (AMRI) surveillance of patients with chronic liver disease for hepatocellular carcinoma (HCC). This surveillance strategy has been proposed as a high-sensitivity alternative to ultrasound for identification of patients with early-stage HCC, particularly in patients with cirrhosis or obesity, in whom sonographic visualization of small tumors may be compromised. Three general AMRI approaches have been developed and studied in the literature - non-contrast AMRI, dynamic contrast-enhanced AMRI, and hepatobiliary phase contrast-enhanced AMRI - each comprising a small number of selected sequences specifically tailored for HCC detection. The rationale, general technique, advantages and disadvantages, and diagnostic performance of each AMRI approach is explained. Additionally, current gaps in knowledge and future directions are discussed. Based on emerging evidence, we cautiously recommend the use of AMRI for HCC surveillance in situations where ultrasound is compromised.

Keywords: Abbreviated magnetic resonance imaging, cirrhosis, Hepatitis B, hepatocellular carcinoma, surveillance, magnetic resonance imaging



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INTRODUCTION

Imaging-based surveillance for hepatocellular carcinoma (HCC) aims to detect early-stage, potentially curable tumors in asymptomatic high-risk patients to prolong life. First introduced about four decades ago, it is now an established part of routine clinical care for patients with chronic hepatitis B or cirrhosis in many countries across the globe. A randomized controlled trial of over 18,000 people with active or chronic hepatitis B showed that semi-annual screening with a combination of ultrasound (US) and serum alpha fetoprotein reduced HCC-related mortality by 37%^[1]. Based on the above findings, other studies^[2,3], cost and availability considerations, US is recommended by most national and international hepatology societies for HCC surveillance^[4-10]. Since surveillance US does not permit a definitive diagnosis of HCC, positive surveillance US exams prompt additional diagnostic tests, usually a contrast-enhanced multiphase computed tomography (CT) or magnetic resonance imaging (MRI). Patients with negative US exams return for routine surveillance US examinations, usually at six-month intervals.

Despite universal recommendation for use of US in HCC surveillance, the efficacy of this modality is disappointing. US has low sensitivity for HCC^[11,12], in particular for patients with early-stage tumors^[12-14], ascites, cirrhosis or obesity^[15-17]. Meta-analyses indicate that the sensitivity of surveillance US to detect small (e.g., ≤ 2 cm) HCCs in patients with cirrhosis is less than 50%, i.e., more than half of patients with potentially curable cancers are missed and may progress to advanced, incurable disease before diagnosis^[14,18,19]. Delayed diagnosis defeats the purpose of surveillance, which aims to detect patients with very early- or early-stage HCC^[20], allowing for curative therapies^[21]. The failure to detect early disease contributes to HCC-related mortality^[22].

A more sensitive surveillance test might improve outcomes in patients at risk for HCC. Compared to US, both CT and MRI have superior reported diagnostic sensitivity to identify patients with HCC^[16,19], including those with early-stage tumors^[15], however they also pose challenges as surveillance tools. CT requires injection of iodinated intravenous contrast agents, which can cause allergic reactions and possibly nephrotoxicity, potentially limiting the use of this modality in certain populations. In addition, CT exposes patients to ionizing radiation, an important consideration in younger or middle-aged adults with well-compensated cirrhosis. Conventional MRI provides higher sensitivity than CT^[16,19], but also requires administration of intravenous contrast material; moreover, long exam duration, interpretation complexity, and high cost hinder its suitability for surveillance.

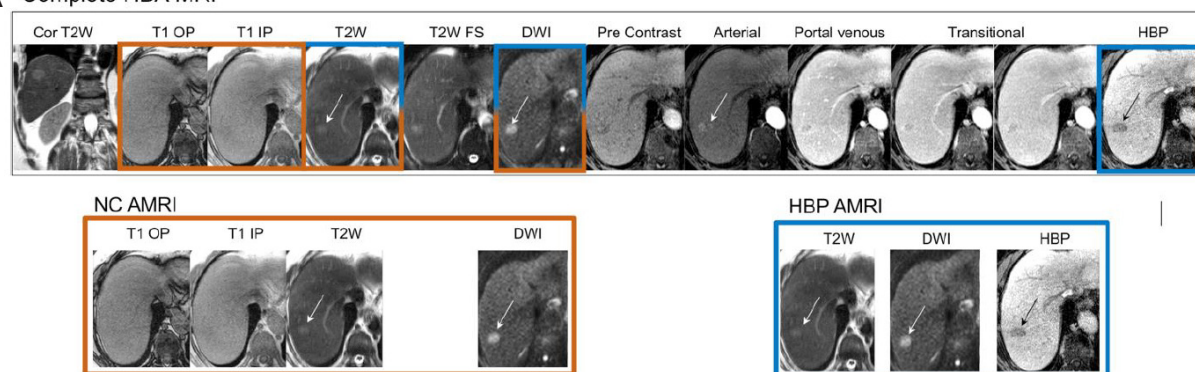
Motivated to provide higher sensitivity than US while avoiding the limitations of CT and conventional MRI, investigators have developed abbreviated MRI (AMRI) protocols that rely on a small number of select sequences specifically tailored for HCC detection^[12,23-36]. The rationale is that reduced scanner time decreases costs and complexity, while improving patient comfort, without significantly compromising HCC detection. AMRI also simplifies workflow and possibly interpretation, while utilizing fewer resources. Recent studies suggest that AMRI might be a high-sensitivity and feasible alternative to US for HCC surveillance, and a recent Markov model-based cost-utility analysis suggested AMRI-based HCC-surveillance may be the most cost-effective strategy^[37].

The purpose of this article is to review emerging concepts on AMRI-based HCC surveillance, including technical aspects, diagnostic performance, current gaps in knowledge, and future directions.

AMRI: APPROACHES

Three general AMRI approaches have been developed: non-contrast AMRI, dynamic contrast-enhanced AMRI, and hepatobiliary phase contrast-enhanced (HBP) AMRI. All can be completed in approximately 10 min or less of scanner time, considerably less than a complete or conventional MRI exam of the liver,

A Complete HBA MRI



B Complete ECA MRI

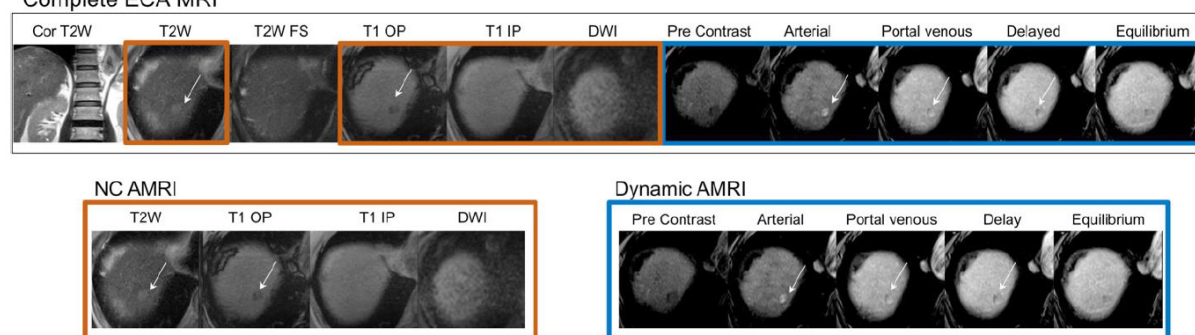


Figure 1. Complete MRI exams (A: HBA MRI; B: ECA) disaggregated into each of the three AMRI approaches (NC-AMRI, HBP AMRI and Dynamic AMRI). MRI: magnetic resonance imaging; NC AMRI: non-contrast abbreviated MRI; HBA: hepatobiliary agents; ECA: extracellular contrast agents; HBP: hepatobiliary phase

which typically requires half an hour or more. [Figure 1](#) illustrates how a complete MRI exam can be disaggregated into each of the three AMRI approaches. The approaches, discussed in detail below, are summarized in [Table 1](#) along with their advantages and disadvantages.

NON-CONTRAST AMRI

Imaging

The simplest approach to MRI-based HCC surveillance is non-contrast abbreviated MRI (NC-AMRI), which implements up to three sequences without administering contrast material:

T1 weighted in-phase and out-of-phase imaging

With current MRI systems, T1-weighted in-phase and out-of-phase images of the liver can be acquired in a single breath-hold. These images can detect HCC nodules that are either hypointense or hyperintense relative to liver, but they generally have low sensitivity for early-stage HCC, which is usually hypointense on this sequence. In-phase and out-of-phase (IP/OOP) images can also provide information on fat [\[Figure 2\]](#) or iron content, which might be useful for differentiating suspicious from benign lesions. In particular, nodules that differ in fat content from background liver (either more fat or less) based on IP/OOP signal characteristics signal characteristics or nodules with lower iron content than background liver (iron sparing) are suspicious for malignancy. By comparison, nodules with higher iron content (siderotic) are usually non-malignant; if only siderotic nodules are detected, the exam is considered negative for HCC.

T2 weighted imaging

The main purpose of including T2 weighted imaging is to help differentiate suspicious from benign lesions. Marked T2 hypointensity or marked T2 hyperintensity suggest that a lesion is non-malignant, whereas

Table 1. AMRI approaches

	Sequences	Pros	Cons
NC-AMRI	T1 weighted in-phase and out-of-phase T2 weighted imaging Diffusion weighted imaging (DWI)	Cheapest approach Avoids risk of GBCA No issues with contrast timing	Relies on unenhanced imaging Heavily dependent on DWI imaging, which is prone to artifacts in the upper abdomen HCC may not exhibit restricted diffusion
Dynamic-AMRI	Pre-contrast imaging Arterial phase imaging Portal venous phase imaging Delayed phase imaging	Allows definitive diagnosis of HCC Allows diagnosis of tumor in vein Cheaper contrast agent options	Inability to detect ancillary features of HCC Risk of miscategorization of vascular pseudolesions Dependence on contrast timing, thus repeat imaging requires repeat dose of GBCA or repeat exam Requires power injector
HBP-AMRI	Hepatobiliary phase imaging T2 weighted imaging DWI (optional)	High contrast-to-noise Contrast material can be hand injected in waiting room Contrast material is retained in the liver for prolonged duration providing a long imaging window and allowing all sequences to be repeated if necessary Established scoring system based on LI-RADS US	Contrast agent is expensive Lesions may be obscured by severe cirrhosis Can detect very early HCCs that cannot be confirmed with currently available call-back tests

AMRI: Abbreviated magnetic resonance imaging; GBCA: gadolinium-based contrast agent; HCC: hepatocellular carcinoma; LI-RADS: Liver Imaging Reporting and Data System; US: ultrasound; HBP: hepatobiliary phase

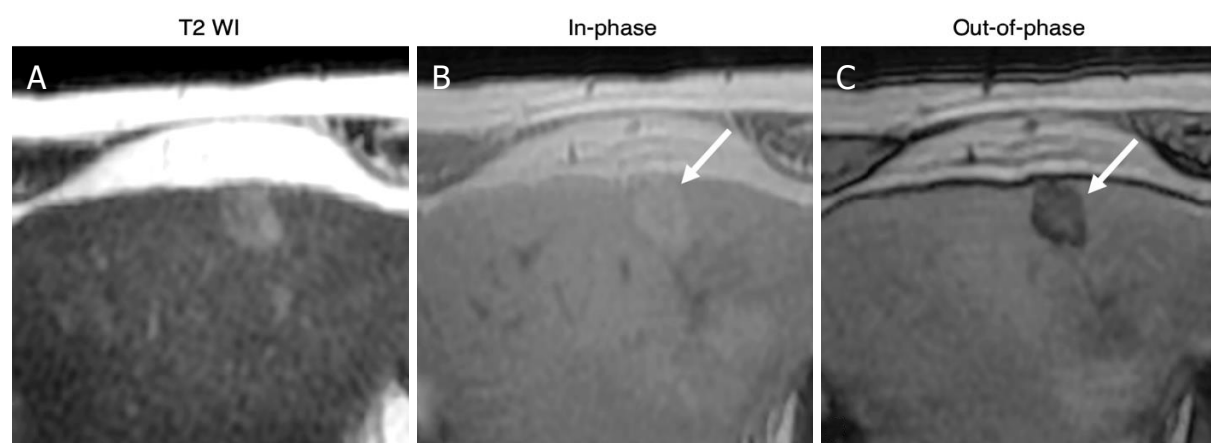


Figure 2. Intraslesional fat: 80-year-old male with HCV cirrhosis. Images show a 18 mm observation in the left lobe. The lesion has ancillary features favoring HCC including mild hyperintense on T2WI (A) as well as intraslesional fat in the mass more than adjacent liver. The latter is characterized by signal drop from In-phase (B) to Out-of-phase (C) images (arrows). HCV: hepatitis C virus; HCC: hepatocellular carcinoma

mild-to-moderately increased T2 signal, relative to the background liver parenchyma, is more concerning for HCC in high-risk patients^[38]. T2-weighted imaging may also improve sensitivity by detecting T2-hyperintense HCC nodules that are difficult to see for various reasons on the other sequences; the incremental benefit is likely to be modest given the relatively low sensitivity of this sequence for small HCC nodules.

Diffusion weighted imaging

Inclusion of diffusion weighted imaging (DWI) increases sensitivity^[39-41] by detecting lesions based on restricted diffusion, which is thought to reflect hypercellularity. Some DWI features may also be used to help differentiate HCC from non-HCC malignancy, such as intrahepatic cholangiocarcinoma (iCCA), which often has a more targetoid appearance^[42,43]. The highest b-values have ranged from 500-800 s/mm² for NC-AMRI studies.

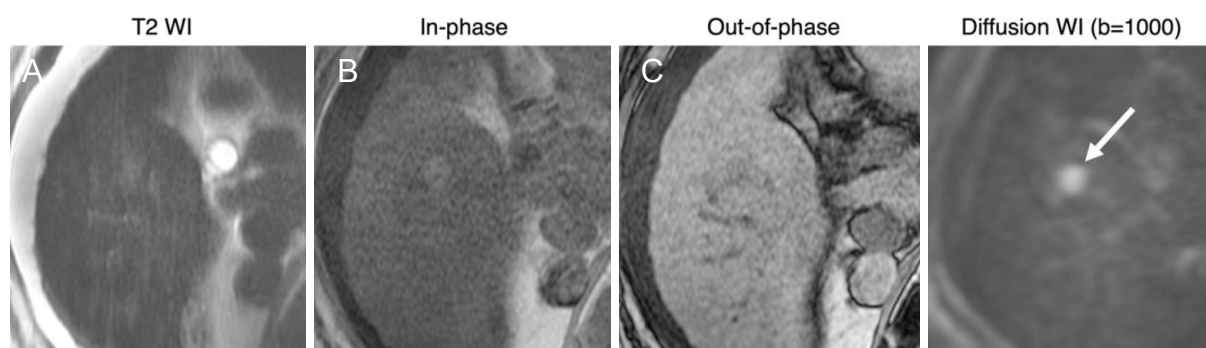


Figure 3. Positive NC-AMRI examinations: 66-year-old male with HCV cirrhosis. Images show a 14 mm observation in seen in the right lobe. While subtle on T2WI (A) and T1WI (B, C), the presence of restricted diffusion (arrow) favors malignancy. HCV: hepatitis C virus; NC AMRI: non-contrast abbreviated magnetic resonance imaging

Reporting

NC-AMRI exams can be interpreted as positive in the setting of a focal observation meeting any of the above described criteria [Figure 3]. A positive examination would warrant a call back diagnostic study to provide a definitive diagnosis of HCC. Features that suggest non-HCC malignancy do not affect the need for call-back but might guide the radiologist's choice of modality and contrast agent.

Advantages

NC-AMRI offers several advantages. By avoiding gadolinium-based contrast agent (GBCA) administration, this approach curtails costs, avoids IV placement, saves time, and simplifies workflow. There is no need for image acquisition timing, and images compromised by respiratory or other motion artefacts can simply be repeated. It also eliminates any GBCA-associated risks, including rare but potentially serious adverse reactions^[31], theoretical concerns about gadolinium deposition in the brain^[44,45], and the remote possibility of nephrogenic systemic sclerosis, a disorder unique to patients with acute kidney injury or severely compromised renal function receiving high doses of certain GBCAs^[46].

Disadvantages

The main disadvantage of NC-AMRI is that it relies exclusively on unenhanced images, which tend to have a relatively low contrast to noise ratio, potentially diminishing the visibility of HCC nodules as compared to post contrast sequences used in the other AMRI approaches. The inclusion of DWI, a high-contrast sequence, can aid in detecting liver lesions^[47], thereby improving sensitivity. However, DWI is technically challenging and often suffers from a variety of artifacts^[48] that can cause blind spots, most often near the liver dome or in the left lobe. Many early stage HCCs may not exhibit restricted diffusion relative to liver. In addition, HCC may be isointense to liver on T2 weighted imaging^[49] or obscured by altered signal in the liver parenchyma in the setting of cirrhosis. Such HCCs may be difficult to visualize on NC-AMRI.

Studies to date

Several studies have retrospectively assessed the performance of a simulated NC-AMRI (derived by extracting only the non-contrasted sequences from a complete MRI), most utilizing all three sequences outlined above^[23,25,32], and some utilizing DWI alone^[34,36] [Table 2]. While these studies found favorable sensitivities ranging from 84%-92% on a per-patient basis, sensitivity was 78% on a per-lesion basis in one study that used liver explant pathology as the reference standard MC^[36]. Most of these were retrospective studies in predominantly hepatitis-B population without advanced cirrhosis, enriched with a high prevalence of malignancy. Only one study thus far prospectively evaluated the performance of NC-AMRI in an HCC surveillance population^[34]. Using DWI alone, this study demonstrated a sensitivity of 83% and sensitivity of 98%. However, a small number of incident HCCs ($n = 6$) and low prevalence of Child Pugh

Table 2. AMRI studies to date

Author	Year	Context of image interpretation	Approach	Country	Target	Design	Intent of source imaging	Liver disease	Reference standard	Sample size	Analysis	Sens.	Spec.	Comments
Kim <i>et al.</i> [23]	2014	Simulation	NC-AMRI	Korea	Malignancy*	Retros	Diagnosis	Mixed**	Path or FU	128 pts	Per-patient	0.92	0.78	All lesions less than 2cm
Han <i>et al.</i> [24]	2018	Simulation	NC-AMRI	Korea	HCC	Retros	Diagnosis	Mixed**	Path, cMRI, FU	247 pts	Per-patient	0.84	0.82	
Chan <i>et al.</i> [25]	2019	Simulation	NC-AMRI	Australia	HCC	Retros	Diagnosis	Cirrhosis	cMRI	44 pts	Per-patient	0.86	0.86	
Sutherland <i>et al.</i> [34]	2016	Clinical practice	NC-AMRI*	Australia	HCC	Prosp	Surveillance	Mixed**	Path, cMRI, CT	192 pts	Per-patient	0.83	0.98	NC-AMRI was DWI only
McNamara [36]	2018	Simulation	NC-AMRI*	USA	HCC	Retros	Surveillance	Mixed**	Explant	37 pts*	Per-lesion*	0.78	0.88	NC-AMRI was DWI only; 17 HCC
Hecht <i>et al.</i> [35]	2006	Simulation	Dyn-AMRI	USA	HCC	Retros	Diagnosis*	Cirrhosis	Explant	50 pts*	Per-lesion*	0.68	0.66	All scans at 1.5 Tesla; 19 HCC
Khatiri <i>et al.</i> [27]	2020	Simulation	Dyn-AMRI	USA	HCC	Retros	Diagnosis	Cirrhosis	Path, cMRI, FU	100 pts	Per-patient	0.92	0.88	Used coronal T2 as localizing sequence
Marks <i>et al.</i> [28]	2015	Simulation	HBP-AMRI	USA	HCC	Retros	Surveillance	Cirrhosis or HBV	cMRI or FU	298 pts	Per-patient	0.83	0.93	
Besa <i>et al.</i> [29]	2017	Simulation	HBP-AMRI	USA	HCC	Retros	Mixed***	Mixed**	Path or cMRI	174 pts	Per-patient	0.80	0.96	
Tillman <i>et al.</i> [30]	2018	Simulation	HBP-AMRI	USA	HCC	Retros	Surveillance	Cirrhosis or HBV	Path of cMRI	79 pts*	Per-lesion*	0.85	NR	27 HCC
Brunsing <i>et al.</i> [31]	2019	Clinical practice	HBP-AMRI	USA	HCC	Retros	Surveillance	Cirrhosis or HBV	cMRI or CT	141 pts	Per-patient	0.91	0.99	

*See comments; **"Mixed" under "Liver Disease" refers to a cohort or population with mixed etiologies of liver disease which is not easily summarized; ***"Mixed" under "Intent of source imaging" indicates that imaging included in the study could have been done either for the purpose of diagnosis or surveillance. AMRI: Abbreviated magnetic resonance imaging; cMRI: complete MRI; CT: computed tomography; DWI: diffusion weighted imaging; Dyn-AMRI: Dynamic abbreviated MRI; FU: follow-up; les: lesions; HCC: hepatocellular carcinoma; NC-AMRI: non-contrasted abbreviated MRI; NR: not reported; Path: histopathology; Prosp: prospective study; pts: patients; Retros: retrospective study; Sens: sensitivity; Spec: specificity; USA: United States

status B or C cirrhosis (< 6%) limit the generalizability of this result. To our knowledge, this study and a HBP-AMRI study discussed below^[31] are the only two studies to-date evaluating the performance of AMRI interpreted prospectively in the clinical setting.

Summary statement

The strengths of NC-AMRI are maximum reduction in cost due to lack of contrast, minimum patient risk, simplified workflow, and the ability to repeat sequences compromised by motion or other resolvable artifacts. However, the generalizability of existing data, in particular to Western surveillance populations, is challenged by the enrichment of study populations with malignant lesions, preponderance of hepatitis B patients, and low prevalence of advanced (e.g., Child-Pugh B) cirrhosis. It is likely that the sensitivities and performance of NC-AMRI may be less favorable in North American or European populations due to differences in body habitus, etiologies of liver disease, and severity of cirrhosis. HCC detection accuracy for full NC-AMRI needs to be validated in prospective studies in surveillance patient cohorts. To this end, a randomized control trial has been initiated in a Korean population directly

comparing NC-AMRI to US for HCC surveillance^[50], but similar studies will be needed in non-Asian populations before this approach can be widely recommended. Ultimately, the performance and clinical utility of this approach will be determined mainly by DWI, which provides higher lesion conspicuity than the other sequences, thus optimizing this sequence will be essential.

DYNAMIC AMRI

Imaging

Dynamic contrast-enhanced AMRI (Dynamic-AMRI), one of two AMRI strategies that utilize GBCAs, acquires dynamic contrast enhanced images using T1-weighted images with fat suppression following administration of an extracellular contrast agent. The dynamic component refers to images acquired at predetermined and successive phases to detect and characterize HCCs based on the vascular alterations of hepatocarcinogenesis. These phases include the following:

Pre-contrast imaging

The pre-contrast images provide a baseline from which all post-contrast images are assessed for contrast enhancement. Pre-contrast images also allow detection of intrinsic T1 hyperintense observations, and for confirming that any hyperintensity on post contrast images represents true contrast enhancement. With modern MRI systems, it is possible to collect IP/OOP images simultaneously with the pre-contrast T1-weighted images (i.e., no additional acquisition is needed). If such images are acquired, they may permit assessment of relative fat or iron content relative to liver, as described for NC-AMRI.

Arterial phase imaging

Arterial phase (AP) is the time point after contrast injection at which tumor enhancement via arterial inflow is expected to be maximal. This usually occurs when portal veins are moderately to fully enhanced but the hepatic veins are not yet enhanced by antegrade flow. Appropriate timing of the AP is essential and can be achieved with reasonable consistency using current bolus-tracking technology or other methods^[51]. This sequence is used to assess arterial phase hyperenhancement (APHE), meaning enhancement greater than background liver parenchyma in the AP. Thought to reflect the arterialization of HCC during hepatocarcinogenesis, APHE is one of the defining imaging features of HCC and is required for imaging-based diagnosis in high-risk patients, per Liver Imaging Reporting and Data System (LI-RADS)^[9].

Portal venous phase imaging

Portal venous phase (PVP) is the time point after contrast injection at which the portal veins are fully enhanced and the hepatic veins are enhanced by antegrade flow^[9], occurring approximately 40 sec after AP when the liver is expected to be at its peak enhancement. Portal and hepatic vein anatomy and patency are assessed on this phase, including the presence of tumor in vein, which indicates macrovascular invasion. Washout appearance and enhancing capsule appearance, other defining imaging features of HCC, may be detected if present.

Delayed phase imaging

Delayed phase (DP) images are usually acquired 2-5 min after injection. Washout appearance and enhancing capsule appearance are usually most conspicuous on the DP images.

Reporting

Reporting of dynamic-AMRI is based on the major features of HCC as defined by LI-RADS [Figure 4]. An exam detecting a mass, meeting criteria for HCC (i.e., LR-5), should be reported as a positive result. The reporting and follow-up recommendations for exams showing indeterminate lesions (i.e., LR-3 or LR-4) based on Dynamic-AMRI has not been standardized.

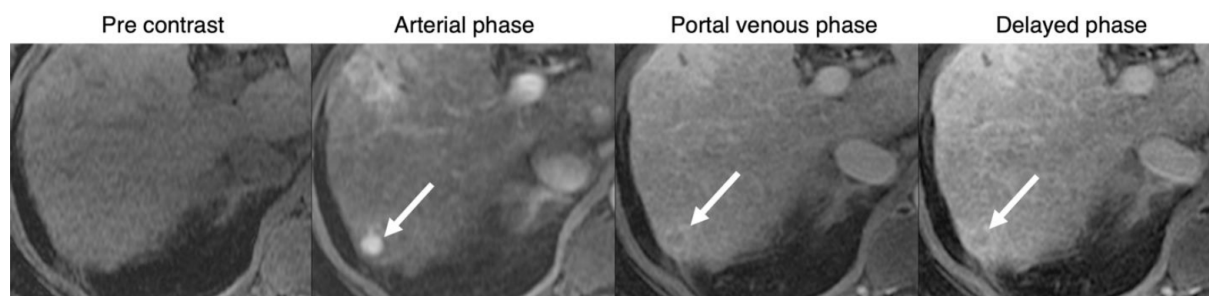


Figure 4. Positive dynamic-AMRI examination: 80-year-old male with HCV cirrhosis, images show a 11 mm observation in segment 7. The lesion has major features of HCC including nonrim APHE, washout and enhancing capsule (arrows) indicating definite HCC (LI-RADS-5). AMRI: abbreviated magnetic resonance imaging; HCV: hepatitis C virus; HCC: hepatocellular carcinoma; LI-RADS: Liver Imaging Reporting and Data System; APHE: arterial phase hyperenhancement

Advantages

Dynamic-AMRI offers unique advantages. The defining imaging features of HCC (i.e., the LI-RADS major features of size, APHE, washout appearance, and enhancing capsule appearance) are determined from dynamic imaging. When a liver observation meets the required diagnostic criteria, dynamic AMRI alone suffices for definitive diagnosis of HCC per LI-RADS (i.e., LR-5). It also permits the diagnosis of tumor in vein (TIV). Additionally, it provides cost benefits, as the contrast agents used in dynamic AMRI are typically less expensive than the contrast agent (gadoteric acid) required for HBP-AMRI^[52]. Some investigators have used coronal T2 imaging for localizer sequences, which can aid in characterizing benign lesions such as simple cysts and hemangiomas.

Disadvantages

The disadvantages of dynamic-AMRI relate to the lack of additional non-contrast sequences, which may provide ancillary imaging features otherwise not available from the dynamic images^[53]. The inability of dynamic-AMRI to evaluate these features may cause miscategorization of observations. In particular, dynamic-AMRI might over-categorize some vascular pseudolesions (e.g., arterio-portal shunts) as indeterminate (LR-3), potentially leading to unnecessarily close follow up. In theory, dynamic-AMRI also might under-categorize some early or small HCCs as LR-3, potentially delaying diagnosis, but the frequency with which this occurs is thought to be low. HCC detection by dynamic-AMRI depends on the timing and quality of arterial-phase imaging, which cannot be repeated if these images are mistimed or degraded by motion artifact or other problems. Finally, dynamic-AMRI requires a power injector for bolus intravenous administration of GBCA, which may not be available at all facilities and introduces complexity.

Studies to date

A few studies to date have retrospectively assessed the performance of a simulated dynamic-AMRI (derived by extracting only the dynamic sequences from a complete MRI) for HCC detection in patients with cirrhosis [Table 2]. These studies have shown that dynamic AMRI is diagnostically similar to complete MRI for HCC detection^[26,27], with per-patient reported sensitivity and specificity of 94% and 88%, respectively^[26]. However, these studies were conducted in diagnostic cohorts, in whom complete MRIs were indicated for known or clinically suspected liver lesions, which may have caused inflation in the sensitivity estimates. Dynamic-AMRI has yet to be tested prospectively in an HCC surveillance population.

Summary Statement

Dynamic-AMRI can characterize the defining imaging features of HCC and allows the detection and diagnosis of HCCs in a single surveillance exam. The absence of T2 weighted and DWI sequences, however, may cause diagnostic uncertainty, particularly for benign vascular pseudolesions, and lead to unnecessary

short interval follow-up or call-back. The requirements for a power injector and for precise arterial phase timing complicate the workflow compared to other AMRI approaches. HCC detection accuracy for dynamic AMRI needs to be validated prospectively in a surveillance patient cohort.

HEPATOBILARY-PHASE AMRI

Imaging

HBP contrast-enhanced AMRI (HBP-AMRI), the other AMRI approach that utilizes GBCA, is performed after administration of the hepatobiliary agent, gadoxetate disodium. The sequences include:

Hepatobiliary phase imaging

Acquired about 15-20 min following the administration of gadoxetate, when parenchymal enhancement with this agent is expected to be maximal, the hepatobiliary phase T1-weighted images provide high contrast-to-noise for lesion detection. In the hepatobiliary phase (HBP masses that are not of benign hepatocellular nature (e.g., HCCs and non-HCC malignant neoplasms) are hypointense relative to the high signal background liver, creating high liver to lesion contrast and increasing sensitivity. Hepatobiliary phase hypointensity is not specific for malignant nodules, however, and can be seen in benign non-hepatocellular entities, such as cysts and hemangiomas. Hence, any detected lesion must be correlated on T2-weighted imaging. If IP/OOP images are acquired, they may permit assessment of relative fat or iron content relative to liver, as described for the other AMRI approaches.

T2 weighted imaging

T2 weighted imaging is included to increase specificity. Benign lesions like cysts or hemangiomas have high intrinsic T2 signal and can be readily identified, while marked T2 darkness also suggests benignity, which helps with reducing unnecessary call-backs. In contrast, HCC tends to be mildly to moderately T2 hyperintense.

Optional: DWI

Similar to NC-AMRI, inclusion of DWI is meant to increase sensitivity for malignancy via a mechanism distinct from HBP imaging. Some DWI features may also be used to help differentiate HCC from non-HCC malignancy, such as intrahepatic cholangiocarcinoma (ICC), as discussed earlier^[43].

Reporting

Reporting of HBP-AMRI is the most developed of all AMRI approaches since HBP-AMRI has been implemented in clinical practice in selected centers in the United States. HBP-AMRI reporting mirrors that of LI-RADS US surveillance reporting with three outcomes: Positive (suspicious nodules ≥ 1 cm), subthreshold (suspicious nodules < 1 cm), and negative (no suspicious nodules)^[49]. Positive examinations prompt call back for diagnostic MRI or CT. The scoring of HBP-AMRI has been reported previously^[31], with an example provided in [Figure 5](#).

Advantages

HBP-AMRI provides several advantages. The core T1-weighted HBP images have high-contrast-to-noise, aiding in lesion detection. Importantly, hepatocytes retain gadoxetate for an extended period of time. Thus, images can be repeated as necessary. The 20-min delay also allows hand injection of contrast while the patient is in the waiting room, which simplifies workflow, reduces the time the patient is on the MRI table, thus reducing the examination cost, and diminishes the chance of contrast extravasation. This also eliminates the need for a power injector. Finally, HBP-AMRI are reported and interpreted using a simple scoring system modeled from LI-RADS US surveillance^[54], which many radiologists are already familiar with, in theory facilitating implementation.

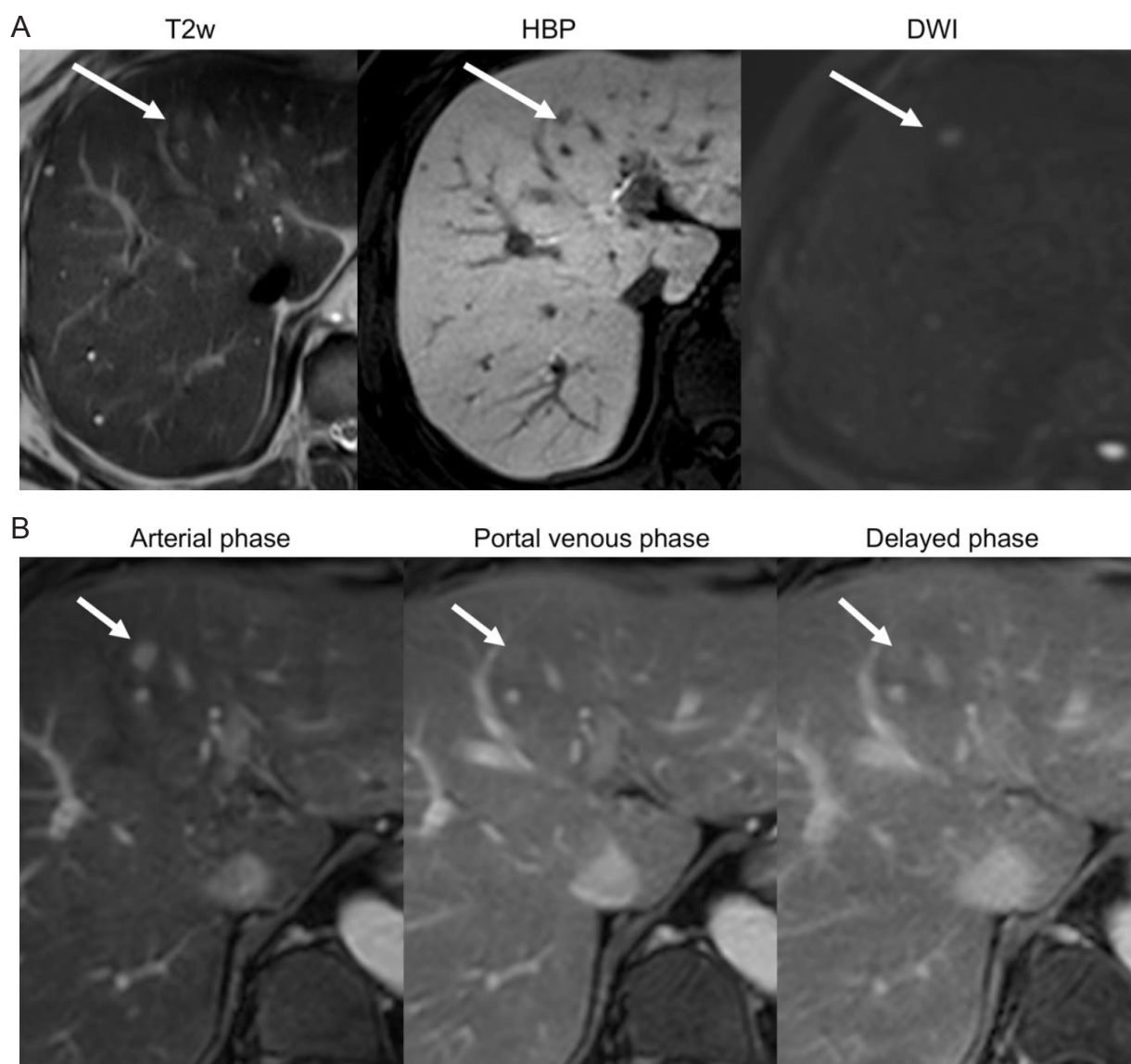


Figure 5. (A) Positive HBP-AMRI examination: 53-year-old male with chronic hepatitis B without cirrhosis. Images show an 8 mm HBP defect in segment 4, with mild T2 hyperintensity and restricted diffusion (arrows). On follow-up extra-cellular contrast MRI dynamic images (B) the lesion exhibits nonrim arterial phase hyperenhancement and capsule. An HCC was confirmed after lesion resection. HBP: hepatobiliary phase; AMRI: abbreviated magnetic resonance imaging; HCC: hepatocellular carcinoma

Disadvantages

The disadvantages of HBP-AMRI center on the contrast agent used and sequelae from cirrhosis. The contrast agent used in HBP-AMRI, gadoxetate, is more expensive than the extracellular agents used for dynamic-AMRI, which may counterbalance some of the cost gains from a simplified workflow. Patients with advanced cirrhosis may have reduced hepatocyte function, which may limit contrast uptake (i.e., reduced liver to lesion contrast), or may have areas of confluent fibrosis, which may reduce the accuracy for HCC detection by obscuring tumors (false negatives) or being mistaken for tumors (false positives). An additional problem is that HBP-AMRI detects HCC based on a very early alteration during hepatocarcinogenesis, namely reduced expression of the OATP transporter, the molecule required for uptake of gadoxetate into hepatocytes^[55], which occurs prior to neoangiogenesis^[56]. This means very early HCC may be detected as hypointense lesions on HBP-AMRI even before they exhibit APHE, making them impossible to definitively characterize as HCC on call back diagnostic imaging^[9,57]. Centers that elect to

apply HBP-AMRI need to be aware of this potential pitfall and understand that HBP-AMRI will detect some patients with HCC precursor nodules prior to overt malignant transformation. Conversely, some reports have shown that occasionally HCCs can be iso- or hyperintense on HBP imaging and may be mistaken for benign lesions^[15,23,40].

Studies to date

Three studies have retrospectively assessed the performance of a simulated HBP-AMRI (derived from a complete MRI with gadoxetate) for HCC detection in patients with cirrhosis or chronic hepatitis B [Table 2], the largest of which was a dual center study in a surveillance population^[28]. These studies have reported per-patient sensitivities in the range of 80%-83%, per-patient specificities in the range of 93%-96%, and a per-lesion sensitivity of 85%. One study evaluated the performance of HBP-AMRI interpreted prospectively in an HCC surveillance population, demonstrating a sensitivity of 91% and sensitivity of 99%^[31]. In this study, 20% of patients had Child Pugh B or C cirrhosis with 12 HCC in the cohort. To our knowledge, this study and the previously discussed study evaluating DWI alone^[34] are the only two studies to date evaluating the performance of AMRI interpreted prospectively in the clinical setting. Clinical trials are underway^[58].

The financial implications of HBP-AMRI have also been studied. By one estimate, HBP-AMRI screening would result in a 30% immediate cost savings relative to complete contrast enhanced-MRI^[29]. In another estimate, an HCC screening strategy using HBP-AMRI had a favorable incremental cost-effectiveness ratio (ICER) (\$3,000) per quality-adjusted life year (QALY) gained compared to US, across a wide range of HCC incidences^[59].

Summary statement

HBP-AMRI, perhaps the most well studied of the AMRI approaches, offers a streamlined workflow with simple, established reporting guidelines, the use of high-contrast sequences that can be repeated if needed, and preliminary studies demonstrating its cost effectiveness and diagnostic performance in surveillance populations. The disadvantages are the potential for reduced accuracy in some patients with advanced cirrhosis, the increased cost of the GBCA used for HBP-AMRI compared to dynamic-AMRI, and the possibility of detecting very early HCCs that cannot be confirmed with currently available diagnostic imaging tests.

CURRENT ISSUES AND GAPS IN KNOWLEDGE

Despite the growing body of literature suggesting AMRI offers superior sensitivity in HCC detection to that reported for surveillance US, there is insufficient evidence to recommend widespread adoption of AMRI by international guidelines. Prospective studies evaluating the performance and cost-effectiveness of AMRI versus US in surveillance populations for detecting HCC and prolonging life will be needed to inform changes to existing guidelines. Although it may take years for that evidence to be generated, AMRI can be of use today. One potential way to integrate AMRI into current practice is to apply it in patients who have severe limitations of their US examinations, such as those with an US LI-RADS visualization score of C^[54], or at the discretion of hepatologists, who might be concerned about the reliability of US imaging for patients with markedly heterogeneous liver parenchyma due to underlying cirrhosis or with poor liver visualization due to large body habitus, ascites, or other factors.

Another challenge of implementing AMRI, at least in the United States, is insurance reimbursement. The overarching goal of AMRI is to leverage the high sensitivity of MRI in a cost-effective manner. Moreover, one of the key elements in evaluating or implementing a surveillance program is the overall cost effectiveness of the approach. However, in order to accurately assess the cost-effectiveness of AMRI there must be a billing mechanism that appropriately reflects the reduced scanner time and other health-economic benefits of the shortened protocols. This mechanism currently does not exist in the United States.

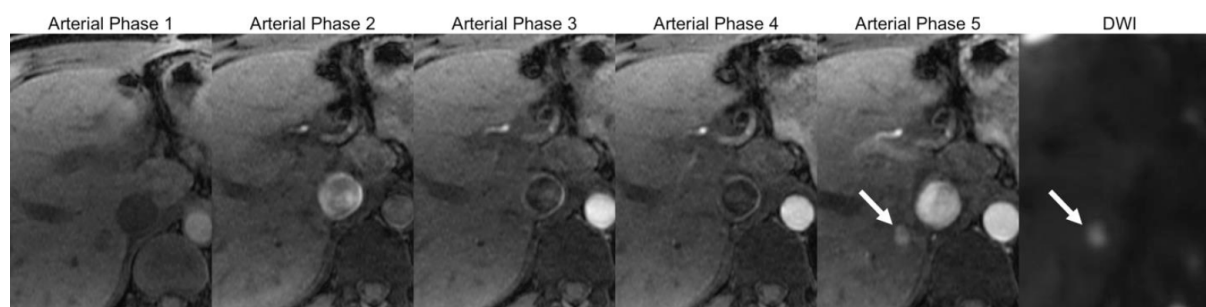


Figure 6. MRI multiarterial phase acquisition (Arterial phase 1-5): the multiphase acquisition in a single breath hold allows capturing the optimally timed arterial phase for HCC detection (in this example, arterial phase 5). A 8 mm observation with nonrim APHE is seen in segment 6, confirmed as a suspicious observation due to restricted diffusion (arrows). MRI: magnetic resonance imaging; HCC: hepatocellular carcinoma; APHE: arterial phase hyperenhancement; DWI: diffusion weighted imaging

Objective assessment and wide-spread implementation of AMRI may require the development of new, exam-specific billing codes, like what was done for MR Elastography in 2019. Other countries will likely have to weigh the efficacy, availability, and relative costs to determine the feasibility of AMRI in practice.

While increasing sensitivity by using AMRI addresses one of the problems of surveillance US, it does not solve the problem of poor compliance with surveillance programs^[60]. The reasons for poor compliance are complicated and not entirely understood. Contributing factors in the United States may include wait times and access to specialists^[60]. It is not clear if a surveillance modality that requires intravenous contrast and screening like MRI would pose an additional barrier for patient compliance. There is the potential that the higher sensitivity of AMRI would allow for less frequent surveillance, perhaps from twice a year (the current standard) to only once a year, as has been previously proposed^[61]. However, increasing the surveillance interval remains a theoretical benefit of AMRI and it is unclear if this would improve compliance^[62]. The impact of AMRI on surveillance compliance should be included in prospective comparative studies.

No study to date has directly compared the different AMRI approaches, and head-to-head studies will be needed to determine the optimal approach. It is possible that no one approach will be best in all patients, and tailored strategies may be needed.

FUTURE DIRECTION: MEETING CHALLENGES OF MRI WITH NEW TECHNOLOGY

Existing data suggests that AMRI techniques maintain the high sensitivity of complete MRI examinations, however there remains room for improvement and innovation^[63]. Human and technical factors can contribute to artifacts and undermine image quality, reducing sensitivity for malignancy, especially small lesions. MRI is extremely versatile with many ways to collect data during image acquisition and continuous development of tools for image reconstruction.

Recent advances^[64-70] that allow acquisition of multiple arterial phases in a single breath hold are finding their way into clinical practice, increasing the chances of capturing an optimally timed arterial phase, when HCC most commonly shows the highest degree of APHE [Figure 6].

Motion artifacts commonly degrade liver MRI quality. Free-breathing MRI tools are being developed for dynamic post-contrast imaging^[71,72], HBP imaging^[73,74], and DWI^[75-77], as are tools to address cardiac motion, which is particularly problematic in the left lobe of the liver^[78-81].

There is great interest in applying artificial intelligence to improve MRI image quality, image registration, and workflow^[73,82-84] all of which are active areas of investigation.

KEY POINTS

There are three variations of AMRI for HCC surveillance (non-contrast, dynamic, and hepatobiliary), each offering unique advantages.

There is a growing body of literature suggesting the sensitivity of AMRI may be higher than US, however existing data does not yet support widespread adoption of AMRI-based HCC surveillance by international guidelines.

Current utilization of AMRI should focus on patients in whom US-based HCC surveillance is compromised.

Clinical trials directly comparing AMRI to US for HCC surveillance in high-risk populations are underway.

Continued evolution of MRI technology is expected to increase the robustness of AMRI for HCC detection.

RECOMMENDATION

We cautiously recommend AMRI in situations where US is compromised. With regard to the exact approach - NC-AMRI, Dynamic-AMRI, or HBP-AMRI - all are reasonable. There is not yet sufficient evidence to recommend one approach over another. Hence, we leave protocol selection to the individual radiologist, referrer, and institution, considering patient preferences.

DECLARATIONS

Authors' contributions

Made substantial contributions to structure and content of the text and figures: Brunsing RL, Fowler KJ, Yokoo T, Cunha GM, Sirlin CB, Marks RM

Made substantial contributions to synthesizing edits from all authors: Brunsing RL

Availability of the data and materials

Not applicable.

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Conflicts of interest

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Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004;130:417-22.
2. Pascual S, Miralles C, Bernabé JM, Irurzun J, Planells M. Surveillance and diagnosis of hepatocellular carcinoma: a systematic review. *World J Clin Cases* 2019;716:2269-86.
3. Ronot M, Pommier R, Dioguardi Burgio M, Purcell Y, Nahon P, et al. Hepatocellular carcinoma surveillance with ultrasound - cost-effectiveness, high-risk populations, uptake. *Br J Radiol* 2018;91:20170436.
4. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236.
5. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, et al. Asia-pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017;11:317-70.
6. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;68:723-50.
7. Kokudo N, Takemura N, Hasegawa K, Takayama T, Kubo S, et al. Clinical practice guidelines for hepatocellular carcinoma: The Japan Society of Hepatology 2017 (4th JSH-HCC guidelines) 2019 update. *Hepatology Res* 2019;4910:1109-13.
8. Korean Liver Cancer Association (KLCA); National Cancer Center (NCC), Goyang, Korea. 2018 Korean Liver Cancer Association-National Cancer Center Korea Practice Guidelines for the Management of Hepatocellular Carcinoma. *Korean J Radiol* 2019;13:227-99.
9. LI-RADS. American College of Radiology; Available from: <https://www.acr.org/Clinical-Resources/Reporting-and-Data-Systems/LI-RADS>. [Last accessed on 13 Jul 2020]
10. Kim TH, Kim SY, Tang A, Lee JM. Comparison of international guidelines for noninvasive diagnosis of hepatocellular carcinoma: 2018 update. *Clin Mol Hepatol* 2019;25:245-63.
11. Colli A, Fraquelli M, Casazza G, Massironi S, Colucci A, et al. Accuracy of ultrasonography, Spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: a systematic review. *Am J Gastroenterol* 2006;10:513-23.
12. Kim SY, An J, Lim YS, Han S, Lee JY, et al. MRI with liver-specific contrast for surveillance of patients with cirrhosis at high risk of hepatocellular carcinoma. *JAMA Oncol* 2017;3:456-63.
13. Singal A, Volk ML, Waljee A, Salgia R, Higgins P, et al. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009;30:37-47.
14. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, et al. Surveillance Imaging and alpha fetoprotein for early detection of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. *Gastroenterology* 2018;154:1706-18.e1.
15. Yu NC, Chaudhari V, Raman SS, Lassman C, Tong MJ, et al. CT and MRI improve detection of hepatocellular carcinoma, compared with ultrasound alone, in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2011;9:161-7.
16. Colli A, Fraquelli M, Casazza G, Massironi S, Colucci A, et al. Accuracy of ultrasonography, Spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: a systematic review. *Am J Gastroenterol* 2006;101:513-23.
17. Samoylova ML, Mehta N, Roberts JP, Yao FY. Predictors of ultrasound failure to detect hepatocellular carcinoma. *Liver Transpl* 2018;24:1171-7.
18. Singal AG, Conjeevaram HS, Volk ML, Fu S, Fontana RJ, et al. Effectiveness of hepatocellular carcinoma surveillance in patients with cirrhosis. *Cancer Epidemiol Biomarkers Prev* 2012;21:793-9.
19. Hanna RF, Milousev VZ, Tang A, Finklestone LA, Brejt SZ, et al. Comparative 13-year meta-analysis of the sensitivity and positive predictive value of ultrasound, CT, and MRI for detecting hepatocellular carcinoma. *Abdom Radiol (NY)* 2016 41:71-90.
20. Forner A, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. *Semin Liver Dis* 2010;30:61-74.
21. Balogh J, Victor D, Asham EH, Burroughs SG, Boktour M, et al. Hepatocellular carcinoma: a review. *J Hepatocell Carcinoma* 2016;3:41-53.
22. Singal AG, Marrero JA, Yopp A. Screening process failures for hepatocellular carcinoma. *J Natl Compr Canc Netw* 2014;12:375-82.
23. Kim YK, Kim YK, Park HJ, Park MJ, Lee WJ, et al. Noncontrast MRI with diffusion-weighted imaging as the sole imaging modality for detecting liver malignancy in patients with high risk for hepatocellular carcinoma. *Magn Reson Imaging* 2014;32:610-8.
24. Han S, Choi JI, Park MY, Choi MH, Rha SE, et al. The diagnostic performance of liver MRI without intravenous contrast for detecting hepatocellular carcinoma: a case-controlled feasibility study. *Korean J Radiol* 2018;19:568-77.
25. Chan MV, McDonald SJ, Ong YY, Mastrocostas K, Ho E, et al. HCC screening: assessment of an abbreviated non-contrast MRI protocol. *Eur Radiol Exp* 2019;3:49.
26. Lee JY, Huo EJ, Weinstein S, Santos C, Monto A, et al. Evaluation of an abbreviated screening MRI protocol for patients at risk for hepatocellular carcinoma. *Abdom Radiol (NY)* 2018;43:1627-33.
27. Khatri G, Pedrosa I, Ananthakrishnan L, de Leon AD, Fetzer DT, et al. Abbreviated-protocol screening MRI vs. complete-protocol diagnostic MRI for detection of hepatocellular carcinoma in patients with cirrhosis: An equivalence study using LI-RADS v2018. *J Magn Reson Imaging* 2020;51:415-25.
28. Marks RM, Ryan A, Heba ER, Tang A, Wolfson TJ, et al. Diagnostic per-patient accuracy of an abbreviated hepatobiliary phase gadoteric

- acid-enhanced MRI for hepatocellular carcinoma surveillance. *AJR Am J Roentgenol* 2015;204:527-35.
29. Besa C, Lewis S, Pandharipande PV, Chhatwal J, Kamath A, et al. Hepatocellular carcinoma detection: diagnostic performance of a simulated abbreviated MRI protocol combining diffusion-weighted and T1-weighted imaging at the delayed phase post gadoxetic acid. *Abdom Radiol* 2017;42:179-90.
30. Tillman BG, Gorman JD, Hru JM, Lee MH, King MC, et al. Diagnostic per-lesion performance of a simulated gadoxetate disodium-enhanced abbreviated MRI protocol for hepatocellular carcinoma screening. *Clin Radiol* 2018;73:485-93.
31. Brunsing RL, Chen DH, Schlein A, Wolfson T, Gamst A, et al. Gadoxetate-enhanced abbreviated MRI for hepatocellular carcinoma surveillance: preliminary experience. *Radiol Imaging Cancer* 2019;1:e190010.
32. Park SH, Kim B, Kim SY, Shim YS, Kim JH, et al. Abbreviated MRI with optional multiphasic CT as an alternative to full-sequence MRI: LI-RADS validation in a HCC-screening cohort. *Eur Radiol* 2020;30:2302-11.
33. Canellas R, Rosenkrantz AB, Taouli B, Sala E, Saini S, et al. Abbreviated MRI protocols for the abdomen. *Radiographics* 2019;39:744-58.
34. Sutherland T, Watts J, Ryan M, Galvin A, Temple F, et al. Diffusion-weighted MRI for hepatocellular carcinoma screening in chronic liver disease: direct comparison with ultrasound screening. *J Med Imaging Radiat Oncol* 2017;61:34-9.
35. Hecht EM, Holland AE, Israel GM, Hahn WY, Kim DC, et al. Hepatocellular carcinoma in the cirrhotic liver: gadolinium-enhanced 3D T1-weighted MR imaging as a stand-alone sequence for diagnosis. *Radiology* 2006;239:438-47.
36. McNamara MM, Thomas JV, Alexander LF, Little MD, Bolus DN, et al. Diffusion-weighted MRI as a screening tool for hepatocellular carcinoma in cirrhotic livers: correlation with explant data-a pilot study. *Abdom Radiol (NY)* 2018;43:2686-92.
37. Lima PH, Fan B, Bérubé J, Cerny M, Olivie D, et al. Cost-utility analysis of imaging for surveillance and diagnosis of hepatocellular carcinoma. *AJR Am J Roentgenol* 2019;17:1-9.
38. Cerny M, Bergeron C, Billiard JS, Murphy-Lavallée J, Olivie D, et al. LI-RADS for MR imaging diagnosis of hepatocellular carcinoma: performance of major and ancillary features. *Radiology* 2018;288:118-28.
39. Park MS, Kim S, Patel J, Hajdu CH, Do RKG, et al. Hepatocellular carcinoma: detection with diffusion-weighted versus contrast-enhanced magnetic resonance imaging in pretransplant patients. *Hepatology* 2012;56:140-8.
40. Zhang T, Huang ZX, Wei Y, Jiang HY, Chen J, et al. Hepatocellular carcinoma: Can LI-RADS v2017 with gadoxetic-acid enhancement magnetic resonance and diffusion-weighted imaging improve diagnostic accuracy? *World J Gastroenterol* 2019;25:622-31.
41. Basha MAA, Refaat R, Mohammad FF, Khamis MEM, El-Maghraby AM, et al. The utility of diffusion-weighted imaging in improving the sensitivity of LI-RADS classification of small hepatic observations suspected of malignancy. *Abdom Radiol* 2019;44:1773-84.
42. Min JH, Kim YK, Choi SY, Jeong WK, Lee WJ, et al. Differentiation between cholangiocarcinoma and hepatocellular carcinoma with target sign on diffusion-weighted imaging and hepatobiliary phase gadoxetic acid-enhanced MR imaging: classification tree analysis applying capsule and septum. *Eur J Radiol* 2017;92:1-10.
43. Park HJ, Kim YK, Park MJ, Lee WJ. Small intrahepatic mass-forming cholangiocarcinoma: target sign on diffusion-weighted imaging for differentiation from hepatocellular carcinoma. *Abdom Imaging* 2013;38:793-801.
44. Kanda T, Ishii K, Kawaguchi H, Kitajima K, Takenaka D. High signal intensity in the dentate nucleus and globus pallidus on unenhanced T1-weighted MR images: relationship with increasing cumulative dose of a gadolinium-based contrast material. *Radiology* 2013;270:834-41.
45. Choi JW, Moon WJ. Gadolinium deposition in the brain: current updates. *Korean J Radiol* 2019;20:134-47.
46. Reiter T, Ritter O, Prince MR, Nordbeck P, Wanner C, et al. Minimizing risk of nephrogenic systemic fibrosis in cardiovascular magnetic resonance. *J Cardiovasc Magn Reson* 2012;14:31.
47. Shankar S, Kalra N, Bhatia A, Srinivasan R, Singh P, et al. Role of diffusion weighted imaging (DWI) for hepatocellular carcinoma (HCC) detection and its grading on 3T MRI: a prospective study. *J Clin Exp Hepatol* 2016;6:303-10.
48. Chandarana H, Taouli B. Diffusion and perfusion imaging of the liver. *Eur J Radiol* 2010;76:348-58.
49. Saito K, Moriyasu F, Sugimoto K, Nishio R, Saguchi T, et al. Histological grade of differentiation of hepatocellular carcinoma: comparison of the efficacy of diffusion-weighted MRI with T2-weighted imaging and angiography-assisted CT. *J Med Imaging Radiat Oncol* 2012;56:261-9.
50. An C, Kim DY, Choi JY, Han KH, Roh YH, et al. Noncontrast magnetic resonance imaging versus ultrasonography for hepatocellular carcinoma surveillance (MIRACLE-HCC): study protocol for a prospective randomized trial. *BMC Cancer* 2018;24:18.
51. Pinho DF, Lev-Cohain N, Awdeh H, Xi Y, Khatri G, et al. Optimization of breathing instructions and timing of late arterial phase acquisition on gadobutrol-enhanced MRI of the liver. *Clinical Imaging* 2016;40:1274-9.
52. Contrast Manual. American College of Radiology; 2020. Available from: <https://www.acr.org/Clinical-Resources/Contrast-Manual>. [Last accessed on 13 Jul 2020]
53. Wald C, Russo MW, Heimbach JK, Hussain HK, Pomfret EA, et al. New OPTN/UNOS policy for liver transplant allocation: standardization of liver imaging, diagnosis, classification, and reporting of hepatocellular carcinoma. *Radiology* 2013;266:376-82.
54. Ultrasound LI-RADS v2017. American College of Radiology. Available from: <https://www.acr.org/Clinical-Resources/Reporting-and-Data-Systems/LI-RADS/Ultrasound-LI-RADS-v2017>. [Last accessed on 13 Jul 2020]
55. Nassif A, Jia J, Keiser M, Oswald S, Modess C, et al. Visualization of hepatic uptake transporter function in healthy subjects by using gadoxetic acid-enhanced MR imaging. *Radiology* 2012;264:741-50.
56. Sano K, Ichikawa T, Motosugi U, Sou H, Muhi AM, et al. Imaging study of early hepatocellular carcinoma: usefulness of gadoxetic acid-enhanced MR imaging. *Radiology* 2011;261:834-44.
57. Rosenkrantz AB, Campbell N, Wehrli N, Triolo MJ, Kim S. New OPTN/UNOS classification system for nodules in cirrhotic livers detected with MR imaging: effect on hepatocellular carcinoma detection and transplantation allocation. *Radiology* 2014;274:426-33.

58. Gadolinium Contrast-enhanced Abbreviated MRI (AMRI) vs. Standard Ultrasound for Hepatocellular Carcinoma (HCC) Surveillance in Patients With Cirrhosis - Full Text View - ClinicalTrials.gov. Available from: <http://clinicaltrials.gov/ct2/show/NCT04288323>. [Last accessed on 13 Jul 2020]
59. Goossens N, Singal AG, King LY, Andersson KL, Fuchs BC, et al. Cost-effectiveness of risk score-stratified hepatocellular carcinoma screening in patients with cirrhosis. *Clin Transl Gastroenterol* 2017;8:e101.
60. Goldberg DS, Taddei TH, Serper M, Mehta R, Dieperink E, et al. Identifying barriers to hepatocellular carcinoma surveillance in a national sample of patients with cirrhosis. *Hepatology* 2017;65:864-74.
61. Chalasani N, Horlander JC, Said A, Hoen H, Kopecky KK, et al. Screening for hepatocellular carcinoma in patients with advanced cirrhosis. *Am J Gastroenterol* 1999;94:2988-93.
62. Goldberg D, Valderrama A, Kamalakar R, Sansgiry S, Babajanyan S, et al. Hepatocellular carcinoma surveillance among cirrhotic patients with commercial health insurance. *J Clin Gastroenterol* 2016;50:258-65.
63. Zech CJ, Ba-Ssalamah A, Berg T, Chandarana H, Chau GY, et al. Consensus report from the 8th international forum for liver magnetic resonance imaging. *Eur Radiol* 2020;30:370-82.
64. Breuer FA, Blaimer M, Mueller MF, Seiberlich N, Heidemann RM, et al. Controlled aliasing in volumetric parallel imaging (2D CAIPIRINHA). *Magn Reson Med* 2006;55:549-56.
65. Low RN, Bayram E, Panchal NJ, Estkowski L. High-resolution double arterial phase hepatic MRI using adaptive 2D centric view ordering: initial clinical experience. *AJR Am J Roentgenol* 2010;194:947-56.
66. Ikram NS, Yee J, Weinstein S, Yeh BM, Corvera CU, et al. Multiple arterial phase MRI of arterial hypervascular hepatic lesions: improved arterial phase capture and lesion enhancement. *Abdom Radiol (NY)*. 2017;42:870-6.
67. Brodsky EK, Bultman EM, Johnson KM, Horng DE, Schelman WR, et al. High spatial and high temporal resolution dynamic contrast-enhanced perfusion imaging of the liver with time-resolved 3D-radial MRI. *Magn Reson Med* 2014;71:934-41.
68. Saranathan M, Rettmann DW, Hargreaves BA, Clarke SE, Vasanawala SS. Differential subsampling with cartesian ordering (DISCO): a high spatio-temporal resolution dixon imaging sequence for multiphasic contrast enhanced abdominal imaging. *J Magn Reson Imaging* 2012;35:1484-92.
69. Shaikh J, Stoddard PB, Levine EG, Roh AT, Saranathan M, et al. View-sharing artifact reduction with retrospective compressed sensing reconstruction in the context of contrast-enhanced liver MRI for hepatocellular carcinoma (HCC) screening. *J Magn Reson Imaging* 2019;49:984-93.
70. Xu B, Spincemaille P, Chen G, Agrawal M, Nguyen TD, et al. Fast 3D contrast enhanced MRI of the liver using temporal resolution acceleration with constrained evolution reconstruction. *Magn Reson Med* 2013;69:370-81.
71. Cheng JY, Zhang T, Ruangwattanapaisarn N, Alley MT, Uecker M, et al. Free-breathing pediatric MRI with nonrigid motion correction and acceleration. *J Magn Reson Imaging* 2015;42:407.
72. Hedderich DM, Weiss K, Spiro JE, Giese D, Beck GM, et al. Clinical evaluation of free-breathing contrast-enhanced T1w MRI of the liver using pseudo golden angle radial k-space Sampling. *Rofo* 2018;190:601-9.
73. Zeng DY, Shaikh J, Holmes S, Brunsing RL, Pauly JM, et al. Deep residual network for off-resonance artifact correction with application to pediatric body MRA with 3D cones. *Magnetic Resonance in Medicine*. *Magn Reson Med* 2019;82:1398-411.
74. Kim YC, Min JH, Kim YK, Lee SJ, Ahn S, et al. Intra-individual comparison of gadolinium-enhanced MRI using pseudo-golden-angle radial acquisition with gadoteric acid-enhanced MRI for diagnosis of HCCs using LI-RADS. *Eur Radiol* 2019;29:2058-68.
75. Choi JS, Kim MJ, Chung YE, Kim KA, Choi JY, et al. Comparison of breathhold, navigator-triggered, and free-breathing diffusion-weighted MRI for focal hepatic lesions. *J Magn Reson Imaging* 2013;38:109-18.
76. Szklaruk J, Son JB, Wei W, Bhosale P, Javadi S, et al. Comparison of free breathing and respiratory triggered diffusion-weighted imaging sequences for liver imaging. *World J Radiol* 2019;11:134-43.
77. Ichikawa S, Motosugi U, Tamada D, Wakayama T, Sato K, et al. Improving the quality of diffusion-weighted imaging of the left hepatic lobe using weighted averaging of signals from multiple excitations. *Magn Reson Med Sci* 2019;18:225-32.
78. Zhang Y, Peña-Nogales Ó, Holmes JH, Hernando D. Motion-robust and blood-suppressed M1-optimized diffusion MR imaging of the liver. *Magn Reson Med* 2019;82:302-11.
79. Rauh SS, Riexinger AJ, Ohlmeyer S, Hammon M, Saake M, et al. A mixed waveform protocol for reduction of the cardiac motion artifact in black-blood diffusion-weighted imaging of the liver. *Magn Reson Imaging* 2020;67:59-68.
80. Aliotta E, Wu HH, Ennis DB. Convex optimized diffusion encoding (CODE) gradient waveforms for minimum echo time and bulk motion-compensated diffusion-weighted MRI. *Magn Reson Med* 2017;77:717-29.
81. Aliotta E, Moulin K, Ennis DB. Eddy current-nulled convex optimized diffusion encoding (EN-CODE) for distortion-free diffusion tensor imaging with short echo times. *Magn Reson Med* 2018;79:663-72.
82. Hasenstab KA, Cunha GM, Higaki A, Ichikawa S, Wang K, et al. Fully automated convolutional neural network-based affine algorithm improves liver registration and lesion co-localization on hepatobiliary phase T1-weighted MR images. *Eur Radiol Exp* 2019;3:43.
83. Chen F, Cheng JY, Taviani V, Sheth VR, Brunsing RL, et al. Data-driven self-calibration and reconstruction for non-cartesian wave-encoded single-shot fast spin echo using deep learning. *J Magn Reson Imaging* 2020;51:841-53.
84. Cunha GM, Hasenstab KA, Higaki A, Wang K, Delgado T, et al. Convolutional neural network-automated hepatobiliary phase adequacy evaluation may optimize examination time. *Eur J Radiol* 2020;124:108837.

Review

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Comparison and analysis of the efficacy of drug therapy for liver cancer

Philippe Merle^{1,2,3}, Miroslava Subic¹

¹Unité d'Hépatologie et Gastroentérologie, Hôpital de la Croix Rousse, Hospices Civils de Lyon, Lyon 69004, France.

²Centre de Recherche en Cancérologie de Lyon, INSERM U1052, Lyon 69003, France.

³Université Claude Bernard Lyon 1, Villeurbanne 69100, France.

Correspondence to: Prof. Philippe Merle, Unité d'Hépatologie, Groupement Hospitalier Lyon Nord, Hospices Civils de Lyon, 103 Grande rue de la Croix-Rousse, Lyon 69004, France. E-mail: philippe.merle@inserm.fr

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Abstract

Hepatocellular carcinoma (HCC) is a poor prognosis tumor when not accessible to potentially curative treatments such as surgical resection, thermal ablations or liver transplantation. Systemic cytotoxic chemotherapies have shown inconsistent clinical benefit. In 2007, sorafenib, a tyrosine kinase inhibitor (TKI), was the first systemic therapy able to significantly improve the outcome of HCC patients non-eligible for curative or loco-regional therapies, despite a modest tolerance and low tumor objective response rate (ORR). Among the newer TKIs approved after 2017, lenvatinib was the first to show a striking ORR and demonstrate non-inferiority vs. sorafenib in the first-line setting. Furthermore, phase 3 trials showed the benefit of other TKIs, regorafenib and cabozantinib, and the anti-angiogenic ramucirumab monoclonal antibody, in systemic second-line therapy. Immune checkpoint inhibitors targeting PD1, achieved striking tumor shrinkage in some patients in monotherapy, seeming to be associated with exciting outcomes. Unfortunately, this occurred in too few patients to improve the median overall survival. More recently, the combination of anti-angiogenic drugs targeting the liver microenvironment with PD-1/PD-L1 inhibitors, such as the combination of bevacizumab and atezolizumab, proved to be substantially effective in phase 3, and other combinations of PD-1/PD-L1 and CTLA-4 inhibitors or TKIs have raised a lot of hopes for the systemic treatment of HCC.

Keywords: Hepatocellular carcinoma, therapy, immune checkpoint inhibitors, tyrosine kinase inhibitors



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INTRODUCTION

Hepatocellular carcinoma (HCC) is a poor prognosis tumor ranking fourth as the leading cause of cancer death worldwide, with about 841,000 new cases and 782,000 deaths annually inventoried in 2018^[1]. Due to the frequently silent clinical character and the low sensitivity of currently available diagnostic biomarkers, HCC is commonly diagnosed at an advanced stage when curative treatments, i.e., surgical resection, ablations, and liver transplantation, or radiologic palliative loco-regional therapies are not feasible. Thus, these patients are eligible for systemic strategies^[2]. Until 2007, treatment options for advanced HCC were lacking. No systemic cytotoxic chemotherapies, including new compounds loaded onto nanoparticles^[3], have ever shown to significantly improve overall survival (OS) of HCC patients. Similarly, hormonotherapy and somatostatin analogs have failed to definitely benefit OS^[2]. The approval in 2007 of the first oral tyrosine kinase inhibitor (TKI) and antiangiogenic agent (AAA), sorafenib, and the more recent development of other TKIs and immune checkpoint inhibitors (ICIs) as well, have completely revolutionized the therapeutic paradigm for HCC. The perspectives for advanced HCC patients have changed from palliative short-term mortality towards long-term survival expectations. Several drugs are now available, and in this review, we will compare their efficacy with respect to OS and other surrogate endpoints as well, keeping in mind that they are still controversial and their pertinence must be carefully discussed. We will only focus on data emerging from positive phase 3 trials, and from those phase 1b/2 studies that led to an early US-FDA approval.

EFFICACY OF DRUGS: ENDPOINTS OF CLINICAL TRIALS

Clinical trials in HCC have been originally designed according to conventional biostatistical rules applied in oncology trials^[4], following the traditional linear model of cancer drug development in which drug activity assessment occurs in randomized confirmatory phase 2 and 3 clinical trials with OS as the most important endpoint for demonstrating clinical benefit. Nevertheless, OS has some disadvantages such as the requirement for long follow-up time, the need for a high number of patients and the possibility to be affected by sequential therapies administrated after tumor progression. The need to achieve a more rapid development of new targeted antitumor agents led to the adoption of innovative clinical trial designs and the identification of surrogate endpoints of survival such as progression-free survival (PFS), time to progression (TTP) and objective response rate (ORR).

Objective response rate

ORR directly reflects the treatment antitumor activity and is usually defined as the sum of complete (CR) and partial response (PR) rates. In HCC, ORR is measured according to Recist (Response Evaluation Criteria In Solid Tumors) version and/or liver modified-Recist (mRecist) criteria^[5]. ORR has been considered to be the primary endpoint for phase 2 studies dealing with local ablations or loco-regional therapies studies in HCC where this endpoint is consistently associated with OS^[6]. Whereas with the introduction of molecularly targeted treatments with TKIs, reliance on ORR needs to be reconsidered because clinically significant survival advantages are reported despite faint ORRs. Of course, long-lasting stable disease with the absence of progression is a beneficial characteristic, as death due to progression would not occur. In contrast, ORR has shown to be a potentially promising endpoint to obtain clinical benefit from some systemic drugs and in particular ICIs in HCC^[7,8].

Although Recist 1.1 and mRecist criteria can both be used to assess ORR in HCC, Recist 1.1 remains the gold-standard in phase 3 trials with systemic therapies. Of course, it is quite simple to apply Recist 1.1 after liver resection or transplantation. In contrast, local thermoablations or loco-regional intra-arterial therapies induce tumor necrosis, and thus, Recist 1.1 is not appropriate any more since it is unable to capture such an effect since relying on size reduction and ignoring necrosis. That is the reason why the EASL introduced criteria including the use of absence of contrast uptake in dynamic imaging to register response^[9],

Table 1. Objective response rate per Recist 1.1 and/or mRecist

	Led to committee approval	Systemic line	ORR (%) (95%CI)	Median duration of response in months (95%CI)	DCR (%)	First author (TRIAL)
Sorafenib (vs. placebo)	US-FDA, EMA	1L	IRF Recist 1.1 2%	ND	IRF Recist 1.1 71%	Llovet <i>et al.</i> ^[10] (Ph 3, SHARP)
Lenvatinib (vs. sorafenib)	US-FDA, EMA	1L	IRF Recist 1.1 18.8% (15.3-22.3) vs. 6.5% (4.3-8.7) IRF mRecist 40.6% (36.2-44) vs. 12.4% (9.4-15.4)	ND	IRF Recist 1.1 72.8% (68.8-76.8) vs. 59.0% (54.6-63.5) IRF mRecist 73.8% (69.9-77.8) vs. 58.4% (54.0-62.8)	Kudo <i>et al.</i> ^[11] (Ph 3, REFLECT)
Regorafenib (vs. placebo)	US-FDA, EMA	2L	Per investigator Recist 1.1 7%	Per investigator mRecist 3.5 (1.9-4.5)	Per investigator Recist 1.1 66%	Bruix <i>et al.</i> ^[12] (Ph 3, RESORCE)
Cabozantinib (vs. placebo)	US-FDA, EMA	2L or 3L	Per investigator Recist 1.1 4%	ND	Per investigator Recist 1.1 64%	Abou-Alfa <i>et al.</i> ^[13] (Ph 3, CELESTIAL)
Ramucirumab (vs. placebo)	US-FDA, EMA	2L	Per investigator Recist 1.1 5%	Per investigator Recist 1.1 at 6 months 59.9%	ND	Zhu <i>et al.</i> ^[14] (Ph 3, REACH-2)
Nivolumab	US-FDA	1L or 2L	Per investigator Recist 1.1 19%	Per investigator Recist 1.1 9.9 months (8.3-NE)	Per investigator Recist 1.1 64%	El-Khoueiry <i>et al.</i> ^[19] (Ph 1/2, CheckMate-040, dose-expansion phase)
Nivolumab (vs. sorafenib)	-	1L	IRF Recist 1.1 15%	IRF Recist 1.1 23.3 (3.1-34.5+) vs. 23.4 (1.9+-28.7+)	IRF Recist 1.1 55% vs. 55%	Yau <i>et al.</i> ^[20] (Ph 3, CheckMate-459)
Pembrolizumab	US-FDA	2L	IRF mRecist 17% (11-26)	IRF mRecist NR (3.1-14.6+)	IRF mRecist 61%	Zhu <i>et al.</i> ^[17] (Ph 2, KEYNOTE-224)
Pembrolizumab - (vs. placebo)	-	2L	IRF Recist 1.1 18.3% (14-23.4)	IRF Recist 1.1 13.8 (1.5+-23.6+)	IRF Recist 1.1 62.2%	Finn <i>et al.</i> ^[18] (Ph 3, KEYNOTE-240)
Atezolizumab + bevacizumab (vs. sorafenib)	Ongoing for US-FDA and EMA	1L	IRF Recist 1.1 27% (23-33) IRF mRecist 33% (28-39)	IRF Recist 1.1 NR IRF mRecist NR	IRF Recist 1.1 74% IRF mRecist 72%	Finn <i>et al.</i> ^[16] (Ph 3, IMbrave150)
Nivolumab + ipilimumab	US-FDA	2L	IRF Recist 1.1 32%	IRF Recist 1.1 17.5 (4.6-30.5+)	IRF Recist 1.1 50%	Yau <i>et al.</i> ^[8] (Ph 1/2 CheckMate-040, Arm A)

US-FDA: American Federal Drug Administration; EMA: European Medicines Agency; NR: not reached; NE: not estimable; ND: not determined; ORR: objective response rate; CR: complete response; PR: partial response; SD: stable disease; DCR: disease control rates: CR+PR+SD; IRF: independent review facility; First (1L), second (2L) or third (3L) systemic therapeutic line; Ph: phase

which corresponds to mRecist criteria. If Recist 1.1 can miss the initial antitumor effect on HCC such as devascularization, no study has definitely demonstrated its correlation with OS. Antiangiogenic agents may prompt a variable degree of vascular shutdown - i.e., sorafenib, regorafenib, cabozantinib, ramucirumab - and have marginal impact in terms of response as *per* Recist 1.1^[10-14].

Further, another issue comes from the inter-observer variability in tumor response assessment *per* Recist 1.1 and mRecist for HCC. However, although it remains poorly known and warrants prospective assessment, it is possible that concordance is good between operators with expertise in liver imaging and lower with non-specifically trained operator, independently of the response criteria^[15].

As detailed in Table 1, in ORR assessed by Recist 1.1, atezolizumab/bevacizumab^[16] and nivolumab/ipilimumab^[8] combinations displayed the best ORR (27% and 32%, respectively), followed by lenvatinib/TKI (18.8%)^[11], ICI monotherapy with pembrolizumab (17%-18.3%)^[17,18] and nivolumab (15%-19%)^[19,20], and at a disappointing lower level all the remaining TKIs such as sorafenib (2%-6.5%)^[10,11], regorafenib (7%)^[12] and cabozantinib (4%)^[13], and finally the monoclonal antibody ramucirumab (5%)^[14]. These data suggest that these latter drugs have mostly a tumor-static rather than tumoricidal activity by comparison to the

former. In those, comprising atezolizumab/bevacizumab^[16] and nivolumab/ipilimumab^[8] combinations, durations of response were long-lasting (median not reached and 17.5 months, respectively) as well as with ICI monotherapies (median 23.3 months with nivolumab^[20], 13.8 months with pembrolizumab^[18]), and unfortunately not determined with lenvatinib^[11].

ORR might be a surrogate endpoint of drug efficacy in some cases. In phase 1/2 trials with ICIs, ORR by Recist 1.1 seemed to deeply correlate with OS of patients treated either with nivolumab monotherapy^[7] or with the nivolumab/ipilimumab combination^[8]. In both cases, tumor responders (CR + PR) had the best OS [median non-reached (NE-NE) for both cases]. Patients in progression disease (PD) did not seem to have any benefit on OS by comparison to well known patients randomized in the placebo arms in controlled trials [8.9 months (7.3-13.4) and 8.3 months (6.6-10.8), respectively]. Intermediately, stable diseases (SD) had better but not striking data [16.7 (13.8-20.2) and 14.5 (8.4-29.6), respectively]. However, it has not been assessed so far in the atezolizumab/bevacizumab phase 3 trial^[16] or other kind of ICI plus AAA combination in phase 1/2 studies, whether ORR has the same predictive value on the outcome of HCC patients. Furthermore, no data on the field are available regarding the correlation between ORR by mRecist and OS of HCC patients treated with ICIs.

These observations do not seem so evident with TKIs, which have the disadvantage of resulting in very low levels of ORR except for lenvatinib^[11]. ORR (and TTP) have been suggested as potential surrogate endpoints for OS in advanced HCC with brivanib^[21,22], and seemed to be confirmed with sorafenib and lenvatinib in REFLECT^[11,23]. However, a weak correlation was reported between ORR, TTP/PFS and OS in SHARP with sorafenib^[10], and with regorafenib in RESORCE^[12,24]. In this later study, since ORR was rather low either by Recist 1.1 (2%) or mRecist (10%), a bootstrap approach was applied to simulate 10,000 trials of patients with advanced HCC from RESORCE ($n = 573$), and the mean simulated results were calculated. A Pearson correlation was calculated between estimated median OS and estimated ORR for regorafenib and placebo arms separately. The Pearson correlation of log-rank test statistics was calculated comparing regorafenib and placebo. The Pearson correlation of log-rank test statistics comparing the two arms for OS was used and the Cochran-Mantel-Haenszel test statistic used to compare the two treatment arms for ORR. Finally, a weak correlation between median OS and ORR was found for regorafenib and placebo in RESORCE, indicating that mRecist/Recist 1.1 ORR may not be a reliable surrogate endpoint for OS in patients with advanced HCC. The same observation was found for TTP in this study.

In summary, ORR could be as a good surrogate marker for OS in HCC patients under lenvatinib or ICI therapy, which give high levels of ORR, whereas it is more complex, debatable and doubtful for drugs with low level of ORR such as sorafenib and regorafenib, keeping in mind that this research has not been performed so far for cabozantinib and ramucirumab.

Progression-free survivals and/or time to radiologic progression

In HCC, progression-free survival (PFS) is frequently used in phase 2 trials. PFS is a composite endpoint that includes: (1) radiologic progression as defined by Recist 1.1 or mRecist; and (2) death due to tumor progression or the terminal natural history of the underlying chronic liver disease. In general, regulatory agencies prefer PFS to TTP for drug approval because the former endpoint may be better correlated with OS^[25]. However, in HCC, PFS might not be reliable because death resulting from the natural history of cirrhosis might confound the detection of potential benefits from effective drugs. The risk of bias in detection of potential benefits from effective antitumor drugs due to death related to liver failure despite a relevant antitumor response can be avoided using restrictive inclusion criteria for evaluation of liver function^[25].

Time to radiologic progression (TTP), on the other hand, is a pure radiologic endpoint^[26], and requires repeated radiologic measurements to capture relevant differences between groups that can be missed if the

Table 2. Median PFS and/or TTP following Recist 1.1 and/or mRecist

	PFS in months (95%CI)	TTP in months (95%CI)	Authors (Trial)
Sorafenib vs. placebo	ND	IRF Recist 1.1 5.5 vs. 2.8; HR = 0.58, 95% CI: 0.45-0.74; $P < 0.001$	Llovet <i>et al.</i> ^[10] (Ph 3, SHARP)
Lenvatinib vs. sorafenib	IRF Recist 1.1 7.3 vs. 3.6 HR = 0.65, 95% CI: 0.56-0.77; $P < 0.0001$ IRF mRecist 7.3 vs. 3.6 HR = 0.64, 95% CI: 0.55-0.75; $P < 0.0001$	IRF Recist 1.1 7.4 vs. 3.7 HR = 0.61, 95% CI: 0.51-0.72; $P < 0.0001$ IRF mRecist 7.4 vs. 3.7 HR = 0.60, 95% CI: 0.51-0.71; $P < 0.0001$	Kudo <i>et al.</i> ^[11] (Ph 3, REFLECT)
Regorafenib vs. placebo	Per investigator Recist 1.1 3.4 vs. 1.5 HR = 0.43, 95%CI: 0.35-0.52; $P < 0.0001$	Per investigator Recist 1.1 3.9 vs. 1.5 HR = 0.41, 95%CI: 0.34-0.51; $P < 0.0001$	Bruix <i>et al.</i> ^[12] (Ph 3, RESORCE)
	Per investigator mRecist 3.1 vs. 1.5 HR = 0.46, 95% CI: 0.37-0.56; $P < 0.0001$	Per investigator mRecist 3.2 vs. 1.5 HR = 0.44, 95% CI: 0.36-0.55; $P < 0.0001$	
Cabozantinib vs. placebo	Per investigator Recist 1.1 5.2 vs. 1.9 HR = 0.44, 95%CI: 0.36-0.52; $P < 0.001$	ND	Abou-Alfa <i>et al.</i> ^[13] (Ph 3, CELESTIAL)
Ramucirumab vs. placebo	Per investigator Recist 1.1 2.8 vs. 1.5 HR = 0.57, 95%CI: 0.47-0.69; $P < 0.0001$	ND	Zhu <i>et al.</i> ^[14] (Ph 3, REACH-2)
Nivolumab	Per investigator Recist 1.1 4.0 (2.9-5.4)	ND	El-Khoueiry <i>et al.</i> ^[19] (Ph 1/2, CheckMate-040, dose-expansion phase)
Nivolumab vs. sorafenib	Per investigator Recist 1.1 3.7 vs. 3.8 HR = 0.93, 95%CI: 0.79-1.10	ND	Yau <i>et al.</i> ^[20] (Ph 3, CheckMate-459)
Pembrolizumab	IRF mRecist 4.9 (3.4-7.2)	IRF mRecist 4.9 (3.9-8.0)	Zhu <i>et al.</i> ^[17] (Ph 2, KEYNOTE-224)
Pembrolizumab vs. placebo	IRF Recist 1.1 3.0 vs. 2.8 HR = 0.72, 95%CI: 0.57-0.90; $P = 0.0022$	IRF Recist 1.1 3.8 vs. 2.8 HR = 0.69, 95%CI: 0.54-0.87; $P = 0.0011$	Finn <i>et al.</i> ^[18] (Ph 3, KEYNOTE-240)
Atezolizumab + bevacizumab vs. sorafenib	IRF Recist 1.1 6.8 vs. 4.3 HR = 0.59, 95%CI: 0.47-0.76; $P < 0.0001$	ND	Finn <i>et al.</i> ^[16] (Ph 3, IMbrave150)
Nivolumab + ipilimumab	ND	ND	Yau <i>et al.</i> ^[8] (Ph 1/2 CheckMate-040, Arm A)

PFS: progression-free survival; TTP: time to radiologic progression; IRF: independent review facility; ND: not determined

intervals between measurements are too long. Symmetric assessment should be ensured between treatment arms. TTP can be recommended as the main time-to-event endpoint to capture possible antitumor benefits in phase 2 trials testing systemic therapies in HCC because it is less vulnerable (only progression is captured) than composite endpoints. However, TTP has been measured less commonly than PFS in HCC phase 3 studies.

In the present review, PFS has been assessed in 7 out of 8 phase 3 studies, and TTP in only 4 of them [Table 2]. When both were available, a close correlation existed between PFS and TTP, thus suggesting that the drugs tested in those trials were not toxic enough to engender death independently of tumor radiologic progression. Taking into account PFS only, atezolizumab/bevacizumab combination clearly gave the best PFS (6.8 months)^[16] as well as lenvatinib (7.3 months)^[11], although comparison of PFS between trials should

be done with considerable caution. However, the long duration of tumor response under atezolizumab/bevacizumab combination as discussed above in “ORR” paragraph, was clearly of huge importance to impact on the long median OS (not reached)^[16], whereas the quite similar PFS under lenvatinib was associated with a much lower OS (13.6 months)^[11]. Unfortunately, the duration of response under lenvatinib has not been assessed, although it is likely shorter than under ICIs and similar to those of other TKIs [Table 1], for instance 3.5 months with regorafenib^[12]. This difference in OS cannot be explained by the disease control rate (DCR by Recist 1.1) since very similar in both trials (74% for atezolizumab/bevacizumab^[16] vs. 72.8% for lenvatinib^[11]) [Table 1].

PFS by Recist 1.1 for monotherapies of ICI gave values around 4 months (3.0-4.9)^[17-20], quite similar to those of TKIs such as sorafenib (3.6 months)^[11] and regorafenib (3.4 months)^[12]. PFS seems to be better with cabozantinib (5.2 months)^[13], but worse with ramucirumab (2.8 months)^[14], which supported a poor prognosis subpopulation. When available, TTP was in accordance with PFS. It is important to underline that ICI monotherapy responders have a long duration of response [Table 1], but the number of responders was too low in the trials to have a significant impact on median PFS or median TTP, and finally on median OS. Differences on PFS/TTP/OS cannot be explained by different DCRs [Table 1] since they were quite the same between ICI and TKI schedules: atezolizumab/bevacizumab (74%)^[16], nivolumab/ipilimumab (50%)^[8], nivolumab (55%-64%)^[19,20], pembrolizumab (61%-62.2%)^[17,18], lenvatinib (72.8%)^[11], sorafenib (59-71%)^[10,11], regorafenib (66%)^[12], and cabozantinib (64%)^[13].

Overall survival

Overall survival (OS), defined as the time from randomization to death, is a direct measure of clinical benefit to a patient and the gold standard primary endpoint to evaluate the outcome in oncologic clinical trials. OS is easily measured, unambiguous, objective, not subjected to researcher bias and it is used by the international authorities worldwide for cancer drug approval. OS is the primary endpoint recommended for all phase 3 studies in HCC. When selecting endpoints in HCC clinical trials, it must be also considered that OS is impacted by liver failure due to both the end stage natural history of underlying chronic liver disease and the HCC loco-regional spread, which in turn promotes liver failure and leads to death. Thus, if the treatment aims to reduce HCC-related death (i.e., the endpoint is cancer-related death), but the competing mortality from progressive liver failure is high in both the active treatment and in the control arms, the risk ratio will be reduced and the required sample size increases. Thus, phase 3 studies in HCC require a larger sample size to include competing risk analysis and assess cancer-related deaths as compared to OS evaluation.

In the present review, regarding OS [Table 3] and taking into account potential confounding factors that may influence the median OS, atezolizumab/bevacizumab therapy has not reached median OS so far taking into account that the median follow-up is only 17 months^[16], the nivolumab/ipilimumab combination (22 months)^[8], nivolumab (16.4 months)^[20], pembrolizumab (12.9-13.9 months)^[17,18], lenvatinib (13.6 months)^[11], sorafenib (10.7 months)^[10], regorafenib (10.6 months)^[12] and cabozantinib (10.2 months)^[13], while worse with ramucirumab (8.5 months) due to the poor prognosis assessed subpopulation^[14]. The 1L or 2L design of the trials does not impact much the spontaneous OS of patients since in the placebo arms of randomized controlled trials, median OS is quite the same in 1L or 2L for HCC patients eligible for systemic therapies with ECOG PS status 0-1 and Child-Pugh A liver functions. Indeed, OS of placebo arms seems similar in 1L phase 3 trials (7.9 months in SHARP^[10], 8.5 months in SEARCH^[27]) by comparison to 2L/3L phase 3 trials (7.9 months in RESORCE^[12], 8 months in CELESTIAL^[13], 10.6 months in KEYNOTE-240^[18], 8.2 months in BRISK-PS^[21], 7.3 months in EVOLVE-1^[28], 7.6 months in REACH^[29], and 9.1 months in METIV-HCC^[30]).

The control arm and subsequent therapies administered after trial withdrawal are of prominent importance. Indeed, OS in HCC randomized controlled trials depends on the target population, the parameters assessed

Table 3. Median overall survival

	OS in months (95%CI)	Subsequent systemic therapy after trial withdrawal	Authors (Trial)
Sorafenib vs. placebo	10.7 vs. 7.9 HR = 0.69, 95%CI: 0.55-0.87; $P < 0.001$	Absence of active drug against HCC	Llovet <i>et al.</i> ^[10] (SHARP)
Lenvatinib vs. sorafenib	13.6 vs. 12.3 HR = 0.92, 95%CI: 0.79-1.06; meeting criteria for non-inferiority	32.6% (sorafenib 25%; investigational therapy) vs. 38.7% (sorafenib 12%; investigational therapy)	Kudo <i>et al.</i> ^[11] (Ph 3, REFLECT)
Regorafenib vs. placebo	10.6 vs. 7.8 HR = 0.63, 95%CI: 0.50-0.79; $P < 0.0001$	ND	Bruix <i>et al.</i> ^[12] (Ph 3, RESORCE)
Cabozantinib vs. placebo	10.2 vs. 8.0 HR = 0.76, 95%CI: 0.63-0.92; $P = 0.005$	25% [anti-PD1/PD-L1 5%; TKI (sorafenib, lenvatinib, regorafenib) 6.5%; systemic chemotherapy 12%; investigational agents 6%] vs. 30% [anti-PD1/PD-L1 6%; TKI (sorafenib, lenvatinib, regorafenib) 3%; systemic chemotherapy 17%; investigational agents 7%]	Abou-Alfa <i>et al.</i> ^[13] (Ph 3, CELESTIAL)
Ramucirumab vs. placebo	8.1 vs. 5.0 HR = 0.69, 95%CI: 0.57-0.84; $P = 0.0002$	26.9% [immunotherapy 6.6%; TKI (regorafenib, sorafenib, cabozantinib, BLU-554, lenvatinib) 13.7%; systemic chemotherapy 11.2%; investigational drug 2.5%; other 0.5%] vs. 28.4% [immunotherapy 6.3%; TKI (regorafenib, sorafenib, cabozantinib, BLU-554, lenvatinib) 6.3%; systemic chemotherapy 15.8%; investigational drug 2.1%; other 1.1%]	Zhu <i>et al.</i> ^[14] (Ph 3, REACH-2)
Nivolumab	NR	ND	El-Khoueiry <i>et al.</i> ^[19] (Ph 1/2, CheckMate-040, dose-expansion phase)
Nivolumab vs. sorafenib	16.4 vs. 14.7 HR = 0.85, 95%CI: 0.72-1.02; $P = 0.0752$	49% (ICI 2%; TKI 36%; systemic chemotherapy 4%; investigational agent 3%; other 1%) vs. 53% (ICI 20%; TKI 23%; systemic chemotherapy 7%; investigational agent 11%; other 1%)	Yau <i>et al.</i> ^[20] (Ph 3, CheckMate-459)
Pembrolizumab	12.9 (95%CI: 9.7-15.5)	ND	Zhu <i>et al.</i> ^[17] (Ph 2, KEYNOTE-224)
Pembrolizumab vs. placebo	13.9 vs. 10.6 HR = 0.78, 95%CI: 0.61-0.99; $P = 0.0238$	41.7% [approved anticancer medication 31.7%; ICI 6.8%, others (lenvatinib, regorafenib, ramucirumab) 31.7%] vs. 47.4% [approved anticancer medication 31.9%, ICI 10.4%, others (lenvatinib, regorafenib, ramucirumab) 31.9%]	Finn <i>et al.</i> ^[18] (Ph 3, KEYNOTE-240)
Atezolizumab + bevacizumab vs. sorafenib	NR vs. 13.2 HR = 0.58, 95%CI: 0.42-0.79; $P = 0.0006$	ND	Finn <i>et al.</i> ^[16] (Ph 3, IMbrave150)
Nivolumab + ipilimumab	22.8 (95%CI: 9.4-NE)	ND	Yau <i>et al.</i> ^[8] (Ph 1/2 CheckMate-040, Arm A)

OS: overall survival; NR: not reached; NE: not evaluable; ND: not determined; TKI: tyrosine kinase inhibitor; ICI: immune checkpoint inhibitor

and reported in the trial, the stratification before randomization in both the active and the control arms. For most HCC trials, the study population is composed of approximately 80% BCLC-C and 20% BCLC-B HCCs, with a good general status (PS ECOG 0-1) and conserved liver functions (Child-Pugh A). A critical element that can substantially affect the interpretation of trial results is whether patients are allowed to receive medications or undergo procedures potentially active against HCC after trial withdrawal.

As far as control arms are considered, the SHARP trial^[10] still represents a paradigm since patients were treated in both arms up to symptomatic progression, and patients could not be treated by other active drugs after radiologic progression since such drugs were not existing [Table 3]. Thus, in SHARP, OS of the control arm (composed of placebo only or subsequent inactive drugs against HCC) was 7.9 months, and sorafenib

increased OS *vs.* placebo with a hazard ratio (HR) of 0.69 (95%CI: 0.55-0.87, $P < 0.0001$)^[10]. REFLECT was a head-to-head comparison of lenvatinib and sorafenib, within a non-inferiority trial^[11], but after trial withdrawal, 33% and 39% of patients received potentially active medications against HCC, likely one of the reasons why the OS of sorafenib improved from SHARP (10.7 months) conducted a couple of years before 2008^[10], towards REFLECT (12.3 months) conducted 10 years later^[11] [Table 3]. In the other 1L systemic therapies with control arm, the same comments can arise from CheckMate-459 comparing nivolumab to sorafenib^[20]. It was an open label trial, and at radiologic progression, patients were withdrawn and received potentially active subsequent medications [Table 3]. That explains, at least in part, the high OS value in the sorafenib arm (14.7 months), that maybe led to conceal the benefit of nivolumab (OS 16.4 months) *vs.* sorafenib (HR 0.85 [95%CI: 0.72-1.02]; $P = 0.0752$)^[20]. The same conclusions can be drawn from the IMBrave150 trial^[16] [Table 3] where OS under sorafenib was surprisingly high at 13.2 months, and the atezolizumab/bevacizumab combination increased OS (not reached) *vs.* sorafenib (13.2 months) with HR of 0.58 (95%CI 0.42-0.79, $P < 0.0006$)^[16], the efficacy of the atezolizumab/bevacizumab combination being high enough to prevent any concealing by the overestimated value of OS in the sorafenib arm.

However, operator experience acquired over time is also likely a relevant factor that has a greater impact on OS in the sorafenib arms

In 2L setting, all control arms were placebo arms, also debatable due to post-withdrawal medications [Table 3]. In spite of the overestimated values of OS in placebo arms in 2L, regorafenib increased OS with HR of 0.63 (95%CI: 0.50-0.79, $P < 0.0001$)^[12], cabozantinib improved OS with HR of 0.76 (95% CI 0.63-0.92, $P < 0.005$)^[13], and ramucirumab improved OS with HR of 0.71 (95%CI: 0.53-0.95, $P = 0.0199$)^[14]. In the KEYNOTE-240 phase 3 study, the trial did not meet the statistical criteria for either of the dual endpoints (OS and PFS) although pembrolizumab improved OS over placebo with HR of 0.78 (95% CI 0.61-0.99, $P = 0.0238$)^[18], but the placebo arm showed abnormally high OS value of 10.6 months, in part due to post-withdrawal trial medication [Table 3].

CONCLUSION

For more than a decade, huge improvements have arisen in the systemic strategy of HCC therapy. The coming 1L will associate atezolizumab and bevacizumab. Of course, a lot a work remains to be done to improve this combination and find some strategies overwhelming primary or secondary resistances. Results are soon expected from other 1L combinations in phase 3: pembrolizumab/lenvatinib (NCT03713593), atezolizumab/cabozantinib (NCT03755791), durvalumab/tremelimumab (NCT03298451), and nivolumab/ipilimumab (NCT 04039607). At the moment, there is also an urgent need for prospective controlled trials to identify the best TKI therapy following progression under any ICI combination schedule. Sorafenib and lenvatinib were the two possible 1L. Will they remain the gold standard after ICI combination schedule failure? If yes, the subsequent TKIs after their own failure would likely remain regorafenib, cabozantinib or ramucirumab, if not used in the prior ICI combination schedules of 1L. Only randomized controlled trials will guide the future ways of research and draw the future therapeutic algorithms to improve more and more the treatment of HCC.

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Authors' contributions

Made substantial contribution to conception and design of the review article, and performed data analysis and interpretation: Merle P, Subic M

Availability of data and materials

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Conflicts of interest

Merle P has to declare AbBoard with Bayer, Ipsen, Exelixis, Eisai, Lilly, Roche, Bristol Myers Squibb, AstraZeneca, and Onxeo. Subic M declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
2. Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. *Gastroenterology* 2016;150:835-53.
3. Merle P, Blanc J, Phelip J, Pelletier G, Bronowicki J, et al. Doxorubicin-loaded nanoparticles for patients with advanced hepatocellular carcinoma after sorafenib treatment failure (RELIVE): a phase 3 randomised controlled trial. *Lancet Gastroenterol Hepatol* 2019;4:454-65.
4. Whitehead J. Stopping clinical trials by design. *Nat Rev Drug Discov* 2004;3:973-7.
5. Vogel A, Cervantes A, Chau I, Daniele B, Llovet J, et al. Hepatocellular carcinoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2018;29:iv238-55.
6. Llovet JM, Real MI, Montaña X, Planas R, Coll S, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002;359:1734-9.
7. El-Khoueiry AB, Melero I, Yau T, Crocenzi TS, Kudo M, et al. Impact of antitumor activity on survival outcomes, and nonconventional benefit, with nivolumab in patients with advanced hepatocellular carcinoma: subanalysis of CheckMate-040. *J Clin Oncol* 2018;36:475.
8. Yau T, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, et al. Nivolumab + ipilimumab combination therapy in patients with advanced hepatocellular carcinoma: results from CheckMate-040. *J Clin Oncol* 2019;37:4012.
9. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL Conference. *J Hepatol* 2001;35:421-30.
10. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-90.
11. Kudo M, Finn RS, Qin S, Han K, Ikeda K, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018;391:1163-73.
12. Bruix J, Qin S, Merle P, Granito A, Huang Y, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;389:56-66.
13. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N Engl J Med* 2018;379:54-63.
14. Zhu AX, Kang Y, Yen C, Finn RS, Galle PR, et al. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α -fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2019;20:282-96.
15. Tovoli F, Renzulli M, Negrini G, Brocchi S, Ferrarini A, et al. Inter-operator variability and source of errors in tumour response assessment for hepatocellular carcinoma treated with sorafenib. *Eur Radiol* 2018;28:3611-20.
16. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, et al; IMbrave150 Investigators. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med* 2020;382:1894-905.
17. Zhu AX, Finn RS, Edeline J, Cattani S, Ogasawara S, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018;19:940-52.
18. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, et al. Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: a randomized, double-blind, phase III trial. *J Clin Oncol* 2020;38:193-202.
19. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
20. Yau T, Park JW, Finn RS, Cheng AL, Mathurin P, et al. CheckMate 459: a randomized, multi-center phase III study of nivolumab vs

- sorafenib as first-line treatment in patients with advanced hepatocellular carcinoma. Barcelona (Spain): European-Society-for-Medical-Oncology (ESMO) Congress; 2019.
21. Llovet JM, Decaens T, Raoul JL, Boucher E, Kudo M, et al. Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase III BRISK-PS study. *J Clin Oncol* 2013;31:3509-16.
 22. Lencioni R, Montal R, Torres F, Park JW, Decaens T, et al. Objective response by mRECIST as a predictor and potential surrogate endpoint of overall survival in advanced HCC. *J Hepatol* 2017;66:1166-72.
 23. Kudo M, Finn RS, Qin SK, Han KH, Ikeda K, et al. Analysis of survival and objective response in patients with hepatocellular carcinoma in a phase III study of lenvatinib (REFLECT). San Francisco: Gastrointestinal Cancers Symposium (ASCO GI) Congress; 2019.
 24. Bruix J, Huang LP, Nakajima K, Han GH, Meinhardt G, et al. Time to progression and response rate are not reliable surrogate endpoints for overall survival in hepatocellular carcinoma: an analysis from the phase 3 RESORCE trial. San Francisco: American Association for the Study of Liver Diseases (AASLD) Congress; 2018.
 25. Llovet JM, Montal R, Villanueva A. Randomized trials and endpoints in advanced HCC: role of PFS as a surrogate of survival. *J Hepatol* 2019;70:1262-77.
 26. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981;47:207-14.
 27. Zhu AX, Rosmorduc O, Evans TR, Ross PJ, Santoro A, et al. SEARCH: a phase III, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2015;33:559-66.
 28. Zhu AX, Kudo M, Assenat E, Cattani S, Kang YK, et al. Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial. *JAMA* 2014;312:57-67.
 29. Zhu AX, Baron AD, Malfertheiner P, Kudo M, Kawazoe S, et al. Ramucirumab as second-line treatment in patients with advanced hepatocellular carcinoma: analysis of REACH trial results by child-pugh score. *JAMA Oncol* 2017;3:235-43.
 30. Rimassa L, Assenat E, Peck-radosavljevic M, Pracht M, Zagonel V, et al. Tivantinib for second-line treatment of MET-high, advanced hepatocellular carcinoma (METIV-HCC): a final analysis of a phase 3, randomised, placebo-controlled study. *Lancet Oncol* 2018;19:682-93.

Review

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Detection of circulating tumor cells in hepatocellular carcinoma: applications in diagnosis, prognosis prediction and personalized treatment

Peng-Xiang Wang, Jian-Wen Cheng, Xin-Rong Yang

Department of Liver Surgery and Transplantation, Liver Cancer Institute, Zhongshan Hospital, Fudan University; Key Laboratory of Carcinogenesis and Cancer Invasion, Ministry of Education, Shanghai 200032, P. R. China.

Correspondence to: Dr. Xin-Rong Yang, Department of Liver Surgery and Transplantation, Liver Cancer Institute, Zhongshan Hospital, Fudan University; Key Laboratory of Carcinogenesis and Cancer Invasion, Ministry of Education, 180 Fenglin Road, Shanghai 200032, P. R. China. E-mail: yang.xinrong@zs-hospital.sh.cn

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide and is associated with poor clinical prognosis, which is mainly caused by tumor recurrence and metastasis. Circulating tumor cells (CTCs) are tumor cells shed into the bloodstream and regarded as the “seeds” of tumor recurrent or metastatic lesions. Over the past decade, the clinical value of CTC analysis has been extensively explored. CTC analysis is a representative form of liquid biopsy, offering a novel solution that can bypass the problems of invasive biopsy procedures, enabling comprehensive, non-invasive, and real-time disease monitoring. In HCC, CTC analysis has facilitated early detection and prognosis prediction, as well as treatment monitoring and therapeutic intervention guiding. In this review, we summarize available literature and provide an overview of CTC biology, detection technologies, and clinical applications in the diagnosis, prognosis prediction, and personalized treatment of HCC.

Keywords: Hepatocellular carcinoma, liquid biopsy, circulating tumor cells, biomarkers, personalized medicine



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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most prevalent malignancy worldwide and is currently listed as the fourth leading cause of cancer-related death^[1]. HCC is a highly aggressive neoplasm; distant organ metastasis can occur at a very early stage^[2]. Thus, early detection of HCC is of great importance in the management of HCC. Surgical resection or liver transplantation remains the primary therapy for HCC patients. However, only approximately 20%-30% of patients are eligible for surgical intervention at the time of the first diagnosis, most patients have already reached an advanced cancer stage^[3]. The 5-year survival rate of HCC with Barcelona Clinic Liver Cancer (BCLC) stage 0-A treated with curative therapeutic modalities is 40%-70%, but the median overall survival (OS) for patients with BCLC stage B-C is only 11-20 months^[4]. Even after curative resection, the five-year cumulative recurrence rate is 50%-70%, which results in the unsatisfactory long-term outcomes of HCC patients^[5,6]. Currently, early detection or monitoring HCC recurrence mainly relies on imaging examinations and serum tumor biomarkers such as alpha-fetoprotein (AFP); however, their diagnostic sensitivity is limited and often fails to foresee the tumor metastatic potential^[7,8]. Therefore, there is an unmet need for reliable biomarkers for early HCC detection and tumor recurrence monitoring.

In recent years, various “liquid biopsy” techniques have emerged and shown significant promise as novel biomarkers for HCC. Liquid biopsy represents a modality of collecting bodily fluids instead of solid tissue for pathophysiological or sequencing analysis. Liquid biopsy offers a solution that can bypass the problems of invasive biopsy procedures, enabling repeated and real-time disease status monitoring^[9]. Circulating tumor cells (CTCs) and circulating tumor DNA are two of the most widely studied biomarkers in liquid biopsy^[10]. CTCs are the cells that derive from the primary or metastatic lesions and migrate into circulation and are regarded as the “seeds” of tumor metastasis^[11]. CTCs represent a unique liquid biopsy form that is different from any of the existing cancer biomarkers, as they are a sampling of the patient’s live tumor cells, carrying comprehensive biological information of the primary tumor, including genomic mutations, cancer subtypes, and drug sensitivity^[12]. CTC research has flourished over the past decade, spanning fields including CTC detection, identification of prognostic significance, and evaluation for treatment response and disease surveillance^[13,14].

In the context of HCC, excellent progress has been made using CTCs as blood-based biomarkers^[9,14,15]. Herein, to gain comprehensive insight into the role of CTCs in HCC, this review article provides an overview of their biology, detection technologies, and clinical significance in HCC.

OVERVIEW OF CTC BIOLOGY

To date, the definition of CTC from CellSearchTM system is considered as the current standard: a CTC is an intact nucleated epithelial cell, expressing epithelial cell adhesion molecule (EpCAM) and/or cytokeratin 8, 18, and 19, and negative for the leukocyte-biomarker CD45^[16]. EpCAM⁺ CTCs were also identified as a stem cell-like subpopulation in HCC, with a highly invasive and metastatic capacity^[17].

Thousands of tumor cells are shed into the bloodstream everyday^[18]; however, most CTCs are eliminated in the bloodstream by shear stress, immune attack, and anoikis^[11,19]. Only a small number of viable and highly metastatic subpopulations of CTCs could eventually survive and develop into metastatic lesions^[20-22]. However, the biological mechanism of how CTCs survive the bloodstream and home to distant organs remains largely unknown. CTCs may undergo several adaptations to survive in a hostile environment. A recent study used *in vivo* genome-wide CRISPR to screen and identify a subset of CTCs with the deregulation of ribosomal protein expression and translation. This CTC subset was significantly associated with enhanced metastatic capabilities and poor clinical outcome^[23]. Meanwhile, one of the key biological events is epithelial-to-mesenchymal transition (EMT), during which CTCs downregulate epithelial markers such as E-cadherin and gain mesenchymal markers, thus acquiring increased ability to invade the

adjacent tissues and survive the environmental stress^[24-26]. Recently, Sun *et al.*^[27] compared EMT-features of CTCs isolated from different vascular sites of HCC patients prior to resection, including peripheral vein, peripheral artery, hepatic veins, infrahepatic inferior vena cava, and portal vein. Single-cell transcriptional characterization demonstrated that CTCs were initially epithelial phenotype at release, but they switched to EMT-activated phenotype during hematogenous transit via Smad2- and b-catenin-related signaling pathways. They suggested such heterogeneous EMT status during the CTC transition may be the result of shear stress in circulation. Nevertheless, while the loss of E-cadherin increased invasion, it also reduced cancer cell proliferation and survival of CTCs in multiple models of breast cancer, indicating the complex phenotypic plasticity of CTCs in EMT status^[28,29].

In addition, CTCs may aggregate to form CTC clusters [also referred to as CTC microemboli (CTM)] and travel together in circulation, with or without fibroblasts, leukocytes, endothelial cells, or platelets, which possess significantly higher invasiveness and increased survival ability compared to individual CTCs^[30]. Notably, clusters of circulating tumor cells (CTCs) possessed an up to 50 times greater metastatic potential compared with single CTCs^[31]. CTC clusters have been identified in many cancer types including HCC; these clinical studies confirmed that CTC clusters are a much stronger prognostic factor for cancer metastasis than single CTCs^[27,32-34]. A recent study comprehensively profiled the DNA methylation landscape of single CTCs and CTC clusters from breast cancer patients and mouse models, and the results revealed that binding sites for stemness- and proliferation-associated transcription factors were specifically hypomethylated in CTC clusters, thus promoting the stemness and metastasis of CTC clusters^[35]. Szczerba *et al.*^[36] identified a specific of CTC-neutrophil cluster in circulation and further confirmed this interaction drove cell cycle progression within the bloodstream and expanded the metastatic potential of CTCs. Targeting this interaction may be rational in treating cancer metastasis. Additionally, the formation of CTC clusters can induce a hypoxic environment that drives hypoxia-inducible factor 1- α -mediated mitophagy, clearing damaged mitochondria, and limiting reactive oxygen species. Such a metabolic switch may support the survival and metastatic spread of CTCs in circulation^[37]. Although the specific mechanism driving CTC cluster formation remains unclear, it is currently considered that CTC clusters arise from oligoclonal tumor cell groupings from the primary tumor or intra-vascular aggregation of single CTCs in circulation^[31,38]. A recent study has proposed that CTC cluster formation was a dynamic event rather than grouped migration derived from the primary tumor by their multi-vascular sampling, in which CTM displayed an “aggregated-apart-aggregated” pattern during the circulatory pathway. They hypothesized that single CTCs might aggregate spontaneously in blood vessels against the unfavorable microenvironment in the bloodstream^[27]. Characterization of the mechanism that determines the cluster formation may identify viable therapeutic targets for inhibiting metastasis.

CTC DETECTION TECHNOLOGY

CTCs are extremely rare and surrounded by numerous blood cells in the bloodstream, thus CTCs are generally required to be firstly enriched from blood samples^[39]. Reliable CTC enrichment technologies are essential to the downstream comprehensive CTC analysis. Many different technologies have been developed for CTC detection in the past years, and they can be classified into two categories based on whether they make use of the physical or biological properties of the target cells [Table 1]^[40]. However, most of these technologies for CTC capture and downstream analyses are designed for scientific research, requiring multiple batch-process steps and having relatively limited throughput. The price of CTC analysis is still on the high side (it generally costs hundreds of dollars per patient). A standardized and easy-to-use platform that facilitates integrated CTC analysis is still needed.

Immunoaffinity-based method

The immunoaffinity-based method for CTC detection relies on the tumor-specific antibodies against cell surface markers, including EpCAM, human epidermal growth factor receptor (EGFR)2, prostate-specific

Table 1. Overview of CTC detection technology

Platform	Enrichment method	Phenotypic Markers	Study cohort	Blood volume	CTC positive rate	Ref.
Immunoaffinity-based method						
CellSearch platform	Immunomagnetic	EpCAM, CK8/18/19	964 metastatic carcinomas, 199 NLD, and 145 HD	7.5 mL	36.0% (≥ 2 CTCs)	[16]
Flow cytometric analysis	Immunomagnetic	CD90	34 HCC, 19 LC, and 19 HD	10 mL	91.2%	[44]
Flow cytometric analysis	Flow cytometry	ICAM-1	60 HCC	NA	50.0%	[46]
ASGPR sorting	Immunomagnetic	ASGPR, Hep Par 1	85 HCC, 37 NLD, 20 HD, and 14 patients with other advanced cancers	5 mL	81.0%	[67]
ASGPR sorting	Density gradient centrifugation and Immunomagnetic	ASGPR, pan-cytokeratin, CPS1	27 HCC patients	5 mL	89.0%	[68]
Glypican-3 sorting	Density gradient centrifugation and Immunomagnetic	Glypican-3	85 HCC patients	8 mL	38.8% (≥ 5 CTCs)	[69]
NanoVelcro CTC assay	Microfluidic device	ASGPR, glypican-3, EpCAM, vimentin	61 HCC, 11 NLD, and 8 HD	4 mL	97.0%	[70]
Biophysical properties-based method						
qRT-PCR-based platform	Density gradient centrifugation and Ficoll-Paque	AFP (mRNA)	44 HCC, and 7 HD	5 mL	72.7%	[39]
qRT-PCR-based platform	Density gradient centrifugation and Ficoll-Paque	Cytokeratin 20 (mRNA)	65 patients with colorectal cancer	10 mL	41.4%	[50]
qRT-PCR-based platform	Density gradient centrifugation and OncoQuick	Cytokeratin 20 (mRNA)	37 patients with gastrointestinal tumors	10 mL	30.0%	[51]
iset platform	Microfiltration	AFP (mRNA)	37 HCC	15 mL	42.9%	[54]
iset platform	Microfiltration	AFP	44 patients with primary liver cancer, 30 patients with chronic active hepatitis, 39 LC, and 38 HD	6 mL	52.3%	[55]
ScreenCell platform	Microfiltration	Cytokeratins	NA	NA	NA	[56]
CTC-Chip	Microfluidic device	EpCAM	116 patients with metastatic cancer	1 mL	99.0%	[58]
HB-Chip	Microfluidic device	EpCAM	15 patients with metastatic cancer	1 mL	93.0%	[59]
CTC-iChip	Microfluidic device	Cytokeratins	NA	8 mL	97.0%	[60]
Cluster-Chip	Microfluidic device	Cytokeratins, Ki67	27 patients with breast cancer, 20 patients with melanoma, and 13 patients with prostate cancer	1 mL	30%-40%	[61]
qRT-PCR-based platform	Density gradient centrifugation and RosetteSep Human CD45 Depletion Cocktail	EpCAM (mRNA)	299 HCC, and 120 HD	5 mL	41.2%	[71]
digital PCR-based platform	Microfluidic device and immunomagnetic	10 liver-specific transcripts (mRNA)	16 HCC, and 31 NLD	4 mL	56.0%	[72]
CanPatrol platform	Microfiltration	EpCAM, CK8/18/19, vimentin, twist (mRNA)	33 HCC, and 10 HD	5 mL	NA	[73]
Labyrinth microfluidic device	Microfluidic device	Glypican-3, Glutamine Synthetase, HepPar-1, CD44	42 HCC	10 mL	88.1%	[74]

CTC: circulating tumor cells; EpCAM: epithelial cell adhesion molecule; CK: cytokeratin; NLD: non-malignant liver disease; HD: healthy donor; HCC: hepatocellular carcinoma; LC: liver cirrhosis; ASGPR: asialoglycoprotein receptor; CPS1: carbamoyl phosphate synthetase 1; qRT-PCR: quantitative reverse transcription polymerase chain reaction; AFP: alpha-fetoprotein

antigen, and so on^[41]. EpCAM is the most frequently used antigen in CTC recognition, as the only FDA-approved semi-automated CTC detection device, CellSearchTM system, is based on the expression of surface EpCAM. CellSearchTM system utilizes anti-EpCAM-coated magnetic beads for CTC sorting in 7.5 mL of blood, and the extracted CTCs are then fixed, stained by antibodies against EpCAM and cytokeratin, and counted. EpCAM and cytokeratin also have been regarded as a clinical standard in CTC labeling among other markers^[16,42]. CellSearchTM system can retain morphological and immunological characters of isolated cells, thus allowing the following fluorescence-based assays. However, CellSearchTM system is incompatible with direct downstream single-cell molecular analysis since these cells have been fixed, which limits their clinical utility in CTC-based comprehensive analysis^[43]. Fluorescence-activated cell sorting is another widely used CTC detecting method that combines conventional flow cytometry technique and immunoaffinity sorting, while this strategy is limited by the makers' selection^[44-46].

Biophysical properties-based method

The biophysical property-based enrichment utilizes various physical properties including density, size, shape, inertia, and electrical property of CTCs to distinguish them from other blood cells^[47]. These so-called “label-free” methods are gaining increasing attention, as they avoid cell loss when choosing specific antigens targeting CTCs. In addition, unlabeled CTCs are generally compatible with a variety of downstream analyses^[48].

Among these strategies, density-based gradient centrifugation is the most commonly used method, which utilizes the differences in specific densities CTCs to separate the target tumor cells and blood cells^[49]; this is also a common pre-processing step integrated with many methods for CTC detection. Ficoll-PaqueTM is a cell separation medium used for the isolation of CTCs in patients with various types of cancer, including liver cancer and colorectal cancer^[39,50]. OncoquickTM has made improvements in the centrifuge tubes that combines filtration and centrifugation and has superior CTC recovery rate and less blood cell contamination^[51]. RosetteSepTM CTC enrichment cocktail is as an example of label-free CTC enrichment, where a mixture of antibodies is used to target and cross-link unwanted blood cells to form immunorosettes, and then density gradient centrifugation is performed to deplete the unwanted cells^[52,53].

Another common method that utilizes the biophysical properties of CTC is size-based filtration. The diameter of tumor cells is generally larger than blood cells, and CTCs would be retained in the filter, while smaller blood cells pass through. Isolation by size of epithelial tumor cells (ISET) was developed as a microfilter to isolate tumor cells by a polycarbonate membrane with calibrated pores^[54]. ISETTM system can visualize and count CTCs and CTC clusters in blood samples obtained from HCC patients^[55]. More recently, ScreenCellTM device was developed as an advanced microfilter to isolate viable CTCs with a high recovery rate. Immunocytochemistry assays for CTCs can be performed directly on the filter^[56]. To conclude, the filtration method is one of the simplest and most widely studied methods for capturing CTCs. However, this method is limited by its low specificity that the products may be contaminated by other cells owing to natural variation in size of leukocytes, and small CTCs may be lost during the filtration^[34].

Alternatively, microfluidic techniques are now increasingly being exploited in CTC isolation, which allows for precise control of fluids in a small volume and rapid sample processing at relatively low cost and high sensitivity^[57]. Microfluidic platforms enable on-chip CTC isolation, identification, and even culturing. The first microfluidic device named “CTC-chip” was developed to capture rare CTCs in 2007, which successfully identified CTCs in the peripheral blood of patients with metastatic lung, prostate, pancreatic, breast, and colon cancer^[58]. In 2010, an improved herringbone-chip was developed. The herringbone-shaped grooves of this chip can generate a microvortex when blood is pumped, which enhances the contact between the chip surface and tumor cell. Its clinical utility was demonstrated in specimens from patients with prostate cancer^[59]. Another novel CTC-iChip platform utilizes the distinct differences between cancer

cells and blood cells in size and deformability, reaching a 97% yield of rare tumor cells^[60]. The Cluster-Chip is a unique 3D microfiltration system, designed specifically to capture CTC clusters^[61]. One obvious advantage of microfluidic chip is that it can isolate CTCs from whole blood in a high-throughput fashion without complicated initial preparation step, thus decreasing the possibility of destruction and loss of CTCs^[62].

Technology developed for detecting CTC in HCC

CTCs in patients with HCC are a highly heterogeneous population; currently, there are no widely accepted antigens specifically targeting HCC CTCs^[27,63]. Although CellSearchTM system is commonly used in CTC detection, it is reported that the EpCAM-based CTC-sorting strategy could only identify approximately 10%-35% of the total amount of tumor cells in blood due to the EMT process and heterogeneous CTC molecular phenotypes^[64,65]. Thus, a clinically relevant subset of CTCs may be missed by singular epithelial markers-based sorting strategies^[29,66]. Some research groups have broadened target epitopes to include alternative candidates specific to hepatocytes. For example, Xu *et al.*^[67] developed a novel CTC enumeration system for HCC by taking advantage of a “biotin and anti-biotin antibody” combination, in which circulating HCC cells were bound by biotinylated asialofetuin, an asialoglycoprotein receptor (ASGPR) ligand and subsequently magnetically labeled by anti-biotin antibody-coated magnetic beads, followed by magnetic separation. This system was able to detect CTCs in 69 out of 85 (81%) HCC patients. The same group used an anti-ASGPR antibody instead of ASGPR ligand with successful CTC detection in 89% of HCC patients^[68]. Glypican-3 (GPC3) is an oncofetal heparan sulfate proteoglycan that is currently used as a pathologic biomarker for HCC diagnosis. It can also be used for the detection of HCC-specific CTCs^[69]. Recently, the novel NanoVelcro CTC assay uses an antibody cocktail targeting the cell-surface markers ASGPR, GPC3, and EpCAM to detect CTCs. It has been showed that multi-marker capture detected greater numbers of CTCs than any individual antibody alone in both cell line and HCC patient samples^[70].

“Label-free” strategies have also exhibited great utilities in HCC CTC enrichment. RosetteSepTM and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) were combined for the optimized CTC detection in HCC patients, yielding a 41.2% positive rate of EpCAM^{mRNA+} CTCs^[71]. Kalinich *et al.*^[72] integrated a microfluidic chip device and RNA-based digital PCR to detect molecular signatures derived from HCC CTCs. Using the identified 10 liver-specific transcripts, 9 out of 16 (56%) untreated HCC cases had detectable CTCs. The CanPatrolTM CTC analysis platform used a two-step technique including microfiltration and subsequent RNA *in situ* hybridization (ISH) assay, to characterize epithelial (EpCAM and cytokeratin 8/9/19) and mesenchymal (Vimentin and Twist) markers of CTCs from patients with HCC^[73]. Wan *et al.*^[74] used a labyrinth microfluidic device to detect CTCs in patients with HCC. They showed that 71.4% of the HCC patients had CTCs positive for cancer stem cell marker CD44, while 55% of the patients had the presence of CTM, which was correlated with advanced HCC stage.

EARLY DETECTION AND PREDICTION OF TUMOR PROGRESSION IN HCC

Research focusing on clinical implications of CTCs in HCC patients has flourished over the past decade. CTCs have shown great potential for the early diagnosis and prognostication of HCC patients^[14]. The clinical applications of CTC detection in patients with HCC are summarized in Table 2.

Currently, EpCAM⁺ CTCs have been extensively investigated in HCC, as immunoaffinity-based CTC enrichment techniques such as CellSearchTM have been widely used to capture EpCAM⁺ CTCs. In 2013, Sun *et al.*^[17] used CellSearchTM to detect EpCAM⁺ CTCs in HCC patients undergoing tumor resection. They found that 66.7% of HCC patients had detectable EpCAM⁺ CTCs preoperatively; moreover, EpCAM⁺ CTCs ≥ 2 per 7.5 mL of blood was the strongest predictor of HCC recurrence. The prognostic value of CTCs was retained in patient subgroups with minor recurrence risk by traditional evaluation. They also found that CTC numbers were significantly correlated to the systemic immune-inflammation index

Table 2. Applications of CTC detection in HCC

Year	Techniques/ Platform	Model	Objective	Main findings	Quality of the evidence (GRADE)	Ref.
2011	Flow cytometric analysis	82 HCC patients	Prognostic significance of stem cell-like CTCs in HCC undergoing liver resection	Circulating cancer stem cells > 0.01% predicted post-hepatectomy HCC recurrence with high accuracy	●●○○ Low	[86]
2012	<i>In vivo</i> flow cytometry	HCC orthotopic metastatic tumor model (mouse)	Monitor CTC dynamics <i>in vivo</i>	The number of CTCs and early metastases rates decreased significantly after the resection of primary tumor	●○○○ Very Low	[99]
2013	CellSearch	123 HCC patients	Prognostic significance of CTCs in HCC undergoing liver resection	A preoperative EpCAM CTCs $\geq 2/7.5$ mL of blood was an independent prognostic factor for tumor recurrence; such prognostic value retained in patient subgroups; a significant decrease of CTC-positive rates and numbers was observed 1 month after resection	●●●○ Moderate	[17]
2013	CellSearch	59 HCC patients	Prognostic significance of CTCs in HCC undergoing liver resection	Presence of EpCAM-positive CTC was associated with intermediate or advanced HCC stage and worse OS	●●○○ Low	[77]
2013	Flow cytometric analysis	60 HCC patients	Prognostic significance of stem cell-like CTCs in HCC undergoing liver resection	Increased numbers of ICAM-1(+) cells in blood samples of HCC patients correlated with worse clinical outcomes	●●○○ Low	[46]
2013	ASGPR sorting	60 HCC patients	Relationship between the EMT status of CTCs and HCC metastasis and prognosis after surgical resection	Twist and Vimentin expression levels in CTCs could serve as promising biomarkers for evaluating metastasis and prognosis in HCC	●●○○ Low	[87]
2014	qRT-PCR- based platform	299 HCC patients	Diagnostic value of CTC; Clinical significance of CTCs in patients treated with surgical resection, TACE and radiotherapy	Negative enrichment and qRT-PCR- based platform can effectively detect CTCs; the platform might be clinically useful in auxiliary diagnosis, treatment response assessment, and early decision making of antitumor strategies for HCC	●●●○ Moderate	[71]
2015	CellSearch	20 HCC patients	Prognostic significance of CTCs and CTC-based DNA signature	CTC detection was associated with elevated AFP, vascular invasion, and poor prognostic factors; sequencing of CTC DNA identified known HCC mutations	●○○○ Very Low	[78]
2015	<i>In vivo</i> flow cytometry	HCC orthotopic metastatic tumor model (mouse)	Monitor CTC dynamics following sorafenib treatment <i>in vivo</i>	CTCs can be a biomarker in predicting disease progression and monitoring therapeutic efficacy in HCC	●○○○ Very Low	[107]
2016	qRT-PCR- based platform	49 HCC patients	Prognostic significance of CTCs and T regulatory cell in the prediction of postoperative recurrence	Combination of EpCAM CTC and T regulatory/CD4(+) cell may be a novel prognostic predictor for HCC patients	●○○○ Very Low	[83]
2016	CanPatrol platform	33 HCC patients	Diagnostic value of CTCs of EMT phenotypes in HCC; relationship between the EMT process of CTCs and HCC	Epithelial-mesenchymal-mixed CTCs might be a vital factor for intrahepatic metastasis, and mesenchymal CTCs predicated extrahepatic metastasis in HCC	●○○○ Very Low	[73]
2016	qRT-PCR- based platform	72 HCC patients	Dynamic monitoring of CTC counts after surgical resection	Increased AFP mRNA(+) CTCs can be a predictor for HCC metastasis before and after hepatectomy	●●○○ Low	[101]
2016	Density gradient centrifugation	59 HCC patients	Determining pERK and pAkt expressions in CTCs isolated from HCC patients	pERK+/pAkt- CTCs were the most sensitive to sorafenib and an independent predictive factor of PFS in HCC patients treated with sorafenib	●●○○ Low	[109]
2016	Microfluidic device and ASGPR sorting	36 HCC patients	Isolation of viable CTCs of HCC for their culture and drug sensitivity assays	The device can accurately enumerate CTCs and release viable CTCs for <i>in</i> <i>vitro</i> culture and further functional assays	●○○○ Very Low	[110]
2017	CellSearch	61 HCC patients	Prognostic significance of CTCs in HCC undergoing liver resection	Detection of CTC prior to curative- intended liver resection disclosed an elevated risk of HCC recurrence	●●○○ Low	[80]
2017	CanPatrol platform	195 HCC patients	EMT phenotypes of CTCs in the early diagnosis of HCC metastasis and progression after surgical resection	CTCs count and EMT classification are correlated with clinical stages and metastasis of HCC	●●○○ Low	[88]

2018	CellSearch	73 HCC patients	Spatial heterogeneity of phenotypic and molecular characteristics of CTCs	Multi-vascular measurement of CTCs facilitates precise prediction of postoperative relapse or metastasis pattern; profound spatial heterogeneity in cellular distribution and biological features of CTCs during circulation	●●●○ Moderate	[27]
2018	CellSearch	139 HCC patients	Prognostic significance of CTCs and effect of liver resection on CTCs	Both CTC detection incidence and mean CTC counts increased postoperatively; increased postoperative CTC numbers were associated with a worse prognosis	●●○○ Low	[79]
2018	CellSearch	97 HCC patients	Prognostic significance of CTCs in predicting survival outcomes of patients with unresectable HCC treated with TACE	High EpCAM-positive CTC count predicts poor survival of patients with unresectable HCC treated with chemoembolization	●●○○ Low	[81]
2018	qRT-PCR-based platform	445 HCC patients	Clinical value of CTCs with stem-like phenotypes for diagnosis, prognosis, and surveillance in HBV-related HCC	CTC panel may be a useful tool in HCC diagnosis, risk prediction, and treatment response monitoring	●●●○ Moderate	[63]
2018	CanPatrol platform	165 HCC patients	Prognostic significance of EMT phenotypes of CTCs after surgical resection	Presence of mesenchymal CTCs predicted the shortest relapse-free survival	●●●○ Moderate	[89]
2018	CanPatrol platform	80 HCC patients	Relationship between expression of Twist in CTCs and HCC clinical parameters	Twist ⁺ CTCs were closely correlated with the rate of metastasis or recurrence and the mortality rate in HCC	●●○○ Low	[90]
2018	CanPatrol platform	42 HCC patients	Prognostic significance of the change of CTC numbers in tumor recurrence and metastasis after surgical resection	Unfavorable changes after surgery in CTC counts may be independent prognostic indicators for PFS in patients with HBV-related HCC	●●○○ Low	[91]
2018	CanPatrol platform	62 HCC patients	Relationship between postoperative circulating tumor cells subtypes and HCC recurrence	HCC patients with positive postoperative peripheral mesenchymal CTCs had a higher risk of early recurrence	●○○○ Very Low	[93]
2018	CanPatrol platform	112 HCC patients	Prognostic significance of EMT phenotypes of CTCs after surgical resection	CTCs were highly correlated with HCC characteristics, representing a novel marker for early diagnosis and early recurrence prediction	●●●○ Moderate	[94]
2018	CanPatrol platform	47 HCC patients	Prognostic significance of EMT phenotypes of CTCs in HCC patients treated with liver transplantation	CTC levels and subtypes were not predictive of HCC recurrence following liver transplantation	●○○○ Very Low	[96]
2018	iFISH platform	30 HCC patients	Prognostic significance of CTCs in HCC patients treated with liver transplantation	iFISH-CTC ≥ 5 may be a good prognostic indicator for patients with HCC undergoing liver transplantation	●○○○ Very Low	[97]
2018	Flow cytometric analysis	43 patients with liver malignant tumor	Effect of percutaneous radiofrequency ablation on CTCs	Liver tumor ablation might increase the level of mesenchymal phenotype CTCs	●○○○ Very Low	[103]
2018	Flow cytometry	HCC orthotopic metastatic tumor model (mouse)	Monitor CTC dynamics following transcatheter arterial embolization	EGFR inhibitor application may reduce circulating cancer cells during transcatheter arterial embolization and improve the therapeutic outcomes for advanced HCC	●○○○ Very Low	[108]
2019	CanPatrol platform	113 HCC patients	Diagnostic value of different EMT phenotypes of CTC in HCC	Total CTCs were more effective than AFP in the diagnosis of HCC; combined use of total CTCs and AFP can enhance the sensitivity of HCC diagnosis	●●○○ Low	[92]
2019	CanPatrol platform	256 HCC patients	Prognostic significance of EMT phenotypes of CTCs after surgical resection	CTC count and EMT classification were not correlated with clinical stages or predictive of HCC recurrence	●○○○ Very Low	[95]
2019	Tapered slit filter platform	105 HCC patients	Monitor CTC before and after surgery and its association with clinical outcomes in early-stage HCC	Count of Δ CTC is predictive of recurrence in patients with early HCC undergoing surgery	●●○○ Low	[100]

2019	FISH analysis	155 HCC patients	Value of serum dickkopf-1 and CTCs in predicting the efficacy and prognosis of TACE treatment in HCC	CTCs levels can predict the efficacy and prognosis of TACE treatment in patients with HCC	●○○○ Very Low	[102]
2020	CanPatrol platform	136 HCC patients	Relationship between CTC status and outcomes of different surgical methods in HCC	Anatomic resection might improve the survival of HCC patients, but only those with low CTC count and negative mesenchymal and epithelial/mesenchymal-CTC phenotypes	●●○○ Low	[98]
2020	PowerMag negative selection system	30 HCC patients	Longitudinal monitoring the treatment response of patients with locally advanced HCC using CTCs	Sequential CTC enumeration during treatment can benefit the management of patients with locally advanced or metastatic HCC, in particular for the AFP-low cases	●○○○ Very Low	[104]

CTC: circulating tumor cells; HCC: hepatocellular carcinoma; EpCAM: epithelial cell adhesion molecule; OS: overall survival; ICAM-1: intercellular cell adhesion molecule-1; EMT: epithelial-to-mesenchymal transition; ASGPR: asialoglycoprotein receptor; TACE: transcatheter arterial chemoembolization; qRT-PCR: quantitative reverse transcription polymerase chain reaction; AFP: alpha-fetoprotein; pERK: phosphorylated ERK; pAkt: phosphorylated Akt; PFS: progression-free survival; HBV: hepatitis B virus; FISH: fluorescence *in situ* hybridization; EGFR: epidermal growth factor receptor

(SII), a novel index based on peripheral lymphocyte, neutrophil, and platelet counts. HCC patients who experienced unfavorable internal inflammatory alterations after radical hepatic resection suffered an earlier recurrence and distant metastasis. Thus, they proposed that the dissemination and colonization of CTCs may be influenced by host inflammatory and immune response status^[75,76]. In 2018, Sun *et al.*^[27] showed that CTC and circulating tumor cluster burden in hepatic veins and peripheral circulation prognosticated postoperative lung metastasis and intrahepatic recurrence in HCC patients by multi-vascular sites sampling, respectively. This study provided new insight that multi-vascular measurement of CTCs could facilitate precise prediction of postoperative relapse or metastasis patterns in HCC. Other studies using CellSearchTM also demonstrated that patients who had EpCAM⁺ CTCs were associated with vascular invasion^[77,78], significantly elevated AFP^[78], more advanced BCLC stage^[77], higher recurrence rate^[79,80], and shorter OS^[77,79]. High EpCAM-positive CTC count also predicted poor survival of patients with unresectable HCC treated with transcatheter arterial chemoembolization (TACE)^[81].

PCR-based methods can detect tumor-specific mRNA in blood cells with high sensitivity and specificity. Yao *et al.*^[82] reported the combination of positive/negative cell sorting methods and RT-PCR could effectively detect AFP mRNA-positive CTCs in HCC patients. Guo *et al.*^[71] established an optimized platform based on negative enrichment and qRT-PCR for the detection of EpCAM mRNA-positive CTCs in HCC. Using their platform, they reported good sensitivity and specificity of PCR-based CTC detection in a cohort of 299 HCC patients. The novel platform exhibited 76.6% consistency with the CellSearchTM system while required a reduced blood volume (5 mL). They also reported that low pretreatment CTC levels were significantly correlated to a better prognosis after curative resection, TACE, or radiotherapy for patients with HCC. Using the same platform, Zhou *et al.*^[83] discovered that patients with high CTC/Treg levels exhibited higher recurrence rates than those with low CTC/Treg counterparts (66.7% *vs.* 10.3%, $P < 0.001$) by combining the measurement of EpCAM^{mRNA+} CTCs and CD4⁺ CD25⁺ Foxp3⁺ Treg cells. Currently, AFP examination remains the most extensively used screening method to indicate early HCC. However, about 30%-40% of HCC patients are AFP negative and the specificity of AFP may be flawed by false-positive results^[84,85]. CTC enumeration can be useful in the early detection of HCC since tumor dissemination can occur at the early stage of tumor development^[2]. In 2018, the same group developed a qRT-PCR-based multimarker (EpCAM, CD90, CD133, and CK19) diagnostic CTC panel for the identification of CTCs with stem-like phenotypes. They obtained a sensitivity of over 70.0% and specificity of over 90.0% in a well-designed multicenter cohort ($n = 1,006$). This panel performed equally well in detecting early-stage and AFP-negative HCC as in differentiating HCC from patients with benign liver diseases. The CTC panel outperformed AFP as a biomarker in terms of differential diagnostic capability, yielding higher area under

curve (AUC) value than AFP alone. This study demonstrated the clinical significance of using CTC panel in diagnosis and real-time risk evaluation for HCC^[63].

Increasing efforts have been made to investigate the correlation between different molecular phenotypes of CTCs and corresponding clinical outcomes. The stem-like phenotype of CTC has been explored as a strong predictor of the clinical outcome of patients with HCC. For instance, circulating CD45⁺ intercellular cell adhesion molecule-1 (+) (ICAM-1⁺) cells were regarded as HCC CTCs with stem cell-like properties. Liu *et al.*^[46] showed that patients with a higher burden of ICAM-1⁺ CTCs had significantly shorter disease-free survival and OS. Fan *et al.*^[86] also reported that circulating cancer stem cells (CD45⁺ CD90⁺ CD44⁺) predicted post-hepatectomy HCC recurrence with high accuracy. Moreover, EMT subtypes of CTCs have been studied for the correlation to clinicopathological features and prognosis of HCC patients. It is reported that a presence or dominance of mesenchymal-like CTCs represented worse clinical outcomes for HCC patients due to earlier tumor relapse and metastasis^[73,87]. The majority of these studies used the CanPatrolTM platform for CTC analysis^[73,88-94]. In a cohort of 113 HCC patients (65% BCLC 0/A) and 57 non-malignant liver diseases patients, the system presented a higher diagnostic value (AUC = 0.774, 95%CI: 0.704-0.834) of HCC than AFP [0.669 (AUC = 0.669, 95%CI: 0.587-0.750)]. A further combination of CTCs and AFP showed the highest diagnostic capability (AUC = 0.821, 95%CI: 0.756-0.886)^[92]. The proportion of mixed EMT status CTCs or mesenchymal CTCs was associated with advanced BCLC stages, higher metastatic tendency, and elevated serum levels of AFP^[88,89]. Yin *et al.*^[90] reported that twist expression in CTCs could serve as a biomarker for evaluating HCC metastasis and prognosis. Similarly, Wang *et al.*^[93] studied 62 HCC patients undergoing surgical resection and found that mesenchymal CTC positivity was an independent risk factor for early recurrence. A similar study using CanPatrolTM platform also found that CTCs undergoing EMT were significantly associated with early recurrence, multi-intrahepatic recurrence, and lung metastasis^[94]. However, a recent study reported that CTCs undergoing EMT were poorly correlated with clinical stages or predictive of recurrence of HCC using the platform^[95]. Another study using this platform also failed to uncover significant associations between change in total CTCs or CTC subtypes and HCC recurrence in a cohort consisting of 47 patients who underwent liver transplantation^[96]. Nevertheless, Xue *et al.*^[97] utilized an iFISH[®] platform to detect CTCs in patients undergoing liver transplantation and found that patients with preoperative iFISH-CTCs ≥ 5 in 7.5 mL of blood had significantly shorter recurrence-free survival than those with lower CTCs. Further large, multicenter studies are still needed to confirm the association between different molecular phenotypes of CTCs and HCC prognosis.

TUMOR MONITORING AND GUIDING PERSONALIZED THERAPEUTIC INTERVENTION IN HCC

Surgical resection remains the most effective therapy for HCC. CTCs could serve as a complementary tool to assess the efficacy of surgical resection and monitor tumor progression^[8,14]. Qi *et al.*^[98] recently compared the outcomes of patients undergoing anatomical or non-anatomical resection according to the number and EMT phenotype of CTCs. They suggested that anatomic resection may improve the survival of HCC patients, for those with low CTC count, negative epithelial/mesenchymal hybrid CTCs, and mesenchymal CTCs. Thus, CTC analysis before surgery can be used to better guide the resection method for HCC. Meanwhile, the decrease of CTC count after surgical treatment often reflects therapeutic efficacy. Fan *et al.*^[99] investigated the effect of liver tumor resection on CTC dynamics using *in vivo* flow cytometry (IVFC) in a green fluorescent protein-transfected HCC orthotopic metastatic mouse model. Their preliminary study found that the number of CTCs and early metastases rates decreased significantly after the resection of the primary tumor. Several clinical studies obtained similar results that CTC load decreased significantly after tumor resection, while increased CTC numbers after surgery were associated with a worse prognosis in patients with HCC^[17,63,71,79,100]. Besides, Jin *et al.*^[101] explored the clinical value of serial postsurgical observation (at 0, 3, 6, 9, and 12 months) of AFP mRNA level of CTCs in assessing the therapeutic effectiveness of hepatectomy.

In addition, the dynamic change of CTC counts reflected the treatment response in patients treated with locoregional therapies, including TACE, radiotherapy, and radiofrequency ablation^[63,71,102,103]. Li *et al.*^[103] found that the total number of CTCs and mesenchymal phenotype CTCs significantly increased three days after percutaneous radiofrequency ablation of liver tumor. However, no significant correlation was identified between changes in CTC levels and all the radiofrequency ablation factors. Recently, Rau *et al.*^[104] demonstrated the clinical utilities of sequential CTC monitoring in a patient cohort ($n = 17$) with locally advanced or metastatic HCC accepted systemic/targeted therapy. They found that a change in the CTC count correlated with the patient treatment response in most of the cases and was particularly useful for monitoring patients without elevated serum AFP levels.

Drug therapy is an important component of the comprehensive treatment of HCC. However, only a few patients are sensitive to chemotherapy drugs. Compared with other liquid biopsy biomarkers, CTCs contain more information on the functional characteristics and biological behaviors of tumor^[105,106]. Thus, CTCs can be a more relevant biomarker in guiding personalized therapeutic intervention for cancer patients. Yan *et al.*^[107] monitored the effect of sorafenib on CTC count in an orthotopic HCC mouse model by IVFC. They showed that the sorafenib treatment could dramatically reduce the number of CTCs, associated with a decreased probability of lung metastasis. Zhu *et al.*^[108] showed that the application of EGFR inhibitor could reduce CTC numbers caused as a side effect of transcatheter arterial embolization. Moreover, Li *et al.*^[109] presented a novel system to simultaneously detect the expressions of the phosphorylated extracellular signal-regulated kinase (pERK) and phosphorylated protein kinase B (pAkt) in CTCs. They showed that CTCs can be used in place of tumor tissue for characterization of pERK/pAkt expression, and HCC patients with pERK+/pAkt- CTCs were more sensitive to sorafenib treatment. Another potential application of CTCs in drug therapy is to test the drug sensitivity by CTC culture. Zhang *et al.*^[110] used a microfluidic chip to isolate and release viable CTCs and then performed chemotherapeutic drug assay. The number of spheroids formed by CTCs declined greatly when cultured with sorafenib or oxaliplatin. Furthermore, the novel single-cell sequencing technology makes individual CTC profiling possible, which may provide more valuable drug target information and guide individualized treatment in the future clinical practice^[12,111,112].

CONCLUSION

CTC analysis is an exciting field that is gaining increasing attention thanks to the significant technological advancements in CTC isolation and detection. As an important component of “liquid biopsy”, CTC analysis enables early cancer detection, prognosis prediction, therapy response monitoring, and novel therapeutic target identification in patients with HCC. However, significant challenges still exist in translating CTC analysis from bench to bedside. Most of the current studies in HCC used different technologies or platforms to detect CTCs in a relatively small, single-centered cohort with widely varying patient demographics, making it difficult to compare studies. Therefore, clinical utilities of CTC should be validated in more multicenter, large, and long-term studies using a standardized CTC assay. Moreover, CTCs hold great promise as a tool to deepen our knowledge of the complicated metastasis process. In recent years, CTCs researches have moved beyond simple CTC enumeration towards more sophisticated molecular analyses. Single-cell sequencing technology may pave the way for using CTCs to understand the underlying mechanisms of cancer metastasis and provide critical insights for new therapeutic strategies. Hopefully, routine CTCs evaluation will become a clinical reality in the near future.

DECLARATIONS

Authors' contributions

Manuscript writing, design, planning: Wang PX, Cheng JW, Yang XR

Manuscript review and editing: Yang XR

Approved the final manuscript: Wang PX, Cheng JW, Yang XR

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020;70:7-30.
2. Klein CA. Parallel progression of primary tumours and metastases. *Nat Rev Cancer* 2009;9:302-12.
3. European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236.
4. Villanueva A, Hernandez-Gea V, Llovet JM. Medical therapies for hepatocellular carcinoma: a critical view of the evidence. *Nat Rev Gastroenterol Hepatol* 2013;10:34-42.
5. Tabrizian P, Jibara G, Shrager B, Schwartz M, Roayaie S. Recurrence of hepatocellular cancer after resection: patterns, treatments, and prognosis. *Ann Surg* 2015;261:947-55.
6. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358-80.
7. Agopian VG, Harlander-Locke MP, Markovic D, Zarrinpar A, Kaldas FM, et al. Evaluation of Patients With Hepatocellular Carcinomas That Do Not Produce alpha-Fetoprotein. *JAMA Surg* 2017;152:55-64.
8. Chen VL, Xu D, Wicha MS, Lok AS, Parikh ND. Utility of liquid biopsy analysis in detection of hepatocellular carcinoma, determination of prognosis, and disease monitoring: a systematic review. *Clin Gastroenterol Hepatol* 2020; doi: 10.1016/j.cgh.2020.04.019.
9. Mann J, Reeves HL, Feldstein AE. Liquid biopsy for liver diseases. *Gut* 2018;67:2204-12.
10. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol* 2014;32:579-86.
11. Massague J, Obenauf AC. Metastatic colonization by circulating tumour cells. *Nature* 2016;529:298-306.
12. Keller L, Pantel K. Unravelling tumour heterogeneity by single-cell profiling of circulating tumour cells. *Nat Rev Cancer* 2019;19:553-67.
13. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin Cancer Res* 2008;14:6302-9.
14. Ahn JC, Teng PC, Chen PJ, Posadas E, Tseng HR, et al. Detection of circulating tumor cells and their implications as a novel biomarker for diagnosis, prognostication, and therapeutic monitoring in hepatocellular carcinoma. *Hepatology* 2020; doi: 10.1002/hep.31165.
15. Ye Q, Ling S, Zheng S, Xu X. Liquid biopsy in hepatocellular carcinoma: circulating tumor cells and circulating tumor DNA. *Mol Cancer* 2019;18:114.
16. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res* 2004;10:6897-904.
17. Sun YF, Xu Y, Yang XR, Guo W, Zhang X, et al. Circulating stem cell-like epithelial cell adhesion molecule-positive tumor cells indicate poor prognosis of hepatocellular carcinoma after curative resection. *Hepatology* 2013;57:1458-68.
18. Chang YS, di Tomaso E, McDonald DM, Jones R, Jain RK, et al. Mosaic blood vessels in tumors: frequency of cancer cells in contact

- with flowing blood. *Proc Natl Acad Sci U S A* 2000;97:14608-13.
19. Mohme M, Riethdorf S, Pantel K. Circulating and disseminated tumour cells - mechanisms of immune surveillance and escape. *Nat Rev Clin Oncol* 2017;14:155-67.
 20. Liberko M, Kolostova K, Bobek V. Essentials of circulating tumor cells for clinical research and practice. *Crit Rev Oncol Hematol* 2013;88:338-56.
 21. Kim MY, Oskarsson T, Acharyya S, Nguyen DX, Zhang XH, et al. Tumor self-seeding by circulating cancer cells. *Cell* 2009;139:1315-26.
 22. Coumans FA, Siesling S, Terstappen LW. Detection of cancer before distant metastasis. *BMC Cancer* 2013;13:1-12.
 23. Ebright RY, Lee S, Wittner BS, Niederhoffer KL, Nicholson BT, et al. Deregulation of ribosomal protein expression and translation promotes breast cancer metastasis. *Science* 2020;367:1468-73.
 24. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008;133:704-15.
 25. Kalluri R. EMT: when epithelial cells decide to become mesenchymal-like cells. *J Clin Invest* 2009;119:1417-9.
 26. Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, et al. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* 2013;339:580-4.
 27. Sun YF, Guo W, Xu Y, Shi YH, Gong ZJ, et al. Circulating tumor cells from different vascular sites exhibit spatial heterogeneity in epithelial and mesenchymal composition and distinct clinical significance in hepatocellular carcinoma. *Clin Cancer Res* 2018;24:547-59.
 28. Padmanaban V, Krol I, Suhail Y, Szczerba BM, Aceto N, et al. E-cadherin is required for metastasis in multiple models of breast cancer. *Nature* 2019;573:439-44.
 29. Thompson EW, Haviv I. The social aspects of EMT-MET plasticity. *Nat Med* 2011;17:1048-9.
 30. Aceto N. Bring along your friends: homotypic and heterotypic circulating tumor cell clustering to accelerate metastasis. *Biomed J* 2020;43:18-23.
 31. Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* 2014;158:1110-22.
 32. Manjunath Y, Upparahalli SV, Suvilesh KN, Avella DM, Kimchi ET, et al. Circulating tumor cell clusters are a potential biomarker for detection of non-small cell lung cancer. *Lung Cancer* 2019;134:147-50.
 33. Wang C, Mu Z, Chervoneva I, Austin L, Ye Z, et al. Longitudinally collected CTCs and CTC-clusters and clinical outcomes of metastatic breast cancer. *Breast Cancer Res Treat* 2017;161:83-94.
 34. Wang L, Li Y, Xu J, Zhang A, Wang X, et al. Quantified postsurgical small cell size CTCs and EpCAM(+) circulating tumor stem cells with cytogenetic abnormalities in hepatocellular carcinoma patients determine cancer relapse. *Cancer Lett* 2018;412:99-107.
 35. Gkoutela S, Castro-Giner F, Szczerba BM, Vetter M, Landin J, et al. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. *Cell* 2019;176:98-112.e14.
 36. Szczerba BM, Castro-Giner F, Vetter M, Krol I, Gkoutela S, et al. Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature* 2019;566:553-7.
 37. Labuschagne CF, Cheung EC, Blagih J, Domart MC, Vousden KH. Cell clustering promotes a metabolic switch that supports metastatic colonization. *Cell Metab* 2019;30:720-34.e5.
 38. Liu X, Taftaf R, Kawaguchi M, Chang YF, Chen W, et al. Homophilic CD44 interactions mediate tumor cell aggregation and polyclonal metastasis in patient-derived breast cancer models. *Cancer Discov* 2019;9:96-113.
 39. Guo J, Yao F, Lou Y, Xu C, Xiao B, et al. Detecting carcinoma cells in peripheral blood of patients with hepatocellular carcinoma by immunomagnetic beads and rt-PCR. *J Clin Gastroenterol* 2007;41:783-8.
 40. Shen Z, Wu A, Chen X. Current detection technologies for circulating tumor cells. *Chem Soc Rev* 2017;46:2038-56.
 41. Joosse SA, Gorges TM, Pantel K. Biology, detection, and clinical implications of circulating tumor cells. *EMBO Mol Med* 2015;7:1-11.
 42. Deng G, Herrler M, Burgess D, Manna E, Krag D, et al. Enrichment with anti-cytokeratin alone or combined with anti-EpCAM antibodies significantly increases the sensitivity for circulating tumor cell detection in metastatic breast cancer patients. *Breast Cancer Res* 2008;10:R69.
 43. Andree KC, van Dalum G, Terstappen LW. Challenges in circulating tumor cell detection by the CellSearch system. *Mol Oncol* 2016;10:395-407.
 44. Yu D, Sun X, Qiu Y, Zhou J, Wu Y, et al. Identification and clinical significance of mobilized endothelial progenitor cells in tumor vasculogenesis of hepatocellular carcinoma. *Clin Cancer Res* 2007;13:3814-24.
 45. Yang ZF, Ngai P, Ho DW, Yu WC, Ng MN, et al. Identification of local and circulating cancer stem cells in human liver cancer. *Hepatology* 2008;47:919-28.
 46. Liu S, Li N, Yu X, Xiao X, Cheng K, et al. Expression of intercellular adhesion molecule 1 by hepatocellular carcinoma stem cells and circulating tumor cells. *Gastroenterology* 2013;144:1031-41.e10.
 47. Harouaka RA, Nisic M, Zheng SY. Circulating tumor cell enrichment based on physical properties. *J Lab Autom* 2013;18:455-68.
 48. Song Y, Tian T, Shi Y, Liu W, Zou Y, et al. Enrichment and single-cell analysis of circulating tumor cells. *Chem Sci* 2017;8:1736-51.
 49. Gertler R, Rosenberg R, Fuehrer K, Dahm M, Nekarda H, et al. Detection of circulating tumor cells in blood using an optimized density gradient centrifugation. 2003;162:149.
 50. Weitz J, Kienle P, Lacroix J, Willeke F, Benner A, et al. Dissemination of tumor cells in patients undergoing surgery for colorectal cancer. *Clin Cancer Res* 1998;4:343-8.
 51. Rosenberg R, Gertler R, Friederichs J, Fuehrer K, Dahm M, et al. Comparison of two density gradient centrifugation systems for the enrichment of disseminated tumor cells in blood. *Cytometry* 2002;49:150-8.

52. Zuccolo J, Unruh TL, Deans JP. Efficient isolation of highly purified tonsil B lymphocytes using RosetteSep with allogeneic human red blood cells. *BMC Immunol* 2009;10:30.
53. Hayes GM, Busch R, Voogt J, Siah IM, Gee TA, et al. Isolation of malignant B cells from patients with chronic lymphocytic leukemia (CLL) for analysis of cell proliferation: validation of a simplified method suitable for multi-center clinical studies. *Leuk Res* 2010;34:809-15.
54. Vona G, Sabile A, Louha M, Sitruk V, Romana S, et al. Isolation by size of epithelial tumor cells: a new method for the immunomorphological and molecular characterization of circulating tumor cells. *Am J Pathol* 2000;156:57-63.
55. Vona G, Estepa L, Beroud C, Damotte D, Capron F, et al. Impact of cytomorphological detection of circulating tumor cells in patients with liver cancer. *Hepatology* 2004;39:792-7.
56. Desitter I, Guerrouahen BS, Benali-Furet N, Wechsler J, Janne PA, et al. A new device for rapid isolation by size and characterization of rare circulating tumor cells. *Anticancer Res* 2011;31:427-41.
57. Dong Y, Skelley AM, Merdek KD, Sprott KM, Jiang C, et al. Microfluidics and circulating tumor cells. *J Mol Diagn* 2013;15:149-57.
58. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature* 2007;450:1235-9.
59. Stott SL, Hsu CH, Tsukrov DI, Yu M, Miyamoto DT, et al. Isolation of circulating tumor cells using a microvortex-generating herringbone-chip. *Proc Natl Acad Sci U S A* 2010;107:18392-7.
60. Karabacak NM, Spuhler PS, Fachin F, Lim EJ, Pai V, et al. Microfluidic, marker-free isolation of circulating tumor cells from blood samples. *Nat Protoc* 2014;9:694-710.
61. Sarioglu AF, Aceto N, Kojic N, Donaldson MC, Zeinali M, et al. A microfluidic device for label-free, physical capture of circulating tumor cell clusters. *Nat Methods* 2015;12:685-91.
62. Qian W, Zhang Y, Chen W. Capturing cancer: emerging microfluidic technologies for the capture and characterization of circulating tumor cells. *Small* 2015;11:3850-72.
63. Guo W, Sun YF, Shen MN, Ma XL, Wu J, et al. Circulating tumor cells with stem-like phenotypes for diagnosis, prognosis, and therapeutic response evaluation in hepatocellular carcinoma. *Clin Cancer Res* 2018;24:2203-13.
64. de Boer CJ, van Krieken JH, Janssen-van Rhijn CM, Litvinov SV. Expression of Ep-CAM in normal, regenerating, metaplastic, and neoplastic liver. *J Pathol* 1999;188:201-6.
65. Yamashita T, Forgues M, Wang W, Kim JW, Ye Q, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. *Cancer Res* 2008;68:1451-61.
66. Nicolazzo C, Gradilone A, Loreni F, Raimondi C, Gazzaniga P. EpCAM(low) circulating tumor cells: gold in the waste. *Dis Markers* 2019;2019:1718920.
67. Xu W, Cao L, Chen L, Li J, Zhang XF, et al. Isolation of circulating tumor cells in patients with hepatocellular carcinoma using a novel cell separation strategy. *Clin Cancer Res* 2011;17:3783-93.
68. Li J, Chen L, Zhang X, Zhang Y, Liu H, et al. Detection of circulating tumor cells in hepatocellular carcinoma using antibodies against asialoglycoprotein receptor, carbamoyl phosphate synthetase 1 and pan-cytokeratin. *PLoS One* 2014;9:e96185.
69. Hamaoka M, Kobayashi T, Tanaka Y, Mashima H, Ohdan H. Clinical significance of glypican-3-positive circulating tumor cells of hepatocellular carcinoma patients: a prospective study. *PLoS One* 2019;14:e0217586.
70. Court CM, Hou S, Winograd P, Segel NH, Li QW, et al. A novel multimarker assay for the phenotypic profiling of circulating tumor cells in hepatocellular carcinoma. *Liver Transpl* 2018;24:946-60.
71. Guo W, Yang XR, Sun YF, Shen MN, Ma XL, et al. Clinical significance of EpCAM mRNA-positive circulating tumor cells in hepatocellular carcinoma by an optimized negative enrichment and qRT-PCR-based platform. *Clin Cancer Res* 2014;20:4794-805.
72. Kalinich M, Bhan I, Kwan TT, Miyamoto DT, Javadi S, et al. An RNA-based signature enables high specificity detection of circulating tumor cells in hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 2017;114:1123-8.
73. Liu YK, Hu BS, Li ZL, He X, Li Y, et al. An improved strategy to detect the epithelial-mesenchymal transition process in circulating tumor cells in hepatocellular carcinoma patients. *Hepatology* 2016;10:640-6.
74. Wan S, Kim TH, Smith KJ, Delaney R, Park GS, et al. New labyrinth microfluidic device detects circulating tumor cells expressing cancer stem cell marker and circulating tumor microemboli in hepatocellular carcinoma. *Sci Rep* 2019;9:18575.
75. Hu B, Yang XR, Xu Y, Sun YF, Sun C, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. *Clin Cancer Res* 2014;20:6212-22.
76. Wang BL, Tian L, Gao XH, Ma XL, Wu J, et al. Dynamic change of the systemic immune inflammation index predicts the prognosis of patients with hepatocellular carcinoma after curative resection. *Clin Chem Lab Med* 2016;54:1963-9.
77. Schulze K, Gasch C, Staufer K, Nashan B, Lohse AW, et al. Presence of EpCAM-positive circulating tumor cells as biomarker for systemic disease strongly correlates to survival in patients with hepatocellular carcinoma. *Int J Cancer* 2013;133:2165-71.
78. Kelley RK, Magbanua MJ, Butler TM, Collisson EA, Hwang J, et al. Circulating tumor cells in hepatocellular carcinoma: a pilot study of detection, enumeration, and next-generation sequencing in cases and controls. *BMC Cancer* 2015;15:206.
79. Yu JJ, Xiao W, Dong SL, Liang HF, Zhang ZW, et al. Effect of surgical liver resection on circulating tumor cells in patients with hepatocellular carcinoma. *BMC Cancer* 2018;18:835.
80. von Felden J, Schulze K, Krech T, Ewald F, Nashan B, et al. Circulating tumor cells as liquid biomarker for high HCC recurrence risk after curative liver resection. *Oncotarget* 2017;8:89978-87.
81. Shen J, Wang WS, Zhu XL, Ni CF. High epithelial cell adhesion molecule-positive circulating tumor cell count predicts poor survival of patients with unresectable hepatocellular carcinoma treated with transcatheter arterial chemoembolization. *J Vasc Interv Radiol* 2018;29:1678-84.

82. Yao F, Guo JM, Xu CF, Lou YL, Xiao BX, et al. Detecting AFP mRNA in peripheral blood of the patients with hepatocellular carcinoma, liver cirrhosis and hepatitis. *Clin Chim Acta* 2005;361:119-27.
83. Zhou Y, Wang B, Wu J, Zhang C, Zhou Y, et al. Association of preoperative EpCAM circulating tumor cells and peripheral Treg cell levels with early recurrence of hepatocellular carcinoma following radical hepatic resection. *BMC Cancer* 2016;16:506.
84. Taketa K. Alpha-fetoprotein: reevaluation in hepatology. *Hepatology* 1990;12:1420-32.
85. Trevisani F, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, et al. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* 2001;34:570-5.
86. Fan ST, Yang ZF, Ho DW, Ng MN, Yu WC, et al. Prediction of posthepatectomy recurrence of hepatocellular carcinoma by circulating cancer stem cells: a prospective study. *Ann Surg* 2011;254:569-76.
87. Li YM, Xu SC, Li J, Han KQ, Pi HF, et al. Epithelial-mesenchymal transition markers expressed in circulating tumor cells in hepatocellular carcinoma patients with different stages of disease. *Cell Death Dis* 2013;4:e831.
88. Chen J, Cao SW, Cai Z, Zheng L, Wang Q. Epithelial-mesenchymal transition phenotypes of circulating tumor cells correlate with the clinical stages and cancer metastasis in hepatocellular carcinoma patients. *Cancer Biomark* 2017;20:487-98.
89. Ou H, Huang Y, Xiang L, Chen Z, Fang Y, et al. Circulating tumor cell phenotype indicates poor survival and recurrence after surgery for hepatocellular carcinoma. *Dig Dis Sci* 2018;63:2373-80.
90. Yin LC, Luo ZC, Gao YX, Li Y, Peng Q, et al. Twist expression in circulating hepatocellular carcinoma cells predicts metastasis and prognoses. *Biomed Res Int* 2018;2018:3789613.
91. Ye X, Li G, Han C, Han Q, Shang L, et al. Circulating tumor cells as a potential biomarker for postoperative clinical outcome in HBV-related hepatocellular carcinoma. *Cancer Manag Res* 2018;10:5639-47.
92. Cheng Y, Luo L, Zhang J, Zhou M, Tang Y, et al. Diagnostic value of different phenotype circulating tumor cells in hepatocellular carcinoma. *J Gastrointest Surg* 2019;23:2354-61.
93. Wang Z, Luo L, Cheng Y, He G, Peng B, et al. Correlation between postoperative early recurrence of hepatocellular carcinoma and mesenchymal circulating tumor cells in peripheral blood. *J Gastrointest Surg* 2018;22:633-9.
94. Qi LN, Xiang BD, Wu FX, Ye JZ, Zhong JH, et al. Circulating tumor cells undergoing EMT provide a metric for diagnosis and prognosis of patients with hepatocellular carcinoma. *Cancer Res* 2018;78:4731-44.
95. Chen Y, Li S, Li W, Yang R, Zhang X, et al. Circulating tumor cells undergoing EMT are poorly correlated with clinical stages or predictive of recurrence in hepatocellular carcinoma. *Sci Rep* 2019;9:7084.
96. Wang S, Zheng Y, Liu J, Huo F, Zhou J. Analysis of circulating tumor cells in patients with hepatocellular carcinoma recurrence following liver transplantation. *J Investig Med* 2018;66:1-6.
97. Xue F, Shi S, Zhang Z, Xu C, Zheng J, et al. Application of a novel liquid biopsy in patients with hepatocellular carcinoma undergoing liver transplantation. *Oncol Lett* 2018;15:5481-8.
98. Qi LN, Ma L, Chen YY, Chen ZS, Zhong JH, et al. Outcomes of anatomical versus non-anatomical resection for hepatocellular carcinoma according to circulating tumour-cell status. *Ann Med* 2020;52:21-31.
99. Fan ZC, Yan J, Liu GD, Tan XY, Weng XF, et al. Real-time monitoring of rare circulating hepatocellular carcinoma cells in an orthotopic model by in vivo flow cytometry assesses resection on metastasis. *Cancer Res* 2012;72:2683-91.
100. Ha Y, Kim TH, Shim JE, Yoon S, Jun MJ, et al. Circulating tumor cells are associated with poor outcomes in early-stage hepatocellular carcinoma: a prospective study. *Hepatol Int* 2019;13:726-35.
101. Jin J, Niu X, Zou L, Li L, Li S, et al. AFP mRNA level in enriched circulating tumor cells from hepatocellular carcinoma patient blood samples is a pivotal predictive marker for metastasis. *Cancer Lett* 2016;378:33-7.
102. Wu X, Yang C, Yu H, Cao F, Shan Y, et al. The predictive values of serum dickkopf-1 and circulating tumor cells in evaluating the efficacy of transcatheter arterial chemoembolization treatment on hepatocellular carcinoma. *Medicine (Baltimore)* 2019;98:e16579.
103. Li Y, Huang N, Wang C, Ma H, Zhou M, et al. Impact of liver tumor percutaneous radiofrequency ablation on circulating tumor cells. *Oncol Lett* 2018;16:2839-50.
104. Rau KM, Liu CT, Hsiao YC, Hsiao KY, Wang TM, et al. Sequential circulating tumor cell counts in patients with locally advanced or metastatic hepatocellular carcinoma: monitoring the treatment response. *J Clin Med* 2020;9.
105. Tayoun T, Faugeron V, Oulhen M, Aberlenc A, Pawlikowska P, et al. CTC-derived models: a window into the seeding capacity of circulating tumor cells (CTCs). *Cells* 2019;8.
106. Baccelli I, Schneeweiss A, Riethdorf S, Stenzinger A, Schillert A, et al. Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. *Nat Biotechnol* 2013;31:539-44.
107. Yan J, Fan Z, Wu X, Xu M, Jiang J, et al. Circulating tumor cells are correlated with disease progression and treatment response in an orthotopic hepatocellular carcinoma model. *Cytometry A* 2015;87:1020-8.
108. Zhu L, Liu R, Zhang W, Qian S, Wang J. Application of EGFR inhibitor reduces circulating tumor cells during transcatheter arterial embolization. *Clin Transl Oncol* 2018;20:639-46.
109. Li J, Shi L, Zhang X, Sun B, Yang Y, et al. pERK/pAkt phenotyping in circulating tumor cells as a biomarker for sorafenib efficacy in patients with advanced hepatocellular carcinoma. *Oncotarget* 2016;7:2646-59.
110. Zhang Y, Zhang X, Zhang J, Sun B, Zheng L, et al. Microfluidic chip for isolation of viable circulating tumor cells of hepatocellular carcinoma for their culture and drug sensitivity assay. *Cancer Biol Ther* 2016;17:1177-87.
111. Aoki M, Shoji H, Kashiwa A, Takeuchi K, Shimizu Y, et al. Prospects for comprehensive analyses of circulating tumor cells in tumor biology. *Cancers (Basel)* 2020;12.
112. D'Avola D, Villacorta-Martin C, Martins-Filho SN, Craig A, Labgaa I, et al. High-density single cell mRNA sequencing to characterize circulating tumor cells in hepatocellular carcinoma. *Sci Rep* 2018;8:1157.

Review

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Evaluation and impact of different biomarkers for early detection of hepatocellular carcinoma

Jan Best¹, Svenja Sydor², Lars P. Bechmann², Ali Canbay²

¹Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University, University Hospital Magdeburg, Magdeburg 39120, Germany.

²Department of Internal Medicine, University Hospital Knappschafts Krankenhaus, Ruhr-University Bochum, Bochum 44892, Germany.

Correspondence to: Dr. Jan Best, Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University, University Hospital Magdeburg, Leipziger Str. 44, Magdeburg 39120, Germany. E-mail: jan.best@med.ovgu.de

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Abstract

Worldwide, hepatocellular carcinoma (HCC) is a frequent complication of liver diseases and remains a major cause of cancer-related mortality. In addition, the prevalence of nonalcoholic steatohepatitis (NASH) as prerequisite of hepatocarcinogenesis, even in the absence of cirrhosis, is rising rapidly. The early detection of HCC has been crucial in improving the survival outcomes of those patients. However, in the mostly obese NASH population, diagnostic sensitivity of ultrasound-based HCC screening approaches is limited. On the other hand, biomarkers for HCC show promising potential to improve early detection, providing reproducible, investigator-independent results that can be used either alone or integrated with other biomarkers for scoring models. In the past, validation has been limited due to a lack of prospective longitudinal cohort studies. At present, large-scale retrospective phase-III- biomarker- development gives hope for the availability of biomarker-based screening approaches in the near future. This review focuses on the potential impact of biomarkers on surveillance strategies, potentially allowing for earlier HCC diagnosis.

Keywords: Nonalcoholic steatohepatitis, hepatocellular carcinoma, alpha fetoprotein, AFP-L3, des-gamma-carboxy-prothrombin, Gender, Age, GALAD-score, Glypican-3, microbiome



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INTRODUCTION

Globally, hepatocellular carcinoma (HCC) is the sixth most frequent malignancy and the second most common cause of cancer-related death^[1]. The global incidence of HCC has tripled since the 1980s^[2]. Despite the availability of numerous locoregional therapies and a plethora of novel systemic treatment options, the overall survival of HCC remains relatively poor^[3]. Furthermore, incidence of HCC in the western world is rising with hepatic steatosis being seen as the major risk factor [Figure 1]. Nonalcoholic fatty liver (NAFL; i.e., hepatic steatosis without significant inflammation) and nonalcoholic steatohepatitis (NASH; i.e., hepatic steatosis associated with hepatic inflammation and hepatocellular ballooning) in particular have increasingly been recognized as risk factors for HCC. Previously, these cases have frequently been classified as cryptogenic cirrhosis^[5]. Moreover, a significant proportion of patients develops HCC in the absence of liver cirrhosis predominantly in the case of predisposing chronic hepatitis B or NASH^[6]. Nevertheless, multiple HCC surveillance guidelines do not sufficiently recognize NASH as major risk factor of hepatocarcinogenesis. Additionally, ultrasound for detection of smaller lesions, such as in early stage HCC, lacks sensitivity and is further impaired when there is underlying cirrhosis, steatosis, or obesity. To address those aforementioned insufficiencies, the definition of patients at risk has to be more concisely defined and further prospective trials have to elucidate whether ultrasound alone has potential to detect a sufficient proportion of HCC at stages when curative treatment options are still available. Several recent trials clearly indicate that biomarker-based surveillance algorithms have potential to complement or even surpass ultrasound as a surveillance strategy. This review aims to provide an overview of current biomarkers with utility in HCC detection and how they could be implemented into current HCC early detection programs.

EARLY HCC DIAGNOSIS DETERMINES PATIENT PROGNOSIS

In patients participating in HCC early detection programs, an initial diagnosis is made in less advanced stages, which results in a clear survival benefit. Successful surveillance, however, requires a reliable screening method and a definition of the risk population based on medical needs.

Targeted HCC monitoring anticipates offering curative intended therapeutic procedures, such as liver resection or transplantation, to the highest possible proportion of patients upon their initial HCC diagnosis. Unfortunately, HCC is diagnosed in these early stages only in a minority of patients^[7]. In a representative German singlecenter cohort, between 1998 and 2009, only 23.5% of over a thousand HCC patients were in an early stage of HCC [Barcelona Clinic Liver Cancer (BCLC) 0/A] at initial diagnosis. Accordingly, less than half of the patients received curative therapies and the median overall survival was only 16 months^[8].

The above-mentioned data are consistent with large international trials where only 10%-23% of the patients were curatively treatable at first diagnosis^[9-12]. Consequently, only 10%-39% of those patients survived at least one year after diagnosis^[9-14].

Since HCC is better suited for early detection programs than most other cancers, and since the risk population to be screened could be well defined, the currently available data is particularly discouraging. International guidelines recommend regular examinations of symptom-free risk patients according to certain criteria.

INTERNATIONAL GUIDELINES PROVIDE DIFFERENT HCC SURVEILLANCE

RECOMMENDATIONS

Globally, there are marked regional variations in the algorithms for HCC early detection [Table 1]. The guideline of the German Society for Digestive and Metabolic Diseases (DGVS) recommends that patients with liver cirrhosis of any etiology, as well as patients with chronic hepatitis B or NASH in the absence of cirrhosis, undergo liver ultrasound. Optionally, the liver tumor marker α -fetoprotein (AFP) can be

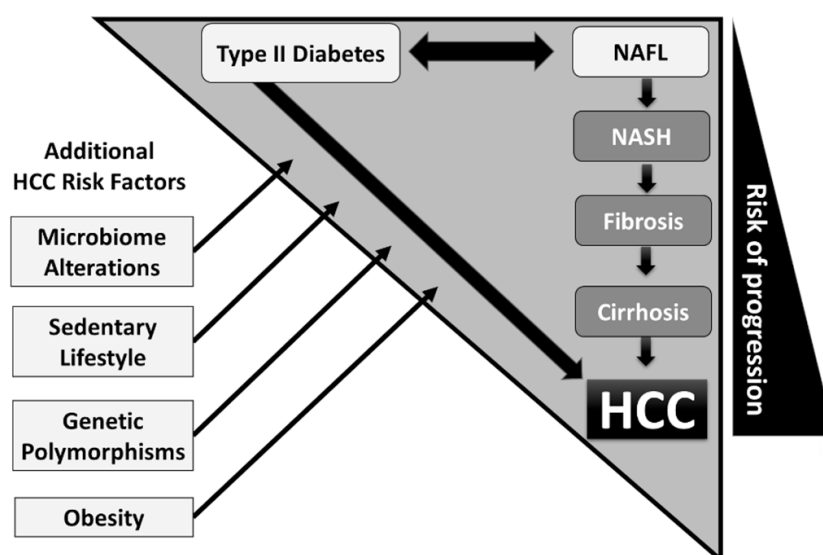


Figure 1. Triangle of hepatocarcinogenesis in metabolic syndrome: hepatocellular carcinoma (HCC) risk in nonalcoholic steatohepatitis (NASH) significantly increases with progression of liver fibrosis. Independently, type II diabetes mellitus promotes progression from nonalcoholic fatty liver (NAFL) to NASH, but needs to be recognized as an individual predisposing HCC-risk factor since peripheral insulin resistance may promote hepatocarcinogenesis even in the absence of cirrhosis^[4]. Microbiome alterations, sedentary lifestyle, genetic polymorphisms, and obesity represent additional factors aggravating HCC risk in the NASH population

Table 1. Overview of international HCC surveillance recommendations

Society	Risk group	Procedure
DGVS ^[15]	Liver cirrhosis of all etiologies: chronic HBV and NASH	US with or without AFP every 6 months, US- quality standards required
AASLD ^[16]	Liver cirrhosis of all etiologies; chronic HBV depending on ethnical background, age, and genetic background	US with or without AFP every 6 months
EASL/EORCT ^[17]	Liver cirrhosis of all etiologies at CTP stage A and B or CTP stage C if listed for oLT; chronic hepatitis B or active hepatitis; chronic HCV with advanced (F3) fibrosis	US every 6 months
APASL ^[18,19]	Liver cirrhosis, chronic HBV and/or HCV	US and AFP every 6 months
JSH ^[20]	High risk: Liver cirrhosis of all etiologies; chronic HBV and/or HCV	US, AFP, AFP-L3 and DCP every 6 months
	Very high risk: Liver cirrhosis with chronic HBV and/or HCV	US, AFP, AFP-L3 and DCP every 3-4 months

DGVS: German Society of Gastroenterology and Metabolic Diseases; EASL: European Association for the Study of the Liver; AASLD: American Association for the Study of Liver Disease; APASL: Asian Pacific Association for the Study of the Liver; JSH: Japanese Society of Hepatology; HBV: hepatitis B virus; NASH: non-alcoholic Steatohepatitis; HCV: hepatitis C virus; US: ultrasound; AFP: alpha fetoprotein; AFP-L3: Lektin reactive α -Fetoprotein; DCP: Des-gamma-carboxy prothrombin also known as Protein-Induced-by-Vitamin-K-Absence-or-Antagonist-II (PIVKA II); CTP: Child Turcotte Pugh

determined. The sonographic examinations should be carried out according to quality criteria of the German Society for Ultrasound in Medicine (DEGUM)^[15].

While the guidelines of the the American Society for the Study of the Liver (AASLD) and the European Association for the Study of the Liver (EASL) do not recommend an AFP determination at all, or only recommend it on an optional basis, the Asian Pacific Association for the Study of the Liver (APASL) proposed a combination of regular ultrasound (US) and AFP determination. All the aforementioned guidelines recommend those examinations every 6 months. The Japanese Society for Hepatology (JSH) also recommends US in combination with complimentary determination of three different biomarkers, namely AFP, lectin-reactive- α -fetoprotein (AFP-L3), and des-gamma-carboxy-prothrombin (DCP). In contrast to all other guidelines, JSH discriminates between high risk and very high risk groups and therefore recommends screening every 6 months and every 3 months, respectively, as depicted in Table 1.

The limitations of ultrasound scanning have been explicitly recognized in previous AASLD guidelines which state “performance characteristics have not been well defined in cirrhotic livers” and “some patients, particularly the obese, are not good candidates (for surveillance) despite their risk”^[21-25]. To address this insufficiency, a recent large meta-analysis, which included thirty-two studies comprising 13,367 patients, characterized sensitivity of imaging with or without AFP for detection of HCC in cirrhotic patients. The authors concluded that US alone has a low sensitivity to detect early stage HCC in patients with cirrhosis and thus the addition of AFP to US significantly increased the sensitivity of early HCC detection^[26]. The latest AASLD guidelines emphasize the importance of determining whether other serum biomarkers (specifically AFP-L3 and DCP) might complement AFP and US in the surveillance setting^[16].

NASH AS MAIN RISK FACTOR OF INCREASING HCC INCIDENCE

The global prevalence of nonalcoholic fatty liver disease (NAFLD) is estimated at 25%^[27]. In up to one third of all NAFL patients, progression to NASH can occur, which in turn may lead to higher-grade fibrosis on the basis of inflammation and, in the final stage, to cirrhosis and HCC^[28,29]. A recent meta-analysis conducted by Younossi *et al.*^[13] even reported a global prevalence of NASH among biopsied NAFLD patients that was almost double the previous studies’ findings at 59.1%. According to the latest epidemiological studies, NASH is regarded as the main cause of the steadily increasing incidence of HCC in western industrialized nations^[30,31]. An analysis of the American liver transplant registry from 2002 to 2007 showed that the prevalence of HCC patients waiting for a liver transplant increased by 15.6% in the past 15 years, from 6.4% in 2002 to 22% in 2017. NASH became the fastest growing cause of HCC. The proportion of NASH as the cause of HCC increased 8.5-fold during the study period, from 2.1% in 2002 to 17.9% in 2017^[31]. It should be noted here that many cases of HCC arise due to predisposing NASH in the absence of cirrhosis, a population that has so far not been internationally screened for the purpose of early detection of HCC. About 20% of all NASH-associated HCC cases occurred in the absence of cirrhosis^[32].

A German single-center study from 2015, which included 1,119 HCC patients of all etiologies, showed that there are relevant differences between patients with HCC with underlying NASH compared to those with other hepatopathies. Patients with NASH-related HCC were older than those with other predisposing hepatopathies (67.6 years *vs.* 65 years) when they were first diagnosed. In addition, the NASH-HCC cohort showed a higher prevalence of obesity (31.1% *vs.* 14.7%) and diabetes mellitus type II (T2DM) (66.7% *vs.* 37.85%) [Figure 1]. Interestingly, NASH-associated HCC shows a trend towards a higher frequency of multifocality (80% *vs.* 69.7%) with overall larger lesions (6 cm *vs.* 4.8 cm) and a tendency towards an increased rate of extrahepatic metastasis at time of initial diagnosis. It is also important to note that the NASH-HCC patients had better global liver function despite a higher tumor burden when diagnosed for the first time. It can be postulated that later HCC diagnosis may be the result of less intensive screening efforts due to lesser extent of hepatic deterioration in NASH patients compared to other liver diseases^[33].

DO BIOMARKERS ENHANCE HCC EARLY DETECTION?

For clinical routine, the diagnostic significance of ultrasound of the liver as part of the HCC screening may exhibit distinct limitations. This includes the comparability with preliminary examinations due to changing investigators and ultrasound devices. Predominantly in the cirrhotic or steatotic liver, small lesions are detected in a limited extent. Determination of HCC biomarkers such as AFP have the advantage of being independent of the investigator. Laboratory tests are subject to strict quality guidelines and deliver reproducible results. For this reason, the addition of AFP is recommended in some guidelines to supplement the US. In the high-risk cohort suffering from chronic viral hepatitis B or C relevant for HCC screening, however, the hepatic inflammation may lead to a false positive AFP value elevation which significantly attenuates the specificity, especially in this scenario^[34,35]. In contrast, if the AFP cut-off values are set higher to improve specificity, 40% of the early stages of HCC are not detected by AFP^[36].

In order to achieve a sensitivity superior to the combination of US and AFP while preserving high specificity in early HCC detection, a large number of studies examined the suitability of different biomarkers, such as Glypican-3, AFP-L3, and DCP for HCC surveillance^[37-40]. The focus during recent years has been on validation of AFP, AFP-L3, and DCP which are already available as certified laboratory tests.

AVAILABLE HCC-BIOMARKERS AND DIAGNOSTIC MODELS

AFP and AFP-L3

The overall AFP, which is usually determined in the clinical routine, consists of the three different isoforms: AFP-L1, AFP-L2, and AFP-L3. AFP is a fetal glycoprotein that can be produced later in life when the hepatocytes are in the process of malignant transformation^[41]. According to various studies, the sensitivity of AFP in HCC detection is between 39% and 65% and the specificity varies between 76% to 94%^[42].

AFP-L3 is a AFP variant that binds to the lectin molecule “Lens culinaris agglutinin” and, in contrast to the overall AFP, is HCC-specific. While the AFP-L3 fraction is produced exclusively by malignant transformed hepatocytes, an AFP-L1 elevation, the non-glycosylated AFP main fraction, can also be caused by viral hepatitis and in this scenario is responsible for an incorrectly increased total AFP level^[43,44]. With a cut-off of 15%, sensitivities between 75%-96.6% and specificities of 90%-92% have been described for AFP-L3^[45,46].

Des-gamma-carboxy prothrombin

Des-gamma-carboxy prothrombin (DCP), also known as Protein-Induced-by-Vitamin-K-Absence-or-Antagonist-II (PIVKA II), is a precursor of prothrombin and is formed in the context of hepatocarcinogenesis due to an impaired vitamin K metabolism. Here, the carboxylation of prothrombin is so impaired that the serum concentration of the DCP increases. Sensitivities between 48% and 62% and specificities between 81% and 98% have been described for the DCP, making DCP a more specific marker than AFP, albeit with lower sensitivity^[47-49].

Combination of AFP, AFP-L3 and DCP

Various clinical trials have clearly demonstrated that there were no correlations among the results of AFP, AFP-L3, and DCP. Thus, some HCC cases can be positive for only one marker at a time while negative for the others. In clinical practice, this means that the combination of the above biomarkers leads to a gradual increase in sensitivity.

Especially when AFP remains the only available marker in clinical routine, the complementary use of AFP-L3 and DCP represents an additional diagnostic option. In a retrospective Japanese single-center study with 270 AFP-negative HCC patients, it was demonstrated that the majority of patients with positive AFP-L3 and/or DCP findings were correctly recorded^[50].

GALAD model

The aforementioned triple combination of the biomarkers AFP, AFP-L3, and DCP demonstrated superior detection of HCC compared to their individual utilization with no significant decrease in specificity in an Asian patient cohort^[51,52]. For further optimization, a statistically based model called GALAD score was developed a few years ago and was extensively validated in several international studies. It is a diagnostic algorithm based on rigorous statistical analysis. The formula is calculated on the measured absolute values of the three markers instead of defining cut-off levels. Thus, they are considered as continuous variables rather than categorical. Gender and age information are also included since older age and male sex represent independent HCC risk factors^[53,54]. A GALAD point value of -0.63 serves as a cut-off value for optimal sensitivity and specificity regardless of the BCLC stage. Using this model in a British cohort, the GALAD model achieved an overall AUROC (area under the receiver operating characteristic curve) of 0.97 in detection of all BCLC stages, and early stage HCC (BCLC 0/A) was detected with an AUROC of

0.92 and a corresponding sensitivity of 86% and specificity of 89%^[55]. A subsequent international multi-center validation study comprising 6,834 patients of different etiologies of HCC achieved an AUROC for GALAD of consistently > 0.90, confirming the efficacy of this model^[56]. A following German single-center study observed that the GALAD score, even in BCLC 0/A stage HCC, achieved an AUROC of 0.92, again demonstrating superiority in HCC detection compared to the triple biomarker combination of AFP, AFP-L3 and DCP without considering gender and age^[57].

To further address the inadequate performance of US-based HCC early detection in NASH, where obesity and sound artifacts due to steatosis further attenuate the diagnostic performance, the utility of the GALAD score in the detection of NASH-associated early HCCs had been tested specifically in this high-risk collective. In a retrospective German multi-center cohort (8 centers) study, the GALAD score was able to detect NASH-HCC patients with an AUROC of 0.96, significantly better than the performance of the biomarkers alone [AFP (AUROC 0.88), AFP-L3 (AUROC 0.86), or DCP (AUROC, 0.87)]. Even for NASH patients in early HCC stages (within the Milan criteria), the GALAD score achieved an AUROC of 0.91. Furthermore, in a prospective Japanese cohort study, it was demonstrated that the average GALAD score in those patients who developed HCC during the observation period was already significantly elevated up to 1.5 years before the initial diagnosis of HCC. The GALAD scores of these HCC patients rose above the cut-off value of 0.63 approximately 200 days before first diagnosis^[58].

This implies that the GALAD model is quite suitable for early detection of HCC of all etiologies, even in NASH. However, a phase IV multi-center prospective study has yet to test whether the GALAD Score can be used in the future as an integral part of HCC screening algorithms in patients at risk.

Osteopontin

Osteopontin (OPN) is an integrin-binding phosphoprotein that is overexpressed in a variety of cancers including lung, breast, colon cancer, and HCC^[39,59]. At a low level, it is also secreted by biliary epithelial cells. OPN mediates cell signaling that controls inflammation as it is the case in hepatitis, HCC tumor progression, and metastasis^[60]. Hepatocarcinogenesis results in elevated OPN levels compared to those patients with chronic liver disease in the absence of HCC^[61]. In a meta-analysis investigating the efficacy of OPN in HCC detection, the sensitivity and specificity of elevated OPN levels have been reported between 75%-87% and 62%-82%, respectively^[62]. In a phase III validation study, OPN outperformed AFP for HCC detection with an AUROC of 0.73 [95%CI: 0.62-0.85] vs. AUROC of 0.68 [95%CI: 0.54-0.82], respectively. The combined utilization of AFP and OPN resulted in a sensitivity of 82% and specificity of 77% for HCC detection; however, the number of patients at BCLC stage 0/A was limited in this study^[63].

Glypican-3

Glypican-3 (GPC-3), is a heparin sulfate proteoglycan playing a pivotal role in cell proliferation and tumor suppression, representing a potential biomarker for the diagnosis of HCC. GPC-3 binds to growth factor receptors and is involved in cell proliferation and tumor suppression^[64]. In healthy hepatocytes it is absent, but during hepatocarcinogenesis it is upregulated, and it is assumed to participate in the canonical Wnt- signaling growth pathway^[65,66]. GPC-3 is present in approximately 33% of HCC patients that were seronegative for both DCP and AFP^[67]. A meta-analysis found that GPC-3 had a sensitivity of 55.1% and a specificity of 97%^[68]. AFP and GPC-3 in concert achieved a sensitivity of 76% even at early stage HCC. In light of these findings, GPC-3 has been proposed to be a complementary serologic biomarker to AFP due to the ability of GPC-3 to accurately distinguish between patients with small, well differentiated HCC and those with underlying cirrhosis^[69].

HCC SURVEILLANCE BASED ON COMBINATION OF BIOMARKERS AND ULTRASOUND

Combination of AFP and ultrasound

The latest HCC guidelines have recently shown a tendency to omit biomarker-based diagnostics in favor of sole ultrasound examinations. In a large American meta-analysis of US, the sensitivity and specificity of US in HCC detection was analyzed with and without additional AFP determination in an HCC high-risk patient group (32 trials/13,367 patients). Ultrasound alone detected an HCC across all stages with a sensitivity of 84% when carried out in accordance with regional guidelines, but there was a dramatic drop in sensitivity to 47% in early stage HCC. The combination of US with AFP was able to improve the sensitivity in the early detection of HCC to at least 63%^[21]. This clearly indicates that ultrasound alone has low sensitivity in detecting early stage HCC in patients with cirrhosis. Hence, the addition of AFP to ultrasound may significantly increase the sensitivity of early HCC detection in future.

Combination of GALAD and ultrasound

Recently, the GALAD model has also been validated in an American US cohort study [single-center cohort of 111 HCC and 180 controls and a multi-center cohort of 233 early HCC and 412 cirrhosis patients from the Early Detection Research Network (EDRN) Phase 2 HCC Study] and the performance has been shown to be clearly superior to sonography for HCC detection. Here the AUROC of GALAD for HCC detection was 0.95, which was clearly superior to the AUROC of ultrasound (0.82). The combination of GALAD and ultrasound (GALADUS score) achieved an AUC of 0.98, clearly superior to US or GALAD used solely^[70]. These very promising data indicate that a combination of ultrasound and biomarker-based scores can significantly improve the performance of current surveillance strategies.

MECHANISMS OF NASH-RELATED HEPATOCARCINOGENESIS AS POTENTIAL TARGETS FOR SURVEILLANCE

Understanding the sequence from NAFLD to HCC and the impact of additive risks such as type II diabetes mellitus [Figure 1], genetic polymorphisms, and stool microbiome are increasingly becoming the focus of current research.

Inflammation per se, which defines NASH, is a clinically relevant trigger of carcinogenesis, even without the basis of cirrhosis of the liver. While an altered lipid and glucose metabolism contributes to hepatic steatosis in the context of the metabolic syndrome, the interplay of genetic variations, mitochondrial dysfunction, altered immune response, and an imbalance of the microbiome cause a progression of simple fatty liver to NASH, and in the “worst case” scenario, HCC.

GENETIC FACTORS

For certain gene polymorphisms, there is a direct relationship between the prevalence of NAFLD and the risk of progression to advanced NASH fibrosis.

The polymorphism of the patatin-like-phospholipase-domain-containing-3 (PNPLA3) gene leads to increased hepatic lipid accumulation and an alteration in retinol storage in the liver. Independent of potential disruptive factors such as body mass index (BMI), diabetes, and advanced fibrosis, there is a 3-fold increased HCC risk due to PNPLA3^[71].

The polymorphism of the transmembrane 6-superfamily member 2 (TM6SF2) gene manifests itself as a transport disorder of pre-VLDL (very low-density lipoprotein) particles. There is a correlation with the extent of steatosis and progression of fibrosis in NASH, regardless of obesity, diabetes, and PNPLA3 genotype. The direct role of this TM6SF2 variant in hepatocarcinogenesis has not yet been fully elucidated; it is possibly the profibrogenic effect that indirectly promotes progression to HCC^[72].

Genetic variability is not completely explained by these common aforementioned variants and many of the phenotypic differences potentially result from gene-environment interactions. NAFLD development and progression are also modulated by epigenetic factors, in particular microRNAs (miRNAs). At the post-transcriptional level, they control many complementary target mRNAs. Their dysregulation have a high predictive value in NAFLD development and progression^[73,74]. Epigenetic changes, which cause aberrant DNA methylation, as well as the expression of various micro-RNAs (e.g., miR-21, miR-29, miR-23, miR-155, miR-221, miR-222, miR-106, miR-93, and miR-519) are additive drivers of carcinogenesis. There is a direct influence on the most relevant tumor-associated signal cascades [transforming growth factor beta, wingless and INT-1 (Wnt)/ β -catenin, mitogenactivated protein kinase, and phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin]^[75].

GUT MICROBIOME ANALYSES AS FUTURE PREDICTORS OF HCC RISK

Distinct changes or shifts in the composition of intestinal bacteria have been described for different intestinal metabolic and inflammatory diseases^[76-78]. Shifts of certain bacterial strains may also affect the formation of different bacterial-derived metabolic active components such as short-chain fatty acids, bile acids, ethanol, cytokines, or other inflammatory metabolites that may affect the host and possibly promote cancer-related risk factors or diseases^[79]. The microbiota of an obese host has an impact on bodyweight and gut permeability, and it affects the release of gut-derived components that may also influence the progression of inflammatory mechanisms and promote the formation of cancer. Mouse models of fecal microbiota transfer (FMT) from obese to lean animals could show that microbiota affect bodyweight^[80]. FMT is a potential therapeutic tool that has been used to treat *Clostridium difficile* infections^[81]. In mouse studies, FMT was shown to be a useful treatment to increase abundances of beneficial bacterial groups such as Christensenellaceae and *Lactobacillus* to alleviate the progression of NASH-development^[82].

The modulation of the gut microbiota by FMT to treat or study different metabolism-related diseases has been taken into account for many years^[83]. It has been shown that antibiotic treatments, in order to modulate the gut microbiota, may reduce the risk of hepatic carcinogenesis^[84]. In a high fat diet mouse model of NASH, antibiotic treatments were associated with a reduction of toxic secondary bile acids^[85].

NAFL and especially its progressive inflammatory form NASH are often related to the formation of HCC. Here, nutrition, metabolic disturbances, and related comorbidities such as diabetes may influence the composition of the gut microbiota. Changes in the abundances of different bacterial groups have been described within different patient groups.

The metabolism of certain bacterial groups affects the mucosal barrier, hepatic inflammation, fibrogenesis, and tumorigenesis^[86]. The gut microbiota has an impact on energy balance, altering the uptake of calories derived from food and even alcohol^[87]. Emerging data indicate that certain characteristic changes in the gut microbiome are associated with NAFLD and even with cirrhosis, which is a main driver of HCC-development^[76,88,89]. It has not only been shown in NAFLD-related HCC, but also in viral hepatitis-related HCC (hepatitis B) that makes a modification of specific gut microbiota, a potential therapeutic option for HCC^[90].

In a study comparing NASH and NASH-HCC patients with or without cirrhosis, alterations in bacterial groups regulating bile acid metabolism had an impact on hepatic fibrogenesis and liver injury. Alterations of the bile acid pool was accompanied with increased abundances of different bacterial strains, especially *Lactobacilli* and *Bacteroides* which were associated with changed liver stiffness and liver injury^[91]. In NASH patients, the abundance of bile salt hydrolase (an enzyme involved in deconjugation of bile acids) expressing bacteria is shifted, which leads to increased bile acid levels as well as an altered composition of the bile acid pool tending to an increased amount of secondary conjugated bile acids^[92]. Changes within the

bile acid composition can be related to advanced fibrosis in NASH-HCC which indicates its important role in fibrosis-related tumorigenesis^[93,94]. In obese children, an increased abundance of *Lactobacillus* strains was associated with NAFLD and NASH. while in colon cancer increased *Lactobacillus* abundance was related to an anti-tumor effect^[95].

FUTURE PERSPECTIVES

In its current state, HCC biomarker research is far from fulfilling its promises. Therefore, it has to be subject of future investigations to elucidate the role of technologies that might complement current biomarker-based surveillance strategies. The identification of a subset of patients at the highest risk is critical to concentrate the effort and resources of regular HCC screening^[96]. Chromosomal aberrations, epigenetic abnormality, and changes of gene expression are involved in hepatocarcinogenesis. Besides microbiome analysis, omics profiling (e.g., transcriptomics, proteomics, metabolomics) has been derived using several candidate HCC risk biomarkers which could refine HCC screening by enabling individual risk-stratified patient management. High-throughput omics technologies have been widely applied, aiming at the discovery of candidate biomarkers. Different types of biomolecules have been explored as sources of information to predict HCC risk. Transcriptomic dysregulations in chronic hepatopathies capture the functional molecular status supporting carcinogenesis. Circulating nucleic acids, proteins, and metabolites could serve as measures of molecular HCC risk. Large amounts of data on genetic and epigenetic abnormalities, gene expression profiles, and proteomics are available. Here, bioinformatics and network medicine increasingly play a pivotal role to organize and analyze the accumulated data^[97]. Those analyses may facilitate the identification of a distinct niche of application for each individual biomarker.

CONCLUSION

HCC surveillance, in line with guidelines, significantly improves survival outcomes due to a higher proportion of patients diagnosed in an earlier stage of HCC, allowing for curative treatment options. According to most international guideline, recommendations have considered ultrasound as the method of choice for screening. US alone lacks sensitivity in the detection of small lesions, particularly in advanced cirrhosis and obesity^[98], factors that are compounded by varying skill levels of investigators and available technology. Nevertheless, numerous guidelines omitted the additional determination of AFP, despite the fact that previous trials clearly demonstrated that the utilization of AFP or biomarker-based scores such as GALAD complimentary to ultrasound resulted in a significant improvement in sensitivity while preserving high level of specificity. During the past decade, several biomarkers such as AFP-L3, DCP, osteopontin, glypican-3, and others were evaluated, however, only few markers reached longitudinal retrospective phase III development. Widely lacking are large multinational phase IV prospective screening trials confirming the benefit of the markers or their combinations for surveillance.

Facing increasing global HCC mortality, NASH has become a major risk factor for HCC development, even in the absence of cirrhosis^[5,99,100], therefore the definition of the population at risk has to be redefined for future HCC diagnostic and treatment guidelines to address this epidemiological shift. Nevertheless, cost-effectiveness analyses do not support surveillance in the entire population with NAFLD who do not have cirrhosis or advanced fibrosis. Here, continued efforts of risk stratification with factors such as gender and age in conjunction with NAFLD-associated HCC risks such as PNPLA3 or TM6SF2 gene polymorphisms or distinct alterations of the microbiome and bile acid metabolism may facilitate a more concise definition of populations at HCC risk within this cohort.

Furthermore, diabetes mellitus needs to be recognized as an individual predisposing risk factor for HCC development in the presence and even in the absence of concomitant NASH.

In the near future, the combination of standard of care ultrasound and biomarker-based screening approaches seem to be the next step to increase sensitivity and specificity of HCC surveillance.

Among the efforts of optimizing screening algorithms, the education of gate keepers such as primary care physicians, health care associates, and also the population at risk per se is another pivotal factor to increase the rate of HCC early detection.

DECLARATIONS

Authors' contributions

Conception or design of the work: Best J, Bechmann LP

Drafting the article: Best J, Sydor S

Final approval of the version to be published: Best J, Sydor S, Bechmann LP, Canbay A

Critical revision of the article: Bechmann LP, Canbay A

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REFERENCES

1. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011;365:1118-27.
2. El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: where are we? Where do we go? *Hepatology* 2014;60:1767-75.
3. Rich NE, Hester C, Odewole M, Murphy CC, Parikh ND, et al. Racial and ethnic differences in presentation and outcomes of hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2019;17:551-9.e1.
4. De Minicis S, Agostinelli L, Rychlicki C, Sorice GP, Saccomanno S, et al. HCC development is associated to peripheral insulin resistance in a mouse model of NASH. *PLoS One* 2014;9:e97136.
5. White DL, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clin Gastroenterol Hepatol* 2012;10:1342-59.e2.
6. Ertle J, Dechene A, Sowa JP, Penndorf V, Herzer K, et al. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *Int J Cancer* 2011;128:2436-43.
7. Kudo M. Early detection and curative treatment of early-stage hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2005;3:S144-8.
8. Weinmann A, Koch S, Niederle IM, Schulze-Bergkamen H, König J, et al. Trends in epidemiology, treatment, and survival of hepatocellular carcinoma patients between 1998 and 2009: an analysis of 1066 cases of a German HCC Registry. *J Clin Gastroenterol* 2014;48:279-89.
9. Shah SA, Smith JK, Li Y, Ng SC, Carroll JE, et al. Underutilization of therapy for hepatocellular carcinoma in the medicare population. *Cancer* 2011;117:1019-26.
10. Sonnenday CJ, Dimick JB, Schulick RD, Choti MA. Racial and geographic disparities in the utilization of surgical therapy for hepatocellular carcinoma. *J Gastrointest Surg* 2007;11:1636-46; discussion 46.

11. Wang Y, Luo Q, Li Y, Deng S, Wei S, et al. Radiofrequency ablation versus hepatic resection for small hepatocellular carcinomas: a meta-analysis of randomized and nonrandomized controlled trials. *PLoS One* 2014;9:e84484.
12. Zheng SS, Xu X, Wu J, Chen J, Wang WL, et al. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 2008;85:1726-32.
13. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73-84.
14. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol* 2009;27:1485-91.
15. Arends J, Bartenstein P, Bechstein W, Bernatik T, Bitzer M, et al. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF): Diagnostik und Therapie des hepatozellulären Karzinoms, Kurzversion 1.0, AWMF Registrierungsnummer: 032-053OL, 2013. Available from: https://www.leitlinienprogramm-onkologie.de/fileadmin/user_upload/Downloads/Leitlinien/HCC/S3-HCC-OL-Langversion-V1.0.pdf. [Last accessed on 14 Aug 2020]
16. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358-80.
17. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;66:908-43.
18. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017;11:317-70.
19. Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, et al. Asian pacific association for the study of the liver consensus recommendations on hepatocellular carcinoma. *Hepatol Int* 2010;4:439-74.
20. Kudo M, Izumi N, Kokudo N, Matsui O, Sakamoto M, et al. Management of hepatocellular carcinoma in Japan: Consensus-Based Clinical Practice Guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. *Dig Dis* 2011;29:339-64.
21. Bruix J, Sherman M, Practice Guidelines Committee AASLD. Management of hepatocellular carcinoma. *Hepatology* 2005;42:1208-36.
22. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53:1020-2.
23. Bolondi L, Sofia S, Siringo S, Gaiani S, Casali A, et al. Surveillance programme of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost effectiveness analysis. *Gut* 2001;48:251-9.
24. Dyson J, Jaques B, Chattopadhyay D, Lochan R, Graham J, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014;60:110-7.
25. Siegel AB, Zhu AX. Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. *Cancer* 2009;115:5651-61.
26. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, et al. Surveillance imaging and alpha fetoprotein for early detection of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. *Gastroenterology* 2018;154:1706-18.e1.
27. Araujo AR, Rosso N, Bedogni G, Tiribelli C, Bellentani S. Global epidemiology of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: what we need in the future. *Liver Int* 2018;38 Suppl 1:47-51.
28. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002;346:1221-31.
29. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011;34:274-85.
30. Wong RJ, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014;59:2188-95.
31. Younossi Z, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, et al, Global Nonalcoholic Steatohepatitis Council. Nonalcoholic steatohepatitis is the fastest growing cause of hepatocellular carcinoma in liver transplant candidates. *Clin Gastroenterol Hepatol* 2019;17:748-55.e3.
32. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, et al. Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease. *Gastroenterology* 2018;155:1828-37.e2.
33. Weinmann A, Alt Y, Koch S, Nelles C, Duber C, et al. Treatment and survival of non-alcoholic steatohepatitis associated hepatocellular carcinoma. *BMC Cancer* 2015;15:210.
34. Di Bisceglie AM, Hoofnagle JH. Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. *Cancer* 1989;64:2117-20.
35. Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, et al. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C trial. *J Hepatol* 2005;43:434-41.
36. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology* 1995;22:432-8.
37. Li D, Mallory T, Satomura S. AFP-L3: a new generation of tumor marker for hepatocellular carcinoma. *Clin Chim Acta* 2001;313:15-9.
38. Lieberman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med* 1984;310:1427-31.
39. Shang S, Plymoth A, Ge S, Feng Z, Rosen HR, et al. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. *Hepatology* 2012;55:483-90.
40. Wang XY, Degos F, Dubois S, Tessitore S, Allegretta M, et al. Glypican-3 expression in hepatocellular tumors: diagnostic value for preneoplastic lesions and hepatocellular carcinomas. *Hum Pathol* 2006;37:1435-41.
41. Abelev GI, Perova SD, Khramkova NI, Postnikova ZA, Irlin IS. Production of embryonal alpha-globulin by transplantable mouse hepatomas. *Transplantation* 1963;1:174-80.
42. Daniele B, Bencivenga A, Megna AS, Tinessa V. Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma.

- Gastroenterology 2004;127:S108-12.
43. Kumada T, Nakano S, Takeda I, Kiriya S, Sone Y, et al. Clinical utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: special reference to imaging diagnosis. *J Hepatol* 1999;30:125-30.
 44. Yamashita F, Tanaka M, Satomura S, Tanikawa K. Prognostic significance of Lens culinaris agglutinin A-reactive alpha-fetoprotein in small hepatocellular carcinomas. *Gastroenterology* 1996;111:996-1001.
 45. Khien VV, Mao HV, Chinh TT, Ha PT, Bang MH, et al. Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. *Int J Biol Markers* 2001;16:105-11.
 46. Taketa K, Okada S, Win N, Hlaing NK, Wind KM. Evaluation of tumor markers for the detection of hepatocellular carcinoma in Yangon General Hospital, Myanmar. *Acta Med Okayama* 2002;56:317-20.
 47. Marrero JA, Su GL, Wei W, Emick D, Conjeevaram HS, et al. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. *Hepatology* 2003;37:1114-21.
 48. Volk ML, Hernandez JC, Su GL, Lok AS, Marrero JA. Risk factors for hepatocellular carcinoma may impair the performance of biomarkers: a comparison of AFP, DCP, and AFP-L3. *Cancer Biomark* 2007;3:79-87.
 49. Yuen MF, Lai CL. Serological markers of liver cancer. *Best Pract Res Clin Gastroenterol* 2005;19:91-9.
 50. Toyoda H, Kumada T, Tada T, Kaneoka Y, Maeda A, et al. Clinical utility of highly sensitive Lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein <20 ng/mL. *Cancer Sci* 2011;102:1025-31.
 51. Choi JY, Jung SW, Kim HY, Kim M, Kim Y, et al. Diagnostic value of AFP-L3 and PIVKA-II in hepatocellular carcinoma according to total-AFP. *World J Gastroenterol* 2013;19:339-46.
 52. Koike Y, Shiratori Y, Sato S, Obi S, Teratani T, et al. Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma: a prospective analysis of 227 patients. *Cancer* 2001;91:561-9.
 53. Lok AS, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, et al. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009;136:138-48.
 54. Johnson PJ, Pirrie SJ, Cox TF, Berhane S, Teng M, et al. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. *Cancer Epidemiol Biomarkers Prev* 2014;23:144-53.
 55. Chan SL, Mo F, Johnson PJ, Siu DY, Chan MH, et al. Performance of serum alpha-fetoprotein levels in the diagnosis of hepatocellular carcinoma in patients with a hepatic mass. *HPB (Oxford)* 2014;16:366-72.
 56. Berhane S, Toyoda H, Tada T, Kumada T, Kagebayashi C, et al. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. *Clin Gastroenterol Hepatol* 2016;14:875-86.e6.
 57. Best J, Bilgi H, Heider D, Schotten C, Manka P, et al. The GALAD scoring algorithm based on AFP, AFP-L3, and DCP significantly improves detection of BCLC early stage hepatocellular carcinoma. *Z Gastroenterol* 2016;54:1296-305.
 58. Best J, Bechmann LP, Sowa JP, Sydor S, Dechene A, et al. GALAD score detects early hepatocellular carcinoma in an international cohort of patients with nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2020;18:728-35.e4.
 59. Fedarko NS, Jain A, Karadag A, Van Eman MR, Fisher LW. Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. *Clin Cancer Res* 2001;7:4060-6.
 60. Zhao J, Dong L, Lu B, Wu G, Xu D, et al. Down-regulation of osteopontin suppresses growth and metastasis of hepatocellular carcinoma via induction of apoptosis. *Gastroenterology* 2008;135:956-68.
 61. Kim J, Ki SS, Lee SD, Han CJ, Kim YC, et al. Elevated plasma osteopontin levels in patients with hepatocellular carcinoma. *Am J Gastroenterol* 2006;101:2051-9.
 62. Wan HG, Xu H, Gu YM, Wang H, Xu W, et al. Comparison osteopontin vs AFP for the diagnosis of HCC: a meta-analysis. *Clin Res Hepatol Gastroenterol* 2014;38:706-14.
 63. Li J, Chen X, Dai M, Huang S, Chen J, et al. Diagnostic accuracy of osteopontin plus alpha-fetoprotein in the hepatocellular carcinoma: a meta-analysis. *Clin Res Hepatol Gastroenterol* 2017;41:543-53.
 64. Filmus J, Capurro M. The role of glypican-3 in the regulation of body size and cancer. *Cell Cycle* 2008;7:2787-90.
 65. Capurro M, Wanless IR, Sherman M, Deboer G, Shi W, et al. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology* 2003;125:89-97.
 66. Capurro MI, Xiang YY, Lobe C, Filmus J. Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. *Cancer Res* 2005;65:6245-54.
 67. Nakatsura T, Yoshitake Y, Senju S, Monji M, Komori H, et al. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. *Biochem Biophys Res Commun* 2003;306:16-25.
 68. Jia X, Liu J, Gao Y, Huang Y, Du Z. Diagnosis accuracy of serum glypican-3 in patients with hepatocellular carcinoma: a systematic review with meta-analysis. *Arch Med Res* 2014;45:580-8.
 69. Qiao SS, Cui ZQ, Gong L, Han H, Chen PC, et al. Simultaneous measurements of serum AFP, GPC-3 and HCCR for diagnosing hepatocellular carcinoma. *Hepatogastroenterology* 2011;58:1718-24.
 70. Yang JD, Addissie BD, Mara KC, Harmsen WS, Dai J, et al. GALAD score for hepatocellular carcinoma detection in comparison to liver ultrasound and proposal of GALADUS score. *Cancer Epidemiol Biomarkers Prev* 2019;28:531-8.
 71. Hassan MM, Kaseb A, Etzel CJ, El-Serag H, Spitz MR, et al. Genetic variation in the PNPLA3 gene and hepatocellular carcinoma in USA: risk and prognosis prediction. *Mol Carcinog* 2013;52 Suppl 1:E139-47.
 72. Tang S, Zhang J, Mei TT, Guo HQ, Wei XH, et al. Association of TM6SF2 rs58542926 T/C gene polymorphism with hepatocellular carcinoma: a meta-analysis. *BMC Cancer* 2019;19:1128.
 73. Dongiovanni P, Meroni M, Longo M, Fargion S, Fracanzani AL. miRNA signature in NAFLD: a turning point for a non-invasive

- diagnosis. *Int J Mol Sci* 2018;19.
74. Zhang C, Wang P, Li Y, Huang C, Ni W, et al. Role of MicroRNAs in the development of hepatocellular carcinoma in nonalcoholic fatty liver disease. *Anat Rec (Hoboken)* 2019;302:193-200.
 75. Kutlu O, Kaleli HN, Ozer E. Molecular pathogenesis of nonalcoholic steatohepatitis- (NASH-) related hepatocellular carcinoma. *Can J Gastroenterol Hepatol* 2018;2018:8543763.
 76. Chassaing B, Etienne-Mesmin L, Gewirtz AT. Microbiota-liver axis in hepatic disease. *Hepatology* 2014;59:328-39.
 77. Konturek PC, Harsch IA, Konturek K, Schink M, Konturek T, et al. Gut(-)Liver Axis: how do gut bacteria influence the liver? *Med Sci (Basel)* 2018;6.
 78. Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 2014;146:1489-99.
 79. Bashiardes S, Shapiro H, Rozin S, Shibolet O, Elinav E. Non-alcoholic fatty liver and the gut microbiota. *Mol Metab* 2016;5:782-94.
 80. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027-31.
 81. Khoruts A, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. *Nat Rev Gastroenterol Hepatol* 2016;13:508-16.
 82. Zhou D, Pan Q, Shen F, Cao HX, Ding WJ, et al. Total fecal microbiota transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of gut microbiota. *Sci Rep* 2017;7:1529.
 83. de Groot PF, Frissen MN, de Clercq NC, Nieuwdorp M. Fecal microbiota transplantation in metabolic syndrome: History, present and future. *Gut Microbes* 2017;8:253-67.
 84. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 2012;21:504-16.
 85. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013;499:97-101.
 86. Ponziani FR, Bhoori S, Castelli C, Putignani L, Rivoltini L, et al. Hepatocellular carcinoma is associated with gut microbiota profile and inflammation in nonalcoholic fatty liver disease. *Hepatology* 2019;69:107-20.
 87. Schonewille M, de Boer JF, Groen AK. Bile salts in control of lipid metabolism. *Curr Opin Lipidol* 2016;27:295-301.
 88. Mouzaki M, Wang AY, Bandsma R, Comelli EM, Arendt BM, et al. Bile acids and dysbiosis in non-alcoholic fatty liver disease. *PLoS One* 2016;11:e0151829.
 89. Neuschwander-Tetri BA, Van Natta ML, Tonascia J, Brunt EM, Kleiner DE. Trials of obeticholic acid for non-alcoholic steatohepatitis - Authors' reply. *Lancet* 2015;386:28-9.
 90. Liu Q, Li F, Zhuang Y, Xu J, Wang J, et al. Alteration in gut microbiota associated with hepatitis B and non-hepatitis virus related hepatocellular carcinoma. *Gut Pathog* 2019;11:1.
 91. Sydor S, Best J, Messerschmidt I, Manka P, Vilchez-Vargas R. Altered microbiota diversity and bile acid signaling in cirrhotic and noncirrhotic NASH-HCC. *Clin Transl Gastroenterol* 2020;11:e00131.
 92. Jiao N, Baker SS, Chapa-Rodriguez A, Liu W, Nugent CA, et al. Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD. *Gut* 2018;67:1881-91.
 93. Bechmann LP, Kocabayoglu P, Sowa JP, Sydor S, Best J, et al. Free fatty acids repress small heterodimer partner (SHP) activation and adiponectin counteracts bile acid-induced liver injury in superobese patients with nonalcoholic steatohepatitis. *Hepatology* 2013;57:1394-406.
 94. Caussy C, Tripathi A, Humphrey G, Bassirian S, Singh S, et al. A gut microbiome signature for cirrhosis due to nonalcoholic fatty liver disease. *Nat Commun* 2019;10:1406.
 95. Nowak A, Paliwoda A, Blasiak J. Anti-proliferative, pro-apoptotic and anti-oxidative activity of *Lactobacillus* and *Bifidobacterium* strains: a review of mechanisms and therapeutic perspectives. *Crit Rev Food Sci Nutr* 2019;59:3456-67.
 96. Fujiwara N, Qian T, Koneru B, Hoshida Y. Omics-derived hepatocellular carcinoma risk biomarkers for precision care of chronic liver diseases. *Hepatol Res* 2020;50:817-30.
 97. Pei Y, Zhang T, Renault V, Zhang X. An overview of hepatocellular carcinoma study by omics-based methods. *Acta Biochim Biophys Sin (Shanghai)* 2009;41:1-15.
 98. Del Poggio P, Olmi S, Ciccarese F, Di Marco M, Rapaccini GL, et al. Factors that affect efficacy of ultrasound surveillance for early stage hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2014;12:1927-33.e2.
 99. Chagas AL, Kikuchi LO, Oliveira CP, Vezozzo DC, Mello ES, et al. Does hepatocellular carcinoma in non-alcoholic steatohepatitis exist in cirrhotic and non-cirrhotic patients? *Braz J Med Biol Res* 2009;42:958-62.
 100. Kawada N, Imanaka K, Kawaguchi T, Tamai C, Ishihara R, et al. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. *J Gastroenterol* 2009;44:1190-4.

Review

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CT and MRI of the liver for hepatocellular carcinoma

Cynthia Santillan

Department of Radiology, University of California San Diego, San Diego, CA 92103, USA.

Correspondence to: Dr. Cynthia Santillan, Department of Radiology, University of California San Diego, 200 W Arbor Dr. MC 8756, San Diego, CA 92103, USA. E-mail: csantillan@health.ucsd.edu

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Abstract

Computed tomography (CT) and magnetic resonance imaging (MRI) are commonly used modalities for the imaging based diagnosis and staging of hepatocellular carcinoma (HCC). The Liver Imaging Reporting and Data System (LI-RADS) was initially released in 2011 in an effort to standardize the interpretation and reporting of these studies in patients at increased risk for the development of HCC. With the release of LI-RADS v2018, LI-RADS has reached two important milestones - 10 years since the formation of the American College of Radiology supported LI-RADS committee and integration of LI-RADS into the 2018 American Association for the Study of Liver Disease practice guidance for HCC. In this article, we will discuss recent changes to LI-RADS with v2018, technical recommendations for the performance of CT and MRI in patients at risk for HCC, and critical imaging features in the LI-RADS algorithm.

Keywords: Hepatocellular carcinoma, Liver Imaging Reporting and Data System, magnetic resonance imaging, computed tomography

INTRODUCTION

LI-RADS was originally developed to provide a consistent method of conveying relative concern for the presence of hepatocellular carcinoma (HCC) or other malignancies based on Computed tomography(CT) or magnetic resonance imaging (MRI) of a patient at increased risk of developing HCC^[1,2]. This relative concern for HCC, malignancy, or tumor in vein is conveyed by the different categories shown in Table 1. Liver Imaging Reporting and Data System (LI-RADS) v2018 has now evolved to also include guidance



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Table 1. LI-RADS v2018 diagnostic categories

Category	Definition
LR-1	Definitely benign
LR-2	Probably benign
LR-3	Intermediate probability of malignancy
LR-4	Probably HCC
LR-5	Definitely HCC
LR-M	Probably or definitely malignancy but not HCC specific
LR-TIV	Definite tumor in vein
LR-NC	Cannot be categorized due to image degradation or omission

LI-RADS: Liver Imaging Reporting and Data System; HCC: hepatocellular carcinoma; LR-TIV: LI-RADS Tumor in Vein

CT/MRI Diagnostic Table

Arterial phase hyperenhancement (APHE)		No APHE		Nonrim APHE		
Observation size (mm)		< 20	≥ 20	< 10	10-19	≥ 20
Count additional major features: • Enhancing “capsule” • Nonperipheral “washout” • Threshold growth	None	LR-3	LR-3	LR-3	LR-3	LR-4
	One	LR-3	LR-4	LR-4	LR-4 LR-5	LR-5
	≥ Two	LR-4	LR-4	LR-4	LR-5	LR-5



Observations in this cell are categorized based on one additional major feature:

- LR-4 – if enhancing “capsule”
- LR-5 – if nonperipheral “washout” **OR** threshold growth

Figure 1. LI-RADS Diagnostic Table. Reproduced with permission from the American College of Radiology LI-RADS version 2018 manual. Available from: <https://www.acr.org/Clinical-resources/Reporting-and-Data-Systems/LI-RADS>^[4]. LI-RADS: Liver Imaging Reporting and Data System

for the evaluation of contrast-enhanced ultrasound, screening ultrasound, and CT and MRI following locoregional therapy for HCC. LI-RADS v2018 also includes technical recommendations for the performance of CT and MRI for the evaluation of HCC. The development of LI-RADS has been in the context of patients with cirrhosis, chronic hepatitis B infection without cirrhosis, or a history of prior HCC. LI-RADS should not be used in patients with vascular causes for their cirrhosis due to the decreased specificity of arterial hyperenhancement for malignancy in these patients^[3].

LI-RADS V2018

The LI-RADS algorithm provides a step by step approach for consistent categorization of observations seen on CT and MR imaging of patients with cirrhosis, chronic hepatitis B viral infection, or a history of HCC^[4]. Observations that are definitely benign (LR-1) or probably benign (LR-2) can be categorized based on typical imaging features associated with specific benign entities, such as hepatic hemangiomas, cysts, or perfusion alterations. Observations that demonstrate imaging features suggestive of malignancies other than HCC are categorized as LR-M. These imaging features include peripheral washout appearance, targetoid restricted diffusion, and rim arterial phase hyperenhancement. Observations that are not initially categorized as LR-NC, LR-1, LR-2, LR-M, or LI-RADS Tumor in Vein (LR-TIV) are then categorized using the LI-RADS Diagnostic Table [Figure 1]. The diagnostic table guides the radiologist to category LR-3, LR-4, or LR-5 based on the presence or absence of major features as discussed in further detail below.

Table 2. LI-RADS v2018 minimum technical recommendations for CT

Feature	Recommendation
Scanner configuration	≥ 8 detector rows
Multiplanar reformations	Suggested
Slice thickness	≤ 5 mm required for axial reconstructions 3-2.5 mm suggested for multiplanar reformations if obtained
Precontrast imaging	Suggested for patients that have had prior locoregional therapy, optional otherwise
Contrast-enhanced phases	Late arterial Portal venous Delayed (2-5 min)
Contrast administration	Injection rate of ≥ 3 mL/s ≥ 300 mgI/mL for dose of 1.5-2.5 mL/kg Saline chaser bolus (30-40 mL)

Adapted with permission from American College of Radiology Liver Imaging Reporting and Data System version 2018 manual. Available from: <https://www.acr.org/Clinical-resources/Reporting-and-Data-Systems/LI-RADS>^[4]. LI-RADS: Liver Imaging Reporting and Data System; CT: computed tomography

Although an update to LI-RADS was released in 2017, a new version was released only a year later to allow for two changes to CT/MRI LI-RADS. Both changes were made to allow for unification between LI-RADS and American Association for the Study of Liver Disease (AASLD) practice guidelines. The first change was the definition of threshold growth. Previously, threshold growth was defined as ≥ 50% size increase of a mass in ≤ 6 months, ≥ 100% increase in size over > 6 months, or a new ≥ 10 mm observation developing in ≤ 24 months. The definition of threshold growth in LI-RADS v2018 is now restricted to only ≥ 50% increase in size of a mass over ≤ 6 months. This stricter definition is now concordant with growth criteria used by the AASLD and the Organ Procurement and Transplantation Network (OPTN). The second change was to the categorization of 10-19 mm observations with arterial phase hyperenhancement and one additional major feature in the LI-RADS Diagnostic Table. Previously, these observations were categorized based on which imaging features were present and required the use of “-g” and “-us” added to the LR-5 to specify the features used. This portion of the table has now been simplified so that if a 10-19 mm observation with arterial phase hyperenhancement also demonstrates nonperipheral “washout” or threshold growth, it is categorized as LR-5. If a 10-19 mm observation with arterial phase hyperenhancement only demonstrates the additional feature of an enhancing “capsule”; however, it is designated as LR-4.

TECHNICAL RECOMMENDATIONS

Consistent imaging techniques are necessary to enable reproducibility of LI-RADS categories between radiologists at different institutions. The wide variety of equipment, technical parameters, and sequences available throughout the world can lead to difficulty in evaluating critical imaging features for LI-RADS category assessment. To address these inconsistencies, the Technique Working Group of LI-RADS has developed minimum technical requirements for the performance of CT and MRI in patients at risk for developing HCC, summarized in [Tables 2 and 3](#)^[5].

Vascular phases for CT and MRI

Multiphase contrast enhanced imaging is required to make the imaging diagnosis of HCC (LI-RADS 5) confidently and adequately evaluate the regional vascular anatomy and patency. Pre-contrast imaging provides information on pre-existing hyperattenuating and T1 hyperintense material, which are often seen as a sequelae of locoregional therapies. This bright material can obscure or mimic enhancement on post-contrast phases. Although pre-contrast T1-weighted imaging is required for MRI, a pre-contrast phase is optional for CT imaging unless the patient has had locoregional therapy due to the low likelihood of pre-existing hepatic high attenuation material in a patient without locoregional therapy and associated increased radiation from an additional CT imaging phase.

Table 3. LI-RADS v2018 minimum technical recommendations for MRI

Feature	Recommendation
Scanner strength	1.5 or 3 Tesla
Coil type	Phased array multichannel torso coil
Slice thickness	≤ 5 mm required for multiphase contrast-enhanced sequences ≤ 8 mm with ≤ 2 mm slice gap for other sequences
Multiphase contrast-enhanced phases (required)	T1 weighted with 3D acquisition and fat suppression strongly recommended Precontrast Late arterial Portal venous Delayed (2-5 min) if using ECA or gadobenate
Contrast administration	Transitional phase (2-5 min) if using gadoxetate Hepatobiliary phase (approx 20 min) if using gadoxetate ECA or gadobenate or gadoxetate, weight adjusted dose Injection rate of 1-2 mL/s Saline chaser bolus (30-40 mL)
Other required sequences	Unenhanced T1-weighted opposed-phase and in-phase imaging T2-weighted imaging (fat suppression optional)
Other suggested sequences	Diffusion weighted imaging Multiplanar acquisitions, such as coronal T2-weighted imaging

Adapted with permission from American College of Radiology Liver Imaging Reporting and Data System version 2018 manual. Available from: <https://www.acr.org/Clinical-resources/Reporting-and-Data-Systems/LI-RADS>^[4]. LI-RADS: Liver Imaging Reporting and Data System; MRI: magnetic resonance imaging; ECA: extracellular contrast agent

The first required post-contrast phase is the late hepatic arterial phase which refers to the phase of contrast when there is enhancement of the portal vein but no antegrade enhancement of the hepatic veins. As some HCCs are not conspicuous until the late hepatic arterial phase, earlier arterial phase imaging can result in reduced sensitivity for HCC^[6]. The arterial phase is also required for evaluation of the LI-RADS major feature of arterial phase hyperenhancement.

The portal venous phase occurs when antegrade enhancement of the portal and hepatic veins is present and there is peak parenchymal enhancement of the liver. Portal venous phase imaging is often the phase that best demonstrates the LI-RADS major feature of “washout appearance” due to the peak enhancement of background liver.

Delayed phase refers to the imaging phase performed after the portal venous phase, typically 3-5 min following the injection of an extracellular contrast agent (ECA) or gadobenate. The vessels and parenchyma all remain enhanced during this phase, but are overall decreased in brightness compared to the portal venous phase. A combination of the portal venous phase and delayed phase can more reliably demonstrate the LI-RADS major features of “washout appearance” and “capsule appearance” than the portal venous phase alone^[7]. Examples of these dynamic imaging phases are shown in [Figure 2](#).

The transitional and hepatobiliary phases are only available on MRI following the administration of hepatobiliary contrast agents (HBAs). Hepatobiliary contrast agents such as gadobenate and gadoxetate are gadolinium contrast agents that have a portion of the contrast agent enter the hepatocytes with subsequent excretion into the biliary system. The transitional phase occurs between the portal venous phase and hepatobiliary phase and demonstrates persistent vascular enhancement with increasing hepatic parenchymal enhancement. Although both gadobenate and gadoxetate are capable of demonstrating a hepatobiliary phase, gadobenate does not have sufficiently strong parenchymal uptake to allow for a transitional phase. The hepatobiliary phase refers to a delayed phase following HBA administration that shows hyperintensity of hepatic parenchyma and relative hypointensity of the hepatic vessels. This phase provides high sensitivity for observations that do not have hepatocytes capable of contrast uptake, which includes a broad spectrum of both benign and malignant entities^[7]. Patients with severe hepatic dysfunction, however, may not have sufficient hepatocyte uptake of the contrast agent to yield an adequate

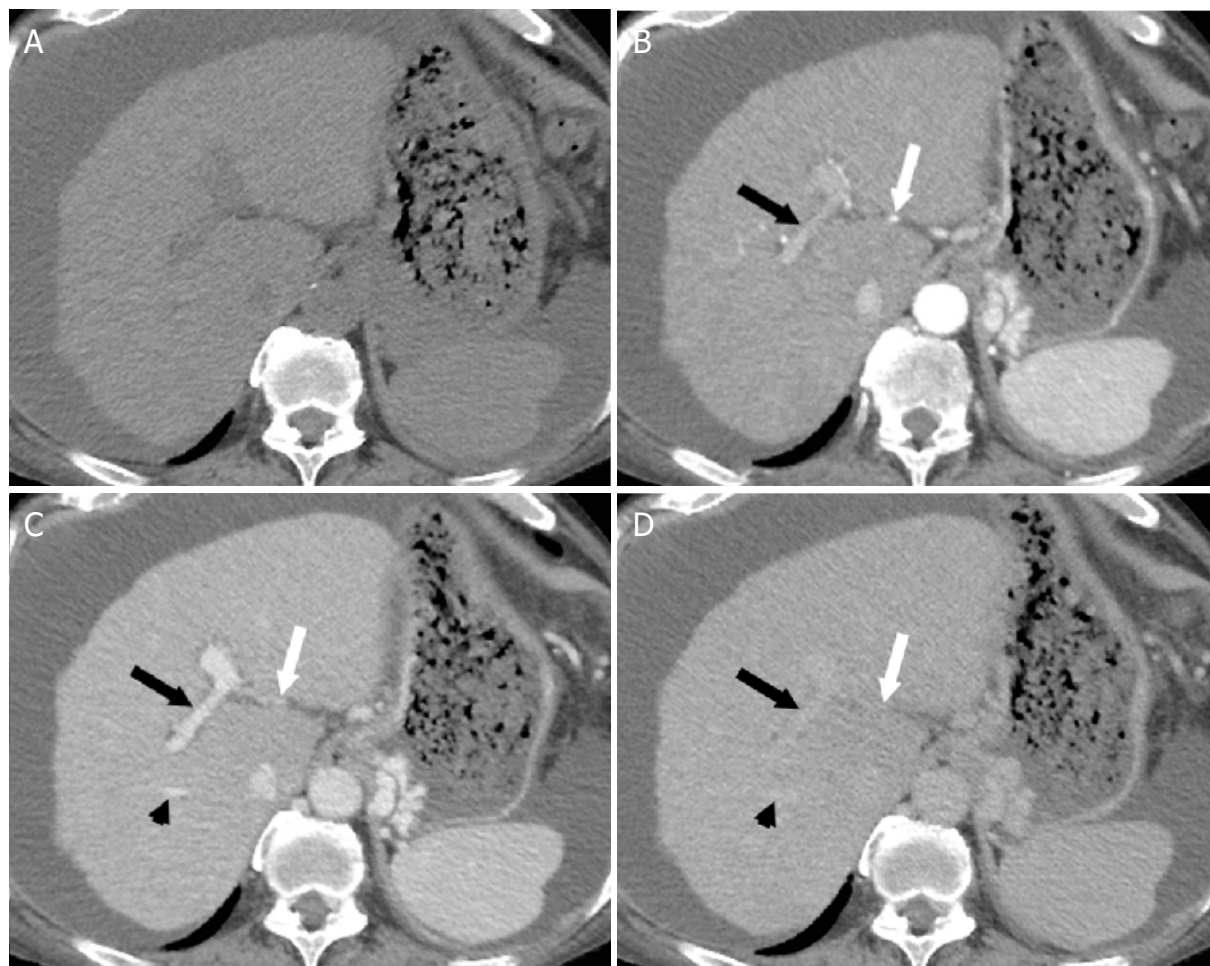


Figure 2. Dynamic Imaging for Evaluation for Hepatocellular Carcinoma. Axial CT images demonstrating the pre-contrast (A), hepatic arterial (B), portal venous (C), and delayed (D) post-contrast imaging phases. Pre-contrast imaging (A) is not required for HCC evaluation unless the patient has undergone local ablation that results in high attenuation material in the liver that may obscure enhancing lesions. The hepatic artery (white arrow) and portal vein (black arrow) demonstrate enhancement during the hepatic arterial phase (B). During the portal venous phase (C), the hepatic veins begin to enhance (black arrowhead). The hepatic veins, portal veins, and hepatic arteries demonstrate progressively decreasing enhancement during the delayed phase (D). HCC: hepatocellular carcinoma; CT: computed tomography

hepatobiliary phase, and a history of prior inadequate hepatobiliary phase imaging should be factored in the selection of contrast agents for future imaging in these patients.

Additional MRI sequences

In addition to dynamic contrast enhanced imaging, T2-weighted and unenhanced T1-weighted opposed-phase and in-phase imaging are required for complete evaluation of the liver in patients at risk for HCC. These sequences provide important information regarding the presence of fat, iron, fibrosis, and edema in observations relative to the background liver parenchyma that can contribute to observation conspicuity and enable evaluation for the presence of ancillary features that can enable modification of the final LI-RADS category.

Diffusion weighted imaging provides information regarding relative cellularity of tissues and is also an important sequence for improving the specificity and sensitivity for HCC^[8]. Despite the widespread recognition of the importance of diffusion weighted imaging in this setting, however, this sequence remains optional since it is not as widely available and robust as other MRI sequences^[5]. An example of an MR exam that meets complete technical requirements for LI-RADS is shown in Figure 3.

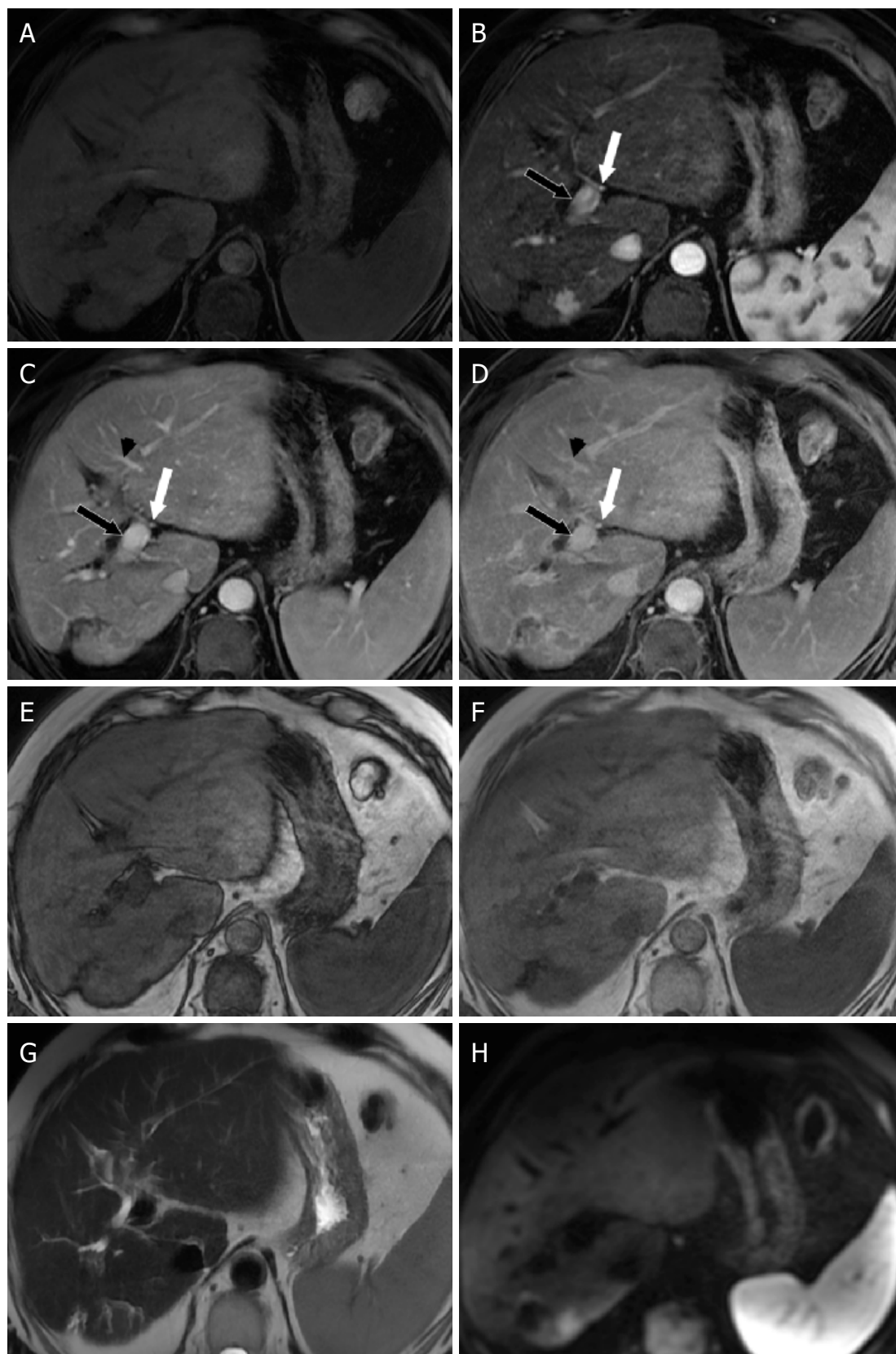


Figure 3. MRI for Evaluation for HCC. Axial MR images of an exam meeting minimum technical requirements for evaluation of HCC. Axial T1-weighted fat saturated images should be obtained pre-contrast (A), and following intravenous contrast during the hepatic arterial (B), portal venous (C), and delayed (D) phases. Additional required imaging sequences include opposed phase (E) and in phase (F) T1-weighted images and T2-weighted images (G). Diffusion weighted imaging (H) is not required, but is suggested if available. White arrow: hepatic artery; Black arrow: portal vein; Black arrowhead: hepatic vein. MRI: magnetic resonance imaging; HCC: hepatocellular carcinoma

IMAGING FEATURES

Major features

LI-RADS major features are the primary imaging features used to categorize observations as LR-3, LR-4, or LR-5. Major features were selected to provide high specificity for hepatocellular carcinoma and are the only features that can be used to categorize an observation as LR-5. These major features are included in the diagnostic table and include arterial phase hyperenhancement, nonperipheral “washout”, enhancing “capsule”, size, and threshold growth [Figure 1].

Arterial phase hyperenhancement refers to enhancement of an observation during the arterial phase that is greater than the background liver and results in signal intensity or attenuation that is higher than the background liver. This feature is best assessed during the late hepatic arterial phase and is present in most HCCs that have progressed^[9,10]. A peripheral pattern to the arterial phase hyperenhancement, however, has been associated with non-HCC malignancies such as cholangiocarcinoma and metastases [Figure 4]^[11]. Therefore, only nonrim arterial phase hyperenhancement should be used for the assignment of LR-5 category to an observation.

The term nonperipheral “washout” refers to reduction in enhancement in whole, or in part, within an observation from an earlier post-contrast imaging phase to a later extracellular post-contrast phase. “Washout” (with quotation marks) refers to visual assessment of washout appearance and does not specifically require measurement of enhancement or construction of an enhancement curve. “Washout” in combination with arterial phase hyperenhancement is a highly specific imaging feature of HCC^[12,13]. If “washout” is present primarily along the margins of the observation, however, the imaging feature is instead considered peripheral “washout” and is not a major feature due to its association with intrahepatic cholangiocarcinoma^[11]. Hypointensity on the transitional or hepatobiliary phases should not be considered “washout” since the high specificity of washout in the literature and its inclusion in LI-RADS has been based on exams performed with ECA. If “washout” is present in an observation prior to the transitional phase on an exam using a hepatobiliary contrast agent, “washout” can be considered present and used as a major feature for LI-RADS categorization.

Enhancing “capsule” describes a smooth uniform border around the majority of an observation margin that is unequivocally thicker or distinct from any fibrotic tissue present elsewhere in the liver. To be considered a major feature, this finding must be present on the portal venous, delayed, or transitional phase of post-contrast imaging [Figure 5]. The term “capsule” is used in place of capsule appearance, since the imaging finding of a “capsule” can be indicative of either a true fibrous capsule or pseudocapsule on histology. Regardless of whether a true capsule or pseudocapsule is present, however, the imaging feature of “capsule” is present in 12%-94% of HCCs^[14-17].

The size of an observation is the largest outer edge to outer edge dimension of an observation and should be measured on the sequence or phase where the margins of the observation are the most clear and distinct. Due to perfusion alterations that can manifest during the arterial phase, size should not be assessed on the arterial phase unless the observation is not visible on any other phase or sequence. Also, due to anatomic distortion that is often present on diffusion weighted imaging (DWI), measurements should be avoided on the DWI sequence unless the observation is not visible on another sequence. If the observation demonstrates capsule appearance, the capsule should be included in the size measurement. Only observations that are 10 mm or larger are eligible to be considered definitely as HCC (LR-5) in combination with arterial phase hyperenhancement and other major features.

Threshold growth in LI-RADS v2018 refers to the size increase of an observation by greater than 50% within six months. Threshold growth only applies to observations that are definitely masses, since perfusion

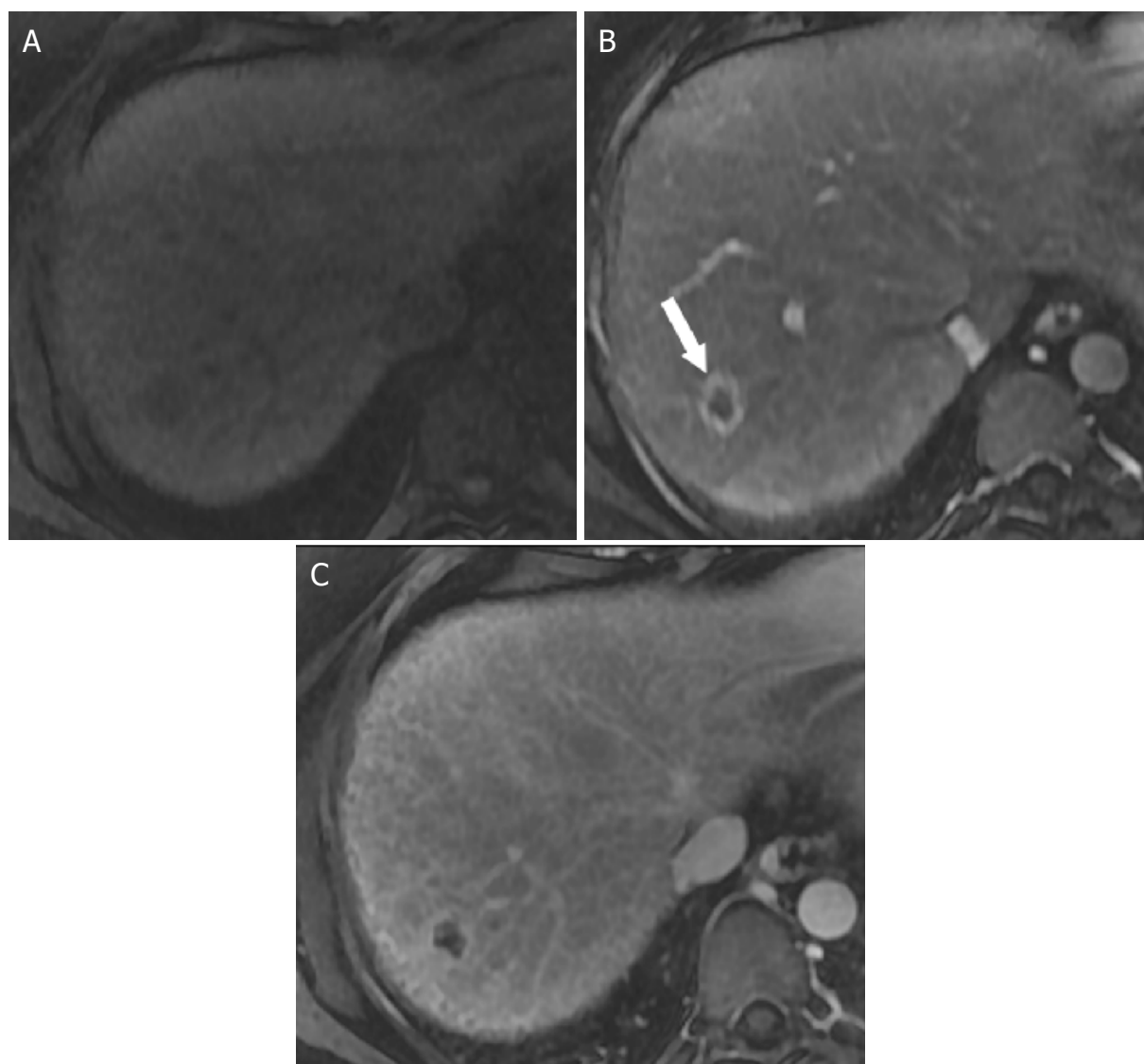


Figure 4. Peripheral Arterial Phase Hyperenhancement. Axial T1-weighted fat saturated MR images of the abdomen prior to (A), and during the arterial phase (B) and delayed phase (C) of dynamic post extracellular contrast images. The 26 mm observation in the right hepatic lobe demonstrates peripheral arterial phase hyperenhancement (white arrow). This feature does not qualify as the major feature of nonrim arterial phase hyperenhancement and should lead the radiologist to assign the category of LR-M (probably or definitely malignancy, but not HCC specific). HCC: hepatocellular carcinoma

alterations can often vary in size from one exam to the next. Also, the comparison prior examination must be a CT or MRI exam that was performed 6 months or less prior to the more recent study [Figure 6]. This definition of threshold growth is different from prior versions of LI-RADS and was changed to achieve congruence with the definition of threshold growth used by the OPTN^[18]. Note that the development of a new observation within 6 months of a prior examination is not considered threshold growth in LI-RADS, as the definition requires that the observation was present on the prior exam.

Ancillary features

Ancillary features are those imaging features that can be used to change the LI-RADS category of an observation after the application of major features. Ancillary features can change the category by one category to reflect either a higher or lower suspicion of malignancy. Ancillary features cannot, however, be used to change the category of an observation from LR-4 to LR-5. Only major features may be used

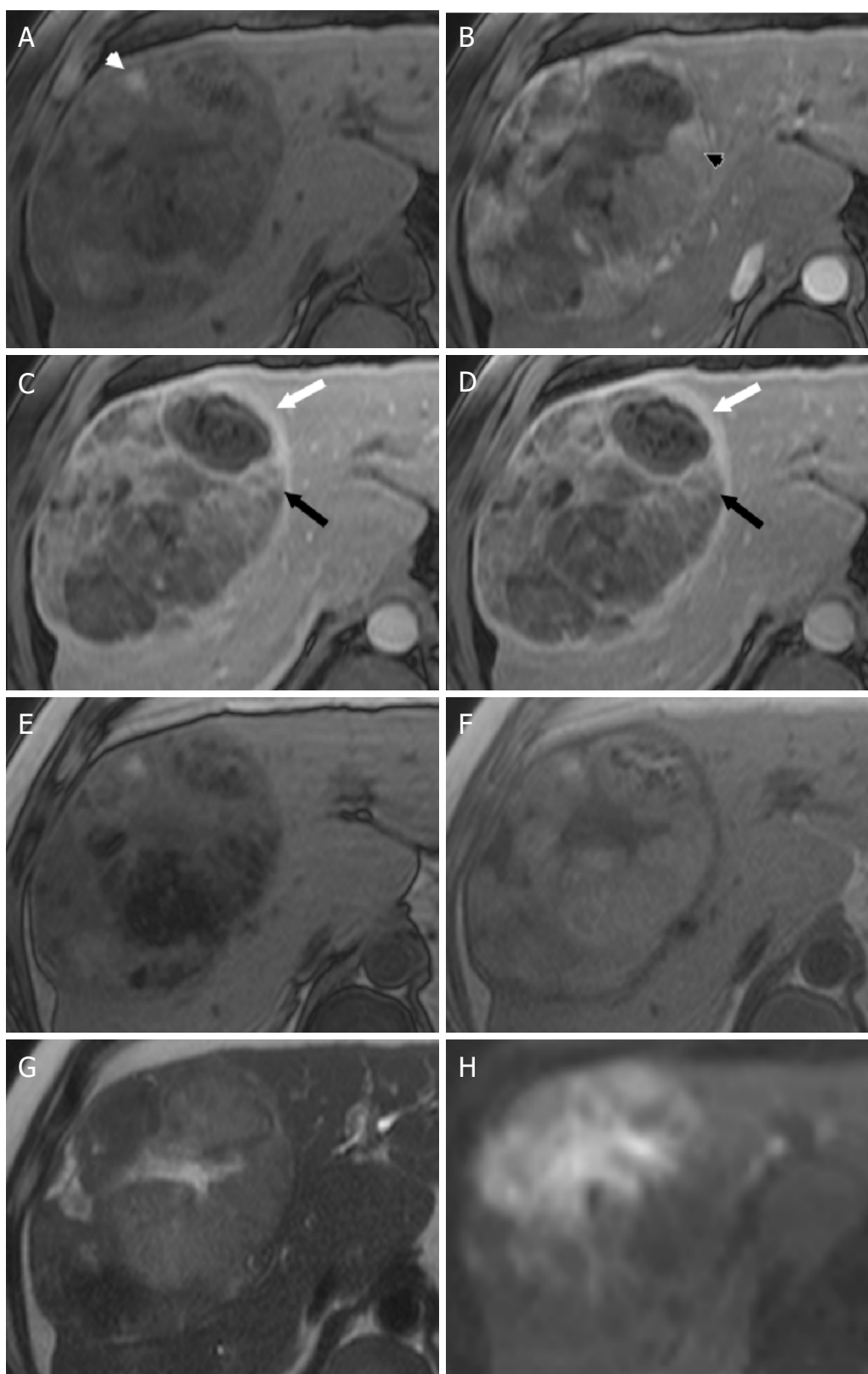


Figure 5. LR-5 - Definitely HCC. Axial MR images of a 14 cm mass at the junction of the right and left hepatic lobes. T1-weighted fat saturated images performed prior to (A) as well as during the arterial phase (B), portal venous phase (C), and delayed phase (D) following contrast administration demonstrate areas of arterial phase hyperenhancement (black arrowhead), “washout” (black arrow), and “capsule” (white arrow). The presence of three major features and size greater than 20 mm lead to LI-RADS 5 as the correct category for this mass. Additionally, the mass has ancillary features favoring HCC including mosaic architecture, fat in mass (seen as loss of signal between in-phase (F) and opposed-phase (E) T1-weighted images), and blood products in mass (white arrowhead, A). Additional ancillary features favoring malignancy, not HCC in particular, are also present including mild-moderate T2 hyperintensity (T2-weighted image, G) and restricted diffusion (diffusion weighted images, H). HCC: hepatocellular carcinoma

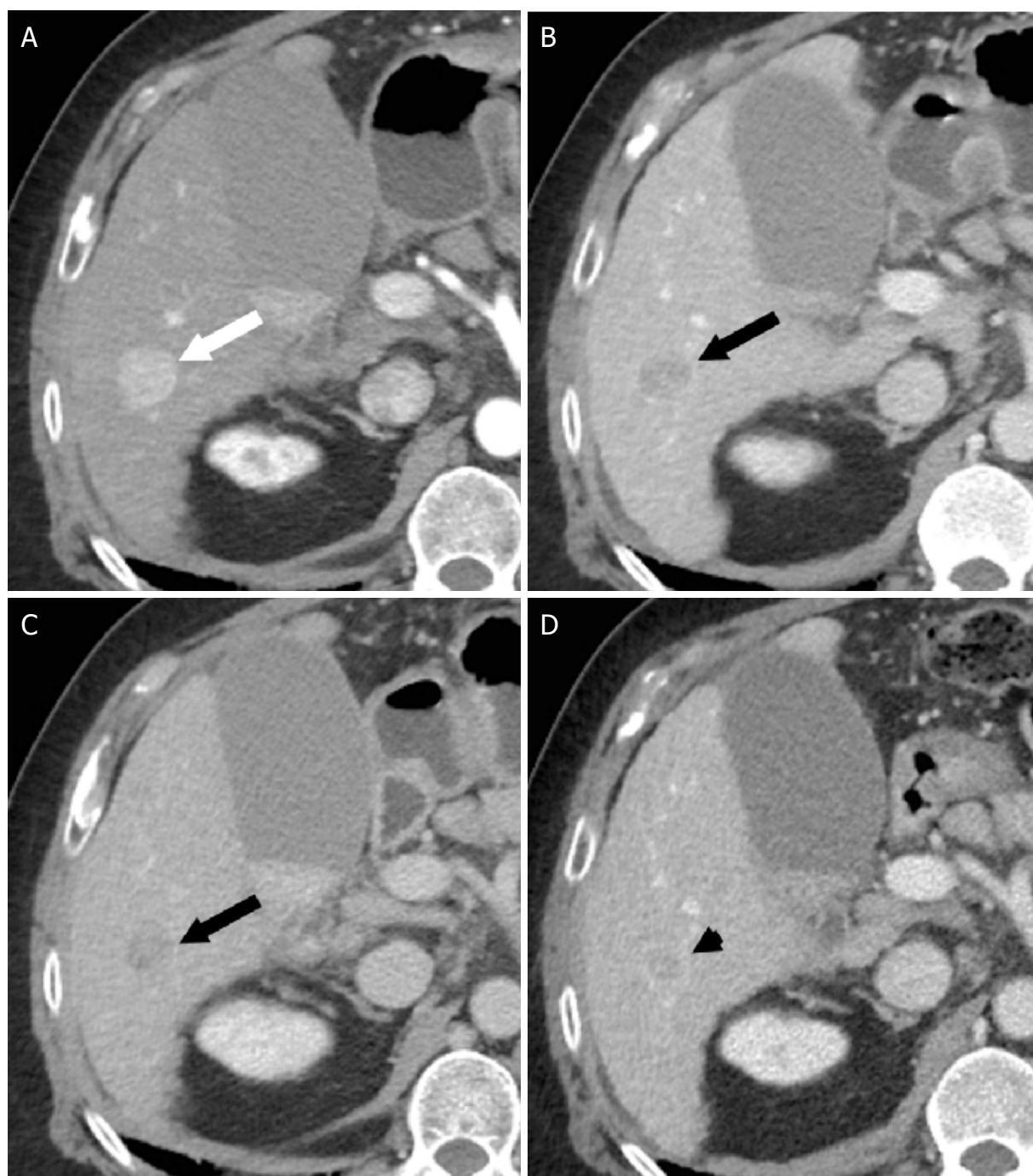


Figure 6. Threshold Growth. Axial CT images of a 21 mm mass in the right hepatic lobe. The mass demonstrates arterial phase hyperenhancement during the arterial phase (white arrow, A), and “washout” with “capsule” (black arrow) on the portal venous (B) and delayed phases (C). Axial CT performed 5 months prior demonstrates a 12 mm mass on the portal venous phase (D, black arrowhead). The interval growth is more than 50% in less than 6 months, therefore, the mass also demonstrates threshold growth. The most appropriate category for this mass is LI-RADS 5. CT: computed tomography; LI-RADS: Liver Imaging Reporting and Data System

to categorize an observation as LR-5 to preserve high specificity for HCC. In LI-RADS v2018, ancillary features are divided into those suggestive of malignancy versus those suggestive of benignity [Table 4]. Ancillary features suggestive of malignancy are further subdivided into those that are and are not specific to HCC. For example, the presence of intralesional fat is considered a specific finding of HCC in those patients at risk for developing HCC, whereas restricted diffusion can be present in many types of malignant

Table 4. LI-RADS v2018 ancillary imaging features

Features favoring malignancy, not HCC in particular	Features favoring HCC in particular	Features favoring benignity
US visibility as discrete nodule	Nonenhancing “capsule”	Size stability ≥ 2 years
Subthreshold growth	Nodule-in-nodule architecture	Size reduction
Corona enhancement	Mosaic architecture	Parallels blood pool enhancement
Fat sparing in solid mass	Fat in mass, more than adjacent liver	Undistorted vessels
Restricted diffusion	Blood products in mass	Iron in mass, more than liver
Mild-moderate T2 hyperintensity		Marked T2 hyperintensity
Iron sparing in solid mass		Hepatobiliary phase isointensity
Transitional phase hypointensity		
Hepatobiliary phase hypointensity		

RADS: Liver Imaging Reporting and Data System; HCC: hepatocellular carcinoma



Figure 7. LR-4 - Probably HCC. Axial MR images of a 9 mm observation in the right hepatic lobe. T1-weighted fat saturated images performed prior to (A) as well as during the arterial phase (B), portal venous phase (C), and delayed phase (D) following contrast administration demonstrate arterial phase hyperenhancement (black arrow) of the observation which persists into the portal venous phase. Based on the size and presence of a single major feature, the appropriate category for this observation is LI-RADS 3. The observation also demonstrates mild T2-hyperintensity (white arrow, E) and restricted diffusion (white arrowhead, F). The presence of ancillary features suggestive of malignancy then allow the radiologist discretion with changing the category to LI-RADS 4 to reflect higher suspicion for HCC. HCC: hepatocellular carcinoma; LI-RADS: Liver Imaging Reporting and Data System

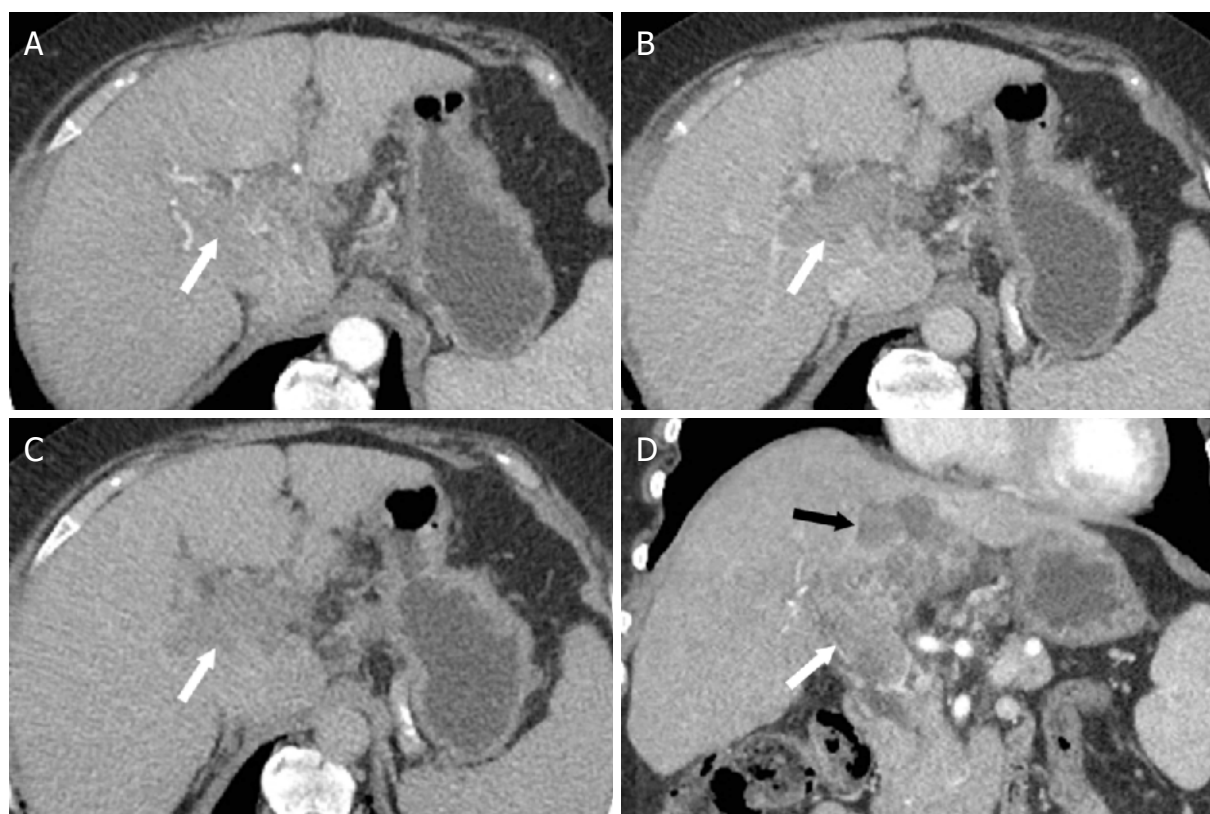


Figure 8. LR-TIV. Axial CT images from the hepatic arterial (A), portal venous (B), and delayed (C) phases following intravenous contrast administration and coronal image from the hepatic arterial phase (D). Enhancing soft tissue is present in the right and main portal veins (white arrow). Due to an associated parenchymal mass (black arrow) with arterial phase hyperenhancement (not shown) and “washout”, the most appropriate category for this finding is LR-TIV, likely due to HCC. CT: computed tomography; LI-RADS: Liver Imaging Reporting and Data System; HCC: hepatocellular carcinoma; LR-TIV: LI-RADS Tumor in Vein

lesions such as metastases from an extrahepatic primary malignancy [Figure 7]^[8]. If an observation has multiple ancillary features for both benignity and malignancy, then the category of the observation should not be adjusted. Finally, the use of ancillary features is optional at the radiologist’s discretion for designating a LI-RADS category.

TUMOR IN VEIN

The category of LR-TIV should be used for the evidence of vascular invasion of the portal veins or hepatic veins. Unequivocal vascular invasion is present when enhancing soft tissue is present within the vessel [Figure 8]. This category does not require the presence of a parenchymal mass. If possible, the radiologist should indicate the most probably etiology between HCC and non-HCC malignancy.

CONCLUSION

Since its initial release in 2011, CT/MR LI-RADS has evolved based on user feedback, ongoing expert review, and the need for unification with other HCC imaging algorithms. CT/MR LI-RADS v2018 provides an algorithm for the standardized reporting and interpretation of findings in patients at risk for HCC, and is now concordant with AASLD practice guidelines. With increasing adoption worldwide, the CT/MR algorithm and associated lexicon enable clearer communication between radiologists, other physicians, and researchers to better provide care for patients at risk for developing HCC.

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The author contributed solely to the article.

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REFERENCE

1. Elsayes KM, Kielar AZ, Chernyak V, Morshid A, Furlan A, et al. LI-RADS: a conceptual and historical review from its beginning to its recent integration into AASLD clinical practice guidance. *J Hepatocell Carcinoma* 2019;6:49-69.
2. Sirlin CB. The LI-RADS adventure-a personal statement. *Abdom Radiol (NY)* 2018;43:1-2.
3. Chernyak V, Santillan CS, Papadatos D, Sirlin CB. LI-RADS® algorithm: CT and MRI. *Abdom Radiol (NY)* 2018;43:111-26.
4. American College of Radiology. Liver Imaging Reporting and Data System version 2018. Available from: <http://www.acr.org/Quality-Safety/Resources/LIRADS>. [Last accessed on 12 Aug 2020]
5. Kambadakone AR, Fung A, Gupta RT, Hope TA, Fowler KJ, et al. LI-RADS technical requirements for CT, MRI, and contrast-enhanced ultrasound. *Abdom Radiol (NY)* 2018;43:56-74.
6. Ichikawa T, Kitamura T, Nakajima H, Sou H, Tsukamoto T, et al. Hypervascular hepatocellular carcinoma: can double arterial phase imaging with multidetector CT improve tumor depiction in the cirrhotic liver? *AJR Am J Roentgenol* 2002;179:751-8.
7. Choi JY, Lee JM, Sirlin CB. CT and MR imaging diagnosis and staging of hepatocellular carcinoma: part II. Extracellular agents, hepatobiliary agents, and ancillary imaging features. *Radiology* 2014;273:30-50.
8. Chernyak V, Tang A, Flusberg M, Papadatos D, Bijan B, et al. LI-RADS® ancillary features on CT and MRI. *Abdom Radiol (NY)* 2018;43:82-100.
9. Matsui O, Kobayashi S, Sanada J, Kouda W, Ryu Y, et al. Hepatocellular nodules in liver cirrhosis: hemodynamic evaluation (angiography-assisted CT) with special reference to multi-step hepatocarcinogenesis. *Abdom Imaging* 2011;36:264-72.
10. Muto J, Shirabe K, Sugimachi K, Maehara Y. Review of angiogenesis in hepatocellular carcinoma. *Hepatol Res* 2015;45:1-9.
11. Fowler KJ, Potretzke TA, Hope TA, Costa EA, Wilson SR. LI-RADS M (LR-M): definite or probable malignancy, not specific for hepatocellular carcinoma. *Abdom Radiol (NY)* 2018;43:149-57.
12. Pawlik TM, Delman KA, Vauthey JN, Nagorney DM, Ng IO, et al. Tumor size predicts vascular invasion and histologic grade: implications for selection of surgical treatment for hepatocellular carcinoma. *Liver Transpl* 2005;11:1086-92.
13. Forner A, Vilana R, Ayuso C, Bianchi L, Solé M, et al. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. *Hepatology* 2008;47:97-104.
14. Kelekis NL, Semelka RC, Worawattanakul S, de Lange EE, Ascher SM, et al. Hepatocellular carcinoma in North America: a multiinstitutional study of appearance on T1-weighted, T2-weighted, and serial gadolinium-enhanced gradient-echo images. *AJR Am J Roentgenol* 1998;170:1005-13.
15. Freeny PC, Baron RL, Teefey SA. Hepatocellular carcinoma: reduced frequency of typical findings with dynamic contrast-enhanced CT in a non-Asian population. *Radiology* 1992;182:143-8.
16. Kadota M, Matsui O, Takashima T, Nonomura A. Hepatocellular carcinoma: correlation of MR imaging and histopathologic findings. *Radiology* 1992;183:819-25.
17. Choi JY, Lee JM, Sirlin CB. CT and MR imaging diagnosis and staging of hepatocellular carcinoma: part I. Development, growth, and

spread: key pathologic and imaging aspects. *Radiology* 2014;272:635-54.

18. Kielar AZ, Chernyak V, Bashir MR, Do RK, Fowler KJ, et al. An update for LI-RADS: Version 2018. Why so soon after version 2017? *J Magn Reson Imaging* 2019;50:1990-1.

Commentary

Open Access



From nonalcoholic fatty liver disease to metabolic dysfunction-associated fatty liver disease: is it time for a change of terminology?

Giovanni Targher¹, Christopher D. Byrne²

¹Department of Medicine, Section of Endocrinology, Diabetes and Metabolism, University of Verona, Verona 37126, Italy.

²Southampton National Institute for Health Research Biomedical Research Centre, University Hospital Southampton, Southampton General Hospital, Southampton SO16 6YD, United Kingdom.

Correspondence to: Prof. Giovanni Targher, Department of Medicine, Section of Endocrinology, Diabetes and Metabolism, University of Verona, Verona, Piazzale A. Stefani 1, Verona 37126, Italy. E-mail: giovanni.targher@univr.it

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Abstract

Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of liver disease in many parts of the world, causing considerable liver-related (steatohepatitis, cirrhosis, liver failure and hepatocellular carcinoma) and extra-hepatic morbidity and mortality (mainly cardiovascular disease, chronic kidney disease or certain types of extra-hepatic cancers). Recently, based on insights gained from the past two decades, an international panel of experts from 22 countries has taken the initiative to propose a new name and definition for NAFLD in adult individuals - that is, metabolic dysfunction-associated fatty liver disease. This proposed change in nomenclature is not simply a semantic revision, but may facilitate improved diagnosis of this common liver disease for health promotion, case identification, patient awareness, ongoing clinical trials and health services delivery. The aim of this commentary is to discuss the proposal for a change in nomenclature of this common and burdensome liver disease and to address the "pros and cons" for changing the name according to the perspective of different stakeholders.

Keywords: Nonalcoholic fatty liver disease, metabolic dysfunction-associated fatty liver disease, liver fat, commentary



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MAIN TEXT

Nonalcoholic fatty liver disease (NAFLD) is a growing global health problem that affects about a quarter of the world's adult population and poses a major health and economic burden to all societies^[1-3]. Recently, two new position articles, published by a panel of international experts from 22 countries, have proposed a change of terminology and definition for NAFLD, called “metabolic dysfunction-associated fatty liver disease” (MAFLD)^[4,5].

As summarized in the schematic [Figure 1](#), over the last two decades there have been concerns expressed by both individual experts and some special conferences of scientific societies (an AASLD 2003 single topic conference and an EASL 2009 special conference, respectively) regarding the inaccuracy and possible “negative” consequences of using the term “NAFLD” to describe a fatty liver disease associated with metabolic dysfunction^[6-13]. Since the initial descriptions in the early 1980s by Ludwig *et al.*^[6], Schaffner and Thaler^[7], who coined the terms of nonalcoholic steatohepatitis (NASH) and NAFLD, respectively, to describe a fatty liver disease arising in the absence of significant alcohol consumption, there have been major conceptual advances in our understanding of the complex pathophysiological mechanisms of this common liver disease.

Although the change of nomenclature from NAFLD to MAFLD that has been recently proposed by the panel of international experts is still under discussion, it is important to underline that this change in terminology is not merely a semantic revision, but can also represent the first step toward a better identification of this common and burdensome metabolic liver disease for improved health promotion, case identification, patient awareness, ongoing clinical trials and health services delivery^[5,6,14-16].

Criteria for MAFLD diagnosis

The current definition of NAFLD is based on the presence of hepatic steatosis (detected by liver biopsy, imaging methods or blood biomarkers/scores) in the absence of significant alcohol consumption (though the currently recommended cut-offs to define “significant” alcohol consumption are arbitrary) and the exclusion of other secondary causes of hepatic steatosis^[17-19]. Interestingly, the newly proposed definition of MAFLD shifts from a liver disease of “exclusion” (i.e., non-alcoholic fatty liver without coexisting known causes of fatty liver) to one of “inclusion”, as the newly proposed diagnostic criteria are based on the presence of hepatic steatosis, in addition to one of the following three criteria (namely overweight/obesity, presence of established type 2 diabetes mellitus, or evidence of metabolic dysregulation), regardless of daily alcohol consumption and other concomitant liver diseases^[5]. A flowchart for the proposed simple criteria for the diagnosis of MAFLD in adult individuals is depicted in [Figure 2](#). It is important to underline that these diagnostic criteria do not apply to paediatric population (< 18 years), because different cut-off points for defining the presence of overweight/obesity and other metabolic risk abnormalities should be used in children and adolescents. As also shown in this figure, the criteria proposed for diagnosing the presence of metabolic dysregulation among lean/normal weight individuals with hepatic steatosis who do not have type 2 diabetes, are the presence of at least two metabolic risk factors from: (1) those risk factors that are widely used to identify the metabolic syndrome (using ethnic- and country-specific cutoff points of increased waist circumference)^[20]; (2) a homeostasis model assessment-estimated insulin resistance score ≥ 2.5 ; or (3) a plasma high sensitivity-C-reactive protein (hs-CRP) level > 2 mg/L. It is well known that the metabolic syndrome is a complex of inter-related risk factors for both type 2 diabetes and cardiovascular disease. These risk factors include dysglycaemia, hypertension (or raised blood pressure), atherogenic dyslipidemia (typically defined by increased plasma triglycerides and low high-density lipoprotein cholesterol) and obesity (particularly increased abdominal adiposity)^[20]. Interestingly, the new definition of MAFLD also includes elevated plasma hs-CRP levels as one of the metabolic risk factors, because it is well established that this plasma inflammatory biomarker (mostly secreted by the liver) is also often increased with cardiometabolic disorders.

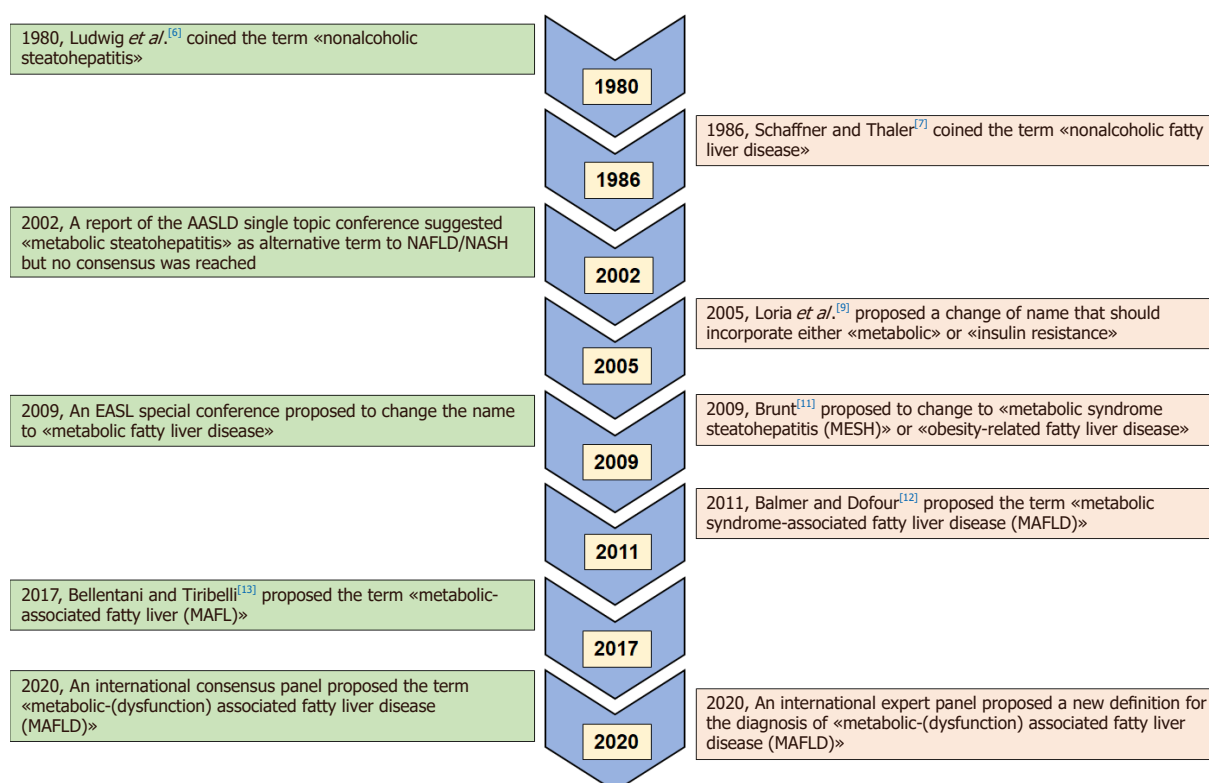


Figure 1. Schematic timeline of key suggestions for the revising the nomenclature of fatty liver disease from NAFLD to MAFLD^[4-13]. NAFLD: nonalcoholic fatty liver disease; MAFLD: metabolic dysfunction-associated fatty liver disease

It is important to highlight that these “positive” diagnostic criteria recognize that MAFLD can also frequently co-exist with other conditions (e.g., significant alcohol consumption, viral hepatitis or other known chronic liver diseases), but the exclusion of these conditions is not a prerequisite for the diagnosis of MAFLD. Indeed, one of the advantages of the proposed diagnostic criteria for MAFLD is that they do not rely on exclusion of significant alcohol intake to establish the diagnosis. Individuals with MAFLD who also have one (or more) of these conditions should be defined as having dual (or more) aetiology of fatty liver disease^[5], and it is likely that these individuals will have a different natural history and response to different treatments. For instance, previous studies have shown that a significant proportion of the general adult population (e.g., up to nearly 1% of United States individuals from the National Health and Nutrition Examination Survey database 2003-2014)^[20] may be affected by both alcoholic disease and NAFLD (referred as BAFLD) and these patients tend to have more severe liver-related outcomes, given the synergistic interactions between alcohol consumption and features of the metabolic syndrome, such as obesity and type 2 diabetes mellitus^[20,21].

The rationale for the use of these “positive” diagnostic criteria for MAFLD largely stem from the fact that there is a strong pathophysiological link between this liver disease and the presence of underlying abnormalities in metabolic health (namely overweight/obesity, type 2 diabetes, insulin resistance or other metabolic risk abnormalities)^[17-19,21-29]. In addition, there is also convincing evidence that the coexistence of these metabolic risk abnormalities is not only one of the strongest risk factors of liver disease progression but also extra-hepatic clinical outcomes (mainly cardiovascular disease, chronic kidney disease and certain types of extra-hepatic cancers) in this patient population^[17-19,23-31]. As depicted in Figure 2, the new diagnostic criteria for MAFLD would also be able to capture the whole phenotypical spectrum of fatty liver disease from that associated with metabolically unhealthy normal weight to metabolically unhealthy obesity.

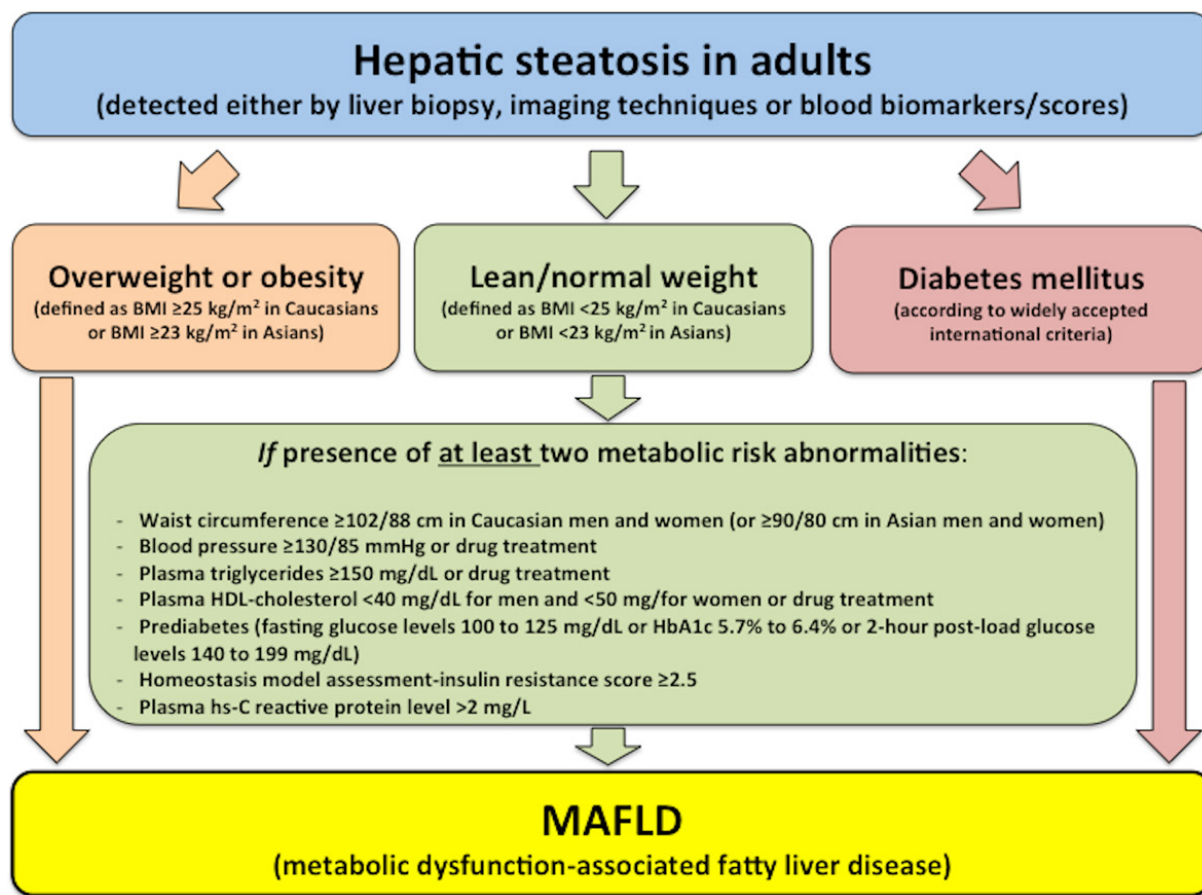


Figure 2. Flowchart for the proposed “positive” diagnostic criteria for metabolic dysfunction-associated fatty liver disease (MAFLD) in adult individuals. These diagnostic criteria are not applicable to the paediatric population. This figure is reproduced with permission from Eslam *et al.*^[5]

The panel of international experts has also proposed that MAFLD should be the single “overarching” term to describe this fatty liver disease^[4,5]. Similar to that accepted for other chronic liver diseases, they proposed that the histological severity of liver disease should be defined by its grade of activity and its stage of fibrosis^[5]. This recognizes that the grade of MAFLD activity is a disease continuum and the all-embracing term of MAFLD should replace the current stratification of liver disease into NASH and non-NASH, which may also have some important limitations (e.g., there is significant inter-observer and intra-observer variability for the histologic confirmation of NASH, particularly for ballooned hepatocytes)^[5]. From a clinical and pathological perspective, this suggestion should also result in improved case identification, while sub-classification of MAFLD (based on the use of liver histology) may also capture histological changes in disease status with relevant impacts on the disease course^[5]. As suggested by the panel of international experts, it is reasonable to assume that MAFLD could be sub-classified in the foreseeable future, based on new epidemiological and experimental data that might suggest the predominant pathophysiological pathway that drives the development of specific features of liver histology (steatosis, necro-inflammation or fibrosis), but which might lead to different hepatic and extra-hepatic clinical complications (e.g., cardiovascular disease, extra-hepatic cancers or chronic kidney disease)^[5].

The expert panel has also proposed a set of diagnostic criteria to define MAFLD-related cirrhosis (in order to avoid the use of the term “cryptogenic cirrhosis” in this patient group) and proposed a conceptual framework to consider other causes of fatty liver disease^[5]. The diagnostic criteria for MAFLD-related cirrhosis include patients with established cirrhosis in the absence of the typical histological signs

suggestive of steatohepatitis, who meet at least one of the following criteria: past or present evidence of metabolic risk abnormalities that meet the criteria to diagnose MAFLD (as described in [Figure 2](#)) with at least one of the following diagnostic criteria, namely documentation of MAFLD on a previous liver biopsy, or historical documentation of hepatic steatosis by imaging methods^[5]. Notably, a history of past alcohol intake should be considered as patients may have a dual disease aetiology with alcohol use disorder^[5].

Finally, the panel of international experts has also proposed the new definition of MAFLD in order to bring more clarity to the distinction between the diagnostic criteria used in clinical practice to define this common liver disease and the inclusion criteria used for future research studies or randomized controlled trials^[5]. However, these considerations in no way detract the conduct from ongoing trials, nor does it affect the diagnostic criteria proposed for MAFLD. As also suggested by the panel of international experts, it is reasonable to hypothesize that the use of the newly proposed “positive” criteria for the diagnosis of MAFLD and the exclusion of individuals with hepatic steatosis who do not have coexisting metabolic dysfunction will render study cohorts less heterogeneous, thereby increasing the probability of detecting a positive effect of clinical approaches targeting MAFLD^[5].

MAFLD and NAFLD: are two terms interchangeable?

It is possible that a consequence of implementing the proposed change in terminology will also highlight some new categories of fatty liver disease. This might also foster new discoveries of the causes, mechanisms, classification and treatment of fatty liver disease^[5].

In line with this hypothesis, Lin *et al.*^[32] have recently compared the characteristics of MAFLD and NAFLD in a nationally representative cohort of nearly 13,000 United States adults with liver ultrasonography and complete laboratory data. Interestingly, using the National Health and Nutrition Examination Survey (NHANES-III) 1988-1994 database, the authors reported that NAFLD was present in 33.2% of participants, while MAFLD was present in 31.2%. In addition, compared to those with NAFLD, patients with MAFLD were more likely to have multiple metabolic comorbidities (e.g., obesity, hypertension and type 2 diabetes) and more cases with advanced liver fibrosis as detected by non-invasive fibrosis scores^[32]. These findings suggest that MAFLD definition is more accurate than NAFLD definition in identifying those patients with hepatic steatosis who are at higher risk of disease progression or have a greater risk of cardiovascular disease.

As depicted in [Figure 3](#), these “real-world” data clearly indicate that there is an excellent concordance between MAFLD and NAFLD (with a calculated Cohen’s kappa coefficient of 0.92)^[33]. However, NAFLD and MAFLD definitions do not identify exactly the same individuals and, therefore, the two terms are not fully interchangeable. Looking at these “real-world” data from adult individuals in the United States, it is evident that not all individuals with NAFLD have MAFLD and vice versa. Indeed, as shown in [Figure 3](#), there are some individuals with MAFLD and coexisting liver diseases who do not have NAFLD. There is also a non-negligible proportion of individuals (620/4,347; nearly 15%) previously deemed as having NAFLD who do not have MAFLD, and that cannot be attributed to significant alcohol consumption (based on self-reported history that might also be under-reported in clinical practice) or other known causes of liver disease^[32]. Notably, as reported in the study by Lin *et al.*^[32], these latter individuals are predominantly of female sex (~60% women), young (mean \pm SD: 35 \pm 13.4 years) and have “lean” NAFLD (mean body mass index: 21.7 \pm 2.1 kg/m²) without coexisting metabolic dysregulation, as defined by the diagnostic criteria of MAFLD. Most importantly, these individuals also have a (relatively) low proportion of advanced liver fibrosis^[32]. Unfortunately, no information is available about patatin-like phospholipase domain-containing protein-3 (PNPLA3) I148M variant, trans-membrane-6 superfamily member 2 (TM6SF2) E167K variant or other NAFLD-related genetic variants in these “lean” NAFLD individuals without metabolic dysregulation.

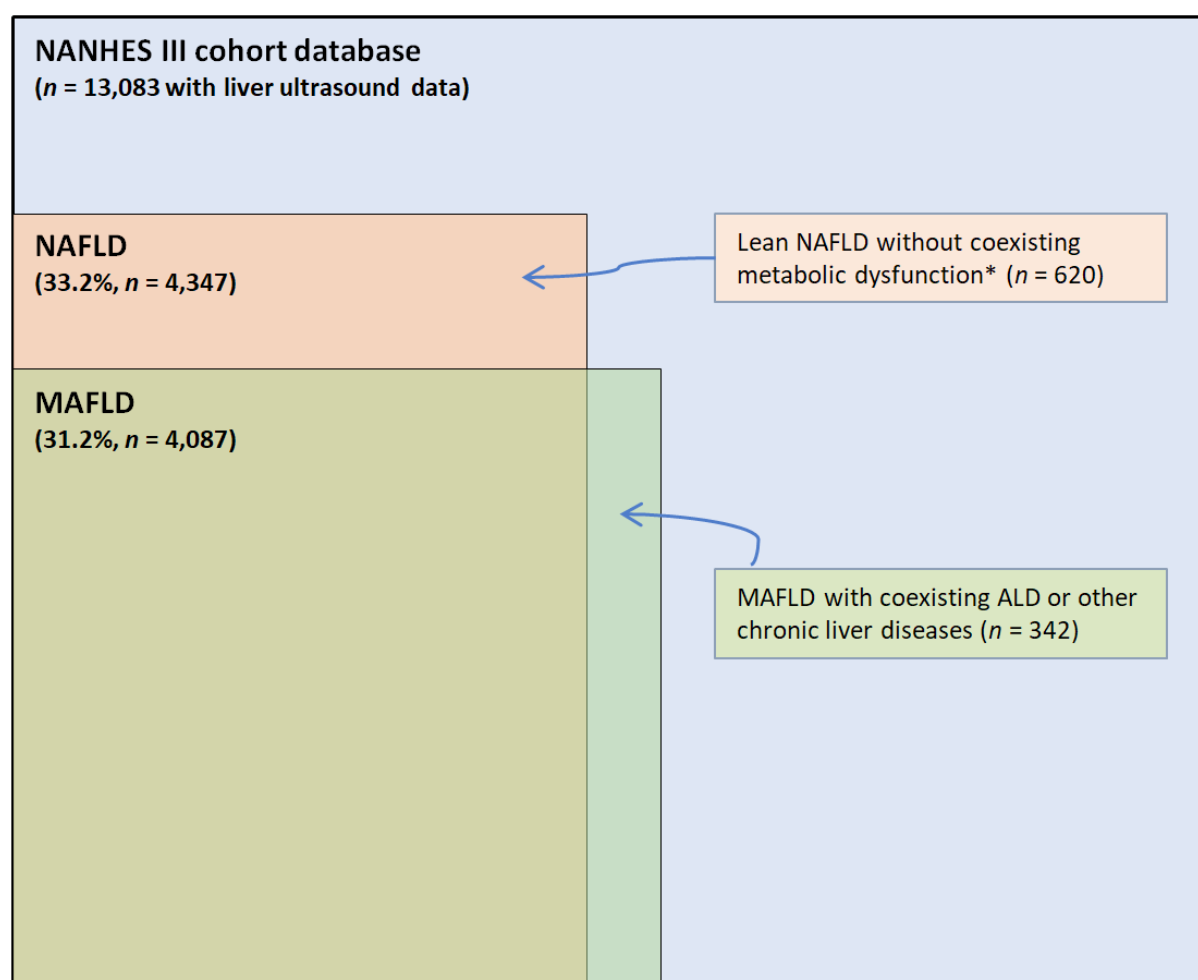


Figure 3. Concordance between MAFLD and NAFLD diagnostic criteria in the Third National Health and Nutrition Examination Survey (NHANES) 1988-1994 database. Data are extrapolated from the study by Lin *et al.*^[32]. This figure is quoted with permission from Targher G.^[33]. *Metabolic dysfunction was defined according to MAFLD diagnostic criteria. MAFLD: metabolic dysfunction-associated fatty liver disease; NAFLD: nonalcoholic fatty liver disease; ALD: alcoholic liver disease

That said, further large cohort studies are needed to validate and corroborate the aforementioned findings in European, American and Asian populations (where the overall prevalence of “lean” NAFLD is greater than in United States)^[34] and, most importantly, to examine the risk of developing adverse liver-related and extra-hepatic outcomes (e.g., cardiovascular disease, chronic kidney disease and extra-hepatic cancers) in patients with MAFLD compared with those with NAFLD. In addition, further research is also needed to better understand the risk of disease progression amongst “lean” NAFLD individuals without any metabolic dysregulation.

CHALLENGES AND THE WAY FORWARD

In addition to the challenges for the MAFLD field that have been outlined in the two new position papers (e.g., definition of “metabolic health”, different disease subtypes)^[4,5], words of caution have also been expressed by a distinguished group of United States experts regarding the possible implications of a premature change in NAFLD terminology^[35]. In particular, although these colleagues are in agreement that the new term MAFLD reflects more accurately the relevant metabolic risk factors than the age-old term NAFLD, they also suggest that a change in terminology for a disease entity is only justified when there is a better understanding of its pathogenesis, risk stratification and molecular phenotyping^[35]. That said, one

good reason to change the nomenclature and the diagnostic criteria for defining this fatty liver disease is that current diagnostic criteria focused on diagnosing NASH by histology only is hindering both the testing of novel treatments for NAFLD and the discovery of new biomarkers. As the change in nomenclature and diagnostic criteria for this fatty liver disease have not been accepted by the major scientific societies, it has been recommended that an international consensus conference should be jointly organized by the major relevant scientific societies along with the patient advocacy organizations, biopharmaceutical industry, regulatory agencies and policy makers to thoughtfully consider any implications of a change in terminology of NAFLD as we know it^[35]. Following the same process that occurred for changing the term “primary biliary cirrhosis” to “primary biliary cholangitis”, all the main scientific societies should approve the different steps, reach a consensus and publish a position paper on MAFLD.

CONCLUSION

Based on pathophysiological insights gained from the past decades, two new articles from a panel of international experts from 22 countries have proposed a new name and definition for NAFLD in adults - i.e., MAFLD^[4,5]. We believe that a change in the nomenclature and definition for this burdensome liver disease affecting nearly one billion people globally is overdue, as knowledge gained from the last two decades has clearly established that MAFLD is a (predominantly) metabolic liver disease. Furthermore, the term “non-alcoholic” over-emphasizes the absence of significant alcohol consumption and does not acknowledge the importance of overweight/obesity, type 2 diabetes mellitus, insulin resistance and other metabolic risk abnormalities that fuel the risk of liver disease progression and the development of serious adverse extra-hepatic outcomes (e.g., cardiovascular disease, extra-hepatic malignancies or chronic kidney disease)^[36-41]. To date, it is known that there is a substantial under-appreciation of NAFLD by both primary care clinicians and patients^[42,43]. We believe that the proposed change of the name from NAFLD to MAFLD holds promise to aid in increasing awareness of this liver disease and decreasing its possible social stigma due to its link to alcohol intake. It is reasonable to propose that this new definition might also promote the establishment of MAFLD clinics run jointly by hepatologists and diabetologists to further improve patient care. To date, however, a number of challenges and uncertainties remain before making this step-change. As discussed above, a change to the nomenclature and diagnostic criteria for NAFLD is a potential first relevant step to acknowledging that NAFLD is an important metabolic liver disease whose aetiology and pathogenesis extend beyond the liver. Indeed, it is known that NAFLD is a novel risk factor for the development of cardiovascular disease, chronic kidney disease, type 2 diabetes and some extra-hepatic malignancies (e.g., colorectal cancers)^[36-41]. In the meantime, an international consensus conference should be jointly organized by the major relevant scientific societies to discuss the implications of a change in terminology from NAFLD to MAFLD and to achieve a consensus regarding any change in nomenclature and definition for this common metabolic liver disease.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and contributed equally to write this commentary article: Targher G, Byrne CD

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Conflicts of interest

Both authors declared that there are no conflicts of interest. Targher G was a coauthor of one of the two recent articles^[5] proposing the change of terminology from NAFLD to MAFLD.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Bellentani S. The epidemiology of non-alcoholic fatty liver disease. *Liver Int* 2017;37 Suppl 1:81-4.
2. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018;15:11-20.
3. Byrne CD, Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015;62:S47-64.
4. Eslam M, Sanyal AJ, George J; International Consensus Panel. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology* 2020;158:1999-2014.e1.
5. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol* 2020;73:202-9.
6. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: mayo clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980;55:434-8.
7. Schaffner F, Thaler H. Nonalcoholic fatty liver disease. *Prog Liver Dis* 1986;8:283-98.
8. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. *Hepatology* 2003;37:1202-19.
9. Loria P, Lonardo A, Carulli N. Should nonalcoholic fatty liver disease be renamed? *Dig Dis* 2005;23:72-82.
10. Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010;53:372-84.
11. Brunt EM. Histopathology of non-alcoholic fatty liver disease. *Clin Liver Dis* 2009;13:533-44.
12. Balmer ML, Dufour JF. Non-alcoholic steatohepatitis - from NAFLD to MAFLD. *Ther Umsch* 2011;68:183-8.
13. Bellentani S, Tiribelli C. Is it time to change NAFLD and NASH nomenclature? *Lancet Gastroenterol Hepatol* 2017;2:547-8.
14. Valenti L, Pelusi S. Redefining fatty liver disease classification in 2020. *Liver Int* 2020;40:1016-7.
15. Fouad Y, Waked I, Bollipo S, Goma A, Ajlouni Y, et al. What's in a name? Renaming 'NAFLD' to 'MAFLD'. *Liver Int* 2020;40:1254-61.
16. Tilg H, Effenberger M. From NAFLD to MAFLD: when pathophysiology succeeds. *Nat Rev Gastroenterol Hepatol* 2020;17:387-8.
17. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1388-402.
18. Italian Association for the Study of the Liver (AISF). AISF position paper on nonalcoholic fatty liver disease (NAFLD): updates and future directions. *Dig Liver Dis* 2017;49:471-83.
19. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American association for the study of liver diseases. *Hepatology* 2018;67:328-57.
20. Khoudari G, Singh A, Nouredin M, Fritze D, Lopez R, et al. Characterization of patients with both alcoholic and nonalcoholic fatty liver disease in a large United States cohort. *World J Hepatol* 2019;11:710-8.
21. Åberg F, Helenius-Hietala J, Puukka P, Färkkilä M, Jula A. Interaction between alcohol consumption and metabolic syndrome in predicting severe liver disease in the general population. *Hepatology* 2018;67:2141-9.
22. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, et al; International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society, International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009;120:1640-5.
23. Bugianesi E, Zannoni C, Vanni E, Marzocchi R, Marchesini G. Non-alcoholic fatty liver and insulin resistance: a cause-effect relationship? *Dig Liver Dis* 2004;36:165-73.
24. Marchesini G, Marzocchi R. Metabolic syndrome and NASH. *Clin Liver Dis* 2007;11:105-17, ix.
25. Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013;10:330-44.
26. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. *Dig Liver Dis* 2015;47:181-90.
27. Lonardo A, Bellentani S, Argo CK, Ballestri S, Byrne CD, et al; Non-alcoholic Fatty Liver Disease Study Group. Epidemiological modifiers of non-alcoholic fatty liver disease: focus on high-risk groups. *Dig Liver Dis* 2015;47:997-1006.

28. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol* 2017;14:32-42.
29. Byrne CD, Patel J, Scorletti E, Targher G. Tests for diagnosing and monitoring non-alcoholic fatty liver disease in adults. *BMJ* 2018;362:k2734.
30. Gastaldello A, Cusi K. From NASH to diabetes and from diabetes to NASH: Mechanisms and treatment options. *JHEP Rep* 2019;1:312-28.
31. Khan RS, Bril F, Cusi K, Newsome PN. Modulation of insulin resistance in nonalcoholic fatty liver disease. *Hepatology* 2019;70:711-24.
32. Lin S, Huang J, Wang M, Kumar R, Liu Y, et al. Comparison of MAFLD and NAFLD diagnostic criteria in real world. *Liver Int* 2020;2082-9.
33. Targher G. Concordance of MAFLD and NAFLD diagnostic criteria in “real-world” data. *Liver Int* 2020; doi: 10.1111/liv.14623.
34. Lu FB, Zheng KI, Rios RS, Targher G, Byrne CD, et al. Global epidemiology of lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2020; doi: 10.1111/jgh.15156.
35. Younossi ZM, Rinella ME, Sanyal A, Harrison SA, Brunt E, et al. From NAFLD to MAFLD: implications of a premature change in terminology. *Hepatology* 2020; doi: 10.1002/hep.31420.
36. Yki-järvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol* 2014;2:901-10.
37. Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut* 2017;66:1138-53.
38. Stefan N, Häring H, Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. *Lancet Diabetes Endocrinol* 2019;7:313-24.
39. Targher G, Byrne CD, Tilg H. NAFLD and increased risk of cardiovascular disease: clinical associations, pathophysiological mechanisms and pharmacological implications. *Gut* 2020;69:1691-705.
40. Byrne CD, Targher G. NAFLD as a driver of chronic kidney disease. *J Hepatol* 2020;72:785-801.
41. Mantovani A, Scorletti E, Mosca A, Alisi A, Byrne CD, et al. Complications, morbidity and mortality of nonalcoholic fatty liver disease. *Metabolism* 2020;154170.
42. Wieland AC, Mettler P, McDermott MT, Crane LA, Cicutto LC, et al. Low awareness of nonalcoholic fatty liver disease among patients at high metabolic risk. *J Clin Gastroenterol* 2015;49:e6-10.
43. Alemany-Pagès M, Moura-Ramos M, Araújo S, Macedo MP, Ribeiro RT, et al. Insights from qualitative research on NAFLD awareness with a cohort of T2DM patients: time to go public with insulin resistance? *BMC Public Health* 2020;20:1142.

Editorial

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Robotic-assisted laparoscopic liver resection in hepatocellular carcinoma

Kevin P. Labadie¹, James O. Park^{1,2}, Jonathan G. Sham^{1,2}

¹Department of Surgery, University of Washington, Seattle, WA 98195, USA.

²Center for Advanced Minimally Invasive Liver Oncologic Therapies (CAMILOT), University of Washington, Seattle, WA 98195, USA.

Correspondence to: Dr. Jonathan G. Sham, Department of Surgery, University of Washington, 1959 Pacific St. NE, Seattle, WA 98195, USA. E-mail: jsham@uw.edu

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Over the past 20 years the use of laparoscopy has revolutionized hepatic surgery and, in recent years, robotic assistance to the laparoscopic approach has been explored by some centres. Since its approval, there has been a prodigious increase in robotic utilization for surgical operations worldwide^[1,2]. As its use continues to increase, assessment of efficacy and outcomes is an area of active study.

Preliminary inquiries into robotic-assistance in surgical procedures began in the late 1980s with a robot designed to perform neurosurgical biopsies with improved precision^[3]. Innovation accelerated shortly thereafter as multiple governmental organizations, including the United States Army and National Air and Space Administration, became interested in telesurgery - the capability for a surgeon to operate on a physically distanced patient using a robotic interface^[4,5]. Expertise and technology devised from these efforts later facilitated development of commercial robotic surgical systems for civilian use, including the ROBODOC^[6], AESOP system^[7], Zeus^[8], ARTEMIS^[9] and the da Vinci Surgical System^[10].

Currently, the most widely used robotic platform worldwide is the da Vinci Surgical system, first approved for use in general laparoscopic surgery by European regulatory agencies in 1999, followed shortly thereafter by the United States Food and Drug Administration in 2000. Initially reserved for smaller operations such as cholecystectomy, the robot is now used for a wide variety of operations ranging from inguinal herniorrhaphy to major hepatectomy. Many surgeons perceive advantages when using robotic systems, which may explain this increased utilization^[11]. These include wristed articulated instruments,



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3-dimensional high definition optics, improved surgeon ergonomics, diminished surgeon fatigue, as well as a shortened learning curve compared to standard laparoscopic liver surgery. Several disadvantages are emphasized by detractors of the robotic technique, including the need for experienced teams, substantial acquisition and operational costs, and concerns over lack of benefit over standard laparoscopic surgery^[12,13].

Robot-assisted laparoscopic liver resection (RALLR) was first described in the 2000s^[14], with the initial landmark case series of 70 patients reported by Guilianotti and colleagues in 2010^[15]. The indications for RALLR mirror those of the standard laparoscopic approach, including resection of both benign and malignant tumours in patients who can tolerate general anaesthesia and prolonged pneumoperitoneum. Over the last two decades, numerous single and multi-institutional case series have reported outcomes of RALLR in high-volume centres, demonstrating it to be safe, feasible, and effective for minor and major hepatectomies^[16]. Compared to standard laparoscopic liver resection, perioperative outcomes including operative time and estimated intraoperative blood loss have been shown to be higher in RALLR, but the open conversion rate, R0 resection rate, hospital length of stay, morbidity and mortality are no different than standard laparoscopic hepatectomy^[17]. Indeed, RALLR recapitulates the main benefits observed with standard laparoscopic hepatectomy in comparison to open hepatectomy, including reduced postoperative complications and shorter length of stay without compromising oncological outcomes such as radical resection rate, overall survival, and disease-free survival rate^[18,19]. The accumulation of evidence supporting RALLR resulted in international consensus guidelines promoting the development and standardization of robot-assisted laparoscopic hepatectomy in 2018^[20].

RALLR is indicated for resection of all liver tumour types including hepatocellular carcinoma (HCC). It has been demonstrated to be safely performed for patients with HCC who have well-compensated cirrhosis without signs of severe portal hypertension. Evidence examining the safety and oncological effectiveness of RALLR for HCC is accumulating, with a systematic review published in 2018 highlighting the favourable short-term outcomes reported in over 300 patients from 10 institutional series^[21]. In the largest series examining RALLR for HCC to date, Chen *et al.*^[22] performed a propensity score matching analysis of 81 RALLR and 81 open liver resections for HCC. In this report, which included approximately 40% major hepatectomies, operative time was longer in the RALLR compared to open cohort (median of 343 min *vs.* 220 min). However, the intraoperative blood loss (median 282 mL *vs.* 263 mL), percent of patients requiring an intra-operative blood transfusion (7.4% *vs.* 3.7%), and postoperative complications (4.9% *vs.* 3.7%) were comparable between the two techniques. Notably, the authors observed a significantly reduced length of hospital stay (median 7.5 days *vs.* 10.1 days), reduced patient-controlled analgesia use on postoperative day 1 and during initial ambulation in the RALLR compared to open hepatectomy cohort. Similar outcomes have been observed in other institutional cohorts; however, it is worth noting that these early published series come from high-volume institutions with extensive experience with laparoscopic liver resection, potentially limiting the generalizability of these findings.

Given the relatively recent adoption of RALLR, studies examining the long-term oncological outcomes in patients after resection for HCC are limited. An early and available outcome that many authors examine as a surrogate for oncological efficacy is the R0 resection rate, as a positive histological margin is known to be associated with a higher incidence of postoperative recurrence in HCC^[23]. R0 resection rate may also serve as a hallmark of technical feasibility, as lower R0 resection may indicate suboptimal technical skill or an inherent limitation of the technology (i.e., inability to palpate tumour with minimally invasive approach). Published RALLR R0 resection rates are high, ranging from 85%-100% in most series^[16]. In regard to long-term oncological outcome data, four of the largest studies examining RALLR for HCC report disease-free survival and overall survival ranging from 72% to 75% and 93% to 98% at 2 and 3 years postoperatively, respectively^[24-26]. These mid-term oncological data are encouraging, but will need confirmation with longer-term outcome reporting.

The modern liver surgeon is afforded with numerous technical approaches to the resection of liver tumours. Although several matched comparisons and meta-analyses have been performed examining the differences between RALLR and several types of laparoscopic liver resection (LLR), many of these are limited by retrospective design and confounded by selection biases^[27]. One of the largest studies comparing RALLR and LLR matched 57 RALLR to 114 LLR based on background liver disease, extent of resection, diagnosis and other patient demographic factors^[28]. The authors concluded that RALLR and LLR displayed similar safety and feasibility for hepatectomies, but that more resections approached robotically were completed totally minimally invasively compared to LLR, with a significant proportion of their LLR cohort requiring hand-assist port. The clinical impact of hand-port utilization on postoperative pain and quality of life is unclear. Magistri *et al.*^[29] compared RALLR and LLR specifically for HCC and reported similar outcomes between both approaches.

A specific indication where RALLR may be advantageous to standard LLR is the resection of liver segments in the posterosuperior segments (S1, S4A, S7, S8), which are difficult to approach by standard LLR. Challenges in these approaches for standard LLR include poor visualization, difficulty in haemorrhage control, as well as deep, curved or angled parenchymal transection planes associated with the posterior location of these segments. Although tumourectomies and segmentectomies of these segments can be feasibly approached laparoscopically, they often require expert skill and invasive manoeuvres (i.e., transpleural approach), potentially limiting their generalizability^[30,31]. Laparoscopic right hepatectomy is often the preferred approach to resections of these lesions since it is technically less challenging. Several studies have evaluated RALLR for parenchymal-sparing resection of lesions in the posterosuperior segments, with authors concluding that RALLR does indeed offer specific advantages to standard LLR and may serve as an alternative to open resection for these lesions^[32-36]. A review of our institutional series of over 250 RALLR similarly corroborate these findings.

Enhanced efficacy of systemic therapies has provided significant benefit for patients with primary and secondary hepatic malignancies and highlighted the importance of parenchymal sparing resections when performing hepatectomy, as more patients may benefit from resection of intrahepatic recurrence. This trend in management has challenged liver surgeons to totally extirpate tumours while preserving maximal liver parenchyma, which is often damaged following numerous rounds of systemic chemotherapy, to allow for future liver-directed therapies. Performing these resections from a minimally invasive approach may be advantageous to survival by minimizing postoperative complications and potentially expediting a return to adjuvant therapy, although additional confirmatory data are needed. Nevertheless, it is appealing to speculate that performing minimally invasive parenchymal-sparing liver resections is advantageous for certain liver cancer patients.

New technologies to improve pre-operative planning, intra-operative decision making, and surgical training are being developed for the da Vinci robotic surgical system^[37]. Advances in image-guided liver surgery, surgical resection maps, 3D modelling, and indocyanine green fluorescence with near-infrared fluorescent imaging and 3D modelling have been developed to assist the surgeon and surgical trainee. In collaboration with Intuitive, our group has helped to develop novel interactive 3D models for pre-operative planning and intra-operative navigation [Figure 1]. Numerous three-dimensional virtual-reality robotic surgery simulators now exist for surgical trainees to improve robotic technique and simulate real-life operative situations. Furthermore, development of novel robotic surgical systems by companies such as Medtronic, Johnson & Johnson, and TransEnterix will only propel ongoing technological innovation. We hope these innovative new technologies translate into improved surgical outcomes for our patients and a well-trained next generation of minimally invasive liver surgeons.

In conclusion, RALLR is a safe and effective approach to the minimally invasive resection of hepatic malignancies. In experienced hands, it is equivalent to the standard laparoscopic approach to anterior

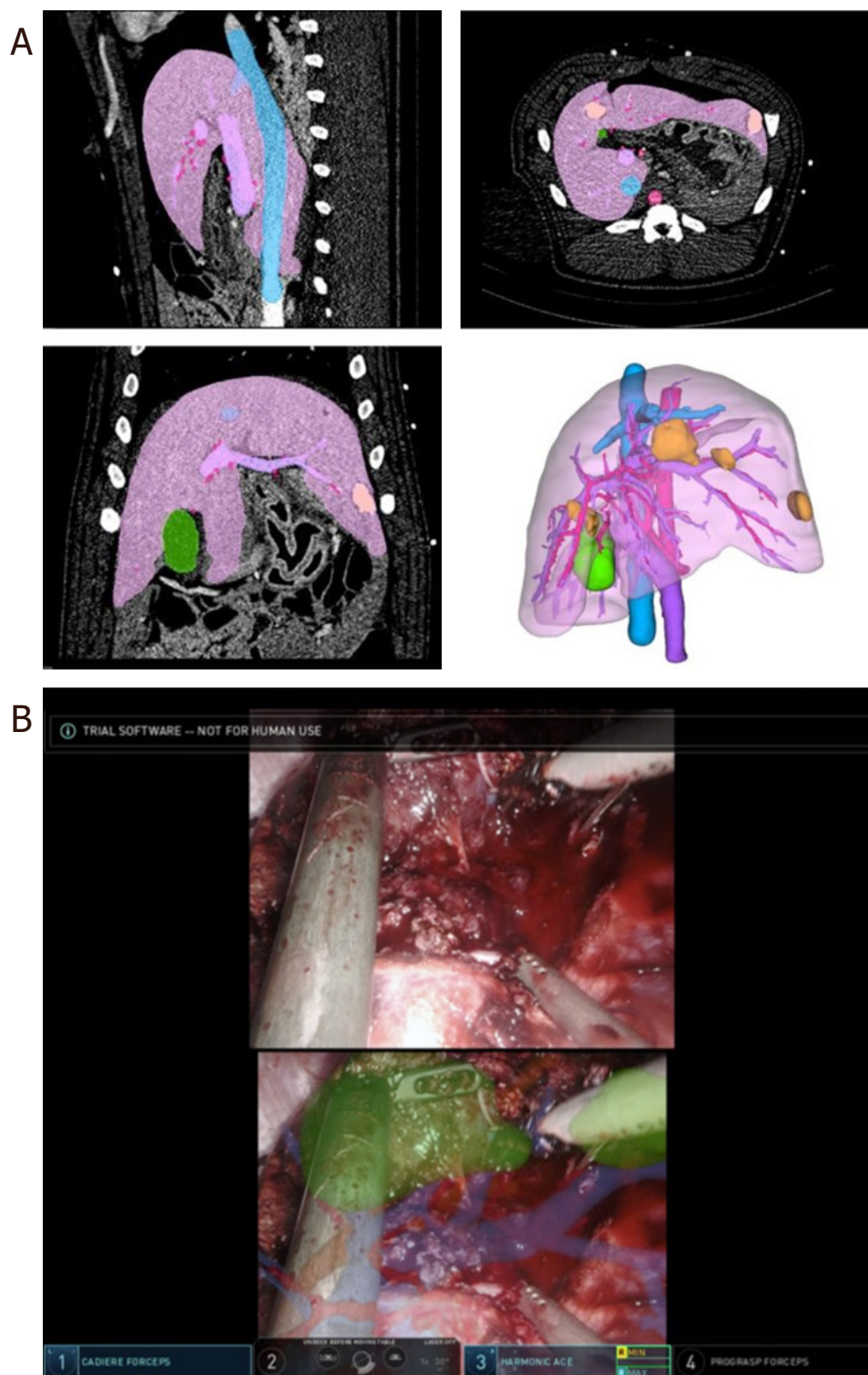


Figure 1. Real time navigation on da Vinci surgeon console. A: interactive three-dimensional anatomic representations are developed from the patient's preoperative imaging; B: 3D representations overlaid onto the surgeon's console in a porcine model to reveal the tumour's location in relation to the vascular and biliary structures in real-time. This technology can enhance the surgeon's ability to visualize the anatomy and perform a margin negative resection. Video and images courtesy of Intuitive. The da Vinci technology presented is still in development, is not 510(k) cleared and the safety and effectiveness of the product has not been established. The technology is not currently for sale in the US

and inferior hepatic segments and may provide benefits over standard laparoscopy during resections in posteriorly and superiorly located tumours.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Labadie KP, Park JO, Sham JG

Performed data acquisition, as well as provided administrative, technical, and material support: Labadie KP, Park JO

Availability of data and materials

Not applicable.

Financial support and sponsorship

Dr. James O. Park received an educational grant of < \$10,000 from Intuitive Surgical Inc for research related to the 3D real time navigation development.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Childers CP, Maggard-Gibbons M. Estimation of the acquisition and operating costs for robotic surgery. *JAMA* 2018;320:835-6.
2. Sheetz KH, Claflin J, Dimick JB. Trends in the adoption of robotic surgery for common surgical procedures. *JAMA Netw Open* 2020;3:e1918911.
3. Kwok YS, Hou J, Jonckheere EA, Hayati S. A robot with improved absolute positioning accuracy for CT guided stereotactic brain surgery. *IEEE Trans Biomed Eng* 1988;35:153-60.
4. Satava RM. Surgical robotics: the early chronicles: a personal historical perspective. *Surg Laparosc Endosc Percutan Tech* 2002;12:6-16.
5. Satava RM. Emerging technologies for surgery in the 21st century. *Arch Surg* 1999;134:1197-202.
6. Bargar WL, Bauer A, Borner M. Primary and revision total hip replacement using the Robodoc system. *Clin Orthop Relat Res* 1998;82-91.
7. Alessandrini M, De Padova A, Napolitano B, Camillo A, Bruno E. The AESOP robot system for video-assisted rigid endoscopic laryngosurgery. *Eur Arch Otorhinolaryngol* 2008;265:1121-3.
8. Marescaux J, Leroy J, Gagner M, Rubino F, Mutter D, et al. Transatlantic robot-assisted telesurgery. *Nature* 2001;413:379-80.
9. Rininsland H. ARTEMIS. A telemanipulator for cardiac surgery. *Eur J Cardiothorac Surg* 1999;16 Suppl 2:S106-11.
10. Ballantyne GH, Moll F. The da Vinci telerobotic surgical system: the virtual operative field and telepresence surgery. *Surg Clin North Am* 2003;83:1293-304, vii.
11. Lanfranco AR, Castellanos AE, Desai JP, Meyers WC. Robotic surgery: a current perspective. *Ann Surg* 2004;239:14-21.
12. Prabhu AS, Carbonell A, Hope W, Warren J, Higgins R, et al. Robotic inguinal vs transabdominal laparoscopic inguinal hernia repair: the RIVAL randomized clinical trial. *JAMA Surg* 2020;155:380-7.
13. Sheetz KH, Norton EC, Dimick JB, Regenbogen SE. Perioperative outcomes and trends in the use of robotic colectomy for medicare beneficiaries from 2010 through 2016. *JAMA Surg* 2019;155:41-9.
14. Choi SB, Park JS, Kim JK, Hyung WJ, Kim KS, et al. Early experiences of robotic-assisted laparoscopic liver resection. *Yonsei Med J* 2008;49:632-8.
15. Giulianotti PC, Coratti A, Sbrana F, Addeo P, Bianco FM, et al. Robotic liver surgery: results for 70 resections. *Surgery* 2011;149:29-39.
16. Nota CL, Rinkes IHB, Molenaar IQ, van Santvoort HC, Fong Y, et al. Robot-assisted laparoscopic liver resection: a systematic review

- and pooled analysis of minor and major hepatectomies. *HPB (Oxford)* 2016;18:113-20.
17. Montalti R, Berardi G, Patriti A, Vivarelli M, Troisi RI. Outcomes of robotic vs laparoscopic hepatectomy: a systematic review and meta-analysis. *World J Gastroenterol* 2015;21:8441-51.
 18. Kingham TP, Leung U, Kuk D, Gonen M, D'Angelica MI, et al. Robotic liver resection: a case-matched comparison. *World J Surg* 2016;40:1422-8.
 19. Beard RE, Khan S, Troisi RI, Montalti R, Vanlander A, et al. Long-term and oncologic outcomes of robotic versus laparoscopic liver resection for metastatic colorectal cancer: a multicenter, propensity score matching analysis. *World J Surg* 2020;44:887-95.
 20. Liu R, Wakabayashi G, Kim HJ, Choi GH, Yiengpruksawan A, et al. International consensus statement on robotic hepatectomy surgery in 2018. *World J Gastroenterol* 2019;25:1432-44.
 21. Magistri P, Tarantino G, Assirati G, Olivieri T, Catellani B, et al. Robotic liver resection for hepatocellular carcinoma: a systematic review. *Int J Med Robot* 2019;15:e2004.
 22. Chen PD, Wu CY, Hu RH, Chou WH, Lai HS, et al. Robotic versus open hepatectomy for hepatocellular carcinoma: a matched comparison. *Ann Surg Oncol* 2017;24:1021-8.
 23. Pawlik TM, Scoggins CR, Zorzi D, Abdalla EK, Andres A, et al. Effect of surgical margin status on survival and site of recurrence after hepatic resection for colorectal metastases. *Ann Surg* 2005;241:715-22, discussion 22-4.
 24. Khan S, Beard RE, Kingham PT, Fong Y, Boerner T, et al. Long-term oncologic outcomes following robotic liver resections for primary hepatobiliary malignancies: a multicenter study. *Ann Surg Oncol* 2018;25:2652-60.
 25. Wang WH, Kuo KK, Wang SN, Lee KT. Oncological and surgical result of hepatoma after robot surgery. *Surg Endosc* 2018;32:3918-24.
 26. Lai EC, Yang GP, Tang CN. Robot-assisted laparoscopic liver resection for hepatocellular carcinoma: short-term outcome. *Am J Surg* 2013;205:697-702.
 27. Qiu J, Chen S, Chengyou D. A systematic review of robotic-assisted liver resection and meta-analysis of robotic versus laparoscopic hepatectomy for hepatic neoplasms. *Surg Endosc* 2016;30:862-75.
 28. Tsung A, Geller DA, Sukato DC, Sabbaghian S, Tohme S, et al. Robotic versus laparoscopic hepatectomy: a matched comparison. *Ann Surg* 2014;259:549-55.
 29. Magistri P, Tarantino G, Guidetti C, Assirati G, Olivieri T, et al. Laparoscopic versus robotic surgery for hepatocellular carcinoma: the first 46 consecutive cases. *J Surg Res* 2017;217:92-9.
 30. Cho JY, Han HS, Yoon YS, Shin SH. Feasibility of laparoscopic liver resection for tumors located in the posterosuperior segments of the liver, with a special reference to overcoming current limitations on tumor location. *Surgery* 2008;144:32-8.
 31. Teramoto K, Kawamura T, Takamatsu S, Noguchi N, Nakamura N, et al. Laparoscopic and thoracoscopic partial hepatectomy for hepatocellular carcinoma. *World J Surg* 2003;27:1131-6.
 32. Troisi RI, Patriti A, Montalti R, Casciola L. Robot assistance in liver surgery: a real advantage over a fully laparoscopic approach? Results of a comparative bi-institutional analysis. *Int J Med Robot* 2013;9:160-6.
 33. Casciola L, Patriti A, Ceccarelli G, Bartoli A, Ceribelli C, et al. Robot-assisted parenchymal-sparing liver surgery including lesions located in the posterosuperior segments. *Surg Endosc* 2011;25:3815-24.
 34. Patriti A, Cipriani F, Ratti F, Bartoli A, Ceccarelli G, et al. Robot-assisted versus open liver resection in the right posterior section. *JSL.S* 2014;18:e2014.00040.
 35. Leung U, Fong Y. Robotic liver surgery. *Hepatobiliary Surg Nutr* 2014;3:288-94.
 36. Nota CL, Woo Y, Raoof M, Boerner T, Molenaar IQ, et al. Robotic versus open minor liver resections of the posterosuperior segments: a multinational, propensity score-matched study. *Ann Surg Oncol* 2019;26:583-90.
 37. Zygomalas A, Kehagias I. Up-to-date intraoperative computer assisted solutions for liver surgery. *World J Gastrointest Surg* 2019;11:1-10.

Original Article

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Stereotactic body radiation therapy for primary liver tumors with adverse factors

Takuya Shimizuguchi¹, Jun Imamura², Simpei Hashimoto¹, Katsuyuki Karasawa¹

¹Department of Radiation Oncology, Cancer and Infectious Diseases Center Komagome Hospital, Tokyo 113-8677, Japan.

²Department of Hepatology, Cancer and Infectious Diseases Center Komagome Hospital, Tokyo 113-8677, Japan.

Correspondence to: Dr. Takuya Shimizuguchi, Department of Radiation Oncology, Cancer and Infectious Diseases Center Komagome Hospital, 3-18-22 Hon-komagome, Bunkyo-ku, Tokyo 113-8677, Japan. E-mail: shimizuguchi@cick.jp

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Abstract

Aim: To test the efficacy and safety of liver stereotactic body radiation therapy (SBRT) in patients who harbor adverse factors.

Methods: We retrospectively evaluated the outcomes of liver SBRT in a single cancer center. We invented criteria consisting of two physical factors and two tumor factors to measure the treatment difficulty in each case. The clinical outcomes and toxicity were evaluated by stratification of the harboring factors.

Results: A total of 24 (23 hepatocellular carcinoma and 1 intrahepatic cholangiocarcinoma) patients were eligible for this study, with a median follow-up duration of 18 months. Of all eligible patients, 21 patients (88%) had one or more factors. The local control, progression-free survival, and overall survival rates for all patients at 2 years were 89%, 42%, and 76% respectively. In the patients with physical and tumor adverse factors, local control/progression-free survival/overall survival rates at 2 years were 100%/42%/69% and 80%/23%/78%, respectively. The subgroup of 11 patients with 2 or more factors showed comparable local control rate at 2 years to the subgroup of 13 patients with 0 to 1 factors (100% vs. 86%, $P = 0.59$). One patient (4.2%) experienced a decline in the Child-Pugh score by 2 points at 3 months after the treatment. Grade 2 to 3 gastrointestinal toxicity was observed in three patients.

Conclusion: SBRT showed a high local control rate with acceptable toxicity for the group of liver cancer patients harboring both physical and tumor adverse factors as long as conducted following patient selection and dose constraints that were used in this study.



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Keywords: Hepatocellular carcinoma, stereotactic body radiation therapy, vulnerable patients

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases in the world and the fourth most common cause of cancer death in Japan^[1,2]. Treatment of HCC consists of surgical resection, radiofrequency ablation, transarterial chemoembolization, and use of systemic anticancer agents^[3,4]. Moreover, the use of stereotactic body radiation therapy (SBRT), a nonsurgical local treatment, has rapidly increased in the past decade owing to its ability to deliver a precise radiation dose with modern radiation oncology devices and techniques^[5]. Referring to the eligibility criteria for prospective liver SBRT trials, the ideal candidate for the treatment is considered to: (1) be in fair general condition; (2) have adequate liver function [Child-Pugh (CP) score 5 to 6]; and (3) a tumor size, number, and location that are amenable to dose constraints for organs at risk (OAR) in treatment planning^[6-8]. However, patients with liver tumors who are referred to SBRT are usually unsuitable for surgery or other local therapies due to comorbidity and impaired liver function. In addition, in daily practice, a considerable proportion of tumors are unfit for typical SBRT planning due to the radiation therapy planning dose constraint. Both physical factors and tumor factors affect the decision of treatment choice. The more patient factors that do not meet clinical trial criteria, the higher tendency to select non-localized treatment or conservative options instead of liver SBRT in clinical practice. To the best of our knowledge, no study has directly tested the impact of those composite adverse factors in liver SBRT. Therefore, this study aimed to evaluate the efficacy and feasibility of liver SBRT for cases with originally defined adverse factors.

METHODS

Patient data

We planned a retrospective study of patients with liver tumors who were treated by liver SBRT at the Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital between 2014 and 2019. The institutional patient database was used to screen patients. The eligibility criteria were as follows: (1) diagnosis of primary liver malignancy; (2) first liver SBRT (subsequent SBRT episodes were excluded); and (3) definitive treatment (not palliative intent). Patient characteristics, laboratory testing, imaging findings, and SBRT parameters were extracted from the medical records, and the comorbidities of the patients were evaluated by the Charlson comorbidity index^[9]. This study was approved by the Institutional Review Board of Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital (#2450).

Treatment procedure

To visualize the liver tumor position, a fiducial marker (Visicoil, RadioMed LLC, California, USA; length: 10 mm, diameter: 0.75 mm) was implanted next to the target by the hand of a hepatologist 2 weeks prior the treatment. Subsequently, a 4-dimensional computed tomography (CT) image with contrast-enhancement was obtained for treatment planning. Treatments were conducted using a Vero 4DRT system (Mitsubishi Heavy Industry, Tokyo, Japan), which equips the tracking system.

Gross tumor volume was defined in the planning CT scan with the guide of contrast-enhanced magnetic resonance imaging image fusion. The clinical target volume (CTV) was obtained by adding a 4-mm margin to the gross tumor volume within the liver volume, while the planning target volume (PTV) was generated by adding a margin to the CTV for uncertainty of setup and tracking (usually a 5-6 mm margin to the CTV).

A total of 40 Gy in 5 fractions over two weeks was prescribed to the 70% isodose fitting to the PTV. The modified prescription was allowed to achieve dose constraints: A reduced prescription dose (down to 32 Gy)

Table 1. Criteria of adverse factors

Factor	Criteria
1. Physical factor	
General condition	ECOG performance status = 2 or above or Charlson comorbidity index = 5 or above
Liver function	CP score = 7 or above or normal liver volume < 1000 mL*
2. Tumor factor	
Tumor persistence	Three or more previous liver tumor treatments**
Planning difficulty	Target is in immediate contact with the gastrointestinal tract or tumor size > 5 cm

*Liver volume subtracted by the gross target volume; **either surgery, radiofrequency ablation, or transarterial chemoembolization for liver tumor. CP score: Child-Pugh score; ECOG: European Clinical Oncology Group

in 5 fractions was selected to reduce the normal liver dose, while a more fractionated schedule (40 Gy in 10 fractions) was selected for cases in which the PTV was in direct contact with the OARs. Dose constraint criteria for the liver were volumes receiving 20 Gy (V20) < 20%. Dose for digestive tube was restricted below 30 Gy in 5 fractions to at most 0.5 cc of the volume.

Evaluation of outcomes and statistical considerations

To measure the difficulty of the treatment in each case, we invented criteria consisting of two physical factors and two tumor factors as shown in Table 1. Each adverse factor was weighted equally, and the patients were categorized by the number of harboring factors.

Patients were usually evaluated by liver function blood test and CT or magnetic resonance imaging every 3 to 6 months after the treatment. Endoscopy was not routinely performed unless the patient had gastrointestinal symptoms. Local control was defined as freedom from radiological progression (> 20% growth in the diameter), and data were censored when patients were lost to follow-up or died without local progression. Progression-free survival was defined as the duration to any liver tumor recurrence and death. Overall survival (OS) was defined as the time until death from any cause. All the indicators were counted from the initial day of SBRT, and rates were estimated by the Kaplan-Meier method. Patients who had two or more adverse factors were compared to those who did not, in terms of local control rate. Toxicity was evaluated using the Common Terminology Criteria for Adverse Events version 4.0. Liver function before and after SBRT was evaluated with the CP scoring system.

RESULTS

A total of 24 patients were eligible for this study between January 2014 and December 2019. The median follow-up duration was 18 months, and the patient characteristics are listed in Table 2. All the patients were pathologically or radiologically diagnosed with primary liver malignancy; one patient was diagnosed with intrahepatic cholangiocarcinoma, the remainder of patients were diagnosed with HCC. A modified SBRT prescription was used in 9 patients (an OAR dose constraint in 3 patients and a liver dose constraint in 6 patients). Of all eligible patients, 21 patients (88%) had one or more adverse factors, 16 patients (67%) had physical factors, 13 patients (54%) had tumor factors, and 11 patients (46%) had two or more adverse factors. The number of patients who met the criteria of the adverse factor was 10 for general condition, 10 for liver function, 9 for tumor persistence, and 7 for planning difficulty.

Outcomes

The local control, progression-free survival, and OS rates for all patients at 2 years were 89%, 42%, and 76%, respectively [Figure 1]. One patient (patient number 8 in Table 2) experienced local progression within the PTV 20 months after SBRT was confirmed on CT. In the patients with physical and tumor adverse factors, local control/progression-free survival/OS rates at 2 years were 100%/42%/69% and 80%/23%/78%, respectively. The subgroup of 11 patients with 2 or more (13 patients with 0 to 1) adverse factors showed

Table 2. Patient baseline characteristics

Characteristic		Value
Age, median (range), years		72 (57-93)
sex	Male	19
	Female	5
ECOG performance status		
0		5
1		16
2		3
Charlson comorbidity index ^[9]		
3 to 4		15
5 or more		9
Underlying liver disease		
HCV infection		7
HBV infection		4
Alcohol		4
Non-alcoholic steatohepatitis		5
Other/none		4
Previous treatment course		
0		9
1 to 2		6
3 or more		9

ECOG: European Oncology Study Group; HVB: hepatitis B virus; HCV: hepatitis C virus

a comparable local control rate at 2 years 100% (86%) ($P = 0.59$) [Figure 2]. In the 11 patients who experienced non-local progression, 9 had intrahepatic recurrences as the first site of recurrence, one had both intrahepatic and regional lymph node recurrence, and one had lung metastasis.

Toxicity

One patient (4.2%) experienced a decline in CP score by 2 points 3 months after the treatment, although it remains unclear whether SBRT directly worsened the liver function since HCC developed rapidly after the treatment. Grade 2 or greater gastrointestinal toxicity was observed in three patients: One patient experienced Grade 3 cholangitis 3 months after the SBRT for S4/8 tumor; another patient experienced Grade 2 esophagitis 2 months after SBRT for S8 tumor; and the other patient with an S5/6 tumor experienced Grade 2 lower gastrointestinal hemorrhage. The list of SBRT treatment planning parameters, liver function before and after treatment, and toxicity are shown in Table 3.

DISCUSSION

The current study evaluated the safety and efficacy of liver SBRT in patients with combined treatment difficulty. An equally high local control rate with acceptable toxicity was demonstrated in patients with both physical and tumor adverse factors.

The patient selection criteria of the prospective liver SBRT trials, and the patient characteristics that were actually included denote the group of patients in which the feasibility of SBRT has been confirmed. Existing prospective studies of liver SBRT mainly recruited patients with CP A diseases, or those with at least CP B score 7^[6-8]. Additionally, most of the patients who participated in those prospective studies were categorized as ECOG PS 0 or 1. Thus, the safety and efficacy of liver SBRT in unfit patients has not been demonstrated in prospective trials. Although tumor location is not necessarily regarded as a crucial factor in liver SBRT eligibility, substantial attention is paid to dose constraints when the tumor is in contiguity with OAR. Furthermore, controversy exists in relation to the dose constraint for the digestive tube, although previous studies encourage clinicians to reduce the dose for the digestive tube below the prescription dose^[10].

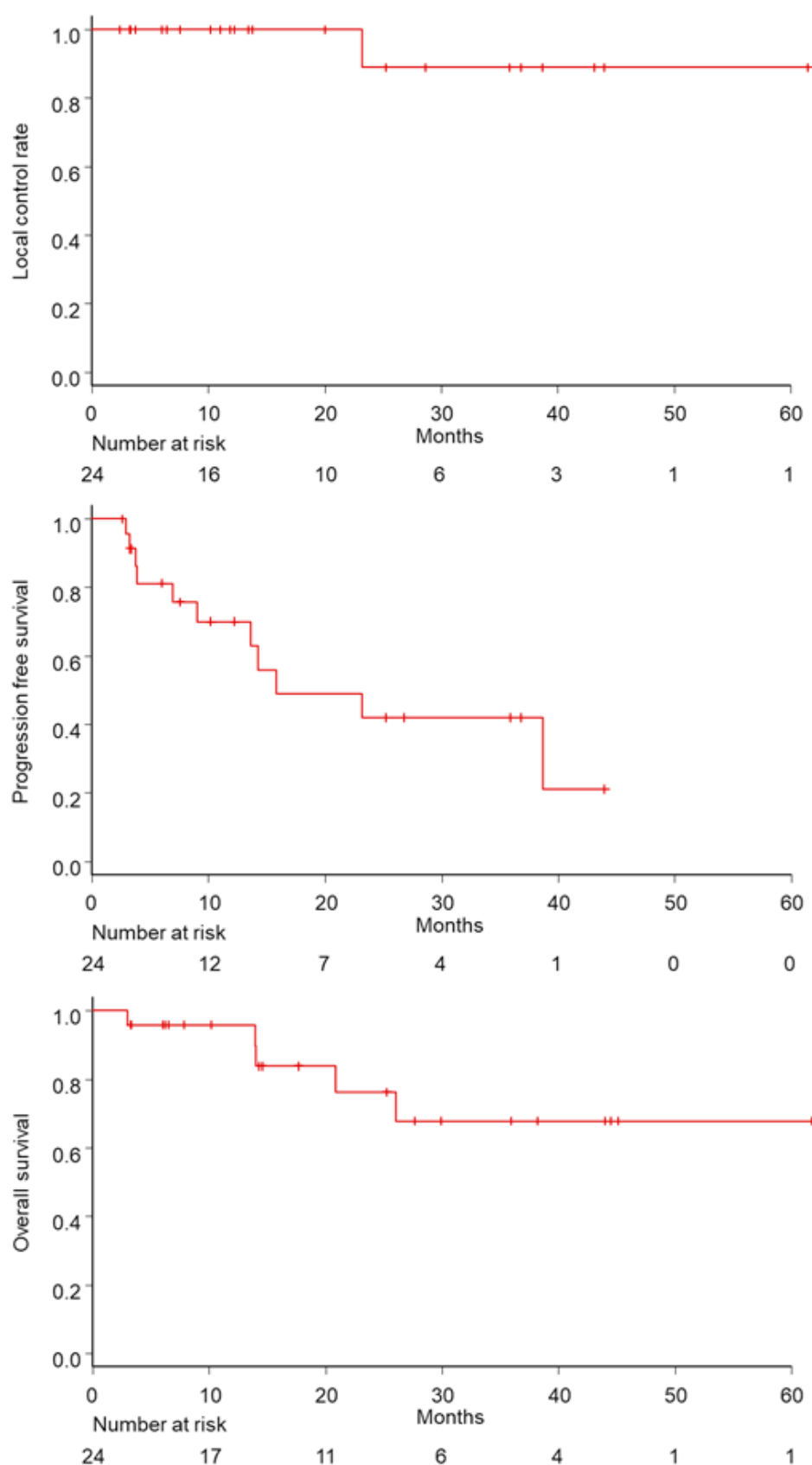


Figure 1. Local control rate (top), progression-free survival rate (middle), and overall survival rate (bottom) for all patients in this study

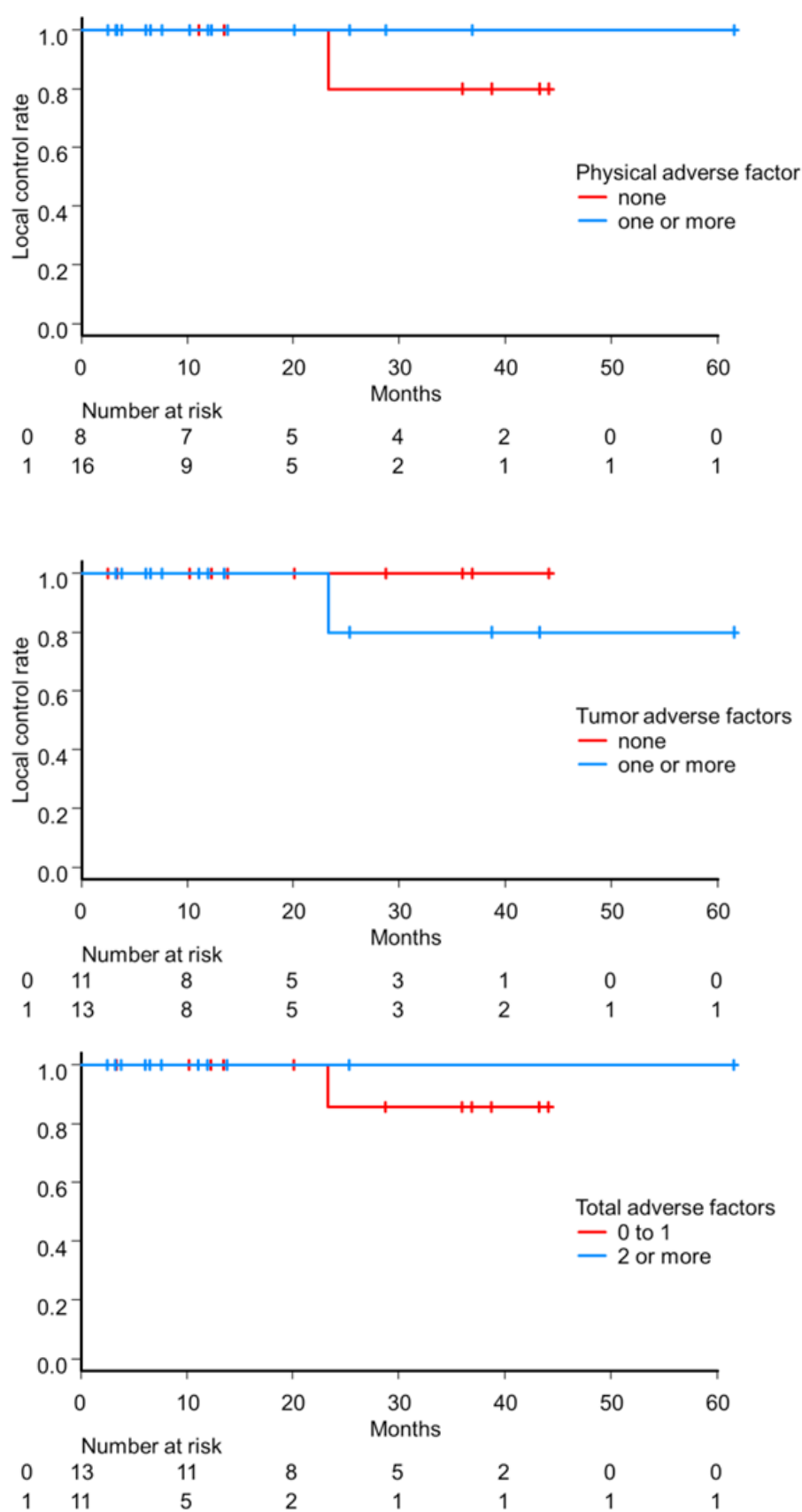


Figure 2. Local control rate for patients with or without physical adverse factor (top), for patients with or without (middle) and for patients with 2 or more total factors or not (bottom)

Table 3. Stereotactic body radiation therapy parameters, liver function, and adverse events

Patient	Tumor size (mm)	Number of physical adverse factor	Number of tumor adverse factor	Total number of adverse factors	Total prescribed dose	Number of fraction	Liver volume - TV (mL)	Mean dose for liver volume - TV (Gy)	Liver V20 Gy (%) [*]	Maximal dose of spared 700 cc of liver	Non-liver adverse events	CP score	
												Baseline	12 months after SBRT
1	20	0	0	0	4000	5	1020	9.0	10.7	11.5		5	NA
2	10	0	0	0	4000	5	1224	6.3	5.2	1.3		5	NA
3	26	0	0	0	4000	5	1065	9.2	20.4	10.0		5	5
4	21	0	1	1	4000	5	1487	6.5	5.2	1.5		5	5
5	28	1	0	1	3500	5	964	10.2	15.6	13.7		5	5
6	12	0	1	1	4000	5	1452	9.9	12.1	6.2		5	5
7	66	0	1	1	3200	5	1047	14.3	26.4	17.7	Biliary tract infection G3	6	5
8	70	0	1	1	3500	5	1026	11.3	22.3	12.4		5	5
9	17	1	0	1	4000	5	987	6.1	5.0	6.7		5	NA
10	39	1	0	1	4000	5	1155	13.8	23.8	14.0		5	5
11	10	1	0	1	4000	5	1457	4.1	3.6	2.4		5	5
12	48	1	0	1	4000	5	1223	10.3	18.7	4.7		5	7
13	45	1	0	1	4000	5	1556	11.6	20.0	4.5		6	5
14	43	0	2	2	4000	10	1203	11.3	14.5	11.1		6	8
15	24	1	1	2	4000	5	792	11.8	18.2	26.6		5	5
16	22	1	1	2	4000	5	862	10.2	14.0	16.6		5	NA
17	34	1	1	2	4000	5	1681	10.5	11.0	5.1		6	5
18	36	2	0	2	3500	5	785	15.4	24.5	31.7		5	5
19	35	2	0	2	4000	5	823	11.8	19.2	23.4		5	NA
20	79	1	1	2	3200	5	1401	12.9	22.2	11.2		5	NA
21	38	1	2	3	4000	10	821	14.5	26.1	30.1		5	NA
22	28	1	2	3	4000	10	699	13.6	20.8	Not applicable		5	5
23	62	2	1	3	3500	5	820	10.5	20.7	27.9	Esophagitis G2	5	NA
24	33	2	1	3	4000	5	1086	8.7	14.2	7.2	Lower gastrointestinal hemorrhage G2	8	NA

^{*}volume receiving 20 Gy. CP score: Child-Pugh score; G: Grade; NA: not available; SBRT: stereotactic body radiation therapy; TV: target volume

A systematic quantitative review including 13 studies on the efficacy of SBRT reported no obvious relationship between the local control rate and the biologically effective dose among patients treated with SBRT for primary liver tumors^[11]. In a Japanese retrospective study, a reduced intensity prescription (35 Gy in 5 fractions) was used for CP B patients, although equally good local control rate was obtained in the standard dose group (40 Gy in 5 fractions). The local control rate at 3 years for 35 Gy and 40 Gy group was 89% and 91% respectively^[12]. From these results, dose-escalation seems unnecessary, especially in cases harboring adverse factors.

Studies involving patients with more advanced liver tumors, or in those with impaired liver function, reported a greater risk of treatment-related toxicity. In the largest prospective Phase I/II study in patients with multiple HCC, 68% of patients had two or more lesions, resulting in a mean liver dose > 20 Gy in a fraction of patients. Seven patients experienced Grade 5 adverse events (liver failure for five patients). Additionally, 29% of the patients experienced deterioration in the CP class 3 months after SBRT^[6]. A study involving patients with poor liver function (CP B or C) reported that the OS rate at 1 year was 32% and 63% of patients experienced a decline in CP score by 2 or more points at 3 months^[13]. For challenging cases, the indication for patient-oriented SBRT should be decided based on HCC prognosis, liver function, patients request, and other options.

The originally defined adverse factors, both physical and tumor factors, in this study did not seem to be a crucial issue in the liver SBRT. Of the total included patients, 88% had at least one and 46% had two or more adverse factors, although a high local control rate and acceptable toxicity were achieved. Thus, our approach appears reasonable in terms of patient selection and toxicity management.

In the future, novel technologies might change the borderline of the indication of liver SBRT. Magnetic resonance imaging linac provides real-time high contrast image-guided radiation therapy, which enables highly accurate dose delivery with a minimal PTV margin^[14]. Moreover, proton beam RT has an advantage on dose distribution over standard proton-based radiation in terms of its physical profile, and some prospective trials have reported the clinical outcome^[15]. However, as there is no direct comparison to date, its clinical advantage over photon-SBRT remains unclear. To clarify this point, a Phase III randomized trial NRG-GI003 (NCT03186898) is open for accruing patients.

Advances in other local liver treatments, including, surgery and radiofrequency ablation, have also provided the opportunity for less invasive local treatment for HCC. Indeed, laparoscopic resection is a recently established method of hepatic resection that is supported by several studies^[16,17]. Furthermore, robotic surgery is a promising modality in the field of surgical resection of malignant disease. With regards to liver tumor resection, due to lack of evidence, robotic surgery is not the standard of care at this time, while oncological efficacy and the perioperative outcome is under evaluation^[18]. In the future, as less invasive treatment options become available, the current indication of local treatment can be overwritten. Discussion in a multi-disciplinary team, consisting of a surgeon, hepatologist, medical oncologist, and radiation oncologist, may lead to better decision making, especially in cases with adverse factors.

This study has several limitations due to the small retrospective study basis. The number of patients and events are not sufficient to perform a reliable statistical test to detect the critical adverse factors related to SBRT. Additionally, there were no highly challenging cases in the current study population; for example, poorer liver function, poorer performance status, or multiple lesions. Thus, the boundary of feasible patients on liver SBRT was not shown in this study.

In conclusion, SBRT was safely and effectively administered to a group of patients harboring both physical and tumor adverse factors as long as conducted following patient selection and dose constraints that were used in this study. Therefore, SBRT seems to be a good treatment option for patients with primary liver tumors.

DECLARATIONS

Authors' contributions

Made contributions to conception and design of the study, performed data analysis and interpretation and manuscript writing: Shimizuguchi T

Performed data acquisition, technical and material support and manuscript editing: Imamura J, Hashimoto S, Karasawa K

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This study was approved by the Institutional Review Board of Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital (#2450).

Consent for publication

Not applicable.

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REFERENCES

1. Akinyemiju T, Abera S, Ahmed M, Alam N. Global burden of disease liver cancer collaboration. the burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. *JAMA Oncol* 2017;3:1683-91.
2. Hori M, Matsuda T, Shibata A, Katanoda K, Sobue T, et al. Cancer incidence and incidence rates in Japan in 2009: a study of 32 population-based cancer registries for the Monitoring of Cancer Incidence in Japan (MCIJ) project. *Jpn J Clin Oncol* 2015;45:884-91.
3. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017;11:317-70.
4. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018;67:358-80.
5. Murray LJ, Dawson LA. Advances in stereotactic body radiation therapy for hepatocellular carcinoma. *Semin Radiat Oncol* 2017;27:247-55.
6. Bujold A, Massey CA, Kim JJ, Brierley J, Cho C, et al. Sequential phase I and II trial stereotactic body radiotherapy for locally advanced hepatocellular carcinoma. *J Clin Oncol* 2013;31:1631-9.
7. Kang JK, Kim MS, Cho CK, Yang KM, Yoo HJ, et al. Stereotactic body radiation therapy for inoperable hepatocellular carcinoma as a local salvage treatment after incomplete transarterial chemoembolization. *Cancer* 2012;118:5424-31.
8. Durand-Labrunie J, Baumann AS, Ayav A, Laurent V, Boleslawski E, et al. Curative irradiation treatment of hepatocellular carcinoma: a multicenter phase 2 trial. *Int J Radiat Oncol Biol Phys* 2020;107:116-25.
9. Charlson ME, Pompei P, Ales KL, MacKenzie CR, Tang GJ, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987;40:373-83.
10. Michel R, Françoise I, Laure P, Anouchka M, Guillaume P, et al. Dose to organ at risk and dose prescription in liver SBRT. *Rep Pract Oncol Radiother* 2017;22:96-102.
11. Ohri N, Tomé WA, Méndez Romero A, Miften M, Ten Haken RK, et al. Local control after stereotactic body radiation therapy for liver tumors. *Int J Radiat Oncol Biol Phys* 2018; doi: 10.1016/j.ijrobp.2017.12.288.
12. Sanuki N, Takeda A, Oku Y, Mizuno T, Aoki Y, et al. Stereotactic body radiotherapy for small hepatocellular carcinoma: a retrospective outcome analysis in 185 patients. *Acta Oncol* 2014;53:399-04.
13. Culleton S, Jiang H, Haddad CR, Kim J, Brierley J, et al. Outcomes following definitive stereotactic body radiotherapy for patients with Child-Pugh B or C hepatocellular carcinoma. *Radiother Oncol* 2014;111:412-7.
14. Rosenberg SA, Henke LE, Shaverdian N, Mittauer K, Wojcieszynski AP, et al. A multi-institutional experience of MR-guided liver stereotactic body radiation therapy. *Adv Radiat Oncol* 2018;4:142-9.
15. Hong TS, Wo JY, Yeap BY, Ben-Josef E, McDonnell EI, et al. Multi-institutional phase ii study of high-dose hypofractionated proton beam therapy in patients with localized, unresectable hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *J Clin Oncol* 2016;34:460-8.
16. Cheung TT, Poon RT, Yuen WK, Chok KS, Jenkins CR, et al. Long-term survival analysis of pure laparoscopic versus open hepatectomy for hepatocellular carcinoma in patients with cirrhosis: a single-center experience. *Ann Surg* 2013;257:506-11.
17. Yoon YI, Kim KH, Kang SH, Kim WJ, Shin MH, et al. Pure laparoscopic versus open right hepatectomy for hepatocellular carcinoma in

- patients with cirrhosis: a propensity score matched analysis. *Ann Surg* 2017;265:856-63.
18. Liu R, Wakabayashi G, Kim HJ, Choi GH, Yiengpruksawan A, et al. International consensus statement on robotic hepatectomy surgery in 2018. *World J Gastroenterol* 2019;25:1432-44.

Original Article

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Prognostic ability of inflammation-based markers in radioembolization for hepatocellular carcinoma

Grant Yoneoka¹, Kliment Bozhilov^{1,2}, Linda L. Wong^{1,2}

¹Transplant Center, The Queen's Medical Center, Honolulu, HI 96813, USA.

²Department of Surgery, University of Hawaii, John A. Burns School of Medicine, Honolulu, HI 96813, USA.

Correspondence to: Dr. Linda L. Wong, Department of Surgery, University of Hawaii, John A. Burns School of Medicine, 550 South Beretania Street, Suite 403, Honolulu, HI 96813, USA. E-mail: hepatoma@aol.com

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Abstract

Aim: Inflammation-based markers, such as the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), have recently been used as prognostic indicators in hepatocellular carcinoma (HCC). We aimed to determine whether NLR and PLR may predict response to yttrium-90 transarterial radioembolization (TARE) as primary treatment for HCC.

Methods: We performed a retrospective review of a prospectively collected database of HCC cases (1994-2019) and selected patients who received TARE as primary treatment ($n = 42$). Laboratory studies were used to calculate NLR and PLR. Response to TARE was determined using the modified response evaluation criteria in solid tumors (mRECIST). Patients were classified as non-responders (stable or progressive disease) or responders (partial or complete response) to treatment based on mRECIST.

Results: Receiver operating characteristic curves identified a pre-treatment NLR cutoff of ≥ 2.83 and a pre-treatment PLR cutoff of ≥ 83 for predicting non-response to treatment. Pre-treatment NLR ≥ 2.83 was the only significant predictor of non-response to TARE in multivariate logistic regression analysis (odds ratio 7.83, $P = 0.036$). On time to progression analysis, both pre-treatment NLR ≥ 2.83 and pre-treatment PLR ≥ 83 were associated with a higher proportion of tumor progression at 6 months post-treatment (43.6% vs. 10.0%, $P = 0.014$, log-rank) and (38.6% vs. 0%, $P = 0.010$, log-rank), respectively.

Conclusion: NLR confers prognostic value and may be superior to PLR in determining response to TARE as primary treatment for HCC. Future studies are necessary to validate these findings in a larger cohort.



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Keywords: Neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, transarterial radioembolization, hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the fourth most common cause of cancer-related death worldwide^[1]. In the United States, the overall prognosis for HCC is poor, with a 5-year survival rate of 10%^[2]. Generally accepted curative therapies include liver resection or transplantation. Unfortunately, patients with advanced disease are usually not amenable to surgical intervention. For patients with unresectable tumors, transarterial chemoembolization (TACE) or transarterial radioembolization (TARE) with yttrium-90 (Y90) can be considered to treat or downstage disease to qualify for curative surgery. Although TACE has been the mainstay of treatment for intermediate-stage tumors, TARE has a distinct advantage in that it can be used in portal venous thrombosis and has a better adverse effect profile with similar efficacy to TACE^[3-5].

Response to TARE is measured by the modified response evaluation criteria in solid tumors (mRECIST) using either contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI)^[6]. Depending on individual center protocols, initial images are performed 1 to 3 months post-treatment. Unfortunately, tumors that are non-responsive to TARE may progress while waiting for subsequent imaging. Therefore, prognostic biomarkers are needed to help predict which patients will benefit from TARE.

The serum marker alpha-fetoprotein (AFP), widely used as a screening tool for HCC, has been shown to have prognostic value in treatment^[7,8]. However, AFP is also elevated in non-tumor environments and is not particularly sensitive for small tumors^[9]. Liquid biopsy, which detects circulating tumor cells or nucleic acids, is a promising alternative to AFP but is not yet widely available^[10]. Recently, inflammatory markers such as the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have gained popularity as prognostic indicators in cancer^[11-14]. While the mechanism behind these markers is not precisely understood, a proinflammatory environment along with thrombocytosis has been associated with tumor growth and survival^[15-17].

Previous studies have highlighted the clinical utility of NLR and PLR as prognostic markers for HCC after liver resection, transplantation, and TACE^[12,14,18-20]. However, the use of NLR in combination with PLR for TARE has not been well established. This study aimed to understand the prognostic value of NLR and PLR in patients who received TARE as a first-line therapy for HCC.

METHODS

Patients

This was a retrospective review from a prospectively collected database of 1,442 patients diagnosed with HCC from 1993 to 2019. All patients were referred to a group of hepatobiliary surgeons affiliated with a tertiary medical center in Hawaii that has the only liver center and liver transplant program in the state. This surgical group evaluates approximately 60%-70% of all the cases of HCC in Hawaii and includes referrals from the American territories of the Pacific Basin. We selected patients who received TARE as a primary treatment for HCC. Patients were excluded if they had a previous liver resection, liver transplant, any systemic therapy or locoregional therapy prior to TARE. Patients were also excluded if they received adjuvant therapy following TARE but prior to follow-up imaging. Patients who had initial follow-up later than 12 months were additionally excluded. We included patients who had two separate TARE treatments for bilateral or extensive disease. These Y90 treatments were typically done about 1 month apart, and

imaging tests were done 3 months after treatment. This retrospective chart and imaging review study was approved by the Institutional Review Board at the Queen's Medical Center and was determined to be exempt from needing informed consent.

The diagnosis of HCC was made with histologic confirmation of HCC with biopsy or with contrast-enhanced imaging (CT or MRI) which demonstrated liver mass or masses with LI-RADS 5 criteria, an arterial phase hyperenhancement and one or more of the following, "washout" on venous phase, an enhancing capsule or threshold growth. These criteria were also consistent with the Organ Procurement and Transplantation class 5 criteria.

Pre-treatment imaging was performed using either CT or MRI. Pre-treatment tumor size was defined as the sum of the diameters of all enhancing lesions. All images were taken within 6 months prior to TARE. All patients were evaluated by an interventional radiologist, hepatologist and surgeon and cases were discussed at a multidisciplinary hepatobiliary conference. Patients were not candidates for TARE if they had a total bilirubin above 2.0 mg/dL or evidence of extrahepatic spread of disease. A Y90 arterial mapping procedure was performed to identify the tumor(s), vascular branches supplying the tumor and degree of lung shunting with ^{99m}Tc -macroaggregated albumin. Patients with greater than 10% lung shunting were not candidates for TARE.

Radioembolization was performed with Y90 delivered via glass microspheres (TheraSphere, Boston Scientific, USA) or resin microspheres (SIR-Spheres, Sirtex Medical, Australia). All procedures were performed by one of seven interventional radiologists who comprise the only group that performs complex hepatobiliary interventions in Hawaii.

Post-treatment imaging was performed at approximately 3-month and 6-month intervals. Response to TARE was determined using mRECIST. Patients with complete response (CR) or partial response (PR) according to mRECIST were further classified into a response group, while patients with stable disease (SD) or progressive disease (PD) were classified into a non-response group. For patients who received both a 3-month and 6-month scan, the 6-month scan was used to determine overall response to treatment.

Data collected

Collected demographic information included patient age, sex and race. Medical history included height, weight, body mass index, diabetes mellitus, hyperlipidemia, hypertension, infection with hepatitis B or hepatitis C, significant alcohol use (> 2 alcoholic beverages daily for 10 years), smoking history, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, ascites, encephalopathy, cirrhosis, AFP and normal AFP (< 20 ng/mL).

Laboratory studies included prothrombin time, international normalized ratio, creatinine, aspartate transaminase, alanine aminotransferase, bilirubin, albumin, white blood cell count, neutrophil count, lymphocyte count and platelet count. Laboratory values were obtained prior to TARE and approximately 2 weeks, 3 months and 6 months post-treatment. NLR was defined as the ratio between the absolute neutrophil count and the absolute lymphocyte count. PLR was defined as the ratio between the absolute platelet count and the absolute lymphocyte count. Date of laboratory draws were used to determine temporal trends in NLR and PLR following treatment. Albumin-bilirubin (ALBI) grade and Child-Pugh class were calculated from baseline laboratory values.

After identifying patients and collecting baseline data from the prospectively collected database, individual charts were queried to obtain detailed imaging reports. Imaging was reviewed and measured retrospectively by a single physician. Collected imaging data included pre-treatment tumor size, post-treatment tumor size, mRECIST, and dates of imaging and treatment.

Table 1. Cohort characteristics

Characteristic	
Mean age in years (s.d.)	66.8 (11.3)
Males	30 (71.4%)
Ethnicity	
Caucasian	8 (19.0%)
Pacific Islander	6 (14.3%)
Asian	26 (61.9%)
Hispanic	2 (4.8%)
Hepatitis B surface Ag positive	5 (11.9%)
Hepatitis B core Ab positive	6 (14.3%)
Hepatitis C positive	19 (45.2%)
Alcohol abuse	14 (33.3%)
NASH/NAFLD	13 (31.0%)
Mean BMI (s.d.)	26.7 (4.9)
BMI 30 or higher	8 (19.0%)
Smoking history	27 (64.3%)
Diabetes mellitus	17 (40.5%)
Hyperlipidemia	19 (45.2%)
Hypertension	32 (76.2%)
ALBI	
Grade 1	19 (45.2%)
Grade 2	20 (47.6%)
Grade 3	3 (7.1%)
Child-Pugh class	
A	33 (78.6%)
B	9 (21.4%)
Mean AFP in ng/mL (s.d.)	2023 (7605)
Normal AFP	24 (57.1%)
Mean total tumor size in cm (s.d.)	7.0 (4.0)
Number of tumors (s.d.)	1.71 (1.07)

s.d.: standard deviation; NASH: non-alcoholic steatohepatitis; NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; ALBI: albumin-bilirubin; AFP: alpha-fetoprotein

Statistical analysis

Receiver operator characteristic (ROC) curves were used to determine optimal NLR and PLR cutoffs. Cutoff points were selected by maximizing Youden's index. Mean comparisons were analyzed using Welch's *t*-test. Categorical comparisons were performed using Fisher's exact test. Independent predictors of response to treatment were determined using univariate logistic regression. Variables that were significant in the univariate analysis were included in the multivariate logistic regression model. Time to progression (TTP) was defined as the date of treatment until the date of PD based on mRECIST. Patients who did not reach the endpoint were censored based on their last imaging date. TTP was analyzed via the Kaplan-Meier method and compared using the log-rank test. All tests were two-tailed, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS version 26 (IBM, USA), Jamovi version 1.0.8 and GraphPad Prism8 (GraphPad Software, USA).

RESULTS

Cohort characteristics

In this cohort of 1,442 patients with HCC, a total of 276 TARE procedures were performed. Of those patients, 77 received TARE as primary treatment for HCC, and 42 patients met criteria for this study. Seven patients received a second TARE procedure within a month of the first: six were for bilateral disease, and one patient had extensive disease that was completed in 2 separate sessions to treat the entire lobe. The characteristics of this cohort are described in Table 1. The mean age of the cohort was 66.8 years [standard deviation (s.d.) 11.3 years]. There were 30 males and 12 females. Asian represented the largest ethnicity

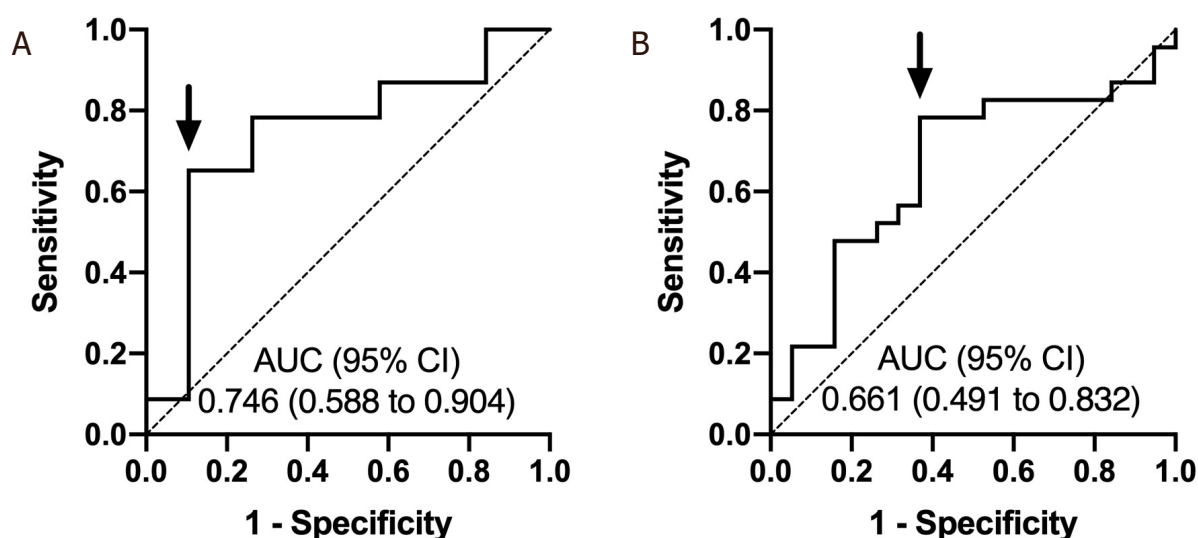


Figure 1. Receiver operating characteristic curves for pre-treatment NLR (A) and PLR (B) in predicting non-response to TARE. The cutoff points for pre-treatment NLR and PLR were 2.83 and 83, respectively. Arrows depict selected cutoff points. NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; AUC: area under the curve; CI: confidence interval; TARE: transarterial radioembolization

(61.9%), followed by Caucasian (19.0%), Pacific Islander (14.3%) and Hispanic (4.8%). There were 19 ALBI grade 1 patients, 20 ALBI grade 2 patients and 3 ALBI grade 3 patients. There were 33 Child-Pugh class A patients and 9 Child-Pugh class B patients. There were no Child-Pugh class C patients. The mean pre-treatment AFP was 2,023 ng/mL (s.d. 7605 ng/mL). Twenty-three patients had normal AFP prior to TARE. The mean total tumor size was 7.0 cm (s.d. 4.0 cm), and the mean number of tumors was 1.71 (s.d. 1.07).

Determination of cutoff points and comparison between groups

ROC analysis identified a pre-treatment NLR cutoff of 2.83 [area under the curve (AUC) = 0.746, 95% confidence interval (CI): 0.588-0.904, sensitivity: 65.2% and specificity: 89.5%] [Figure 1A] and a pre-treatment PLR cutoff of 83 (AUC = 0.661, 95%CI: 0.491-0.832, sensitivity: 78.3% and specificity: 63.2%) for predicting non-response to TARE [Figure 1B].

The mean age was higher in the pre-treatment NLR ≥ 2.83 group than the pre-treatment NLR < 2.83 group (72.2 vs. 63.1, $P = 0.008$) [Table 2]. Pre-treatment NLR ≥ 2.83 was associated with ALBI grade ≥ 2 ($P = 0.029$). The pre-treatment NLR ≥ 2.83 group had a higher mean neutrophil count ($3.97 \times 10^9/\text{L}$ vs. $2.51 \times 10^9/\text{L}$, $P = 0.001$) but lower mean lymphocyte count ($0.98 \times 10^9/\text{L}$ vs. $1.71 \times 10^9/\text{L}$, $P = 0.001$) compared to the pre-treatment NLR < 2.83 group.

The mean age was higher in the pre-treatment PLR ≥ 83 group than the pre-treatment PLR < 83 group (72.1 vs. 59.0, $P = 0.001$) [Table 3]. Pre-treatment PLR ≥ 83 was associated with hyperlipidemia ($P = 0.004$) and Child-Pugh class B ($P = 0.006$). The pre-treatment PLR ≥ 83 group had a higher mean platelet count ($186.2 \times 10^9/\text{L}$ vs. $97.5 \times 10^9/\text{L}$, $P = 0.001$) but lower mean lymphocyte count ($1.24 \times 10^9/\text{L}$ vs. $1.67 \times 10^9/\text{L}$, $P = 0.048$) compared to the pre-treatment PLR < 83 group.

Response to treatment

The change in response to TARE over time is shown in Figure 2. There were 15 responders to treatment (4 CR, 11 PR) and 25 non-responders to treatment (18 SD, 7 PD) at 3-month follow-up. At 6-month follow-up, there were 14 responders to treatment (6 CR, 8 PR) and 4 non-responders to treatment (4 SD). In total, using the latest available scan to determine overall response, there were 19 responders to treatment (7 CR,

Table 2. Comparison of pre-treatment NLR < 2.83 and NLR ≥ 2.83 groups

	Pre-treatment NLR < 2.83 (n = 25)	Pre-treatment NLR ≥ 2.83 (n = 17)	P-value
Mean age in years (s.d.)	63.1 (11.0)	72.2 (9.7)	0.008
Male sex	18 (72.0%)	12 (70.6%)	1.000
Hepatitis B	5 (20.0%)	0 (0%)	0.070
Hepatitis C	14 (56.0%)	5 (29.4%)	0.120
Alcohol abuse	8 (32.0%)	6 (35.3%)	1.000
NASH/NAFLD	5 (20.0%)	8 (47.1%)	0.092
Mean BMI (s.d.)	26.2 (4.4)	27.4 (5.5)	0.448
Smoking history	14 (56.0%)	13 (76.5%)	0.207
Diabetes mellitus	8 (32.0%)	9 (52.9%)	0.212
Hyperlipidemia	8 (32.0%)	11 (64.7%)	0.059
Hypertension	18 (72.0%)	14 (82.4%)	0.490
ALBI grade ≥ 2	10 (40.0%)	13 (76.5%)	0.029
Child-Pugh class B	4 (16.0%)	5 (29.4%)	0.446
Mean neutrophils (10 ⁹ /L) (s.d.)	2.51 (0.98)	3.97 (1.18)	0.001
Mean lymphocytes (10 ⁹ /L) (s.d.)	1.71 (0.66)	0.98 (0.42)	0.001
Mean AFP in ng/mL (s.d.)	2157 (8765)	1826 (5734)	0.883
Normal AFP	15 (60.0%)	9 (52.9%)	0.755
Mean total tumor size in cm (s.d.)	6.1 (3.6)	8.3 (4.3)	0.088
Number of tumors (s.d.)	1.76 (1.01)	1.65 (1.17)	0.748

NLR: neutrophil-to-lymphocyte ratio; s.d.: standard deviation; NASH: non-alcoholic steatohepatitis; NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; ALBI: albumin-bilirubin; AFP: alpha-fetoprotein

Table 3. Comparison of pre-treatment PLR < 83 and PLR ≥ 83 groups

	Pre-treatment PLR < 83 (n = 17)	Pre-treatment PLR ≥ 83 (n = 25)	P-value
Mean age in years (s.d.)	59.0 (9.1)	72.1 (9.6)	0.001
Males	14 (82.4%)	16 (64.0%)	0.300
Hepatitis B	2 (11.8%)	3 (12.0%)	1.000
Hepatitis C	11 (64.7%)	8 (32.0%)	0.059
Alcohol abuse	5 (29.4%)	9 (36.0%)	0.747
NASH/NAFLD	3 (17.6%)	10 (40.0%)	0.179
Mean BMI (s.d.)	26.9 (4.4)	26.5 (5.3)	0.772
Smoking history	12 (70.6%)	15 (60.0%)	0.531
Diabetes mellitus	5 (29.4%)	12 (48.0%)	0.339
Hyperlipidemia	3 (17.6%)	16 (64.0%)	0.004
Hypertension	12 (70.6%)	20 (80.0%)	0.714
ALBI grade ≥ 2	7 (41.2%)	16 (64.0%)	0.209
Child-Pugh class B	0 (0.0%)	9 (36.0%)	0.006
Mean platelets (10 ⁹ /L) (s.d.)	97.5 (51.1)	186.2 (75.2)	0.001
Mean lymphocytes (10 ⁹ /L) (s.d.)	1.67 (0.68)	1.24 (0.62)	0.048
Mean AFP in ng/mL (s.d.)	516 (1163)	3048 (9757)	0.211
Normal AFP	10 (58.8%)	14 (56.0%)	1.000
Mean total tumor size in cm (s.d.)	6.3 (4.0)	7.5 (4.0)	0.359
Number of tumors (s.d.)	2.06 (1.09)	1.48 (1.00)	0.090

PLR: platelet-to-lymphocyte ratio; s.d.: standard deviation; NASH: non-alcoholic steatohepatitis; NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; ALBI: albumin-bilirubin; AFP: alpha-fetoprotein

12 PR) and 23 non-responders to treatment (16 SD, 7 PD). Of the causes of progression in the 7 patients with PD, 1 had new intrahepatic lesions, 4 had an increase in size of existing intrahepatic lesion(s) and 2 had both an increase in size of an existing intrahepatic lesion and a new intrahepatic lesion.

NLR and PLR for non-responders and responders

The mean values of NLR and PLR at pre-treatment, 2 weeks post-treatment, 3 months post-treatment and 6 months post-treatment are shown in [Figure 3](#). The mean pre-treatment NLR for non-responders was

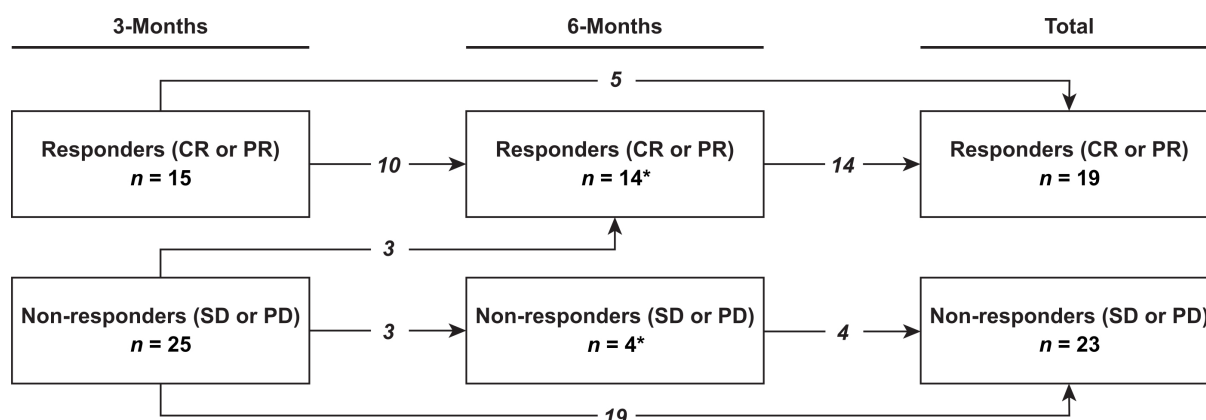


Figure 2. Changes in response to transarterial radioembolization over time. Response was defined as complete response (CR) or partial response (PR) using modified response evaluation criteria in solid tumors. Non-response was defined as stable disease (SD) or progressive disease (PD). Arrows depict changes in response between 3-month and 6-month imaging. The total box represents the overall count of responders and non-responders to treatment. Two patients (one responder and one non-responder) did not receive 3-month imaging, and initial response was evaluated at 6-month follow-up instead (asterisk)

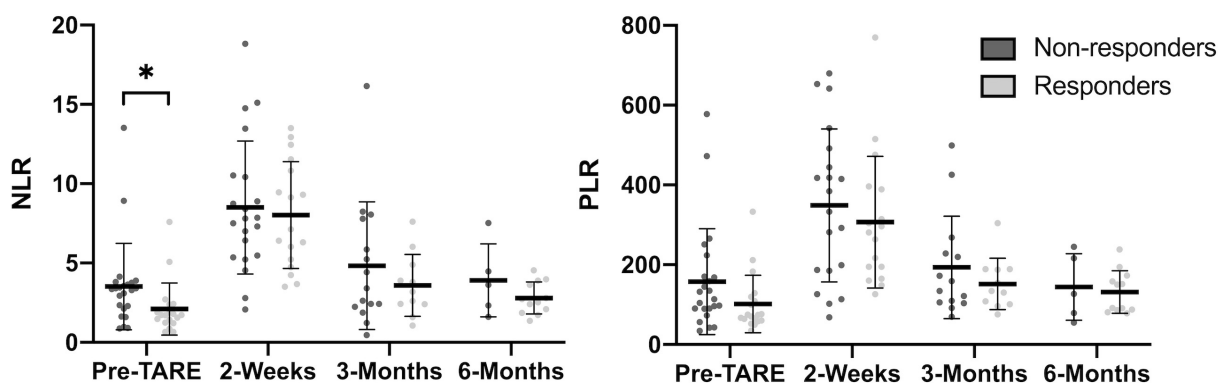


Figure 3. Mean NLR and PLR for non-responders and responders to TARE. The mean pre-treatment NLR was higher for non-responders than for responders (3.5 vs. 2.1, $P = 0.045$) (asterisk). Error bars represent the standard deviation. NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; TARE: transarterial radioembolization

significantly higher than that for responders (3.5 vs. 2.1, $P = 0.045$). There were no statistically significant differences in PLR or NLR for other time points.

Predictors of response to TARE

Predictors of response to treatment are shown in Table 4. Univariate predictors of non-response to TARE included age ≥ 65 [odds ratio (OR) = 4.06, 95%CI: 1.12-14.80, $P = 0.034$], ALBI grade ≥ 2 (OR = 6.14, 95%CI: 1.60-23.50, $P = 0.008$), pre-treatment NLR ≥ 2.83 (OR = 15.94, 95%CI: 2.92-87.06, $P = 0.001$) and pre-treatment PLR ≥ 83 (OR = 6.17, 95%CI: 1.58-24.05, $P = 0.009$). On multivariate analysis, pre-treatment NLR ≥ 2.83 was a significant variable associated with non-response to TARE (OR = 7.83, 95%CI: 1.14-53.61, $P = 0.036$), while pre-treatment PLR ≥ 83 was not a significant variable associated with non-response to TARE (OR = 3.01, 95%CI: 0.49-18.34, $P = 0.232$).

Time to progression

TTP for pre-treatment NLR and pre-treatment PLR is shown in Figure 4. Pre-treatment NLR ≥ 2.83 was associated with a higher proportion of tumor progression than pre-treatment NLR < 2.83 at 6 months post-TARE (43.6% vs. 10.0%, $P = 0.014$, log-rank). Pre-treatment PLR ≥ 83 was also associated with a higher proportion of tumor progression than pre-treatment PLR < 83 at 6 months post-TARE (38.6% vs. 0%, $P = 0.010$, log-rank). Median TTP was not reached in any group.

Table 4. Predictors of non-response to TARE

	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P-value	OR (95%CI)	P-value
Age ≥ 65	4.06 (1.12-14.80)	0.034	1.45 (0.21-10.17)	0.709
Male sex	0.82 (0.21-3.16)	0.769		
Hepatitis B	0.17 (0.02-1.68)	0.130		
Hepatitis C	0.86 (0.25-2.90)	0.801		
Alcohol abuse	1.80 (0.48-6.74)	0.383		
NASH/NAFLD	1.49 (0.39-5.67)	0.556		
BMI ≥ 30	1.48 (0.31-7.21)	0.626		
Smoking history	1.09 (0.31-3.88)	0.890		
Diabetes mellitus	1.32 (0.38-4.58)	0.663		
Hyperlipidemia	2.82 (0.79-10.04)	0.110		
Hypertension	2.19 (0.52-9.33)	0.288		
ALBI grade ≥ 2	6.14 (1.60-23.50)	0.008	4.15 (0.80-21.52)	0.090
Child-Pugh class B	1.04 (0.24-4.59)	0.957		
Normal AFP	0.42 (0.12-1.50)	0.183		
Total tumor size ≥ 10 cm	3.00 (0.53-17.02)	0.215		
Multiple tumors	0.59 (0.17-2.06)	0.410		
Pre-treatment NLR ≥ 2.83	15.94 (2.92-87.06)	0.001	7.83 (1.14-53.61)	0.036
Pre-treatment PLR ≥ 83	6.17 (1.58-24.05)	0.009	3.01 (0.49-18.34)	0.232

OR: odds ratio; CI: confidence interval; NASH: non-alcoholic steatohepatitis; NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; ALBI: albumin-bilirubin; AFP: alpha-fetoprotein; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; TARE: transarterial radioembolization

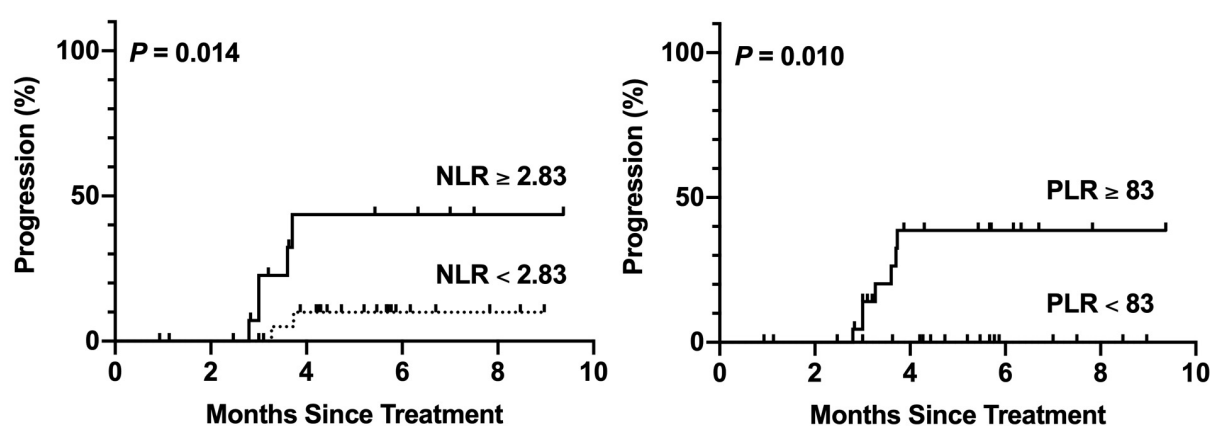


Figure 4. Kaplan-Meier curves for time to progression grouped according to pre-treatment NLR and pre-treatment PLR cutoff values. Censored events are represented by vertical lines. NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio

DISCUSSION

Traditional ways of monitoring response to TARE have relied on imaging techniques such as CT or MRI. While imaging has been the best modality to demonstrate changes in tumor size, it may require months to see a visible response. Patients who did not respond to therapy during this time may have had disease progression. Therefore, it would be advantageous to find prognostic markers that can predict tumor response or progression prior to subsequent imaging. Inflammation-based markers, such as NLR and PLR, may provide an ideal solution as they are relatively easy to obtain from routine laboratory results and have established prognostic value in previous studies on HCC^[11-14].

This study sought to determine the ability of NLR and PLR to predict response to TARE as primary treatment for HCC. We demonstrated that a pre-treatment NLR ≥ 2.83 was associated with non-response to TARE in both univariate and multivariate analysis. These findings were in agreement with Taussig *et al.*^[21],

who previously used a similar grouping system based on mRECIST to demonstrate that an elevated NLR is associated with tumor progression after intra-arterial therapy. Although other studies have shown that an elevated NLR was associated with poor overall survival following TARE, none of these studies reported specifically on tumor progression based on imaging^[22,23]. These results taken together suggest that NLR may be a valuable prognostic marker in TARE.

Notably, we found that an elevated pre-treatment PLR predicted non-response to TARE in univariate analysis but was not a significant variable in our multivariate model. This suggests that the pre-treatment NLR may be superior to pre-treatment PLR in predicting non-response to TARE. Nonetheless, this result may be limited by our small sample size. To our knowledge, this was the first study to examine the prognostic capabilities of PLR in TARE based specifically on tumor response to therapy. D'emic *et al.*^[24] previously suggested in their study of 116 patients who received selective internal radiation therapy that pre-treatment PLR > 78 was the most predictive serum marker associated with improved overall survival. However, it is difficult to make definitive conclusions about NLR and PLR in HCC as their study also included other cancer types and only 37 patients had HCC. Future studies are therefore needed to compare the prognostic capabilities of PLR compared to NLR in TARE.

On TTP analysis, we found that pre-treatment NLR ≥ 2.83 and pre-treatment PLR ≥ 83 were both associated with a higher proportion of tumor progression at 6 months post-TARE. The median TTP was not yet reached in all groups. This is consistent with previous results published by Salem *et al.*^[3], who found that the median TTP for radioembolization was greater than 26 months. On the basis of these results, both the pre-treatment NLR and pre-treatment PLR may have utility in predicting tumor progression at 6 months following TARE. Nonetheless, the prognostic value of NLR could have a distinct advantage over PLR because pre-treatment NLR < 2.83 was also associated with response to treatment in our multivariate logistic regression analysis. NLR may therefore have greater clinical utility than PLR as pre-treatment NLR was predictive of both tumor progression and potential response to therapy in our cohort. In comparison, pre-treatment PLR was only predictive of tumor progression in our TTP analysis.

The ALBI grade was a newer model proposed by Johnson *et al.*^[25] that offered better discriminatory capabilities compared to the Child-Pugh class. While other studies reported that the ALBI grade was predictive of survival following TARE, our multivariate model did not find the ALBI grade helpful in predicting response to TARE^[26,27]. Since the ALBI grade likely reflects underlying liver function, it may be more suitable for determining longer-term overall survival following TARE, rather than predicting specific tumor response to treatment.

The underlying mechanism behind NLR and PLR is not well understood. However, it is generally recognized that inflammation plays a key role in the development of cancer^[15,17]. Neutrophils can favor a pro-mutagenic state with the abundant release of reactive oxygen species and proteases^[28]. In addition, platelets may support a pro-tumor microenvironment with the release of angiogenic factors such as vascular endothelial growth factor and basic fibroblastic growth factor^[29]. These observations, coupled with the fact that lymphopenia has been associated with advanced disease in various tumors, may be reflected in systemic inflammation-based markers such as NLR and PLR^[30]. Nonetheless, more research is needed to better understand the basis of these two markers.

This study was limited in that this was a single center study with a small sample size. This study was also retrospective, and the exact timings of imaging and laboratory studies were not collected consistently as part of a study protocol. Missing data in some patients may have also contributed to our small sample size. In addition, several patients may have had laboratory data collected for other medical issues unrelated to TARE, which may have influenced NLR and PLR.

Despite these limitations, the results of this study suggest that the pre-treatment NLR may predict response to TARE as primary treatment for HCC. Furthermore, the pre-treatment NLR may also have better prognostic value than the pre-treatment PLR or ALBI grade in predicting tumor response to therapy. These findings may help clinicians identify patients who are expected to respond poorly to TARE prior to treatment and enable them to consider additional or alternative therapies. However, future studies that examine NLR and PLR in a larger cohort prospectively will be necessary to draw definitive conclusions about the prognostic capabilities of these two inflammation-based markers.

DECLARATIONS

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Authors' contributions

Drafting of manuscript: Yoneoka G

Data collection: Yoneoka G, Bozhilov K, Wong LL

Data analysis: Yoneoka G, Wong LL

Critical review of manuscript: Bozhilov K, Wong LL

Conception: Wong LL

Availability of data and materials

Data inquiries may be forwarded to the corresponding author.

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Conflicts of interest

Mr. Grant Yoneoka and Dr. Kliment Bozhilov declared that there are no conflicts of interest. Dr. Linda Wong is on the Speakers Bureau for Eisai.

Ethical approval and consent to participate

This study was approved by the Institutional Review Board at the Queen's Medical Center and was determined to be exempt from needing informed consent.

Consent for publication

Not applicable.

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REFERENCES

1. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, et al. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019;16:589-604.
2. McGlynn KA, London WT. The global epidemiology of hepatocellular carcinoma: present and future. *Clin Liver Dis* 2011;15:223-43, vii-x.
3. Salem R, Gordon AC, Mouli S, Hickey R, Kallini J, et al. Y90 radioembolization significantly prolongs time to progression compared with chemoembolization in patients with hepatocellular carcinoma. *Gastroenterology* 2016;151:1155-63.e2.
4. Moreno-Luna LE, Yang JD, Sanchez W, Paz-Fumagalli R, Harnois DM, et al. Efficacy and safety of transarterial radioembolization versus chemoembolization in patients with hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 2013;36:714-23.

5. Carr BI, Kondragunta V, Buch SC, Branch RA. Therapeutic equivalence in survival for hepatic arterial chemoembolization and yttrium 90 microsphere treatments in unresectable hepatocellular carcinoma: a two-cohort study. *Cancer* 2010;116:1305-14.
6. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010;30:52-60.
7. Duvoux C, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, et al; Liver Transplantation French Study Group. Liver transplantation for hepatocellular carcinoma: a model including α -fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012;143:986-94.e3; quiz e14-5.
8. Berry K, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. *Liver Transpl* 2013;19:634-45.
9. Tateishi R, Yoshida H, Matsuyama Y, Mine N, Kondo Y, et al. Diagnostic accuracy of tumor markers for hepatocellular carcinoma: a systematic review. *Hepatol Int* 2008;2:17-30.
10. Li J, Han X, Yu X, Xu Z, Yang G, et al. Clinical applications of liquid biopsy as prognostic and predictive biomarkers in hepatocellular carcinoma: circulating tumor cells and circulating tumor DNA. *J Exp Clin Cancer Res* 2018;37:213.
11. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst* 2014;106:dju124.
12. Qi X, Li J, Deng H, Li H, Su C, et al. Neutrophil-to-lymphocyte ratio for the prognostic assessment of hepatocellular carcinoma: a systematic review and meta-analysis of observational studies. *Oncotarget* 2016;7:45283-301.
13. Templeton AJ, Ace O, McNamara MG, Al-Mubarak M, Vera-Badillo FE, et al. Prognostic role of platelet to lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2014;23:1204-12.
14. Li C, Wen TF, Yan LN, Li B, Wang WT, et al. Postoperative neutrophil-to-lymphocyte ratio plus platelet-to-lymphocyte ratio predicts the outcomes of hepatocellular carcinoma. *J Surg Res* 2015;198:73-9.
15. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010;140:883-99.
16. Buegry D, Wenz F, Groden C, Brockmann MA. Tumor-platelet interaction in solid tumors. *Int J Cancer* 2012;130:2747-60.
17. Greten FR, Grivennikov SI. Inflammation and Cancer: Triggers, mechanisms, and consequences. *Immunity* 2019;51:27-41.
18. Motomura T, Shirabe K, Mano Y, Muto J, Toshima T, et al. Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. *J Hepatol* 2013;58:58-64.
19. Pinato DJ, Sharma R. An inflammation-based prognostic index predicts survival advantage after transarterial chemoembolization in hepatocellular carcinoma. *Transl Res* 2012;160:146-52.
20. Tian XC, Liu XL, Zeng FR, Chen Z, Wu DH. Platelet-to-lymphocyte ratio acts as an independent risk factor for patients with hepatitis B virus-related hepatocellular carcinoma who received transarterial chemoembolization. *Eur Rev Med Pharmacol Sci* 2016;20:2302-9.
21. Taussig MD, Irene Koran ME, Mouli SK, Ahmad A, Geervarghese S, et al. Neutrophil to lymphocyte ratio predicts disease progression following intra-arterial therapy of hepatocellular carcinoma. *HPB (Oxford)* 2017;19:458-64.
22. Soydal C, Araz M, Nak D, Akkus P, Baltacıoğlu MH, et al. Analysis of prognostic factors in patients receiving transarterial radioembolization for unresectable hepatocellular carcinoma. *Nucl Med Commun* 2020;41:73-7.
23. Sukato DC, Tohme S, Chalhoub D, Han K, Zajko A, et al. The prognostic role of neutrophil-to-lymphocyte ratio in patients with unresectable hepatocellular carcinoma treated with radioembolization. *J Vasc Interv Radiol* 2015;26:816-24.e1.
24. D'Emic N, Engelman A, Molitoris J, Hanlon A, Sharma NK, et al. Prognostic significance of neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in patients treated with selective internal radiation therapy. *J Gastrointest Oncol* 2016;7:269-77.
25. Johnson PJ, Berhane S, Kagebayashi C, Satomura S, Teng M, et al. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. *J Clin Oncol* 2015;33:550-8.
26. Gui B, Weiner AA, Noshier J, Lu SE, Foltz GM, et al. Assessment of the albumin-bilirubin (ALBI) grade as a prognostic indicator for hepatocellular carcinoma patients treated with radioembolization. *Am J Clin Oncol* 2018;41:861-6.
27. Mohammadi H, Abuodeh Y, Jin W, Frakes J, Friedman M, et al. Using the albumin-bilirubin (ALBI) grade as a prognostic marker for radioembolization of hepatocellular carcinoma. *J Gastrointest Oncol* 2018;9:840-6.
28. Coffelt SB, Wellenstein MD, de Visser KE. Neutrophils in cancer: neutral no more. *Nat Rev Cancer* 2016;16:431-46.
29. Yan M, Jurasz P. The role of platelets in the tumor microenvironment: from solid tumors to leukemia. *Biochim Biophys Acta* 2016;1863:392-400.
30. Ménétrier-Caux C, Ray-Coquard I, Blay JY, Caux C. Lymphopenia in cancer patients and its effects on response to immunotherapy: an opportunity for combination with cytokines? *J Immunother Cancer* 2019;7:85.

Review

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Immunotherapy of hepatocellular carcinoma with infection of hepatitis B or C virus

Cecilia Monge Bonilla, Nicole A. McGrath, Jianyang Fu, Changqing Xie

Thoracic and Gastrointestinal Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

Correspondence to: Dr. Changqing Xie, Thoracic and Gastrointestinal Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Building 10, 10 Center Drive, Bethesda, MD 20892, USA.
E-mail: changqing.xie@nih.gov

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Abstract

Hepatocellular carcinoma (HCC) has one of highest mortalities globally amongst cancers, but has limited therapeutic options once in the advanced stage. Hepatitis B or C virus infection are the most common drivers for HCC carcinogenesis, triggering chronic liver inflammation and adding to the complexity of the immune microecosystem of HCC. The emergence of immunotherapy has afforded a new avenue of therapeutic options for patients with advanced HCC with a history of hepatitis B or C virus infection. This article reviews the change of immunity elicited by hepatitis B or C virus infection, the immune feature of HCC, and the clinical evidence for immunotherapy in advanced HCC and discusses future directions in this field.

Keywords: Hepatocellular carcinoma, hepatitis B virus, hepatitis C virus, immunotherapy

INTRODUCTION

Liver cancers are the fourth leading cause of cancer-related mortality worldwide^[1,2], and there are over 800,000 new primary liver cancer cases around the world each year^[3]. Hepatocellular carcinoma (HCC) accounts for 75%-85% of these cases and is one of the most aggressive liver cancers^[1]. The incidence of HCC is increasing in many high-income countries^[2]. The majority of HCC occurs in patients with underlying chronic liver diseases triggered by various risks dependent on geographic area, sex, age, and degree of liver damage^[4]. Furthermore, males are twice as likely as females to develop HCC^[5].



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HCC can be caused by both viral and non-viral factors. HCC develops secondary to chronic infection with the hepatitis B virus (HBV) or hepatitis C virus (HCV). High serum levels of HBV DNA and HCV RNA viral load are considered to be independent risk factors for developing HCC in patients infected by these diseases^[6,7]. HBV vaccination has greatly reduced the incidence of HCC in certain geographic areas^[8]. Moreover, improved screening and treatment of HCV infection has also reduced virus-related HCC cases in non-epidemic regions^[9].

Non-viral risk factors for the development of HCC include excessive alcohol consumption, environmental exposure to aflatoxin, metabolic disorders, non-alcoholic steatohepatitis, and genetic disorders^[10]. It is unsurprising that non-viral risk factors are more common causes of HCC in countries such as the USA, UK, and other high income countries. Frequently, viral infection is complicated with non-viral risk factors leading to HCC development. Systematic treatment is the standard approach to control advanced HCC, given that most patients present with advanced stage disease, which limits curative approaches such as surgical resection, liver transplantation, and local liver-directed therapy. Recent molecular landscape analysis has led to the development of systematic targeted therapies for advanced HCC, including sorafenib^[11] and lenvatinib^[12] in the first line setting, and regorafenib^[13], cabozantinib^[14], and ramucirumab^[15] as second line options. The breakthrough of cancer immunology research has provided effective immunotherapy by blocking immunosuppressive mechanisms and enhancing host immune surveillance. This leads to the recognition of tumour and execution of a tumour-specific response capable of treating malignancy, including HCC^[16]. HBV- or HCV-related HCC represents a special entity compared to non-viral HCC. This review discusses the immune response to HBV and HCV infection, the immunology of HCC, and summarizes the current status of immunotherapy in HCC in the context of HBV or HCV infection.

HBV INFECTION AND IMMUNE TOLERANCE

Studies have shown that HBV not only has a direct carcinogenic effect through the integration of viral DNA and the oncoprotein HBV-encoded X protein (HBx), but also has an indirect carcinogenic effect due to chronic immune suppression^[17]. HBV has been considered as a stealth virus and acute infection does not lead to a strong activation of interferon (IFN) and pro-inflammatory responses^[18-22]. Liver resident macrophage Kupffer cells are able to interact with hepatitis B surface antigen (HBsAg) and produce pro-inflammatory cytokines, but Toll-like receptor expression is down-regulated by HBeAg^[23,24]. Indirect activation of natural killer (NK) cells can occur via Kupffer cell derived IL-12 and IL-18^[23,25], evidenced by the increased expression of activation markers CD69 and NKG2D and lower levels of inhibitory markers NKG2A^[26,27], but these are functionally suppressed^[28]. These suggest that NK cells are unable to clear the infection on their own. The weakness of the innate response does not impair the induction of a vigorous HBV-specific CD4⁺ T cell response^[29], that subsequently generates a large number of cytokines necessary for the effective development of cytotoxic CD8⁺ T cells and B cell antibody production^[30]. Potent HBV antigen-specific CD8⁺ T cell responses can control HBV replication and reduce it to undetectable levels during acute HBV infection^[31]. In chronic HBV infection, the antiviral functionality of NK cells is also impaired, evidenced by an alteration of the phenotype and the receptors of NK cells^[32]. This inhibition of NK cell activity is mainly mediated by myeloid-derived suppressor cells (MDSCs) via NKP30 receptor on NK cells^[33] and pro-inflammatory cytokines^[34]. In addition, accumulated liver MDSCs due to HBV infection suppress CD8⁺ T cell function and promote systemic CD8⁺ T cell exhaustion^[35], characterized by high expression levels of inhibitory receptors such as CTLA-4, PD-1, and TIM-3^[36,37]. Furthermore, they inhibit CD4⁺ T cells and metabolically regulate HBV-related liver damage^[38]. MDSCs can induce the development of immunosuppressive regulatory T cells (Tregs) during chronic HBV infection primarily via a TGF β and the IL-10-dependent signalling pathway^[39]. Tregs specifically inhibit CD8⁺ T cell activity; further blocking HBV-specific immune responses, leading to HBV persistence. On the other hand, low levels of HBV activity controlled by HBV antigen-specific CD8⁺ T cells lead to sustained liver inflammation

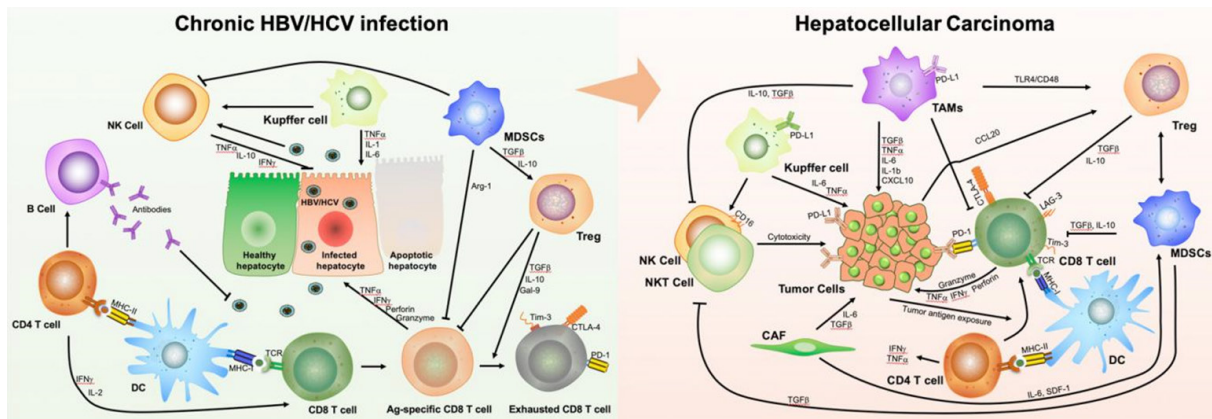


Figure 1. Schematic mechanism of immune evasion across the spectrum from inflammation by chronic hepatitis B (HBV) and C virus (HCV) infection to resultant hepatocellular carcinoma (HCC). The complexity of the mechanism involves multiple immune cells and various collections of cytokines. A: immune tolerance induced by HBV and HCV infection; B: immune evasion driven by the crosstalk between tumour cells and immune cells in HCC. DC: dendritic cells; MDSCs: myeloid-derived suppressor cells; TAMs: tumour-associated macrophages; NK: natural killer; NKT: natural killer T; Treg: regulatory T-cells; CAF: cancer-associated fibroblast; HBV: hepatitis B virus; HCV: hepatitis C virus; MHC: major histocompatibility complex; TCR: T-cell receptor; IL: interleukin; IFN γ : interferon gamma; TNF α : tumour necrosis factor receptor alpha; TGF- β : transforming growth factor beta; CCL: C-C motif chemokine ligand; CXCL: C-X-C motif ligand 1; Gal-9: galactin-9; PD-1: programmed cell death protein; PD-L1: programmed cell death ligand 1; CTLA: cytotoxic T-lymphocyte-associated protein; IL: interleukin; Arg-1: arginase-1; Tim-3: T cell immunoglobulin and mucin domain 3; LAG-3: lymphocyte-activation gene 3; SDF-1: Stromal cell-derived factor 1

and the functional depletion of HBV antigen-specific CD8⁺ T cells^[40-42]. Hence, immunotherapies targeting these inhibitory receptors may modulate the progression of HCC [Figure 1]. Moreover, the exhausted CD8⁺ T cells experience impaired metabolic function and DNA repair capacity that further deteriorates their functions^[43]. This highlights a complex interaction among the abovementioned immune cells during HBV infection, sustaining immune disorders and inflammation in the liver, which predispose patients to HCC development.

HCV INFECTION AND IMMUNE TOLERANCE

The dysregulation in immune surveillance triggered by HCV infection is also thought to be one of the mechanisms by which HCV causes HCC. During acute HCV infections, NK cells are activated with enhanced cytotoxicity and IFN production^[44]. However, 70% of HCV-infected patients progress to chronic infection^[45], partially due to decreased NK cell levels and function^[46]. HCV antigen-specific CD8⁺ T cells participate in controlling HCV infection^[47]. However, non-synonymous mutations in HCV are common, resulting in an escape from CD8⁺ T-cell recognition^[48,49]. Moreover, HCV antigen-specific T cells undergo massive apoptosis during the chronic phase^[50]. It has been reported that CD8⁺ T cell exhaustion develops following prolonged exposure to HCV antigens^[51-53]. During chronic infection, HCV activates monocytes and macrophages, leading to the secretion of pro-inflammatory cytokines^[54]. The released pro-inflammatory cytokines IL-6 and TNF not only promote macrophage apoptosis^[55], but also aggravate liver disease progression and HCC development^[56]. In the setting of HCV infection, impaired macrophage phagocytosis may contribute to chronic infection and subsequent uncontrolled inflammation that promotes liver disease. Similar to HBV, HCV infection is also linked to the presence of MDSCs^[57] and an expansion of Tregs via IL-10^[58] and IL-12^[59,60]. Tregs both suppress the HCV antigen-specific CD8⁺ T cell response in chronic infection and control memory cells. In addition, HCV impedes dendritic cell (DC) function by altering the adaptive response of CD4⁺ and CD8⁺ T cells, and cytokine release^[61,62]. This suggests that HCV often disturbs antigen presentation along with humoral and cell-mediated immune response, resulting in chronic HCV infection and progressive liver damage [Figure 1].

IMMUNE EVASION MECHANISMS OF HCC ASSOCIATED WITH HBV/HCV

Following persistent chronic liver inflammation due to HBV and HCV infection and immune imbalances, HCC develops with specific immunological features. There were 22% of 196 HCC samples displaying high or moderate levels of lymphocyte infiltration from an analysis of TCGA HCC samples, with high expression of immunosuppressive molecules and enriched Tregs, resting DCs and undifferentiated M0 macrophages compared to normal livers. This indicates an immunosuppressed microenvironment in this group of HCC patients. HBV/HCV infection status appeared not to be significantly associated with these observations^[63]. There was also T-cell enrichment with heterogenous clonal expansion of CD8⁺ T-cell populations with exhausted characteristics based on the sequencing of T-cell receptors (TCR) in TILs^[64,65]. Interestingly, a further study showed CD8⁺ resident memory cells were enriched in HBV-related HCC with higher PD-1 expression and functionally more exhausted than non-virus-related HCC^[66]. Increased numbers of CD14⁺ HLA-DR[−]/low MDSCs were found to be related to HCC progression^[67]. Furthermore, infiltrating MDSCs not only suppress T-cell proliferation via arginase to deplete arginine^[67], but also promote Treg expansion through the production of IL-10 and TGF- β , and inhibit effector T cells through PD-L1^[67]. In addition, high IL-10 secretion by MDSCs results in the skewing of resident tumour-associated macrophages (TAMs) and monocytes to an immunosuppressive phenotype^[68]. They release TGF- β and VEGF to promote tumour growth and development, promoting cancer stem cells and metastasis^[69], stimulating Tregs, and suppressing NK cells^[70]. Noticeably, Tregs are enriched in HCC^[64]. This enrichment is prominent in HBV-related HCC with greater expression of PD-1 and increased suppressive function, which represents a more immunosuppressive and exhausted immune microenvironment in HBV-related HCC compared to the non-virus-related HCC^[66]. The increased Tregs not only suppressed HBV antigen-specific immune responses, but also suppressed HCC tumour antigen-specific immune responses^[71]. DCs are severely dysregulated in HCC, with a subset of CD14⁺ DCs expressing high levels of CTLA-4 which indicates an inhibitory phenotype^[72]. In addition to these immune cells, several other stromal cells, such as NK cells, endothelial cells and cancer-associated fibroblasts, orchestrate immune evasion in HCC^[73]. For example, endothelial cells in cancer tissues reportedly produce the C-X-C motif chemokine ligand 12, facilitating the recruitment of MDSCs^[74]. Together, these data suggest that HCC is an immunogenic malignancy, rendering it an attractive target for immunotherapy [Figure 1].

CURRENT IMMUNOTHERAPY OF HBV- AND HCV-RELATED HCC

Immunotherapy, specifically immune checkpoint inhibition, has been considered a useful treatment option for HCC, evidenced by both pembrolizumab (anti-PD-1) and nivolumab (anti-PD-1) with or without ipilimumab (anti-CTLA4) approved as second line therapy, and atezolizumab (anti-PD-L1) with bevacizumab approved as first line treatment options. In addition to immune checkpoint inhibitors (ICIs), several immunotherapy approaches are in development, including antibodies targeting specific tumour-associated antigens (TAAs), adoptive cell therapy, vaccination based on TAAs or mutation-associated neoantigens (MANAs) and oncolytic viruses. Although the infection of HBV and HCV is highly associated with HCC development, data on response outcomes specifically in this population included in trials is scarce.

IMMUNE CHECKPOINT INHIBITORS

Tremelimumab, a CTLA-4 inhibitor, was the first immune checkpoint inhibitor (ICI) that showed encouraging results in patients with advanced HCC. In a phase II study including patients with advanced HCC and chronic HCV infection, tremelimumab showed an objective response rate (ORR) of 17.6%, a disease control rate (DCR) of 76.4%, a median time to progression of 6.48 months, and a median overall survival (OS) of 8.2 months^[75]. Importantly, in this study, tremelimumab also exhibited antiviral effects evidenced by a significant decline in viral load. There were no treatment-related deaths and the treatment was mostly well tolerated.

Table 1. Approved treatments for advanced HCC

Treatment	Benefit	Level of evidence	Comments
Atezolizumab and bevacizumab ^[78]	↑ survival	1A	Non-curative treatment, superior to first line sorafenib In unresectable HCC, bevacizumab-atezolizumab has a better OS and PFS compared to sorafenib Improved OS with bevacizumab-atezolizumab at 6 months (84.8%) and 12 months (67.2%) vs. 72.2% and 54.6% respectively with sorafenib PFS is longer with atezolizumab-bevacizumab (median 6.8 months) than with sorafenib (median 4.3 months) Most common grade 3 or 4 AEs: hypertension, AST increase, ALT increase, fatigue, proteinuria, diarrhoea, decreased appetite, pyrexia
Nivolumab ^[67]	No survival benefit	1A	Treatment of advanced HCC previously treated with sorafenib Durable ORR of 14%, median duration of response 17 months Median OS as second-line therapy: 15.6 months, non-curative Well-tolerated In front line setting vs. sorafenib did not show increase in OS (phase III study)
Pembrolizumab ^[70]	No survival benefit	1A	Treatment of advanced HCC previously treated with sorafenib Overall durable response rate of 17%, PFS 4.9 months, non-curative treatment Well tolerated
Nivolumab and ipilimumab ^[68]	↑ survival	1A	Treatment of advanced HCC after failure of sorafenib treatment Objective response 31%, median duration of response 17 months Most common AEs: fatigue, diarrhoea, rash, pruritus, nausea, musculoskeletal pain, pyrexia, cough, decreased appetite, vomiting, abdominal pain, dyspnoea, upper respiratory tract infection, arthralgia, headache, hypothyroidism, decreased weight, dizziness More than 50% of patients may require systemic steroids to manage AEs

Evidence-based classification adapted from the National Cancer Institute. 1 = Randomized controlled trial or meta-analysis; 2 = Non-randomized controlled trial; 3 = Case series; A = Survival endpoint; B = Cause-specific mortality; C = Quality of life; D = Indirect surrogates. OS: overall survival; AEs: adverse events; HCC: hepatocellular carcinoma; ORR: objective response rate; AST: aspartate transaminase; ALT: alanine aminotransferase

In the open-label phase I/II CheckMate 040 trial, nivolumab was assessed as first-line therapy in patients with advanced HCC. The protocol had three concurrent cohorts of patients, including non-viral infected, HBV, and HCV infected advanced HCC. The results showed an ORR of 15%, a DCR of 58%, and a median OS of 15.6 months in the dose-escalation phase. The six-month OS was 83%, the nine-month OS was 74%, and the median duration of response (DOR) was 17 months in the dose-expansion phase. The most common treatment-related adverse events (TRAEs) were rash (23%) and pruritus (19%)^[76]. Hepatitis flares were not reported. However, in the phase III randomized, double blind, multicentre CheckMate-459 trial, nivolumab failed to show statistical significance in OS benefit though there was a clear trend of improvement in OS for patients treated with nivolumab compared to sorafenib [Table 1]^[77]. The viral infection history of the patient population remains unclear. Nivolumab is also being studied in the phase III CheckMate-9DX study as adjuvant treatment after curative therapy (surgery or ablation) for HCC in patients with a high risk of recurrence compared with placebo (NCT03383458). Recent reports from the combination of nivolumab and ipilimumab in patients with advanced or metastatic HCC showed an ORR of 33% with an 8% complete response (CR) among a total of 49 patients. There was a median DOR of 17 months with TRAEs of grade 3 or higher in 34% of patients. Among the trial cohort, 57% had an active HBV infection and 8% had an active HCV infection, and no evidence of viral hepatitis reactivation was detected^[68]. Nivolumab and ipilimumab in the neoadjuvant setting (NCT03222076) have shown promising preliminary results of 29% pathologic CR with 34% TRAEs (5 HCV-positive and 1 HBV infected patients were reported).

Pembrolizumab is a recombinant monoclonal human antibody for human PD-1. A non-randomized, multicentre, open-label phase II study (KEYNOTE-224) tested the efficacy and safety of pembrolizumab in patients with advanced HCC as a second line treatment option, showing an ORR of 17% and a median OS of 12.9 months. HCV positive ($n = 26$) and HBV positive ($n = 22$) patients did not have reactivation of viral

Table 2. Key immunotherapy trials

Drug name	Trial number	Phase	Comments
Camrelizumab ^[72]	NCT02989922	II	Response rate 14%. Median OS 14.4 months (predominantly HBV-positive patients)
Tislelizumab	NCT02412773	III	Active recruiting
Pembrolizumab (Keynote 937)	NCT03062358	III	Active recruiting in Asia
Pembrolizumab (Keynote 394)	NCT03062358	III	Active accrual in Asia
Nivolumab (Checkmate 9DX)	NCT03383458	III	Currently recruiting
Nivolumab and Ipilimumab	NCT03222076	II	Currently recruiting
Cemiplimab ^[73]	NCT03916627	II	Currently recruiting
Tislelizumab	NCT03412773	III	Results pending
Durvalumab with tremelimumab and ablation ^[75]	NCT02821754	I/II	Response rate 20% Median PFS 7.8 months
Durvalumab with tremelimumab (HIMALAYA)	NCT03298451	III	Currently recruiting

PFS: progression-free survival; OS: overall survival

hepatitis^[78]. However, the subsequent phase III randomized control trial KEYNOTE-240 of pembrolizumab as second line treatment in advanced HCC failed to show a statistically significant improvement in progression-free survival (PFS) or OS. Even so, pembrolizumab showed a reduced risk of death by 22% and an improved PFS compared with placebo. 25.9%, 15.5%, and 58.6% patients were affected by HBV, HCV, or non-infected in the pembrolizumab treatment cohort, respectively, in comparison to 21.5%, 21%, and 85% in the placebo cohort. A subgroup analysis indicated that patients with HBV infection treated with pembrolizumab had a superior median OS compared to those treated with placebo; there was no OS benefit in the group of HCV infected or non-infected patients^[79]. There are two on-going phase III trials of pembrolizumab, including KEYNOTE-394, to evaluate pembrolizumab in Asian HCC patients, and KEYNOTE-937 to evaluate pembrolizumab as an adjuvant therapy in HCC patients after curative treatment.

Other PD-1 antibodies, including tislelizumab (BGB-A317), camrelizumab (SHR-1210) and cemiplimab (REGN2810), also have shown anti-tumour activity in HCC, with response rates of 16.7% (all responders were HBV infected)^[80], 13.8%^[81] and 19.2%^[82], respectively. Interestingly, in the trial of camrelizumab, 83% of patients enrolled were infected with HBV. An increase in HBV titre was noted in 46 participants, but the majority of these occurred after disease progression or after the last dose of treatment. Conversion to HBsAg positive from negative status was not reported during the treatment^[81]. A phase III trial (RATIONALE 301) of tislelizumab versus sorafenib as first-line treatment in patients with unresectable HCC is currently underway (NCT02412773) [Table 2].

Durvalumab (MEDI4736) is an anti-PD-L1 monoclonal antibody. In a phase I/II trial of Child-Pugh class A advanced HCC patients, durvalumab achieved an OS rate of 10.3% in 39 patients in the second line setting. There was comparable ORR of 25% in patients with HCV infections and similar rates of TRAEs^[83]. Furthermore, the combination of durvalumab with tremelimumab in patients with advanced HCC in the second line setting showed an ORR of 20% (2 responders were HCV infected), median PFS of 7.8 months, and median OS of 15.9 months amongst the 10 patients (7 HCV and 1 HBV infected)^[84]. However, the other study reported this combination in advanced HCC showing no response in 9 patients with HCV infection, 1 responder in 11 patients with HBV infection, and an ORR of 35% among 20 uninfected patients with an overall ORR of 20%^[85]. The on-going phase 3 HIMALAYA study evaluating durvalumab and tremelimumab compared with sorafenib or durvalumab monotherapy in the first-line setting in unresectable HCC (NCT03298451) may provide further information regarding the response status of HBV- or HCV-infected patients following anti-PD-L1 treatment.

It remains unknown whether virally-induced HCC is more prone to immune attack either secondary to the presence of foreign viral antigens or an immune response to the virus, compared to non-viral associated HCC. A recent pooled analysis assessed the efficacy of anti-PD1 or PDL1 in HBV infected HCC patients in comparison to non HBV infected HCC patients^[86]. The results indicated that patients with HBV infection achieved ORRs similar to their non-infected counterparts, and this was seen with single and multi-agent treatment regimens. A lower disease control rate (DCR) was reported in HBV-infected HCC patients; stable disease was more likely to be seen in non-viral HCC, but this observation was not statistically significant. Drug efficacy evaluated as ORR and DCR of HCV-infected HCC patients compared to HBV positive HCC and non-viral HCC was similar, and reached statistical significance. Although clinical activity was observed for the most part in non-viral associated HCC patients, the interpretation of potential differences in response based on viral aetiology remains limited by the small number of patients and would require further evaluation with prospective, randomized, and double-blind clinical trials.

Given the profound immunomodulatory effect of the vascular epithelial growth factor (VEGF) pathway and dominant presence of angiogenesis in HCC, there increasing interest in testing the anti-tumour efficacy of ICIs in combination with anti-angiogenetic agents. For example, the anti-PD-L1 antibody atezolizumab was studied in a phase Ib study in combination with bevacizumab in the first-line setting for advanced HCC with Child-Pugh B liver disease^[87]. This study showed promising early findings, resulting in an ORR of 34% with one CR^[87]. This led to the multicentre, open-label, randomized phase III trial IMbrave 150, which evaluated this combination compared with sorafenib^[88]. This study enrolled 336 patients; 49% were infected with HBV, 21% were infected with HCV, and 30% were non-viral in the combination cohort. In the sorafenib cohort 165 patients were enrolled; 46% had HBV, 22% had HCV, and 32% did not have hepatitis viral infections. The reported 12-month OS was 67.2% in the atezolizumab with bevacizumab group and 54.6% in the sorafenib cohort. Grade 3 or greater adverse events were reported in 56.5% of patients who received at least one dose of the combination treatment, and in 55.1% of patients in the sorafenib cohort. Interestingly, the subgroup analysis showed a superior OS benefit in patients with either HBV or HCV infection treated with combination therapy^[88]. The FDA has approved the combination of atezolizumab and bevacizumab for the treatment of patients with unresectable HCC as a first line treatment option [Table 1]. There are other reports using ICIs in combination with anti-angiogenic therapies including pembrolizumab and lenvatinib^[89], durvalumab with ramucirumab^[90], nivolumab with ipilimumab and cabozantinib^[91], as well as avelumab with axitinib^[92]. There are on-going trials with the same strategy, including atezolizumab and cabozantinib (COSMIC-312, NCT03755791), pembrolizumab with lenvatinib (LEAP-002, NCT03713593), SHR-1210 and apatinib (NCT03764293), and sintilimab (anti-PD-1) with bevacizumab biosimilar (ORIENT-32, NCT03794440).

The combined use of locoregional therapies such as ablation and transcatheter arterial chemoembolization (TACE) could improve the effectiveness of immunotherapies against HCC^[93]. There are on-going phase III trials evaluating the outcome of the combination of ICIs with these modalities. For example, durvalumab and bevacizumab, or placebo with TACE in both intermediate HCC (EMERALD-1, NCT03778957) and high-risk HCC (EMERALD-2, NCT03847428), pembrolizumab with stereotactic body radiation therapy (NCT03316872), pembrolizumab following TACE (PETAL, NCT03397654) or Y90 (NCT03099564), and nivolumab with Y90 (NCT03033446).

Other immune checkpoint molecules, such as LAG3, TIM-3, 4-1BB, CD40, and OX40, can also be targeted and combined with PD-1/PD-L1 or CTLA-4 blockade in patients with HCC (NCT03005782, NCT03099109, NCT03241173). Biphasic antibodies to target PD-1 and other immune checkpoints concurrently are being studied as well (NCT03517488, NCT03752398).

CELL-BASED IMMUNOTHERAPY

There are several cell-based immunotherapies being studied in patients with advanced HCC, including chimeric antigen receptor T (CAR-T) cells, cytokine-induced killer cells (CIKs) and T cell receptor (TCR)-engineered T cells.

Proteins found in HCC currently being investigated as targets in CAR-T cell research in early stage studies include GPC3 (NCT02905188, NCT03084380, NCT03130712, NCT03198546, and NCT03302403), AFP (NCT03349255), EpCAM (NCT03013712), c-Met/PD-L1 (NCT03672305), MUC-1 (NCT03198546), and DR5, c-Met or EGFRvIII (NCT03638206). The earliest study in CEA positive liver metastases treated with CAR-T cells was reported in a phase I trial. Anti-CEA CAR-T administered through hepatic artery infusion with or without systemic IL-2 treatment resulted in one case of stable disease (SD)^[94]. HCC patients were not included, however. A phase I trial with anti-GPC3 CAR-T cells for relapsed or refractory GPC3-positive HCC showed one PR and three SD observed among 6 patients, respectively. No dose-limiting toxicity was identified and only one grade 3 fever was reported^[95].

Cytokine-induced killer cells (CIKs) are a mixture of heterogeneous immune cells generated by the *ex vivo* expansion of peripheral blood mononuclear cells with the support of IL-2, IFN γ , and anti-CD3 monoclonal antibodies. A randomized phase II trial in treatment-naïve patients with HCC (over 50% patients had HBV infection) demonstrated that CIK therapy prolonged OS and PFS, compared to standard of care^[96]. A multicentre open-label randomized phase III trial in patients with HCC after curative treatment demonstrated that CIK therapy prolonged recurrence-free survival and OS, though a significant proportion of patients with CIK infusion developed adverse events. In this trial, CIK infusion seemed to benefit the HBV-infected population (over 80% of the patient population) more than the HCV-infected or the uninfected group. No information of hepatitis flares or conversion was reported^[97].

TCR-engineered T cells are generated by integrating a cloned tumour antigen-specific TCR into T cells. Phase I trials are currently evaluating genetically modified T cells expressing AFP-specific TCRs in patients with advanced HCC (NCT03132792) and an autologous TCR-engineered T cell therapy targeting MAGEA1 in solid tumours including HCC (NCT03441100). Since HBV-DNA integration is often seen in HBV-related HCC, cell based therapy studies in HCC have looked into the possibility of using the HBV antigens expressed in HCC cells as a target for autologous TCR redirected therapy^[98,99]. Vector-mediated gene transfer may be a means to introduce HLA-A2-restricted, HBV-specific TCRs into T cells of chronic HBV- and HBV-related HCC patients. Through TCR gene transfer, it has been demonstrated that TCR transduced T cells have the capacity of recognizing HCC cell lines expressing HBV antigens. This data showed that HBV-specific T cell clones cause apoptosis of HCC tumour cells that express the HBV X protein, proving that HBV proteins are identified by the immune system as non-self-tumour antigens^[100]. Nevertheless, HBV antigens were expressed in HCC metastases and there is published evidence of the recognition of tumour cells by lymphocytes engineered to express HBV-specific receptor TCR with HCC autologous T cells genetically modified to express and HBV-specific TCR and treat chemo-resistant metastatic HCC^[101]. These findings suggest that autologous TCR therapy redirected against HBV-associated HCC may have therapeutic potential in the future.

VACCINES

Vaccines against HBV and HCV reduce the likelihood of developing HCC. Vaccine therapy in HCC is an area of important on-going research with the goal of improving the immune response against malignant cells through tumour specific antigens and subsequent T cell activation^[102]. Clinical study protocols including different stages of HCC have been conducted by the Cancer Vaccine development for the HCC Consortium (HEPAVAC)^[103].

Both RNA and peptide-based vaccines are under investigation. A phase I/II trial for advanced solid tumours including HCC treated with NCI-4650, an mRNA-based vaccine, was terminated due to slow accrual (NCT03480152). Peptide-based vaccines for HCC utilize shared TAAs. A phase I trial evaluated the anti-tumour efficacy of an AFP-derived peptide vaccine subcutaneously injected in 15 patients with HCC; 10 HCV and 2 HBV infected patients. The study showed that the vaccine was well tolerated and 33% of the patients had an AFP-specific cytotoxic CD8+ T cell response. One patient had a CR for over 2 years and 8 patients had stable disease^[104]. GPC3 is another antigen that is highly expressed in HCC. In a phase I trial of 33 patients (8 HBV and 15 HCV infected), the GPC3 peptide vaccine was well tolerated and induced a GPC3-specific T cell response. There was one PR (HCV infected) and 19 showing SD. GPC3-specific T cell frequency correlated with OS while higher GPC3-specific T cell frequency showed longer OS^[105]. The additional PD-1 blockade seemed to augment the efficacy of the GPC3 vaccine by increasing the number of vaccine-induced cytotoxic T lymphocytes^[106]. A phase II trial of a TERT-derived peptide vaccine in combination with low dose cyclophosphamide showed no effective antitumor response in 40 advanced HCC patients^[107]. A study utilizing IMA970A with CV8102 vaccines has completed but the results have not yet been published (NCT03203005). Current vaccine trials include the hepcortespensilisimut-L vaccine (NCT02256514, NCT02232490), pneumonia vaccine (NCT03942328), heat shock protein-peptide complex vaccine (NCT04206254), Quilt-2.025 NANT neoepitope yeast vaccine (NCT03552718), DNAJB1-PRKACA fusion kinase peptide vaccine (NCT04248569), personalize DC vaccine (NCT03674073, NCT04147078) and multiple signals loaded DC vaccine (NCT04317248). The results of these trials will be instructive for the next generation of vaccine trial design.

ONCOLYTIC VIRUSES

Oncolytic viruses have attracted lots of attention with the hope of tumour eradication through selective direct viral replication within tumour cells and activation of cell-mediated, tumour-specific immunity^[108]. For example, JX-594 (Pexa-Vec, pexastimogene devacirepvec), derived from a strain of vaccinia, has been studied in HCC^[109,110]. In a randomized phase 2 study with 20% HCV infected and 40% HBV infected patients among the 40 enrolled participants, JX-594 resulted in one CR and three PR^[109]. Nevertheless, it also showed high-dose JX-594 doubled OS to 14.7 months from 6.7 months in the low-dose treatment group. All patients in the study experienced minimal TRAEs. In contrast, a phase 2b trial in 129 HCC patients in the second line setting, including 51.1% HBV- and 14.0% HCV-infected, did not show an OS benefit among 129 patients, compared to those treated with best supportive care^[111]. Patients are presently being recruited for a clinical trial to test JX-594 with nivolumab (NCT03071094) and with sorafenib (NCT02562755), for treatment of advanced HCC as a first-line treatment.

FUTURE DIRECTIONS

HCC is a heterogenic disease in terms of aetiology. HBV or HCV infection add to the complexity of the immune response in HCC. There are emerging data to illuminate the immune landscape, pathway, and mutation profiles of HCC that may provide aetiology-directed study design to obtain the best combination with immunotherapy in the future. Information about the specific immune and genetic landscape of HCV-related HCC is limited, however. In addition, the availability of reported response outcome from patients with different aetiologies in completed clinical trials would provide important data. The ultimate goal is to create aetiology-specific or even personalized therapies for HCC patients.

Furthermore, the schedule and sequence of this combination approach needs further evaluation to determine the optimal timing in order to obtain maximal tumour-directed immunological cell killing, whilst avoiding off-target effects. With more evidence available from other cancer types, especially haematological malignancies, utilizing a maintenance strategy versus moving to a first line or neoadjuvant approach for curative therapy in early HCC is also an interesting topic. In addition, along with the

illumination of the effect of the gastrointestinal tract microbiome in HCC^[112], novel strategies in combination with antimicrobial therapy might be part of future treatment regimens (NCT03785210), such as chemotherapy, targeted therapy and radiation.

The overall clinical response to cell-based immunotherapy has not been robust, which indicates that this therapy may be more helpful when there is a lower disease burden or these precisely designed cells need to be used concurrently with other therapies in order to control HCC, e.g., in combination with ICIs. Moreover, there are subtle but substantial aspects of cell-based immunotherapy that need further evaluation, including virus antigen specific TCR therapy. A further example requiring better understanding is the mechanism by which trafficking of CAR-T cells into HCC cells to execute anti-tumour effects in situ can be achieved. This is a distinct problem observed in solid tumours that is not encountered in CAR-T technology in haematological malignancies.

Lastly, since the overall response to immunotherapy in HCC is suboptimal, it would be critical to identify responder candidates before treatment begins in order to improve the outcomes in patients with HCC associated with HBV or HCV infection. Though tumour mutation burden, PD-L1 expression, TILs, IFN signature and circulating tumour DNA have been indicated as predictive markers in other types of tumours, there has not been strong evidence showing that these markers are valuable in HCC. Therefore, further efforts to identify the predictive biomarkers that may help guide the selection of patients with HCC who are appropriate for ICIs are needed, such as microbiome and TCR repertoire targets. Along with this, intelligent, correlative studies from paired tumour biopsies will be helpful to identify the best therapeutic approaches, timing, and sequences, and improve outcomes of patients with HCC.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception: Monge Bonilla C, Xie C

Manuscript writing and organizing, collect data and interpretation: Monge Bonilla C, McGrath NA, Fu J, Xie C

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCE

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 2006;3:e442.

2. Arnold M, Abnet CC, Neale RE, Vignat J, Giovannucci EL, et al. Global burden of 5 major types of gastrointestinal cancer. *Gastroenterology* 2020;159:335-49.e15.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
4. Lok AS, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, et al; HALT-C Trial Group. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009;136:138-48.
5. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. Global cancer statistics. *CA Cancer J Clin* 2011;61:69-90.
6. Chen CJ, Yang HI, Su J, Jen CL, You SL, et al; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006;295:65-73.
7. Lee MH, Yang HI, Lu SN, Jen CL, Yeh SH, et al. Hepatitis C virus seromarkers and subsequent risk of hepatocellular carcinoma: long-term predictors from a community-based cohort study. *J Clin Oncol* 2010;28:4587-93.
8. Chang MH, You SL, Chen CJ, Liu CJ, Lee CM, et al; Taiwan Hepatoma Study Group. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. *J Natl Cancer Inst* 2009;101:1348-55.
9. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, et al. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. *Gastroenterology* 2017;153:996-1005.e1.
10. Fujiwara N, Friedman SL, Goossens N, Hoshida Y. Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. *J Hepatol* 2018;68:526-49.
11. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-90.
12. Kudo M, Finn RS, Qin S, Han K, Ikeda K, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018;391:1163-73.
13. Bruix J, Qin S, Merle P, Granito A, Huang Y, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;389:56-66.
14. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N Engl J Med* 2018;379:54-63.
15. Zhu AX, Kang Y, Yen C, Finn RS, Galle PR, et al. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α -fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2019;20:282-96.
16. Postow MA, Harding J, Wolchok JD. Targeting immune checkpoints: releasing the restraints on anti-tumor immunity for patients with melanoma. *Cancer J* 2012;18:153-9.
17. Belloni L, Pollicino T, De Nicola F, Guerrieri F, Raffa G, et al. Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function. *Proc Natl Acad Sci U S A* 2009;106:19975-9.
18. Dunn C, Peppas D, Khanna P, Nebbia G, Jones M, et al. Temporal analysis of early immune responses in patients with acute hepatitis B virus infection. *Gastroenterology* 2009;137:1289-300.
19. Sato S, Li K, Kameyama T, Hayashi T, Ishida Y, et al. The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. *Immunity* 2015;42:123-32.
20. Shlomai A, Schwartz RE, Ramanan V, Bhatta A, de Jong YP, et al. Modeling host interactions with hepatitis B virus using primary and induced pluripotent stem cell-derived hepatocellular systems. *Proc Natl Acad Sci U S A* 2014;111:12193-8.
21. Stacey AR, Norris PJ, Qin L, Haygreen EA, Taylor E, et al. Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. *J Virol* 2009;83:3719-33.
22. Wieland S, Thimme R, Purcell RH, Chisari FV. Genomic analysis of the host response to hepatitis B virus infection. *Proc Natl Acad Sci U S A* 2004;101:6669-74.
23. Boltjes A, van Montfort N, Biesta PJ, Op den Brouw ML, Kwekkeboom J, et al. Kupffer cells interact with hepatitis B surface antigen in vivo and in vitro, leading to proinflammatory cytokine production and natural killer cell function. *J Infect Dis* 2015;211:1268-78.
24. Wu J, Meng Z, Jiang M, Pei R, Trippler M, et al. Hepatitis B virus suppresses toll-like receptor-mediated innate immune responses in murine parenchymal and nonparenchymal liver cells. *Hepatology* 2009;49:1132-40.
25. Tu Z, Bozorgzadeh A, Pierce RH, Kurtis J, Crispe IN, et al. TLR-dependent cross talk between human Kupffer cells and NK cells. *J Exp Med* 2008;205:233-44.
26. Fisicaro P, Valdatta C, Boni C, Massari M, Mori C, et al. Early kinetics of innate and adaptive immune responses during hepatitis B virus infection. *Gut* 2009;58:974-82.
27. Lunemann S, Malone DF, Hengst J, Port K, Grabowski J, et al. Compromised function of natural killer cells in acute and chronic viral hepatitis. *J Infect Dis* 2014;209:1362-73.
28. Oliviero B, Varchetta S, Paudice E, Michelone G, Zaramella M, et al. Natural killer cell functional dichotomy in chronic hepatitis B and chronic hepatitis C virus infections. *Gastroenterology* 2009;137:1151-60, 1160.e1-7.
29. Asabe S, Wieland SF, Chattopadhyay PK, Roederer M, Engle RE, et al. The size of the viral inoculum contributes to the outcome of hepatitis B virus infection. *J Virol* 2009;83:9652-62.
30. Rehmann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 2005;5:215-29.
31. Rehmann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat Med* 1996;2:1104-8.

32. Sun C, Sun HY, Xiao WH, Zhang C, Tian ZG. Natural killer cell dysfunction in hepatocellular carcinoma and NK cell-based immunotherapy. *Acta Pharmacol Sin* 2015;36:1191-9.
33. Hoechst B, Voigtlaender T, Ormandy L, Gamrekelashvili J, Zhao F, et al. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the Nkp30 receptor. *Hepatology* 2009;50:799-807.
34. Peppas D, Micco L, Javaid A, Kennedy PT, Schurich A, et al. Blockade of immunosuppressive cytokines restores NK cell antiviral function in chronic hepatitis B virus infection. *PLoS Pathog* 2010;6:e1001227.
35. Kong X, Sun R, Chen Y, Wei H, Tian Z. $\gamma\delta$ T cells drive myeloid-derived suppressor cell-mediated CD8⁺ T cell exhaustion in hepatitis B virus-induced immunotolerance. *J Immunol* 2014;193:1645-53.
36. Park JJ, Wong DK, Wahed AS, Lee WM, Feld JJ, et al; Hepatitis B Research Network. Hepatitis B virus--specific and global T-cell dysfunction in chronic hepatitis B. *Gastroenterology* 2016;150:684-95.e5.
37. Wherry EJ. T cell exhaustion. *Nat Immunol* 2011;12:492-9.
38. Pallett LJ, Gill US, Quaglia A, Sinclair LV, Jover-Cobos M, et al. Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells. *Nat Med* 2015;21:591-600.
39. Pal S, Nandi M, Dey D, Chakraborty BC, Shil A, et al. Myeloid-derived suppressor cells induce regulatory T cells in chronically HBV infected patients with high levels of hepatitis B surface antigen and persist after antiviral therapy. *Aliment Pharmacol Ther* 2019;49:1346-59.
40. Kim GA, Lim YS, Han S, Choi J, Shim JH, et al. High risk of hepatocellular carcinoma and death in patients with immune-tolerant-phase chronic hepatitis B. *Gut* 2018;67:945-52.
41. Maini MK, Boni C, Lee CK, Larrubia JR, Reignat S, et al. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* 2000;191:1269-80.
42. Mason WS, Gill US, Litwin S, Zhou Y, Peri S, et al. HBV DNA integration and clonal hepatocyte expansion in chronic hepatitis B patients considered immune tolerant. *Gastroenterology* 2016;151:986-98.e4.
43. Fiscaro P, Barili V, Montanini B, Acerbi G, Ferracin M, et al. Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B. *Nat Med* 2017;23:327-36.
44. Amadei B, Urbani S, Cazaly A, Fiscaro P, Zerbini A, et al. Activation of natural killer cells during acute infection with hepatitis C virus. *Gastroenterology* 2010;138:1536-45.
45. Axley P, Ahmed Z, Ravi S, Singal AK. Hepatitis C virus and hepatocellular carcinoma: a narrative review. *J Clin Transl Hepatol* 2018;6:79-84.
46. Rosen HR, Golden-Mason L. Control of HCV infection by natural killer cells and macrophages. *Cold Spring Harb Perspect Med* 2019;a037101.
47. Gremion C, Grabscheid B, Wölk B, Moradpour D, Reichen J, et al. Cytotoxic T lymphocytes derived from patients with chronic hepatitis C virus infection kill bystander cells via Fas-FasL interaction. *J Virol* 2004;78:2152-7.
48. Erickson AL, Kimura Y, Igarashi S, Eichelberger J, Houghton M, et al. The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. *Immunity* 2001;15:883-95.
49. Ruhl M, Knuschke T, Schewior K, Glavinic L, Neumann-Haefelin C, et al; East German HCV Study Group. CD8⁺ T-cell response promotes evolution of hepatitis C virus nonstructural proteins. *Gastroenterology* 2011;140:2064-73.
50. Radziejewicz H, Ibegbu CC, Hon H, Osborn MK, Obideen K, et al. Impaired hepatitis C virus (HCV)-specific effector CD8⁺ T cells undergo massive apoptosis in the peripheral blood during acute HCV infection and in the liver during the chronic phase of infection. *J Virol* 2008;82:9808-22.
51. Rutebemberwa A, Ray SC, Astemborski J, Levine J, Liu L, et al. High-programmed death-1 levels on hepatitis C virus-specific T cells during acute infection are associated with viral persistence and require preservation of cognate antigen during chronic infection. *J Immunol* 2008;181:8215-25.
52. Owusu Sekyere S, Suneetha PV, Kraft AR, Zhang S, Dietz J, et al. A heterogeneous hierarchy of co-regulatory receptors regulates exhaustion of HCV-specific CD8 T cells in patients with chronic hepatitis C. *J Hepatol* 2015;62:31-40.
53. McMahan RH, Golden-Mason L, Nishimura MI, McMahon BJ, Kemper M, et al. Tim-3 expression on PD-1⁺ HCV-specific human CTLs is associated with viral persistence, and its blockade restores hepatocyte-directed in vitro cytotoxicity. *J Clin Invest* 2010;120:4546-57.
54. Chattergoon MA, Latanich R, Quinn J, Winter ME, Buckheit RW 3rd, et al. HIV and HCV activate the inflammasome in monocytes and macrophages via endosomal Toll-like receptors without induction of type 1 interferon. *PLoS Pathog* 2014;10:e1004082.
55. Liu Y, Wang W, Zou Z, Hu Z, Fan Q, et al. Hepatitis C virus entry into macrophages/monocytes mainly depends on the phagocytosis of macrophages. *Dig Dis Sci* 2019;64:1226-37.
56. He G, Karin M. NF- κ B and STAT3 - key players in liver inflammation and cancer. *Cell Res* 2011;21:159-68.
57. Shin EC, Sung PS, Park SH. Immune responses and immunopathology in acute and chronic viral hepatitis. *Nat Rev Immunol* 2016;16:509-23.
58. Ren JP, Wang L, Zhao J, Wang L, Ning SB, et al. Decline of miR-124 in myeloid cells promotes regulatory T-cell development in hepatitis C virus infection. *Immunology* 2017;150:213-20.
59. Farooqi ZU, Tareen SH, Ahmed J, Zaidi NU. Logical analysis of regulation of interleukin-12 expression pathway regulation during HCV infection. *Protein Pept Lett* 2016;23:581-9.
60. Piconese S, Timperi E, Pacella I, Schinzari V, Tripodo C, et al. Human OX40 tunes the function of regulatory T cells in tumor and nontumor areas of hepatitis C virus-infected liver tissue. *Hepatology* 2014;60:1494-507.
61. Della Bella S, Crosignani A, Riva A, Presicce P, Benetti A, et al. Decrease and dysfunction of dendritic cells correlate with impaired

- hepatitis C virus-specific CD4⁺ T-cell proliferation in patients with hepatitis C virus infection. *Immunology* 2007;121:283-92.
62. Ulsenheimer A, Gerlach JT, Gruener NH, Jung MC, Schirren CA, et al. Detection of functionally altered hepatitis C virus-specific CD4 T cells in acute and chronic hepatitis C. *Hepatology* 2003;37:1189-98.
63. Cancer Genome Atlas Research Network. Electronic address: wheeler@bcm.edu. Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell* 2017;169:1327-41.e23.
64. Zheng C, Zheng L, Yoo JK, Guo H, Zhang Y, et al. Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing. *Cell* 2017;169:1342-56.e16.
65. Kim HD, Song GW, Park S, Jung MK, Kim MH, et al. Association between expression level of PD1 by tumor-infiltrating CD8⁺ T cells and features of hepatocellular carcinoma. *Gastroenterology* 2018;155:1936-50.e17.
66. Lim CJ, Lee YH, Pan L, Lai L, Chua C, et al. Multidimensional analyses reveal distinct immune microenvironment in hepatitis B virus-related hepatocellular carcinoma. *Gut* 2019;68:916-27.
67. Hoechst B, Ormandy LA, Ballmaier M, Lehner F, Krüger C, et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. *Gastroenterology* 2008;135:234-43.
68. Lin EY, Li JF, Gnatovskiy L, Deng Y, Zhu L, et al. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res* 2006;66:11238-46.
69. Ugel S, De Sanctis F, Mandruzzato S, Bronte V. Tumor-induced myeloid deviation: when myeloid-derived suppressor cells meet tumor-associated macrophages. *J Clin Invest* 2015;125:3365-76.
70. Li H, Han Y, Guo Q, Zhang M, Cao X. Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1. *J Immunol* 2009;182:240-9.
71. Zhang HH, Mei MH, Fei R, Liu F, Wang JH, et al. Regulatory T cells in chronic hepatitis B patients affect the immunopathogenesis of hepatocellular carcinoma by suppressing the anti-tumour immune responses. *J Viral Hepat* 2010;17 Suppl 1:34-43.
72. Han Y, Chen Z, Yang Y, Jiang Z, Gu Y, et al. Human CD14⁺ CTLA-4⁺ regulatory dendritic cells suppress T-cell response by cytotoxic T-lymphocyte antigen-4-dependent IL-10 and indoleamine-2,3-dioxygenase production in hepatocellular carcinoma. *Hepatology* 2014;59:567-79.
73. Prieto J, Melero I, Sangro B. Immunological landscape and immunotherapy of hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2015;12:681-700.
74. Chen Y, Huang Y, Reiberger T, Duyverman AM, Huang P, et al. Differential effects of sorafenib on liver versus tumor fibrosis mediated by stromal-derived factor 1 alpha/C-X-C receptor type 4 axis and myeloid differentiation antigen-positive myeloid cell infiltration in mice. *Hepatology* 2014;59:1435-47.
75. Sangro B, Gomez-Martin C, de la Mata M, Iñarrairaegui M, Garralda E, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol* 2013;59:81-8.
76. El-khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
77. Yau T, Park J, Finn R, Cheng A, Mathurin P, et al. CheckMate 459: a randomized, multi-center phase III study of nivolumab (NIVO) vs sorafenib (SOR) as first-line (1L) treatment in patients (pts) with advanced hepatocellular carcinoma (aHCC). *Ann Oncol* 2019;30:v874-5.
78. Zhu AX, Finn RS, Edeline J, Cattani S, Ogasawara S, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018;19:940-52.
79. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, et al; KEYNOTE-240 investigators. Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: a randomized, double-blind, phase III trial. *J Clin Oncol* 2020;38:193-202.
80. Yen C, Markman B, Chao Y, Hill A, Kang J, et al. Preliminary results of a phase 1A/1B study of BGB-A317, an anti-PD-1 monoclonal antibody (mAb), in patients with advanced hepatocellular carcinoma (HCC). *Ann Oncol* 2017;28:iii54.
81. Qin S, Ren Z, Meng Z, Chen Z, Chai X, et al. Camrelizumab in patients with previously treated advanced hepatocellular carcinoma: a multicentre, open-label, parallel-group, randomised, phase 2 trial. *Lancet Oncol* 2020;21:571-80.
82. He A, Weiss G, Falchook G, Yee N, Gil-martin M, et al. Cemiplimab, a human monoclonal anti-PD-1, in patients (pts) with advanced or metastatic hepatocellular carcinoma (HCC): Data from an expansion cohort (EC) in a phase I study. *Ann Oncol* 2018;29:x26..
83. Wainberg ZA, Segal NH, Jaeger D, Lee KH, Marshall J, et al. Safety and clinical activity of durvalumab monotherapy in patients with hepatocellular carcinoma (HCC). *J Clin Oncol* 2017;35:4071.
84. Floudas CS, Xie C, Brar G, Morelli MP, Fioravanti S, et al. Combined immune checkpoint inhibition (ICI) with tremelimumab and durvalumab in patients with advanced hepatocellular carcinoma (HCC) or biliary tract carcinomas (BTC). *JCO* 2019;37:336.
85. Kelley RK, Abou-alfa GK, Bendell JC, Kim T, Borad MJ, et al. Phase I/II study of durvalumab and tremelimumab in patients with unresectable hepatocellular carcinoma (HCC): Phase I safety and efficacy analyses. *JCO* 2017;35:4073.
86. Li B, Yan C, Zhu J, Chen X, Fu Q, et al. Anti-PD-1/PD-L1 blockade immunotherapy employed in treating hepatitis B virus infection-related advanced hepatocellular carcinoma: a literature review. *Front Immunol* 2020;11:1037.
87. Pishvaian MJ, Lee MS, Ryoo B, Stein S, Lee K, et al. Updated safety and clinical activity results from a phase Ib study of atezolizumab 1 bevacizumab in hepatocellular carcinoma (HCC). *Ann Oncol* 2018;29.
88. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, et al; IMbrave150 Investigators. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med* 2020;382:1894-905.
89. Ikeda M, Sung MW, Kudo M, Kobayashi M, Baron AD, et al. A phase 1b trial of lenvatinib (LEN) plus pembrolizumab (PEM) in patients (pts) with unresectable hepatocellular carcinoma (uHCC). *JCO* 2018;36:4076.

90. Bang Y, Golan T, Lin C, Dahan L, Fu S, et al. Ramucirumab (Ram) and durvalumab (Durva) treatment of metastatic non-small cell lung cancer (NSCLC), gastric/gastroesophageal junction (G/GEJ) adenocarcinoma, and hepatocellular carcinoma (HCC) following progression on systemic treatment(s). *JCO* 2019;37:2528.
91. Yau T, Kang Y, Kim T, El-khoueiry AB, Santoro A, et al. Nivolumab (NIVO) + ipilimumab (IPI) combination therapy in patients (pts) with advanced hepatocellular carcinoma (aHCC): results from CheckMate 040. *JCO* 2019;37:4012.
92. Kudo M, Motomura K, Wada Y, Inaba Y, Sakamoto Y, et al. First-line avelumab + axitinib in patients with advanced hepatocellular carcinoma: Results from a phase 1b trial (VEGF Liver 100). *JCO* 2019;37:4072.
93. Duffy AG, Ulahannan SV, Makorova-Rusher O, Rahma O, Wedemeyer H, et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *J Hepatol* 2017;66:545-51.
94. Katz SC, Burga RA, McCormack E, Wang LJ, Mooring W, et al. Phase I hepatic immunotherapy for metastases study of intra-arterial chimeric antigen receptor-modified T-cell therapy for CEA+ liver metastases. *Clin Cancer Res* 2015;21:3149-59.
95. Zhai B, Shi D, Gao H, Qi X, Jiang H, et al. A phase I study of anti-GPC3 chimeric antigen receptor modified T cells (GPC3 CAR-T) in Chinese patients with refractory or relapsed GPC3+ hepatocellular carcinoma (r/r GPC3+ HCC). *JCO* 2017;35:3049.
96. Yu X, Zhao H, Liu L, Cao S, Ren B, et al. A randomized phase II study of autologous cytokine-induced killer cells in treatment of hepatocellular carcinoma. *J Clin Immunol* 2014;34:194-203.
97. Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, et al. Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. *Gastroenterology* 2015;148:1383-91.e6.
98. Tan AT, Yang N, Lee Krishnamoorthy T, Oei V, Chua A, et al. Use of expression profiles of HBV-DNA integrated into genomes of hepatocellular carcinoma cells to select T cells for immunotherapy. *Gastroenterology* 2019;156:1862-76.e9.
99. Wisskirchen K, Metzger K, Schreiber S, Asen T, Weigand L, et al. Isolation and functional characterization of hepatitis B virus-specific T-cell receptors as new tools for experimental and clinical use. *PLoS One* 2017;12:e0182936.
100. Gehring AJ, Xue SA, Ho ZZ, Teoh D, Ruedl C, et al. Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines. *J Hepatol* 2011;55:103-10.
101. Qasim W, Brunetto M, Gehring AJ, Xue SA, Schurich A, et al. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient. *J Hepatol* 2015;62:486-91.
102. Sahin U, Türeci Ö. Personalized vaccines for cancer immunotherapy. *Science* 2018;359:1355-60.
103. Buonaguro L; HEPAVAC Consortium. Developments in cancer vaccines for hepatocellular carcinoma. *Cancer Immunol Immunother* 2016;65:93-9.
104. Nakagawa H, Mizukoshi E, Kobayashi E, Tamai T, Hamana H, et al. Association between high-avidity T-cell receptors, induced by alpha-fetoprotein-derived peptides, and anti-tumor effects in patients with hepatocellular carcinoma. *Gastroenterology* 2017;152:1395-406.e10.
105. Sawada Y, Yoshikawa T, Nobuoka D, Shirakawa H, Kuronuma T, et al. Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival. *Clin Cancer Res* 2012;18:3686-96.
106. Sawada Y, Yoshikawa T, Shimomura M, Iwama T, Endo I, et al. Programmed death-1 blockade enhances the antitumor effects of peptide vaccine-induced peptide-specific cytotoxic T lymphocytes. *Int J Oncol* 2015;46:28-36.
107. Greten TF, Forner A, Korangy F, N'Kontchou G, Barget N, et al. A phase II open label trial evaluating safety and efficacy of a telomerase peptide vaccination in patients with advanced hepatocellular carcinoma. *BMC Cancer* 2010;10:209.
108. Harrington K, Freeman DJ, Kelly B, Harper J, Soria JC. Optimizing oncolytic virotherapy in cancer treatment. *Nat Rev Drug Discov* 2019;18:689-706.
109. Heo J, Reid T, Ruo L, Breitbach CJ, Rose S, et al. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. *Nat Med* 2013;19:329-36.
110. Park B, Hwang T, Liu T, Sze DY, Kim J, et al. Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial. *Lancet Oncology* 2008;9:533-42.
111. Moehler M, Heo J, Lee HC, Tak WY, Chao Y, et al. Vaccinia-based oncolytic immunotherapy Pexastimogene Devacirepvec in patients with advanced hepatocellular carcinoma after sorafenib failure: a randomized multicenter Phase IIb trial (TRAVERSE). *Oncoimmunology* 2019;8:1615817.
112. Ma C, Han M, Heinrich B, Fu Q, Zhang Q, et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science* 2018;360:eaan5931.

Review

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Beneficial effects of coffee in non-alcoholic fatty liver disease: a narrative review

Alessio Calabrò¹, Anna Caterina Procopio², Francesco Primerano³, Tiziana Larussa², Francesco Luzzza², Laura Di Renzo⁴, Antonino De Lorenzo⁴, Paola Gualtieri⁴, Ludovico Abenavoli²

¹Independent Nutritionist, Viale dei Bizantini 25, Catanzaro 88100, Italy.

²Department of Health Sciences, University "Magna Graecia" of Catanzaro, Viale Europa, Catanzaro 88100, Italy.

³Independent Biomedical Engineer, Viale degli Aurunci 9, 88060 Montepaone, Catanzaro 88100, Italy.

⁴Department of Biomedicine and Prevention, University of Rome "Tor Vergata", Rome 00133, Italy.

Correspondence to: Prof. Ludovico Abenavoli, Department of Health Sciences, University "Magna Graecia" of Catanzaro, Viale Europa, Catanzaro 88100, Italy. E-mail: l.abenavoli@unicz.it

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases and is related to unhealthy lifestyle habits, characterized by a diet rich in sugars and fats leading to excessive calorie intake, and lack of exercise. In recent years, there is a growing incidence of this pathology, raising the attention of hepatologists, endocrinologists, diabetologists, and nutritionists. In this context, the alimentary regimen adopted by patients with NAFLD has become an increasingly scrutinised parameter. Diet is now considered a crucial factor in the treatment of NAFLD since it has been observed that some functional foods play a beneficial role. These include coffee whose health effects have already been amply demonstrated. Here we describe the beneficial effects of coffee consumption reported in the NAFLD literature.

Keywords: Caffeine, steatosis, functional food, liver disease, antioxidant, chlorogenic-acid

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common liver pathology characterized by fat accumulation in the liver, following a sequence of steatosis, possible evolution in fibrosis, Non-alcoholic steatohepatitis



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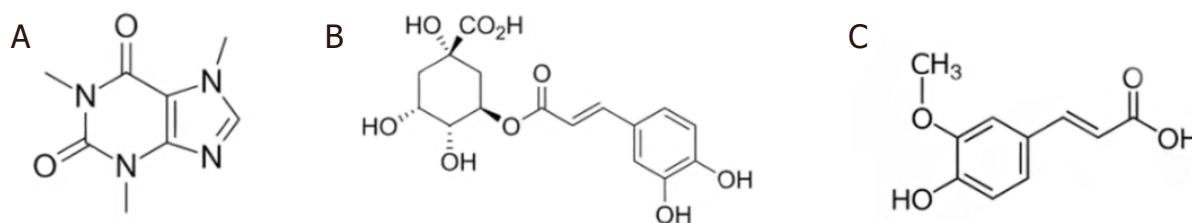


Figure 1. 2D chemical structures of caffeine (A), chlorogenic acid (B), ferulic acid (C)

(NASH), and finally in cirrhosis. Today liver diseases and in particular NAFLD, affects about 25% of the population worldwide, representing the main cause of chronic liver disease in industrialized countries^[1-4].

Lifestyle modifications such as maintaining a healthy diet and regular physical activity are key in reducing the onset of NAFLD^[5-9]. In particular, it is most important to remove all dietary compounds that can promote NAFLD, for example fructose, saturated fatty acids, carbohydrates with a high glycemic index, and foods with a high sodium content^[2,3]. Promotion of a balanced diet and loss of excess weight are also important in the context of this clinical condition. The reduction of at least 5% body weight is effective in reducing hepatic fat accumulation^[10,11].

In recent years, the Mediterranean diet has also been accredited as a therapeutic standard in the treatment of NAFLD^[12-17]. Different studies have showed how functional foods can positively influence our health^[5,18-22].

Among the functional foods, we find coffee the most promising; a drink appreciated and consumed all over the world^[23]. Different beneficial effects have been related to moderate and regular consumption of coffee. In particular, the intake of this drink has proven effective in reducing the risk of type II diabetes mellitus, gastrointestinal disorders, Parkinson disease, cardiovascular problems, and gallbladder stones^[24-28].

The present review aims to describe the beneficial effects of coffee consumption in patients with NAFLD.

CHEMICAL COMPONENTS OF COFFEE

Coffee, one of the most consumed beverages in the world, is composed of a large number of substances and polyphenols that contribute to making coffee a real functional food thanks to their beneficial activities. Among the main polyphenols in coffee, chlorogenic acid and ferulic acid [Figure 1] have shown promising antioxidative properties.

The most representative and known compound of coffee is undoubtedly caffeine [Figure 1], an alkaloid naturally present in leaves, seeds, and fruits of coffee, tea, cocoa, cola, and guarana plants. Caffeine is absorbed in the stomach and first portion of the intestine within 10 min after ingestion, reaching a maximum concentration in the bloodstream after 45-60 min. Once absorbed by the body, caffeine is metabolized in the liver where it is converted into three dimethylxanthines: paraxanthine, theobromine, theophylline which contribute to enhancing its effects^[29].

In recent years, natural compounds have been the subject of several studies in order to identify their beneficial activities, and in this regard promising antifibrotic effects have been attributed to caffeine [Figure 2].

In particular, caffeine showed promising antifibrotic effect resulting from a series of biochemical processes initiated by liver cell stimulation causing induction of intracellular F-actin and cAMP expression, inhibition

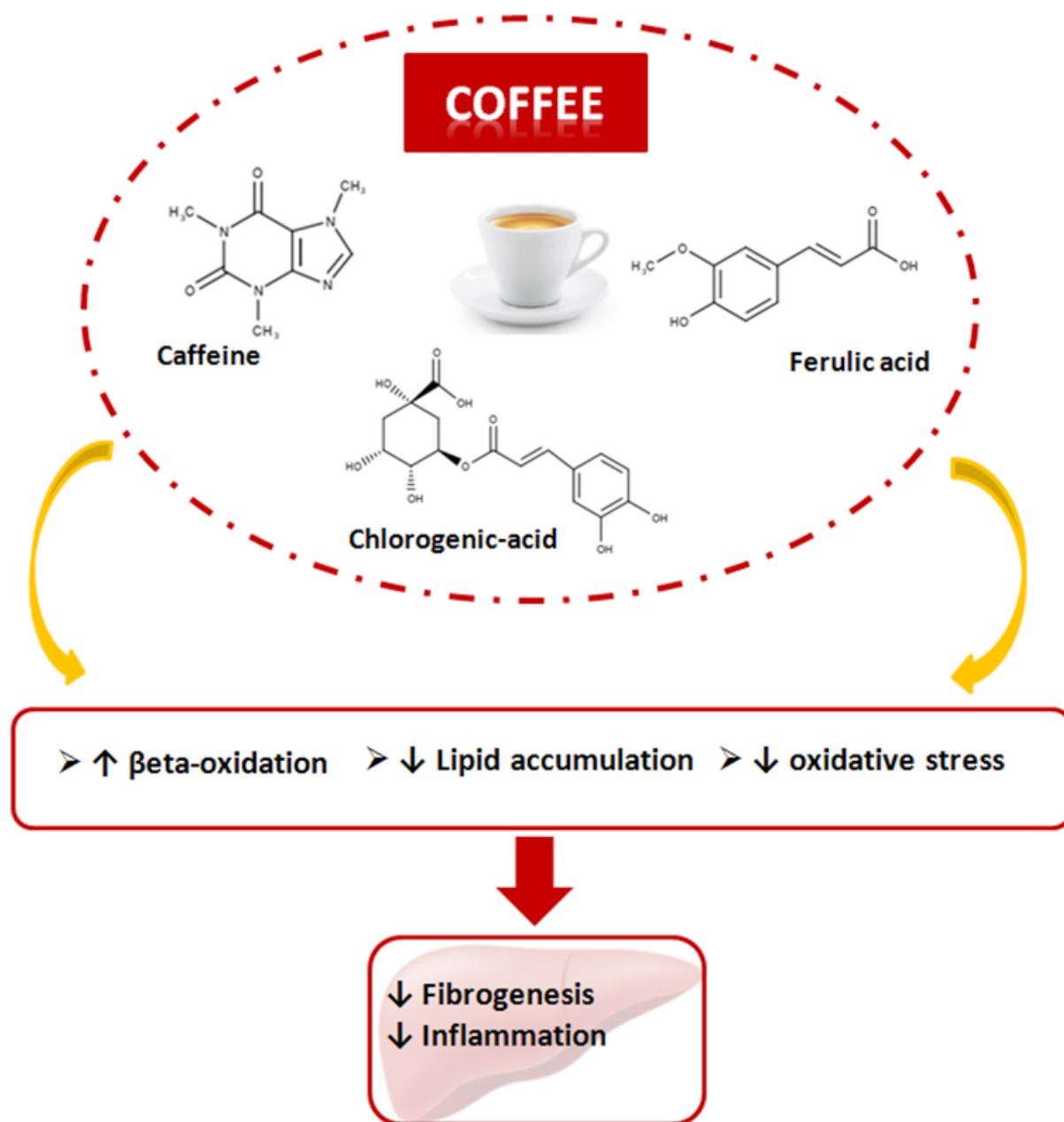


Figure 2. Beneficial effects of coffee on the liver

of focal adhesion kinase, inhibition of α -smooth muscle actin (α -sma), and up-regulation of the PPAR- α receptor (Peroxisome proliferator-activated receptor alpha) with action on fat deposits, as shown in Figure 2^[30,31]. Quan *et al.*^[32] showed that caffeine is able to reduce the gene expression of the transcription factors Sterol regulatory element-binding protein 1c and 2 (SREBP1c and SREBP2) in HepG2 cells which are involved in the synthesis of triglycerides and cholesterol in the liver. This down-regulation represents a particularly promising finding as it can facilitate the reduction of hepatic lipid accumulation typically associated with NAFLD. In addition, caffeine also causes the reduction of 3-hydroxy 3-methylglutaryl CoA reductase and low density lipoprotein receptor in a dose dependent manner.

Furthermore, Helal *et al.*^[33] conducted a study in animal models in order to demonstrate that the use of caffeine can improve liver damage induced by a high-fat diet (HFD). The rats were divided into

four groups and treated for 16 weeks as follows: control group; HFD Group; HFD group and 20 mg of caffeine; HFD group and 30 mg of caffeine. HFD-induced liver injury was determined by evaluating the alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, bilirubin, triglycerides and cholesterol. Results indicated that caffeine treatment reduced elevated serum levels of ALT, AST, bilirubin and hepatic mRNA expression of fatty acid synthase and acetyl CoA carboxylase.

Chlorogenic acid (CGA) is a phenolic compound that is obtained from the combination of caffeic acid and (*L*)-quinic acid. It belongs to the family of polyphenols, substances with powerful antioxidant actions. It is well known that the polyphenols present in coffee as chlorogenic acid increase the production of antioxidants agents^[34]. Moreover, caffeine increases peroxiredoxin-1 which has positive effects on ROS, reducing oxidative stress at the level of hepatocytes^[34].

The synergistic effect of polyphenols and caffeine on hepatocytes also decreases insulin resistance, while polyphenols contained in caffeine exerts an antifibrotic effect on the liver, an action that has been widely highlighted in studies carried out on obese rats^[35]. It is present in high concentrations in green coffee and in other typical foods of the Mediterranean diet^[36,37]. Coffee roasting produces a compound that takes the name of hydroxy-hydroquinone, which however reduces its bioavailability. It is estimated that one liter of coffee provides between 500 and 800 mg of CGA^[38].

Ferulic acid (FA) is a phenolic acid found in coffee, in some cereals such as oats, wheat and rice, in artichokes and in some types of fruit. FA is a derivative of trans-cinnamic acid and is able to interfere with the expression and activity of cytotoxic enzymes such as nitric oxide synthase, caspases and the cyclooxygenase-2. Ferulic acid has been proposed as treatment for neurodegenerative, cardiovascular and diabetic disorders.

Furthermore, it must be emphasized that the interest in this compound has increased due to its potential therapeutic effects to reduce the deposition of triglycerides and cholesterol in hepatocytes, anti-microbial, anti-inflammatory, and anti-tumor properties^[39,40].

COFFEE AND NAFLD

Among the several studies that analyzed the activities of coffee in NAFLD, Hosseinabadi *et al.*^[41] evaluated the effects of green coffee extract (GCE) on the lipid profile and adiponectin levels in patients with NAFLD. The randomized double-blind study was conducted on 48 patients aged between 20 and 60 years with a body mass index (BMI) between 25-35 kg/m². In this study the patients were divided into two groups, one group has been treated with 400 mg/die GCE and the other with placebo. The liver enzymes, lipid profile, adiponectin concentration, and degree of hepatic steatosis were analyzed at the beginning and at the end of the trial. The results showed that GCE supplementation significantly reduced BMI (MD: -0.57; 95%CI: -0.84 to -0.29; $P < 0.001$). Moreover the study also showed an increase in HDL levels (MD: 7.06; 95%CI: 0.25 to 13.87; $P < 0.05$) and a reduction in serum blood triglyceride levels (MD: -37.91; 95%CI: -72.03 to -3.80; $P = 0.03$) and total cholesterol (MD: -13.33; 95%CI: -26.04 to -0.61; $P < 0.05$) compared to the control group [Table 1].

Graeter *et al.*^[42] have assessed the impact of caffeine consumption on liver fat concentration and ALT values in a total population sample of 1,452 subjects, including 789 women, and 663 men with an average age of 42.3 years. After completing an informative questionnaire on personal data and lifestyle with particular focus on coffee consumption, the liver health of all patients were examined using ultrasound. Univariate logistic regression was used to evaluate the association between caffeine consumption and hepatic steatosis. From the results, a significant association emerged between hepatic steatosis and the male gender ($P < 0.0001$), the same significance was also detected for advanced age subjects ($P < 0.0001$) and the high body-

Table 1. Studies on coffee in liver diseases

Author	Year	Design	Population	Disease	Methods	Country	Findings
Hosseiniabadi <i>et al.</i> ^[41]	2020	Randomized, controlled trial	48 ($n = 24$ GCE 400 mg) ($n = 24$ placebo)	NAFLD	Ultrasonographic	Iran	GCE supplementation improved serum lipid profile and BMI in individuals with NAFLD. GCE may be useful in controlling NAFLD risk factors
Graeter <i>et al.</i> ^[42]	2015	Cross-sectional	1,223	NAFLD	Ultrasonographic	Germany	No evidence for an association between caffeine consumption and either the prevalence of hepatic steatosis or serum ALT concentrations
Bambha <i>et al.</i> ^[43]	2014	Cross-sectional	782	NAFLD/ NASH/ diabetes	Hepatic histological data	USA	Coffee intake is associated with decreased odds of advanced fibrosis among patients with less insulin resistance
Anty <i>et al.</i> ^[44]	2012	Cohort	195	NAFLD/ NASH/ obesity	Liver biopsies	France	Consumption of regular coffee but not espresso is an independent protective factor for liver fibrosis in severely obese European patients
Zelber-Sagi <i>et al.</i> ^[45]	2015	Cross-sectional	494	NAFLD	Hepato renal index (HRI) and Steato test	Israel	No association was demonstrated between coffee consumption and the new onset of non-alcoholic fatty liver, but coffee intake may exert beneficial effects on fibrosis progression
Hodge <i>et al.</i> ^[46]	2017	Retrospective	1,018	NAFLD/ HCV/ HBV	Transient elastography (TE)	Australia	Coffee consumption decreases liver stiffness, which may indicate less fibrosis and inflammation, independent of the disease state. This study adds further evidence to the notion of coffee, which may be beneficial in patients with liver disease
Alferink <i>et al.</i> ^[47]	2017	Cohort	2,424	NAFLD	Elastography, ultrasound	Netherlands	In the general population, frequent coffee and herbal tea consumption were inversely related with liver stiffness but not steatosis

NAFLD: non-alcoholic fatty liver disease; HCV: hepatitis C virus; HBV: hepatitis B virus; GCE: green coffee extract; ALT: alanine aminotransferase

mass index (BMI; $P < 0.0001$). However, no association was identified between caffeine consumption and liver fat levels, nor between caffeine consumption and high levels of ALT concentrations.

Bambha *et al.*^[43] have analyzed the effects of coffee intake in 782 NAFLD patients ($n = 295$ men) with an average age of 48 ± 12 years and an average BMI of 33.5 kg/m^2 with low levels of insulin resistance. Coffee intake was measured by cups per day (cpd), and was represented as follows: 0 cpd, $n = 230$; < 1 cpd, $n = 219$; $1 - < 2$ cpd, $n = 116$; ≥ 2 cpd, $n = 217$. During the study, IR was assessed using HOMA-IR and the association between coffee intake and NAFLD histological severity was modeled using multiple logistic regression. The study found that the effect of coffee on fibrosis varied with the degree of insulin resistance (IR) (interaction $P = 0.001$). Coffee consumers with less IR, defined as $\text{HOMA-IR} < 4.3$, had a lower probability of fibrosis (OR = 0.64; 95%CI: 0.46 to 0.88; $P = 0.001$). However, there was no protective effect of coffee on advanced fibrosis among individuals with higher HOMA-IR (OR = 1.06; 95%CI: 0.87 to 1.28; $P = 0.6$).

In the study of Anty *et al.*^[44] the influence of coffee and other caffeinated drinks on liver fibrosis of severely obese patients was assessed. A total of 195 patients with severe obesity, were enrolled for this trial and were given a specific questionnaire that analyzed the consumption of various types of coffee and other beverages containing caffeine and chocolate. The questionnaires showed that classic coffee and espresso were consumed in 30.8% and 50.2% of patients, respectively. According to logistic regression analysis, coffee consumption represents a protective factor for fibrosis [OR: 0.752 (0.578-0.980); $P = 0.035$] in a model that

included the level of AST [(OR: 1.04 (1.004-1.076); $P = 0.029$] and presence of NASH [OR: 2.41 (1.007-5.782); $P = 0.048$].

Zelber-Sagi *et al.*^[45], evaluated the association between coffee consumption and hepatic steatosis onset in a cohort of 347 patients, comparing them with a control group. Hepatic steatosis was quantified by ultrasound and SteatoTest and the degree of fibrosis was assessed by FibroTest. During recruitment, a questionnaire relating to coffee consumption was filled. The study found that neither the incidence nor the prevalence of steatosis was associated with coffee consumption. Moreover, coffee consumption was associated with a lower clinically significant percentage of fibrosis $\geq F2$ (8.8% vs. 16.3%; $P = 0.038$) and the multivariate logistic regression analysis related the high coffee consumption with a lower probability of fibrosis (probability ratio = 0.49, 95% confidence interval, 0.25-0.97; $P = 0.041$). In a retrospective study by Hodge *et al.*^[46], a total of 1,018 patients (441 women, 577 men) affected by NAFLD, Hepatitis C (HCV), and hepatitis B (HBV) were recruited for determining the effects of coffee and tea intake on liver stiffness (SE). Data showed that SE was higher in males than in females ($P < 0.05$). HBV patients had a lower SE than those with HCV and NAFLD. Those who drank 2 or more cups of coffee a day had a lower SE ($P = 0.044$). Tea consumption had no effect on SE ($P = 0.9$). The study showed that coffee consumption reduces the SE with a decrease of fibrosis and inflammation.

Alferink *et al.*^[47] recruited 2,424 participants (age 66.5 ± 7.4 ; 43% male) who were asked to fill in a questionnaire on eating habits and lifestyle. All patients were examined by liver elastography and ultrasonography to evaluate liver stiffness and the degree of liver steatosis. Coffee and tea consumption were classified into: no intake (0), moderate ($> 0-3$) or frequent intake ≥ 3) (cups/day). Fibrosis was defined as a measure of SE. During course of the study, univariate linear and logistic regression analyses were performed to examine the association between coffee, herbal /green/black tea consumption and SE. For the regression models, multivariable adjustments were made taking into consideration age, sex, BMI, insulin resistance, steatosis, serum ALT, alcohol intake, smoking, use of cream in coffee, use of sugar in tea or coffee and physical activity. The results showed that the SE was inversely related to a higher consumption of coffee (7.8%, 6.9% and 4.1% for non consumption, moderate and frequent respectively; $P = 0.006$). In general, frequent coffee consumption was inversely related to SE but not to steatosis. More in-depth analysis and studies are needed to validate the underlying mechanisms. The daily consumption of three or more cups of coffee was linked to the presence of a low SE, regardless of many other lifestyle and environmental factors.

CONCLUSION

Although the protective role of coffee in NAFLD is still controversial, several studies have shown that coffee consumption in patients with NAFLD can be protective against liver steatosis, progression of fibrosis and liver damage^[48]. The antioxidant capacity of coffee has been demonstrated in animal models of fatty liver, in which caffeine intake improves insulin resistance and reduces the production of inflammatory cytokines. Moreover, the weight of the animals and the intrahepatic levels of glucose were reduced with coffee consumption. However, not all the studies were concordant on the protective effect of coffee against NAFLD: controversial results may be due to the heterogeneity of the patients analyzed, the different protocols of research, and the different methods of collecting data. Furthermore, it should be emphasized that the content of caffeine and the different compounds typical of coffee can be influenced by the type of coffee examined, the type of roasting, the volume of water used, and the type of grinding: all these elements should therefore be standardized to avoid variations in the concentration of the most active compounds.

In conclusion, the overall trend emerging from the literature review suggests that regular coffee consumption might be a protective factor against the evolution of NAFLD complications, especially liver fibrosis. Additional studies should be carried out to further characterize the correlation between coffee and

NAFLD. Many studies are still controversial by designs, methods, amount of caffeine or type of coffee used, measurements, different population investigated but research in this area should also focus on assessing the potential in taking this drink, quantifying the methods and number of intakes that generate an effective advantage. Pending further research, we can suggest that moderate consumption of 2-3 cups of coffee a day in patients with NAFLD should be encouraged.

DECLARATIONS

Authors' contributions

Wrote, reviewed and edited the manuscript: Calabrò A, Procopio AC, Gualtieri P

Provided the tables: Calabrò A, Procopio AC, Gualtieri P

Reviewed and approved the final manuscript as submitted: Primerano F, Larussa T, Luzzza F

Read and approved the final manuscript: Di Renzo L, De Lorenzo A, Abenavoli L

Conceptualized and designed the review: Abenavoli L

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. *J Hepatol* 2019;70:151-71.
2. Abenavoli L, Procopio AC, Medić-Stojanoska M, Luzzza F. Non-alcoholic fatty liver disease and primary care physicians. *Minerva Gastroenterol Dietol* 2020;66:4-5.
3. Abenavoli L, Milanović M, Milić N, Luzzza F, Giuffrè AM. Olive oil antioxidants and non-alcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2019;13:739-49.
4. Rinella M, Charlton M. The globalization of nonalcoholic fatty liver disease: Prevalence and impact on world health. *Hepatology* 2016;64:19-22.
5. Abenavoli L, Peta V, Milic N. Lifestyle changes associated with a new antioxidant formulation in non-alcoholic fatty liver disease: a case series. *Ann Hepatol* 2015;14:121-6.
6. Michel M, Schattenberg JM. Effectiveness of lifestyle interventions in NAFLD (nonalcoholic fatty liver disease) - how are clinical trials affected? *Expert Opin Investig Drugs* 2020;29:93-7.
7. Lonardo A, Mantovani A, Lugari S, Targher G. NAFLD in some common endocrine diseases: prevalence, pathophysiology, and principles of diagnosis and management. *Int J Mol Sci* 2019;20:2841.
8. Gelli C, Tarocchi M, Abenavoli L, Di Renzo L, Galli A, et al. Effect of a counseling-supported treatment with the Mediterranean diet and physical activity on the severity of the non-alcoholic fatty liver disease. *World J Gastroenterol* 2017;23:3150-62.
9. Abenavoli L, Milanovic M, Procopio AC, Spampinato G, Maruca G, et al. Ancient wheats: beneficial effects on insulin resistance. *Minerva Med* 2020 Jul 28; [Online ahead of print].
10. Brunner KT, Henneberg CJ, Wilechansky RM, Long MT. Nonalcoholic Fatty Liver Disease and Obesity Treatment. *Curr Obes Rep* 2019;8:220-8.

11. Hamurcu Varol P, Kaya E, Alphan E, Yilmaz Y. Role of intensive dietary and lifestyle interventions in the treatment of lean nonalcoholic fatty liver disease patients. *Eur J Gastroenterol Hepatol* 2020;32:1352-7.
12. Cai J, Zhang XJ, Li H. Progress and challenges in the prevention and control of nonalcoholic fatty liver disease. *Med Res Rev* 2019;39:328-48.
13. Abenavoli L, Di Renzo L, Boccuto L, Alwardat N, Gratteri S, et al. Health benefits of Mediterranean diet in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2018;12:873-81.
14. Abenavoli L, Boccuto L, Federico A, Dallio M, Loguercio C, et al. Diet and non-alcoholic fatty liver disease: the mediterranean way. *Int J Environ Res Public Health* 2019;16:3011.
15. Abenavoli L, Milic N, Luzzza F, Boccuto L, De Lorenzo A. Polyphenols Treatment in Patients with Nonalcoholic Fatty Liver Disease. *J Transl Int Med* 2017;5:144-7.
16. Abenavoli L, Milic N, Di Renzo L, Preveden T, Medić-Stojanoska M, et al. Metabolic aspects of adult patients with nonalcoholic fatty liver disease. *World J Gastroenterol* 2016;22:7006-16.
17. Di Renzo L, Cioccoloni G, Bernardini S, Abenavoli L, Aiello V, et al. A hazelnut-enriched diet modulates oxidative stress and inflammation gene expression without weight gain. *Oxid Med Cell Longev* 2019;2019:4683723.
18. Araújo AR, Rosso N, Bedogni G, Tiribelli C, Bellentani S. Global epidemiology of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: what we need in the future. *Liver Int* 2018;38 Suppl 1:47-51.
19. Luk JM, Wang X, Liu P, Wong KF, Chan KL, et al. Traditional Chinese herbal medicines for treatment of liver fibrosis and cancer: from laboratory discovery to clinical evaluation. *Liver Int* 2007;27:879-90.
20. Philips CA, Augustine P, Rajesh S, Y PK, Madhu D. Complementary and alternative medicine-related drug-induced liver injury in Asia. *J Clin Transl Hepatol* 2019;7:263-74.
21. Richmond JA, Bailey DE, Patel K, Jezsik JA, Muir A, et al. The use of complementary and alternative medicine by patients with chronic hepatitis C. *Complement Ther Clin Pract* 2010;16:124-31.
22. Henson JB, Brown CL, Chow SC, Muir AJ. Complementary and alternative medicine use in United States adults with liver disease. *J Clin Gastroenterol* 2017;51:564-70.
23. Nieber K. The impact of coffee on health. *Planta Med* 2017;83:1256-63.
24. Costa J, Lunet N, Santos C, Santos J, Vaz-Carneiro A. Caffeine exposure and the risk of Parkinson's disease: a systematic review and meta-analysis of observational studies. *J Alzheimers Dis* 2010;20 Suppl 1:S221-38.
25. Crippa A, Discacciati A, Larsson SC, Wolk A, Orsini N. Coffee consumption and mortality from all causes, cardiovascular disease, and cancer: a dose-response meta-analysis. *Am J Epidemiol* 2014;180:763-75.
26. Nordestgaard AT, Stender S, Nordestgaard BG, Tybjaerg-Hansen A. Coffee intake protects against symptomatic gallstone disease in the general population: a Mendelian randomization study. *J Intern Med* 2020;287:42-53.
27. Lopez-Garcia E, van Dam RM, Li TY, Rodriguez-Artalejo F, Hu FB. The relationship of coffee consumption with mortality. *Ann Intern Med* 2008;148:904-14.
28. Freedman ND, Park Y, Abnet CC, Hollenbeck AR, Sinha R. Association of coffee drinking with total and cause-specific mortality. *N Engl J Med* 2012;366:1891-904.
29. Nehlig A, Daval J, Debry G. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res Rev* 1992;17:139-70.
30. Shim SG, Jun DW, Kim EK, Saeed WK, Lee KN, et al. Caffeine attenuates liver fibrosis via defective adhesion of hepatic stellate cells in cirrhotic model. *J Gastroenterol Hepatol* 2013;28:1877-84.
31. Gressner OA, Lahme B, Rehbein K, Siluschek M, Weiskirchen R, et al. Pharmacological application of caffeine inhibits TGF-beta-stimulated connective tissue growth factor expression in hepatocytes via PPARgamma and SMAD2/3-dependent pathways. *J Hepatol* 2008;49:758-67.
32. Quan HY, Kim DY, Chung SH. Caffeine attenuates lipid accumulation via activation of AMP-activated protein kinase signaling pathway in HepG2 cells. *BMB Rep* 2013;46:207-12.
33. Helal MG, Ayoub SE, Elkashefand WF, Ibrahim TM. Caffeine affects HFD-induced hepatic steatosis by multifactorial intervention. *Hum Exp Toxicol* 2018;37:983-90.
34. Perumpail BJ, Li AA, Iqbal U, Sallam S, Shah ND, et al. Potential therapeutic benefits of Herbs and supplements in patients with NAFLD. *Diseases* 2018;6:80.
35. Watanabe S, Takahashi T, Ogawa H, Uehara H, Tsunematsu T, et al. Daily coffee intake inhibits pancreatic beta cell damage and nonalcoholic steatohepatitis in a mouse model of spontaneous metabolic syndrome, tsumura-suzuki obese diabetic mice. *Metab Syndr Relat Disord* 2017;15:170-7.
36. Di Renzo L, Marsella LT, Carraro A, Valente R, Gualtieri P, et al. Changes in LDL oxidative status and oxidative and inflammatory gene expression after red wine intake in healthy people: a randomized trial. *Mediators Inflamm* 2015;2015:317348.
37. Colica C, Di Renzo L, Trombetta D, Smeriglio A, Bernardini S, et al. Antioxidant effects of a hydroxytyrosol-based pharmaceutical formulation on body composition, metabolic state, and gene expression: a randomized double-blinded, placebo-controlled crossover trial. *Oxid Med Cell Longev* 2017;2017:2473495.
38. Meng S, Cao J, Feng Q, Peng J, Hu Y. Roles of chlorogenic acid on regulating glucose and lipids metabolism: a review. *Evid Based Complement Alternat Med* 2013;2013:801457.
39. Zduńska K, Dana A, Kolodziejczak A, Rotsztein H. Antioxidant properties of ferulic acid and its possible application. *Skin Pharmacol Physiol* 2018;31:332-6.

40. Sarwar T, Zafaryab M, Husain MA, Ishqi HM, Rehman SU, et al. Redox cycling of endogenous copper by ferulic acid leads to cellular DNA breakage and consequent cell death: A putative cancer chemotherapy mechanism. *Toxicol Appl Pharmacol* 2015;289:251-61.
41. Hosseinabadi S, Rafrat M, Asghari S, Asghari-Jafarabadi M, Vojouhi S. Effect of green coffee extract supplementation on serum adiponectin concentration and lipid profile in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. *Complement Ther Med* 2020;49:102290.
42. Graeter T, Niedermayer PC, Mason RA, Oeztuerk S, Haenle MM, et al; EMIL-Study group. Coffee consumption and NAFLD: a community based study on 1223 subjects. *BMC Res Notes* 2015;8:640.
43. Bambha K, Wilson LA, Unalp A, Loomba R, Neuschwander-Tetri BA, et al; Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN). Coffee consumption in NAFLD patients with lower insulin resistance is associated with lower risk of severe fibrosis. *Liver Int* 2014;34:1250-8.
44. Anty R, Marjoux S, Iannelli A, Patouraux S, Schneck AS, et al. Regular coffee but not espresso drinking is protective against fibrosis in a cohort mainly composed of morbidly obese European women with NAFLD undergoing bariatric surgery. *J Hepatol* 2012;57:1090-6.
45. Zelber-Sagi S, Salomone F, Webb M, Lotan R, Yeshua H, et al. Coffee consumption and nonalcoholic fatty liver onset: a prospective study in the general population. *Transl Res* 2015;165:428-36.
46. Hodge A, Lim S, Goh E, Wong O, Marsh P, et al. Coffee intake is associated with a lower liver stiffness in patients with non-alcoholic fatty liver disease, Hepatitis C, and Hepatitis B. *Nutrients* 2017;9:56.
47. Alferink LJM, Fittipaldi J, Kieffe-de Jong JC, Taimr P, Hansen BE, et al. Coffee and herbal tea consumption is associated with lower liver stiffness in the general population: the Rotterdam study. *J Hepatol* 2017;67:339-48.
48. Furtado KS, Prado MG, Aguiar E, Silva MA, Dias MC, et al. Coffee and caffeine protect against liver injury induced by thioacetamide in male Wistar rats. *Basic Clin Pharmacol Toxicol* 2012;111:339-47.

Review

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Systemic therapy for advanced cholangiocarcinoma: new options on the horizon

Saleh A. Alqahtani^{1,2}, Massimo Colombo³

¹Liver Transplant Center, King Faisal Specialist Hospital & Research Center, Riyadh 12713, Saudi Arabia.

²Division of Gastroenterology and Hepatology, Johns Hopkins University, Baltimore, MD 21287, USA.

³Liver Center, San Raffaele Hospital, Milan 20132, Italy.

Correspondence to: Dr. Saleh A. Alqahtani, Division of Gastroenterology and Hepatology, Johns Hopkins University, 600 North Wolfe St, Blalock Suite 412, Baltimore, MD 21287, USA. E-mail: salqaht1@jhmi.edu

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Abstract

Patients with unresectable cholangiocarcinoma (CCA) face a poor prognosis, and there are few effective treatment options for the disease. The standard of care for patients with locally advanced or metastatic CCA is chemotherapy with a gemcitabine-based doublet. Unfortunately, the clinical benefit obtained with these regimens is modest, with a median overall survival of about one year. For CCA that is chemotherapy-refractory or recurs after first-line chemotherapy, the treatment options are even more limited, and no relevant randomized controlled data are available. In recent years, molecular profiling has shed light on the molecular basis of CCA and identified subgroups of patients that might benefit from a personalized treatment approach. These efforts resulted in the recent FDA approval of the fibroblast growth factor receptor (FGFR) inhibitor, pemigatinib, as a second-line treatment for patients with advanced CCA harboring an FGFR2-fusion or rearrangement. Several other targeted agents also are under evaluation in patients with CCA, of which the isocitrate dehydrogenase inhibitor has had the most promising results. Finally, immunotherapy is being explored as a new treatment approach for advanced CCA patients; indeed, the immune checkpoint inhibitor pembrolizumab can already be used to treat CCAs that are mismatch repair deficient. This review is a comprehensive overview of the treatment options for CCA and offers a glimpse into what the future could hold for these patients.

Keywords: Cholangiocarcinoma, fibroblast growth factor receptor inhibitor, isocitrate dehydrogenase inhibitor, immune checkpoint inhibitor



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INTRODUCTION

Cholangiocarcinoma (CCA) refers to a group of malignancies that arise from epithelial cells along the biliary tree^[1]. It is a rare tumor type that accounts for less than 1% of all human cancers^[1]. Based on the location of the tumor, CCAs are divided into three categories: Intrahepatic CCAs (iCCAs) are within the liver parenchyma (i.e., proximal to the second-degree bile ducts), whereas perihilar CCAs (pCCAs) and distal CCAs (dCCAs) are outside the liver, with the cystic duct as the boundary between the two types^[1]. Most CCAs are extrahepatic; iCCAs account for only 10%-20% of cases^[2,3]. CCAs are classified histopathologically as adenocarcinomas, but rare histologic subtypes can be encountered^[4].

Over the last decades, the incidence of CCA has increased in Western countries^[3], which is the basis for large studies that have looked into risk factors for cancer development^[5,6]. The most prominent risk factors identified in these analyses are liver cirrhosis, viral hepatitis, metabolic syndrome, and diabetes mellitus^[5-8]. However, the best-known risk factors for CCA are pre-existing conditions, such as choledochal cysts, inflammatory bowel disease, and primary sclerosing cholangitis^[9,10]. The incidence of CCA is highest in Southeast Asia, where there is a strong relationship between infections with the hepatobiliary flukes *Opisthorchis viverrini* and *Clonorchis sinensis* and CCA^[10].

The only potentially curative treatment for patients with CCA is radical surgical resection of the lesion combined with lymphadenectomy^[3]. Unfortunately, however, surgical resection is feasible in only about 30% of patients^[3,11], and recurrence after surgery is frequent; thus the prospects of long-term survival after resection are poor^[2,3,11]. To counter the high rates of local and distant recurrence after surgery for CCA, several adjuvant treatment strategies have been explored, with mixed results^[3,12-16]. In about 70% of patients, the disease is unresectable or metastatic at the time of diagnosis^[1]. For these patients, the treatment options are usually limited to systemic therapies^[1,3]. Only in a minority of advanced CCA patients are palliative loco-regional therapies beneficial, and the use of this approach is restricted mainly to patients with iCCA whose disease spread is limited to the liver. In this setting, small studies have demonstrated that transarterial chemo- or radioembolization can provide local disease control, with a survival benefit comparable to that of supportive care^[17-21]. More recently, insight into the molecular basis of CCA and understanding of the interplay between tumor cells and the immune system have led to the development of targeted treatments. The most promising results in this area have come from studies evaluating inhibitors of mutated forms of the *FGFR* or isocitrate dehydrogenase (*IDH*) and from studies evaluating immune checkpoint inhibitors.

This review provides an overview of the systemic treatment options for patients with advanced CCA. Data from the use of chemotherapy regimens in initial treatment and of recurrent disease, as well as a summary of the clinical trials evaluating molecularly targeted agents or immunotherapy, are presented.

IS THERE A PLACE FOR ADJUVANT CHEMOTHERAPY IN PATIENTS WITH SURGICALLY RESECTED CCA?

As discussed above, surgical resection of CCA is associated with a high rate of disease recurrence and a poor long-term survival rate. In a series of 564 CCA patients who were operated on between 1973 and 2004, the five-year overall survival (OS) rate was only 18% (30% in patients in whom an R0 resection was possible; median OS 15 months for all patients, 28 months for R0 patients)^[2]. The survival rate was better in patients with more proximal tumors (i.e., five-year OS rates for R0 patients with iCCA, pCCA, and dCCA of 63%, 30%, and 27%, respectively)^[2]. Data from the Memorial Sloan-Kettering Cancer Center confirm the high risk of disease recurrence after surgical resection of CCAs; in their series, the median disease-specific survival of patients with resected CCA was only 36 months, and almost two-thirds of patients had disease relapse during a median follow-up of 26 months^[11]. In attempts to improve the dismal prognosis of resected

CCA patients, adjuvant treatment strategies have been explored, including chemotherapy, radiotherapy, and combination chemoradiotherapy.

A systematic review and meta-analysis of data of 6,712 patients with CCA of the gallbladder or biliary ducts who received adjuvant therapy with either chemotherapy, radiotherapy, or chemoradiotherapy after surgery revealed a non-significant OS improvement compared to OS with surgery alone ($P = 0.06$)^[16]. Patients who received chemo- or chemoradiotherapy had a significantly greater survival benefit than patients who received adjuvant radiotherapy alone (OR = 0.39, 0.61, and 0.98, respectively, $P = 0.02$)^[16]. A second meta-analysis, reported by Ghidini *et al.*^[22] also found a survival benefit from adjuvant chemo(radio)therapy in patients with resected biliary tract cancers; in that analysis ($n = 22,499$), adjuvant therapy was associated with a significant (4.3 months) prolongation in median OS. Compared with surgery alone, adjuvant chemo (radio)therapy was associated with a 41% reduced risk of death (HR = 0.59, 95%CI: 0.49-0.71, $P < 0.001$)^[22]. More recently, however, two prospective, randomized phase III trials of CCA patients found no clinical benefit from adjuvant chemotherapy. The PRODIGE-12 trial randomized 196 patients with localized biliary tract cancer to observation or adjuvant chemotherapy with the GEMOX regimen (gemcitabine 1000 mg/m² on Day 1 and oxaliplatin 85 mg/m² infused on Day 2 of a two-week cycle for 12 cycles). Patients who received adjuvant GEMOX had a median recurrence-free survival of 30.4 months compared with 18.5 months for patients randomized to the control arm. However, this numerical difference did not meet the threshold for statistical significance (HR = 0.88, 95%CI: 0.62-1.25, $P = 0.48$). In addition, there was no significant difference in OS (median OS: 75.8 months vs. 50.8 months; HR = 1.08, 95%CI: 0.70-1.66, $P = 0.74$)^[12]. Similarly, a randomized phase III trial comparing adjuvant gemcitabine alone to observation in 225 patients with resected bile duct cancer found no difference in OS (median OS: 62.3 months vs. 63.8 months; HR = 1.01, 95%CI: 0.70-1.15, $P = 0.96$) or recurrence-free survival (median: 36.0 months vs. 39.9 months; HR = 0.93, 95%CI: 0.66-1.32, $P = 0.69$)^[13].

In the phase III BILCAP trial, 447 patients with histologically confirmed CCA or muscle-invasive gallbladder cancer who underwent a complete resection were randomized to receive oral capecitabine (1250 mg/m² BID on Days 1-14 of a 21-day cycle, for eight cycles) or observation. After a median follow-up of 60 months, the median OS for patients in the adjuvant chemotherapy arm was 51.1 months, which was almost 15 months longer than the 36.4 median OS in the observation arm. In a protocol-specified sensitivity analysis, this difference in OS was statistically significant, with an HR of 0.71 (95%CI: 0.55-0.92, $P = 0.010$)^[14]. Although the trial failed to meet the primary endpoint of improving OS in the intention-to-treat population, the prespecified sensitivity and per-protocol analyses showed signals of capecitabine efficacy and could be considered for adjuvant care. Based on these results, capecitabine has become the preferred adjuvant chemotherapy regimen in patients with resected CCA. In addition, the American Society of Clinical Oncology endorsed this adjuvant chemotherapy regimen in its 2019 practice guidelines update^[23]. In line with endorsement, the ongoing ACTICCA-1 trial, evaluating adjuvant gemcitabine-cisplatin in patients with resected CCA or muscle-invasive gallbladder cancer, amended its protocol and changed its control arm from observation to capecitabine^[15]. The results of this trial are awaited.

CHEMOTHERAPY FOR UNRESECTABLE OR METASTATIC CCA

First-line therapy

As long ago as 1996, it was established that chemotherapy could improve the survival rate and quality of life of patients with advanced biliary tract cancer^[24]. In the early 2000s, gemcitabine monotherapy was often used as a frontline regimen for patients with advanced CCA. In a phase II trial ($n = 23$), gemcitabine (1000 mg/m² over 60 min once a week in a two-weeks on/one-week off schedule) resulted in a median progression-free survival (PFS) of 8.1 months with a median OS of 13.1 months^[25]. However, in a retrospective case series of 100 patients with advanced CCA, the results obtained with gemcitabine monotherapy did not match the results of this phase II trial, with a median OS of only 7.3 months, and only one, out of five patients, was alive after one year^[26].

Table 1. Chemotherapy in the first-line treatment of patients with advanced CCA: an overview of pivotal clinical trials

Trial	Phase	Regimen	N	Median OS [HR (95%CI)]	Response rate
ABC-02 ^[29]	III	Gem-Cis vs. Gem	410	11.7 months vs. 8.1 months 0.640 (0.52-0.80)	DCR: 81.4% vs. 71.8%
Okusaka <i>et al.</i> ^[30]	II	Gem-Cis vs. Gem	83	11.2 months vs. 7.7 months 0.69 (0.42-1.13)	ORR: 19.5% vs. 11.9% DCR: 68.3% vs. 50.0%
FUGA-BT ^[32]	III	Gem-Cis vs. Gem-S1	354	13.4 months vs. 15.1 months 0.945 (0.78-1.15)*	ORR: 29.8% vs. 32.4%
GERCOR ^[33] #	II	GEMOX	33	15.4 months	ORR: 36%
Kim <i>et al.</i> ^[34]	III	GEMOX vs. XELOX	222	10.4 months vs. 10.6 months NR	ORR: 24.6% vs. 15.7% DCR: 63.2% vs. 58.3%
Shroff <i>et al.</i> ^[36]	II	Gem-Cis + nab-paclitaxel	60	19.2 months	ORR: 45% DCR: 84%
KHBO1401-MITSUBA ^[37]	III	Gem-Cis vs. Gem-Cis-S1	246	13.5 months vs. 12.6 months 0.79 (0.60-1.04)	ORR: 41.5% vs. 15%

*90% confidence interval. Study met threshold for non-inferiority; #only data for Group A (good performance status) are listed here. CCA: cholangiocarcinoma; DCR: disease control rate; ORR: overall response rate; OS: overall survival

Table 1 lists the pivotal clinical trials which study various chemotherapy options in the first-line treatment of patients with advanced CCA.

Gemcitabine-based doublet chemotherapy

To improve on these outcomes, numerous gemcitabine-based combination regimens have been tested. The most prominent consists of the gemcitabine-cisplatin (Gem-Cis) doublet. Two phase-II trials of the combination produced efficacy signals in patients with advanced CCA and had a favorable toxicity profile^[27,28]. These encouraging findings formed the rationale for comparing the Gem-Cis doublet to gemcitabine alone in a randomized phase III trial: In the ABC-02 study, 410 patients with locally advanced or metastatic CCA, gallbladder cancer, or ampullary cancer were randomly assigned to receive cisplatin (25 mg/m²) followed by gemcitabine (1000 mg/m² on Days 1 and 8, every three weeks for eight cycles) or gemcitabine alone (1000 mg/m² on Days 1, 8, and 15, every four weeks for six cycles) for a total of 24 weeks. Study patients who received the combination treatment had a median OS of 11.7 months, which was significantly longer than 8.1 months in the gemcitabine-treated cohort (HR = 0.64, 95%CI: 0.52-0.80, $P < 0.001$); the PFS also was significantly longer (median PFS: 8 months vs. 5 months; $P < 0.001$) in patients treated with the chemotherapy doublet, and the tumor was controlled in significantly more patients (81.4% vs. 71.8%; $P = 0.049$)^[29]. Toxicity was similar with the two treatments, but the addition of cisplatin to the regimen resulted in more Grade 3/4 neutropenia (25% vs. 17%) and a higher incidence of Grade 3/4 liver abnormalities (27% vs. 17%)^[29]. A similar efficacy benefit of Gem-Cis over gemcitabine alone was reported by Okusaka *et al.*^[30], with a median OS of 11.2 months with the combination compared with 7.7 months with gemcitabine alone (HR = 0.69, 95%CI: 0.42-1.13); at the one-year mark, this difference translated into an absolute survival difference of 8%^[30]. A subsequent meta-analysis of these two studies indicated that, compared to gemcitabine alone, Gem-Cis was associated with a 35% reduced death risk (HR = 0.65, 95%CI: 0.54-0.78, $P < 0.001$) and a 36% reduced risk for disease progression (HR = 0.64, 95%CI: 0.53-0.76, $P < 0.001$). The benefit of Gem-Cis over gemcitabine monotherapy was present irrespective of the location of the primary tumor (i.e., gallbladder or CCA). These findings established Gem-Cis as the reference first-line treatment for patients with advanced CCA. However, a subgroup analysis of the performance status found that the superiority of Gem-Cis over gemcitabine alone was mainly in patients with good performance status, whereas patients with a European Cooperative Oncology Group (ECOG) performance status of 2 or more benefited less^[31].

A second gemcitabine-based doublet regimen that has gained momentum in recent years is the combination of gemcitabine and S-1, an oral fluoropyrimidine that includes three agents (tegafur, gimeracil,

and oteracil) (Gem-S). In the Japanese FUGA-BT trial, 354 chemotherapy-naïve patients with recurrent or unresectable biliary tract cancer and an ECOG performance status of 0-1 were randomized to treatment with gemcitabine (1000 mg/m² on Days 1 and 8) in combination with either S-1 (60, 80, or 100 mg per day on Days 1-14 of a 21-day cycle) or cisplatin (25 mg/m² IV on Days 1 and 8). In this study, Gem-S was non-inferior to Gem-Cis, with a median OS of 15.1 and 13.4 months, respectively (HR = 0.945, 90%CI: 0.78-1.15, non-inferiority $P = 0.046$)^[32]. In addition, Gem-Cis and Gem-S yielded similar results in PFS (median PFS: 5.8 months vs. 6.8 months; HR = 0.86, 95%CI: 0.70-1.07) and overall response rate (ORR): 32.4% vs. 29.8%. Clinically significant adverse events (AEs) were reported by 35.1% of patients enrolled in the Gem-Cis compared with 29.9% in the Gem-S arm. Based on these findings, Gem-S, when available, is a feasible first-line alternative to Gem-Cis in patients with advanced CCA.

Other gemcitabine-based doublets have also been evaluated in patients with advanced CCA. In the phase II GERCOR trial, 33 patients with newly diagnosed advanced biliary tract cancer and good performance status were treated with a combination of gemcitabine (1000 mg/m² as a 10 mg/m²/min infusion on Day 1) and oxaliplatin (100 mg/m² as a 2-h infusion on Day 2), every two weeks (GEMOX). The GEMOX regimen induced an ORR of 36% and had a median PFS and OS of 5.7 months and 15.4 months, respectively^[33]. Recently, the GEMOX regimen was evaluated in a phase III setting, where it was compared to a combination of capecitabine (1000 mg/m² BID on Days 1-14) and oxaliplatin (130 mg/m² on Day 1) (XELOX). In that non-inferiority trial, with 222 patients with advanced biliary cancer, GEMOX and XELOX were given every three weeks for eight cycles^[34]. The median PFS for GEMOX and XELOX was 5.3 months and 5.8 months, respectively, translating to a five-month PFS rate of 44.5% with GEMOX and 46.7% with XELOX. OS was not significantly different in the two arms, with a median OS of 10.4 and 10.6 months for GEMOX and XELOX, respectively^[34]. These two studies established the clinical efficacy of GEMOX in the frontline treatment of patients with advanced biliary tract cancer, and the drug has become widely used in the treatment of patients with advanced CCA. This practice was fueled by oxaliplatin having a more favorable toxicity profile than that of cisplatin. However, whether GEMOX is non-inferior or superior to Gem-Cis has not been established (no head-to-head comparisons).

A final gemcitabine-based doublet that was explored in patients with advanced CCA is gemcitabine plus nab-paclitaxel. In 2018, Sahai *et al.*^[35] reported the results of a multicenter phase II trial in which 74 patients with advanced CCA were treated with nab-paclitaxel (125 mg/m² IV) followed by gemcitabine (1000 mg/m² on Days 1, 8, and 15) in 28-day treatment cycles^[35]. The regimen was effective, with an ORR of 30% and a median OS of 12.4 months. The most common high-grade AEs with the gemcitabine nab-paclitaxel combination were neutropenia (43% Grade ≥ 3) and fatigue (14% Grade ≥ 3). These findings are promising, but validation in a randomized comparison with the current standard of care (Gem-Cis or Gem-S) is required before this regimen can be used routinely.

Treatment intensification: the more, the merrier?

Several clinical trials have evaluated whether a more intensive treatment strategy would result in better treatment outcomes than those of the current two-drug standard in the frontline treatment of advanced biliary tract cancer. A phase II trial, conducted at the MD Anderson Cancer Center and the Mayo Clinic, tested a triple regimen of gemcitabine, cisplatin, and nab-paclitaxel (initially 1000, 25, and 125 mg/m², respectively, on Days 1 and 8 of 21-day cycles, with a later reduction to 800, 25, and 100 mg/m², respectively, to mitigate the hematological toxicity) in 60 patients with advanced biliary tract cancer (78% CCA)^[36]. The results with the triplet regimen were promising, with a median PFS of 11.8 months and a median OS of 19.2 months. Forty-five percent of patients obtained a partial response, and 39% had disease stabilization. As could be expected, this benefit came with the cost of substantial toxicity, with 58% of patients experiencing Grade ≥ 3 AEs (Grade ≥ 3 AE neutropenia was most common, present in 33% of patients). Sixteen percent of patients withdrew from the treatment because of toxicity^[36]. This regimen will be evaluated further in a phase III randomized trial (NCT03768414).

A second interesting treatment-intensification study in patients with biliary tract cancer is the phase III KHBO1401-MITSUBA trial. In that, 246 chemotherapy-naïve patients with advanced biliary tract adenocarcinoma were randomly assigned to treatment with Gem-Cis or a triple combination of Gem-Cis and S-1 (GCS). The addition of S-1 to Gem-Cis resulted in a lengthening of the median OS from 12.6 months to 13.5 months (HR = 0.79, 95%CI: 0.60-1.04, $P = 0.046$). At the one-year mark, this translated into an absolute survival difference of 5.7% in favor of GCS (59.4% vs. 53.7%). In addition, the median PFS was significantly longer with GCS than with Gem-Cis (7.4 months vs. 5.5 months; HR = 0.75, 95%CI: 0.58-0.97, $P = 0.0015$), and the rate of patients who had a treatment response was almost tripled (41.5% vs. 15.0%)^[37]. Based on these results, the authors concluded that GCS could become a new standard treatment for patients with advanced biliary tract cancer.

Patients with poor performance status: can we do good enough with a little bit less?

As indicated above, poor performance status seemed to be associated with a lower likelihood of treatment benefit from a Gem-Cis doublet. For these patients, gemcitabine monotherapy can be considered. 5-fluorouracil (5-FU) monotherapy is not recommended in patients with biliary tract cancer because of the low response rate (ORR: 20%)^[38], but response rates were slightly higher when leucovorin was used in combination with 5-FU: in 28 patients with biliary tract cancer, leucovorin-modulated 5-FU resulted in an ORR of 32.1%, with a median OS of six months^[39]. Similar results were reported by Chen *et al.*^[40] in a series of 19 biliary tract cancer patients (ORR: 33%, median OS 7.0 months).

A second alternative for gemcitabine monotherapy in CCA patients with a poor performance status could be capecitabine monotherapy. In a study from the MD Anderson Cancer Center^[41], 63 patients with advanced hepatocellular carcinoma ($n = 37$), gallbladder cancer ($n = 8$), or CCA ($n = 18$) were treated with capecitabine monotherapy (1000 mg/m² BID for 14 days, in 21-day cycles). Among the CCA patients in this trial, a median OS of 8.1 months was reported. Although the response rate in this trial was modest, CCA patients have been reported to survive long term with capecitabine monotherapy^[41].

Second-line therapy

Few studies have been conducted in patients with advanced CCA and progression after first-line therapy, so there is no established standard of care for these persons. There are also few data on selecting patients who might benefit from second-line therapy; the available studies consistently required good performance status to initiate second-line therapy^[42-45]. Other prognostic factors are the treatment effect in first-line therapy (disease control or not), a low CA19-9 level, and the absence of peritoneal carcinomatosis^[44,45].

In 2014, Lamarca *et al.*^[46] published a systematic review of clinical studies that evaluated second-line chemotherapy (fluoropyrimidine, irinotecan, docetaxel, and gemcitabine) in patients with advanced biliary tract cancer. A platinum compound was often used in second-line therapy in patients who received a fluoropyrimidine in first-line treatment; the median OS in this analysis was 7.2 months, with a median PFS of 3.2 months. The response rate to second-line chemotherapy was only 7.7%^[46]. Currently, the most frequently used second-line treatment for patients with advanced CCA, who have failed first-line Gem-Cis, is the FOLFOX regimen (oxaliplatin plus 5-FU). This practice is based on the results of the randomized, phase III ABC-06 trial. One hundred and sixty-two patients with locally advanced or metastatic biliary tract cancer, who were previously treated with Gem-Cis, were randomly assigned to active symptom control with or without modified FOLFOX (mFOLFOX) regimen, containing L-folinic acid (175 mg) (or folinic acid 350 mg), 5-FU (400 mg/m² bolus and 2400 mg/m² infusion), and oxaliplatin (85 mg/m²) (all every 14 days, up to 12 cycles)^[47]. Only patients with an ECOG performance status of 0-1 were eligible for the study, and 72% of patients in the study cohort had advanced CCA. The use of mFOLFOX led to only a modest prolongation in the median OS, from 5.3 to 6.2 months (HR: 0.69). However, the absolute OS rates at 6 and 12 months were more impressive: at 6 and 12 months, the OS rate for patients in the mFOLFOX

arm was 50.6% and 25.9%, respectively, compared with 5.5% and 11.4% for active symptom control alone. mFOLFOX was well tolerated, with only a manageable increase in the rate of Grade ≥ 3 neutropenia and fatigue^[47].

The most prominent alternative for mFOLFOX in second-line treatment is a combination of irinotecan and capecitabine (XELIRI). The XELIRI regimen (irinotecan 180 mg/m² on Day 1 and capecitabine 1000 mg/m² BID on Days 1-10 of 14-day cycles) was compared to irinotecan alone in a phase II trial that had 60 patients with Gem-Cis pretreated biliary tract cancer^[48]. In that trial, XELIRI doubled the nine-month OS rate from 32% to 60.9% (no significant difference in median OS: 10.1 months vs. 7.3 months; $P = 0.107$) and increased the disease control rate from 50% to 63.3%. This benefit came at the cost of only a modest increase in toxicity, but there was a higher rate of palmar-plantar erythrodysesthesia with XELIRI (6.7% vs. 0%)^[48].

A third option for advanced CCA patients who failed first-line Gem-Cis is 5-FU-based therapy. Unfortunately, there are no randomized data for comparison of 5-FU to FOLFOX or XELIRI for this indication. The best data come from a large retrospective series of 321 advanced CCA patients^[49]. In that series, 5-FU-based chemotherapy was modestly effective as second-line chemotherapy for patients with advanced biliary tract cancer who failed on first-line Gem-Cis (ORR 8% for 5-FU platinum combinations and 1% for 5-FU alone). A 5-FU-platinum combination was not associated with a better OS or PFS than those outcomes with 5-FU monotherapy^[49].

Molecularly targeted therapy for CCA

CCA has a high level of intra- and intertumoral heterogeneity^[50]. As a result, clinical trials testing molecularly targeted agents in unselected patients with CCA have consistently yielded negative results. In recent years, however, advances in whole-exome and transcriptome sequencing have shed light on the genetic landscape of the CCA subtypes, opening the door to tailored treatment approaches^[51]. In fact, recent biomarker-driven clinical trials in CCA patients have reported positive outcomes. These results have prompted FDA approval of pemigatinib as the first targeted treatment for patients with previously treated, advanced CCA who harbor an *FGFR2*-fusion or rearrangement. We now present an overview of the molecularly targeted agents that are under clinical evaluation in CCA patients.

FGFR-directed therapy

Gene fusions involving *FGFR2* have been reported in 10-20% of iCCA patients^[52,53]. The fusions result in constitutive activation of *FGFR2*, ultimately leading to activation of oncogenesis-promoting signaling pathways, such as RAS-RAF-MEK^[54]. Several *FGFR*-targeting agents have been evaluated for the treatment of advanced CCA. As indicated above, pemigatinib recently became the first FDA-approved molecularly targeted agent for treating patients with CCA, specifically those with previously treated tumors and an *FGFR2* rearrangement. This approval was based on the results of phase II, multicenter FIGHT-202 study. In that trial, 146 patients with locally advanced or metastatic CCA were treated with pemigatinib at a dose of 13.5 mg/day in a two-weeks on/one-week off schedule^[55]. Most of the patients ($n = 107$) had an *FGFR2* fusion or rearrangement; after a median follow-up of 17.8 months, 36% of these patients had an objective response to the therapy, and the responses were durable, with a median duration of response of 9.1 months, median PFS of 6.9 months, and median OS an impressive 21.1 months. Grade ≥ 3 AEs occurred in 64% of patients in the trial, with 45% having a serious AE (most frequently abdominal pain and pyrexia)^[55]. The clinical benefit of pemigatinib in this setting will be further evaluated in a randomized trial with an active comparator arm.

The pan-FGFR inhibitor infigratinib (BGJ398) was evaluated in a phase II study with CCA patients^[56]. In that trial, including 61 previously treated patients with advanced CCA and an *FGFR* alteration, infigratinib induced an ORR of 14.8% (18.8% in the cohort of patients with an *FGFR2*-fusion/alteration). An additional

60.6% of patients experienced disease stabilization under therapy, for an overall disease control rate of 75.4% (83.3% in *FGFR2*-fusion/rearrangement patients). The estimated median PFS in this study was 5.8 months. Grade ≥ 3 AEs occurred in 41% of patients, with hyperphosphatemia (16.4%), stomatitis (6.6%), and palmar-plantar erythrodysesthesia (4.9%) being the most common high-grade toxicities^[56]. A phase III clinical trial comparing infigratinib to Gem-Cis in the first-line treatment of patients with locally advanced/metastatic CCA and an *FGFR2*-fusion/rearrangement is ongoing (NCT03773302).

During the 2020 annual meeting of the American Society of Clinical Oncology (ASCO), phase II data were presented for the irreversible *FGFR1-4* inhibitor futibatinib^[57]. In total, 103 patients with unresectable or metastatic iCCA and an *FGFR2* fusion (or another rearrangement involving this gene) with disease progression after at least one prior systemic therapy (including Gem-Cis) were treated with futibatinib at a dose of 20 mg/day. The data presented at ASCO included the first 67 patients with at least six months of follow-up. An objective response was obtained in 37.3% of patients, with an additional 44.8% of patients experiencing disease stabilization (disease control rate 82.1%). Responses also proved to be durable, with a median duration of response of 8.3 months. The median PFS was reported at 7.2 months, with a 6- and 12-month PFS rates of 61.0% and 39.4%, respectively. Grade ≥ 3 AEs were reported in 56.7% of patients, with 10.4% of treatment-related severe AEs. Only one patient had to discontinue the therapy for reasons of toxicity. The most common Grade 3 AE with futibatinib consisted of hyperphosphatemia (26.9%)^[57]. Based on these excellent results, futibatinib is also being compared to Gem-Cis in a randomized phase III trial, including previously untreated CCA patients (NCT04093362).

In other work, the pan-*FGFR* inhibitor erdafitinib demonstrated clinical activity in patients with *FGFR*-mutated solid tumors. In a phase I basket trial, erdafitinib induced a partial response in 3 of 11 CCA patients with an *FGFR2*-fusion or rearrangement^[58].

A fifth pan-*FGFR* inhibitor that demonstrated potential in patients with CCA is derazantinib. In a multicenter phase I/II trial of 29 patients with unresectable iCCA and an *FGFR2*-fusion, it was associated with an ORR of 20.7% and a disease control rate of 82.8%. The estimated PFS was 5.7 months, and 27.6% of patients had Grade ≥ 3 AEs^[59]. A pivotal trial of derazantinib in patients with iCCA is ongoing (NCT03230318).

IDH-directed therapy

Mutations in *IDH-1* and *-2* are present in 15-20% of patients with iCCA^[51,60]. These mutations profoundly affect cell differentiation and cell growth, and they are involved in tumorigenesis^[61]. Several inhibitors of mutant IDH proteins have been developed in recent years. Ivosidenib (AG-120) is a first-in-class, oral, small-molecule inhibitor of the mutant IDH1 protein. In the randomized, phase III ClarIDHy trial, 185 previously treated patients with advanced CCA and an *IDH1* mutation were randomly assigned (2:1) to receive either ivosidenib (500 mg per day) or matching placebo^[62]. The study met its primary endpoint, with a significantly longer median PFS for patients in the ivosidenib arm than in the placebo arm (2.7 months *vs.* 1.4 months; HR = 0.37, 95%CI: 0.25-0.54, $P < 0.001$). At the 6- and 12-month marks, 32% and 21.9% of patients, respectively, treated with ivosidenib were free of progression, whereas none of the patients in the placebo arm was progression-free at six months. The ORR with ivosidenib was low (2.4%), with 50.8% of patients having disease stabilization. The median OS among patients treated with ivosidenib was 10.8 months. Patients in the placebo arm had a median OS of 9.7 months, but this OS was significantly influenced by 57% of placebo patients crossing over to ivosidenib. Overall, 46% of patients experienced a Grade 3/4 AE on ivosidenib as compared with 36% with placebo; the most common AEs seen with ivosidenib were nausea (32.1%), diarrhea (28.8%), and fatigue (23.7%)^[62]. The results of phase III ClarIDHy trial are especially important in CCA treatment as they provide level A evidence for the efficacy of targeted therapy in this setting and establish a role for molecular profiling in this cancer type. Several other

inhibitors of mutant IDH proteins are under clinical evaluation in patients with *IDH*-mutant solid tumors (including CCA), e.g., enasidenib (NCT02273739), IDH305 (NCT02381886), and AG-881 (NCT02481154).

Targeting *ROS1* and *NTRK* fusions

Gene fusions involving *ROS1* have been reported in about 8% of CCA patients^[63], and an oncogenic role for ROS kinase fusions was established in a CCA mouse model^[64]. These findings make *ROS1* an interesting therapeutic target. The *ALK* and *ROS1* inhibitors ceritinib and crizotinib are already being used in patients with metastatic non-small cell lung cancers that harbor *ALK* and *ROS1* fusions. Phase II studies are ongoing to investigate their potential in patients with *ROS1*- and/or *ALK*-mutated CCA (NCT02374489 and NCT02034981). Recently, the FDA gave an agnostic approval to the *NTRK* inhibitor larotrectinib to treat patients with solid tumors harboring an *NTRK* gene fusion. This approval was based on the results of three multicenter, single-arm trials (LOXO-TRK-1400, SCOUT, and NAVIGATE) of patients with solid tumors and an *NTRK* fusion. Larotrectinib induced an ORR of 75%, with 71% of responses ongoing at one year^[65]. These studies also included two patients with CCA, one of whom experienced disease stabilization under larotrectinib^[65]. In August 2019, the FDA approved the *NTRK* inhibitor entrectinib for treating patients with solid tumors and *NTRK* gene fusions. This approval followed an integrated analysis of the pivotal Phase II STARTRK-2, Phase I STARTRK-1, and Phase I ALKA-372-001 trials^[66]. In these studies, in which several *NTRK*-positive advanced CCA were included, entrectinib induced an ORR of 57%, with a median duration of response of 10 months. Notwithstanding the rarity of *NTRK* fusions in CCA patients, the fact that this alteration is now actionable with effective targeted therapies justifies screening for it in patients with advanced CCA.

EGFR-directed therapy

Patients with CCA often harbor mutations in *EGFR*^[52]. The mutations are more common in patients with pCCA and dCCA (about 15%) than in iCCA patients^[67]. *EGFR* inhibitors in patients with biliary tract cancer, either as monotherapy or in combination with chemotherapy, have been studied. Unfortunately, the trials consistently yielded disappointing results^[68,69]. The only phase III trial of *EGFR* inhibitors in this condition studied the addition of erlotinib to gemcitabine and oxaliplatin as first-line treatment for patients with metastatic biliary cancer; although the response rate was significantly increased, this did not translate into a longer PFS or OS^[70]. Similarly, the addition of panitumumab to Cis-Gem did not improve the ORR, PFS, or OS in a phase II trial of 62 patients with *KRAS*-wildtype biliary tract cancer^[71], and no improvement in ORR or PFS was found in a phase II/III TreeTopp trial that evaluated the addition of the pan-HER inhibitor varlitinib to capecitabine as a second-line treatment for patients with biliary tract cancer.

Angiogenesis-directed therapy

The results of preclinical studies suggest that several angiogenic factors are important in the tumorigenesis of biliary tract cancers^[72,73]. Thus, angiogenesis-directed therapy has been explored as a therapeutic strategy in patients with these tumor types. In a phase II trial of 35 patients with advanced biliary tract cancer, the combination of GEMOX with bevacizumab had promising antitumor activity (median PFS: seven months; six-month PFS rate: 63%) with a tolerable safety profile^[74]. In a randomized phase II trial ($n = 57$), the addition of bevacizumab to GEMOX significantly prolonged the median PFS, from 3.72 to 6.48 months ($P = 0.049$), with only a small increase in toxicity^[75]. In contrast with those results, a randomized phase II trial, presented during the 2020 annual Gastrointestinal Cancers Symposium, found that the addition of the VEGF inhibitor ramucirumab did not improve the ORR, PFS, or OS in patients with biliary tract cancer^[76]. Similarly, a phase I study evaluating a combination of ramucirumab and the immune checkpoint inhibitor pembrolizumab found a limited clinical effect in patients with previously treated biliary tract cancer^[77].

Emerging targets

Several other promising new drugs are in early clinical development for the treatment of patients with CCA. Constituted JAK/STAT activation is a recurrent finding in CCA, making it a potential therapeutic

target^[78,79]. In a phase I trial, the STAT3 inhibitor ABC294640 showed activity in CCA, and the inhibitor is under further evaluation in a phase II trial (NCT03377179).

Amplification and overexpression of *MET* have been described in CCA, with associated poor prognosis^[80]. Clinical studies evaluating *MET* inhibitors in monotherapy revealed limited clinical activity. In contrast, phase I data of a study evaluating the combination of the *MET* inhibitor tivantinib with gemcitabine in patients with metastatic solid cancers suggest the presence of antitumor activity^[81]. However, phase II data failed to show an ORR, PFS, or OS benefit from the addition of the *MET* inhibitor merestinib to first-line Gem-Cis in patients with advanced biliary tract cancer^[76].

IMMUNE CHECKPOINT INHIBITION FOR CCA

Over the last decade, immune checkpoint inhibitors have revolutionized the treatment landscape of many different cancer types, and this strategy is being explored in CCA.

Pembrolizumab is a monoclonal antibody directed against the programmed death-1 receptor (PD-1). In 2017, the FDA had approved pembrolizumab for the treatment of microsatellite instability-high solid tumors. In the phase II Keynote-158 trial, the antibody had robust clinical activity in patients with non-colorectal microsatellite instability-high/mismatch repair-deficient solid tumors. Two hundred and thirty-three patients, including 22 with advanced CCA, were enrolled in the study. Pembrolizumab was administered at a dose of 200 mg once every three weeks for a maximum of two years^[82]. An overall response rate of 34.3% was reported, with a median duration of response that was not yet reached after a median follow-up of 13.4 months. Among the cohort of CCA patients, the ORR was 49%, and two patients had a complete response; the median OS in CCA patients was 24.3 months. Treatment-related AEs - most commonly fatigue (14.6%), pruritus (12.9%), and diarrhea (12.0%) - occurred in 64.8% of patients; Grade ≥ 3 treatment-related AEs occurred in 14.6% of patients, and 23.2% experienced an immune-related AE^[82]. Thus, these data establish pembrolizumab as an effective and safe treatment option for patients with CCA and mismatch repair deficiency. According to Silva *et al.*^[83], 5%-10% of patients with CCA meet this criterion.

Pembrolizumab was also evaluated in non-mismatch repair-deficient CCA patients. In the large multi-cohort, phase Ib Keynote-028 trial, 24 patients with PD-L1-positive CCA were treated with pembrolizumab at a dose of 10 mg/kg every two weeks for up to two years^[84]. In this cohort, four patients (17%), three with CCA and one with gallbladder cancer, had a partial response, and four patients (17%) had disease stabilization; at 12 months, 27.6% of patients were still alive. The rate of Grade 3 toxicities was 16.7%, with no reported Grade ≥ 4 toxicities^[84].

The PD-1 inhibitor nivolumab has also been evaluated in patients with biliary tract cancer. In a study of 30 patients with metastatic disease, nivolumab was associated with an ORR of 20% and a disease control rate of 60%; the median PFS was 3.1 months^[85]. Preliminary results of an ongoing phase II trial in patients with advanced refractory biliary tract cancer indicate an ORR of 22%, with a disease control rate of 60%. The median OS in this study was 14.2 months, with 6- and 12-month OS rates of 71.4% and 52.3%, respectively. At six months, 35.2% of patients were free of progression; the rate was 24.1% at 12 months. The safety profile was in line with that of previous reports on nivolumab. Importantly, this trial did not select for PD-L1 expression at study entry^[86]. In a Japanese trial, nivolumab was evaluated as monotherapy or combined with chemotherapy in 60 patients with biliary tract cancer; the monotherapy was associated with a median OS of 5.2 months, a median PFS of 1.4 months, and a low ORR, with only one patient obtaining a response. In the combined therapy cohort, median OS (5.4 months) and median PFS (4.2 months) were longer, and 11 of 30 patients had an objective response^[87].

A third immune checkpoint inhibitor under evaluation in CCA is the PD-L1 inhibitor durvalumab. In a phase I trial, durvalumab was evaluated as monotherapy ($n = 42$) or in combination with the CTLA-4 inhibitor tremelimumab ($n = 65$) to treat patients with previously advanced biliary tract cancer^[88]. At 12 weeks, durvalumab monotherapy was associated with a disease control rate of 16.7%; with the durvalumab-tremelimumab combination, this metric increased to 32.2%. The median duration of response with durvalumab alone was 9.7 months; with the combination, it was 8.5 months. The median OS of patients in the monotherapy cohort was 8.1 months; with durvalumab plus tremelimumab, it was 10.1 months. The treatments were generally well-tolerated, with Grade ≥ 3 treatment-related AEs in 19% and 23% of patients treated with monotherapy and combination, respectively^[88]. Thus, these findings reveal promising clinical activity of durvalumab, both as monotherapy and in combination with tremelimumab in patients with advanced biliary tract cancer. Durvalumab is also being studied in combination with chemotherapy: in the randomized phase III TOPAZ trial, the combination of durvalumab with Gem-Cis is under evaluation as a first-line treatment for patients with advanced biliary cancer (NCT03875235).

CONCLUSION

Patients with advanced CCA face a poor prognosis. The standard of care for these patients is gemcitabine-based doublet chemotherapy (Gen-Cis or GemS), which has a median OS of about one year. For patients with disease progression after first-line therapy, there is no universal standard of care. Small steps have been made towards a personalized treatment approach for patients with CCA. The most promising approach is the recently FDA-approved *FGFR* inhibitor pemigatinib in the second-line treatment of patients with previously treated advanced CCA harboring an *FGFR2* fusion or rearrangement. For patients with an *IDH1* mutation, ivosidenib treatment has been found to show progression-free efficacy. Several other targeted therapies are being explored in molecularly oriented clinical trials of CCA: promising data have been generated with the immune checkpoint inhibitors pembrolizumab, nivolumab, and durvalumab in patients with advanced CCA, and it appears that immunotherapy will become an important strategy in the treatment of these patients. The response of mismatch repair-deficient CCA patients to pembrolizumab treatment is especially promising.

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Authors' contributions

Made equal and substantial contribution to the conception of idea, literature review, and drafting and finalization of manuscript: Alqahtani SA, Colombo M

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REFERENCES

1. Razumilava N, Gores GJ. Cholangiocarcinoma. *Lancet* 2014;383:2168-79.
2. DeOliveira ML, Cunningham SC, Cameron JL, Kamangar F, Winter JM, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007;245:755-62.
3. Valle JW, Borbath I, Khan SA, Huguet F, Gruenberger T, et al. Biliary cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2016;27:v28-37.
4. Nakanuma Y, Sato Y, Harada K, Sasaki M, Xu J, et al. Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. *World J Hepatol* 2010;2:419-27.
5. Petrick JL, Yang B, Altekruse SF, Van Dyke AL, Koshiol J, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based study in SEER-Medicare. *PLoS One* 2017;12:e0186643.
6. Massarweh NN, El-Serag HB. Epidemiology of hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *Cancer Control* 2017;24:1073274817729245.
7. Palmer WC, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. *J Hepatol* 2012;57:69-76.
8. Lee BS, Cha BH, Park EC, Roh J. Risk factors for perihilar cholangiocarcinoma: a hospital-based case-control study. *Liver Int* 2015;35:1048-53.
9. Massironi S, Pilla L, Elvevi A, Longarini R, Rossi RE, et al. New and emerging systemic therapeutic options for advanced cholangiocarcinoma. *Cells* 2020;9:688.
10. Tyson GL, El-Serag HB. Risk factors for cholangiocarcinoma. *Hepatology* 2011;54:173-84.
11. Endo I, Gonen M, Yopp AC, Dalal KM, Zhou Q, et al. Intrahepatic cholangiocarcinoma: rising frequency, improved survival, and determinants of outcome after resection. *Ann Surg* 2008;248:84-96.
12. Edeline J, Benabdelghani M, Bertaut A, Watelet J, Hammel P, et al. Gemcitabine and oxaliplatin chemotherapy or surveillance in resected biliary tract cancer (PRODIGE 12-ACCORD 18-UNICANCER GI): a randomized phase III study. *J Clin Oncol* 2019;37:658-67.
13. Ebata T, Hirano S, Konishi M, Uesaka K, Tsuchiya Y, et al. Randomized clinical trial of adjuvant gemcitabine chemotherapy versus observation in resected bile duct cancer. *Br J Surg* 2018;105:192-202.
14. Primrose JN, Fox RP, Palmer DH, Malik HZ, Prasad R, et al. Capecitabine compared with observation in resected biliary tract cancer (BILCAP): a randomised, controlled, multicentre, phase 3 study. *Lancet Oncol* 2019;20:663-73.
15. Stein A, Arnold D, Bridgewater J, Goldstein D, Jensen LH, et al. Adjuvant chemotherapy with gemcitabine and cisplatin compared to observation after curative intent resection of cholangiocarcinoma and muscle invasive gallbladder carcinoma (ACTICCA-1 trial) - a randomized, multidisciplinary, multinational phase III trial. *BMC Cancer* 2015;15:564.
16. Horgan AM, Amir E, Walter T, Knox JJ. Adjuvant therapy in the treatment of biliary tract cancer: a systematic review and meta-analysis. *J Clin Oncol* 2012;30:1934-40.
17. Kiefer MV, Albert M, McNally M, Robertson M, Sun W, et al. Chemoembolization of intrahepatic cholangiocarcinoma with cisplatin, doxorubicin, mitomycin C, etidolol, and polyvinyl alcohol: a 2-center study. *Cancer* 2011;117:1498-505.
18. Kuhlmann JB, Euringer W, Spangenberg HC, Breidert M, Blum HE, et al. Treatment of unresectable cholangiocarcinoma: conventional transarterial chemoembolization compared with drug eluting bead-transarterial chemoembolization and systemic chemotherapy. *Eur J Gastroenterol Hepatol* 2012;24:437-43.
19. Park SY, Kim JH, Yoon HJ, Lee IS, Yoon HK, et al. Transarterial chemoembolization versus supportive therapy in the palliative treatment of unresectable intrahepatic cholangiocarcinoma. *Clin Radiol* 2011;66:322-8.
20. Hoffmann RT, Paprottka PM, Schön A, Bamberg F, Haug A, et al. Transarterial hepatic yttrium-90 radioembolization in patients with unresectable intrahepatic cholangiocarcinoma: factors associated with prolonged survival. *Cardiovasc Intervent Radiol* 2012;35:105-16.
21. Rafi S, Piduru SM, El-Rayes B, Kauh JS, Kooby DA, et al. Yttrium-90 radioembolization for unresectable standard-chemorefractory intrahepatic cholangiocarcinoma: survival, efficacy, and safety study. *Cardiovasc Intervent Radiol* 2013;36:440-8.
22. Ghidini M, Tomasello G, Botticelli A, Barni S, Zabbialini G, et al. Adjuvant chemotherapy for resected biliary tract cancers: a systematic review and meta-analysis. *HPB (Oxford)* 2017;19:741-8.
23. Shroff RT, Kennedy EB, Bachini M, Bekaii-Saab T, Crane C, et al. Adjuvant therapy for resected biliary tract cancer: ASCO clinical practice guideline. *J Clin Oncol* 2019;37:1015-27.
24. Glimelius B, Hoffman K, Sjöden PO, Jacobsson G, Sellström H, et al. Chemotherapy improves survival and quality of life in advanced pancreatic and biliary cancer. *Ann Oncol* 1996;7:593-600.
25. Park JS, Oh SY, Kim SH, Kwon HC, Kim JS, et al. Single-agent gemcitabine in the treatment of advanced biliary tract cancers: a phase II study. *Jpn J Clin Oncol* 2005;35:68-73.
26. Suzuki E, Furuse J, Ikeda M, Okusaka T, Nakachi K, et al. Treatment efficacy/safety and prognostic factors in patients with advanced biliary tract cancer receiving gemcitabine monotherapy: an analysis of 100 cases. *Oncology* 2010;79:39-45.
27. Thongprasert S, Napapan S, Charoentum C, Moonprakan S. Phase II study of gemcitabine and cisplatin as first-line chemotherapy in inoperable biliary tract carcinoma. *Ann Oncol* 2005;16:279-81.
28. Giuliani F, Gebbia V, Maiello E, Borsellino N, Bajardi E, et al. Gemcitabine and cisplatin for inoperable and/or metastatic biliary tree

- carcinomas: a multicenter phase II study of the Gruppo Oncologico dell'Italia Meridionale (GOIM). *Ann Oncol* 2006;17:vii73-7.
29. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010;362:1273-81.
 30. Okusaka T, Nakachi K, Fukutomi A, Mizuno N, Ohkawa S, et al. Gemcitabine alone or in combination with cisplatin in patients with biliary tract cancer: a comparative multicentre study in Japan. *Br J Cancer* 2010;103:469-74.
 31. Valle JW, Furuse J, Jitlal M, Beare S, Mizuno N, et al. Cisplatin and gemcitabine for advanced biliary tract cancer: a meta-analysis of two randomised trials. *Ann Oncol* 2014;25:391-8.
 32. Morizane C, Okusaka T, Mizusawa J, Katayama H, Ueno M, et al. Combination gemcitabine plus S-1 versus gemcitabine plus cisplatin for advanced/recurrent biliary tract cancer: the FUGA-BT (JCOG1113) randomized phase III clinical trial. *Ann Oncol* 2019;30:1950-8.
 33. André T, Tournigand C, Rosmorduc O, Provent S, Maindrault-Goebel F, et al. Gemcitabine combined with oxaliplatin (GEMOX) in advanced biliary tract adenocarcinoma: a GERCOR study. *Ann Oncol* 2004;15:1339-43.
 34. Kim ST, Kang JH, Lee J, Lee HW, Oh SY, et al. Capecitabine plus oxaliplatin versus gemcitabine plus oxaliplatin as first-line therapy for advanced biliary tract cancers: a multicenter, open-label, randomized, phase III, noninferiority trial. *Ann Oncol* 2019;30:788-95.
 35. Sahai V, Catalano PJ, Zalupski MM, Lubner SJ, Menge MR, et al. Nab-paclitaxel and gemcitabine as first-line treatment of advanced or metastatic cholangiocarcinoma: a phase 2 clinical trial. *JAMA Oncol* 2018;4:1707-12.
 36. Shroff RT, Javle MM, Xiao L, Kaseb AO, Varadhachary GR, et al. Gemcitabine, cisplatin, and nab-paclitaxel for the treatment of advanced biliary tract cancers: a phase 2 clinical trial. *JAMA Oncol* 2019;5:824-30.
 37. Sakai D, Kanai M, Kobayashi S, Eguchi H, Baba H, et al. 615ORandomized phase III study of gemcitabine, cisplatin plus S-1 (GCS) versus gemcitabine, cisplatin (GC) for advanced biliary tract cancer (KHBO1401-MITSUBA). *Ann Oncol* 2018;29.
 38. Takada T, Kato H, Matsushiro T, Nimura Y, Nagakawa T, et al. Comparison of 5-fluorouracil, doxorubicin and mitomycin C with 5-fluorouracil alone in the treatment of pancreatic-biliary carcinomas. *Oncology* 1994;51:396-400.
 39. Choi CW, Choi IK, Seo JH, Kim BS, Kim JS, et al. Effects of 5-fluorouracil and leucovorin in the treatment of pancreatic-biliary tract adenocarcinomas. *Am J Clin Oncol* 2000;23:425-8.
 40. Chen JS, Jan YY, Lin YC, Wang HM, Chang WC, et al. Weekly 24 h infusion of high-dose 5-fluorouracil and leucovorin in patients with biliary tract carcinomas. *Anticancer Drugs* 1998;9:393-7.
 41. Petekkaya I, Gezgen G, Roach EC, Solak M, Gullu I. Long-term advanced cholangiocarcinoma survivor with single-agent capecitabine. *J buon* 2012;17:796.
 42. Schweitzer N, Kirstein MM, Kratzel AM, Mederacke YS, Fischer M, et al. Second-line chemotherapy in biliary tract cancer: Outcome and prognostic factors. *Liver Int* 2019;39:914-23.
 43. Fornaro L, Cereda S, Aprile G, Di Girolamo S, Santini D, et al. Multivariate prognostic factors analysis for second-line chemotherapy in advanced biliary tract cancer. *Br J Cancer* 2014;110:2165-9.
 44. Brieau B, Dahan L, De Rycke Y, Boussaha T, Vasseur P, et al. Second-line chemotherapy for advanced biliary tract cancer after failure of the gemcitabine-platinum combination: a large multicenter study by the Association des Gastro-Entérologues Oncologues. *Cancer* 2015;121:3290-7.
 45. Neuzillet C, Casadei Gardini A, Brieau B, Vivaldi C, Smolenschi C, et al. Prediction of survival with second-line therapy in biliary tract cancer: actualisation of the AGEO CT2BIL cohort and European multicentre validations. *Eur J Cancer* 2019;111:94-106.
 46. Lamarca A, Hubner RA, David Ryder W, Valle JW. Second-line chemotherapy in advanced biliary cancer: a systematic review. *Ann Oncol* 2014;25:2328-38.
 47. Lamarca A, Palmer DH, Wasan HS, Ross PJ, Ma YT, et al. ABC-06 | A randomised phase III, multi-centre, open-label study of active symptom control (ASC) alone or ASC with oxaliplatin / 5-FU chemotherapy (ASC+mFOLFOX) for patients (pts) with locally advanced / metastatic biliary tract cancers (ABC) previously-treated with cisplatin/gemcitabine (CisGem) chemotherapy. *J Clin Oncol* 2019;37:4003.
 48. Zheng Y, Tu X, Zhao P, Jiang W, Liu L, et al. A randomised phase II study of second-line XELIRI regimen versus irinotecan monotherapy in advanced biliary tract cancer patients progressed on gemcitabine and cisplatin. *Br J Cancer* 2018;119:291-5.
 49. Kim BJ, Yoo C, Kim KP, Hyung J, Park SJ, et al. Efficacy of fluoropyrimidine-based chemotherapy in patients with advanced biliary tract cancer after failure of gemcitabine plus cisplatin: retrospective analysis of 321 patients. *Br J Cancer* 2017;116:561-7.
 50. Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, et al. Genomic spectra of biliary tract cancer. *Nat Genet* 2015;47:1003-10.
 51. Rizvi S, Khan SA, Hallemeier CL, Kelley RK, Gores GJ. Cholangiocarcinoma - evolving concepts and therapeutic strategies. *Nat Rev Clin Oncol* 2018;15:95-111.
 52. Churi CR, Shroff R, Wang Y, Rashid A, Kang HC, et al. Mutation profiling in cholangiocarcinoma: prognostic and therapeutic implications. *PLoS One* 2014;9:e115383.
 53. Ross JS, Wang K, Gay L, Al-Rohil R, Rand JV, et al. New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing. *Oncologist* 2014;19:235-42.
 54. Wu YM, Su F, Kalyana-Sundaram S, Khazanov N, Ateeq B, et al. Identification of targetable FGFR gene fusions in diverse cancers. *Cancer Discov* 2013;3:636-47.
 55. Abou-Alfa GK, Sahai V, Hollebecque A, Vaccaro G, Melisi D, et al. Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study. *Lancet Oncol* 2020;21:671-84.
 56. Javle M, Lowery M, Shroff RT, Weiss KH, Springfield C, et al. Phase II study of BGJ398 in patients with FGFR-altered advanced cholangiocarcinoma. *J Clin Oncol* 2018;36:276-82.
 57. Goyal L, Meric-Bernstein F, Hollebecque A, Valle J, Morizane C, et al. FOENIX-CCA2: a phase II, open-label, multicenter study of futibatinib in patients (pts) with intrahepatic cholangiocarcinoma (iCCA) harboring FGFR2 gene fusions or other rearrangements. *J Clin*

- Oncol 2020;38:108.
58. Nishina T, Takahashi S, Iwasawa R, Noguchi H, Aoki M, et al. Safety, pharmacokinetic, and pharmacodynamics of erdafitinib, a pan-fibroblast growth factor receptor (FGFR) tyrosine kinase inhibitor, in patients with advanced or refractory solid tumors. *Invest New Drugs* 2018;36:424-34.
 59. Mazzaferro V, El-Rayes BF, Droz Dit Busset M, Cotsoglou C, Harris WP, et al. Derazantinib (ARQ 087) in advanced or inoperable FGFR2 gene fusion-positive intrahepatic cholangiocarcinoma. *Br J Cancer* 2019;120:165-71.
 60. Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, et al. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. *Oncologist* 2012;17:72-9.
 61. Dang L, Yen K, Attar EC. IDH mutations in cancer and progress toward development of targeted therapeutics. *Ann Oncol* 2016;27:599-608.
 62. Abou-Alfa GK, Macarulla T, Javle M, Kelley RK, Lubner SJ, et al. ClarIDHy: a global, phase 3, randomized, double-blind study of ivosidenib (IVO) vs placebo in patients with advanced cholangiocarcinoma (CC) with an isocitrate dehydrogenase 1 (IDH1) mutation. *Ann Oncol* 2019;30:Abstract LBA 10_PR.
 63. Gu TL, Deng X, Huang F, Tucker M, Crosby K, et al. Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. *PLoS One* 2011;6:e15640.
 64. Saborowski A, Saborowski M, Davare MA, Druker BJ, Klimstra DS, et al. Mouse model of intrahepatic cholangiocarcinoma validates FIG-ROS as a potent fusion oncogene and therapeutic target. *Proc Natl Acad Sci U S A* 2013;110:19513-8.
 65. Drilon A, Laetsch TW, Kummar S, DuBois SG, Lassen UN, et al. Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. *N Engl J Med* 2018;378:731-9.
 66. Doebele RC, Drilon A, Paz-Ares L, Siena S, Shaw AT, et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1-2 trials. *Lancet Oncol* 2020;21:271-82.
 67. Galdy S, Lamarca A, McNamara MG, Hubner RA, Cella CA, et al. HER2/HER3 pathway in biliary tract malignancies; systematic review and meta-analysis: a potential therapeutic target? *Cancer Metastasis Rev* 2017;36:141-57.
 68. Jensen LH. Clinical aspects and perspectives of erlotinib in the treatment of patients with biliary tract cancer. *Expert Opin Investig Drugs* 2016;25:359-65.
 69. Rizzo A, Frega G, Ricci AD, Palloni A, Abbati F, et al. Anti-EGFR monoclonal antibodies in advanced biliary tract cancer: a systematic review and meta-analysis. *In Vivo* 2020;34:479-88.
 70. Lee J, Park SH, Chang HM, Kim JS, Choi HJ, et al. Gemcitabine and oxaliplatin with or without erlotinib in advanced biliary-tract cancer: a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* 2012;13:181-8.
 71. Vogel A, Kasper S, Bitzer M, Block A, Sinn M, et al. PICCA study: panitumumab in combination with cisplatin/gemcitabine chemotherapy in KRAS wild-type patients with biliary cancer-a randomised biomarker-driven clinical phase II AIO study. *Eur J Cancer* 2018;92:11-9.
 72. Möbius C, Demuth C, Aigner T, Wiedmann M, Wittekind C, et al. Evaluation of VEGF A expression and microvascular density as prognostic factors in extrahepatic cholangiocarcinoma. *Eur J Surg Oncol* 2007;33:1025-9.
 73. Benckert C, Jonas S, Cramer T, Von Marschall Z, Schäfer G, et al. Transforming growth factor beta 1 stimulates vascular endothelial growth factor gene transcription in human cholangiocellular carcinoma cells. *Cancer Res* 2003;63:1083-92.
 74. Zhu AX, Meyerhardt JA, Blaszkowsky LS, Kambadakone AR, Muzikansky A, et al. Efficacy and safety of gemcitabine, oxaliplatin, and bevacizumab in advanced biliary-tract cancers and correlation of changes in 18-fluorodeoxyglucose PET with clinical outcome: a phase 2 study. *Lancet Oncol* 2010;11:48-54.
 75. Bréchon M, Dior M, Dréanic J, Briau B, Guillaumot MA, et al. Addition of an antiangiogenic therapy, bevacizumab, to gemcitabine plus oxaliplatin improves survival in advanced biliary tract cancers. *Invest New Drugs* 2018;36:156-62.
 76. Valle J, Bai LY, Orlova R, Van Cutsem E, Adeva J, et al. Ramucirumab (RAM) or merestinib (MER) or placebo (PL) plus gemcitabine (GEM) and cisplatin (CIS) as first-line treatment for advanced or metastatic biliary tract cancer (BTC): a randomized, double-blind, phase II study. *J Clin Oncol* 2020;38:Abstract 477.
 77. Arkenau HT, Martin-Liberal J, Calvo E, Penel N, Krebs MG, et al. Ramucirumab plus pembrolizumab in patients with previously treated advanced or metastatic biliary tract cancer: nonrandomized, open-label, phase I trial (JVDF). *Oncologist* 2018;23:1407-e136.
 78. Yang XW, Li L, Hou GJ, Yan XZ, Xu QG, et al. STAT3 overexpression promotes metastasis in intrahepatic cholangiocarcinoma and correlates negatively with surgical outcome. *Oncotarget* 2017;8:7710-21.
 79. Dokduang H, Techasen A, Namwat N, Khuntikeo N, Paironkul C, et al. STATs profiling reveals predominantly-activated STAT3 in cholangiocarcinoma genesis and progression. *J Hepatobiliary Pancreat Sci* 2014;21:767-76.
 80. Miyamoto M, Ojima H, Iwasaki M, Shimizu H, Kokubu A, et al. Prognostic significance of overexpression of c-Met oncoprotein in cholangiocarcinoma. *Br J Cancer* 2011;105:131-8.
 81. Pant S, Saleh M, Bendell J, Infante JR, Jones S, et al. A phase I dose escalation study of oral c-MET inhibitor tivantinib (ARQ 197) in combination with gemcitabine in patients with solid tumors. *Ann Oncol* 2014;25:1416-21.
 82. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase II KEYNOTE-158 study. *J Clin Oncol* 2020;38:1-10.
 83. Silva VW, Askan G, Daniel TD, Lowery M, Klimstra DS, et al. Biliary carcinomas: pathology and the role of DNA mismatch repair deficiency. *Chin Clin Oncol* 2016;5:62.
 84. Bang YJ, Ueno M, Malka D, Chung HC, Nagrial A, et al. Pembrolizumab (pembro) for advanced biliary adenocarcinoma: results from

- the KEYNOTE-028 (KN028) and KEYNOTE-158 (KN158) basket studies. *J Clin Oncol* 2019;37:4079.
85. Gou M, Zhang Y, Si H, Dai G. Efficacy and safety of nivolumab for metastatic biliary tract cancer. *Onco Targets Ther* 2019;12:861-7.
 86. Kim RD, Kim DW, Alese OB, Li D, Shah N, et al. A phase II study of nivolumab in patients with advanced refractory biliary tract cancers (BTC). *J Clin Oncol* 2019;37:4097.
 87. Ueno M, Ikeda M, Morizane C, Kobayashi S, Ohno I, et al. Nivolumab alone or in combination with cisplatin plus gemcitabine in Japanese patients with unresectable or recurrent biliary tract cancer: a non-randomised, multicentre, open-label, phase 1 study. *Lancet Gastroenterol Hepatol* 2019;4:611-21.
 88. Ioka T, Ueno M, Oh DY, Fujiwara Y, Chen JS, et al. Evaluation of safety and tolerability of durvalumab (D) with or without tremelimumab (T) in patients (pts) with biliary tract cancer (BTC). *J Clin Oncol* 2019;37:387.

Original Article

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Post liver transplant recurrence in patients with hepatocellular carcinoma: not necessarily the end of the road!

Prashant Bhangui, Sanjay Yadav, AS Soin

Medanta Institute of Liver Transplantation and Regenerative Medicine, Medanta-The Medicity, Gurgaon, Delhi NCR 122001, India.

Correspondence to: Dr. Prashant Bhangui, Senior Consultant Hepatobiliary and Liver Transplant Surgeon, Medanta Institute of Liver Transplantation and Regenerative Medicine, Medanta-The Medicity, Sector 38, Gurugram, Delhi NCR 122001, India. E-mails: pbhangui@gmail.com; prashant.bhangui@medanta.org

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Abstract

Aim: We analysed outcomes using multimodality therapy in patients with hepatocellular carcinoma (HCC) recurrence post living donor liver transplantation (LDLT).

Methods: Of 2363 LDLT's performed between 2005 to mid 2018, 435 (18.4%) were for HCC within our expanded selection criteria (absence of extrahepatic disease and vascular invasion, irrespective of tumor size and number). Survival after recurrence, and prognostic factors for these patients were studied.

Results: Of 435 LDLT patients, 51% had HCC beyond Milan and 43% beyond UCSF criteria at the time of LDLT. pre-LT AFP > 100 ng/mL and tumour FDG-18 PET avidity predicted overall survival (OS), whereas pre-LT AFP > 100 ng/mL, UCSF criteria, and FDG-18 PET avidity predicted recurrence-free survival. Hundred patients (23%) developed HCC recurrence at a median time of 16 months (range 2-108 months) post LDLT. Lungs (53%), liver (37%), and bone (21%) were the most common sites of recurrence. Ninety-five patients received tyrosine kinase inhibitors (TKI) after recurrence and 62 received mTOR inhibitors (protocol-based after LDLT, or post recurrence). Surgical resection of metastases was performed in 14 patients, 15 received stereotactic body radiotherapy, and 18 underwent ablation (radiofrequency, microwave ablation, transarterial chemoembolisation, or percutaneous ethanol injection). One- and 3-yr OS after recurrence were 57%, and 24% respectively, with a maximum post recurrence survival of 7.5 years. HCC recurrence within one year after LDLT ($P = 0.004$, HR = 2.38, 95%CI: 1.325-



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4.276), AFP > 200 ng/mL at the time of recurrence ($P = 0.02$, HR = 2.075, 95%CI: 1.121-3.841), and recurrence at multiple sites ($P = 0.047$, HR = 1.831, 95%CI: 1.009-3.321) were poor prognostic factors for post recurrence survival. Multimodality management of recurrence using combined medical, surgical, ablative treatments and radiotherapy significantly improved survival compared to the use of TKI's or mTORi's alone, or in combination.

Conclusion: In patients accepted for LDLT beyond the conventional size-number criteria, even after HCC recurrence, an aggressive approach using multimodality therapy, when possible, aids in further prolongation of survival.

Keywords: Living donor liver transplantation, hepatocellular carcinoma recurrence, multimodality treatment, outcomes, prognostic factors

INTRODUCTION

Liver transplantation (LT) is now accepted as the best curative option for hepatocellular carcinoma (HCC) in patients with decompensated liver disease as it achieves oncological clearance, and also treats the underlying chronic liver disease (CLD)^[1]. This however holds true when selection of patients for LT adheres to either the conventional Milan^[2] and UCSF^[3] criteria, or other expanded criteria^[4-9] which have also yielded similar long term outcomes. Over the years, the focus of selection has shifted from size and number of tumours on pre-LT imaging, to tumour biology. Inclusion of tumour markers like alpha fetoprotein (AFP) and des-gamma-carboxy- prothrombin (DGCP), grade of the tumour, and FDG-18 PET scan avidity in the selection criteria, indicates the increased recognition that tumour biology is crucial and dictates long term outcome^[10-13]. The main determinant of long term outcomes in HCC patients after LT is indeed tumour recurrence, and it continues to be the Achilles heel of this curative strategy. With an expansion of criteria, the chance of recurrence tends to be higher. It is still believed that recurrence leads to early death in most cases, since management of post-LT HCC recurrence to prolong survival remains very challenging. Recurrences frequently occur within the first 2 years after LT, however very late recurrence after a 10 year period have also been reported^[14-16]. Furthermore, recurrences tend to occur as extrahepatic disease (as high as 71%), followed by intrahepatic recurrence alone, and both intra- plus extrahepatic recurrence^[17]. Given that recurrence more often occurs at multiple sites, treatment of recurrence is even more difficult, and mostly limited to systemic therapy alone. Tumour burden at the time of recurrence is also a major determinant of outcomes (single vs. multiple nodules at the time of recurrence). The options available for systemic therapy are few, including tyrosine kinase inhibitors, and the recently introduced checkpoint inhibitors. Results using these systemic agents are far from satisfactory, and they are known to prolong survival only by a few months.

In this study, we analyzed our results in managing patients with HCC recurrence post-LDLT with the use of multimodality treatment including surgical resection, ablative treatment, radiotherapy, modulation of immunosuppression, and systemic agents.

METHODS

Selection criteria for LT in patients with cirrhosis and HCC

At our center, we accept medically fit HCC patients for living donor liver transplantation (LDLT) irrespective of tumor size and number, provided they have no extrahepatic disease, or major vascular invasion. Listing for deceased donor liver transplantation is based on the UCSF criteria. Pre-LT recipient evaluation in HCC patients includes a whole body FDG-18 PET scan with triphasic CT angiography abdomen (or dynamic contrast MRI in recipients with borderline renal function), and whole body Tc-99m bone scan to rule out extent of hepatic, as well as extrahepatic spread. Alpha-fetoprotein (AFP) levels are also measured.

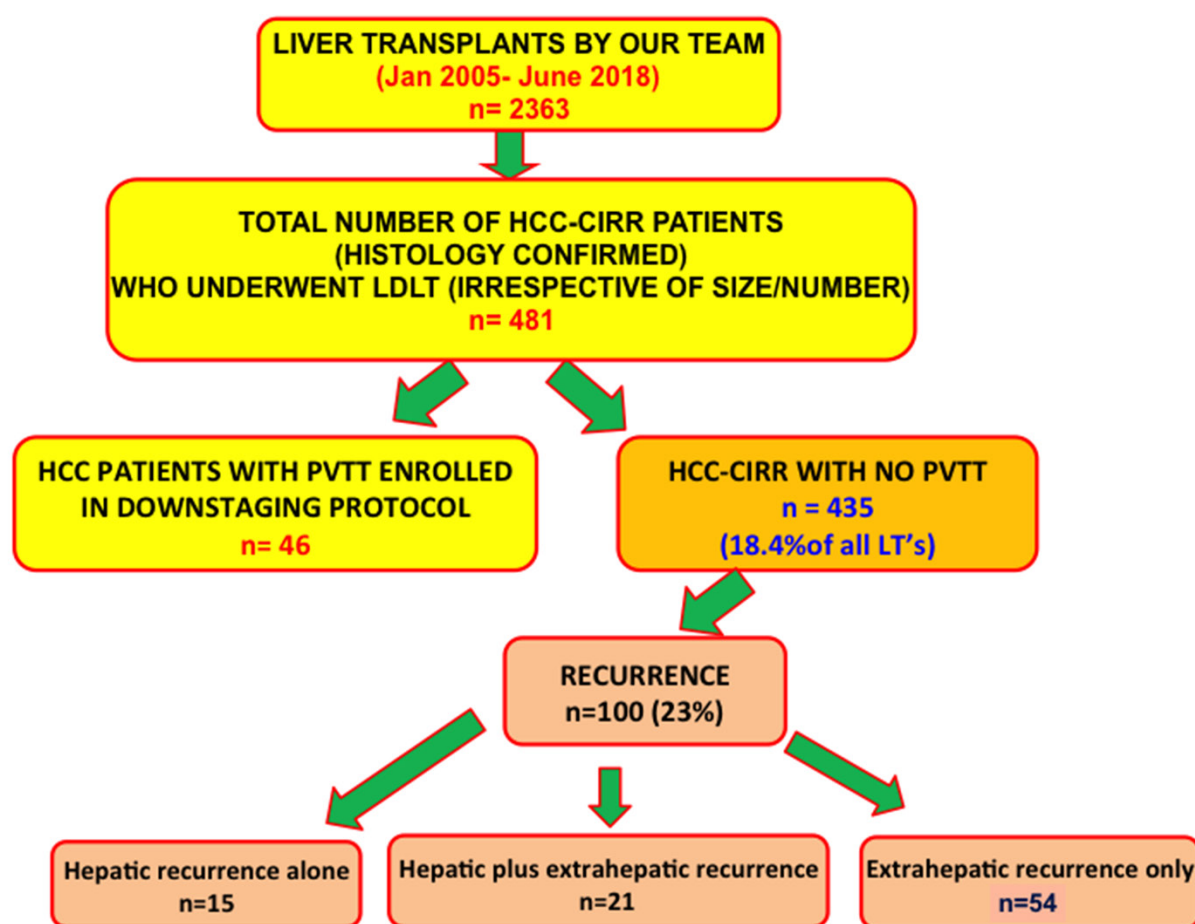


Figure 1. Patient Cohort

Study cohort

From our prospectively maintained database of 2,363 LDLT's (2005 to mid 2018 so as to have a minimum follow up of 2 years post LDLT at the time of data analysis), we studied outcomes in 435 histologically confirmed (on the explant specimen) cirrhosis and HCC (HCC-cirr) patients (18.4% of all LDLTs performed during this period). During this period, 481 LDLT's were performed in HCC-cirr patients. Forty-six had segmental or lobar portal vein tumour thrombosis, and underwent downstaging before LDLT as per our Institutional protocol^[18]. Those 46 patients were excluded from this study. Hundred patients developed recurrence, and their management and outcomes were further analysed [Figure 1].

Post LDLT immunosuppression and follow up

Post LDLT, patients were maintained on the standard 3 drug immunosuppressive regimen consisting of CNI inhibitor (Tacrolimus or cyclosporine), mycophenolate mofetil and steroid, the doses of which were slowly tapered, and steroid and mycophenolate were gradually omitted (usually by 6 months and 2 years, respectively). Since 2014, we followed a policy of early switch to mTOR inhibitor based immunosuppression with CNI reduction, 4-6 weeks after LDLT.

In addition to the routine follow up of all transplanted patients, follow up for tumour recurrence in HCC-cirr patients included USG and AFP level at 3 months and 6 months, then once every 6 months till 2 years after LT. After this USG and AFP were repeated once annually. MDCT abdomen (or PET-scan if the tumours were initially PET-avid) was done 6 monthly for the first two years after LDLT, and then yearly.

Symptoms suggestive of metastases such as chest symptoms or bone pains were evaluated as indicated. All cases with suspected recurrence were discussed at a multidisciplinary meeting. Patients with an increased AFP underwent triphasic PET CT scans for radiological confirmation of HCC recurrence; histological confirmation was obtained only where the diagnosis was doubtful.

Management of recurrence

Different treatment options were discussed in a multidisciplinary team meeting which included surgeons, radiologists, hepatologists, and oncologists. All patients were treated with sorafenib (after 2009), either alone (when other loco regional therapy or surgical resection were not possible), or in combination with surgery/ablation/radiotherapy. Resection was proposed in patients whenever technically feasible, especially in patients with isolated metastases in the lungs [video assisted thoracoscopic surgery (VATS), liver, bone, or scar site. Ablative therapy included transarterial chemoembolisation (TACE) for liver recurrence, or radiofrequency ablation (RFA)] for liver, lung or bone lesions. Stereotactic body radiotherapy was used for bone, lung, or hepatic recurrence in some cases.

Statistical analysis

Continuous variables are expressed as mean \pm SD or median (range, IQR) as appropriate. Categorical data are presented as frequency and percentage. For outcomes post LDLT, overall survival (OS) was calculated from the date of transplant to the date of death or last follow up, whereas recurrence-free survival (RFS) was calculated from the date of transplant to the date of first recurrence, or last follow up whichever was earlier. Post-recurrence was calculated from the time of detection of recurrence till death, or last follow up. The Chi-square test was used for categorical variables, and independent *t*-test or ANNOVA for continuous variables. The Kaplan-Meier method was used for survival analysis, and the survival curves were compared by using the log-rank test. Univariate and multivariate analysis of risk factors associated with post recurrence survival was performed using Cox proportional hazard model. A *P* value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 20.0 for Windows statistical software (SPSS Inc., Chicago, IL, USA).

The study was performed in accordance with the Declaration of Helsinki. Since this was a retrospective review and analysis of prospectively maintained data at our Institute, no consent was required for inclusion in this study. However, all patients who had undergone LDLT or any procedure/therapy for recurrence were consented at every stage.

RESULTS

The overall and recurrence-free 5 year survival in our cohort of 435 patients was 64%, and 70%, respectively [Figure 2A and B]. Prognostic factors for OS included pre-LT AFP > 100 ng/mL and tumour FDG-18 PET avidity. Predictors of recurrence following multivariate analysis included UCSF criteria on imaging, serum AFP level > 100 ng/mL before LDLT, and tumour FDG-18 PET avidity.

The baseline clinico-pathological characteristics of 100 HCC-cirr patients who developed recurrence post LDLT are summarised in Table 1. The mean age of the studied population was 53 years of which 88% were males. The most common underlying causes of cirrhosis were HCV (34%) and HBV (32%) cirrhosis. The median pre-LDLT AFP was 96 ng/mL (range 1-11200), and mean was 788 ± 1985 ng/mL; 64% of patients had tumours beyond UCSF, and 71% beyond Milan criteria. 67% of tumours were FDG-18 PET scan avid. The characteristics in the entire cohort of 435 patients are detailed in Supplementary Table 1.

In the 100 patients with recurrence, the median time to HCC recurrence after LDLT was 16 months (range 2-108 months), mean AFP at recurrence was $80 \pm 11,796$ ng/mL and majority of the recurrences (68%) were detected within first two years after LDLT. At the time of diagnosis, most recurrences were extrahepatic

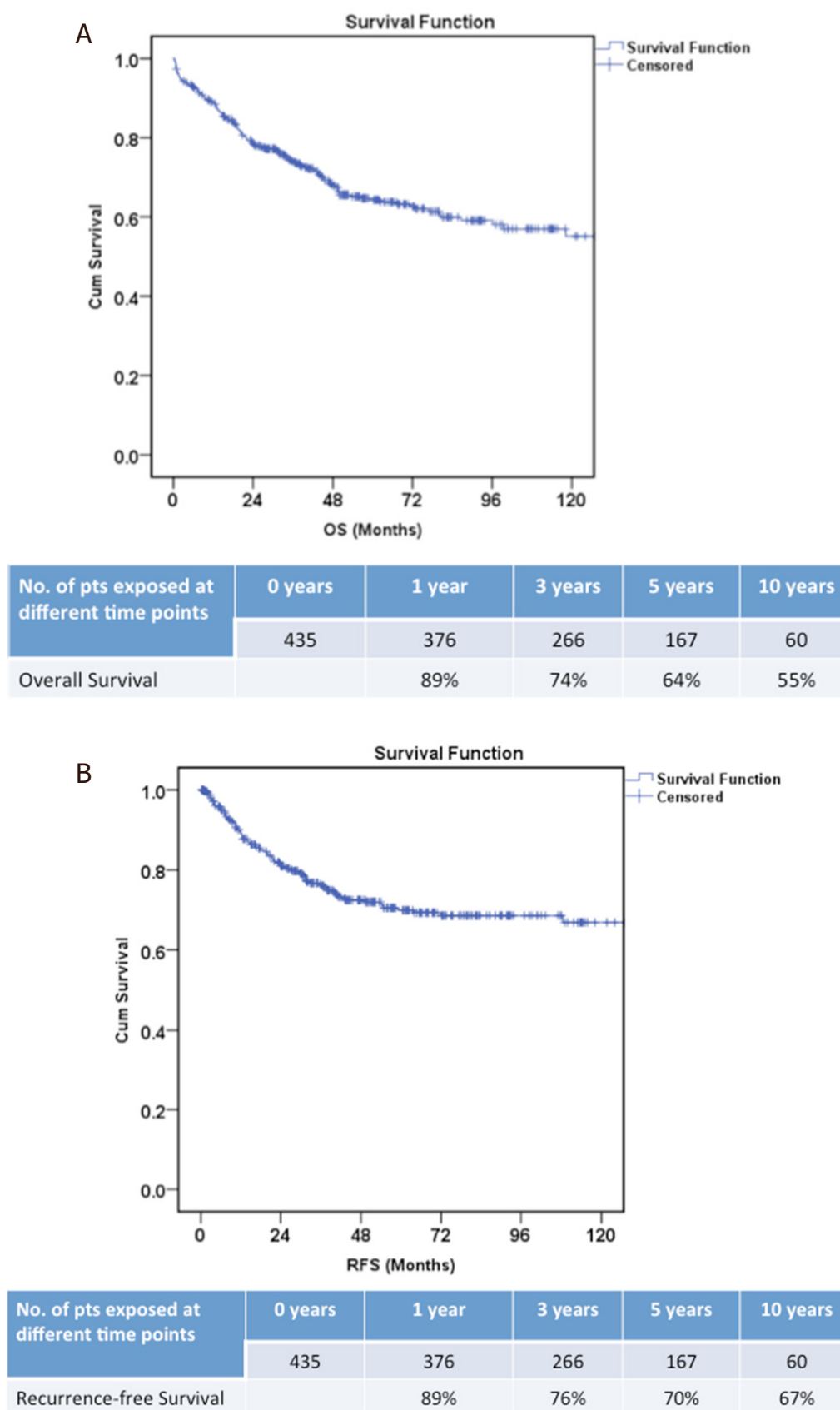


Figure 2. Overall survival (A) and recurrence-free survival (B) in our cohort of 435 patients with hepatocellular carcinoma who underwent living donor liver transplantation

Table 1. Patient demographics and tumour characteristics in 100 patients with HCC recurrence post LDLT

Patient demographics and tumour characteristics (n = 100)	Values
Age in years at the time of LDLT (mean ± standard deviation)	53 ± 8
Gender	
Male/Female	88/12
Etiology of underlying cirrhosis	
HBV/HCV/HBV + HCV	32%/34%/4%
Cryptogenic	6%
Ethanol	12%
NASH/NAFLD	10%
Others	25%
Non tumour MELD score (mean ± standard deviation)	12 ± 6
Pre-LT treatment for HCC	
TACE	22
RFA	6
Resection	6
Pre-LT AFP ng/mL Mean ± SD	788 ± 1,985
Tumour characteristics	
Within UCSF/Beyond UCSF	36%/64%
Within Milan/Beyond Milan	29%/71%
Tumour FDG-18 PET avidity Non Avid vs. Avid in those who had a PET scan before LT	23%/67%

HCC: hepatocellular carcinoma; HBV: hepatitis B virus; HCV: hepatitis C virus; NASH: non alcoholic steatohepatitis; NAFLD: non alcoholic fatty liver disease; MELD: Model for End Stage Liver Disease; TACE: transarterial chemoembolization; RFA: radiofrequency ablation; AFP: alpha feto-protein; UCSF: University of California, San Francisco; FDG-18 PET: 18-fluoro deoxy-glucose positron emission tomography; LT: liver transplantation

(n = 54; 54%), liver only recurrence was seen in 15 patients, and in 21 patients, recurrence occurred in the liver as well as extrahepatic sites. Thirty-six patients presented with HCC recurrence at more than one site and the three most common sites of disease recurrence were lungs (53%), liver (37%), and bone (21%). Thirty-two percent of patients had a single nodule recurrence, whereas 68% of patients presented with two or more tumour nodules (at one or multiple sites) when they recurred [Table 2].

One patient developed an abdominal wall squamous cell carcinoma post LDLT and subsequently underwent excision. Two other HCC patients (in the overall cohort of 435 patients) developed *de novo* malignancies during follow up (one patient had an inflammatory myofibroblastic tumour of the colon, and another developed a brain cancer).

Management of HCC recurrence

Ninety five patients with recurrence were put on sorafenib of which in 20 patients dose adjustment was required due to adverse effects. mTOR inhibitors were used in 62 patients. In 38 patients, they were started “de principe” within 6 months of LDLT, as our protocol from 2012 onwards included switch to mTORI and mycophenolate based immunosuppression with CNI reduction early after LDLT. The other 24 patients were switched over to mTORi's later, either pre emptively (11 patients), or after developing recurrence (13 patients). All patients except one received Sirolimus (1 patient received Everolimus). Thirty-two patients received tyrosine kinase inhibitors (TKI's) alone, and 36 received both TKI's and mTORi's [Table 2].

Surgical excision of lung metastases was performed using VATS in 5 patients, and limited hepatectomy was performed for liver recurrence in 3 patients. Scar site excision (n = 3), laminectomy, mediastinal tumour excision, and supraadrenal metastatectomy (one each), were the other surgical procedures performed. Fifteen patients received stereotactic radiotherapy (for bone/lung/liver/soft tissue lesions), 10 underwent RFA or microwave ablation (MWA) of liver/lungs/bone metastases, 5 underwent TACE for liver lesions, and 3 had percutaneous ethanol injection in metastatic lymph nodes [Table 2].

Thirty-two patients received tyrosine kinase inhibitors with/without mTORi's and other modalities (surgery and/or ablative therapy and/or radiotherapy).

Table 2. Characteristics of tumour recurrence and treatment modalities

Characteristics (n = 100)	Values
Time to recurrence after LDLT	16 months
Median (range)	(2-108 months)
AFP at the time of recurrence (ng/mL) Mean \pm SD	80 \pm 11,796
Site of tumour recurrence (number of patients)	
Lungs	53
Liver	37
Bone	21
Lymph nodes	7
Brain	2
Scar site	2
More than one site of recurrence	36
Treatment for recurrence	
Kinase inhibitors alone (Sorafenib, Regorafenib, Lenvatinib)	32 patients
Kinase inhibitors with mTORi's (sirolimus, everolimus) only	36 patients
Medical (Kinase inhibitors with/without mTORi's) plus other modalities (surgery, radiotherapy, ablation)	32 patients
Other modalities used in addition to kinase inhibitors and mTORi's to treat metastases	
Radiotherapy	15
TACE for liver metastases	5
VATS for lung metastases	5
Surgical resection of metastases (liver, scar site, mediastinal metastases, laminectomy, supra adrenal metastases)	9
Percutaneous ethanol injection in lymph node	3
Radiofrequency ablation and microwave ablation for liver, lung, or bone metastases	10
More than 1 modality used in one patient	11

LDLT: living donor liver transplantation; mTORi: mammalian target of rapamycin inhibitor; TACE: transarterial chemoembolization; VATS: video assisted thoracoscopic surgery

When we compared the tumour characteristics at the time of recurrence between the three treatment groups, we found that there was no statistically significant difference in AFP levels, occurrence of metastases at single *vs.* multiple sites, single *vs.* multiple nodules at recurrence, or intrahepatic recurrence only *vs.* intrahepatic and extrahepatic recurrence [Supplementary Table 2]. However, on post hoc analysis we did find that more patients in the Kinase inhibitor + mTOR inhibitor group had HCC recurrence within 1 year of LDLT ($P = 0.05$), and had multiple nodules at the time of recurrence (*vs.* single nodule, $P = 0.04$) as compared to the combined therapy group.

Survival post recurrence

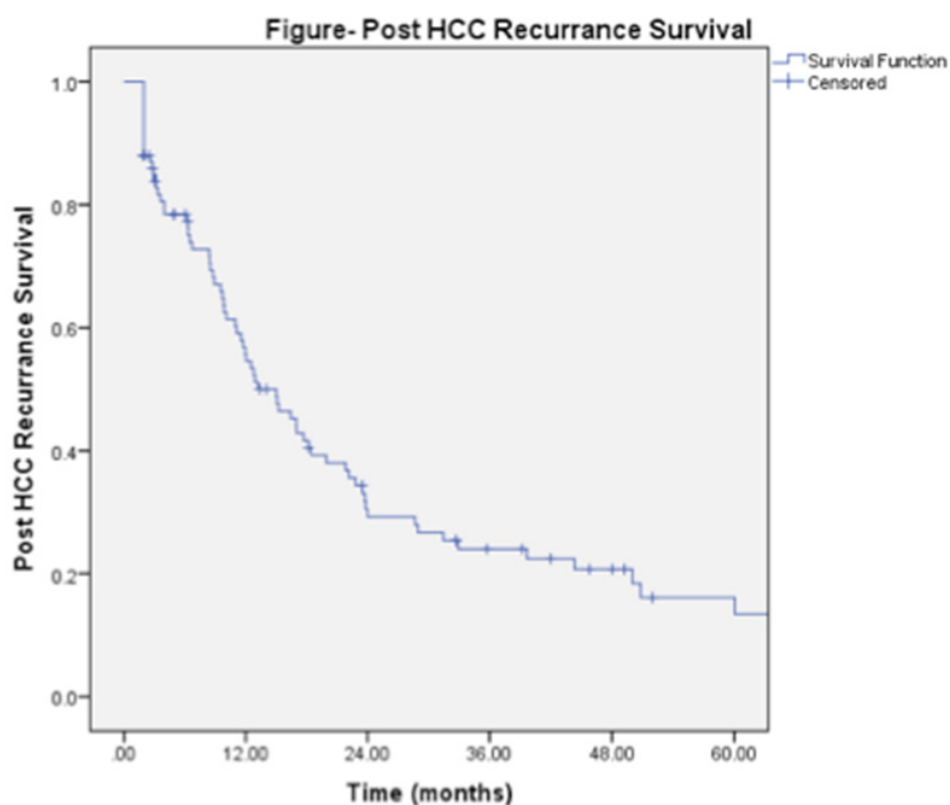
One-year, 2-year, and 3-year post recurrence survival were 57%, 31%, and 24%, respectively, with a median survival of 12 months (IQR 4-24 months) [Figure 3]. The maximum post recurrence survival was 88 months (7½ years), and the longest survivor still alive is 77 months post recurrence.

With regards to predictors of survival after recurrence, HCC recurrence within one year after LDLT ($P = 0.004$, HR = 2.38, 95%CI: 1.325-4.276), AFP > 200 ng/mL at the time of recurrence ($P = 0.02$, HR = 2.075, 95%CI: 1.121-3.841), and recurrence at multiple sites ($P = 0.047$, HR = 1.831, 95%CI: 1.009-3.321), were poor prognostics factors for post recurrence survival [Table 3].

Post recurrence survival rates in the tyrosine kinase inhibitor only group (1-year, 3-year OS of 38% and 15%), as well as the tyrosine kinase with mTORi group (1-year, 3-year OS of 56% and 19%) were significantly inferior to those who received multimodality treatment using combined medical and surgical/ablative/radiotherapy (1-year, 3-year OS of 77% and 39%); $P = 0.017$ [Figure 4].

DISCUSSION

Post- transplant HCC recurrence is seen in 10%-20% of the patients and this has remained stable over the years, despite repeated efforts to refine the selection criteria for transplant to achieve best outcomes^[6]. This is much higher when the selection criteria are expanded, especially in the LDLT setting. A 2015 systematic



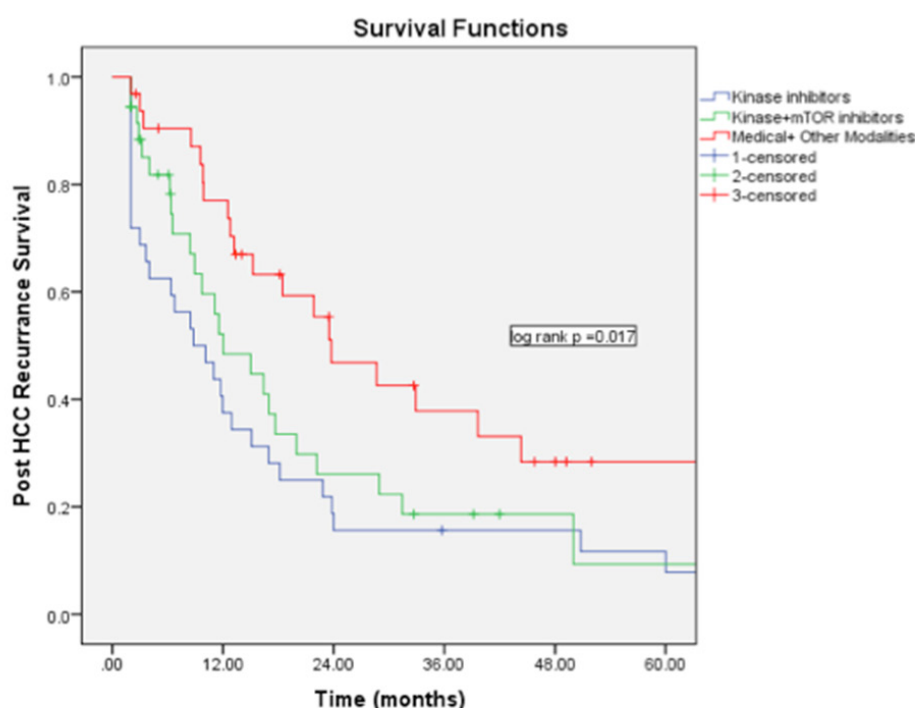
No. of pts exposed at different time points	1 year	2 years	3 years	5 years
Overall Survival	57%	31%	24%	17%

Figure 3. Overall survival post hepatocellular carcinoma recurrence in 100 patients

Table 3. Cox regression analysis for survival post HCC recurrence

Parameters	Univariate analysis			Multivariate analysis		
	Hazard ratio	95%CI	P value	Hazard ratio	95%CI	P value
Age at the time of recurrence	1.02	0.98-1.05	0.34			
Gender - Male	0.90	0.46-1.78	0.77			
HCC within <i>vs.</i> outside UCSF criteria at the time of LDLT	1.06	0.66-1.70	0.82			
Type of recurrence: recurrence at single <i>vs.</i> multiple sites	1.70	1.06-2.73	0.029	1.831	1.009-3.321	0.047
Tumour burden: single nodule <i>vs.</i> multiple nodules	2.23	1.13-4.40	0.022	1.462	0.719-2.970	0.294
Intrahepatic only <i>vs.</i> extrahepatic or Intrahepatic plus extrahepatic metastases	0.58	0.29-1.13	0.11			
Alpha fetoprotein at the time of recurrence AFP < 200 <i>vs.</i> > 200 ng/mL	2.59	1.42-4.71	0.002	2.075	1.121-3.841	0.020
Time to HCC recurrence: > 1 year after LDLT <i>vs.</i> within 1 year of LDLT	2.49	1.56-3.98	0.001	2.381	1.325-4.276	0.004

HCC: hepatocellular carcinoma; LDLT: living donor liver transplantation; UCSF: University of California, San Francisco; mTORi: mammalian target of rapamycin inhibitor



Overall Survival	1 year	2 years	3 years	5 years
Tyrosine kinase inhibitors alone	38%	19%	15%	12%
Tyrosine kinase and mTOR inhibitors	56%	28%	19%	10%
Medical plus other treatment modalities	77%	48%	39%	28%

Figure 4. Comparative post-recurrence survival in patients with hepatocellular carcinoma recurrence based on therapeutic modalities used

review including a heterogeneous group of 61 studies demonstrated a mean recurrence rate of 16%, and mean time from transplant to HCC recurrence of 13 months (range 2-132 months)^[19]. The median time to recurrence in our series was 16 months (IQR 8-29 months). As centers worldwide transplant patients with HCC beyond Milan criteria, the magnitude of HCC recurrence may be larger than proposed by these estimates^[20]. On the other hand, in patients slightly beyond conventional criteria, LT remains the best treatment option, since ablative or systemic therapy are often not possible due to deranged liver function, and LT is the best chance for oncological cure.

Post LT HCC recurrence is the key determinant of survival, and managing patients with recurrence continues to be a major challenge, as none of the treatment options guarantee long term survival. Predominant extrahepatic recurrence, and recurrence at multiple sites, are two main issues that make management difficult.

Early HCC recurrence (within the first year of LT), usually portends worse prognosis^[21,22]. Our findings were similar, with recurrence within the first year predicting worse survival. Early recurrence could occur due to non-detectable extrahepatic metastases that may be present before LT, or due to circulating HCC tumour cells engrafting and growing in a target organ after LT. A strict post-LT surveillance protocol during the 1-2 year period post-LT (which covers most recurrences), could help in early detection when metastases are amenable to curative treatment, especially if they are single and resectable. Surveillance and staging should include regular imaging of lungs, bone and liver graft (most common sites of recurrences),

and should be performed using modalities that are highly sensitive and specific (whole body FDG-18 PET scan with triphasic CT angiography abdomen, or contrast computed tomography abdomen and chest with bone scan). In our study, we found that 68% of recurrences occurred within two years following LT, which is in accordance with other studies (median time of recurrence 7-36 months)^[23-27].

Sorafenib is known to confer some survival benefit in patients with disseminated recurrence (median survival of 12 months)^[28-33], but is also associated with some drug limiting side effects. Combined with mTOR inhibitors, the synergistic effect shows a trend towards better tumour response. High dose CNI exposure has been shown to be a risk factor for HCC recurrence. Hence, an mTORi based immunosuppression strategy with reduced CNI has been proposed in HCC patients post LT to reduce recurrence^[34] and improve survival benefit as demonstrated in a meta analysis^[35]. Sirolimus is generally well tolerated. Recently, studies using everolimus in both the deceased donor and living donor LT scenario have shown a tendency towards lower recurrence^[36]. A recent meta analysis comparing everolimus and sirolimus^[37] showed lower recurrence rates in patients on everolimus compared to those on sirolimus or calcineurin inhibitors. However, everolimus-treated recipients had a shorter follow-up time and fewer advanced tumours in the above study. Though data on mTOR inhibitor therapy for established recurrence after liver transplantation remain scarce, a combination of either sirolimus or everolimus with reduced-dose tacrolimus may prove beneficial in addition to sorafenib^[36,38,39]. Our study showed that recurrence at multiple sites was a poor prognostic factor for survival post recurrence. In patients with recurrence at multiple sites where no form of directed therapy is possible, combining TKI's with mTORi's, with initial reduction in CNI dose and then stopping it, may prolong survival. In patients with recurrence who are not already on mTORi's, therapy is initiated (initially at a dose of 0.75 mg BD Everolimus or 1 mg OD of Sirolimus) and maintained with trough levels of 3-5 ng/mL. CNI's doses are gradually reduced and then omitted. If required, the patient is additionally maintained on mycophenolate mofetil in a low dose and titrated based on liver function.

We tried to analyze the change in AFP levels (response to treatment) in our cohort of patients. We looked at the initial AFP (pre-LDLT) level, AFP at recurrence, and evolution with treatment. AFP was high (> 50 ng/mL in 57 patients, > 100 ng/mL in 47 patients) in only 50-60% of patients at the time of LDLT (AFP secretors) itself. Of the 48 patients with AFP > 100 ng/mL at the time of LDLT, only 20 patients presented with high AFP levels at the time of recurrence, and only 12 patients had AFP levels that were similar to or higher than those pre LDLT. This could probably be due to early detection of recurrences using a stringent follow up protocol. Due to this low number of patients with high AFP levels at the time of recurrence, we could not draw any definite conclusions with respect to change in AFP levels post treatment. Patients who had high AFP levels were those who had recurrence at multiple sites. Patients whose AFP levels responded (decreased) to treatment had a longer survival, and most had been treated with a combination of Sorafenib and Sirolimus.

In patients who progress on sorafenib, regorafenib may be used as a second-line agent^[40]. The utility of checkpoint inhibitors as immunotherapy in transplant recipients may appear promising, but also poses challenges due to the potentially increased risk of allograft rejection and graft loss^[41].

Surgical resection or ablation of HCC recurrence (as opposed to systemic or palliative therapy alone) has been shown to be an independent predictor of long-term survival if recurrence is isolated to a single organ, whether it is in the liver or an extrahepatic site^[14,22-24,42-44]. Post-LT HCC recurrences develop in non-cirrhotic livers without portal hypertension, thus qualifying most patients for surgery. Most studies have shown that resection is safe and significantly prolongs survival after HCC recurrence as compared to palliative treatment. In a recent study, Fernandez-Sevilla *et al.*^[22] reported a median survival of 35 months (vs. 15 months) in patients with resection (vs. non resection) of intra- and extrahepatic recurrences. Actual

benefit following surgery in patients with HCC recurrence post LT could of course be challenged, as only some patients with a limited disease burden may be amenable to resection.

Lung is the most common site of recurrence, probably owing to proximity to the liver at the time of hepatectomy, and the lymphatic drainage^[45]. In our study, we also observed that lungs were the most frequent site of HCC recurrence. Resection of pulmonary metastases is often not attempted owing to their multiplicity, frequent multiorgan involvement, and unclear impact on survival. However, some centers have reported good outcomes of pulmonary metastases resection (PMR) in patients treated by liver resection or ablative procedures for the liver recurrence^[24,46,47]. In a large series of pulmonary metastatectomy cases post LT, Hwang *et al.*^[48] showed that those patients who were selected for surgery based on the feasibility of complete resection and sufficient pulmonary function after surgery, the 5-year survival rate was significantly better. A large Italian multicenter study^[45] found that pulmonary metastatectomy with a low complication rate was feasible in patients who were judged operable when they developed pulmonary metastasis as the first metastasis after LT for HCC. One year, 3-yr, and 5-yr cumulative overall survival rates of 100%, 66%, and 43%, respectively, were reported in this study, with a median OS of 51 months. They selected patients with lung only metastasis, and a good liver function for surgery. Repeat metastatectomy for recurrence after the first surgery has also been proposed^[49-53]. In the 5 patients who underwent VATS resection of pulmonary metastases in our series, the median survival post recurrence was 18 months (range 10-52 months). When pulmonary metastatectomy is precluded by inadequate lung function, SBRT is considered an alternative^[54].

Ablative therapy in the form of RFA or MWA^[55], TACE^[56], and TARE using Yttrium-90 microspheres (Y-90) for unresectable intrahepatic HCC recurrence^[57], have also occasionally shown good results. A retrospective cohort study compared results in 15 patients who were treated with surgery *vs.* 11 who underwent RFA for intrahepatic recurrence^[58]. A similar 3-year (51% *vs.* 51%, $P = 0.88$) and 5-year (35% *vs.* 28%, $P = 0.88$) overall survival was reported in the two groups. Zhou *et al.*^[59] prospectively compared TACE *vs.* systemic therapy in patients with unresectable intrahepatic recurrence. Survival benefits were achieved in the TACE arm ($P = 0.013$), indicating that regional control could have contributed to the improvement in overall survival.

Ablative treatment modalities are usually safe and well tolerated, and may be repeated multiple times or combined in a multimodality approach. Some also propose that early intrahepatic HCC recurrences may be better “tested” by a locoregional treatment, prior to performing a resection, provided no further disease appears in the mean time^[60].

Use of non-invasive radiotherapy such as SBRT is also effective for local control of pulmonary and skeletal oligo-recurrences. Similarly, focused ablation of intrahepatic HCC recurrence spares the adjacent normal liver parenchyma. A higher dose of radiation is delivered while the risk of collateral damage is minimized^[61]. An abbreviated duration of treatment with SBRT (usually completed in 1-5 fractions) compared to the 10-20 days for conventional radiotherapy (during which systemic therapy is usually deferred) is an added advantage. It is now also established in pre-clinical models that stereotactic radiation may up regulate antitumour immunity^[62-64].

Results of our analysis also emphasized the utility of multimodality treatment. including systemic therapy combined with surgery/ablation/radiotherapy. In 32 patients undergoing resection or ablative treatment in addition to systemic therapy, the post recurrence survival was superior to systemic therapy alone. This is in line with the limited studies that have been published on this topic^[65-67]. It is however also true that resection or ablation is not possible in all patients with recurrence, especially in those with recurrence at multiple sites or multiple nodules at the time of recurrence. One may argue that due to this reason,

the results are biased towards the multimodality treatment group. We recommend surgical resection of isolated metastasis (either in extra hepatic or intrahepatic location) in carefully selected patients with good functional status. If resection is not possible, ablative therapy and SBRT should be used especially in liver, lung or bone metastases. Systemic therapy with Kinase inhibitors (mainly sorafenib) is advisable in all patients. Furthermore, combination therapy with mTOR inhibitors (sirolimus, everolimus) in order to reduce CNI doses, may have some benefit over kinase inhibitors alone, as seen in our study.

In conclusion, multimodality treatment for post LT HCC recurrence has shown to further improve survival rates, which calls for an aggressive approach while formulating a treatment regime for these patients.

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Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Bhangui P, Yadav S

Drafted the manuscript and substantively revised it: Bhangui P, Yadav S, Soin AS

Availability of data and materials

The authors can provide data if required, this data however is a part of the entire HCC database of the Institute based on which a manuscript is currently undergoing review in another Journal.

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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REFERENCES

1. European Association for the Study of the Liver. Corrigendum to "EASL Clinical Practice Guidelines: management of hepatocellular carcinoma". *J Hepatol* 2018;69:182-236.
2. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996;334:693-9.
3. Yao FY, Xiao L, Bass NM, Kerlan R, Ascher NL, et al. Liver transplantation for hepatocellular carcinoma: validation of the UCSF expanded criteria based on pre-operative imaging. *Am J Transpl* 2007;7:2587-96.

4. Herrero JI, Sangro B, Quiroga J, Pardo F, Herraiz M, et al. Influence of tumor characteristics on the outcome of liver transplantation among patients with liver cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2001;7:631-6.
5. Duffy JP, Vardanian A, Benjamin E, Watson M, Farmer DG, et al. Liver transplantation criteria for hepatocellular carcinoma should be expanded. A 22-year experience with 467 patients at UCLA. *Ann Surg* 2007;246:502-11.
6. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009;10:35-43.
7. D'Amico F, Schwartz M, Vitale A, Tabrizian P, Roayaie S, et al. Predicting recurrence after liver transplantation in patients with hepatocellular carcinoma exceeding the up-to-seven criteria. *Liver Transpl* 2009;15:1278-87.
8. Sugawara Y, Tamura S, Makuuchi M. Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. *Dig Dis* 2007;25:310-2.
9. Lee SG, Hwang S, Moon DB, Ahn CS, Kim KH, et al. Expanded indication criteria of living donor liver transplantation for hepatocellular carcinoma at one large-volume center. *Liver Transpl* 2008;14:935-45.
10. Duvoux C, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, et al; Liver Transplantation French Study Group. Liver transplantation for hepatocellular carcinoma: a model including α -fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012;143:986-94.
11. Kaido T, Ogawa K, Mori A, Fujimoto Y, Ito T, et al. Usefulness of the Kyoto criteria as expanded selection criteria for liver transplantation for hepatocellular carcinoma. *Surgery* 2013;154:1053-60.
12. Taketomi A, Sanefuji K, Soejima Y, Yoshizumi T, Uchiyama H, et al. Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. *Transplantation* 2009;87:531-7.
13. Ito T, Takada Y, Ueda M, Haga H, Maetani Y, et al. Expansion of selection criteria for patients with hepatocellular carcinoma in living donor liver transplantation. *Liver Transpl* 2007;13:1637-44.
14. Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, et al. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012;13:e11-22.
15. Alshahrani AA, Ha SM, Hwang S, Ahn CS, Kim KH, et al. Clinical features and surveillance of very late hepatocellular carcinoma recurrence after liver transplantation. *Ann Transplant* 2018;23:659-65.
16. Chok KS, Chan SC, Cheung TT, Chan AC, Fan ST, et al. Late recurrence of hepatocellular carcinoma after liver transplantation. *World J Surg* 2011;35:2058-62.
17. Foerster F, Hoppe-Lotichius M, Vollmar J, Marquardt JU, Weinmann A, et al. Long-term observation of hepatocellular carcinoma recurrence after liver transplantation at a European transplantation centre. *United European Gastroenterol J* 2019;7:838-49.
18. Soin AS, Bhangui P, Kataria T, Baijal SS, Piplani T, et al. Experience with LDLT in patients with hepatocellular carcinoma and portal vein tumor thrombosis postdownstaging. *Transplantation* 2020; doi: 10.1097/TP.0000000000003162.
19. de'Angelis N, Landi F, Carra MC, Azoulay D. Managements of recurrent hepatocellular carcinoma after liver transplantation: a systematic review. *World J Gastroenterol* 2015;21:11185-98.
20. Mazzaferro V, Sposito C, Zhou J, Pinna AD, De Carlis L, et al. Metroticket 2.0 model for analysis of competing risks of death after liver transplantation for hepatocellular carcinoma. *Gastroenterology* 2018;154:128-39.
21. Liang W, Wu L, Ling X, Schroder PM, Ju W, et al. Living donor liver transplantation versus deceased donor liver transplantation for hepatocellular carcinoma: a meta-analysis. *Liver Transpl* 2012;18:1226-36.
22. Fernandez-Sevilla E, Allard MA, Selten J, Golse N, Vibert E, et al. Recurrence of hepatocellular carcinoma after liver transplantation: Is there a place for resection? *Liver Transpl* 2017;23:440-7.
23. Sapisochin G, Goldaracena N, Astete S, Laurence JM, Davidson D, et al. Benefit of treating hepatocellular carcinoma recurrence after liver transplantation and analysis of prognostic factors for survival in a large Euro-American series. *Ann Surg Oncol* 2015;22:2286-94.
24. Kornberg A, Küpper B, Tannapfel A, Katenkamp K, Thrum K, et al. Long-term survival after recurrent hepatocellular carcinoma in liver transplant patients: clinical patterns and outcome variables. *Eur J Surg Oncol* 2010;36:275-80.
25. Valdivieso A, Bustamante J, Gastaca M, Uriarte JG, Ventoso A, et al. Management of hepatocellular carcinoma recurrence after liver transplantation. *Transplant Proc* 2010;42:660-2.
26. Escartin A, Sapisochin G, Bilbao I, Vilallonga R, Bueno J, et al. Recurrence of hepatocellular carcinoma after liver transplantation. *Transplant Proc* 2007;39:2308-10.
27. Kim HR, Cheon SH, Rha SY, Lee S, Han KH, et al. Treatment of recurrent hepatocellular carcinoma after liver transplantation. *Asia Pac J Clin Oncol* 2011;7:258-69.
28. Wang Y, Speeg KV, Washburn WK, Half G. Sorafenib plus sorafenib in treating HCC recurrence after liver transplantation: a case report. *World J Gastroenterol* 2010;16:5518-22.
29. Bhoori S, Toffanin S, Sposito C, Germini A, Pellegrinelli A, et al. Personalized molecular targeted therapy in advanced, recurrent hepatocellular carcinoma after liver transplantation: a proof of principle. *J Hepatol* 2010;52:771-75.
30. Gomez-Martin C, Bustamante J, Castroagudin JF, Salcedo M, Garralda E, et al. Efficacy and safety of sorafenib in combination with mammalian target of rapamycin inhibitors for recurrent hepatocellular carcinoma after liver transplantation. *Liver Transpl* 2012;18:45-52.
31. Sposito C, Mariani L, Germini A, Reyes MF, Bongini M, et al. Comparative efficacy of sorafenib versus best supportive care in recurrent hepatocellular carcinoma after liver transplantation: a case-control study. *J Hepatol* 2013;59:59-66.
32. Weinmann A, Niederle IM, Koch S, Hoppe-Lotichius M, Heise M, et al. Sorafenib for recurrence of hepatocellular carcinoma after liver transplantation. *Dig Liver Dis* 2012;44:432-37.
33. Yoon DH, Ryoo BY, Ryu MH, Lee SG, Hwang S, et al. Sorafenib for recurrent hepatocellular carcinoma after liver transplantation. *Jpn J*

- Clin Oncol 2010;40:768-73.
34. Geissler EK, Schnitzbauer AA, Zülke C, Lamby PE, Proneth A, et al. Sirolimus use in liver transplant recipients with hepatocellular carcinoma: a randomized, multicenter, open-label phase 3 trial. *Transplantation* 2016;100:116-25.
 35. Zhang ZH, Li LX, Li P, Lv SC, Pan B, et al. Sirolimus in liver transplant recipients with hepatocellular carcinoma: an updated meta-analysis. *J Invest Surg* 2019;32:632-41.
 36. Jeng LB, Lee SG, Soin AS, Lee WC, Suh KS, et al. Efficacy and safety of everolimus with reduced tacrolimus in living-donor liver transplant recipients: 12-month results of a randomized multicenter study. *Am J Transplant* 2018;18:1435-46.
 37. Cholongitas E, Mamou C, Rodríguez-Castro KI, Burra P. Mammalian target of rapamycin inhibitors are associated with lower rates of hepatocellular carcinoma recurrence after liver transplantation: a systematic review. *Transpl Int* 2014;27:1039-49.
 38. Vivarelli M, Dazzi A, Zanella M, Cucchetti A, Cescon M, et al. Effect of different immunosuppressive schedules on recurrence-free survival after liver transplantation for hepatocellular carcinoma. *Transplantation* 2010;89:227-31.
 39. Zhou J, Wang Z, Wu ZQ, Qiu SJ, Yu Y, et al. Sirolimus-based immunosuppression therapy in liver transplantation for patients with hepatocellular carcinoma exceeding the Milan criteria. *Transplant Proc* 2008;40:3548-53.
 40. Iavarone M, Invernizzi F, Czauderna C, Sanduzzi-Zamparelli M, Bhoori S, et al. Preliminary experience on safety of regorafenib after sorafenib failure in recurrent hepatocellular carcinoma after liver transplantation. *Am J Transplant* 2019;19:3176-84.
 41. Kittai AS, Oldham H, Cetnar J, Taylor M. Immune checkpoint inhibitors in organ transplant patients. *J Immunother* 2017;40:277-81.
 42. Roayaie S, Schwartz JD, Sung MW, Emre SH, Miller C, et al. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl* 2004;10:534-40.
 43. Regalia E, Fassati LR, Valente U, Pulvirenti A, Damilano I, et al. Pattern and management of recurrent hepatocellular carcinoma after liver transplantation. *J Hepatobiliary Pancreat Surg* 1998;5:29-34.
 44. Bodzin AS, Lunsford KE, Markovic D, Harlander-Locke MP, Busuttill RW, et al. Predicting mortality in patients developing recurrent hepatocellular carcinoma after liver transplantation: impact of treatment modality and recurrence characteristics. *Ann Surg* 2017;266:118-25.
 45. Invenizzi F, Iavarone M, Donato MF, Mazzucco A, Torre M, et al. Pulmonary resection for metastasis of hepatocellular carcinoma recurring after liver transplant: an Italian multicenter experience. *Front Oncol* 2020;10:381.
 46. Tomimaru Y, Sasaki Y, Yamada T, Eguchi H, Takami K, et al. The significance of surgical resection for pulmonary metastasis from hepatocellular carcinoma. *Am J Surg* 2006;192:46-51.
 47. Bates MJ, Farkas E, Taylor D, McFadden PM. Pulmonary resection of metastatic hepatocellular carcinoma after liver transplantation. *Ann Thorac Surg* 2008;85:412-5.
 48. Hwang S, Kim YH, Kim DK, Ahn CS, Moon DB, et al. Resection of pulmonary metastases from hepatocellular carcinoma following liver transplantation. *World J Surg* 2012;36:1592-602.
 49. Nakajima J, Tanaka M, Matsumoto J, Takeuchi E, Fukami T, et al. Appraisal of surgical treatment for pulmonary metastasis from hepatocellular carcinoma. *World J Surg* 2005;29:715-8.
 50. Poon RT, Fan ST, O'Suilleabhain CB, Wong J. Aggressive management of patients with extrahepatic and intrahepatic recurrences of hepatocellular carcinoma by combined resection and locoregional therapy. *J Am Coll Surg* 2002;195:311-8.
 51. O'Suilleabhain CB, Poon RT, Lau CW, Fan ST. Repeated resections of extrahepatic metastases after hepatic resection: an aggressive approach to hepatocellular carcinoma. *Hepatogastroenterology* 2004;51:825-9.
 52. Gwak GY, Jung JO, Sung SW, Lee HS. Long-term survival after pulmonary metastatectomy of hepatocellular carcinoma; treatment outcome or natural history? *Hepatogastroenterology* 2004;51:1428-33.
 53. Han KN, Kim YT, Yoon JH, Suh KS, Song JY, et al. Role of surgical resection for pulmonary metastasis of hepatocellular carcinoma. *Lung Cancer* 2010;70:295-300.
 54. Aoki M, Hatayama Y, Kawaguchi H, Hirose K, Sato M, et al. Stereotactic body radiotherapy for lung metastases as oligo-recurrence: a single institutional study. *J Radiat Res* 2016;57:55-61.
 55. Zhai H, Liang P, Yu XL, Cheng Z, Han ZY, et al. Microwave ablation in treating intrahepatic recurrence of hepatocellular carcinoma after liver transplantation: an analysis of 11 cases. *Int J Hyperthermia* 2015;31:863-8.
 56. Ko HK, Ko GY, Yoon HK, Sung KB. Tumor response to transcatheter arterial chemoembolization in recurrent hepatocellular carcinoma after living donor liver transplantation. *Korean J Radiol* 2007;8:320-7.
 57. Rivera L, Giap H, Miller W, Fisher J, Hillebrand DJ, et al. Hepatic intra-arterial infusion of yttrium-90 microspheres in the treatment of recurrent hepatocellular carcinoma after liver transplantation: a case report. *World J Gastroenterol* 2006;12:5729-32.
 58. Huang J, Yan L, Wu H, Yang J, Liao M, et al. Is radiofrequency ablation applicable for recurrent hepatocellular carcinoma after liver transplantation? *J Surg Res* 2016;200:122-30.
 59. Zhou B, Shan H, Zhu KS, Jiang ZB, Guan SH, et al. Chemoembolization with lobaplatin mixed with iodized oil for unresectable recurrent hepatocellular carcinoma after orthotopic liver transplantation. *J Vasc Interv Radiol* 2010;21:333-8.
 60. Toso C, Mentha G, Majno P. Integrating sorafenib into an algorithm for the management of post-transplant hepatocellular carcinoma recurrence. *J Hepatol* 2013;59:3-5.
 61. Sanuki N, Takeda A, Kunieda E. Role of stereotactic body radiation therapy for hepatocellular carcinoma. *World J Gastroenterol* 2014;20:3100-11.
 62. Sharabi AB, Nirschl CJ, Kochel CM, Nirschl TR, Francica BJ, et al. Stereotactic radiation therapy augments antigen-Specific PD-1-mediated antitumor immune responses via cross-presentation of tumor antigen. *Cancer Immunol Res* 2015;3:345-55.
 63. Parker JJ, Jones JC, Strober S, Knox SJ. Characterization of direct radiation-induced immune function and molecular signalling changes

- in an antigen presenting cell line. *Clin Immunol* 2013;148:44-55.
64. Gupta A, Probst HC, Vuong V, Landshammer A, Muth S, et al. Radiotherapy promotes tumor-specific effector CD8+ T cells via dendritic cell activation. *J Immunol* 2012;189:558-66.
 65. Pfiffer TEF, Seehofer D, Nicolaou A, Neuhaus R, Riess H, et al. Recurrent hepatocellular carcinoma in liver transplant recipients: parameters affecting time to recurrence, treatment options and survival in the sorafenib era. *Tumori* 2011;97:436-41.
 66. Roh YN, David Kwon CH, Song S, Shin M, Man Kim J, et al. The prognosis and treatment outcomes of patients with recurrent hepatocellular carcinoma after liver transplantation. *Clin Transplant* 2014;28:141-8.
 67. Guerrini GP, Berretta M, Tarantino G, Magistri P, Pecchi A, et al. Multimodal oncological approach in patients affected by recurrent hepatocellular carcinoma after liver transplantation. *Eur Rev Med Pharmacol Sci* 2017;21:3421-35.

Original Article

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The transcontinental variability of nonalcoholic fatty liver disease

Claudia P. Oliveira^{1,2}, Angelo Paredes³, Mohammed Siddiqui⁴, Lawrence Serfaty⁵, Abhijit Chowdhury^{6,7}, Jose Tadeu Stefano², Denise Siqueira Vanni², Sherry Boyett⁴, Arun J. Sanyal⁴

¹Department of Gastroenterology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP 01000-000, Brazil.

²Laboratório de Gastroenterologia Clínica e Experimental (LIM-07) do Departamento de Gastroenterologia e Hepatologia do Hospital das Clínicas HCFMUSP da Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP 01000-000, Brazil.

³Internal Medicine, Gastroenterology and Hepatology Services, Brooke Army Medical Center, San Antonio, TX 78234, USA.

⁴Div. of Gastroenterology, Hepatology and Nutrition, Dept. of Internal Medicine, Virginia Commonwealth University School of Medicine, Richmond, VA 23298-0341, USA.

⁵Viral and Metabolic Liver Unit, Department of Hepatology, Saint-Antoine Hospital, 75012 Paris, Paris.

⁶Department of Hepatology, School of Digestive and Liver Disease, Institute of Post Graduate Medical Education & Research, Kolkata 700020, India.

⁷Disease Biology, John C Martin Centre for Liver Research and Innovations Sonarpur, Kolkata 7000150, India.

Correspondence to: Dr. Arun J. Sanyal, Professor of Medicine, Physiology and Molecular Pathology, Virginia Commonwealth University School of Medicine, MCV Box 980341, Richmond, VA 23298-0341, USA. E-mail: asanyal@mcvh-vcu.edu

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Abstract

Aim: To compare the phenotype of lean versus overweight (OW) and obese (OB) subjects with non-alcoholic fatty liver disease (NAFLD) across multiple continents.

Methods: A retrospective study of histologically defined subjects from a single center each in France (Fr), Brazil (Br), India (In) and United States (US) was performed.

Results: A total of 70 lean [body mass index (BMI) < 25 kg/m²] subjects (Fr:Br:In:US: 16:19:22:13) with NAFLD were compared to 136 OW (BMI > 25 kg/m², BMI < 29 kg/m²) ($n = 28:33:52:23$) and 224 OB subjects (BMI > 29 kg/m²) ($n = 81:11:22:103$). Lean French subjects had the lowest incidence of type 2 diabetes while those from Brazil ($P < 0.01$) had the highest. Lean subjects had similar low-density lipoprotein-cholesterol, but higher high-density lipoprotein-cholesterol compared to obese subjects in all regions. In both lean and obese subjects, there were both insulin-sensitive and insulin-resistant subjects. Lean French subjects were most insulin-sensitive while those from



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Brazil were mostly insulin-resistant. For each weight category, subjects from India were more insulin-sensitive than those from other regions. Disease activity increased from lean to overweight to obese in France but was similar across weight categories in other regions.

Conclusion: The phenotype of NAFLD in lean subjects varies by region. Some obese subjects with NAFLD are insulin-sensitive. We hypothesize that genetics and region-specific disease modifiers account for these differences.

Keywords: Non-alcoholic fatty liver disease, phenotype, epidemiology, demographics

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a major cause of liver-related morbidity and mortality^[1]. Nonalcoholic steatohepatitis (NASH), the more aggressive form of NAFLD, can progress to cirrhosis and end-stage liver disease more frequently than non-alcoholic fatty liver (NAFL)^[2]. The principal risk factors for NAFLD include obesity and insulin resistance (IR) and its associated conditions such as type 2 diabetes^[3-5].

Numerous reports from Asia and other parts of the developing world have identified NAFLD, including NASH, in lean subjects without biochemically obvious IR^[6,7]. NAFLD has also been described in lean subjects in western countries^[8-10]. There is however only limited information on the clinical, laboratory, and histological profile of lean versus obese subjects with NAFLD in the West. It is also not known if the histological spectrum of NAFLD and its relationship to IR is similar from one continent to another when subjects of identical body mass index (BMI) are considered. Similarly, when considering afflicted individuals with similar degree of IR, the distribution of histological findings from one continent to another is not known. These are likely to be relevant for either continuing a “one set of risk factors fits all” paradigm globally or informing development of “region-specific” risk stratification approaches.

In this study, we report data from four cohorts of subjects from the USA, Brazil, France and India to address the trans-continental drifts in disease phenotype across the spectrum of BMI and IR. The specific aims were to: (1) define the similarities and differences in disease expression between lean *vs.* obese subjects with NAFLD in the countries represented in this study; and (2) to compare the disease phenotype within specific weight and IR strata from one country to the next.

METHODS

The current study is a retrospective cross-sectional analysis of a cohort of subjects with NAFLD at the participating centers. The participating centers were in Virginia, USA (PI: AJS), Brazil (PI: CPO), France (PI: LS) and India (PI: AC). The data sheets were developed at EUA: (2008-2013); France: (1999-2014); Brazil: (2009-2014); India (2008-2014); and analyzed by the investigators. The study involved an anonymized data set from an existing larger data set and was therefore considered exempt from IRB review.

Patient population

Subjects with biopsy proven NAFLD with a full set of histological and laboratory data were included in this analysis from each site. Exclusion criteria included age < 18 years, absence of full data set including measures of IR, liver injury and function, anthropometric data, lipid profile and liver histology. Subjects with concomitant presence of alternate causes of liver disease, e.g., hepatitis C were also excluded to avoid their confounding effects. Finally, those with drug-induced NAFLD, TPN-associated NAFLD, and bariatric surgery within last 5 years or known infectious, e.g., HIV or known genetic disorders, e.g., abetalipoproteinemia associated with NAFLD, were excluded to keep the analysis focused on “garden-variety” NAFLD.

Assessment of NAFLD

The liver histology was read by an experienced hepatopathologist at each site with multiple prior publications related to NAFLD. The presence of NAFLD was defined by the presence of hepatic steatosis (> 5%) confirmed by a liver biopsy in all instances; the nonalcoholic nature was established by clinical assessment of alcohol consumption to be less than 2 units daily for women and 3 units daily for men over the last 2 years^[11]. Steatohepatitis was defined by the presence of steatosis (> 5%) along with lobular inflammation and cytological ballooning^[11]. The severity of individual histological features of NAFLD was scored using the NIDDK NASH CRN criteria^[12].

Assessment and stratification of body mass index

Lean, overweight and obese states were defined according to the World Health Organization and Modified National Cholesterol Education Program, Adult Treatment Panel III guidelines^[13]. For subjects from India, two separate analyses were performed. In the first, the same body mass index cutoffs used in the West were used to define these states to directly relate the clinical-laboratory-histological findings across continents matched for BMI. A second analysis using the thresholds used for the Asian subcontinent was used in order to evaluate those who were lean versus obese using physiologically relevant parameters for the region^[14,15].

Assessment and stratification of IR

IR was derived from fasting blood glucose and insulin. The Homeostatic Model of Assessment for IR (HOMA-IR) was calculated from the formula: $[22.5 \times \text{fasting insulin (mU/mL)} \times \text{glucose (mmol/L)}]$ ^[16].

To further visualize the relationship of glucose and insulin, the plasma insulin was plotted as a function of fasting plasma glucose. The resulting plot was divided into four quadrants based on a fasting glucose threshold of 100 mg/dL and fasting insulin of 12 μ U/mL. A fasting glucose below 100 mg/dL represents euglycemia and a fasting insulin level above 12 μ U/mL has been associated with IR^[17,18].

Subjects with a glucose < 100 mg/dL and a fasting insulin < 12 μ U/mL were considered insulin sensitive. Those with a glucose < 100 mg/dL but insulin levels > 12 μ U/mL were considered to have IR with relatively intact beta cell function. Those with higher plasma glucose (> 100 mg/dL) and insulin > 12 μ U/mL were considered to have severe IR. When the fasting plasma glucose levels were higher than 100 mg/dL and the corresponding insulin levels declined below 12 μ U/mL subjects were considered to have advanced IR with beta cell failure. Type 2 diabetes was defined by a fasting blood glucose > 126 mg/dL in patients who had a hemoglobin A1c level of 6.5% or greater, an FPG level of 126 mg/dL or greater^[19].

Plan of analysis

Data were analyzed using SPSS version 2. Descriptive statistics of subjects from each site was performed. Data for lean versus overweight versus obese subjects were compared for each country individually. Numerical data were compared using analysis of variance for normally distributed data. Quantitative variables with asymmetric distribution were described as median and interquartile range and compared between groups using the Kruskal-Wallis test. Next, subjects within each BMI strata from each country were compared to other countries correcting for multiple comparisons using Tukey's test. A similar analysis of overweight or obese subjects was also performed. In another analysis, the cohort was stratified by the HOMA scores of < 2, 2-4 and > 4 and a similar analysis performed. Finally, a multivariable regression analysis was performed to evaluate the interactions between BMI, IR and other clinical parameters on liver histology in lean subjects with NAFLD in each region. Significance was set at a *P* value of 0.05.

RESULTS

A total of 430 subjects with biopsy-proven NAFLD was enrolled (USA:Brasil:France:India 139:125:96:63)

Table 1. Demographics and clinical profiles across-country in subjects with NAFLD

Parameter	USA	France	Brazil	India
Age (years)	53.3 ± 0.92	49.7 ± 1.2*	55.08 ± 0.2	38.06 ± 1.6*
Females (%)	71.2	30.9	46.5	66.2
T2DM (%)	21.1	21.6	53.3*	31.0
Hypertension (%)	43.7	25.3	67.4*	11.6
Triglycerides (mg/dL)	204.9 ± 10.3	112.5 ± 11.4*	218.0 ± 7.9	198.3 ± 12.4
Total Cholesterol (mg/dL)	197.8 ± 4.2	206.5 ± 5.5	193.5 ± 4.2	165.8 ± 7.7
LDL-XOL (mg/dL)	125.8 ± 4.5	135.6 ± 7.1	115.5 ± 3.2	93.3 ± 6.6
HDL-XOL (mg/dL)	44.0 ± 5.4	43.0 ± 5.2	46.0 ± 5.5	40.0 ± 4.7

*statistical significance. T2DM: type 2 diabetes mellitus; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; NAFLD: non-alcoholic fatty liver disease

[Table 1]. They included 70 lean ($\text{BMI} < 25 \text{ kg/m}^2$) subjects (Fr:Br:In:US 22:16:19:13), 136 overweight ($\text{Is this BMI} > 25 \text{ kg/m}^2$, $\text{BMI} < 29 \text{ kg/m}^2$) ($n = 52:28:33:23$) and 224 obese subjects ($\text{BMI} > 29 \text{ kg/m}^2$) ($n = 22:81:11:103$). Subjects in the Indian and French cohorts were younger (mean 38.06 ± 1.6 ; 49.7 ± 1.2 , respectively) compared to those from US and Brazil (mean 53.3 ± 0.92 ; 55.08 ± 0.2 , respectively). In France about 70% of the subjects were male while in Brazil and USA approximately 70% were female. In the Brazilian cohort the prevalence of type 2 diabetes mellitus (T2DM) and hypertension were globally higher than other countries.

Comparison of lean vs. overweight and obese subjects

Demographic and clinical profiles

In the US cohort, obese subjects were younger than overweight and lean subjects, respectively (51.9 ± 1.0 ; 53.3 ± 2.1 ; 61.8 ± 1.9 ; $P = 0.02$) [Table 1]. While the proportion of subjects with hypertension or requiring lipid-lowering therapy were similar across the different weight strata, overweight subjects had less type 2 diabetes compared to lean and obese subjects (3.7% vs. 22.2% vs. 28.4%, $P = 0.01$). In the Brazilian cohort the prevalence of T2DM was high in lean subjects, approaching 66% [Figure 1]. In the French cohort, the proportion of individuals with features of the metabolic syndrome increased progressively from lean to overweight to the obese groups. The Indian cohort had more males in the lean group ($P < 0.001$ vs. other groups) and had a progressively greater proportion of subjects with T2DM with progressively higher weight strata.

Insulin resistance

The distribution of insulin and fasting glucose values yielded interesting insights in all regions [Figure 2]. In the US, approximately 20% of lean subjects had a fasting blood glucose $< 100 \text{ mg/dL}$ and a fasting insulin less than 12 mIU/mL . The remaining subjects had evidence of increasing IR with 4 subjects demonstrating IR with beta cell failure, i.e., low fasting insulin ($< 12 \text{ mIU/mL}$) despite a fasting glucose $> 100 \text{ mg/dL}$. As expected, obese subjects had a substantially greater number of insulin resistant subjects with and without beta cell dysfunction. Interestingly, 11 (11.45%) obese subjects had both low fasting glucose and insulin levels suggesting that they were relatively insulin sensitive.

In the Brazilian cohort, 4 (25%) lean subjects had relatively low fasting insulin and glucose levels while the rest showed IR with or without beta cell failure [Figure 2]. As noted in the US cohort, a subset of subjects in the overweight and obese categories also were relatively insulin sensitive (fasting plasma glucose $< 100 \text{ mg/dL}$, fasting plasma insulin $< 12 \text{ } \mu\text{U/mL}$). In France, the majority of lean subjects were relatively insulin sensitive and IR increased progressively from lean-overweight-obese subjects and most overweight and obese subjects have more advanced IR. Subjects from India had lower fasting insulin levels compared to the other cohorts especially those from the US and Brazil even amongst obese subjects. Lean and obese subjects in the Indian cohort had similar insulin sensitivity.

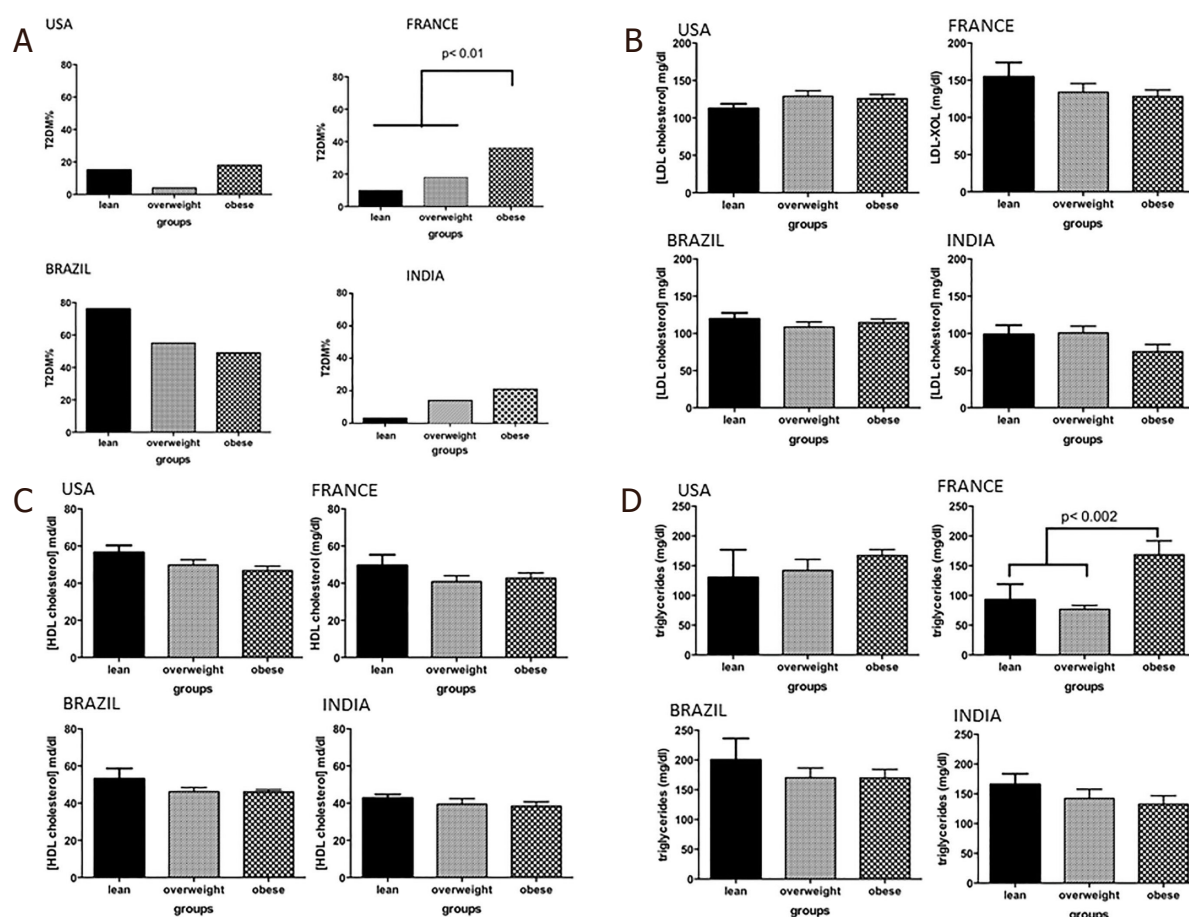


Figure 1. Comorbidities of lean vs. overweight vs. obese subjects with nonalcoholic fatty liver disease. A: type 2 diabetes mellitus (T2DM); B: LDL cholesterol; C: HDL cholesterol; D: triglycerides. LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol

Histology in lean versus obese subjects

The steatosis grade was similar across lean, overweight, and obese subjects in the US cohort [Figure 3]. Similarly, the inflammation, ballooning grades and fibrosis stage were similar across the weight strata. The spectrum of liver histology in subjects from Brazil was similar to that seen in the US. Importantly, the severity of steatosis, inflammation and ballooning were similar across the three weight strata in this country.

On the other hand, obese subjects in the French cohort had significantly greater steatosis grade compared to both lean and overweight subjects ($P < 0.002$). There was also a stepwise increase in inflammation grade from lean to overweight to obese subjects which reached significance (lean vs. obese $P < 0.01$). There was a trend for lower ballooning scores in the lean subjects ($P = 0.06$). Lean and overweight subjects also had a significantly lower fibrosis stage compared to the obese subjects ($P < 0.01$ for both).

Lean Indian subjects had similar degrees of steatosis compared to overweight and obese subjects. The lobular inflammation grades were also similar across the three weight groups. However, in contrast to other regions, the ballooning grade was significantly lower in lean and overweight subjects compared to obese subjects ($P < 0.01$ for both). The fibrosis stage was similar across the three weight categories. Recalibrating BMI threshold for Indians to 22 kg/m^2 for the diagnosis of obesity did not alter the results qualitatively.

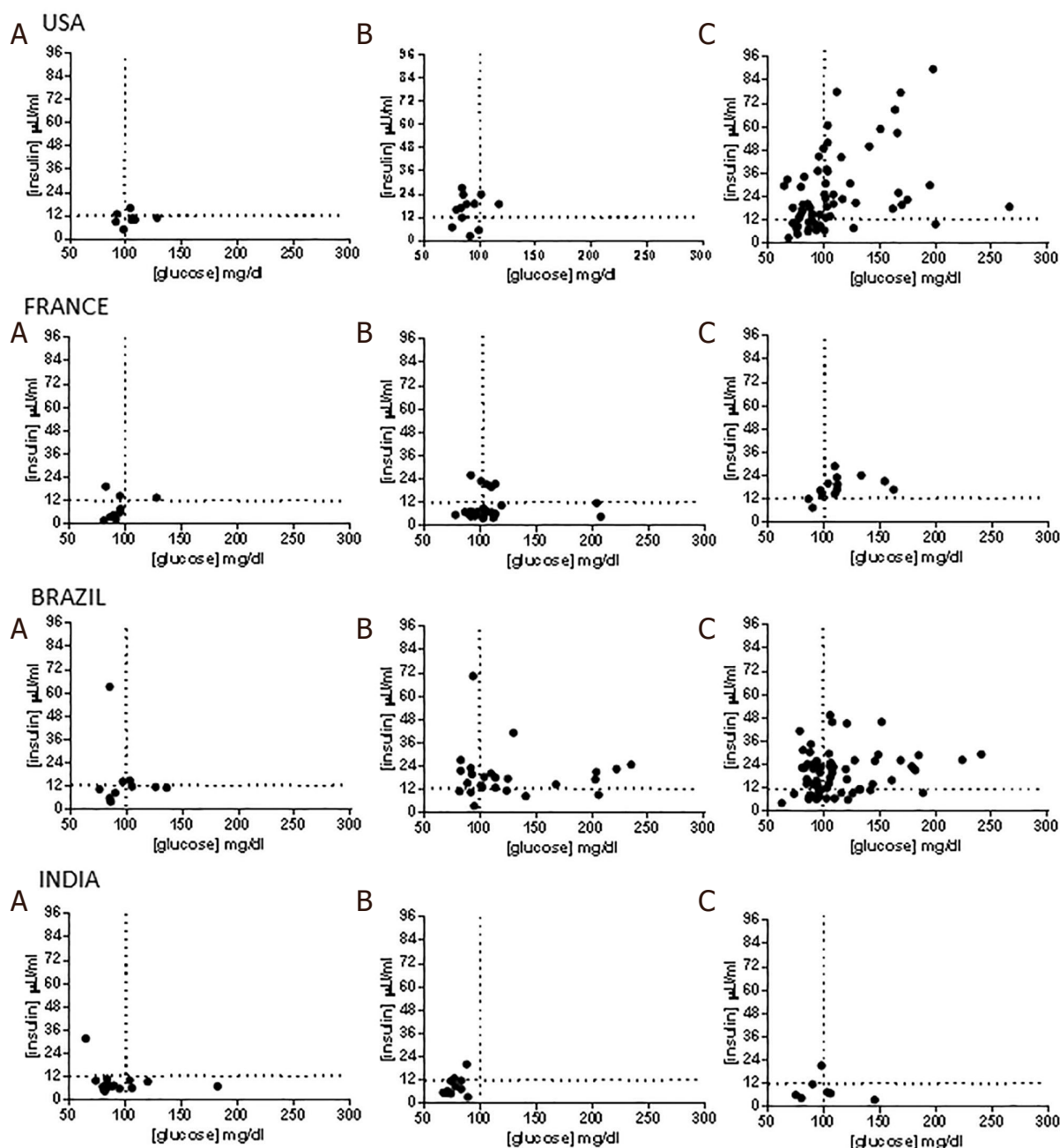


Figure 2. Insulin resistance and insulin sensitivity in nonalcoholic fatty liver disease lean subjects vs. overweight vs. obese across regions. A: lean subjects; B: overweight subjects; C: obese subjects

Clinical-laboratory-histological variability within similar weight strata

Lean subjects

Lean subjects in the US were also more likely to be hypertensive (55.6%) compared to those from Brazil (33.3%), France (14.3%), and India (15.8%). Only 9.5% of lean US subjects had T2DM compared to 26% of subjects in India and 66% of subjects in Brazil [Table 2]. There were no differences in the lipid profile of lean subjects across the various cohorts. However, subjects in Brazil were more insulin resistant than those in the other groups.

In line with greater IR, lean subjects from Brazil (mean grade 2.8) had a higher steatosis grade ($P < 0.0005$)

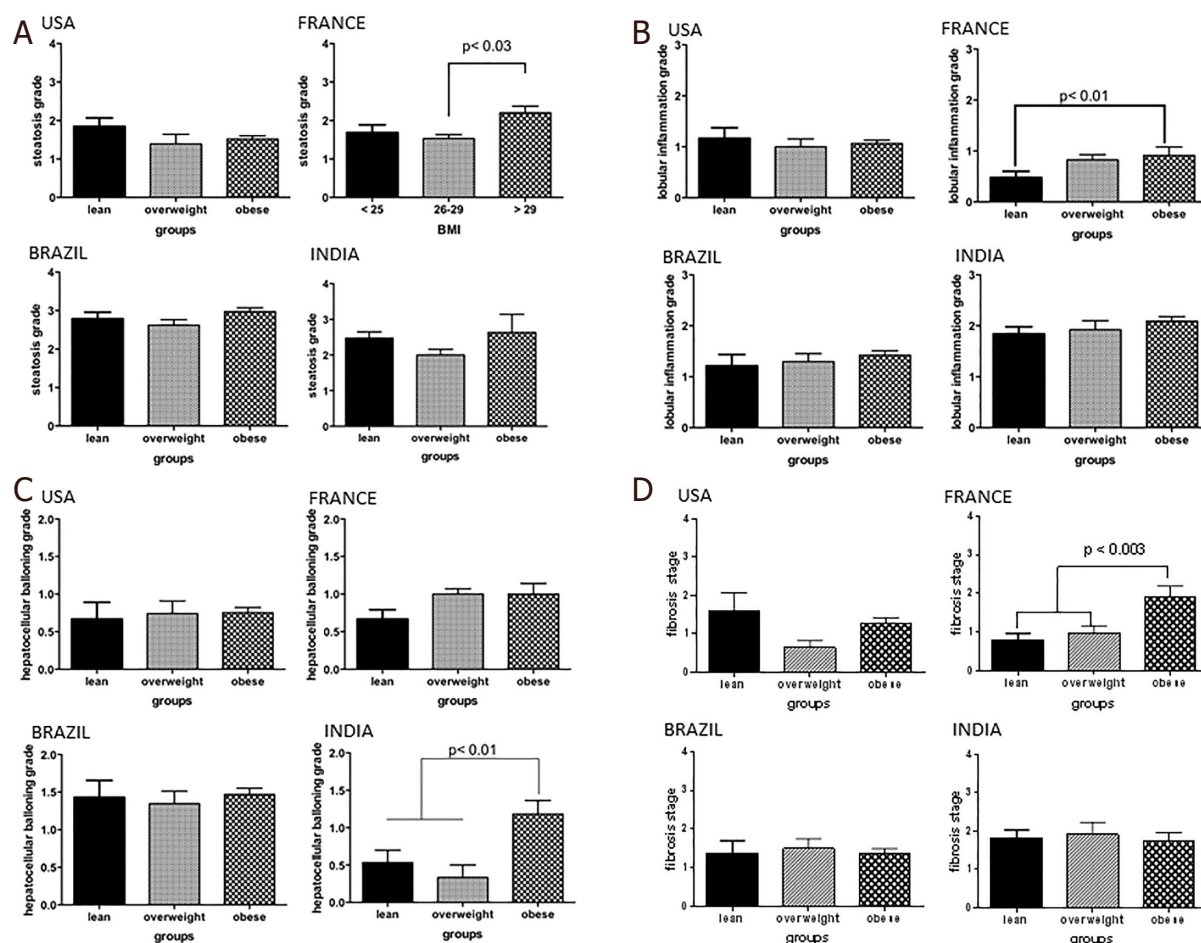


Figure 3. The histological spectrum of nonalcoholic fatty liver disease in lean vs. overweight vs. obese across regions. A: steatosis grade; B: lobular inflammation grade; C: hepatocellular ballooning; D: fibrosis stage

compared to subjects from the US (mean grade 1.9), France (mean grade 1.8), and India (mean grade 2.2). Lean subjects from Brazil also had greater lobular inflammation (mean grade 1.5) compared to subjects from the other countries (mean grade range 0.5-0.7) ($P < 0.0001$). The ballooning grade was similar across the US, Brazilian, and Indian cohorts but was lower in lean subjects from the French cohort ($P < 0.0001$). Similarly, the mean fibrosis stage was similar across the US, Brazil, and India but higher than that seen in the French group ($P < 0.0013$).

Overweight subjects

The prevalence of hypertension and T2DM was higher in the subjects from Brazil. The mean HOMA scores were similar across the four groups. As noted in the lean subjects, a proportion of subjects were still relatively insulin sensitive whereas a proportion was insulin resistant with beta cell failure. Subjects from Brazil had more advanced IR with a majority of subjects with a fasting plasma glucose > 100 mg/dL. Interestingly, the ballooning grade was more severe in overweight subjects from India compared to those from the USA and France (1.7 vs. 1 vs. 0.8, $P < 0.0001$). Subjects from Brazil had intermediate degrees of ballooning injury (mean grade 1.2). The fibrosis stage in overweight subjects in the Indian cohort was modestly higher than the other cohorts (mean: 1.8 vs. 0.6 vs. 1 vs. 1.2 India vs. US vs. France vs. Brazil) [Table 3].

Obese subjects

In Brazil almost 80% of the obese subjects were hypertensive while 49.5%, 44.4%, and 18.2% of obese

Table 2. Comparison of lean subjects across study cohorts

Parameter	USA	Brazil	France	India
Age (years)	58.3 ± 0.7	57.7 ± 4	46 ± 6	40.5 ± 3
Males (%)	0	33.3	71.4	84.2
Type 2 diabetes (%)	22.2	55.6	9.5	26.3
Hypertension (%)	55.6	33.3	14.3	15.8
Dyslipidemia (%)	44.4	55.6	33.3	31.6
AST (IU/L)	50 (40-78)	35 (20-93)	29 (19-47)	45 (24-60)
ALT (IU/L)	49 (30-114)	52 (22-101)	60 (45-79)	56 (31-84)
GGT (IU/L)	170 (134-276)	50 (25-286)	75 (50-134)	45 (36-72)
Triglycerides (mg/dL)	83 (83)	129 (99-221)	52 (22-100)	132 (111-187)
HDL cholesterol (mg/dL)	47 (47)	51 (38-66)	44 (37-63)	43 (35-49)
INR	1 (0.9-1)	1 (1-1)	1.03 (1-1.10)	1.2 (1.17-1.22)
Steatohepatitis (%)	55.6	77.8	60	84
Steatosis grade - 0/1/2/3 (%)	0/33/44/23	0/22/55/22	0/55/20/25	10.5/36.8/47.4/5.3
Lobular inflammation - 0/1/2/3 (%)	12.5/62.5/12.5/12.5	33/22/33/12	50/40/10/0	26.3/63.2/10.5/0
Ballooning - 0/1/2 (%)	44.4/22.2/33.4	11/77/12	35/60/5	53/40/7
Fibrosis stage - 0-none/1-perisinusoidal/2-portal-periportal/3-bridging/4-cirrhosis (%)	11.1/33.3/0/33.3/22.2	0/44/44/12/0	21.4/50/14.3/14.3/0	57.9/15.8/15.8/10.5/0
Glucose (mg/dL)	95 (87-107)	88 (85-126)	90 (83-95)	84 (80-95)
Insulin (mU/mL)	10 (7-12)	11 (6-15)	5 (3-14)	7 (6-10)
HOMA	2.35	2.39	1.11	1.45

ALT: alanine aminotransferase; AST: aspartate aminotransferase; HOMA: homeostatic model assessment; INR: international normalized ratio

Table 3. Comparison of overweight subjects across study cohorts

Parameter	USA	Brazil	France	India
Age (years)	54.1 ± 6	55.1 ± 2	51 ± 7	34.1 ± 5
Males (%)	30	48.6	70.2	46.2
Type 2 diabetes (%)	3.7	56.8	19.3	14.3
Hypertension (%)	30	45.9	23.6	0
Dyslipidemia (%)	44.4	73.0	21.4	30.8
AST (IU/L)	50 (39-84)	30 (22-41)	31 (23-48)	40 (29-56)
ALT (IU/L)	60 (46-95)	36 (27-57)	63 (48-80)	45 (38-79)
GGT (IU/L)	83 (38-156)	66 (30-160)	50 (27-103)	46 (33-55)
Triglycerides (mg/dL)	141 (76-195)	150 (107-241)	66 (40-108)	120 (112-155)
HDL cholesterol (mg/dL)	50 (40-61)	48 (38-52)	40 (30-52)	37 (32-45)
INR	1 (1-1)	0.98 (0.95-1)	1.08 (1.02-1.13)	1.2 (1.14-1.27)
Steatohepatitis (%)	60	73	80	70
Steatosis grade - 0/1/2/3 (%)	25/30/15/30	5.4/40.5/43.2/10.8	0/50/40/10	15.4/69.2/15.4/0
Lobular inflammation - 0/1/2/3 (%)	20.8/58.3/20.8/0	14.3/54.3/25.7/5.7	19.6/64.3/14.3/1.8	23.1/61.5/15.4/0
Ballooning - 0/1/2 (%)	45.8/37.5/16.7	60/40/10	12.5/71.5/16	67/33/0
Fibrosis stage - 0-none/1-perisinusoidal/2-portal-periportal/3-bridging/4-cirrhosis (%)	0/57.7/19.4/15.4/7.7	36.1/13.9/27.8/17.7/5.6	26.2/35.7/19.0/7.1/11.9	53.8/7.7/30.8/7.7/0
Glucose (mg/dL)	91 (83-102)	110 (94-141)	103 (94-113)	78 (72-84)
Insulin (mU/mL)	16 (10-19)	16 (12-21.5)	7 (5-16)	7 (6-12)
HOMA	3.6	4.35	1.78	1.35

ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyl transferase; HOMA: homeostatic model assessment; HDL: high-density lipoprotein cholesterol; INR: international normalized ratio

subjects in US, France, and India respectively were hypertensive. 100% of the obese subjects in the Indian cohort had T2DM [Table 4]. The lipid profiles were comparable amongst obese subjects from different countries.

As expected, most subjects had IR. Interestingly, a subset of obese subjects from each country had a fasting

Table 4. Comparison of obese subjects across study cohorts

Parameter	USA	Brazil	France	India
Age (years)	52.4 ± 2	55.1 ± 5	50.7	38.4 ± 5
Males (%)	38	24.7	66.7	9.1
Type 2 diabetes (%)	28.4	51.9	44.4	100
Hypertension (%)	50	79.2	44.4	18.2
Dyslipidemia (%)	38	70.1	61.1	9.1
AST (IU/L)	40 (27-63)	35 (20-93)	48 (29-58)	36 (23-62)
ALT (IU/L)	53 (34-83)	52 (22-101)	76 (57-99)	33 (29-78)
GGT (IU/L)	38 (27-66)	53 (27-87)	55 (36-128)	33 (28-46)
Triglycerides (mg/dL)	149 (107-211)	129 (99-221)	141 (107-256)	126 (99-155)
HDL cholesterol (mg/dL)	43 (36-50)	51 (38-66)	43 (36-48)	38 (32-42)
INR	1 (0.97-1.0)	1 (1.0-1.0)	1.04(0.96-1.06)	1.3 (1.2-1.3)
Steatohepatitis (%)	56.8	89.6	81.3	90
Steatosis grade - 0/1/2/3 (%)	6.5/44.6/33.7/15.2	5.3/20.0/45.3/10.8	0/13/57/30	0/36.4/63.6/0
Lobular inflammation - 0/1/2/3 (%)	8.8/76.9/14.3/0	8/46.7/37.3/8	12.5/68.8/18.8/0	0/90/10/0
Ballooning - 0/1/2 (%)	38.6/45.5/15.9	46.6/52/1.3	12.5/68.8/18.8	9/63.6/27.4
Fibrosis stage - 0-none/1-perisinusoidal/2-portal-periportal/3-bridging/4-cirrhosis (%)	36.6/28.9/8.9/21.1/4.4	22.7/44/10.7/16/6.7	12.5/18.8/31.3/12.5/25.0	45.5/36.4/18.2/0/0
Glucose (mg/dL)	101 (86-124)	103 (90-127)	104 (96-143)	93 (81-106)
Insulin (mU/mL)	20 (14-37)	17 (11-26)	17 (14-20)	7 (5-20)
HOMA	4.99	4.32	4.37	1.65

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyl transferase; HDL: high-density lipoprotein cholesterol; INR: international normalized ratio; HOMA: homeostatic model assessment

plasma glucose < 100 mg/dL as well as a fasting plasma insulin < 12 µU/mL indicating that they were relatively insulin sensitive. The proportion of such individuals was greatest in the cohort from India. The US cohort had somewhat lower steatosis grade whereas subjects from India had more inflammation and ballooning. The fibrosis stages were similar across the groups.

Transcontinental variability in histological severity in those with similar degrees of IR

A potential reason for the variability in histology from one country to another could be the variable degree of IR in each weight strata. In order to evaluate this, subjects were stratified into those with mild, moderate and severe IR (HOMA: 0-2, 2.1-4 and > 4 respectively). The severity of individual histological features was then assessed across the four study cohorts.

As expected, the severity of steatosis was largely similar across the four groups with the exception of somewhat lower steatosis in the Indian group in those with moderate IR ($P < 0.01$ vs. France). On the other hand, the group in Brazil had lower scores for lobular inflammation compared to other groups across multiple IR strata. They also had lower ballooning grades especially in those with moderate or severe IR. This was accompanied by less fibrosis across all strata of IR.

DISCUSSION

Body weight and IR are two of the best-known risk factors for NAFLD. While it is known that many subjects with NAFLD in Asia are lean and do not have the usual biochemical features of IR, it was not known if a similar profile was also seen in lean subjects in other regions of the world. It is however generally believed that the relationship between body weight and severity of IR on one hand, versus liver histology is generally similar in all parts of the world. The current study challenges this “one size fits all” approach and demonstrates substantial differences and heterogeneity from countries in one continent versus even within similar weight strata.

The prevalence of obesity, T2DM and hypertension are higher in the USA and Brazil than in some

European countries like France^[20-23]. In Brazil, the prevalence of arterial hypertension found was 21.4% (95%CI: 20.8-22.0) using the self-reported criterion, 22.8% (95%CI: 22.1-23.4) for measured arterial hypertension, and 32.3% (95%CI: 31.7-33.0) for hypertension arterial pressure and / or report of medication use^[24]. Furthermore, according IDF Atlas, in Brazil and USA, the prevalence of T2DM in 2019 was 8.5% and 11.1% respectively, while in Europe was 6.3%. USA is the third-highest country with patients with T2DM in the world, and Brazil the fifth^[20]. Also, caloric consumption in American countries is higher than in some countries and in Europe^[25]. In addition, patients in Brazil and the USA come from large tertiary-level university hospitals, where patients of greater severity are referred to them.

An important observation in this study is the remarkable range of laboratory-histological findings when comparing lean subjects with overweight and obese subjects in the different countries where this study was conducted. In France, a clear stepwise worsening of IR and severity of liver histology was noted with increasing BMI. In contrast, lean subjects in Brazil were more insulin-resistant and like overweight and obese subjects with respect to both the severity of their IR and liver histology. Subjects from the United States and India had an intermediate relationship with Indian subjects tending to demonstrate generally lower insulin levels than those from the other cohorts at all weight strata. These corroborate the concept that the severity of NAFLD is not a simple function of increasing body mass and that these relationships can be variable from one region to another.

The current study further demonstrates that the spectrum of IR and liver histology is variable from one country to another even when the body mass is accounted for. Lean subjects in the Brazilian cohort were more insulin-resistant and had greater steatosis than subjects from other regions. However, the severity of cytological ballooning was similar in the US, Brazil, and India and higher than that seen in the French subjects. These data are in line with the concept that development of liver injury is more than a simple function of IR and that additional factors are likely to play a role. While theoretically possible, we believe it is unlikely that these data purely represent differences in how the histology was read by the local pathologists since all of the pathologists involved are senior and experienced pathologists who have previously published in the field.

Another noteworthy finding in this study is that even in Western countries, a subset of overweight and even obese subjects with NAFLD were relatively insulin sensitive, i.e., with a fasting insulin < 12 μ U/mL and [glucose] < 100 mg/dL. The lower blood glucose could not be attributed to the use of anti-diabetic therapy alone although it may have played a role in a few subjects. The only potential exception was the obese Indian cohort where all subjects were known to have diabetes and many also had a fasting plasma glucose < 100 mg/dL. While the presence of insulin sensitive obese individuals is well established^[26], there is a general perception that these so called “fit-fat” subjects do not develop end organ diseases typically associated with IR. The current study further demonstrates that the distribution of liver histology in these subjects includes the full spectrum of NAFLD. The mechanisms underlying the liver disease in these subjects are not well understood. Unfortunately, due to the retrospective nature of this current analysis, the subjects were not genotyped and their PNPLA3 and TM6SF2 and other SNPs associated with the metabolic syndrome were not available. This is now a logical future direction of research in the field.

It is also not known if obese insulin-sensitive subjects respond to insulin sensitizers and improve their liver histology in the same manner as obese insulin resistant subjects. In the PIVENS trial, baseline HOMA scores did not predict histological response to pioglitazone^[27,28]. This study however did not evaluate assess the insulin sensitivity status by HOMA alone but by graphical analysis of the relationship of glucose and insulin. It is also recognized that an euglycemic hyperinsulinemic clamp is the gold standard for measurement of insulin sensitivity^[29]. In this retrospective analysis, this was not possible. However, it will be valuable to perform these in obese subjects with NAFLD who have a low fasting glucose and

insulin level in the future to be certain that they are indeed insulin sensitive. Regardless, more information about the pathophysiological mechanisms underlying disease development and progression in obese or overweight insulin sensitive subjects will help future efforts to tailor specific therapies that are most likely to engage the relevant therapeutic targets in these individuals.

There is a difference in the aggressiveness of NAFLD between American and European countries, mainly Mediterranean countries. One interesting example is the number of liver transplantations in USA secondary NASH compared with Europe countries^[30]. In Brazil there has also been an increase in the numbers of liver transplantations secondary to NASH. Several hypotheses can be considered for the greater severity of fibrosis in American countries, including lean NASH: (1) Higher caloric intake in the Americas, diet rich in fructose and trans lipids^[31] low consumption of Mediterranean diet, including fiber and omega 3; (2) greater sedentary lifestyle in American countries; (3) presence of genetic factors such as PNPLA3^[32]; and (4) dysbiosis, even in Lean NASH patients^[33]. Furthermore, in the USA and Brazil, there is also admixture with genes from non-caucasian Hispanic and native American genes. American Hispanics have more severe NASH. The complex genetic background may be another potential explanation. Unfortunately given the sample size of this initial assessment, it is not feasible to address the role of genetics. Similarly, there may be differences in diet and also microbiome functionality that are not captured by current methods that could play a role. NASH is a complex disorder driven by gene environment interactions.

There were also differences in the spectrum of other end organ diseases within lean subjects with NAFLD. These corroborate the concept that the pathogenesis of each of these conditions is complex and there are regional variations in the prevalence rates and severity. All of these data further attest to the trans-continental drifts in phenotype development of this cluster of diseases.

There are several potential explanations for the observed variations in NAFLD phenotype and associated conditions across the four cohorts studied. These include genetic differences in susceptibility, regional variation in diet patterns, differences in the intestinal microbiome, exercise, environmental exposures etc. While a detailed analysis of these is beyond the scope of this manuscript, such studies are now indicated to better understand the variations in phenotype development in different regions.

Perhaps the most relevant implication of the current study is that it provides proof of concept that there are differences in both the histological phenotype and associated clinical features in subjects with NAFLD in different regions of the world and that region-specific data are now needed to provide optimal guidance for clinical care on various regions. Also, it raises the possibility that therapeutic responses in one region may or may not be similar from one region to another. A clearer understanding of variable disease mechanisms underlying the differences in phenotype development should help targeting therapeutics towards the most relevant targets in a given region and phenotype in the future.

The principal limitations of the current study are the relatively modest size of lean subjects with NAFLD in various regions and the potential for ascertainment bias. The small numbers of lean subjects however reflect the fact that they represent a small fraction of all subjects with NAFLD. Also, another limitation is that we report the prevalence of fibrosis stage in patients with NAFLD who had a biopsy at the centers in this study. Since this was done according to standard of care, the selection of patients for a biopsy was variable across centers. Further, the distribution of fibrosis could also be impacted by ascertainment bias due to the nature of the center. It was seen in the Indian cohort probably because increasing age is linked to higher stages of fibrosis and is generally considered to be due to longer exposure to disease state. It is potentially possible that the low fibrosis stage in India reflected the lower age of the population studied. It is noted in the section about variations across centers and as a potential explanation for the differences in fibrosis stage in India vs. other sites. However, most published studies of NAFLD also have the same ascertainment bias associated with tertiary care academic medical centers. These limitations notwithstanding, the current study demonstrates substantial variability in disease phenotype from one region to another.

In summary, the current study provides novel information on the variability in disease phenotype in lean as well as overweight and obese subjects in different parts of the world. It challenges the paradigm that all lean subjects with NAFLD have mild IR and have mild forms of liver disease. Conversely, it also demonstrates that a fraction of obese subjects with the full spectrum of NAFLD may be relatively insulin sensitive. Future studies to define the mechanistic basis for these differences may inform therapeutic choices in subjects in different regions.

DECLARATIONS

Authors' contributions

Design: Paredes A, Siddiqui M, Boyett S, Sanyal AJ

Performance: Oliveira CP, Paredes A, Siddiqui M, Serfaty L, Chowdhury A, Stefano JT, Vanni DS, Boyett S, Sanyal AJ

Analysis: Oliveira CP, Paredes A, Serfaty L, Chowdhury A, Sanyal AJ

Writing: Oliveira CP, Serfaty L, Chowdhury A, Stefano JT, Vanni DS, Sanyal AJ

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

This was a retrospective study, approval from the study number 164.116 at the local Ethics Committee.

Consent for publication

Not applicable.

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REFERENCES

1. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. *Hepatology* 2017;65:1557-65.
2. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015;148:547-55.
3. Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* 2009;29:113-9.
4. Ercin CN, Dogru T, Genc H, Celebi G, Aslan F, et al. Insulin resistance but not visceral adiposity index is associated with liver fibrosis in nondiabetic subjects with nonalcoholic fatty liver disease. *Metab Syndr Relat Disord* 2015;13:319-25.
5. Dumas ME, Kinross J, Nicholson JK. Metabolic phenotyping and systems biology approaches to understanding metabolic syndrome and fatty liver disease. *Gastroenterology* 2014;146:46-62.
6. Feng RN, Du SS, Wang C, Li YC, Liu LY, et al. Lean-non-alcoholic fatty liver disease increases risk for metabolic disorders in a normal weight Chinese population. *World J Gastroenterol* 2014;20:17932-40.
7. Akyuz U, Yesil A, Yilmaz Y. Characterization of lean patients with nonalcoholic fatty liver disease: potential role of high hemoglobin levels. *Scand J Gastroenterol* 2015;50:341-6.
8. Younossi ZM, Stepanova M, Negro F, Hallaji S, Younossi Y, et al. Nonalcoholic fatty liver disease in lean individuals in the United States. *Medicine (Baltimore)* 2012;91:319-27.
9. Margariti E, Deutsch M, Manolakopoulos S, Papatheodoridis GV. Non-alcoholic fatty liver disease may develop in individuals with

- normal body mass index. *Ann Gastroenterol* 2012;25:45-51.
10. Das K, Chowdhury A. Lean NASH: distinctiveness and clinical implication. *Hepatol Int* 2013;7 Suppl 2:806-13.
 11. Younossi ZM, Stepanova M, Rafiq N, Makhlof H, Younossi Z, et al. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. *Hepatology* 2011;53:1874-82.
 12. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41:1313-21.
 13. Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA* 2001;285:2486-97.
 14. Misra A. Revisions of cutoffs of body mass index to define overweight and obesity are needed for the Asian-ethnic groups. *Int J Obes Relat Metab Disord* 2003;27:1294-6.
 15. Misra A, Chowbey P, Makkar BM, Vikram NK, Wasir JS, et al. Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. *J Assoc Physicians India* 2009;57:163-70.
 16. Ascaso JF, Pardo S, Real JT, Lorente RI, Priego A, et al. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. *Diabetes Care* 2003;26:3320-5.
 17. Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* 2005;48:634-42.
 18. Arata M, Nakajima J, Nishimata S, Nagata T, Kawashima H. Nonalcoholic steatohepatitis and insulin resistance in children. *World J Diabetes* 2014;5:917-23.
 19. Menke A, Casagrande S, Geiss L, Cowie CC. Prevalence of and trends in diabetes among adults in the United States, 1988-2012. *JAMA* 2015;314:1021-9.
 20. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, et al. IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* 2018;138:271-81.
 21. Matta J, Carette C, Rives Lange C, Czernichow S. French and worldwide epidemiology of obesity. *Presse Med* 2018;47:434-8.
 22. Martins-Silva T, Vaz JDS, Mola CL, Assunção MCF, Tovo-Rodrigues L. Prevalence of obesity in rural and urban areas in Brazil: national health survey, 2013. *Rev Bras Epidemiol* 2019;22:e190049.
 23. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014;384:766-81.
 24. Malta DC, Gonçalves RPF, Machado Í, Freitas MIF, Azeredo C, et al. Prevalence of arterial hypertension according to different diagnostic criteria, national health survey. *Rev Bras Epidemiol* 2018;21:e180021.
 25. UN Food and Agricultural Organization FAOstat food balance sheets. Chapter 3: Calculation Of The Energy Content Of Foods - Energy Conversion Factors. Food and Agriculture Organization of the United Nations. Available from: <http://www.fao.org/docrep/006/Y5022E/y5022e04.htm>. [Last accessed on 24 Sep 2020]
 26. Duncan GE. The “fit but fat” concept revisited: population-based estimates using NHANES. *Int J Behav Nutr Phys Act* 2010;7:47.
 27. Pacana T, Sanyal AJ. Vitamin E and nonalcoholic fatty liver disease. *Curr Opin Clin Nutr Metab Care* 2012;15:641-8.
 28. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010;362:1675-85.
 29. Borai A, Livingstone C, Ferns GA. The biochemical assessment of insulin resistance. *Ann Clin Biochem* 2007;44:324-42.
 30. Younossi ZM, Stepanova M, Ong J, Trimble G, AlQahtani S, et al. Nonalcoholic steatohepatitis is the most rapidly increasing indication for liver transplantation in the United States. *Clin Gastroenterol Hepatol* 2020; doi: 10.1016/j.cgh.2020.05.064.
 31. Vilar L, Oliveira CP, Faintuch J, Mello ES, Nogueira MA, et al. High-fat diet: a trigger of non-alcoholic steatohepatitis? Preliminary findings in obese subjects. *Nutrition* 2008;24:1097-102.
 32. Mazo DF, Malta FM, Stefano JT, Salles APM, Gomes-Gouveia MS, et al. Validation of PNPLA3 polymorphisms as risk factor for NAFLD and liver fibrosis in an admixed population. *Ann Hepatol* 2019;18:466-71.
 33. Duarte SMB, Stefano JT, Miele L, Ponziani FR, Souza-Basqueira M, et al. Gut microbiome composition in lean patients with NASH is associated with liver damage independent of caloric intake: a prospective pilot study. *Nutr Metab Cardiovasc Dis* 2018;28:369-84.

Review

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Cutaneous toxicities of targeted therapies in the treatment of hepatocellular carcinoma

Tarik Silk¹, Jennifer Wu²

¹Internal Medicine, New York University School of Medicine, New York, NY 10016, USA.

²Hematology and Medical Oncology, Perlmutter Cancer Center, New York University School of Medicine, New York, NY 10016, USA.

Correspondence to: Dr. Jennifer Wu, Hematology and Medical Oncology, Perlmutter Cancer Center, New York University School of Medicine, New York, NY 10016, USA. E-mail: jennifer.wu@nychhc.org

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Abstract

Liver cancer accounts for 4.7% of all newly diagnosed cancers and 8.2% of cancer deaths annually. Hepatocellular carcinoma (HCC) accounts for the majority of primary liver cancers. There are 2 curative strategies in HCC: resection and transplant. Unfortunately, 50% of patients who undergo resection will relapse in 2 years and many patients on transplant lists become ineligible for transplant due to disease progression. The majority of patients still require systemic therapies. Tyrosine kinase inhibitors have successfully extended the overall survival in patients with hepatocellular carcinoma. However, these treatments have been noted to cause severe side effects including liver toxicity, hypertension, gastrointestinal toxicity and cutaneous adverse effects. This article will focus on the adverse skin reactions seen during the treatment of hepatocellular carcinoma by various tyrosine kinase inhibitors. The focus will be symptomatology, management, and whether the development of cutaneous toxicities can be prognostic.

Keywords: Hepatocellular cancer, tyrosine kinase inhibitors, cutaneous toxicity, hand foot reaction syndrome

BACKGROUND

Liver cancer accounts for 4.7% of all newly diagnosed cancers and 8.2% of cancer deaths annually^[1]. Hepatocellular carcinoma (HCC) accounts for the majority of primary liver cancers^[2]. As much as 50% of patients with HCC are diagnosed at early stages when curative treatments are possible^[3]. There are



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2 curative strategies in HCC: resection and transplant. Unfortunately, 50% of patients who undergo resection will relapse in 2 years and many patients on transplant lists become ineligible for transplant due to disease progression. The majority of patients still require systemic therapies. In 2008, the FDA approved the tyrosine kinase inhibitor (TKI) Sorafenib as the first systemic therapy for patients with inoperable hepatocellular cancer. Its approval was based on the overall survival (OS) benefit of Sorafenib compared to placebo in the first-line setting 10.7 months vs. 7.9 months, respectively^[4]. Since Sorafenib's approval, several other tyrosine kinase inhibitors have been added to the armamentarium against late-stage HCC. These new TKIs include Lenvatinib, Cabozantinib, and Regorafenib^[5-7]. Cutaneous toxicities are frequently observed during targeted treatment of cancer, as many targeted therapies, including TKIs, inhibit growth factors found in the skin^[8].

In this review, we will focus on the adverse skin reactions seen during the treatment of hepatocellular carcinoma by various tyrosine kinase inhibitors. The focus will be on symptomatology and management of these reactions. Additionally, this review will discuss whether the development of cutaneous toxicities can be prognostic.

Symptomatology and treatment for particular cutaneous toxicities are discussed with each major toxicity. The cutaneous toxicities of particular TKIs will be discussed within each TKI's section along with the toxicity's onset and suggested dose modifications of the TKI.

HAND FOOT REACTION SYNDROME

Risk factors and incidence

The incidence of hand foot reaction syndrome (HFRS) in the treatment of HCC varies depending on the TKI^[9]. Predisposing factors to HFRS seen in TKI treatments include female gender, ECOG status 2 or lower, two or more organs involved, baseline lung/liver metastases, a baseline white blood count above 5.5×10^9 cells/L, and the duration of the TKI therapy^[10]. Frequency is also higher in Asian populations^[11,12]. Whereas incidences of HFRS are varied, symptomatology of HFRS is shared between TKIs.

Symptoms

Patients affected by HFRS experience a prodromal tingling sensation on their palms or soles which progresses to burning pain. Hand and foot erythema and edema develops with tense blistering and peeling with lesions evolving into areas of callus-like hyperkeratosis with surrounding erythema^[13-16].

Lesions tend to develop over pressure-bearing surfaces, which may be related to the pathophysiology of their development.

Pathophysiology

Although competing hypotheses exist^[9,17,18], the TKI dual inhibition of different receptors, such as VEGF and PDGFR, may be required to trigger dermatological symptoms. Damage occurs when TKIs block signaling pathways resulting in the alteration of repair mechanisms or distortion to microvascular structure in areas where there is frequent trauma or friction like the palms and soles^[12,14,18].

Toxicity grading and treatment

Management of HFRS is based on expert knowledge from oncologists and dermatologists. Before TKI treatment begins, proper consultation with podiatry and dermatology should be made. A full-body skin examination with special attention paid to the hands and feet should be performed. Evidence of abnormal weight bearing can be corrected with mechanical support. Areas of baseline hyperkeratotic skin can be removed with manicures and pedicures^[19,20]. During treatment, twice daily prophylactic application of a 20% urea-based cream has also been suggested by some experts to prevent HFRS^[20,21]. When treatment

is started patients should be advised to avoid tight footwear or gloves. Patients should protect their feet with insole cushions, shock-absorbing soles, and padded socks and wear gloves to protect their hands. Emollients can be used liberally.

At night, patients should continue to wear cotton gloves and socks to retain moisture. They should also avoid extreme temperatures and wash with tepid water^[14,15,18,20,22].

Further recommendations are based upon the grade of HFRS severity. The toxicity grading of HFRS is characterized according to the National Cancer Institute. Grade 1 toxicity is characterized by minimal skin changes or dermatitis (i.e., edema, redness, or hyperkeratosis) but without pain. Grade 2 toxicity develops with skin changes (i.e., peeling, blisters, bleeding, fissures, edema, or hyperkeratosis) with pain and limits daily activities. Grade 3 toxicity is marked by severe skin changes (i.e., peeling, blisters, bleeding, fissures, edema, or hyperkeratosis) with pain that limits self-care^[23].

Grade 1 toxicities can be managed with topical therapy usually with the continuation of the TKI dose^[19,20,22]. Topical treatments are similar to those used for prophylactic care. 20%-40% urea creams should be used on areas of hyperkeratosis twice a day^[15,18,20,22].

Grade 2 toxicities are managed with topical therapy and dose reductions if necessary. Areas of hyperkeratosis should be treated with urea base keratolytic^[16]. 0.05% Clobetasol ointment should be applied to erythematous areas twice daily^[15,20,22]. Topical 2% lidocaine cream can be used for analgesia^[20,22].

For Grade 3 toxicities in addition to the above treatments, dose interruption and reduction is required. Other strategies include the use of pyridoxine with doses of 50-150 mg/day^[20,22,24].

Of note, in one uncontrolled study of 12 patients who developed HFRS when taking Sorafenib, the majority of patients that were treated twice daily with 40% urea cream in combination with tazarotene 0.1% cream or fluorouracil 5% cream saw a ≥ 2 -grade improvement in symptoms^[25] [Figure 1].

RASH

Symptoms

Rashes observed with TKI treatment vary and can develop as a macule, papule, maculopapular, erythematous, and/or pruritic. Rashes often develop on the trunk and extremities or scrotum^[15,26-28]. Facial and scalp erythema similar to seborrheic dermatitis has been noted with Sorafenib.

Treatment

The treatment of rashes seen during TKI therapy is once again based upon the toxicity grade. Grade 1 toxicity is described as macules/papules covering $< 10\%$ body surface area (BSA) with or without symptoms (e.g., pruritus, burning, tightness). Grade 2 toxicity is characterized by macules/papules covering 10%-30% BSA with or without symptoms (e.g., pruritus, burning, tightness); limiting instrumental activities of daily living (ADL), or a rash covering $> 30\%$ BSA with or without mild symptoms. Grade 3 toxicity is defined by macules/papules covering $> 30\%$ BSA with moderate or severe symptoms; limiting self-care ADL^[23].

In general, patients with rashes should be advised to use perfume-free soaps, apply moisturizers, and wear loose comfortable clothing^[22,29]. Topical corticosteroids and antihistamines may also be useful with mild rashes^[22,30]. Dose reductions and interruptions of the TKI should also occur based on prescribing information for severe rashes. With proper management, most rashes have been noted to resolve in less than 6 weeks^[27]. Additionally, some rashes may resolve spontaneously without symptomatic treatment as can be seen in patients with facial and scalp erythema^[26,27] [Figure 2].



Figure 1. Clinical spectrum of HFRS of grade 1 (A,D) grade 2 (B,E) and grade 3(C,F). Used with permission from Lipworth *et al.*^[13], Copyright © 2009 Karger Publishers, Basel, Switzerland. HFRS: hand foot reaction syndrome



Figure 2. Example of rash on the trunk with TKI treatment. Used with permission under the Creative Commons Attribution License. (<http://creativecommons.org/licenses/by/2.0>)^[31]. TKI: tyrosine kinase inhibitor



Figure 3. Subungual splinter hemorrhages in a patient on Sorafenib. Reprinted from Ishak *et al.*^[33]

SCROTAL ECZEMA

Symptoms

Scrotal eczema appears as erythematous macules^[32].

Treatment

Scrotal lesions seen with TKIs have been treated with athletic supporters to reduce friction and barrier ointments/pastes such as zinc oxide and menthol. Topical steroids can also be used^[15,32].

SUBUNGUAL SPLINTER HEMORRHAGES

Symptoms

Subungual splinter hemorrhages appear as multiple subcentimeter longitudinal brown to black lines beneath the distal nail plate. They are asymptomatic^[26].

Treatment

Subungual splinter hemorrhages do not require treatment as they tend to spontaneously resolve^[15,26,27] [Figure 3].

ALOPECIA

Symptoms

Alopecia may occur with thinning or patchy hair loss. Complete alopecia has been seen in patients with renal cell carcinoma treated with Sorafenib. Alopecia can involve loss of hair on the body as well as the scalp^[26].

Treatment

Alopecia produced by TKIs although distressing to patients has not been shown to require dose modification. Alopecia has spontaneously resolved in some patients despite continued treatment^[26].



Figure 4. Example of stomatitis seen with TKI treatment. Used with permission under Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>)^[39]. TKI: tyrosine kinase inhibitor

STOMATITIS

Symptoms

Stomatitis or oral mucositis may manifest as dry mouth, oral sensitivity, dysphagia, or taste changes^[29,34]. Ulcers may also occur^[22].

Toxicity grading and treatment

Like HFRS, prevention may be the best strategy. Prophylactic measures include good oral hygiene by brushing teeth with a soft-headed toothbrush and fluoride toothpaste after meals and before sleep. Flossing once a day and using alcohol-free mouth rinses four times daily is encouraged. Dentures should be cleaned daily. Patients need to avoid spicy and sticky foods, as well as alcohol and tobacco^[29,34,35].

Further recommendations are based upon the grade of stomatitis severity. Adverse events of stomatitis are characterized according to the National Cancer Institute. Grade 1 toxicities are described as asymptomatic or mild symptoms. Grade 2 toxicities are characterized by moderate pain or ulcers that do not interfere with oral intake. Grade 3 toxicities involve severe pain interfering with oral intake^[23].

When adverse events do occur, grade 1/2 events can be managed with magic mouthwash or swish spit rinses with 0.9% sodium bicarbonate or 0.9% saline- containing mouthwash^[36,37]. Magic mouthwashes typically contain a mixture of diphenhydramine, viscous lidocaine, nystatin, dyclonine magnesium hydroxide, or aluminum hydroxide, and occasionally corticosteroids. However, bland saline rinses may be just as effective as magic mouthwashes^[36]. Topical anesthetics, mucosal coating agents, and/or benzydamine HCl may be administered as needed for pain relief, but patients should be advised to avoid eating or performing oral hygiene when their mouth is numb. Grade 3 events require dose interruption and reduction^[22,34,38] [Figure 4].



Figure 5. Example of erythema multiforme. Image author James Heilman, MD. Obtained under Creative Commons license Attribution-Share Alike 3.0 Unported. <https://creativecommons.org/licenses/by-sa/3.0/deed.en>

ERYTHEMA MULTIFORME

Symptoms

Erythema Multiforme is typically a self-limited acute skin reaction that has been reported to occur within the first week of TKI treatment for HCC^[40-42]. Lesions described in the case reports were targetoid erythematous lesions spread over the trunk and extremities. In one report, a patient developed painful oral lesions and, in general, oral lesions are found in up to 60% of people with erythema multiforme^[42,43]. Erythema multiforme is diagnosed clinically based on the patient's history and physical examination. It usually has fixed lesions for a minimum of 7 days.

Treatment

Treatment involves medication discontinuation. Additionally for mild disease oral antihistamines with or without topical steroids are prescribed^[42,44]. If painful mucosal erosions are present, they can be treated with high potency topical corticosteroid gels and magic mouthwashes. If oral lesions prevent sufficient oral intake, studies have recommended systemic glucocorticoids, including prednisone 40-60 mg daily with dosage taper over 2-4 weeks^[42,44] [Figure 5].

STEVENS-JOHNSON SYNDROME AND TOXIC EPIDERMAL NECROLYSIS

Symptoms

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) have both been reported to occur in patients with HCC treated with TKIs^[40,45]. These are both life-threatening reactions that usually begin 4-28 days after taking the offending medication. SJS and TEN are a spectrum of epidermal necrolysis with SJS occurring with less than 10% skin detachment and TEN when there is greater than 30%. Anything in between is called SJS-TEN^[46,47]. Flu-like symptoms usually precede cutaneous manifestations. Initial lesions are usually erythematous, irregularly shaped, and can first appear on the face, upper trunk, and proximal extremities. Necrotic lesions coalesce and slough off either spontaneously or with applied lateral pressure (as seen in Nikolsky's sign) revealing the red underlying dermis.



Figure 6. Example of Toxic Epidermal Necrolysis. Image author Afro Brazilian. Obtained with permission under Creative Commons license Attribution-Share Alike 3.0 Unported. <https://creativecommons.org/licenses/by-sa/3.0/deed.en>

Treatment

Treatment involves a multidisciplinary approach. The TKI must be stopped and the patient should be transferred to the intensive care unit or burn unit. Supportive care measures involve thermoregulation with an ambient temperature of 28-32 degrees Celsius. If a respiratory compromise is suspected, these patients can be intubated. Administration of fluid replacement is advised at 0.7 mL/kg/(% affected area) along with a 5% albumin solution at 1 mL/kg/(% affected area)^[46,47]. Systemic corticosteroids are the most common treatment for SJS/TEN, a suggested protocol is intravenous dexamethasone at 1.5 mg/kg pulse therapy (given for 30-60 min) for 3 consecutive days^[46,48]. Cyclosporine and Etanercept have also shown benefit^[49,50]. Daily skin treatment is recommended with a daily antiseptic bath containing a solution of chlorhexidine 1/5000 or with a chlorhexidine spray. Skin debridement should be avoided because necrolytic sheets act as a natural biological dressing. Nonadhesive dressings are used to cover pressure points^[49,50] [Figure 6].

TYROSINE KINASE INHIBITORS USED FOR HCC TREATMENT

Tyrosine kinase inhibitors, including Sorafenib, Lenvatinib, Cabozantinib, and Regorafenib are small molecules that attack cancers by inhibiting the activity of receptor tyrosine kinases which are responsible for tumor-promoting pathways such as proliferation and angiogenesis^[51].

SORAFENIB

Sorafenib cutaneous toxicities and toxicity incidence

Sorafenib was FDA approved for the treatment of HCC in 2008 after a phase three randomized placebo-controlled trial showed that it extended patient survival. Safety results of that study reported that 80% of patients taking Sorafenib experienced an adverse event with the majority of patients experiencing grade 1 or 2 dermatological events. These events and the percentage of patients reporting them included: alopecia (14%, grade 3: 0%), dry skin (8%, grade 3: 0%), HFRS (24%, grade 3: 8%), pruritus (8%, grade 3: 0%), rash and/or desquamation (16%, grade 3: 1%)^[4]. Similar adverse events were also seen in another study that investigated Sorafenib therapy in an Asian-Pacific population^[11]. Other cutaneous toxicities reported with Sorafenib include sublingual splinter hemorrhage which occurs in as many as 60%-70% of patients as well as case reports of scrotal eczema^[32,52].

The onset of cutaneous toxicity with Sorafenib

HFRS occurs within days or months after starting Sorafenib, but most commonly manifests during the first 6 weeks of therapy^[22,26,27]. Rashes usually present on the extremities and/or the trunk within the first to the second month of treatment^[22]. Scrotal rashes have been seen between the 2nd and 12th weeks of therapy^[22,27]. Alopecia observed in patients treated with Sorafenib occurs within four months of treatment^[26,27]. The subungual splinter hemorrhages appear within the first 2 months of treatment^[26,27].

It is recommended that physicians see their patients in 2-week intervals for the first 2 months of Sorafenib treatment to manage skin toxicities^[20,22].

Dose reductions

The starting dose of Sorafenib is 800 mg daily. Dose reductions for skin toxicity are seen in [Tables 1, 2, and 3](#)^[38].

LENVATINIB

Lenvatinib cutaneous toxicity and toxicity incidence

In a phase 3 trial, Lenvatinib was shown to be non-inferior to Sorafenib for OS in the first-line treatment of HCC that was not amenable to curative or local therapy^[13]. Cutaneous toxicities seen with Lenvatinib include: HFRS (27%, grade 3: 3%), alopecia (3% grade 3: 0%) and rash (10%, grade 3: 0%)^[7].

The onset of cutaneous toxicity with Lenvatinib

The onset of Lenvatinib's cutaneous toxicities for the treatment of HCC has not been described. However, their onset has been documented during the use of Lenvatinib for thyroid cancer^[28]. The median time to the first onset of HFSR was 5.9 weeks and 7.3 weeks for rash. Most rashes occurred during the first cycle of therapy. Whereas HFRS could occur throughout therapy.

Dose reductions

Lenvatinib for HCC is dosed depending on body weight with patients 60 kg or greater started at 12 mg daily whereas those less than 60 kg starting at 8 mg daily. Dose reductions for skin toxicities are displayed in [Tables 1, 2 and 3](#)^[53].

CABOZANTINIB

Cabozantinib cutaneous toxicity and toxicity incidence

Cabozantinib has been approved as second-line therapy for patients with HCC who have progressed on Sorafenib. In phase 3 CELESTIAL trial, Cabozantinib treated patients' median OS was 10.2 months (95%CI: 9.1-12.0) compared to 8.0 months (95%CI: 6.8-9.4) in patients treated with placebo. Cabozantinib cutaneous toxicities and incidences include: HFRS (46%, grade 3: 17%), stomatitis (13%, grade 3: 2%), and rash (12%, grade 3 < 1%)^[6].

Table 1. Hand foot reaction syndrome and tyrosine kinase inhibitor treatment modification

Hand and Foot Reaction Syndrome	Tyrosine kinase inhibitor			
	Sorafenib	Lenvatinib	Cabozantinib	Regorafenib
I: Minimal skin changes or dermatitis (i.e., edema, redness, or hyperkeratosis) without pain	No modification, use topical relief agents			
II: skin changes (i.e., peeling, blisters, bleeding, fissures, edema, or hyperkeratosis) with pain and limiting daily activities	1st occurrence: Continue treatment and consider topical therapy for symptomatic relief. If no improvement within 7 days, see below No improvement, 2nd or 3rd occurrence: Interrupt treatment until toxicity resolves to Grade 0-1. When resuming treatment, decrease dose by one dose level (400 mg daily or 400 mg every other day) 4th occurrence: Discontinue	1st occurrence: withhold until improves to Grade 0-1 or baseline. Resume at 8 mg if ≥ 60 kg, 4 mg if < 60 kg 2nd occurrence: withhold until improves to Grade 0-1 or baseline. Resume at 4 mg if ≥ 60 kg, 4 mg Q.O.D. if < 60 kg 3rd occurrence: Withhold until improves to Grade 0-1 or baseline. Resume at 4 mg Q.O.D. if ≥ 60 kg, discontinue < 60 kg	1st occurrence: withhold until improves to Grade 1 or baseline. Resume at 40 mg daily 2nd occurrence: Withhold until improves to Grade 1 or baseline. Resume at 20 mg daily 3rd occurrence: withhold until improves to Grade 1 or baseline. Resume at 20 mg daily if tolerated or discontinue	1st occurrence: Reduce dose to 120 mg daily and start supportive therapy. If the toxicity does not improve within 7 days interrupt therapy until the toxicity resolves or improves to grade 1. Then can restart at 120 mg daily 2nd occurrence: Interrupt and institute supportive measures until toxicity resolves or improves to grade 1. Reduce dose to 80 mg daily 3rd occurrence: Discontinue if failure to tolerate 80 mg daily dose
III: Moist desquamation, ulceration, blistering, or severe pain of the hands or feet, resulting in inability to work or perform activities of daily living	1st occurrence: Interrupt treatment until toxicity resolves to Grade 0-1. When resuming treatment, decrease dose by one dose level (400 mg daily or 400 mg every other day) 2nd occurrence: Interrupt treatment until toxicity resolves to Grade 0-1. When resuming treatment, decrease dose by one dose level (400 mg daily or 400 mg every other day) 3rd occurrence: Discontinue	Proceed the same as for grade II toxicity		1st occurrence: Interrupt treatment and start supportive measures. Treatment must be interrupted until toxicity resolves or improves to grade 1. But treatment must be stopped for a minimum of 7 days. Resume dose at 120 mg daily 2nd occurrence: Interrupt treatment and start supportive measures. Treatment must be interrupted until toxicity resolves or improves to grade 1. But treatment must be stopped for a minimum of 7 days. Resume dose at 80 mg daily 3rd occurrence: Discontinue

Table 2. Rash and tyrosine kinase inhibitor treatment modifications

Rash	Tyrosine kinase inhibitor			
	Sorafenib	Lenvatinib	Cabozantinib	Regorafenib
I: Macules/papules covering $< 10\%$ body surface area (BSA) with or without symptoms	No modification, use topical relief agents			
II: Macules/papules covering 10 - 30% BSA with or without symptoms (e.g., pruritus, burning, tightness); limiting instrumental activities of daily living (ADL); rash covering $> 30\%$ BSA with or without mild symptoms	1st occurrence: Continue treatment and consider topical therapy for symptomatic relief. If no improvement within 7 days, see below No improvement, 2nd or 3rd occurrence: Interrupt treatment until toxicity resolves to Grade 0-1. When resuming treatment, decrease dose by one dose level (400 mg daily or 400 mg every other day) 4th occurrence: Discontinue	1st occurrence: withhold until improves to Grade 0-1 or baseline. Resume at 8 mg if ≥ 60 kg, 4 mg if < 60 kg 2nd occurrence: withhold until improves to Grade 0-1 or baseline. Resume at 4 mg if ≥ 60 kg, 4 mg Q.O.D. if < 60 kg 3rd occurrence: Withhold until improves to Grade 0-1 or baseline. Resume at 4 mg Q.O.D. if ≥ 60 kg, discontinue < 60 kg	1st occurrence: withhold until improves to Grade 1 or baseline. Resume at 40 mg daily 2nd occurrence: Withhold until improves to Grade 1 or baseline. Resume at 20 mg daily 3rd occurrence: withhold until improves to Grade 1 or baseline. Resume at 20 mg daily if tolerated or discontinue	1st occurrence: Reduce dose to 120 mg daily and start supportive therapy. If the toxicity does not improve within 7 days interrupt therapy until the toxicity resolves or improves to grade 1. Then can restart at 120 mg daily 2nd occurrence: Interrupt and institute supportive measures until toxicity resolves or improves to grade 1. Reduce dose to 80 mg daily 3rd occurrence: Discontinue if failure to tolerate 80 mg daily dose

III: Macules/papules covering > 30% BSA with moderate or severe symptoms; limiting self care ADL	1st occurrence: Interrupt treatment until toxicity resolves to Grade 0-1. When resuming treatment, decrease dose by one dose level (400 mg daily or 400 mg every other day) 2nd occurrence: Interrupt treatment until toxicity resolves to Grade 0-1. When resuming treatment, decrease dose by one dose level (400 mg daily or 400 mg every other day) 3rd occurrence: Discontinue	Proceed the same as for grade II toxicity	1st occurrence: Interrupt treatment and start supportive measures. Interrupt until toxicity resolves or improves to grade 1. But treatment must be stopped for a minimum of 7 days. Resume dose at 120 mg daily 2nd occurrence: Interrupt treatment and start supportive measures. Interrupted until toxicity resolves or improves to grade 1. Stop for a minimum of 7 days. Resume dose at 80 mg daily 3rd occurrence: Discontinue
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Table 3. Stomatitis and tyrosine kinase inhibitor treatment modifications

Stomatitis	Tyrosine kinase inhibitor			
	Sorafenib	Lenvatinib	Cabozantinib	Regorafenib
I: asymptomatic or mild symptoms	No modification, use topical relief agents			
II: moderate pain or ulcers that do not interfere with oral intake	1st occurrence: Continue treatment and consider topical therapy for symptomatic relief. If no improvement within 7 days, see below No improvement, 2nd or 3rd occurrence: Interrupt treatment until toxicity resolves to Grade 0-1. When resuming treatment, decrease dose by one dose level (400 mg daily or 400 mg every other day) 4th occurrence: Discontinue	1st occurrence: withhold until improves to Grade 0-1 or baseline. Resume at 8 mg if ≥ 60 kg, 4 mg if < 60 kg 2nd occurrence: withhold until improves to Grade 0-1 or baseline. Resume at 4 mg if ≥ 60 kg, 4 mg Q.O.D. if < 60 kg 3rd occurrence: Withhold until improves to Grade 0-1 or baseline. Resume at 4 mg Q.O.D if ≥ 60 kg, discontinue < 60 kg	1st occurrence: withhold until improves to Grade 1 or baseline. Resume at 40 mg daily 2nd occurrence: Withhold until improves to Grade 1 or baseline. Resume at 20 mg daily 3rd occurrence: withhold until improves to Grade 1 or baseline. Resume at 20 mg daily if tolerated or discontinue	1st occurrence: Reduce dose to 120 mg daily and start supportive therapy. If the toxicity does not improve within 7 days interrupt therapy until the toxicity resolves or improves to grade 1. Then can restart at 120 mg daily 2nd occurrence: Interrupt and institute supportive measures until toxicity resolves or improves to grade 1. Reduce dose to 80 mg daily 3rd occurrence: Discontinue if failure to tolerate 80 mg daily dose
III: Severe pain interfering with oral intake	1st occurrence: Interrupt treatment until toxicity resolves to Grade 0-1. When resuming treatment, decrease dose by one dose level (400 mg daily or 400 mg every other day) 2nd occurrence: Interrupt treatment until toxicity resolves to Grade 0-1. When resuming treatment, decrease dose by one dose level (400 mg daily or 400 mg every other day) 3rd occurrence: Discontinue	Proceed the same as for grade II toxicity		1st occurrence: Interrupt treatment and start supportive measures. Treatment must be interrupted until toxicity resolves or improves to grade 1. But treatment must be stopped for a minimum of 7 days. Resume dose at 120 mg daily 2nd occurrence: Interrupt treatment and start supportive measures. Treatment must be interrupted until toxicity resolves or improves to grade 1. But treatment must be stopped for a minimum of 7 days. Resume dose at 80 mg daily 3rd occurrence: Discontinue

The onset of cutaneous toxicity with Cabozantinib

The onset of cutaneous toxicity with the use of Cabozantinib to treat HCC has not been described. However, the onset has been documented in the treatment of progressive urothelial carcinoma by Zuo *et al.*^[15] and in renal clear cell carcinoma by Choueiri *et al.*^[54] About half of the patients in each study treated with Cabozantinib developed HFRS with a median onset of 4-5 weeks. Additionally In the study by Zuo *et al.*^[15] skin changes, including xerosis and scrotal erythema, developed within approximately 5 weeks of Cabozantinib treatment.

Dose reduction

The starting dose of Cabozantinib is 60 mg daily for the treatment of HCC. Dose reductions for skin toxicities are displayed in [Tables 1, 2 and 3](#)^[55].

REGORAFENIB

Regorafenib Cutaneous Toxicity and Toxicity Incidence

Regorafenib is another TKI approved for second-line therapy of HCC. The RESORCE trial showed that in patients whose HCC progressed on Sorafenib, those subsequently treated with Regorafenib had improved median OS to 10.6 months (95%CI: 9.1-12.1) compared to 7.8 months (95%CI: 6.3-8.8) in those treated with placebo^[5]. A follow up study also found that patients sequentially treated with Regorafenib after Sorafenib had improved OS from the start of Sorafenib treatment to death on study compared to placebo control. 26.0 months (95%CI: 22.6-28.1) for Regorafenib and 19.2 months (95%CI: 16.3-22.8) for placebo^[56].

In the RESORCE study, all patients receiving Regorafenib experienced an adverse event. Cutaneous toxicities related to Regorafenib and their incidences include HFRS (53%, grade 3: 13%) and stomatitis (13% grade 3: 1%). Of note, 2% of patients suffering from HFRS discontinued Regorafenib treatment^[5].

The onset of cutaneous toxicity with Regorafenib

Regorafenib induced HFRS occurs early in treatment with one study showing a median time to the first occurrence of 15 days^[57]. Onset of stomatitis usually occurs between 5 and 14 days after treatment initiation^[58].

Monitoring of patients should occur frequently especially early in treatment. Patients should be seen at least every 1-2 weeks during the first two cycles and every 4-6 weeks thereafter^[14,59-61].

Dose reduction

The recommended starting dose of Regorafenib is 160 mg daily for 21 days followed by 7 days of a dosing free interval to complete a 28-day cycle. Treatment is continued until disease progression or unacceptable toxicity. Dose reductions with Regorafenib are seen in [Tables 1, 2, and 3](#)^[62].

The incidences of the most common cutaneous toxicities and their toxicity grade are summarized for each tyrosine kinase inhibitor in [Table 4](#).

ADVERSE EVENTS AND EFFICACY OF TKIS

The relationship between patients experiencing adverse events and treatment efficacy has been noted by multiple investigators. In a study of 65 patients treated with Sorafenib, patients who developed at least grade 1 skin toxicity had tumor control rates of 48.3% versus 19.4% in patients who did not develop skin toxicity^[63]. Another study of Sorafenib showed a positive association between higher-grade skin toxicity (> grade 2) and disease control when compared to patients who developed lower grade toxicity or had no skin toxicity^[64]. An association was also seen with adverse events and overall survival in patients treated

Table 4. Incidence of TKI induced cutaneous toxicities during HCC therapy

Tyrosine kinase inhibitor	Cutaneous toxicity	Incidence of grade 1 or 2 toxicity	Incidence of grade 3 toxicity
Sorafenib	Hand foot reaction syndrome	24%	8%
	Rash	16%	1%
	Alopecia	14%	0%
Lenvatinib	Hand foot reaction syndrome	27%	3%
	Rash	10%	0%
	Alopecia	3%	0%
Cabozantinib	Hand foot reaction syndrome	46%	17%
	Rash	12%	< 1%
	Stomatitis	13%	2%
Regorafenib	Hand foot reaction syndrome	53%	13%
	Stomatitis	13%	1%

TKI: tyrosine kinase inhibitor; HCC: hepatocellular carcinoma

Table 5. Recommendations for adverse event management

Adverse event	Recommended management strategies	
	Prophylactic management	Adverse event management
Hand and foot reaction syndrome	Baseline full body skin examination Removal of any pre-treatment hyperkeratotic skin Consider use of prophylactic 20% urea based cream applied twice a day Avoid tight footwear or gloves Use of emollients liberally	Topical 20%-40% urea creams applied to areas of hyperkeratosis twice daily Clobetasol 0.05% ointment applied to erythematous areas twice daily Topical 2% lidocaine cream for analgesia
Rash	Use perfume free soap Loose comfortable clothing Liberal use of moisturizers	Corticosteroids Antihistamines
Scrotal eczema	Athletic supporter	Zinc oxide and menthol barrier ointment Topical corticosteroids
Stomatitis	Brush teeth after meals with soft toothbrush Mouth rinses without alcohol Avoid spicy foods, alcohol and tobacco	Magic mouthwashes or oral rinses with 0.9% saline Topical mucosal anesthetics
Erythema multiforme	None	Antihistamines Topical corticosteroids Systemic glucocorticoids
Stevens johnson syndrome	None	Transfer to intensive care unit or burn unit Thermoregulation of body temperature to 28-32 Celsius Fluid replacement Systemic corticosteroids Antiseptic baths

with Lenvatinib^[65]. Similar results were also noted for Cabozantinib with patients who experienced any grade HFRS having improved median overall survival and progression-free survival compared to those who did not develop HFRS^[66]. The development of HFRS and rash was also associated with overall survival in patients treated with Regorafenib for metastatic colorectal cancer^[67]. Although these associations have been documented with TKIs, a physiologic relationship has not been described. But it has been postulated that the association between cutaneous toxicity and treatment efficacy could be caused by variations in pharmacokinetics as both the toxicity and the response may be dose-dependent^[65]. It has also been suggested that patients who develop skin toxicities may have tyrosine kinase polymorphisms that are more sensitive to drug inhibition which results in greater anti-tumor control but more skin toxicity^[61]. More investigation is warranted but if treatment efficacy is dose-dependent then symptomatic relief of cutaneous toxicity is warranted to maintain medication compliance to achieve maximum results. However, if treatment efficacy is pre-determined by tyrosine kinase polymorphism then further genetic screening is warranted to determine who will benefit most from TKI therapy.

CONCLUSION

Tyrosine kinase inhibitors have successfully extended the overall survival in patients with hepatocellular carcinoma^[4-7]. However these treatments have been noted to cause severe side effects including liver toxicity, hypertension, gastrointestinal toxicity and the discussed cutaneous toxicities^[68]. These cutaneous toxicities tend to occur during the first and second months of treatment and can be managed with symptomatic treatment and dose reductions if necessary. Additional prophylactic measures can help prevent the manifestation of the some of most common cutaneous toxicities including HFRS and stomatitis. It is in these authors' opinions on and others that the monitoring of TKI treated patients for cutaneous toxicities should be conducted every 2 weeks for the first few months of treatment and a baseline dermatological exam is also necessary^[22,58,68]. A summary of recommendations for the management of cutaneous adverse events are displayed in Table 5. As cutaneous toxicities have also been seen with more recently developed TKIs, including Afatinib and Dorafenib, it appears as more TKIs are used to treat HCC that these cutaneous toxicities will remain as treatment side effects requiring careful management^[69,70].

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Made substantial contributions to conception and design of the review article: Silk T, Wu J

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
2. Villanueva A. Hepatocellular carcinoma. *N Engl J Med* 2019;380:1450-62.
3. Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol* 2018;15:599-616.
4. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378-90.
5. Bruix J, Qin S, Merle P, Granito A, Huang Y, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;389:56-66.
6. Abou-Alfa GK, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, et al. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. *N Engl J Med* 2018;379:54-63.
7. Kudo M, Finn RS, Qin S, Han K, Ikeda K, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018;391:1163-73.
8. Reyes-Habito CM, Roh EK. Cutaneous reactions to chemotherapeutic drugs and targeted therapy for cancer: Part II. Targeted therapy. *J*

- Am Acad Dermatol 2014;71:217.e1-11; quiz 227-8.
9. Chu D, Lacouture ME, Fillos T, Wu S. Risk of hand-foot skin reaction with sorafenib: a systematic review and meta-analysis. *Acta Oncol* 2008;47:176-86.
 10. Dranitsaris G, Vincent MD, Yu J, Huang L, Fang F, et al. Development and validation of a prediction index for hand-foot skin reaction in cancer patients receiving sorafenib. *Ann Oncol* 2012;23:2103-8.
 11. Cheng A, Kang Y, Chen Z, Tsao C, Qin S, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009;10:25-34.
 12. Zhang HL, Qin XJ, Wang HK, Gu WJ, Ma CG, et al. Clinicopathological and prognostic factors for long-term survival in Chinese patients with metastatic renal cell carcinoma treated with sorafenib: a single-center retrospective study. *Oncotarget* 2015;6:36870-83.
 13. Lipworth AD, Robert C, Zhu AX. Hand-foot syndrome (hand-foot skin reaction, palmar-plantar erythrodysesthesia): focus on sorafenib and sunitinib. *Oncology* 2009;77:257-71.
 14. McLellan B, Ciardiello F, Lacouture ME, Segar S, Van Cutsem E. Regorafenib-associated hand-foot skin reaction: practical advice on diagnosis, prevention, and management. *Ann Oncol* 2015;26:2017-26.
 15. Zuo RC, Apolo AB, DiGiovanna JJ, Parnes HL, Keen CM, et al. Cutaneous adverse effects associated with the tyrosine-kinase inhibitor cabozantinib. *JAMA Dermatol* 2015;151:170-7.
 16. Reed N, Glen H, Gerrard G, Good J, Lei M, et al. Expert consensus on the management of adverse events during treatment with lenvatinib for thyroid cancer. *Clin Oncol (R Coll Radiol)* 2020;32:e145-53.
 17. Jacobi U, Waibler E, Schulze P, Schouli J, Oskay-Ozelik G, et al. Release of doxorubicin in sweat: first step to induce the palmar-plantar erythrodysesthesia syndrome? *Ann Oncol* 2005;16:1210-1.
 18. Lacouture ME, Wu S, Robert C, Atkins MB, Kong HH, et al. Evolving strategies for the management of hand-foot skin reaction associated with the multitargeted kinase inhibitors sorafenib and sunitinib. *Oncologist* 2008;13:1001-11.
 19. Ai L, Xu Z, Yang B, He Q, Luo P. Sorafenib-associated hand-foot skin reaction: practical advice on diagnosis, mechanism, prevention, and management. *Expert Rev Clin Pharmacol* 2019;12:1121-7.
 20. Anderson R, Jatoi A, Robert C, Wood LS, Keating KN, et al. Search for evidence-based approaches for the prevention and palliation of hand-foot skin reaction (HFSR) caused by the multikinase inhibitors (MKIs). *Oncologist* 2009;14:291-302.
 21. Ren Z, Zhu K, Kang H, Lu M, Qu Z, et al. Randomized controlled trial of the prophylactic effect of urea-based cream on sorafenib-associated hand-foot skin reactions in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2015;33:894-900.
 22. Brose MS, Frenette CT, Keefe SM, Stein SM. Management of sorafenib-related adverse events: a clinician's perspective. *Semin Oncol* 2014;41 Suppl 2:S1-16.
 23. Common Terminology Criteria for Adverse Events (CTCAE) v5. US Department of health and human services. National Institute of Health. 2017. Available from: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf. [Last accessed on 30 Sep 2020]
 24. Vukelja SJ, Lombardo FA, James WD, Weiss RB. Pyridoxine for the palmar-plantar erythrodysesthesia syndrome. *Ann Intern Med* 1989;111:688-9.
 25. Lacouture ME, Reilly LM, Gerami P, Guitart J. Hand foot skin reaction in cancer patients treated with the multikinase inhibitors sorafenib and sunitinib. *Ann Oncol* 2008;19:1955-61.
 26. Autier J, Escudier B, Wechsler J, Spatz A, Robert C. Prospective study of the cutaneous adverse effects of sorafenib, a novel multikinase inhibitor. *Arch Dermatol* 2008;144:886-92.
 27. Lee WJ, Lee JL, Chang SE, Lee MW, Kang YK, et al. Cutaneous adverse effects in patients treated with the multitargeted kinase inhibitors sorafenib and sunitinib. *Br J Dermatol* 2009;161:1045-51.
 28. Haddad RI, Schlumberger M, Wirth LJ, Sherman EJ, Shah MH, et al. Incidence and timing of common adverse events in Lenvatinib-treated patients from the SELECT trial and their association with survival outcomes. *Endocrine* 2017;56:121-8.
 29. Wood LS. Managing the side effects of sorafenib and sunitinib. *Commun Oncol* 2006;3:558-62.
 30. Pérez-Soler R, Delord JP, Halpern A, Kelly K, Krueger J, et al. HER1/EGFR inhibitor-associated rash: future directions for management and investigation outcomes from the HER1/EGFR inhibitor rash management forum. *Oncologist* 2005;10:345-56.
 31. Sarkodie T, Ross P. Late presentation of sorafenib-associated rash: a case report. *J Med Case Rep* 2010;4:338.
 32. Çalışkan S. Sorafenib-induced Scrotal Eczema. *Rev Urol* 2015;17:250-1.
 33. Ishak RS, Aad SA, Kyei A, Farhat FS. Cutaneous manifestations of anti-angiogenic therapy in oncology: review with focus on VEGF inhibitors. *Crit Rev Oncol Hematol* 2014;90:152-64.
 34. De Wit M, Boers-Doets CB, Saettini A, Vermeersch K, de Juan CR, et al. Prevention and management of adverse events related to regorafenib. *Support Care Cancer* 2014;22:837-46.
 35. Harris DJ, Eilers J, Harriman A, Cashavelly BJ, Maxwell C. Putting evidence into practice: evidence-based interventions for the management of oral mucositis. *Clin J Oncol Nurs* 2008;12:141-52.
 36. Bensinger W, Schubert M, Ang KK, Brizel D, Brown E, et al; NCCN Task Force Report. Prevention and management of mucositis in cancer care. *J Natl Compr Canc Netw* 2008;6 Suppl 1:S1-21.
 37. Califano R, Tariq N, Compton S, Fitzgerald DA, Harwood CA, et al. Expert consensus on the management of adverse events from EGFR tyrosine kinase inhibitors in the UK. *Drugs* 2015;75:1335-48.
 38. Bayer HealthCare Pharmaceuticals Inc. Nexavar (Sorafenib) [package insert]. U.S. Food and Drug Administration website. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/021923s0201bl.pdf. [Last accessed on 24 Sep 2017]
 39. Guggina LM, Choi AW, Choi JN. EGFR inhibitors and cutaneous complications: a practical approach to management. *Oncol Ther*

- 2017;5:135-48.
40. Sohn KH, Oh SY, Lim KW, Kim MY, Lee SY, et al. Sorafenib induces delayed-onset cutaneous hypersensitivity: a case series. *Allergy Asthma Immunol Res* 2015;7:304-7.
41. Mantovani A, Álvares-Da-Silva MR. Anaphylaxis preceded by erythema multiforme with sorafenib: first case report. *Ann Hepatol* 2019;18:777-9.
42. Traves KP, Love G, Studdiford JS. Erythema multiforme: recognition and management. *Am Fam Physician* 2019;100:82-88.
43. Ayangco L, Rogers RS. Oral manifestations of erythema multiforme. *Dermatol Clin* 2003;21:195-205.
44. Sokumbi O, Wetter DA. Clinical features, diagnosis, and treatment of erythema multiforme: a review for the practicing dermatologist. *Int J Dermatol* 2012;51:889-902.
45. Choi MK, Woo HY, Heo J, Cho M, Kim GH, et al. Toxic epidermal necrolysis associated with sorafenib and tosufloxacin in a patient with hepatocellular carcinoma. *Ann Dermatol* 2011;23:S404-7.
46. Dodiuk-Gad RP, Chung WH, Valeyrie-Allanore L, Shear NH. Stevens-johnson syndrome and toxic epidermal necrolysis: an update. *Am J Clin Dermatol* 2015;16:475-93.
47. Duong TA, Valeyrie-allanore L, Wolkenstein P, Chosidow O. Severe cutaneous adverse reactions to drugs. *Lancet* 2017;390:1996-2011.
48. Kardaun SH, Jonkman MF. Dexamethasone pulse therapy for Stevens-Johnson syndrome/toxic epidermal necrolysis. *Acta Derm Venereol* 2007;87:144-8.
49. Valeyrie-Allanore L, Wolkenstein P, Brochard L, Ortonne N, Maître B, et al. Open trial of ciclosporin treatment for Stevens-Johnson syndrome and toxic epidermal necrolysis. *Br J Dermatol* 2010;163:847-53.
50. Paradisi A, Abeni D, Bergamo F, Ricci F, Didona D, et al. Etanercept therapy for toxic epidermal necrolysis. *J Am Acad Dermatol* 2014;71:278-83.
51. Kannaiyan R, Mahadevan D. A comprehensive review of protein kinase inhibitors for cancer therapy. *Expert Rev Anticancer Ther* 2018;18:1249-70.
52. Autier J, Mateus C, Wechsler J, Spatz A, Robert C. Cutaneous side effects of sorafenib and sunitinib. *Ann Dermatol Venereol* 2008;135:148-53; quiz 147, 154.
53. Eisai Inc. Lenvima (Lenvatinib) [package insert]. U.S. Food and Drug Administration website. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/206947s0071bl.pdf. [Last accessed on 24 Sep 2020]
54. Choueiri TK, Escudier B, Powles T, Tannir NM, Mainwaring PN, et al. Cabozantinib versus everolimus in advanced renal cell carcinoma (METEOR): final results from a randomised, open-label, phase 3 trial. *Lancet Oncol* 2016;17:917-27.
55. Exelixis. Cabometyx (Cabozantinib) [package insert]. U.S. Food and Drug Administration website. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/208692s0031bl.pdf. [Last accessed on 24 Sep 2020]
56. Finn RS, Merle P, Granito A, Huang YH, Bodoky G, et al. Outcomes of sequential treatment with sorafenib followed by regorafenib for HCC: additional analyses from the phase III RESORCE trial. *J Hepatol* 2018;69:353-8.
57. Grothey A, Sobrero AF, Siena S, Falcone A, Ychou M, et al. Time profile of adverse events (AEs) from regorafenib (REG) treatment for metastatic colorectal cancer (mCRC) in the phase III CORRECT study. Available from: https://doi.org/10.1200/jco.2013.31.15_suppl.3637. [Last accessed on 30 Sep 2020]
58. Sastre J, Argilés G, Benavides M, Feliú J, García-Alfonso P, et al. Clinical management of regorafenib in the treatment of patients with advanced colorectal cancer. *Clin Transl Oncol* 2014;16:942-53.
59. Khan G, Moss RA, Braiteh F, Saltzman M. Proactive strategies for regorafenib in metastatic colorectal cancer: implications for optimal patient management. *Cancer Manag Res* 2014;6:93-103.
60. Grothey A, George S, van Cutsem E, Blay JY, Sobrero A, et al. Optimizing treatment outcomes with regorafenib: personalized dosing and other strategies to support patient care. *Oncologist* 2014;19:669-80.
61. Mitchell J, Khokkaz T, McNeal D, Brent L. Adverse event management strategies: optimizing treatment with regorafenib in patients with metastatic colorectal cancer. *Clin J Oncol Nurs* 2014;18:E19-25.
62. Bayer HealthCare Pharmaceuticals Inc. Stivarga (Regorafenib). U.S. Food and Drug Administration website. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/2043691bl.pdf. [Last accessed on 24 Sep 2020]
63. Vincenzi B, Santini D, Russo A, Addeo R, Giuliani F, et al. Early skin toxicity as a predictive factor for tumor control in hepatocellular carcinoma patients treated with sorafenib. *Oncologist* 2010;15:85-92.
64. Shomura M, Kagawa T, Shiraishi K, Hirose S, Arase Y, et al. Skin toxicity predicts efficacy to sorafenib in patients with advanced hepatocellular carcinoma. *World J Hepatol* 2014;6:670-6.
65. Sung MW, Finn RS, Qin S, Han K, Ikeda K, et al. Association between overall survival and adverse events with lenvatinib treatment in patients with hepatocellular carcinoma (REFLECT). *JCO* 2019;37:317.
66. Abou-alfa GK, Meyer T, Cheng A, Cicin I, Bolondi L, et al. Association of adverse events (AEs) with efficacy outcomes for cabozantinib (C) in patients (pts) with advanced hepatocellular carcinoma (aHCC) in the phase III CELESTIAL trial. *JCO* 2019;37:4088.
67. Wakatsuki T, Shinozaki E, Suenaga M, Nakayama I, Matsushima T, et al. Associations between regorafenib-induced adverse events (AEs) and efficacy in metastatic colorectal cancer (mCRC). *JCO* 2017;35:556.
68. Rimassa L, Danesi R, Pressiani T, Merle P. Management of adverse events associated with tyrosine kinase inhibitors: Improving outcomes for patients with hepatocellular carcinoma. *Cancer Treat Rev* 2019;77:20-8.
69. Kong Y, Sun L, Hou Z, Zhang Y, Chen P, et al. Apatinib is effective for treatment of advanced hepatocellular carcinoma. *Oncotarget* 2017;8:105596-605.
70. Liu J, Li X, Zhang H, Chen G, Chen H, et al. Safety, pharmacokinetics and efficacy of donafenib in treating advanced hepatocellular carcinoma: report from a phase 1b trial. *Pharmazie* 2019;74:688-93.

Review

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The role of genetic factors in HBV-related HCC: perspectives from local genetic backgrounds and clinical epidemiology

Dar-In Tai^{1,2}, Jennifer Tai²

¹Liver Research Center, Chang Gung Memorial Hospital, Linkou Medical Center, Taoyuan 33305, Taiwan.

²Department of Internal Medicine, Chang Gung Memorial Hospital, Linkou Medical Center, Taoyuan 33305, Taiwan.

Correspondence to: Dr. Dar-In Tai, Liver Research Center, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, 199 Tung-Hwa North Road, Taipei 10591, Taiwan. E-mails: tai48978@cgmh.org.tw; imjenkid@gmail.com

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Abstract

Familial clustering of hepatitis B surface antigen carriers (HBsAg) and hepatocellular carcinoma (HCC) has led to the evaluation of the role of genetics in hepatitis B-related diseases. Consistent reports indicate that the HLA-DP and -DQ loci are associated with persistent hepatitis B virus (HBV) infection. However, for hepatocarcinogenesis, existing studies have low power and conflicting data. Global single nucleotide polymorphism (SNP) data was collected from the 1000 Genomes Project and correlated with local epidemiological information. Southeastern Asia has a higher prevalence of HBsAg than Northeastern Asia; this was used in the evaluation of persistent HBV infection. The higher incidence of HCC in West Africa compared with East Africa was used in the evaluation of hepatocarcinogenesis. The allele frequencies for SNPs were significantly different between East Asians and Africans. Therefore, SNPs that have been identified in persistent HBV infections in East Asia may not be completely applicable in Africa. SNPs in NTCP, CTF19, and the HLA-DQ and -DP loci showed North-to-South allele frequency changes in East Asia. These findings confirm the role of genetics in persistent HBV infection. Some of the SNPs in the HLA loci show a trend of West-to-East allele frequency changes in Africa, indicating they may participate in hepatocarcinogenesis. Among the non-HLA related SNPs, rs2596542 in MICA shows a strong trend of allele frequency changes and is correlated with HCC incidence in Africa. SNPs in KIF1, IL-1A, and STAT4 also show, albeit with low statistical power, allele frequency trends compatible with HCC incidence. Taken together, there are strong correlations between background genetics in HLA-DP and -DQ loci with persistent HBV infection and hepatocarcinogenesis. The correlations were weak-positive in non-HLA loci.



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Keywords: Genetic polymorphism, genome-wide associated studies, hepatitis B virus, hepatocellular carcinoma

INTRODUCTION

Hepatitis B is a global disease that results in an increased risk of liver cirrhosis and hepatocellular carcinoma^[1,2]. A strong familial clustering of chronic hepatitis B carriers and hepatocellular carcinomas (HCC) has been well-reported^[3-6]. This could be related to the high intrafamilial spread of hepatitis B virus (HBV) infection. Infection of HBV in the early stage of life will result in chronic persistent infection^[4]. Throughout the course of this disease, intermittent relapsing liver necroinflammation will occur. This process is a defensive response to eliminate HBV replication and/or clear the virus. In general, by 80 years of age, half of chronic hepatitis B carriers will have suppressed HBV replication and cleared hepatitis B surface antigens (HBsAg)^[7].

However, some patients may develop liver cirrhosis as a result of repeated liver inflammation and fibrogenesis^[8]. Liver necroinflammation can induce chromosomal damage^[9], and HBV is able to integrate into the human genome^[10]. Such an injury to the host genome can induce chromosomal instability and promote hepatocarcinogenesis. Genetic factors associated with HCC have been considered because of its familial tendency. Many candidate genes and genome-wide associated studies (GWAS) have revealed nearly one hundred genes to be associated with chronic persistent infection or hepatocarcinogenesis^[11-14]. Previous extensive and elegant meta-analysis reports have addressed these issues. However, not all of these HBV/HCC related genes have been confirmed and replicated by subsequent studies, demonstrating the difficulties in sorting HCC-associated genes. This is partly due to the fact that HBV-host interactions are not simply a genetic problem, as well as the fact that there are differences in the genetic backgrounds of study populations.

Therefore, in this study, we shall try to understand HBV-related single nucleotide polymorphisms (SNPs) by performing correlations between genetic backgrounds and two well-known epidemiological datasets. The genetic backgrounds will be obtained from the 1000 Genomes Project (<http://www.1000genomes.org/>)^[15]. Epidemiological concerns about a higher prevalence of HBsAg in southern compared to Northeastern Asia will be used in the evaluation for persistent HBV infection^[16]. A higher prevalence of HCC in West Africa compared to East Africa will also be used for evaluation of hepatocarcinogenesis (World Health Organization, <http://gco.iarc.fr/today>). With these viewpoints, we may obtain additional information independent of the observations in reported studies about HBV-related genetic polymorphisms.

CHRONIC PERSISTENT HBV INFECTION

Hepatocarcinogenesis in chronic HBV infection is not a purely genetic disease; it depends on host and virus interactions. Persistent HBV infection is the first stage toward hepatocarcinogenesis.

HBV clearance

When humans are exposed to HBV, either acute hepatitis with spontaneous viral clearance or chronic persistent infection may develop [Figure 1]. Both the timing and transmission route of infection are important in persistent infections. Individuals who are infected in the early stages of life and via vertical transmission are more likely to develop persistent infection^[4]. In GWAS studies, HLA-DP and -DQ have been shown to consistently be associated with persistent HBV infection in East Asians^[17-23]. However, such high-risk alleles are relatively rare in Africans^[24]. Therefore, HBV infection elicited in the early stages of life remains an important mechanism of persistent HBV infection. It is independent of genetic polymorphisms in the HLA-DP and -DQ loci.

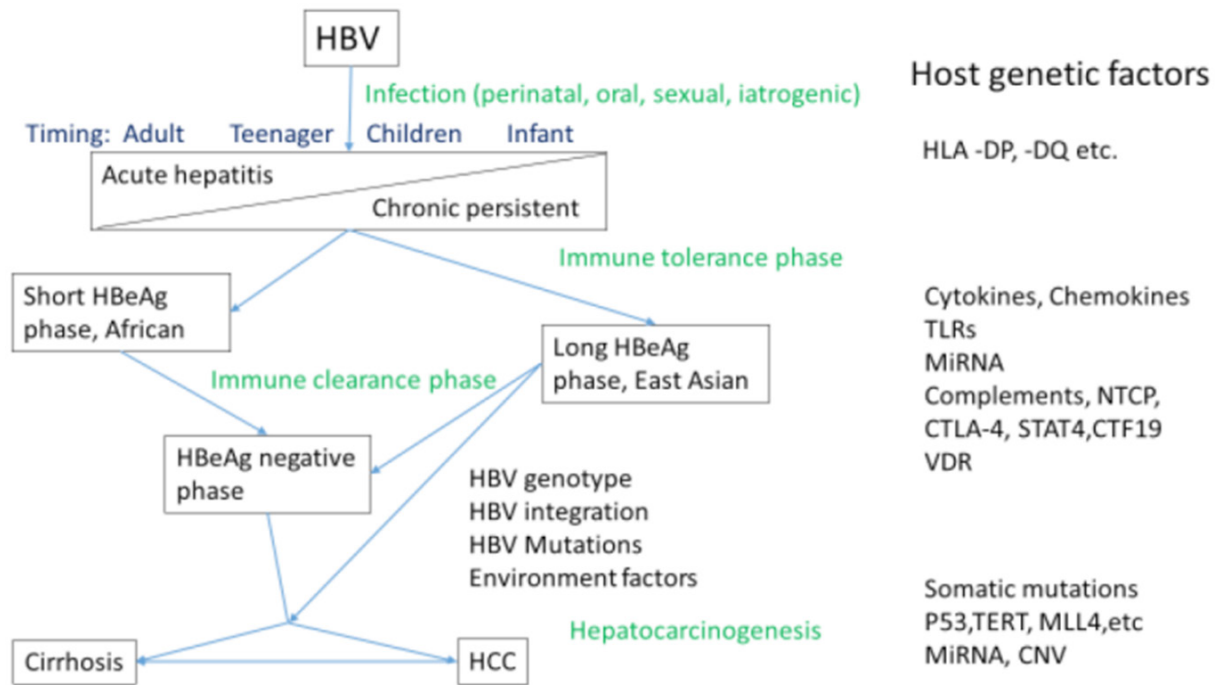


Figure 1. Summary of hepatitis B virus (HBV) and host interactions. When humans are exposed to HBV, either acute hepatitis with subsequent virus clearance or persistent infection may develop. Those who are infected in the early stages of life, and via vertical transmission, are prone to progress to persistent infection. HLA-DP and -DQ loci are associated with persistent HBV infection in East Asians. Chronic persistent HBV infection starts with the presence of hepatitis B e-antigen (HBeAg), known as the immune tolerance stage. In this phase, Africans tend to clear HBeAg before puberty while East Asians tend to clear HBeAg between the second and fourth decades of life. About 5% of HBeAg-negative patients still present with active viral replication. Many immune-related genes participate in HBV clearance. Inability to effectively clear HBV may result in liver cirrhosis and increased HBV mutations and integrations into the human genome. All of these events and some genetic polymorphisms in the host may promote hepatocarcinogenesis. HCC: hepatocellular carcinoma

Persistent HBV infection

Chronic persistent HBV infections start with the presence of the hepatitis B e-antigen (HBeAg). During the HBeAg-positive immune tolerance phase, the HBV DNA level is high with low levels of liver inflammation^[25]. Due to some unclear triggering factors, immune clearance of HBV will develop between the second and fourth decades of life in East Asians. There is a difference between Africans and East Asians regarding the duration of the HBeAg-positive period^[26-29]. A rapid clearance of HBeAg before puberty can be found in Africans but rarely occurs in East Asians^[30,31]. This difference may be associated with variations in the HLA-DP and -DQ genotypes^[24]. Certain SNPs in HLA-DP and -DQ loci may prolong the HBV replication phase in adults of East Asian descent. A high HBV DNA level in parents will increase the risk of persistent infection in their offspring (submitted for publication).

HBV genotypes C and D are associated with a more persistent liver necroinflammation^[28]. Many immune-related genes may participate in this immune clearance phase [Figure 1]. Patients either in the HBeAg-positive or -negative phase who are unable to suppress HBV replication may develop liver cirrhosis due to repeated liver inflammation and fibrogenesis.

HBV integration and host interaction

A prolonged HBV replication phase and liver cirrhosis increases the risk of HCC^[25]. The mechanism may be related to hepatitis Bx proteins^[32], increased endoplasmic reticulum stress^[9], HBV integration^[10,33] and inflammation-related chromosome damage.

Soon after HBV infection, part of the HBV genome can be integrated into the host genome. The mechanism of HBV integration is not fully understood. From the *in vitro* study done using the Na⁺-taurocholate co-transporting polypeptide (NTCP) transfected hepatoma cell line, HBV integration can be detected randomly in the host genome shortly after infection^[33]. This observation implies that HBV integration is independent of immune-related inflammation. These HBV integrations are mostly harmless but may produce genomic instability. After decades, some of the integrations may become more prominent. In the presence of additional factors, such as inflammation, HBV mutations, or environmental carcinogens, a segment of the hepatocytes carrying HBV integrations may become clonal and develop into HCC. Several integration hot sites, including TERT, KMT2B, DDX11L1, CCNE1, and CCNA2, can be found more frequently in HCC than in non-HCC tissues^[34], while FN1 is commonly found in non-tumour tissues. A prolonged HBeAg phase and high viral load carry a higher frequency of HBV integrations^[35].

HBV mutants and host interaction

The wild-type HBV genome and its proteins are not directly cytopathic. Host immune responses and inflammation are induced to clear HBV during the second to fourth decades of life in East Asians. If the HBV immune clearance process is unsuccessful and prolonged, complicated HBV mutations may develop and escape immune surveillance. Through repeat necroinflammation, several mutation hot spots in the EnhII (C1653T)/BCP(A1762T/G1764A, T1753V)/PC(G1896A) regions were found more frequently in HCC^[36]. In addition, some of the pre-S/S mutations or truncations may become directly cytopathic and/or carcinogenic^[37].

HLA SNPS IN RELATION TO HBV INFECTION AND HEPATOCARCINOGENESIS

Global allele frequency of HBV-related SNPs in HLA loci

Based on GWAS studies, HLA-DP and -DQ loci are associated with persistent HBV infection. These SNPs have been reported quite consistently from different centres in East Asia.

To understand the global allele frequency of the HBV-related SNPs, we collected data from the 1000 Genomes Project, with the results listed in Table 1. Although HBsAg prevalence in East Asians is as high as in Africans, the two populations did not show similar allele frequencies on these SNPs. In general, the allele frequencies of these SNPs are significantly different between East Asians and other global populations. Only 5/19 (26.3%) SNPs showed similar allele frequencies between populations from East Asia and Africa [Table 1]. Therefore, these HBV-related SNPs in the HLA-DP and -DQ loci may not completely explain the high prevalence of HBsAg in Africa.

We suspect that the evolution of these SNPs may be related to human migration^[24]. The Indo-China peninsula and southern China are mountainous and forested areas. Such geographic environments are associated with a great diversity of microorganisms, insects, plants, and animals. People who can survive in this milieu may need some adjustments to their immunity, lest they succumb to a cytokine storm after exposure to multiple unfamiliar microorganisms. Because of modified antigen presentation resulting from HLA-DP and -DQ loci, immunity may be decreased or separated into several stages to avoid the development of cytokine storm. Unfortunately, such immunity may also allow HBV infection to become chronic and persistent. HBV clearance is delayed, but clearance may finally occur several decades later. As a matter of fact, only a minority of HBsAg carriers die of acute or chronic liver disease, and around half of chronic HBsAg carriers clear HBsAg by 80 years of age^[7].

Mechanism of persistent HBV infection in HLA-DP and- DQ SNPs

Both rs3077 and rs9277535 were identified by GWAS to be associated with persistent HBV infection in Japanese patients^[18]. Allele A of rs3077 and rs9277535 are associated with a higher mRNA expression than allele G^[38]. The prevalence of the A allele is lower in East Asia compared to other geographic areas [Table 1].

Table 1. Allele frequency differences between African and East Asian regions on HBV-related SNPs at HLA regions

Gene	SNP	Variant	Allele	Allele frequency					P value AFR vs. EAS
				AFR n = 1,322	AMR n = 694	EUR n = 1,008	SAS n = 978	EAS n = 1,008	
<i>LOC107987449/ LOC107987459</i>	rs9272105	Intron	G	0.417	0.467	0.535	0.447	0.575	NS
<i>HLADQA2-DQB1</i>	rs9275319	Intergenic	G	0.113	0.290	0.163	0.091	0.135	NS
<i>HLADQA2-DQB1</i>	rs2856718	Intergenic	T	0.359	0.38	0.351	0.508	0.528	< 0.001
<i>HLADQA2-DQB1</i>	rs9275572	Intergenic	A	0.399	0.314	0.4	0.281	0.254	< 0.001
<i>HLA-DQA2</i>	rs9276370	2KB Upstream	G	0.711	0.367	0.408	0.215	0.159	< 0.001
<i>HLA-DQB2</i>	rs7756516	3 Prime UTR	C	0.631	0.432	0.468	0.335	0.194	< 0.001
<i>HLA-DQB2</i>	rs7453920	Intron	A	0.305	0.274	0.384	0.192	0.127	< 0.001
<i>HLA-DPA1</i>	rs3077	3 Prime UTR	A	0.419	0.716	0.811	0.633	0.320	< 0.001
<i>HLA-DPA1</i>	rs9277341	Intron	T	0.257	0.597	0.682	0.41	0.165	< 0.001
<i>HLA-DPA1/HLA-DPB1</i>	rs3135021	Intron	A	0.356	0.421	0.279	0.435	0.255	< 0.001
<i>HLA-DPB1</i>	rs9277535	3 Prime UTR	G	0.191	0.288	0.271	0.297	0.612	< 0.001
<i>HLA-DPB1</i>	rs9277542	3 Prime UTR	T	0.402	0.669	0.686	0.634	0.38	NS
<i>HLA-DPB1</i>	rs10484569	Downstream	A	0.048	0.036	0.04	0.025	0.39	< 0.001
<i>HLA-DPA2 (Pseudogene)</i>	rs3128917	Downstream HLADPB1	G	0.511	0.265	0.271	0.281	0.535	NS
<i>HLA-DPA2 (Pseudogene)</i>	rs2281388	Downstream HLADPB1	A	0.002	0.017	0.024	0.024	0.378	< 0.001
<i>HLA-DPA2 (Pseudogene)</i>	rs3117222	Downstream HLADPB1	T	0.509	0.264	0.271	0.28	0.537	NS
<i>HLA-DPB2 (Pseudogene)</i>	rs9380343	2KB Upstream	T	0.048	0.032	0.043	0.028	0.394	< 0.001
<i>LOC105375021</i>	rs9366816	Intron	C	0.157	0.375	0.231	0.177	0.46	< 0.001

AFR: African; AMR: American; EUR: European; SAS: South Asian; EAS: East Asian; UTR: un-transcript region; SNP: single nucleotide polymorphism; HLA: human leukocyte antigen; NS: no significance

This may suggest that the antigen presentation and immune response of Allele G are weaker than those of Allele A. Such behaviours may favour a persistent HBV infection.

The SNP rs7756516 in HLA-DQB2 was associated with persistent HBV infection^[23]. We checked potential mRNA binding using the SegalLab tool (http://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html) and found that microRNA-550 may bind to the G allele of this SNP. The binding of miR-RNA-550 decreased mRNA stability, a potential reason why the mRNA level is low and weak function of antigen presentation. The allele frequency of the G allele is 0.806 in East Asians [Table 1], which is much higher than in other areas worldwide (0.369-0.665).

HBV-related SNPs in the HLA region among East Asians

The human migration theory was based on a geographic block on the Indo-China peninsula. After crossing this region, the ancestors of East Asians spread to Northern China, Korea, and Japan.

Northern China is generally a grassland and is associated with a lower prevalence of HBsAg than southern China^[16]. With this in mind, we examined the allele frequencies in East Asian populations. We proposed that a lower HBsAg prevalence in northeast Asia could be related to genetic polymorphisms.

The allele frequencies of HBV-related SNPs in East Asia were obtained from the 1000 Genomes Project [Table 2]. A zone in the HLA-DP and -DQ regions showed a trend of allele frequency changes according to HBsAg prevalence and geographic location. On the other hand, background genetics may explain a lower prevalence of HBsAg in North versus Southeast Asians. This observation may be due to the race differences between North- and Southeast Asia. While plausible, such trends were not found in pseudogene regions [Table 2]. We therefore suggest that only active genes participated in the environmental evolution or adaptation. This is additional evidence that supports the role of geographic blocks in the evolution of HBV-related SNPs in HLA regions.

Table 2. Allele frequency trends among east Asian regions according to geographic location on HBV-related SNPs in HLA regions

Gene	SNP	Allele	JPT n = 208	CHB n = 206	CHS n = 210	KHV n = 198	CDX n = 186	P value χ^2 for trend
HLADQA1-DRB1	rs9272105	A	0.404	0.471	0.49	0.586	0.371	NS
HLADQA2-DQB1	rs9275319	G	0.269	0.141	0.105	0.076	0.075	< 0.0000001
HLA-DQB1	rs2856718	T	0.452	0.519	0.605	0.424	0.645	0.01035
HLA-DQA2/HLA-DQB1	rs9275572	A	0.341	0.311	0.214	0.232	0.161	6.133E-06
HLA-DQA2	rs9276370	G	0.221	0.228	0.09	0.152	0.097	0.00005987
HLA-DQB2	rs7756516	C	0.346	0.248	0.09	0.172	0.108	0.001622
HLA-DQB2	rs7453920	A	0.207	0.189	0.071	0.101	0.059	2.09E-07
HLA-DPA1	rs3077	A	0.413	0.379	0.271	0.303	0.226	0.00001509
HLA-DPA1	rs9277341	T	0.111	0.228	0.129	0.222	0.134	NS
HLA-DPA1/HLA-DPB1	rs3135021	A	0.409	0.301	0.214	0.182	0.156	< 0.0000001
HLA-DPB1	rs9277535	G	0.558	0.539	0.614	0.722	0.634	0.001622
HLA-DPB1	rs9277542	T	0.442	0.461	0.39	0.242	0.355	0.0002659
HLA-DPB1	rs10484569	A	0.375	0.374	0.386	0.394	0.425	0.000229
HLA-DPA2 (Pseudogene)	rs3128917	G	0.553	0.461	0.519	0.606	0.538	NS
HLA-DPA2 (Pseudogene)	rs2281388	A	0.365	0.335	0.371	0.399	0.425	NS
HLA-DPA2 (Pseudogene)	rs3117222	T	0.558	0.461	0.519	0.606	0.543	NS
HLA-DPB2 (Pseudogene)	rs9380343	T	0.375	0.379	0.39	0.394	0.435	NS
LOC105375021	rs9366816	C	0.466	0.481	0.429	0.439	0.489	NS

JPT: Japanese in Tokyo, Japan; CHB: Han Chinese in Beijing, China; CHS: Southern Han Chinese; KHV: Kinh in Ho Chi Minh City, Vietnam; CDX: Chinese Dai in Xishuangbanna, China; SNP: single nucleotide polymorphism; HLA: human leukocyte antigen; HBV: hepatitis B virus; NS: no significance

Hepatocarcinogenesis in HLA loci

Hepatocarcinogenesis is a multifactorial process. There is strong evidence for the role of genetics in persistent HBV infections. However, controversy exists over genetic reports on HBV-related hepatocarcinogenesis. When we examined the global incidence of HBV-related HCC, a higher incidence of HCC could be found in West Africa versus East Africa. Based on this trend, we examined the allele frequency distribution of the reported SNPs and correlated these with HCC incidence in different geographic regions in Africa. One should be notice that the mechanism of hepatocarcinogenesis can be diverse among regions. For example, aflatoxin or other environmental factors may be important in Africans^[39]. On the other hand, a long active HBV replication phase is the key factor in East Asians^[26-29].

In HLA HBV-related SNPs, five SNPs (rs2856718, rs9275572, rs3077 and rs9277341) showed a trend of West-to-East allele frequencies change in Africans ($P < 0.00001$; Table 3). These SNPs were mainly located in HLA-DQ and HLA DPA1 regions; the distribution of these HCC-related SNPs in HLA regions was similar to that observed in persistent HBV infection [Table 2]. All of these SNPs, except rs9277341, were reported to be associated with a greater risk of HCC. The rs9277341 allele had a significant difference in frequency between West and East ($P < 10^{-7}$), but no study had examined its effect on the risk of developing HCC. Further studies regarding this may be needed. The mechanism of hepatocarcinogenesis is probably related to persistent HBV replication and repeated liver necroinflammation.

Non-HLA SNPs in relation to HBV infection and hepatocarcinogenesis

Many SNPs in non-HLA loci were also reported to be associated with HBV infection and/or hepatocarcinogenesis. The associations with HBV infection reported in non-HLA SNPs were generally weaker than those in HLA regions. However, many SNPs related to HBV persistence or carcinogenesis could not be replicated in other studies. This is at least in part due to the different genetic backgrounds across study populations. We saw significant allele frequency differences between Africans and East Asians. Only 2/20 (10%) SNPs showed a similar allele frequency between the two populations [Table 4].

Table 3. Allele frequency trends among African regions according to geographic location on HBV-related SNPs in HLA regions

Gene	SNP	Allele	GWD n = 226	MSL n = 170	YRI n = 216	ESN n = 198	LWK n = 198	P value χ^2 for trend
HLADQA1-DRB1	rs9272105	G	0.367	0.329	0.394	0.535	0.384	NS
HLADQA2-DQB1	rs9275319	G	0.111	0.235	0.106	0.061	0.091	0.008894
HLA-DQB1	rs2856718	T	0.482	0.371	0.343	0.354	0.288	8.64E-05
HLA-DQA2/HLA-DQB1	rs9275572	A	0.204	0.412	0.472	0.449	0.449	1.95E-07
HLA-DQA2	rs9276370	G	0.743	0.812	0.722	0.742	0.631	0.004733
HLA-DQB2	rs7756516	C	0.588	0.659	0.713	0.662	0.530	NS
HLA-DQB2	rs7453920	A	0.296	0.324	0.282	0.323	0.283	NS
HLA-DPA1	rs3077	A	0.358	0.318	0.264	0.46	0.561	1.26E-06
HLA-DPA1	rs9277341	T	0.15	0.182	0.185	0.268	0.399	< 0.0000001
HLA-DPA1/HLA-DPB1	rs3135021	A	0.403	0.353	0.25	0.328	0.434	NS
HLA-DPB1	rs9277535	G	0.173	0.188	0.116	0.212	0.212	NS
HLA-DPB1	rs9277542	T	0.403	0.312	0.292	0.414	0.48	NS
HLA-DPB1	rs10484569	A	0.018	0.018	0.037	0.086	0.061	0.000635
HLA-DPA2 (Pseudogene)	rs3128917	G	0.491	0.571	0.648	0.505	0.46	NS
HLA-DPA2 (Pseudogene)	rs2281388	A	0	0	0	0	0	NS
HLA-DPA2 (Pseudogene)	rs3117222	T	0.491	0.571	0.648	0.495	0.46	NS
HLA-DPB2 (Pseudogene)	rs9380343	T	0.08	0.082	0.009	0.03	0.02	0.000223
LOC105375021	rs9366816	C	0.159	0.135	0.088	0.197	0.192	NS

GWD: Gambian in Western Divisions in the Gambia; MSL: Mende in Sierra Leone; YRI: Yoruba in Ibadan, Nigeria; ESN: Esan in Nigeria; LWK: Luhya in Webuye, Kenya; SNP: single nucleotide polymorphism; HLA: human leukocyte antigen; HBV: hepatitis B virus; NS: no significance

Table 4. Allele frequency differences between African and East Asian regions in HBV-related SNPs at non-HLA regions

Position	Gene	SNP	Variant	Allele	AFR	AMR	EUR	SAS	EAS	AFR vs. EAS
1:10325413	<i>KIF1</i>	rs17401966	Intron	G	0.057	0.32	0.309	0.272	0.288	< 0.001
2:112774138	<i>IL-1A/MIR-122; DELINS</i>	rs16347	Indel 3 prime UTR	TGAA	0.195	0.477	0.322	0.286	0.704	< 0.001
2:112836810	<i>IL-10</i>	rs1800872	5 prime UTR	G	0.564	0.667	0.76	0.542	0.324	< 0.001
2:191099907	<i>STAT4</i>	rs7574865	Intron	T	0.116	0.362	0.23	0.30	0.347	< 0.001
2:203867624	<i>CTLA4</i>	rs5742909	Upstream	T	0.003	0.063	0.084	0.029	0.097	< 0.001
2:203867991	<i>CTLA4</i>	rs231775	Missense	A	0.612	0.537	0.641	0.69	0.363	< 0.001
2:211380366-7	<i>ERBB4</i>	rs6147150	Indel 3 prime UTR	TG	0.591	0.365	0.399	0.244	0.284	< 0.001
3:157429779	<i>VEPH1</i>	rs2120243	intron	A	0.332	0.308	0.43	0.327	0.348	NS
4:186082920	<i>TLR-3</i>	rs3775291	Missense	T	0.026	0.305	0.324	0.263	0.328	< 0.001
6:31162816	<i>CTF19</i>	rs1419881	3 Prime UTR	G	0.363	0.496	0.547	0.55	0.433	< 0.001
6:31398818	<i>MICA-AS1/MICA</i>	rs2596542	Intron/Upstream	T	0.541	0.519	0.396	0.369	0.275	< 0.001
6:31575254	<i>TNFA</i>	rs1800629	Upstream	A	0.12	0.069	0.134	0.053	0.059	< 0.001
6:31575324	<i>TNFA</i>	rs361525	Upstream	A	0.038	0.082	0.064	0.105	0.031	NS
7:100103553	<i>AP4M1/MCM7/MIR-106b</i>	rs999885	Intron/Upstream	G	0.78	0.411	0.472	0.34	0.192	< 0.001
11:86921775	<i>PRSS23/LOC107984428</i>	rs1048338	intron/NCT	C	0.3	0.254	0.14	0.303	0.397	< 0.001
11:112164265	<i>IL-18</i>	rs187238	2KB Upstream	G	0.20	0.314	0.278	0.184	0.121	< 0.001
13:32014688	<i>GRIK1</i>	rs455804	Intron	A	0.416	0.228	0.236	0.195	0.313	< 0.001
14:69778476	<i>NTCP</i>	rs2296651	Missense	A	0.000	0.000	0.000	0.000	0.071	< 0.001
14:69796098	<i>NTCP</i>	rs4646287	Intron	T	0.001	0	0	0.018	0.101	< 0.001
18:5900774	<i>TMEM200C</i>	rs2212522	2KB Upstream	T	0.586	0.344	0.232	0.41	0.502	< 0.001

AFR: African; AMR: American; EUR: European; SAS: South Asian; EAS: East Asian; UTR: un-transcript region; NCT: non-coding transcript; SNP: single nucleotide polymorphism; HLA: human leukocyte antigen; HBV: hepatitis B virus; NS: no significance

HBV-related SNPs in the non-HLA region among East Asians

Most of the persistent HBV infection-related SNPs in non-HLA regions were reported in East Asia. We examined whether these SNPs also showed geographical differences in allele frequencies between Northern and Southern regions. We found that only 2 of 20 (10%) SNPs showed significant North-to-South allele frequency trend in East Asians [Table 5]. Among these, NTCP, a functional receptor of hepatitis B^[40], showed the highest trend ($P = 2.23 \times 10^{-6}$).

Table 5. Allele frequency trends among east Asian regions according to geographic location on HBV-related SNPs in non-HLA regions

Gene	SNP	Allele	JPT	CHB	CHS	KHV	CDX	P value χ^2 for trend
<i>KIF1</i>	rs17401966	G	0.293	0.286	0.367	0.273	0.21	NS
<i>IL-1A/MIR-122; DELINS</i>	rs16347	TGAA	0.726	0.631	0.729	0.747	0.688	NS
<i>IL-10</i>	rs1800872	G	0.361	0.257	0.314	0.343	0.349	NS
<i>STAT4</i>	rs7574865	T	0.327	0.354	0.352	0.354	0.349	NS
<i>CTLA4</i>	rs5742909	T	0.096	0.117	0.114	0.086	0.07	NS
<i>CTLA4</i>	rs231775	A	0.375	0.311	0.343	0.338	0.457	NS
<i>ERBB4</i>	rs6147150	TG	0.226	0.252	0.286	0.323	0.339	0.003133
<i>VEPH1</i>	rs2120243	A	0.274	0.335	0.376	0.354	0.409	0.007567
<i>TLR-3</i>	rs3775291	T	0.293	0.291	0.329	0.389	0.344	0.05213
<i>CTF19</i>	rs1419881	G	0.5	0.495	0.448	0.414	0.29	1.08E-05
<i>MICA-AS1/MICA</i>	rs2596542	C	0.332	0.272	0.229	0.308	0.231	NS
<i>TNFA</i>	rs1800629	A	0.019	0.092	0.057	0.056	0.07	NS
<i>TNFA</i>	rs361525	A	0.014	0.034	0.038	0.056	0.011	NS
<i>AP4M1/MCM7/MIR-106b</i>	rs999885	G	0.168	0.184	0.19	0.197	0.226	NS
<i>RSS23/LOC107984428</i>	rs1048338	C	0.399	0.456	0.333	0.389	0.409	NS
<i>IL-18</i>	rs187238	G	0.159	0.083	0.114	0.116	0.134	NS
<i>GRIK1</i>	rs455804	A	0.216	0.345	0.343	0.298	0.371	0.01208
<i>NTCP</i>	rs2296651	A	0.024	0.029	0.081	0.111	0.118	2.23E-06
<i>NTCP</i>	rs4646287	T	0.154	0.102	0.071	0.086	0.091	0.035
<i>TMEM200C</i>	rs2212522	T	0.433	0.519	0.5	0.515	0.548	0.04036

JPT: Japanese in Tokyo, Japan; CHB: Han Chinese in Beijing, China; CHS: Southern Han Chinese; KHV: Kinh in Ho Chi Minh City, Vietnam; CDX: Chinese Dai in Xishuangbanna, China; SNP: single nucleotide polymorphism; HLA: human leukocyte antigen; HBV: hepatitis B virus; NS: no significance

The T allele of rs2296651 is a missense mutation^[41] and is found only in East Asians [Table 4]. There is a higher T-allele frequency in Southern East Asia (0.111-0.118) than in Northern East Asia (0.024-0.029) [Table 5]. However, the T allele is known to protect against persistent HBV infection. A higher T-allele frequency in a region with a high prevalence of HBsAg requires an explanation. We propose that the T allele is an evolutionary mechanism to defend against persistent HBV infection in the presence of a weakened antigen presentation system.

The rs1419881 in transcription factor 19 (CTF19) shows significant allele frequency differences between the Northern and Southern regions ($P = 1.08 \times 10^{-5}$ Table 5). This GWAS-identified SNP was found to be associated with persistent HBV infection in Korea^[21]. This SNP was validated in China but was not associated with persistent HBV infection in the Thai population^[42]. The G allele is the risk-associated allele, which showed a higher frequency in Japanese people in Tokyo, Japan (JPT; 0.5) than in Chinese Dai people in Xishuangbanna, China (CDX; 0.29). This association was inversely related to HBsAg prevalence [Table 5]. CTF19 mainly plays a role in the transcription of genes required in the later stages of cell cycle progression. Its mechanism in persistent HBV infection is unclear. Whether it is also similar to NTCP, which is associated with an increased defensive response in people living in regions with a high HBsAg prevalence, will require future studies.

Hepatocarcinogenesis in non-HLA loci

The major histocompatibility complex class I-related chain A (MICA) was reported to be associated with HCV-related HCC^[43]. In non-HLA HBV-related SNPs, only rs2596542 in MICA showed a significant trend ($P = 0.00011$; Table 6). Its C allele frequency is lower in West Africa than in East Africa. The C allele is protective against hepatocarcinogenesis, whereas the T allele is a risk factor^[44]. These findings correlate with a higher incidence of HCC in West than in East Africa. The MICA molecule is a ligand of the natural killing group 2 member D molecule, which is involved in nature killer cell function. Some of the tumour cell may relieve soluble MICA molecules to block immune surveillance^[45,46].

Table 6. Allele frequency trends among African regions according to geographic location on HBV-related SNPs in non-HLA regions

Gene	SNP	Allele	GWD	MSL	YRI	ESN	LWK	P value X ² for trend
<i>KIF1</i>	rs17401966	G	0.04	0.035	0.037	0.051	0.096	0.009852
<i>IL-1A/MIR-122; DELINS</i>	rs16347	TGAA	0.15	0.159	0.204	0.202	0.263	0.002744
<i>IL-10</i>	rs1800872	G	0.527	0.524	0.532	0.551	0.606	NS
<i>STAT4</i>	rs7574865	T	0.071	0.129	0.125	0.131	0.131	0.05661
<i>CTLA4</i>	rs231775	A	0.593	0.706	0.648	0.616	0.52	0.05029
<i>ERBB4</i>	rs6147150	TG	0.659	0.594	0.588	0.505	0.641	NS
<i>VEPH1</i>	rs2120243	A	0.296	0.335	0.343	0.364	0.293	NS
<i>TLR-3</i>	rs3775291	T	0.018	0.012	0.009	0.005	0.035	NS
<i>CTF19</i>	rs1419881	G	0.429	0.347	0.361	0.288	0.379	NS
<i>MICA-AS1/MICA</i>	rs2596542	C	0.429	0.565	0.491	0.657	0.586	0.00011
<i>TNFA</i>	rs1800629	A	0.142	0.159	0.102	0.126	0.086	0.05056
<i>TNFA</i>	rs361525	A	0.084	0.047	0.005	0.01	0.061	0.04351
<i>HLADQA1-DRB1</i>	rs9272105	G	0.062	0.129	0.199	0.253	0.242	< 0.0000001
<i>HLADQA2-DQB1</i>	rs9275319	G	0.111	0.235	0.106	0.061	0.091	0.008894
<i>AP4M1/MCM7/MIR-106b</i>	rs999885	G	0.752	0.835	0.755	0.813	0.838	0.07368
<i>PRSS23/LOC107984428</i>	rs1048338	C	0.412	0.3	0.259	0.273	0.273	0.001384
<i>IL-18</i>	rs187238	G	0.248	0.166	0.204	0.207	0.162	0.006079
<i>GRIK1</i>	rs455805	A	0.358	0.382	0.472	0.49	0.399	0.08
<i>NTCP</i>	rs4646287	T	0.0	0.0	0.0	0.0	0.005	NS
<i>TMEM200C</i>	rs2212522	T	0.615	0.671	0.528	0.581	0.581	NS

GWD: Gambian in Western Divisions in the Gambia; MSL: Mende in Sierra Leone; YRI: Yoruba in Ibadan, Nigeria; ESN: Esan in Nigeria; LWK: Luhya in Webuye, Kenya; SNP: single nucleotide polymorphism; HLA: human leukocyte antigen; HBV: hepatitis B virus; NS: no significance

The rest of non-HLA-related SNPs show weak or absent West-to-East allele frequency trend. This also confirms a low power of hepatocarcinogenesis in each HBV-related SNP.

The rs17401966 SNP in Kinesin Family Member 1B is a tumour suppressor gene. It has been identified by GWAS to be associated with HCC^[47], although there is some controversy in the subsequent validation studies. A meta-analysis has revealed that the G allele is a protective allele in the Chinese population^[48]. This G allele shows a higher prevalence in East Asia than in Africa (0.288 vs. 0.057, $P < 0.001$; Table 4). It is interesting to find that there is a trend of a higher G allele frequency in the Luhya people in Webuye, Kenya (LWK), east Africa, than in the Gambian people in Western Divisions in the Gambia (GWD), West Africa (0.096 vs. 0.04, $P = 0.009852$; Table 6). This is compatible with a lower incidence of HCC in East Africa versus in West Africa.

STATs pathway and associated cytokines play roles on hepatitis clearance and fibrogenesis^[49,50]. The rs7574865 SNP in signal transducer and activator of transcription 4 (STAT4) is related to persistent HBV infection and HCC. The T allele shows a lower prevalence in HCC than in chronic hepatitis B^[51]. There is a weak East-to-West T-allele frequency trend in Africa (0.131 in LWK and 0.071 in GWD; $P = 0.05561$; Table 6). However, the higher incidence of HCC in lower T-allele frequency areas supports the conclusion made by the meta-analysis.

The SNP rs16347 in the 3'-untranslated regions of interleukin-1alpha (IL-1A) carries a miRNA-122 binding site. A variant with a TGAA insertion decreases miRNA-122 binding and increases IL-1A mRNA expression^[52]. The prevalence of this insertion variant is low in Southern Chinese patients with HCC. However, another study from China has not shown this result but instead associates the insertion variant with HBV genome mutants^[53]. When we looked at the allele frequency in Africa, the TGAA insertion variant was lower in West Africa than in East Africa. This allele frequency correlated with the lower

incidence of HCC in East Africa than in West Africa [Table 6]. It should be noted that the function of miRNA-122 is complicated and that the frequency of this insertion variant in East Asians (0.704) is much higher than in Africans (0.199; Table 4). If this SNP is associated with HCC, then it will have a significant impact in East Asians.

Rs187238 is located upstream of interleukin-18 (IL-18) (-148 G>C). The G allele induces increased IL-18 mRNA expression compared to the C allele^[54]. The frequency of the G allele is lower in HCC cases than in non-HCC cases. This implies that those with a stronger immunity may be able to control HBV and hepatocarcinogenesis. However, a follow-up study did not validate the allele differences between HCC and non-HCC5 cases^[55]. In this review, the G-allele frequency was higher in West than in East Africa (0.248 to 0.152; Table 6), contradicting the higher incidence of HCC in West Africa. The incident epidemiology does not support rs187238 playing a role in hepatocarcinogenesis in Africans. This does not necessarily provide evidence against the association of this SNP with hepatocarcinogenesis in East Asians. Termination of HBV replication is more important in East Asians than in Africans.

Rs1048338 in PRSS23 has been identified by GWAS to be associated with HCC in China^[56]. However, no other report has validated this observation. A trend of a higher C allele frequency in West compared to East Africa (0.412 to 0.273, $P = 0.001384$; Table 6) has also been observed. This finding is not against the association rs1048338 with hepatocarcinogenesis. However, more studies are needed to confirm its association with HBV-related HCC.

CONCLUSION

We confirm that there are significant differences in genetic background between Africa and East Asia. By correlating genetic backgrounds with clinical epidemiology, we have found that the allele frequency of HLA-DQ and -DP loci do explain a higher prevalence of HBsAg in Southeastern compared to Northeastern Asia. Some of these SNPs also showed West-to-East changes in allele frequency in Africa and are correlated with HCC incidence. For the non-HLA loci, SNPs in NTCP and CTF19 showed allele frequency trends from North-to-South in East Asians, supporting their association with fighting in persistent HBV infection. There is a strong correlation between allele frequency and HCC incidence on SNPs located in MICA and weak positive correlations in KIF1, STAT4, and IL1A. The studies concerning genetic factors and hepatocarcinogenesis are difficult since multiple factors are involved and different genetic backgrounds exist among the study populations.

DECLARATIONS

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Authors' contributions

Designed the study and wrote the manuscript: Tai DI

Collected and organized data: Tai J

Availability of data and materials

The data source is from the 1000 Genomes Project (<http://www.1000genomes.org/>).

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Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

- Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet* 2015;386:1546-55.
- Chen CJ, Tai J, Tai DI. Hepatocellular carcinoma occurred in a Hepatitis B carrier clinic cohort during a mean follow up of 10 years. *Hepatoma Res* 2019;5:25.
- Yu MW, Chang HC, Liaw YF, et al. Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. *J Natl Cancer Inst* 2000;92:1159-64.
- Burk RD, Hwang LY, Ho GY, Shafritz DA, Beasley RP. Outcome of perinatal hepatitis B virus exposure is dependent on maternal virus load. *J Infect Dis* 1994;170:1418-23.
- Tai DI, Chen CH, Chang TT, et al. Eight-year nationwide survival analysis in relatives of patients with hepatocellular carcinoma: role of viral infection. *J Gastroenterol Hepatol* 2002;17:682-9.
- Chen CH, Chen YY, Chen GH, et al. Hepatitis B virus transmission and hepatocarcinogenesis: a 9 year retrospective cohort of 13676 relatives with hepatocellular carcinoma. *J Hepatol* 2004;40:653-9.
- Tai DI, Tsay PK, Chen WT, Chu CM, Liaw YF. Relative roles of HBsAg seroclearance and mortality in the decline of HBsAg prevalence with increasing age. *Am J Gastroenterol* 2010;105:1102-9.
- Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. *Hepatology* 1988;8:493-6.
- Cardin R, Picocchi M, Bortolami M, et al. Oxidative damage in the progression of chronic liver disease to hepatocellular carcinoma: an intricate pathway. *World J Gastroenterol* 2014;20:3078-86.
- Liu WC, Wu IC, Lee YC, et al. Hepatocellular carcinoma-associated single-nucleotide variants and deletions identified by the use of genome-wide high-throughput analysis of hepatitis B virus. *J Pathol* 2017;243:176-92.
- Zhang Z, Wang C, Liu Z, et al. Host genetic determinants of hepatitis B virus infection. *Front Genet* 2019;10:696.
- Akcaay IM, Katrinli S, Ozdil K, Doganay GD, Doganay L. Host genetic factors affecting hepatitis B infection outcomes: insights from genome-wide association studies. *World J Gastroenterol* 2018;24:3347-60.
- Zhu H, Wu J, Shen X. Genome-wide association study: new genetic insights into HBV/HCV-related hepatocellular carcinoma genomes. *Scand J Gastroenterol* 2017;52:209-15.
- Hai H, Tamori A, Kawada N. Role of hepatitis B virus DNA integration in human hepatocarcinogenesis. *World J Gastroenterol* 2014;20:6236-43.
- 1000 Genomes Project Consortium, Auton A, Brooks LD, et al. A global reference for human genetic variation. *Nature* 2015;526:68-74.
- Xia GL, Liu CB, Cao HL, et al. Prevalence of hepatitis B and C virus infections in the general Chinese population. Results from a nationwide cross-sectional seroepidemiologic study of hepatitis A, B, C, D, and E virus infections in China, 1992. *Int Hepatol Comm* 1996;5:62-73.
- Kamatani Y, Watanapokayakit S, Ochi H, et al. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. *Nat Genet* 2009;41:591-5.
- Mbarek H, Ochi H, Urabe Y, et al. A genome-wide association study of chronic hepatitis B identified novel risk locus in a Japanese population. *Hum Mol Genet* 2011;20:3884-92.
- Hu Z, Liu Y, Zhai X, et al. New loci associated with chronic hepatitis B virus infection in Han Chinese. *Nat Genet* 2013;45:1499-503.
- Nishida N, Sawai H, Matsuura K, et al. Genome-wide association study confirming association of HLA-DP with protection against chronic hepatitis B and viral clearance in Japanese and Korean. *PLoS One* 2012;7:e39175.
- Kim YJ, Kim HY, Lee JH, et al. A genome-wide association study identified new variants associated with the risk of chronic hepatitis B. *Hum Mol Genet* 2013;22:4233-8.
- Al-Qahtani AA, Al-Anazi MR, Abdo AA, Sanai FM, Al-Hamoudi W, et al. Association between HLA variations and chronic hepatitis B virus infection in Saudi Arabian patients. *PLoS One* 2014;9:e80445.
- Chang SW, Fann CS, Su WH, et al. A genome-wide association study on chronic HBV infection and its clinical progression in male Han-Taiwanese. *PLoS One* 2014;9:e99724.
- Tai DI, Jeng WJ, Lin CY. A global perspective on HBV-related SNPs and evolution during human migration. *Hepatol Commun* 2017;1:1005-13.
- Liaw YF, Chu CM. Hepatitis B virus infection. *Lancet* 2009;373:582-92.
- Chang MH, Hsu HY, Hsu HC, et al. The significance of spontaneous hepatitis B e antigen seroconversion in childhood: with special

- emphasis on the clearance of hepatitis B e antigen before 3 years of age. *Hepatology* 1995;22:1387-92.
27. Hsu YS, Chien RN, Yeh CT, et al. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. *Hepatology* 2002;35:1522-7.
 28. Lin CL, Kao JH. Natural history of acute and chronic hepatitis B: The role of HBV genotypes and mutants. *Best Pract Res Clin Gastroenterol* 2017;31:249-55.
 29. Chang MH, Sung JL, Lee CY, et al. Factors affecting clearance of hepatitis B e antigen in hepatitis B surface antigen carrier children. *J Pediatr* 1989;115:386-90.
 30. Hadziyannis SJ. Natural history of chronic hepatitis B in Euro-Mediterranean and African countries. *J Hepatol* 2011;55:183-91.
 31. Iorio R, Giannattasio A, Cirillo F, D' Alessandro L, Vegnente A. Long-term outcome in children with chronic hepatitis B: a 24-year observation period. *Clin Infect Dis* 2007;45:943-9.
 32. Liu S, Koh SS, Lee CG. Hepatitis B virus X protein and hepatocarcinogenesis. *Int J Mol Sci* 2016;17:E940.
 33. Tu T, Budzinska MA, Vondran FWR, Shackel NA, Urban S. Hepatitis B virus DNA integration occurs early in the viral life cycle in an in vitro infection model via sodium taurocholate cotransporting polypeptide-dependent uptake of enveloped virus particles. *J Virol* 2018;92:e02007-17.
 34. Budzinska MA, Shackel NA, Urban S, Tu T. Cellular genomic sites of hepatitis B virus DNA integration. *Genes (Basel)* 2018;9:E365.
 35. Yang L, Ye S, Zhao X, et al. Molecular characterization of HBV DNA integration in patients with hepatitis and hepatocellular carcinoma. *J Cancer* 2018;9:3225-35.
 36. An P, Xu J, Yu Y, Winkler CA. Host and viral variation in HBV-related hepatocellular carcinoma. *Front genet* 2018;9:261.
 37. Lazarevic I. Clinical implications of hepatitis B virus mutations: recent advances. *World J Gastroenterol* 2014;20:7653-64.
 38. O'Brien TR, Kohaar I, Pfeiffer RM, et al. Risk alleles for chronic hepatitis B are associated with decreased mRNA expression of HLA-DPA1 and HLA-DPB1 in normal human liver. *Genes Immun* 2011;12:428-33.
 39. Yang JD, Hainaut P, Gores GJ, et al. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019;16:589-604.
 40. Yan H, Zhong G, Xu G, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife* 2012;1:e00049.
 41. Li N, Zhang P, Yang C, et al. Association of genetic variation of sodium taurocholate cotransporting polypeptide with chronic hepatitis B virus infection. *Genet Test Mol Biomarkers* 2014;18:425-9.
 42. Posuwan N, Payungporn S, Tangkijvanich P, et al. Genetic association of human leukocyte antigens with chronicity or resolution of hepatitis B infection in Thai population. *PLoS One* 2014;9:e86007.
 43. Kumar V, Kato N, Urabe Y, et al. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. *Nat Genet* 2011;43:455.
 44. Luo X, Wang Y, Shen A, Deng H, Ye M. Relationship between the rs2596542 polymorphism in the MICA gene promoter and HBV/HCV infection-induced hepatocellular carcinoma: a meta-analysis. *BMC Med Genet* 2019;20:142.
 45. Diefenbach A, Jensen ER, Jamieson AM, Raulet DH. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature* 2001;413:165-71.
 46. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature* 2002;419:734-8.
 47. Zhang H, Zhai Y, Hu Z, et al. Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. *Nat Genet* 2010;42:755-8.
 48. Luo YY, Zhang HP, Huang AL, Hu JL. Association between KIF1B rs17401966 genetic polymorphism and hepatocellular carcinoma susceptibility: an updated meta-analysis. *BMC Med Genet* 2019;20:59.
 49. Kong XN, Horiguchi N, Mori M, Guo B. Cytokines and STATs in liver fibrosis. *Front Physiol* 2012;3:69.
 50. Gao B. Cytokines, STATs and liver disease. *Cell Mol Immunol* 2005;2:92-100.
 51. Shi H, He H, Ojha SC, et al. Association of STAT3 and STAT4 polymorphisms with susceptibility to chronic hepatitis B virus infection and risk of hepatocellular carcinoma: a meta-analysis. *Biosci Rep* 2019;39:BSR20190783.
 52. Gao Y, He Y, Ding J, et al. An insertion/deletion polymorphism at miRNA-122-binding site in the interleukin-1 α 3' untranslated region confers risk for hepatocellular carcinoma. *Carcinogenesis* 2009;30:2064-9.
 53. Du Y, Han X, Pu R, et al. Association of miRNA-122-binding site polymorphism at the interleukin-1 α gene and its interaction with hepatitis B virus mutations with hepatocellular carcinoma risk. *Front Med* 2014;8:217-26.
 54. Kim YS, Cheong JY, Cho SW, et al. A functional SNP of the Interleukin-18 gene is associated with the presence of hepatocellular carcinoma in hepatitis B virus-infected patients. *Dig Dis Sci* 2009;54:2722-8.
 55. Zhu SL, Zhao Y, Hu XY, et al. Genetic polymorphisms -137 (rs187238) and -607 (rs1946518) in the interleukin-18 promoter may not be associated with development of hepatocellular carcinoma. *Sci Rep* 2016;6:39404.
 56. Qu LS, Jin F, Guo YM, et al. Nine susceptibility loci for hepatitis B virus-related hepatocellular carcinoma identified by a pilot two-stage genome-wide association study. *Oncol Lett* 2016;11:624-32.

Perspective

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Should selection criteria for HCC be the same (or different) between LDLT and DDLT?

Flavia Feier^{1,2}

¹Liver Transplantation Department, Hospital de Clinicas de Porto Alegre, Porto Alegre 90035-903, Brazil.

²Pediatric Liver Transplantation Department, Irmandade Santa Casa de Porto Alegre, Porto Alegre 90020-090, Brazil.

Correspondence to: Dr. Flavia Feier, Liver Transplantation Department, Hospital de Clinicas de Porto Alegre, Rua Ramiro Barcelos 2350, Porto Alegre 90035-903, Brazil. E-mail: flavia.feier@gmail.com

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Abstract

Since the Milan Criteria (MC) were adopted in many countries as the allocation policy criteria for patients with hepatocellular carcinoma to be transplanted, many groups started to expand it to provide a chance for patients with tumors outside the MC who could achieve similar survival rates. With the scarcity of deceased donors, Asian countries improved the results with living donor liver transplantation, allowing patients outside MC to be transplanted with a living donor. Newer prognostic models and a more profound understanding of tumor behavior are targeting better patient selection. Currently, patients are unevenly selected for liver transplantation and mostly separated into those fulfilling the MC and transplanted with a deceased donor and those with expanded criteria and transplanted with a living donor. In this paper, insight is brought into this debate.

Keywords: Living donor, hepatocellular carcinoma, alpha-fetoprotein

Liver transplantation (LT) is considered a curative treatment for patients with hepatocellular carcinoma (HCC) not amenable to surgical resection or ablative curative therapies. HCC was the indication for LT in 20.4% of the recipients in the USA in 2018^[1], 14.4% in Europe^[2] and 7.6% and 19% for patients transplanted with deceased donors and living donors, respectively, in Japan^[2].

To provide a real chance for these patients to be transplanted with a deceased donor, exception points (outside the MELD score) have been given all over the world. However, in countries with organ shortage,



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living donor liver transplantation (LDLT) has progressively expanded as the best alternative for such patients. In Asian countries, it is the most prevalent form of LT - 95% of LT in Japan^[2] as opposed to 4.4% in the USA^[1], with excellent outcomes in both recipients and donors^[3,4]. Certainly, donor safety is an ongoing concern, as a healthy individual will undergo a major surgical procedure. However, current morbidity and mortality rates following living liver donation have been reported to be 15%-25% and 0.5%, respectively^[5]. Driven by these results, the indications for LDLT in HCC patients were gradually expanded. The premise was that the survival benefit potentially achieved for those patients, within these expanded criteria, would not compromise the liver transplant allocation policy.

The inclusion criteria for HCC patients has dominated the scientific debate in the last years. Decades ago, the results from Mazzafero's group helped establish criteria to indicate LT for patients with HCC. The so-called Milan Criteria (MC) (single tumor ≤ 5 cm, or less than 3 tumors ≤ 3 cm without major vessel involvement) reported a post-LT 4-year survival of 75% for patients meeting these criteria^[6]. This expected survival was comparable to that of patients transplanted for other indications, justifying organ allocation for patients with HCC.

However, several centers around the world considered the MC to be too strict, possibly excluding patients that still could benefit from LT. The University of California San Francisco expanded the criteria to a single nodule with a maximum diameter of 65 mm, or two or three tumors, each with a maximum diameter of 45 mm, and a sum tumor diameter ≤ 80 mm, with a 5-year survival rate of 75%^[7]. Also achieving a similar survival (71.2%), came the up-to-seven criteria [the total of the size of the largest tumor (in cm) and the number of tumors no larger than 7]^[8], among others^[9].

According to cultural and religious particularities, deceased donor liver transplantation (DDLT) is precluded, or rarely performed in some Eastern countries. In that scenario, centers developed criteria to indicate LDLT for HCC patients. Tokyo has implemented the 5-5 rule (up to five nodules with a maximum diameter of 5 cm), with an overall 5-years survival rate of 75%^[10]. The South Korea criteria expanded the Tokyo criteria to 6 nodules, with a 5-year survival rate of 76.3%^[3]. Choi *et al.*^[11] expanded the criteria for LDLT even more (up to seven tumors with the greatest diameter ≤ 70 mm) reporting a 5-year survival rate of 72%.

Going further, it did not seem logical to restrict the indications only to size and number of nodules without considering the biological behavior of the tumor. Alpha-fetoprotein (AFP) level was included in a couple of studies, with different cutoff values - less than 400 ng/mL, or less than 1000 ng/mL - and were associated with tumor number or diameter^[12]. Shimamura *et al.*^[10] included AFP levels to select patients for LDLT, calling the 5-5-500 criteria (tumor size ≤ 5 cm diameter, ≤ 5 nodules, AFP ≤ 500 ng/mL), and achieved a 5-year recurrence-free survival of 90%.

Other markers are recently being included, such as des-gamma-carboxy prothrombin (DCP), also called protein induced by vitamin K absence or antagonist II. This marker was also associated with the presence of microvascular invasion^[3]. DCP measurement was included in the Kyoto group LDLT criteria (the number of HCC nodules up to ten in addition to the largest diameter ≤ 5 cm and serum DCP level ≤ 400 mAU/mL). The 5-year disease-free and overall survival rates were 93 and 82%, respectively^[13]. In the same direction, Taketomi *et al.*^[14] included all HCCs with a diameter of ≤ 5 cm and DCP < 300 mAU/mL, achieving a 5-year recurrence-free survival rate of 80%. Both Hangzhou and Toronto groups included the HCC biopsy result in their selection criteria^[12].

The MoRAL score was developed in Korea to predict HCC recurrence after LDLT, and includes serum tumor markers, AFP and DCP. The MoRAL score has a high predictive power for tumor recurrence in

patients transplanted with LDLT outside MC (c-statistic = 0.8). In fact, patients within MC and with high MoRAL scores had higher recurrence rates than patients outside MC and with a low MoRAL score^[3]. When compared to other existent scores, it had the best performance in predicting tumor recurrence after LDLT. However, it is important to state that 70%-80% of HCC patients have hepatitis B virus infection in South Korea, so it is difficult to expand these results to Western countries, where hepatitis C dominates the etiologies for HCC and where most of the transplants are with deceased donors. It would be important to validate this model in the West, and for DDLT, to aid in the identification of highly selected patients beyond MC, who are at a low risk of tumor recurrence^[15].

To predict the risk of death after LT for HCC with DDLT, Mazzafero's group developed the Metroticket 2.0 score. They concluded that to achieve a 5-year survival of 70%, AFP levels should be below 200 ng/mL, and the sum of number and size (in cm) of tumors should not exceed 7. These values change according to the AFP levels, and the prediction of survival after LT for HCC can be calculated online^[8].

The role of studying tumor biology (biopsy, tumor markers, tumor behavior) and the presence of microvascular invasion, which is a surrogate marker of worse survival^[4], could help in patient selection, selecting those patients with less aggressive tumors^[16]. This approach could expand safely the indications for LT, keeping the expected 5-year survival above 70%^[3,8].

In contrast to DDLT, recipient selection for LDLT is not limited by organ allocation systems. When the first results after LDLT for HCC were published, recurrence rates were higher than with DDLT. Several concerns were raised about the influence of liver regeneration and suboptimal oncological resection in LDLT, suggesting that it could affect recurrence^[17].

Recently, Zhang *et al.*^[18] conducted a meta-analysis comparing recurrence of HCC in patients transplanted with liver from living or deceased donors and found a higher recurrence rate of HCC in patients submitted to LDLT (HR 1.5). In the selected studies, 5-year recurrence-free survival in DDLT patients was between 42% and 100%, and with living donors between 61.6% and 89%. Waiting time was shorter for LDLT in the majority of the studies, which may be responsible for the higher recurrence rate in LDLT, speculating that patients with biologically higher risk tumors were selected. Selecting patients with a more aggressive tumor biology for LDLT, an effect of a shorter waiting list time and also expanding the criteria for LDLT are also factors that could have an influence on the reported higher recurrence rates in those patients.

Goldaracena *et al.*^[19] tried to answer the question regarding the observed higher recurrence rates in patients transplanted using living donors and conducted an intention to treat analysis. The patients were analyzed according to their inclusion status in the transplant list, as a potential LDLT (pLDLT) or potential DDLT (pDDLT). Patients were included if they fulfilled the Extended Toronto Criteria - total tumor volume $\leq 115 \text{ m}^3$ and AFP $\leq 400 \text{ ng/mL}$, no macrovascular invasion and no extrahepatic disease. The waiting list time was significantly shorter for patients listed as pLDLT, with better 5-year survival rates (68% vs. 57%). In the multivariate regression analysis, having a living donor available was a protective factor against death (HR 0.67). The survival benefit appeared to be predominantly in patients whose tumors fulfilled the MC: 70% in the pLDLT group and 53% in the pDDLT group. Among patients beyond MC, 5-year survival rates were 62% for those outside MC in both groups. For those patients actually transplanted with a living donor, 5-year recurrence-free survival rates were 80% vs. 72% for those actually transplanted with a deceased donor.

Results are still conflicting, but HCC recurrence and post-LT survival appear to correlate with biological tumor behavior and patient selection rather than if the patient was transplanted with a living or deceased donor. Reports from Eastern countries^[3,9,13], where the majority of patients are transplanted with living

donors and with expanded criteria, show excellent disease-free and overall survivals. Even in the West, where there is a predominance of DDLT, the study by Goldaracena *et al.*^[19] showed that having a living donor available had a protective effect, even if in the end, the patient is transplanted with a deceased donor.

As already stated, outcomes in LDLT for HCC can be affected by several factors, including time to transplant, the possibility to perform the transplant in expanded criteria patients, and the possibility of the liver regeneration adversely affecting tumor growth^[20].

Because of the limited number of deceased donors particularly in Asian countries, patients beyond MC were rarely submitted to DDLT. In the West, however, expanding LDLT can provide an alternative to DDLT, allowing many centers to indicate LT for patients beyond MC. Currently, organ allocation systems provide exception points for patients with early HCC, with favorable prognosis. As for recipients from living donors, organ availability is driven by the living donor. In that case, usually the transplant happens when the donor is ready, shortening the waiting time, allowing patients with more aggressive tumors to be transplanted. We are dealing with uneven criteria to indicate LT for HCC, defining these criteria not only on the basis of individual patient prognosis but by the fact of whether there is a living donor available.

If one looks at the results from more than a decade ago, it would be reasonable to address different selection criteria for patients undergoing DDLT or LDLT. However, on the basis of recent research and a better understanding of tumor behavior, the discussion should be “how do we adequately select HCC patients for LT?”, rather than to which transplant modality will the patient be submitted (LDLT, DDLT, Split Liver or Domino Transplantation).

Future directions are pointing to better patient selection - looking deeper into tumor biological behavior and addressing the presence of microvascular invasion, and not indicating LT to patients with aggressive tumors with a worse prognosis. It is urgent to validate existing models that predict survival in LDLT and DDLT, such as the MoRAL Score^[3], including such criteria in the organ allocation policy, currently restricted in many countries to the MC of 1996^[6]. The practice reported by the Toronto group^[19], having the same criteria to indicate LT on the basis of patient prognosis, irrespectively if the patient will be assigned a living or deceased donor, follows the principle of equality. After being included in the waiting list, if there is a living donor available, the better it is for the patient and for the donor pool. If such a policy could be embraced by Western countries, perhaps LDLT in the West could reach comparable results as those achieved by our Asian fellows.

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Authors' contributions

The author contributed solely to the article.

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Conflicts of interest

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Ethical approval and consent to participate

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Not applicable.

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REFERENCES

1. Kwong A, Kim WR, Lake JR, et al. OPTN/SRTR 2018 annual data report: liver. *Am J Transplant* 2020;20 Suppl s1:193-299.
2. Umeshita K, Eguchi S, Egawa H, et al. Liver transplantation in Japan: registry by the Japanese liver transplantation society. *Hepatol Res* 2019;49:964-80.
3. Lee JH, Cho Y, Kim HY, et al. Serum tumor markers provide refined prognostication in selecting liver transplantation candidate for hepatocellular carcinoma patients beyond the Milan Criteria. *Ann Surg* 2016;263:842-50.
4. Bhatti ABH, Dar FS, Qureshi AI, et al. Living donor liver transplantation for hepatocellular carcinoma: a single-center experience from Pakistan. *J Clin Exp Hepatol* 2019;9:704-9.
5. Goldaracena N, Barbas AS. Living donor liver transplantation. *Curr Opin Organ Transplant* 2019;24:131-7.
6. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996;334:693-9.
7. Yao FY, Xiao L, Bass NM, et al. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. *Am J Transplant* 2007;7:2587-96.
8. Mazzaferro V, Llovet JM, Miceli R, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009;10:35-43.
9. Toso C, Meeberg G, Hernandez-Alejandro R, et al. Total tumor volume and alpha-fetoprotein for selection of transplant candidates with hepatocellular carcinoma: a prospective validation. *Hepatology* 2015;62:158-65.
10. Shimamura T, Akamatsu N, Fujiyoshi M, et al; Japanese Liver Transplantation Society. Expanded living-donor liver transplantation criteria for patients with hepatocellular carcinoma based on the Japanese nationwide survey: the 5-5-500 rule - a retrospective study. *Transpl Int* 2019;32:356-68.
11. Choi HJ, Kim DG, Na GH, Hong TH, You YK. Extended criteria for living donor liver transplantation in patients with advanced hepatocellular carcinoma. *Transplant Proc* 2012;44:399-402.
12. Levi Sandri GB, Rayar M, Qi X, Lucatelli P. Liver transplant for patients outside Milan criteria. *Transl Gastroenterol Hepatol* 2018;3:81.
13. Ito T, Takada Y, Ueda M, et al. Expansion of selection criteria for patients with hepatocellular carcinoma in living donor liver transplantation. *Liver Transpl* 2007;13:1637-44.
14. Taketomi A, Sanefuji K, Soejima Y, et al. Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. *Transplantation* 2009;87:531-7.
15. Chang Y, Cho Y, Lee JH, et al. Comparison of models for tumor recurrence after liver transplantation for the patients with hepatocellular carcinoma: a multicenter long-term follow-up study. *Cancers* 2019;11:1295.
16. Limkemann AJP, Abreu P, Sapisochin G. How far can we go with hepatocellular carcinoma in living donor liver transplantation? *Curr Opin Organ Transplant* 2019;24:644-50.
17. Fisher RA, Kulik LM, Freise CE, et al; A2ALL Study Group. Hepatocellular carcinoma recurrence and death following living and deceased donor liver transplantation. *Am J Transplant* 2007;7:1601-8.
18. Zhang HM, Shi YX, Sun LY, Zhu ZJ. Hepatocellular carcinoma recurrence in living and deceased donor liver transplantation: a systematic review and meta-analysis. *Chin Med J* 2019;132:1599-609.
19. Goldaracena N, Gorgen A, Doyle A, et al. Live donor liver transplantation for patients with hepatocellular carcinoma offers increased survival vs. deceased donation. *J Hepatol* 2019;70:666-73.
20. Sugawara Y. Living-donor liver transplantation for patients with hepatocellular carcinoma in Japan: current situations and challenge. *Hepatobiliary Pancreat Dis Int* 2020;19:1-2.

Review

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Living donor liver transplantation for patients with advanced hepatocellular carcinoma

J. Michael Cullen¹, Paola Vargas^{1,2}, Nicolas Goldaracena^{1,2}

¹Department of Surgery, University of Virginia, Charlottesville, VA 22908, USA.

²Division of Transplantation, Department of Surgery, University of Virginia, Charlottesville, VA 22908, USA.

Correspondence to: Dr. Nicolas Goldaracena, Department of Surgery, University of Virginia, Health System, PO BOX 800709, Charlottesville, VA 22908, USA. E-mail: n.goldaracena@virginia.edu

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Abstract

Liver transplantation (LT) is the treatment of choice for patients with hepatocellular carcinoma (HCC) and underlying liver disease. Given the organ scarcity, LT for patients with HCC have been restricted to those patients associated with the highest survivals. However, many patients with extended criteria HCC can still benefit from LT, but due to deceased organ shortage, they are not offered that opportunity. Living donor liver transplantation (LDLT) emerged as a successful strategy to overcome organ shortage around the world and as LDLT experience grows, this technique might offer the opportunity to expand the indications of LT to patients with advanced HCC. Therefore, since LDLT is not competing for deceased donor organs, many patients with extended criteria HCC who could still benefit from transplantation may have access to this treatment option. In this review, we will discuss the role of LDLT for patients with advanced-stage HCC and how LDLT allows for safe expansion of HCC transplant criteria.

Keywords: Living donor liver transplantation, hepatocellular carcinoma, liver transplantation, transplant oncology, clinical outcomes

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third leading cause of death worldwide and the most common primary liver cancer^[1,2]. Resection provides the best treatment option for disease confined to the liver.



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However, the approach and final treatment depends on tumor size, number of tumors and their location, as well as liver function and performance status. Liver transplantation (LT) has the advantage of removing the underlying liver disease, reducing the risk for postoperative liver failure and *de novo* HCC development. Therefore, LT is the best treatment option for patients with HCC and underlying liver cirrhosis. However, due to organ scarcity, LT for HCC has been restricted to patients with the highest prospective 5-year survival and recurrence rates^[3].

Many have proposed that these criteria might be too restrictive, and that many patients with more advanced disease would still benefit from LT if organs would be available^[4,5].

Living donor liver transplantation (LDLT) emerged as a successful strategy to overcome organ shortage around the world^[6]. As LDLT experience grows, the application of this technique might offer the opportunity to expand the boundaries of LT to settings in which the organ shortage presents a limitation. Because the transplant candidate is not competing for deceased donor organs, patients with extended criteria HCC who could still benefit from LT may have access to the treatment. Moreover, many studies have already proven that LDLT might even offer a benefit over deceased donor liver transplantation (DDLT) to patients fulfilling restrictive transplant criteria^[7]. It is the scope of this study to review the role of LDLT for patients with more advanced HCC and how LDLT allows for safe expansion of HCC transplant criteria.

Staging systems for hepatocellular carcinoma

Currently, there is no universal consensus regarding the best staging system for HCC and its management. Among the many proposed, two main classification systems are used. The Barcelona Clinic Liver Cancer (BCLC) classification, the most used in Western countries, and the Hong Kong Liver Cancer (HKLC) staging system, which was created as a treatment guidance for Asian patients with HCC and is mainly used in Asian countries. Both classification systems use parameters such as presence of extrahepatic vascular invasion, tumor size, and number of nodules to stage HCC and provide management recommendations^[8-11].

Despite both classifications being widely used, their differences complicate the decision-making process for the transplant team when presented with an advanced HCC. In a recent Brazilian study^[9], 519 patients diagnosed with HCC were staged according to the BCLC and HKLC system with the aim to analyze therapeutic approach for different stages. The authors found that between both systems, there was high general agreement regarding therapeutic management of HCC in the Western population. The highest agreement was between stages HKLC-I and BCLC-0 (100%) and HKLC-IV and BCLC-C (98.7%). However, agreement was low in intermediate HCC cases (BCLC-B). The authors found that according to the HKLC, more than 50% of the BCLC-B stage could have been candidates for curative treatment rather than palliative treatment recommended by BCLC. Other authors agreed that BCLC is outdated, highly restrictive with a trend to limit treatment options for more advanced tumors, and needs re-evaluation in order to achieve a proper classification of these patients with a management plan according to current practices^[10].

It is crucial that HCC staging be conducted in an individualized manner, taking into account biological and etiological heterogeneity among populations in order to provide the patient with the best treatment option available in their case^[9].

LIVING LIVER DONATION FOR HEPATOCELLULAR CARCINOMA

Surgical resection is generally recommended for Child-Pugh Class A cirrhotic patients without significant portal hypertension or those with early-stage disease and a single HCC lesion^[12]. However, multiple studies have shown that transplantation can provide superior long-term outcomes over resection^[3,12,13].

The available literature comparing outcomes of LDLT and DDLT for HCC is limited^[14-16]. Early reports showed recurrence rates were higher after LDLT than DDLT, but this has been contradicted by recent work^[17,18]. An important advantage of LDLT is that it effectively allows HCC patients to not be in direct competition with liver failure patients whose Model for End-Stage Liver Disease score afford them higher wait list priority. Goldaracena *et al.*^[16] found patients undergoing LDLT had faster access to transplant and shorter wait times compared with DDLT. In this recent intention-to-treat analysis of 219 LDLTs and 632 DDLTs for HCC, patients with a potential live donor had a 33% reduction in risk of death from the time of listing due to shorter wait time and decreased waitlist dropout risk. LDLT actually offered a survival benefit over DDLT in this analysis.

Another important aspect unique to LDLT is that each graft is a private gift and not subject to the allocation system. Donation can be direct or altruistic. Patients with advanced HCC should be evaluated on an individual basis so that those outside conventional criteria who would benefit from a transplant can receive one without the risks of disease progression while on the waitlist and its concomitant mortality.

CONVENTIONAL CRITERIA FOR LIVER TRANSPLANT FOR HEPATOCELLULAR CARCINOMA

The Milan criteria established the benchmark for acceptable outcomes for liver transplantation in HCC. Mazzaferro *et al.*^[19] reported recurrence rates less than 15% and a 5-year survival of 75% when transplanting HCC patients with (a) one tumor less than 5 cm or (b) three tumors each less than 3 cm. The University of California at San Francisco (UCSF) liberalized these size limitations and selected patients with (a) one solitary tumor up to 6.5 cm or (b) three tumors with the largest 4.5 cm or less and total tumor diameter 8 cm or less^[20]. Using these expanded criteria, the UCSF group matched Milan outcomes with recurrence and 5-year survival rates of 10% and 75.2%, respectively. In 2008, the Asan Medical Center group in Seoul, South Korea, further expanded eligibility criteria to include patients with up to 5 tumors and size less than 6 cm and achieved a 5-year survival rate of 81.6%^[21].

TRANSPLANTATION BEYOND CONVENTIONAL CRITERIA

Many centers have sought to expand these traditional criteria, specifically by emphasizing tumor biology and behavior as opposed to a reliance on tumor size and number^[22]. The extended Toronto Criteria places no restrictions on number of tumors or tumor size and offers transplantation to patients without systemic cancer-related symptoms, extrahepatic disease, vascular invasion, or poorly differentiated tumors^[5]. Sapisochin *et al.*^[5] further validated these criteria with a prospective study including 105 patients outside Milan and 76 beyond UCSF criteria, and reported a 5-year survival of 69% which did not differ significantly from patients within Milan. An important aspect of this study was the authors found an alpha-fetoprotein (AFP) level greater than 500 ng/mL to be predictive of poor outcomes.

In Asia, LDLT predominates over DDLT, and this region has been an epicenter for criteria expansion. In 2016, the National Cancer Center - Korea reported an 85.2% overall 5-year survival and 84% disease free survival after LDLT for 164 patients with total tumor size of 10 cm or less and a negative 18F-fluorodeoxyglucose positron emission tomography scan^[23]. The Kyushu Criteria, published in 2017, demonstrated a 75.9% 5-year survival rate when performing LDLT for 161 patients with any number of tumors, but with size less than 5 cm or des-gamma carboxy prothrombin levels less than 300 mAU/mL^[24]. An interesting aspect of this study was that it showed LDLT had a survival benefit when compared with DDLT in an intention to treat sub-analysis, despite LDLT patients having more advanced tumor stage.

LIVING DONOR LIVER TRANSPLANTATION FOR ADVANCED HEPATOCELLULAR CARCINOMA

Because deceased donor organs are allocated to patients on the waiting list expected to survive the longest after transplant, advanced HCC often precludes DDLT. Many patients outside transplantable criteria are

treated with palliative therapies that carry a 3-year survival rate of just 30%^[25]. LDLT is now an option for these patients that offers superior outcomes compared with palliative therapy.

A concise pre-operative evaluation is critical. Imaging techniques including CT and MRI are among the most used diagnostic tools. However, characterization of tumor properties such as invasion into segmental branches of the portal vein can be difficult to assess with these modalities alone^[26]. While biomarkers and cutoff levels vary among centers and within the literature, the most widely used are AFP, PIVKA-II, γ -GT/ALT ratio, and recently proposed inflammatory markers including CRP, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio^[22,26-28].

A multicenter retrospective study in South Korea^[28] evaluated the outcomes of LT for extremely advanced HCC patients ($n = 169$), using AFP and PIVKA-II as a selection strategy to decide whether those patients were suitable for LT. The authors found that patients with AFP + PIVKA-II ≤ 300 showed a 5-year overall and recurrence-free survival rate of 47.8% and 53.4%, respectively. These values were significantly superior to those of patients with AFP (ng/mL) + PIVKA-II (mAU/mL) > 300 (21.0% and 10.8%, respectively; $P < 0.001$). The authors concluded that if tumor biology is favorable regardless of tumor size and number, acceptable long-term survival after LT can be expected.

Recently, Kornberg *et al.*^[27] analyzed 119 LT patients with advanced HCC to determine the prognostic impact of clinical and histopathologic factors including pre-LT serum AFP and CRP values. The authors identified that pre-transplant serum levels of AFP > 100 ng/mL (OR = 13.31) and CRP > 0.8 mg/dL (OR = 13.97) were independent predictors of HCC recurrence. The group proposed a serological risk index based on the mentioned biomarkers. A cumulative risk of HCC relapse at 5 years post-LT was determined to be 2.3% in low serological tumor activity (STA) index (AFP ≤ 100 ng/mL + CRP ≤ 0.8 mg/dL), 17.1% in intermediate STA (AFP ≤ 100 ng/mL or CRP ≤ 0.8 mg/dL), and 91.6% in high STA index (AFP > 100 ng/mL + CRP > 0.8 mg/dL; $P < 0.001$). Prospective randomized studies that provide validation and present reproducible results are needed.

Despite being a promising therapeutic approach for advance HCC, evidence suggest that absolute contraindications for transplant, includes presence of extrahepatic metastasis such as suspicious porta hepatic nodal diseases, massive infiltrative type, major vascular invasion, and cases with ruptured HCC^[29,30].

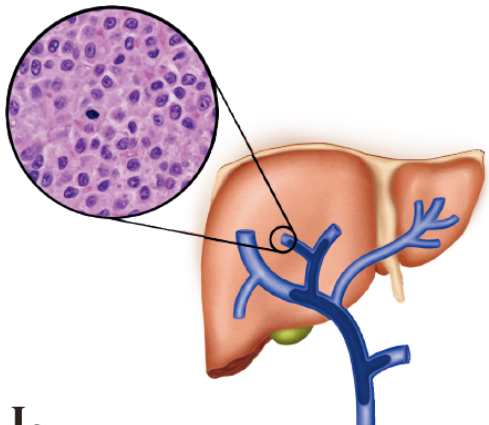
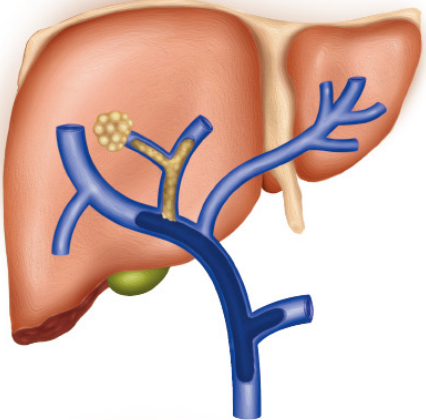
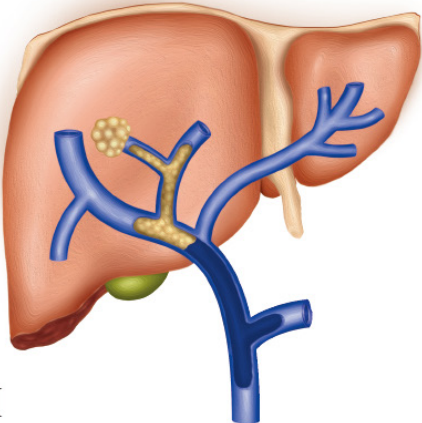
Vascular invasion

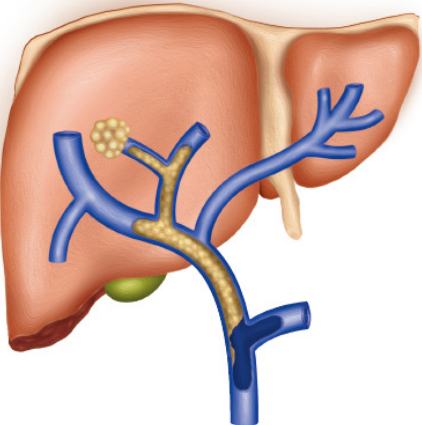
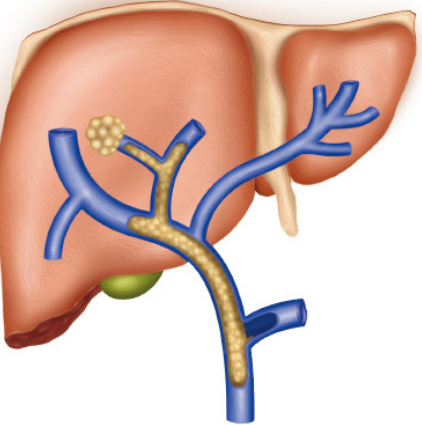
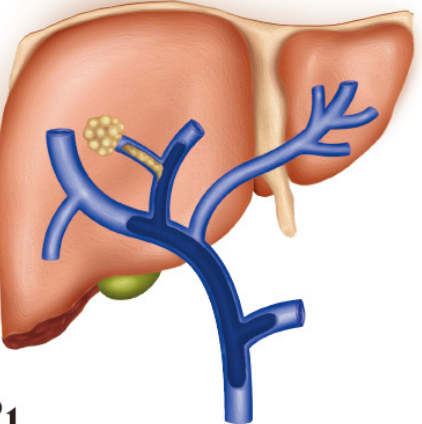
Portal vein tumor thrombosis (PVTT) is seen in up to 60% of HCC patients, and considered a marker of systemic disease associated with a high rate of post-transplant recurrence^[31-33]. Because of this, PVTT has been traditionally considered a contraindication to liver transplantation. However, new studies are challenging this convention based upon the PVTT classification. There are several classifications for PVTT based on the portal vein segment involved. However, two classifications are currently valid to effectively assess prognosis and provide guidance for surgical treatment^[34]. The Liver Cancer Study Group of Japan differentiates PVTT in four main categories of portal vein invasion, VP1, VP2, VP3, and VP4^[34-36]. In a similar fashion, Cheng *et al.*^[34] proposed a four-category classification, with the distinction of a category for microscopic portal vein invasion and the allusion of the extension of PVTT involving the superior mesenteric vein or inferior vena cava^[37,38] [Figure 1].

The prognosis of patients with HCC and PVTT is known to be deleterious^[39], and the BCLC recommendation indicates Sorafenib as the only treatment option^[33,40]. However, depending on the PVTT classification, different treatment options are available and survival rates vary among the literature. A summary of treatment strategies according to PVTT is provided in Figure 1^[26,34,35,38,41]. In terms of LDLT, within the scope of the present review, recent studies demonstrated LDLT is a viable option to improve

Most commonly used classifications for Portal Vein Tumor Thrombosis

Cheng's classification of portal vein tumor thrombosis (37)

Type	Description	Recommended treatment
<p>I_o</p> 	<p>Tumor thrombus formation found under microscopy</p>	
<p>I</p> 	<p>Tumor thrombus involving segmental branches of portal vein or above</p>	<p>For resectable Liver tumor:</p> <ul style="list-style-type: none"> • Surgical resection <ul style="list-style-type: none"> ◦ Segmental hepatectomy ◦ Hemi-hepatectomy • Postoperative adjuvant TACE • Sorafenib orally postoperative to reduce recurrence rate
<p>II</p> 	<p>Tumor thrombus involving right or left portal vein</p>	<p>For non-resectable Liver tumor:</p> <ul style="list-style-type: none"> • TACE • External Beam Radiotherapy. • TARE • Sorafenib • LT*

Type	Description	Recommended treatment
 <p>III</p>	Tumor thrombus involving the main portal vein trunk	<p>For resectable Liver tumor:</p> <ul style="list-style-type: none"> Operative treatment only after downstaging via preoperative radiotherapy. <ul style="list-style-type: none"> Hemi-hepatectomy Hepatectomy plus thrombectomy, embolectomy or en bloc resection (with or without reconstruction of the main portal vein) <p>For non-resectable Liver tumor:</p> <ul style="list-style-type: none"> TACE (under specific conditions, controversial use). External Beam Radiotherapy TARE Sorafenib LT*
 <p>IV</p>	Tumor thrombus involving the superior mesenteric vein	<ul style="list-style-type: none"> External Beam radiotherapy. TARE Sorafenib <p>**Not enough support:</p> <ul style="list-style-type: none"> Systemic chemotherapy using different regimens including: 5-FU, cisplatin, leucovorin, methotrexate, IFN-alpha 2b. LT*
 <p>VP₁</p>	Tumor thrombus distal to the second-order branches of the portal vein (Segmental branch)	<ul style="list-style-type: none"> Sorafenib TACE Surgical resection: Segmental hepatectomy LT*

Japan's PVTT portal vein invasion (VP) classification (34, 35)

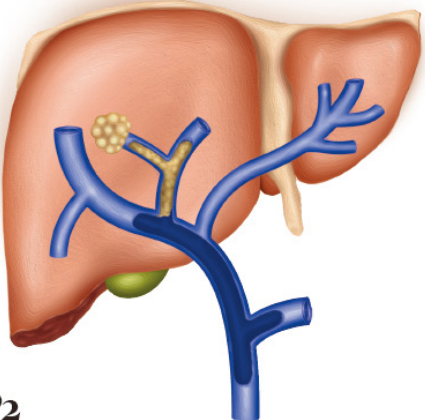
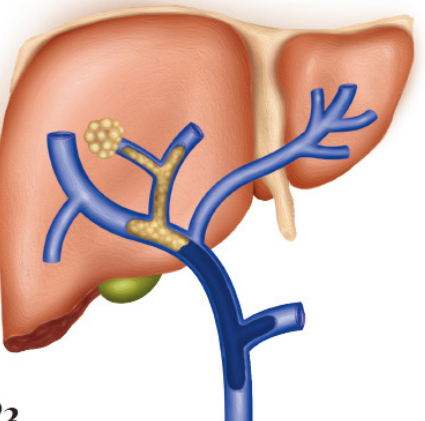
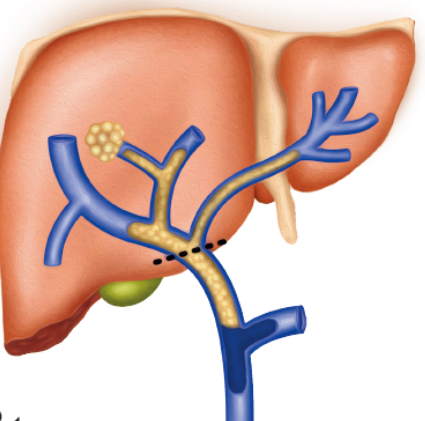
Type	Description	Recommended treatment
 <p>VP2</p>	Tumor thrombus in the second-order branches of the portal vein	<ul style="list-style-type: none"> • Sorafenib • TACE • Surgical resection: Segmental hepatectomy (after downstaging via preoperative Neoadjuvant radiotherapy) • LT*
 <p>VP3</p>	Tumor thrombus in the first-order branches of the portal vein	<ul style="list-style-type: none"> • Sorafenib • TACE • HAIC • Radiotherapy • Surgical resection: Hemihepatectomy (after downstaging via preoperative Neoadjuvant radiotherapy) • LT*
 <p>VP4</p>	Tumor thrombus in the main portal vein trunk or extending to a branch on the opposite side to the primarily involved lobe)	<ul style="list-style-type: none"> • Sorafenib • TACE • HAIC • Radiotherapy • Neoadjuvant RT for downstaging previous to surgery. • Surgical resection: <ul style="list-style-type: none"> °Extended hemihepatectomy °Hepatectomy plus thrombectomy or en bloc resection (with or without reconstruction of the main portal vein) • LT*

Figure 1. Most commonly used classifications for Portal Vein Tumor Thrombosis (PVTT). Cheng's classification of portal vein tumor thrombosis^[37], Japan's PVTT portal vein invasion (VP) classification^[34,35]. HAIC: hepatic arterial infusion chemotherapy; IFN- α 2b: interferon α -2b; LT: liver transplantation; RT: radiation therapy; TACE: transarterial chemoembolization; TARE: transarterial radioembolization; 5-FU: 5-fluorouracil. *Under certain conditions; **not enough support^[26,34,35,38,37,40,41]

long-term survival outcomes among carefully selected patients under specific criteria such as: (a) PVTT in a segmental branch, (b) AFP level < 100ng/mL, (c) low AP score, and (d) patients with HCC and PVTT who were successfully downstaged^[36,42,43].

In a paper in 2017, Choi *et al.*^[36] evaluated PVTT recurrence, disease-free survival (DFS), and overall survival (OS) in a cohort undergoing LDLT to treat HCC. Among the 242 patients, microvascular invasion was apparent in two patients, segmental PVTT was noted in 27, while lobar PVTT was seen in 7 patients. The authors aimed to define the branch level in which PVTT presence was acceptable in LT and found no difference in DFS rate between microvascular invasion and segmental PVTT group, while those with lobar PVTT exhibited poorer DFS and OS. No significant difference in the maximal or total tumor diameter or tumor number was detected between groups. The authors found that in the segmental PVTT group, the 5-year DFS and OS rates were 63.9% and 50.3%, respectively, and did not differ significantly from those of the microvascular invasion group. Based on these results and similar 5-year survival rates between patients, transplantation should not be restricted, and mutual donor and recipient interest should guide decisions to proceed with transplantation.

Other LDLT experiences with patients with vascular involvement include the one by Lee *et al.*^[43], which reported successful LDLT in 11 HCC patients with macrovascular invasion. Recurrence-free survival at 5 years was 45.5%, and 5-year overall survival was 63.6%. The authors found tumor thrombus extension into the main portal vein, elevated AFP, and tumor size greater than 7 cm to be independent risk factors for recurrence. In the largest study to date, 46 patients with PVTT underwent LDLT after a downstaging protocol using stereotactic body radiation in combination with transarterial chemo-embolization or transarterial radio-embolization^[42]. Five-year recurrence free survival and overall survival rates were 51% and 57%, respectively. Furthermore, the authors found several favorable prognosticators, including lower initial AFP levels, significant decrease in AFP after downstaging, and low tumor grade. The authors proposed offering their downstaging protocol to “all comers” with HCC and PVTT without extrahepatic disease or extensive thrombosis of the main portal or superior mesenteric veins. Once again, this offers the surgeon an opportunity to maximize the benefit of LDLT by allowing timing of the operation when the disease is most under control and when it is most likely to be successful for the recipient.

Strategies before and after LDLT in advanced HCC patients

Downstaging and adjuvant therapy administration are commonly used strategies in advanced HCC patients^[44,45]. The goals of each vary depending on the treatment modality to be performed^[44]. When applied before transplantation (versus before resection), downstaging strategies are used to convert a patient outside accepted criteria for transplantation into an acceptable candidate, by reducing tumor burden. It is also used as a way to select patients with low rates of recurrence among those who would have been excluded based on current criteria. On the other hand, adjuvant therapy is used in patients following surgical management to help decrease the incidence of HCC recurrence, and thus, improve their outcomes^[30,44,45].

Locoregional therapies

Locoregional therapies provide several benefits: tumors outside Milan can be downstaged or downsized so that criteria are met, patients within Milan with disease progression can be bridged to avoid waitlist dropout, and tumor necrosis can be induced which reduces intraoperative tumor spread. Additionally, response to these therapies reflects favorable disease biology and indicates recurrence risk.

The most commonly used locoregional techniques are stereotactic body radiation, transarterial chemo-embolization, transarterial radio-embolization, and radiofrequency ablation. Aravinthan *et al.*^[46] reported their outcomes after liver transplantation in patients with advanced HCC within extended Toronto Criteria.

Sixty-five percent of the patients underwent a protocol of bridging therapy, which led to significant reductions in tumor burden and disease progression prior to transplant. A recurrence rate of 35% was observed and 3-year survival was 56%, representing superior survival compared with palliative treatments. Positive outcomes were also reported by Jeong *et al.*^[47] in their experience with 17 HCC patients with major vascular invasion who underwent LDLT after combined treatment with transarterial chemo-embolization and radiotherapy. The authors found that the 1- and 3-years DFS were 70.6% and 57.8% and the OS rates at 1- and 3-years were 87.4% and 60.5%, respectively. Even though long-term results are not provided, the authors concluded that acceptable oncologic outcomes can be achieved in select HCC patients with major vascular invasion, and suggest that LDLT might be a therapeutic option for these patients if the tumors are downstaged considering that 1-year survival rate for HCC patients with PVTT is less than 10%. Even though it has been reported that the use of downstaging strategies in the presence of extended criteria of HCC for LDLT has decreased in Asian countries, published experience among Asian institutions revealed comparable 5-year overall survival rates in patients who received LDLT after downstaging versus those without downstaging^[48].

A benefit to pre-operative therapy is the additional time for proper surgical planning. Where the price of DDLT might be a gap in planning, locoregional therapies create an ideal setting for LDLT in that it provides better selection and timing, allowing for a set date for the surgery when it is most likely to succeed when the disease response is most appropriate after locoregional therapy.

Adjuvant therapy

HCC recurrence remains the leading cause of death after liver transplantation, and patients with advanced disease are at particular risk^[49]. Early recurrences are commonly seen in patients with associated negative prognosis factors such as vascular invasion^[45]. In order to help decrease the incidence of HCC recurrence post-transplantation, numerous strategies have been proposed as adjuvant therapy; however, none have been able to provide enough support regarding prolonged recurrence-free survival and none have been widely accepted^[50]. Other factors complicating the standardization of an adjuvant regimen for HCC is the heterogeneity among HCC between different regions. Thus, targeting recurrence risk factors should be analyzed in an individual manner. Currently published evidence include experience with systemic therapy such as oral anti-HBV agents, tyrosine kinase inhibitors, IFN-alpha, TACE, anti-PD-1 antibodies (e.g., Nivolumab, pembrolizumab, atezolizumab), molecular-targeted agents, and chemotherapy agents (e.g., Oxaliplatin plus fluorouracil/leucovorin)^[50].

Select immunosuppressive medications may portend an oncologic benefit, specifically mammalian target of rapamycin (mTOR) inhibitors such as everolimus and sirolimus^[51,52]. Inhibition of tumor growth mediated by properties such as antiangiogenic and antiproliferative effects makes mTOR an extraordinary class of immunosuppressants^[53]. In 2016, a prospective-randomized open-label international trial compared recurrence-free survival in sirolimus-containing versus mTOR inhibitor-free immunosuppression patients undergoing LT for HCC. The authors found that sirolimus in LT recipients with HCC did not improve long-term recurrence free survival beyond 5 years. However, a benefit in the first 3 to 5 years for recurrence free survival and OS was evident. Regarding adverse events, both groups reported similar frequencies^[53,54].

Clinicians should be aware of the increased risk of post-transplant HCC recurrence with calcineurin inhibitors^[54]. In a systematic review of 42 publications, patients on everolimus had significantly lower recurrence rates of HCC versus calcineurin inhibitors and sirolimus^[52]. Sorafenib, a multikinase inhibitor, has also shown promise as an adjuvant therapy. In a study of 30 patients with HCC beyond Milan, sorafenib reduced recurrence of post-transplant HCC compared with capecitabine^[55]. However, contrasting experiences have been published. A recent publication evaluating the role of Sorafenib on HCC recurrence and survival in 45 patients with advance HCC on explant pathology after LT found that adjuvant

treatment with sorafenib in LT recipients with high-risk features does not improved HCC recurrence-free or overall survival^[56]. When used, it is crucial to be aware of reported early and severe toxicities such as hepatotoxicity, thrombocytopenia, anorexia, fatigue, hand-foot syndrome, and diarrhea^[57].

An ongoing phase 3 study [IMbrave150 study (NCT03434379)] is being done to compare atezolizumab with bevacizumab combination therapy with the standard of care (Sorafenib) among a large cohort of patients with systemic treatment-naïve unresectable HCC. Preliminary phase Ib results showed that the proposed combination resulted in improved progression-free survival compared with atezolizumab monotherapy and had a tolerable safety profile^[58]. Other clinical trials evaluating treatment combinations with acceptable safety profile are currently being developed^[59].

While promising, additional studies will be necessary to further elucidate the potential benefits of specific immunosuppressive regimens as an adjuvant therapy after LDLT for advanced HCC.

Recurrence risk surveillance and useful prognosis tools

Tumor recurrence after LT is seen in approximately 15%-20% of HCC patients despite being within the Milan criteria^[54]. These data are even more worrisome in patients with advanced HCC, which supports the notion that identification of risk factors for recurrence and a proper evaluation for LT plays a pivotal role in the management of these patients.

Risk factors related to recurrence of HCC after LT include tumor factors such as staging, vascular invasion, differentiation, and AFP level, among others. Relevant patient factors such as obesity, viral etiology, HCV treatment, and NAFLD are also important, as well as factors related to the transplant, including percutaneous tumor biopsy, waiting time, bridging therapy, donor's age, ischemia time, surgical technique, and post-transplant immunosuppression regimen and adjuvant therapy.

There is no consensus regarding the protocol for monitoring recurrence after LT. However, important scores have been proposed and should be taken into account when performing follow-up for these patients^[60].

MORAL score

The Model of Recurrence After Liver (MORAL) transplant for HCC is a score developed by Halazun *et al.*^[61] at Columbia University Medical Center to predict recurrence and risk-stratification pre- and post-operatively. Variables included in the MORAL scoring system include pre-operative NLR ≥ 5 , maximum AFP > 200 ng/mL, largest tumor size > 3 cm, grade 4 tumors, vascular invasion, largest size on path > 3 cm, and tumor number on path > 3 . The recurrence risk stratification according to Pre-MORAL score divide the risk of recurrence in low risk (0-2), medium risk (3-6), high risk (7-10) and very high risk (> 10).

Other authors, including a group from South Korea, have published variations of this model. Their variation was derived using PIVKA II levels and AFP to stratify risk and predict HCC recurrence and was developed including 205 patients with HCC beyond Milan criteria undergoing LDLT. The authors found that a low MORAL score (≤ 314.8) was associated with significantly longer recurrence-free and overall survivals in the beyond Milan criteria group. The 5-year recurrence-free and OS rates among these patients were 66.3% and 82.6%, respectively. They conclude that their model provides advanced prognostication and support the idea that HCC patients beyond Milan criteria without extrahepatic metastasis and low MORAL score might be potential candidates for LDLT^[62].

Metroticket 2.0 model

The model for Analysis of Competing Risks of Death After Liver Transplantation (Metroticket 2.0) for HCC is a web-based calculator recognized by the European Liver and Intestine Transplant Association

and the International Liver Transplantation, developed by Mazzaferro *et al.*^[63]. The tool is used to predict post-transplantation HCC specific survival, considering as events of interest only those deaths caused by tumor recurrence. The objective is to provide an upgraded prognostic tool and refine selection criteria for liver transplantation in patients with HCC. Outcome prediction was conducted using pre-operative determinants of prognosis frequently associated with higher risk of pre-transplantation tumor progression and poorer post-transplantation survival. The calculator is a free accessible tool found at www.hcc-olt-metroticket.org^[63].

CONCLUSION

Until proven otherwise, surgical resection is the treatment of choice for patients with HCC confined to the liver. It has been shown that even patients with advanced HCC can achieve cure with a more radical resection as liver transplantation offers. Therefore, it is crucial that the transplant community changes the paradigm and improves transplant criteria for patients with HCC. We should focus more on tumor biology than size restrictions. In addition, tumor markers such as AFP and pathologic grade must be assessed. Response to pre-transplant locoregional therapies might be a surrogate marker of more favorable disease, which may impart lower post-transplant recurrence risk. In addition, current data proposes that PVT should not necessarily be considered a contraindication.

However, the question as to who should receive a liver transplant still persists. An important component to answering this question will be the transplant surgeons who continue to push the limits and boundaries by offering liver transplantation to advanced HCC patients. Nevertheless, the most crucial components to what can be achieved are the patients and the donors; their willingness to accept the risks will make it possible.

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Authors' contributions

Participated in conception and design of the study; generation, collection, assembly, interpretation and critical revision of the data; drafting of the manuscript; approval of the final version of the manuscript: Cullen JM

Participated in the generation, collection, assembly, interpretation and critical revision of the data; drafting of the manuscript; approval of the final version of the manuscript: Vargas P

Participated in conception and design of the study; generation, collection, assembly, interpretation and critical revision of the data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; approval of the final version of the manuscript: Goldaracena N

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All authors declared that there are no conflicts of interest.

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REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359-86.
2. Berumen J, Hemming A. Liver transplantation for hepatocellular carcinoma. *Abdom Radiol (NY)* 2018;43:185-92.
3. Proneth A, Zeman F, Schlitt HJ, Schnitzbauer AA. Is resection or transplantation the ideal treatment in patients with hepatocellular carcinoma in cirrhosis if both are possible? A systematic review and metaanalysis. *Ann Surg Oncol* 2014;21:3096-107.
4. Mulligan DC. The ongoing quest to find the appropriate patients to transplant with hepatocellular carcinoma: Milan to san Francisco to Toronto and beyond. *Hepatology* 2016;64:1853-5.
5. Sapisochin G, Goldaracena N, Laurence JM, et al. The extended Toronto criteria for liver transplantation in patients with hepatocellular carcinoma: a prospective validation study. *Hepatology* 2016;64:2077-88.
6. Raia S, Nery JR, Mies S. Liver transplantation from live donors. *Lancet* 1989;2:497.
7. Wong TCL, Ng KKC, Fung JYY, et al. Long-term survival outcome between living donor and deceased donor liver transplant for hepatocellular carcinoma: intention-to-treat and propensity score matching analyses. *Ann Surg Oncol* 2019;26:1454-62.
8. Cheung TTT, Kwok PCH, Chan S, et al. Hong Kong consensus statements for the management of unresectable hepatocellular carcinoma. *Liver Cancer* 2018;7:40-54.
9. de Freitas LBR, Longo L, Santos D, Grivicich I, Álvares-da-Silva MR. Hepatocellular carcinoma staging systems: Hong Kong liver cancer vs Barcelona clinic liver cancer in a Western population. *World J Hepatol* 2019;11:678-88.
10. De Souza Martins Fernandes E, Rodrigues PD, Álvares-da-Silva MR, et al. Treatment strategies for locally advanced hepatocellular carcinoma. *Transl Gastroenterol Hepatol* 2019;4:12.
11. Xie DY, Ren ZG, Zhou J, Fan J, Gao Q. 2019 Chinese clinical guidelines for the management of hepatocellular carcinoma: updates and insights. *Hepatobiliary Surg Nutr* 2020;9:452-63.
12. Iwatsuki S, Starzl TE, Sheahan DG, et al. Hepatic resection versus transplantation for hepatocellular carcinoma. *Ann Surg* 1991;214:221-9.
13. Bigourdan JM, Jaeck D, Meyer N, et al. Small hepatocellular carcinoma in Child A cirrhotic patients: hepatic resection versus transplantation. *Liver Transpl* 2003;9:513-20.
14. Bhangui P, Vibert E, Majno P, et al. Intention-to-treat analysis of liver transplantation for hepatocellular carcinoma: living versus deceased donor transplantation. *Hepatology* 2011;53:1570-9.
15. Azoulay D, Audureau E, Bhangui P, et al. Living or brain-dead donor liver transplantation for hepatocellular carcinoma. *Ann Surg* 2017;266:1035-44.
16. Goldaracena N, Gorgen A, Doyle A, et al. Live donor liver transplantation for patients with hepatocellular carcinoma offers increased survival vs. deceased donation. *J Hepatol* 2019;70:666-73.
17. Xiao GQ, Song JL, Shen S, Yang JY, Yan LN. Living donor liver transplantation does not increase tumor recurrence of hepatocellular carcinoma compared to deceased donor transplantation. *World J Gastroenterol* 2014;20:10953-9.
18. Fisher RA, Kulik LM, Freise CE, et al. Hepatocellular carcinoma recurrence and death following living and deceased donor liver transplantation. *Am J Transplant* 2007;7:1601-8.
19. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996;334:693-9.
20. Yao FY, Ferrell L, Bass NM, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001;33:1394-403.
21. Lee SG, Hwang S, Moon DB, et al. Expanded indication criteria of living donor liver transplantation for hepatocellular carcinoma at one large-volume center. *Liver Transpl* 2008;14:935-45.
22. Cillo U, Giuliani T, Polacco M, et al. Prediction of hepatocellular carcinoma biological behavior in patient selection for liver transplantation. *World J Gastroenterol* 2016;22:232-52.
23. Lee SD, Lee B, Kim SH, et al. Proposal of new expanded selection criteria using total tumor size and (18)F-fluorodeoxyglucose - positron emission tomography/computed tomography for living donor liver transplantation in patients with hepatocellular carcinoma: The National Cancer Center Korea criteria. *World J Transplant* 2016;6:411-22.
24. Uchiyama H, Itoh S, Yoshizumi T, et al. Living donor liver transplantation for hepatocellular carcinoma: results of prospective patient selection by Kyushu University Criteria in 7 years. *HPB (Oxford)* 2017;19:1082-90.
25. Llovet JM, Real MI, Montaña X, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002;359:1734-9.
26. Wang JC, Xia AL, Xu Y, Lu XJ. Comprehensive treatments for hepatocellular carcinoma with portal vein tumor thrombosis. *J Cell Physiol* 2019;234:1062-70.

27. Kornberg A, Schernhammer M, Kornberg J, Friess H, Thrum K. Serological risk index based on alpha-fetoprotein and C-reactive protein to indicate futile liver transplantation among patients with advanced hepatocellular carcinoma. *Dig Dis Sci* 2019;64:269-80.
28. Lee HW, Song GW, Lee SG, et al. Patient selection by tumor markers in liver transplantation for advanced hepatocellular carcinoma. *Liver Transpl* 2018;24:1243-51.
29. Lin CC, Chen CL. Living donor liver transplantation for hepatocellular carcinoma achieves better outcomes. *HepatoBiliary Surg Nutr* 2016;5:415-21.
30. Lin C, Elsarawy AMAA, Chen C. Living donor liver transplantation for hepatocellular carcinoma. In: Abdeldayem HM, editor. InTech; 2017.
31. Minagawa M, Makuuchi M. Treatment of hepatocellular carcinoma accompanied by portal vein tumor thrombus. *World J Gastroenterol* 2006;12:7561-7.
32. Lee YH, Hsu CY, Huang YH, et al. Vascular invasion in hepatocellular carcinoma: prevalence, determinants and prognostic impact. *J Clin Gastroenterol* 2014;48:734-41.
33. Chen ZH, Zhang XP, Lu YG, et al. Actual long-term survival in HCC patients with portal vein tumor thrombus after liver resection: a nationwide study. *Hepatol Int*; 2020.
34. Cheng S, Yang J, Shen F, et al. Multidisciplinary management of hepatocellular carcinoma with portal vein tumor thrombus - Eastern Hepatobiliary Surgical Hospital consensus statement. *Oncotarget* 2016;7:40816-29.
35. Cerrito L, Annicchiarico BE, Iezzi R, et al. Treatment of hepatocellular carcinoma in patients with portal vein tumor thrombosis: Beyond the known frontiers. *World J Gastroenterol* 2019;25:4360-82.
36. Choi HJ, Kim DG, Na GH, et al. The clinical outcomes of patients with portal vein tumor thrombi after living donor liver transplantation. *Liver Transpl* 2017;23:1023-31.
37. Shi J, Lai EC, Li N, et al. A new classification for hepatocellular carcinoma with portal vein tumor thrombus. *J Hepatobiliary Pancreat Sci* 2011;18:74-80.
38. Peng SY, Wang XA, Huang CY, et al. Better surgical treatment method for hepatocellular carcinoma with portal vein tumor thrombus. *World J Gastroenterol* 2018;24:4527-35.
39. Huo L, Wei W, Yan Z, et al. Short-term and long-term outcomes of liver resection for HCC patients with portal vein tumor thrombus. *Cell Biosci* 2019;9:23.
40. Cheng S, Chen M, Cai J; National Research Cooperative Group for Diagnosis and Treatment of Hepatocellular Carcinoma with Tumor Thrombus. Chinese expert consensus on multidisciplinary diagnosis and treatment of hepatocellular carcinoma with portal vein tumor thrombus: 2016 edition. *Oncotarget* 2017;8:8867-76.
41. Moriguchi M, Furuta M, Itoh Y. A review of non-operative treatments for hepatocellular carcinoma with advanced portal vein tumor thrombus. *J Clin Transl Hepatol* 2017;5:177-83.
42. Soin AS, Bhangui P, Kataria T, et al. Experience with LDLT in patients with hepatocellular carcinoma and portal vein tumor thrombosis postdownstaging. *Transplantation* 2020; doi: 10.1097/TP.0000000000003162.
43. Lee KW, Suh SW, Choi YR, et al. Macrovascular invasion is not an absolute contraindication for living donor liver transplantation. *Liver Transplant* 2017;23:19-27.
44. Toso C, Mentha G, Kneteman NM, Majno P. The place of downstaging for hepatocellular carcinoma. *J Hepatol* 2010;52:930-6.
45. Akateh C, Black SM, Conteh L, et al. Neoadjuvant and adjuvant treatment strategies for hepatocellular carcinoma. *World J Gastroenterol* 2019;25:3704-21.
46. Aravinthan AD, Bruni SG, Doyle AC, et al. Liver transplantation is a preferable alternative to palliative therapy for selected patients with advanced hepatocellular carcinoma. *Ann Surg Oncol* 2017;24:1843-51.
47. Jeong Y, Shin MH, Yoon SM, et al. Liver transplantation after transarterial chemoembolization and radiotherapy for hepatocellular carcinoma with vascular invasion. *J Gastrointest Surg* 2017;21:275-83.
48. Yoon YI, Lee SG. Living donor liver transplantation for hepatocellular carcinoma: an asian perspective. *Dig Dis Sci* 2019;64:993-1000.
49. Todo S, Furukawa H; Japanese Study Group on Organ Transplantation. Living donor liver transplantation for adult patients with hepatocellular carcinoma: experience in Japan. *Ann Surg* 2004;240:451-9.
50. Zhu XD, Li KS, Sun HC. Adjuvant therapies after curative treatments for hepatocellular carcinoma: current status and prospects. *Genes Dis* 2020;7:359-69.
51. Toso C, Meeberg GA, Bigam DL, et al. De novo sirolimus-based immunosuppression after liver transplantation for hepatocellular carcinoma: long-term outcomes and side effects. *Transplantation* 2007;83:1162-8.
52. Cholongitas E, Mamou C, Rodríguez-Castro KI, Burra P. Mammalian target of rapamycin inhibitors are associated with lower rates of hepatocellular carcinoma recurrence after liver transplantation: a systematic review. *Transpl Int* 2014;27:1039-49.
53. Geissler EK, Schnitzbauer AA, Zölke C, et al. Sirolimus use in liver transplant recipients with Hepatocellular carcinoma: a randomized, Multicenter, open-label phase 3 Trial. *Transplantation* 2016;100:116-25.
54. Filgueira NA. Hepatocellular carcinoma recurrence after liver transplantation: Risk factors, screening and clinical presentation. *World J Hepatol* 2019;11:261-72.
55. Huang L, Li GM, Zhu JY, et al. Efficacy of sorafenib after liver transplantation in patients with primary hepatic carcinoma exceeding the Milan criteria: a preliminary study. *Onco Targets Ther* 2012;5:457-62.
56. Satapathy SK, Das K, Kocak M, et al. No apparent benefit of preemptive sorafenib therapy in liver transplant recipients with advanced hepatocellular carcinoma on explant. *Clin Transplant* 2018;32:e13246.
57. Noda S, Hira D, Osaki R, et al. Sorafenib exposure and its correlation with response and safety in advanced hepatocellular carcinoma:

- results from an observational retrospective study. *Cancer Chemother. Pharmacol* 2020;86:129-39.
58. Lee MS, RyooBY, Hsu CH, et al. Atezolizumab with or without bevacizumab in unresectable hepatocellular carcinoma (GO30140): an open-label, multicentre, phase 1b study. *Lancet Oncol* 2020;21:808-20.
 59. Kim R, Tan E, Wang E, et al. A phase i trial of trametinib in combination with sorafenib in patients with advanced hepatocellular cancer. *Oncologist* 2020; doi: 10.1634/theoncologist.2020-0759.
 60. Berenguer M, Burra P, Ghobrial M, et al. Posttransplant management of recipients undergoing liver transplantation for hepatocellular carcinoma. Working Group Report from the ILTS Transplant Oncology Consensus Conference. *Transplantation* 2020;1143-9.
 61. Halazun KJ, Najjar M, Abdelmessih RM, et al. Recurrence after liver transplantation for hepatocellular carcinoma. *Ann Surg* 2017;265:557-64.
 62. Lee JH, Cho Y, Kim HY, et al. Serum tumor markers provide refined prognostication in selecting liver transplantation candidate for hepatocellular carcinoma patients beyond the milan criteria. *Ann Surg* 2016;263:842-50.
 63. Mazzaferro V, Sposito C, Zhou J, et al. Metroticket 2.0 model for analysis of competing risks of death after liver transplantation for hepatocellular carcinoma. *Gastroenterology* 2018;154:128-39.

Review

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Advances in minimally invasive surgery for hepatocellular carcinoma

Cun-Zhen Zhang, Nan Li

Department of Hepatic Surgery, Shanghai Eastern Hepatobiliary Surgery Hospital, Shanghai 200438, China.

Correspondence to: Dr. Nan Li, Department of Hepatic Surgery, Shanghai Eastern Hepatobiliary Surgery Hospital, 255 Changhai Road, Yangpu District, Shanghai 200438, China. E-mail: liparislisi@aliyun.com

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related death worldwide. Surgical resection is still regarded as the first choice of treatment for HCC. With advances in technology and techniques, minimally invasive surgery has now become the standard of care in almost every field in general surgery, including hepatectomy surgery. This review focuses on the latest advances in minimally invasive surgical treatment of HCC, including laparoscopic hepatectomy (LH), robotic hepatectomy (RH) and other minimally invasive treatment technologies. Although some limitation in LH or RH exists, these minimally invasive techniques may be performed for hepatectomy with benefits, and have a promising future. With the development of technology and the improvement of surgical operations, patients will benefit from this.

Keywords: Hepatocellular carcinoma, minimally invasive surgery, laparoscopic hepatectomy, robotic hepatectomy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related death worldwide^[1-4]. Because its early symptoms are not typical, most liver cancers clinically diagnosed belong to the advanced stage^[5]. In addition, most of these patients are accompanied by portal vein tumor thrombus or pulmonary metastasis^[6,7]. Surgical resection is still regarded as the first choice of HCC treatment^[8-12]. However, due to intrahepatic metastasis and early recurrence, postoperative patients' prognosis is poor^[13-18], and the 5-year survival rate is relatively low^[19]. The high recurrence and vascular



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invasion of HCC limit the long-term survival of HCC patients. The recurrence rate of HCC is as high as 80% within 5 years after radical resection^[20]. However, due to a relatively insidious onset, most of the patients are in the middle and late stages when they seek medical treatment, with only 10% at a stage amenable to surgical intervention^[21]. Moreover, the postoperative recurrence rate is high. In recent years, a variety of new technologies and therapies have emerged constantly, which have posed a challenge to the surgical resection of HCC. In addition to liver transplantation, surgical resection of tumor has been accepted as an effective local treatment for HCC. The innovation of HCC treatment technology and the progress of surgical technology have improved the safety of HCC resection^[22].

With advances in technology and techniques, minimally invasive surgery has now become the standard of care in almost every field in general surgery, including hepatectomy surgery. Since the first laparoscopic pericystectomy for hepatic echinococcosis carried out in France in 1991, there have been increasing reports on the laparoscopic treatments for HCC. The da Vinci Surgical System (Intuitive Surgical Inc., United States) is an advanced and minimally invasive surgery tool, which has been shown to have advantages in complex hepatectomy. Liu *et al.*^[23] formulated international consensus statement on robotic hepatectomy surgery. On the basis of ensuring the therapeutic effect and safety, the development of new surgical treatment is conducive to the prognosis of patients and the development of the whole treatment system.

This review focuses on the latest advances in minimally invasive surgical treatment of HCC, including laparoscopic hepatectomy (LH), robotic liver resection and other minimally invasive treatment technologies.

LAPAROSCOPIC HEPATECTOMY

As far as the traditional treatment viewpoint is concerned, surgical resection is the first choice for the treatment of liver cancer^[24]. With the update of treatment viewpoints and the innovation of technical equipment, laparoscopic technology is widely used in various surgical treatment fields. LH as one of the minimally invasive treatment methods for hepatocellular carcinoma, is increasingly used in clinical surgery by more and more surgeons. In 1991, Reich *et al.*^[25] completed the first laparoscopic hepatectomy in the world, and the laparoscopic technique was gradually applied to the surgical treatment of hepatobiliary diseases. In 1994, Zhou *et al.*^[26] reported the first laparoscopic hepatectomy in China. Owing to the differences and gaps in the mastery of technical difficulties and risk awareness, the rich liver blood flow, laparoscopic hepatic portal occlusion that is more difficult than laparotomy, and lack of open operation feel and rapid reactions, accurate hemostasis and control of bleeding are the key to the success of laparoscopic hepatectomy, so the clinical development of laparoscopic hepatectomy is still in the development stage. The classification of laparoscopic hepatectomy in domestic and foreign guidelines mainly includes pure laparoscopic liver resection, hand-assisted laparoscopic liver resection and laparoscopic assisted hepatectomy (hybrid procedures). The indications of laparoscopic hepatectomy vary globally. In 2013, the domestic expert consensus^[27] proposed that the indications of laparoscopic hepatectomy include symptomatic benign diseases, intra- and extra-hepatic bile duct calculi and liver malignant tumors. However, there are still controversies about laparoscopic hepatectomy. According to a survey conducted in Japan in 2013, more than half of the surveyed medical centers routinely perform laparoscopic hepatectomy, but the indications are not consistent, and preoperative liver function reserve and intraoperative bleeding control occupy the primary position. More than two-thirds of medical centers hold that laparoscopic hepatectomy can be used for living donor liver transplantation^[28]. Limited by the surgical instrument and technical mastery, small-scale hepatectomy or anatomical hepatectomy can be performed if the lesion location of liver cancer is superficial and limited, and does not invade large blood vessels and bile ducts according to the traditional surgical viewpoint^[28]. Due to the limitation of the laparoscopic visual field and operation equipment activities, hand-assisted laparoscopic liver resection or laparotomy is the best choice for some larger tumors requiring complex hepatectomy or large-scale hepatectomy. At present, the

industry still maintains a cautious attitude towards the transition to laparotomy because intraoperative bleeding is difficult to effectively control for the safe integration of laparoscopic large-scale hepatectomy or postoperative bleeding. Recent evidence from some centers has shown that laparoscopic hepatectomy is acceptable for HCC patients who meet the surgical criteria, and there is no significant difference in postoperative survival time^[29,30]. Rao *et al.*^[31] compared 700 patients who underwent pure laparoscopic liver resection and laparotomy. Compared with laparotomy, the overall incidence of complications after LH was lower and the length of stay was shorter. For liver cancer patients with obvious liver cirrhosis, the studies from Memeo *et al.*^[32] showed that, compared with laparotomy, patients who underwent laparoscopic liver resection had shorter operation times and complete surgical margins, but there was no significant difference in overall survival rate and disease-free survival time. The 1-, 5-, and 10-year survival rate of laparoscopic liver resection (LLR) group reached 88%, 59%, and 12%, and that of open liver resection (OLR) group was 63%, 44%, and 22%, respectively ($P = 0.27$). However, there is no evidence that patients with liver cancer who undergo laparoscopic liver resection are afforded a better prognosis. In a recent study, Ahmed El-Gendi *et al.*^[33] compared the therapeutic effects of OLR with LLR in patients with liver cirrhosis (Child A) with solitary small (< 5 cm) peripheral HCC. The results showed that LLR had significantly less operative time (120.32 ± 21.58 min *vs.* 146.80 ± 16.59 min, $P < 0.001$) and shorter duration of hospital stay (2.40 ± 0.58 days *vs.* 4.28 ± 0.79 days, $P < 0.001$), with comparable overall complications (25% *vs.* 28%, $P = 0.02$). LLR had comparative resection time (66.56 ± 23.80 min *vs.* 59.56 ± 14.74 min, $P = 0.218$), amount of blood loss (250 mL *vs.* 230 mL, $P = 0.915$), transfusion rate ($P = 1.00$), and R0 resection rate when compared with OLR. There was no significant difference between the two groups in terms of postoperative tumor-free survival time^[33]. In a Japanese study, information on patients undergoing liver cancer surgery was collected from 31 centers between 2000 and 2010, and divided into LLR ($n = 436$) and OLR ($n = 2969$) groups. 387 patients were matched by propensity score matching. There were no significant differences in overall survival and disease-free survival between LLR and OLR. Patients undergoing LLR have shorter hospital stays (13 days *vs.* 16 days, $P < 0.001$) and fewer postoperative complications (6.7% *vs.* 13.0%, $P = 0.003$)^[34].

ROBOTIC LIVER RESECTION

With the progress and development of technology, the surgical treatment of liver cancer has gradually entered the era of minimally invasive precision treatment, mainly including laparoscopic and robotic minimally invasive treatment. The development of minimally invasive surgical techniques for liver tumors is limited by the characteristics of its own organs, a crisp texture, abundant blood supply, high numbers of structural variations of blood vessels and bile ducts, and a close relationship with surrounding organs. Although laparoscopic liver resection has made great progress on the basis of the improvement of laparotomy, it is easy to cause clamping or traction bleeding when the deep lesions and special liver segments are exposed. Therefore, the control of intraoperative bleeding, the exposure of the operative visual field and the effective hemostasis of liver section are the key points of minimally invasive surgery, and also the biggest problem of laparoscopic liver resection^[35,36]. In this case, the robot assisted system is gradually improved. In 2000, Da Vinci robot assisted surgery system was approved for clinical use. It is one of the most mature surgical auxiliary operating systems, which is widely used in the field of surgery. With its unique advantages, it gradually fills the gap in the minimally invasive field of liver tumor treatment. Traditional laparoscopic liver resection has some problems, such as inflexible fixation of the operating instruments and a large swing of the visual field leverage effect^[37,38]. The robot assisted surgery system has no such limitations, and the highly bionic operation can greatly simulate the fine operation of the open hand, provide stable, fixed strength and angle traction and exposure, and even surpass the human operation to a certain extent^[39], and complete the accurate resection and suturing of malignant liver tumors^[40]. In 2003, Giulianotti *et al.*^[38] {Giulianotti, 2003 #170} reported the first robotic liver resection in the world. Under this background, robotic surgery technology has been developing rapidly. At present, the Da Vinci surgical assistant system is widely used in various benign and malignant liver diseases, including for the indications of laparoscopic hepatectomy^[41]. For some special liver segment tumors, robot assisted surgery

is more advantageous. Traditionally, it has been considered that if the tumor located in a location difficult to dissect, intraoperative hemorrhage is anticipated to be difficult to control, or the tumor is large, these are relative contraindications to the use of minimally invasive surgery. The advantages of robot assisted surgery system can solve these problems, and the operation position is no longer the taboo of minimally invasive surgery. For tumors in the upper right posterior lobe or caudate lobe of the liver, robot assisted surgery has a better visual field than laparoscopic liver resection, and the process is more smooth^[42,43]. Hu *et al.*^[37] performed a meta-analysis of 17 case-control studies with different surgical methods, including 487 robotic cases and 902 laparoscopic cases. The results showed that the amount of blood loss in robotic liver resection was significantly increased and the operation time was significantly longer than that in traditional laparoscopic surgery, and there was no significant difference in blood transfusion volume and blood transfusion rate between 33 cases with robotic surgery and 25 cases with laparoscopic liver resection. There was no significant difference in hospital stay, conversion rate to laparotomy, R0 resection rate, complications, or mortality between the two groups. Zhao *et al.*^[44] reported that successful robotic radical resection of hepatic echinococcosis located in posterosuperior liver segments, and robotic isolated partial and complete hepatic caudate lobectomy^[45]. In addition, Hu *et al.*^[46] indicated that robotic and laparoscopic hemi-hepatectomies were associated with less intraoperative blood loss, better postoperative recovery and a lower pain score. Compared with laparoscopic hemi-hepatectomy, robotic hemi-hepatectomy was associated with significantly less intraoperative blood loss and a shorter operative time. In terms of economic benefits, the medical cost of laparoscopic hepatectomy was relatively lower. There was no significant difference in 5-year overall survival rate and disease-free survival rate between the two groups (65% vs. 48%, 42% vs. 38%). Therefore, the high cost of treatment, the high requirements of operation technology and the difficulty in popularizing technical equipment may be the biggest shortcomings in the development of robotic surgery.

CONCLUSION

We have presented a comparison of published evidence for open, laparoscopic, and robotic liver resection, including systematic review and meta-analysis evidence summarizing the advantages and disadvantages of the three techniques. One of the analyses included 26 studies, including 2,630 patients (950 robotic laparoscopic surgery and 1,680 laparoscopic patients). The amount of bleeding in patients undergoing robotic hepatectomy was significantly reduced (286 mL vs. 301 mL, $P < 0.001$). There is no significant difference in postoperative complications of the various surgical methods. However, there is one thing worthy of our attention that patients undergoing robotic liver resection have a higher readmission rates ($P = 0.005$). Compared with traditional open surgery, minimally invasive surgery (robot and laparoscopic) is more expensive, but there is no statistical difference. It can significantly reduce intraoperative blood loss and shorten the length of stay; the tumor volume of patients undergoing laparoscopic liver resection was significantly reduced than patients in the open surgery group^[47-49]. Minimally invasive surgery for HCC is not a simple minimally invasive technology, but a process of minimally invasive concept and comprehensive treatment. For example, the ALPPS procedure (associating liver partition and portal vein ligation for staged hepatectomy) was proposed by Schnitzbauer *et al.*^[50] in 2015. In order to obtain sufficient liver function reserve, create conditions for surgery, and solve the problem of insufficient residual liver volume, it is necessary to make the impossible possible. However, because of its higher rate of complications, the mortality rate is as high as 12%, and, therefore, this kind of operation has caused great controversy. Currently, there is no clear clinical data to support its effect, as well as many postoperative complications, which cannot be popularized in clinical practice. Other studies have shown that the recurrence rate of ALPPS is increased^[51]. Moreover, the key objective of ALPPS is to block the blood supply of the tumor, promote the growth of residual liver, and avoid postoperative liver failure. However, PVE can play a similar role in clinical practice. At present, there is no clinical evidence to prove the overall prognosis of ALPPS. With the development of technology and the improvement of surgical techniques, patients will increasingly benefit from minimally invasive surgical precision treatments for liver pathologies.

DECLARATIONS

Authors' contributions

Conceived the review: Zhang CZ, Li N

Availability of data and materials

Not applicable.

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Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Venook AP, Papandreou C, Furuse J, de Guevara LL. The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. *Oncologist* 2010;15:5-13.
2. Alazawi W, Cunningham M, Dearden J, Foster GR. Systematic review: outcome of compensated cirrhosis due to chronic hepatitis C infection. *Aliment Pharmacol Ther* 2010;32:344-55.
3. Berzigotti A, Reig M, Abraldes JG, Bosch J, Bruix J. Portal hypertension and the outcome of surgery for hepatocellular carcinoma in compensated cirrhosis: a systematic review and meta-analysis. *Hepatology* 2015;61:526-36.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
5. Kaiser K, Mallick R, Butt Z, Mulcahy MF, Benson AB, Cella D. Important and relevant symptoms including pain concerns in hepatocellular carcinoma (HCC): a patient interview study. *Support Care Cancer* 2014;22:919-26.
6. Chaiteerakij R, Roberts LR. Genetic alterations in advanced HBV-related HCC with portal vein tumor thrombosis: insights from next generation DNA sequencing. *J Hepatol* 2013;58:1042-4.
7. Zhang XP, Gao YZ, Chen ZH, et al. An eastern hepatobiliary surgery hospital/portal vein tumor thrombus scoring system as an aid to decision making on hepatectomy for hepatocellular carcinoma patients with portal vein tumor thrombus: a multicenter study. *Hepatology* 2019;69:2076-90.
8. Lee HS. Management of patients with hepatocellular carcinoma and extrahepatic metastasis. *Dig Dis* 2011;29:333-8.
9. Wong R, Frenette C. Updates in the management of hepatocellular carcinoma. *Gastroenterol Hepatol (N Y)* 2011;7:16-24.
10. Toyoda H, Kumada T, Tada T. Highly sensitive Lens culinaris agglutinin-reactive α -fetoprotein: a new tool for the management of hepatocellular carcinoma. *Oncology* 2011;81:61-5.
11. Au JS, Frenette CT. Management of hepatocellular carcinoma: current status and future directions. *Gut Liver* 2015;9:437-48.
12. Bodzin AS, Busuttil RW. Hepatocellular carcinoma: advances in diagnosis, management, and long term outcome. *World J Hepatol* 2015;7:1157-67.
13. Liu Y, Wang YR, Ding GH, et al. Influence of surgical resection of hepatocellular carcinoma(HCC) for hematogenous dissemination of HCC cells and its effect on recurrence and metastasis: 3 years prospective study. *Neoplasma* 2015;62:635-40.
14. Lacaze L, Scotté M. Surgical treatment of intra hepatic recurrence of hepatocellular carcinoma. *World J Hepatol* 2015;7:1755-60.
15. Arima K, Chikamoto A, Hashimoto D, et al. Education and imaging. hepatology: hepatocellular carcinoma with duodenal metastasis. *J Gastroenterol Hepatol* 2015;30:1115.
16. Fang JH, Zhou HC, Zhang C, et al. A novel vascular pattern promotes metastasis of hepatocellular carcinoma in an epithelial-mesenchymal transition-independent manner. *Hepatology* 2015;62:452-65.

17. Hanley KL, Feng GS. A new VETC in hepatocellular carcinoma metastasis. *Hepatology* 2015;62:343-5.
18. Portolani N, Coniglio A, Ghidoni S, et al. Early and late recurrence after liver resection for hepatocellular carcinoma: prognostic and therapeutic implications. *Ann Surg* 2006;243:229-35.
19. Lee SS, Shin HS, Kim HJ, et al. Analysis of prognostic factors and 5-year survival rate in patients with hepatocellular carcinoma: a single-center experience. *Korean J Hepatol* 2012;18:48-55.
20. Eguchi S, Kanematsu T, Arai S, et al. Recurrence-free survival more than 10 years after liver resection for hepatocellular carcinoma. *Br J Surg* 2011;98:552-7.
21. Bruix J. Treatment of hepatocellular carcinoma. *Hepatology* 1997;25:259-62.
22. Imamura H, Seyama Y, Kokudo N, et al. One thousand fifty-six hepatectomies without mortality in 8 years. *Arch Surg* 2003;138:1198-206.
23. Liu R, Wakabayashi G, Kim HJ, et al. International consensus statement on robotic hepatectomy surgery in 2018. *World J Gastroenterol* 2019;25:1432-44.
24. Vibert E, Perniceni T, Levard H, Denet C, Shahri NK, Gayet B. Laparoscopic liver resection. *Br J Surg* 2006;93:67-72.
25. Reich H, McGlynn F, DeCaprio J, Budin R. Laparoscopic excision of benign liver lesions. *Obstet Gynecol* 1991;78:956-8.
26. Zhou WP, SunZH, Wu MC, et al. The first report of laparoscopic liver lobectomy. *J Hepat Surg* 1994;2:82. (In Chinese)
27. Chen XP. Expert consensus on laparoscopic hepatectomy (2013 version). *J Huazhong Univ Sci Technol Med Sci* 2013;33:791-7.
28. Mise Y, Sakamoto Y, Ishizawa T, et al. A worldwide survey of the current daily practice in liver surgery. *Liver Cancer* 2013;2:55-66.
29. Lee DH, Kim D, Park YH, Yoon J, Kim JS. Long-term surgical outcomes in patients with hepatocellular carcinoma undergoing laparoscopic vs. open liver resection: A retrospective and propensity score-matched study. *Asian J Surg* 2020; doi: 10.1016/j.asjsur.2020.05.028.
30. Takaoka M. A commentary on: "Physical recovery after laparoscopic vs. open liver resection - A prospective cohort study". *Int J Surg* 2020;74:22.
31. Rao A, Rao G, Ahmed I. Laparoscopic vs. open liver resection for malignant liver disease. A systematic review. *Surgeon* 2012;10:194-201.
32. Memeo R, de'Angelis N, Compagnon P, et al. Laparoscopic vs. open liver resection for hepatocellular carcinoma of cirrhotic liver: a case-control study. *World J Surg* 2014;38:2919-26.
33. El-Gendi A, El-Shafei M, El-Gendi S, Shawky A. Laparoscopic versus open hepatic resection for solitary hepatocellular carcinoma less than 5 cm in cirrhotic patients: a randomized controlled study. *J Laparoendosc Adv Surg Tech A* 2018;28:302-10.
34. Takahara T, Wakabayashi G, Beppu T, et al. Long-term and perioperative outcomes of laparoscopic versus open liver resection for hepatocellular carcinoma with propensity score matching: a multi-institutional Japanese study. *J Hepatobiliary Pancreat Sci* 2015;22:721-7.
35. Yan Y, Cai X, Geller DA. Laparoscopic liver resection: a review of current status. *J Laparoendosc Adv Surg Tech A* 2017;27:481-6.
36. Milone L, Daskalaki D, Fernandes E, Damoli I, Giulianotti PC. State of the art in robotic hepatobiliary surgery. *World J Surg* 2013;37:2747-55.
37. Hu L, Yao L, Li X, Jin P, Yang K, Guo T. Effectiveness and safety of robotic-assisted versus laparoscopic hepatectomy for liver neoplasms: a meta-analysis of retrospective studies. *Asian J Surg* 2018;41:401-16.
38. Giulianotti PC, Coratti A, Angelini M, et al. Robotics in general surgery: personal experience in a large community hospital. *Arch Surg* 2003;138:777-84.
39. Nota CL, Rinkes IHB, Molenaar IQ, van Santvoort HC, Fong Y, Hagendoorn J. Robot-assisted laparoscopic liver resection: a systematic review and pooled analysis of minor and major hepatectomies. *HPB (Oxford)* 2016;18:113-20.
40. Idrees K, Bartlett DL. Robotic liver surgery. *Surg Clin North Am* 2010;90:761-74.
41. Gonzalez-Ciccarelli LF, Quadri P, Daskalaki D, Milone L, Gangemi A, Giulianotti PC. Robotic approach to hepatobiliary surgery. *Chirurg* 2017;88:19-28.
42. Casciola L, Patriti A, Ceccarelli G, Bartoli A, Ceribelli C, Spaziani A. Robot-assisted parenchymal-sparing liver surgery including lesions located in the posterosuperior segments. *Surg Endosc* 2011;25:3815-24.
43. Pessaux P, Diana M, Soler L, Piardi T, Mutter D, Marescaux J. Towards cybernetic surgery: robotic and augmented reality-assisted liver segmentectomy. *Langenbecks Arch Surg* 2015;400:381-5.
44. Zhao ZM, Yin ZZ, Meng Y, Jiang N, Ma ZG, Pan LC, et al. Successful robotic radical resection of hepatic echinococcosis located in posterosuperior liver segments. *World J Gastroenterol* 2020;26:2831-8.
45. Zhao ZM, Yin ZZ, Pan LC, Hu MG, Tan XL, Liu R. Robotic isolated partial and complete hepatic caudate lobectomy: a single institution experience. *Hepatobiliary Pancreat Dis Int* 2020; doi: 10.1016/j.hbpd.2020.05.003.
46. Hu M, Chen K, Zhang X, Li C, Song D, Liu R. Robotic, laparoscopic or open hemihepatectomy for giant liver haemangiomas over 10 cm in diameter. *BMC Surg* 2020;20:93.
47. Kamarajah SK, Bundred J, Manas D, Jiao LR, Hilal MA, White SA. Robotic versus conventional laparoscopic liver resections: a systematic review and meta-analysis. *Scand J Surg* 2020; doi: 10.1177/1457496920925637.
48. Ziogas IA, Giannis D, Esagian SM, Economopoulos KP, Tohme S, Geller DA. Laparoscopic versus robotic major hepatectomy: a systematic review and meta-analysis. *Surg Endosc* 2020; doi: 10.1007/s00464-020-08008-2.
49. Gavrilidis P, Roberts KJ, Aldrighetti L, Sutcliffe RP. A comparison between robotic, laparoscopic and open hepatectomy: a systematic review and network meta-analysis. *Eur J Surg Oncol* 2020;46:1214-24.
50. Schnitzbauer AA, Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA, et al. Right portal vein ligation combined with in situ

splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg* 2012;255:405-14.

51. Matayoshi T, Oya Y. Current strategy for management of resistant hypertension. *Nihon Rinsho* 2011;69:1987-92.

Review

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Prevalence and incidence of intra- and extrahepatic complications of NAFLD in patients with type 2 diabetes mellitus

Alessandro Mantovani¹, Giorgia Beatrice¹, Roberta Stupia², Andrea Dalbeni²

¹Section of Endocrinology, Diabetes and Metabolism, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona 37126, Italy.

²Section of General Medicine, Hypertension and Liver Unit, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona 37126, Italy.

Correspondence to: Dr. Alessandro Mantovani, Section of Endocrinology, Diabetes and Metabolism, University and Azienda Ospedaliera Universitaria Integrata of Verona, Piazzale Stefani, 1, Verona 37126, Italy. E-mail: alessandro.mantovani@univr.it

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is linked to abdominal obesity, insulin resistance and type 2 diabetes mellitus (T2DM). The association of NAFLD with T2DM is bidirectional. In fact, evidence suggests that abdominal obesity, T2DM and metabolic syndrome play a part in the development and progression of NAFLD. Alternatively, NAFLD is associated with an increased risk of having T2DM and metabolic syndrome. According to this background, it is unsurprising that patients with T2DM also have a higher prevalence of NAFLD than those with no T2DM, as well as an increased risk of developing liver-related and extrahepatic complications, mainly cardiovascular and renal diseases. Seeing the relationship of NAFLD with insulin resistance, obesity and T2DM, recent consensus proposes a change in nomenclature from NAFLD to metabolic associated fatty liver disease. In this review, we will discuss the prevalence and incidence of NAFLD (as detected by imaging techniques or liver biopsy) in patients with type T2DM with particular regard to hepatic and extrahepatic complications.

Keywords: Nonalcoholic fatty liver disease; NASH, metabolic associated fatty liver disease, diabetes, type 2 diabetes



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INTRODUCTION

In the last decades, it became clearly evident that nonalcoholic fatty liver disease (NAFLD) is associated with insulin resistance, abdominal obesity and type 2 diabetes mellitus (T2DM)^[1-5]. Specifically, the association between NAFLD and T2DM is intricate, and of note, it appears to be even bidirectional^[1-5]. In fact, convincing data now indicate that abdominal obesity, T2DM and metabolic syndrome (MetS) can synergistically play a part in the development of NAFLD and its advanced forms^[6,7]. Despite that, NAFLD is linked to a higher risk of T2DM and MetS, as well as to a poorer glycemic control in diabetic patients^[6]. Based on these data, it is therefore unsurprising that patients with T2DM have an increased prevalence of NAFLD, when compared to those without T2DM, as well as a higher risk of developing serious liver-related [including nonalcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma] and extrahepatic complications, such as cardiovascular and renal diseases [Figure 1]^[2,4,8].

Since NAFLD, obesity, insulin resistance and T2DM are strictly linked, some researchers in this field have proposed and recommended a change in nomenclature from NAFLD to MAFLD, i.e., metabolic associated fatty liver disease^[9-11]. This specific issue has not yet reached a consensus and is discussed in another article published in this journal.

In this review, we will discuss the prevalence and incidence of NAFLD (as detected by imaging techniques or liver biopsy) in patients with T2DM with particular regard to hepatic and extrahepatic complications.

Search strategy and selection criteria

In the PubMed-Medline database, we used the following terms: “fatty liver” or “NAFLD” or “NASH” and “diabetes mellitus” or “type 2 diabetes” (concluding research on the 30th July 2020). We did not apply any publication date or language restrictions. Finally, we used specific references of reviews to identify other relevant articles.

Prevalence of NAFLD in patients with diabetes mellitus

As reported in Table 1, in the last five years, several population-based studies and hospital-based studies have reported that in adult patients with T2DM the prevalence of NAFLD, as detected by imaging techniques or liver biopsy, ranged from 30% to 70% and from 50% to 70%, respectively^[12-75]. These data strongly support the assertion that NAFLD is much more frequent in patients with T2DM, when compared to the general population or other patient groups^[1-4]. In particular, regarding the observational studies using liver ultrasound for the diagnosis of NAFLD, which is the recommended first-line imaging method for detecting hepatic steatosis in clinical practice^[76], the prevalence of NAFLD was approximately 70%-75% in patients with T2DM, with however some exceptions. For instance, in a large cohort study involving nearly 5,500 South Korean patients with T2DM, Choe *et al.*^[58] documented that the prevalence of NAFLD was 46%. In another population-based study of 8,571 Chinese hospitalized patients with T2DM, Guo *et al.*^[24] showed that the prevalence of NAFLD was approximately 51%. Contrariwise, in a cross-sectional study including 222 Italian outpatients with T2DM, who were regularly seen at a specific diabetes clinic, Mantovani *et al.*^[45] reported that the prevalence of NAFLD was more than 70%. In addition, in a small study of 106 Australian patients with T2DM belonging a tertiary diabetes center, Williams *et al.*^[22] documented that the prevalence of NAFLD was even higher (84%). Interestingly, in a recent cross-sectional study enrolling 137 patients with non-insulin-treated T2DM who underwent liver ultrasound and liver stiffness measurement (LSM) using vibration-controlled transient elastography (FibroScan®), Mantovani *et al.*^[65] showed that the proportion of T2DM patients with hepatic steatosis (on ultrasound) was 74% and that the proportion of individuals with significant liver fibrosis was approximately 18% with an LSM cut-off ≥ 7 kPa and nearly 10% with an LSM cut-off ≥ 8.7 kPa.

The severity of NAFLD on ultrasound is usually graded using a 4-point scoring system: normal, mild, moderate and severe. In the literature, information regarding the prevalence of different grades of liver

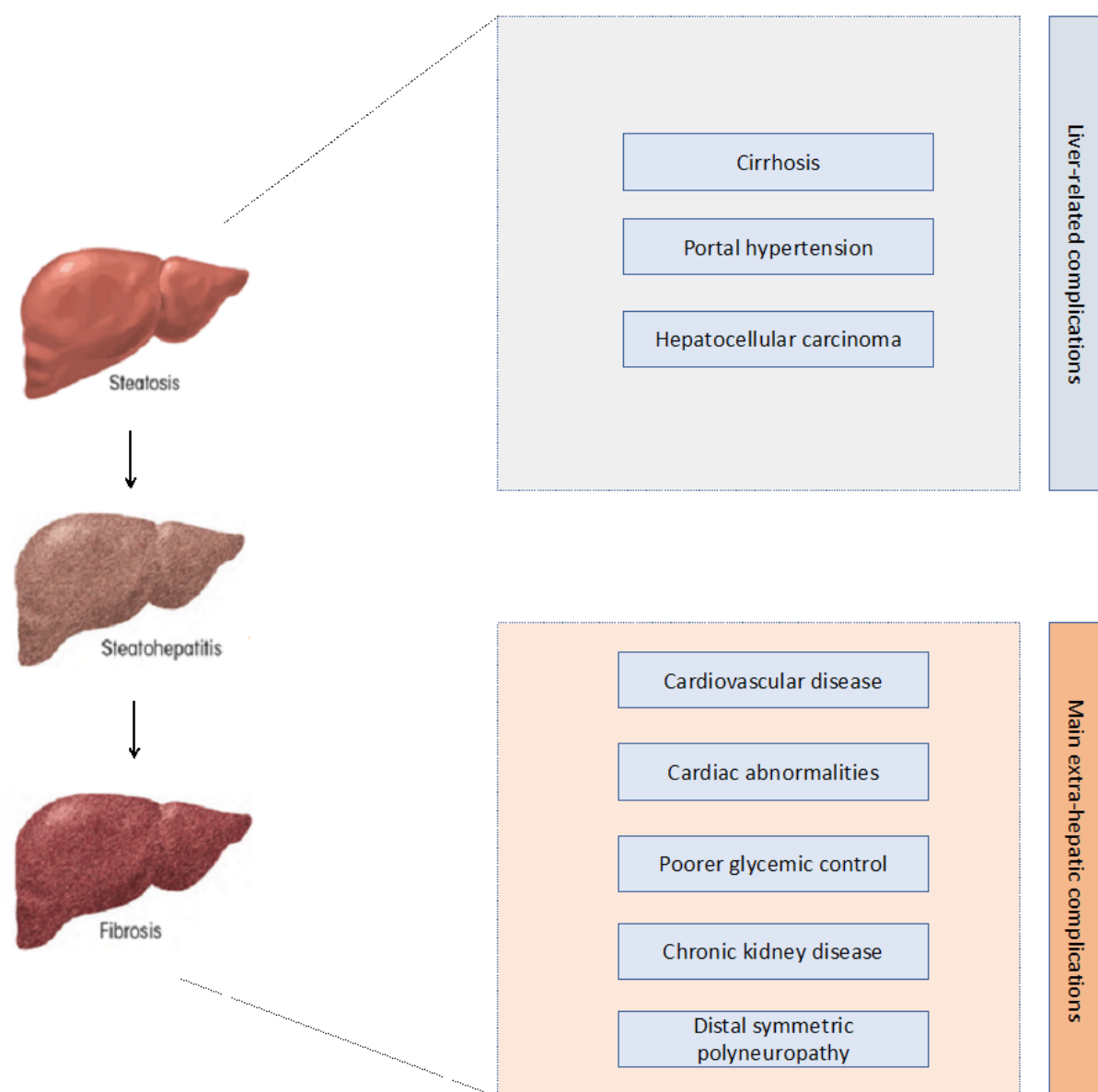


Figure 1. Main hepatic and extrahepatic complications associated with Nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus

steatosis on ultrasound in patients with T2DM are also available^[77,78]. For instance, in a cross-sectional study of 874 diabetic and non-diabetic patients, Wang *et al.*^[77] documented that the prevalence of T2DM among patients with mild liver steatosis (on ultrasound) was 7.5%, whereas the percentage of T2DM individuals among those with moderate-to-severe liver steatosis was 23.4% ($P < 0.05$).

These results clearly show that among the observational studies using liver ultrasound for the diagnosis of NALFD, the prevalence of NAFLD varies across different countries and clinical settings and it is influenced by different prevalence of obesity and degree of insulin resistance, as well as by the fact that ultrasound is an operator-dependent imaging technique. Indeed, the observational studies involving European or USA patients have reported a prevalence of NAFLD higher than that observed in the studies involving Asian patients. Additional factors that might explain these findings are different lifestyles (i.e., sedentary, physical inactivity), diets (i.e., high caloric diets, especially in the form of carbohydrates and

Table 1. Principal studies evaluating the prevalence of NAFLD in patients with type 2 diabetes mellitus published in the last five years

Author	Characteristics of study	Results
Portillo-Sanchez <i>et al.</i> ^[12]	103 patients with T2DM from USA recruited from responses to local newspaper advertisements or from people attending clinics in various USA medical Centers Mean age: 60 years; mean BMI: 33 kg/m ² ; 87% males	Prevalence of NAFLD on MRI was 50%
Kwok <i>et al.</i> ^[13]	1,918 patients with T2DM from Hong Kong, who attended diabetic complications screening Mean age 60 years; mean BMI 33 kg/m ² ; 87% males	Prevalence of NAFLD on US was 73%
Arab <i>et al.</i> ^[14]	133 Chilean patients with T2DM invited by diabetologists and family physicians for liver disease surveillance assessment Mean age 60 years; mean BMI 30 kg/m ² , 47% males	Prevalence of NAFLD on MRI was 64%
Jung <i>et al.</i> ^[15]	186 South Korean patients with T2DM admitted to the Endocrinology Division of a university hospital Mean age 58 years; mean BMI 25 kg/m ² ; 30% Males	Prevalence of NAFLD on US was 53%
Masarone <i>et al.</i> ^[16]	63 Italian patients with T2DM admitted to a tertiary center of internal medicine and hepatology Mean age 57 years, mean BMI 34.4 kg/m ² , 52.3% males	Prevalence of NAFLD on liver biopsy was 100%
Petit <i>et al.</i> ^[17]	264 French patients with T2DM screened prospectively at an endocrinology department Mean age 60 years, mean BMI 34 kg/m ² , 49% males	Prevalence of NAFLD on MRI was 63%
Wilman HR <i>et al.</i> ^[18]	226 British patients with T2DM who provided written informed consent to the study in the United Kingdom Biobank imaging enhancement	Prevalence of NAFLD on MRI was 50%
Doycheva <i>et al.</i> ^[19]	100 patients with T2DM from USA recruited via newspaper advertisement and from primary care practices Mean age 60 years, mean BMI 31 kg/m ² , 53% males	Prevalence of NAFLD on MRI was 65%
Ding <i>et al.</i> ^[20]	1,648 Chinese patients with T2DM who lived in a specific district of Shanghai Mean age 62 years, mean BMI 25 kg/m ² , 49% males	Prevalence of NAFLD on US was 41.6%
Al Rifai <i>et al.</i> ^[21]	517 patients with T2DM of different ethnicity, from different areas of USA, without known CVD at the time of enrollment	Prevalence of NAFLD on CT was 28.4%
Williams <i>et al.</i> ^[22]	106 Australian patients with T2DM from a tertiary diabetes center Mean age 63 years, mean BMI 30 kg/m ² , 61% males	Prevalence of NAFLD on US was 84%
Cusi <i>et al.</i> ^[23]	385 patients with T2DM from USA who had liver fat content evaluated by MRI in four phase 3 studies of basal insulin peglispro (BIL) Mean age 60 years, mean BMI 32 kg/m ² , 62% males	Prevalence of NAFLD on MRI was 75.6% in insulin-naïve T2DM patients, 61.7% in insulin-treated T2DM patients
Guo <i>et al.</i> ^[24]	8,571 Chinese patients with T2DM hospitalized in the department of endocrinology and metabolism of a hospital in Shanghai Mean age 60 years, mean BMI 25 kg/m ² , 56% males	Prevalence of NAFLD on US was 50.6%
Herath <i>et al.</i> ^[25]	233 Sri Lankan patients with T2DM followed up at a diabetes center Mean age 58 years, mean BMI 25 kg/m ² , 47% males	Prevalence of NAFLD on US was 62.6%
Sberna <i>et al.</i> ^[26]	179 French patients with T2DM referred to a tertiary diabetes department Mean age 60 years, mean BMI 34 kg/m ² , 47% males	Prevalence of NAFLD on MRI was 68.7%
Krishan <i>et al.</i> ^[27]	100 Indian patients with T2DM who underwent a routine health check-up Mean age 51 years, mean BMI 28 kg/m ² , 82% males	Prevalence of NAFLD on US was 65%
Su <i>et al.</i> ^[28]	445 Chinese patients with T2DM who visited the hospital for evaluation or treatment of T2DM Mean age 60 years, mean BMI 25 kg/m ² , 53% males	Prevalence of NAFLD on US was 41.4%
Vanjiappan <i>et al.</i> ^[29]	300 Indian patients with T2DM attending the outpatient department of a tertiary care hospital Mean age 54 years, mean BMI 25 kg/m ² , 54% males	Prevalence of NAFLD on US was 61%
He <i>et al.</i> ^[30]	331 hospitalized patients from China with T2DM Mean age 57 years, mean BMI 26 kg/m ² , 50% males	Prevalence of NAFLD on US was 63%
Kabir <i>et al.</i> ^[31]	258 Bangladeshi patients with T2DM included in this observational study in the department of medicine of a hospital in Bangladesh	Prevalence of NAFLD on US was 64.7%
Lai <i>et al.</i> ^[32]	557 Malaysian patients with T2DM attending the diabetes clinic of a university hospital Mean age 61 years, 41% males	Prevalence of NAFLD on US was 72.4%
Hashimoto <i>et al.</i> ^[33]	145 Japanese patients with T2DM recruited from the outpatient clinic of a university hospital in Kyoto Mean age 66 years, mean BMI 25 kg/m ² , 55 % males	Prevalence of NAFLD on US was 67%
Zou <i>et al.</i> ^[34]	2646 Chinese patients with T2DM recruited from a local health examination center for diabetes, newly diagnosed diabetics or with a previous diagnosis of T2DM Mean age 61 years, mean BMI 26 kg/m ² , 42% males	Prevalence of NAFLD on US was 50.9%

Afolabi <i>et al.</i> ^[35]	80 Nigerian patients with T2DM recruited from the endocrinology clinic of a hospital Mean age 61 years, mean BMI 26 kg/m ² , 38% males	Prevalence of NAFLD on US was 68.8%
Moh Moh <i>et al.</i> ^[36]	172 South Korean patients with T2DM who were admitted for glucose control to the endocrinology division of a university hospital Mean age 57 years, mean BMI 25 kg/m ² , 50% males	Prevalence of NAFLD on US was 51%
Cosma <i>et al.</i> ^[37]	60 Italian patients with T2DM enrolled in the study during their routine visit at a diabetes Centre Mean age 65 years, BMI 32 kg/m ² , 42% males	Prevalence of NAFLD on US was 88%
Almobarak <i>et al.</i> ^[38]	167 Sudanese patients with T2DM recruited from the outpatient of a diabetic center 87% subjects were aged 40-70 years, 8% subjects > 70 years and 5% subjects < 40 years, mean 46.7% males	Prevalence of NAFLD on US was 50.3%
Yan <i>et al.</i> ^[39]	212 Chinese patients with T2DM recruited from a metabolic disease hospital Mean age 54 years, mean BMI 27 kg/m ² , 57% males	Prevalence of NAFLD on US was 67.5%
Alsabaani <i>et al.</i> ^[40]	245 Arab patients with T2DM recruited from primary healthcare centers Mean age 57 years, 66% males	Prevalence of NAFLD on US was 72.8%
Gutierrez-Buey <i>et al.</i> ^[41]	56 Spanish patients with well-controlled T2DM (HbA1c < 7%) Mean age 64 years, mean BMI 28 kg/m ² , 80% males	Prevalence of NAFLD on US or CT was 73.2%
Zawdie <i>et al.</i> ^[42]	96 Ethiopian patients with T2DM attending a diabetic clinic Mean BMI 23 kg/m ² , 47% males	Prevalence of NAFLD on US was 73%
Dvorak <i>et al.</i> ^[43]	180 Czech patients with T2DM Mean age 64 years, mean BMI 32 kg/m ² , 63% males	Prevalence of NAFLD on US was 79%
Hua <i>et al.</i> ^[44]	1,037 Chinese patients with T2DM who visited for different medical reasons a specific hospital in the city of Nanjing were enrolled Mean age 57 years, mean BMI 24 kg/m ² , 59% males	Prevalence of NAFLD on US was 56.3%
Mantovani <i>et al.</i> ^[45]	222 Italian patients with T2DM, who regularly attended a specific diabetes clinic Mean age 67 years, mean BMI 29 kg/m ² , 70% males	Prevalence of NAFLD on US was 71.2%
Olusanya <i>et al.</i> ^[46]	168 Nigerian patients with T2DM evaluated at an endocrine clinic Mean age 53 years, mean BMI 28 kg/m ² , 35% males	Prevalence of NAFLD on US was 16.7%
Chang <i>et al.</i> ^[47]	97 Chinese patients with T2DM who agreed to participate in the study Mean age 47 years, mean BMI 26 kg/m ² , 71% males	Prevalence of NAFLD on US was 69%
Zhao <i>et al.</i> ^[48]	2,042 Chinese patients with T2DM hospitalized in an endocrinology and metabolism department Mean age 60 years, mean BMI 25 kg/m ² , 47% males	Prevalence of NAFLD on US was 40.2%
Zhang <i>et al.</i> ^[49]	175 Chinese patients with newly diagnosed T2DM enrolled in this study from the endocrinology department of a hospital in Wuhan Mean age 52 years, mean BMI 24 kg/m ² , 56% males	Prevalence of NAFLD on US was 53%
Johansen <i>et al.</i> ^[50]	120 Danish patients with T2DM recruited at the diabetes outpatient clinic at a university hospital Mean age 63 years, mean BMI 30 kg/m ² , 73% males	Prevalence of NAFLD on MRI was 48 %
Fan <i>et al.</i> ^[51]	541 Chinese patients with T2DM recruited from a department of endocrinology and metabolism Mean age 58 years, mean BMI 25 kg/m ² , 50% Males	Prevalence of NAFLD on US was 56.6%
García Díaz <i>et al.</i> ^[52]	58 Spanish patients with T2DM whose diabetes was treated in any medical center of Lanzarote Mean age 55 years, mean BMI 31 kg/m ² , 61% males	Prevalence of NAFLD on US was 57.8%
Demir <i>et al.</i> ^[53]	124 Turkish patients with T2DM invited to undergo a liver US for screening Mean age 53 years, mean BMI 33 kg/m ² , 37% males	Prevalence of NAFLD on US was 94.3%
Petit <i>et al.</i> ^[54]	308 French patients screened at an endocrinology department to participate in the study to determine whether a specific polymorphism was involved in the development of NAFLD Mean age 60 years, mean BMI 34 kg/m ² , 51% males	Prevalence of NAFLD on MRI was 65.2%
Zhao <i>et al.</i> ^[55]	629 Chinese patients with T2DM enrolled to evaluate their liver conditions Mean age 44 years, mean BMI 26 kg/m ² , 91% males	Prevalence of NAFLD on US was 67.7%
Mantovani <i>et al.</i> ^[56]	330 Italian patients with T2DM who regularly attended a diabetes clinic and had undergone a first 24-hour Holter monitoring for clinical reasons Mean age 70 years, mean BMI 29 kg/m ² , 65% males	Prevalence of NAFLD on US was 72.1%
Barchetta <i>et al.</i> ^[57]	62 Italian patients with T2DM referred to diabetes outpatient clinics who underwent clinical work-up Mean age 59 years, mean BMI 30 kg/m ² , 72% males	Prevalence of NAFLD on MRI was 56.5%
Choe <i>et al.</i> ^[58]	5,507 South Korean patients with T2DM diagnosed at the Huh's Diabetes Center in Seoul, who underwent an abdominal US Mean age 57 years, mean BMI 24 kg/m ² , 51% males	Prevalence of NAFLD on US was 46.4%
Silaghi <i>et al.</i> ^[59]	336 Romanian patients with T2DM who regularly attended diabetes evaluation in the same clinic Mean age 56 years, mean BMI 32 kg/m ²	Prevalence of NAFLD on US was 86%

Lee <i>et al.</i> ^[60]	606 South Korean patients with T2DM, aged ≤ 50 years who had undergone short insulin tolerance test, liver US, and Doppler echocardiography at a specific diabetes center were enrolled in the study protocol Mean age 63 years, mean BMI 26 kg/m ² , 24% males	Prevalence of NAFLD on US was 58.6%
Seo <i>et al.</i> ^[61]	4,210 South Korean patients with T2DM were recruited from the Seoul Metabolic Syndrome Cohort Mean age 57 years, mean BMI 25 kg/m ² , 51% males	Prevalence of NAFLD on US was 30.4%
Yeung <i>et al.</i> ^[62]	1763 Chinese patients with T2DM recruited from the Hong Kong Diabetes Registry Mean age 61 years, mean BMI 26 kg/m ² , 56% males	Prevalence of NAFLD on US was 53.2%
Bellan <i>et al.</i> ^[63]	328 Italian patients with T2DM evaluated at a diabetes clinic were offered to participate in this study, according to the protocol Mean age 65 years, mean BMI 27 kg/m ² , 74% males	Prevalence of NAFLD on US was 59.5%
Tuong <i>et al.</i> ^[64]	307 Vietnamese patients with T2DM or previously unknown diabetes admitted for medical check-up Mean age 57 years, mean BMI 25 kg/m ² , 54% males	Prevalence of NAFLD on US was 73.3%
Mantovani <i>et al.</i> ^[65]	137 Italian patients with non-insulin-treated T2DM and no known liver disease consecutively attending a diabetes outpatients' service Mean age 70 years, mean BMI 28 kg/m ² , 48% males	Prevalence of NAFLD on US was 73.7%
Choi <i>et al.</i> ^[66]	302 South Korean patients with T2DM who underwent regular outpatient clinic follow-up Mean age 58 years, mean BMI 25 kg/m ² , 38% males	Prevalence of NAFLD on US was 62%
Heidari <i>et al.</i> ^[67]	255 Iranian patients with T2DM who were referred to some specific endocrine clinics enrolled in the study by consecutive sampling Mean age 51 years, mean BMI 31 kg/m ² , 32% males	Prevalence of NAFLD on US was 86.7%
Mandal <i>et al.</i> ^[68]	210 Nepalese patients with T2DM treated as outpatients at a hospital in Nepal Mean age 56 years, mean BMI 29 kg/m ² , 57% males	Prevalence of NAFLD on US was 55.7%
Motta <i>et al.</i> ^[69]	173 Italian patients with T2DM enrolled in the study by invitation letter Mean age 67 years, mean BMI 30 kg/m ² , 51% Males	Prevalence of NAFLD on US was 24.9%
Sporea <i>et al.</i> ^[70]	534 Romanian patients with T2DM scheduled for a medical visit at the Department of Diabetes and Metabolic Diseases who agreed to be evaluated by liver elastography Mean age 61 years, mean BMI 32 kg/m ² , 47% males	Prevalence of NAFLD of US was 76%
Lombardi <i>et al.</i> ^[71]	394 Italian outpatients with T2DM attending five Italian diabetes centers who underwent liver ultrasonography, FibroScan and extensive evaluation of macrovascular and microvascular diabetic complications Mean age 68 years, mean BMI 29 kg/m ² , 52% Males	Prevalence of NAFLD on US was 89%
Hamid <i>et al.</i> ^[72]	203 Pakistani patients with T2DM diagnosed during the last 6 months were enrolled Mean age 53 years, 51% males	Prevalence of NAFLD on US was 71.9%
Lee <i>et al.</i> ^[73]	1,992 Taiwanese patients with T2DM who participated in a disease management program at two specialized diabetes outpatient clinics. Mean age 66 years, mean BMI 26 kg/m ² , 34% males	Prevalence of NAFLD on US was 55.9%
El-Ashmawy <i>et al.</i> ^[74]	270 Egyptian patients with T2DM invited to take part in the study. Mean age 53 years, mean BMI 26 kg/m ² , 53% males	Prevalence of NAFLD on US was 73.3%
Mangla <i>et al.</i> ^[75]	96 patients with T2DM from USA recruited in a specific area of California who underwent a research study visit. Mean age 62 years, mean BMI 30 kg/m ² , 54% males	Prevalence of NAFLD on MRI was 65.6%

BMI: body mass index; CT: computed tomography; MRI: magnetic resonance imaging; NAFLD: nonalcoholic fatty liver disease; T2DM: type 2 diabetes; US: ultrasound

fats) and genetic factors^[1-4]. It is known that overeating, physical inactivity and scarce aerobic fitness are associated with reduced triglyceride export, increased *de novo* lipogenesis and increased fatty acid uptake in the liver^[79,80]. All these elements may lead to additional hepatic lipid storage with relevant metabolic consequences^[79,80]. Regarding genetic factors, NAFLD congregates in families with specific variants, such as patatin-like phospholipase domain-containing 3 gene (*PNPLA3*) and transmembrane 6 superfamily member 2 (*TM6SF2*)^[81]. One of the main genetic variants associated with NAFLD is a mutation [I148M] in *PNPLA3*^[81]. *PNPLA3* plays a key role in hepatic fat accumulation in GG homozygous individuals^[81]. In addition, these patients are at higher risk of developing the more severe forms of NAFLD, independent of T2DM^[81]. Interestingly, the single variant in the *PNPLA3* gene (I148M) has the highest prevalence in Hispanics, followed by non-Hispanic whites, Asians, and African Americans^[81].

Regarding the observational studies using magnetic resonance imaging (MRI) for the diagnosis of NAFLD, it is possible to observe that the prevalence of NAFLD ranges from 50% to 70%. For instance, in a study enrolling 103 patients with T2DM and normal plasma aminotransferase levels, Portillo-Sanchez *et al.*^[12] reported that the prevalence of NAFLD was approximately 50%. Moreover, in that study, the authors reported a high prevalence of NASH in a subgroup of patients who underwent liver biopsy^[12]. Indeed, approximately 55% of patients with NAFLD on MRI had histological features suggestive of NASH^[12]. These data strongly support the assertion that patients with T2DM have a high risk of developing severe forms of NAFLD, such as NASH and advanced fibrosis, which are the histological features more closely associated with hepatic and extrahepatic complications^[1-4]. Also in this regard, Bazick *et al.*^[82] found in an observational study involving approximately 350 patients with T2DM who underwent liver biopsy, that the prevalence of NASH and advanced fibrosis was 69 and 41%, respectively. In another study including 108 patients with biopsy-proven NAFLD, McPherson *et al.*^[83] reported that approximately 80% of patients who had had progression in fibrosis were diabetics, whereas among non-progressor patients only 25% had diabetes mellitus. The association between T2DM and the more severe forms of NAFLD was also reported by Loomba *et al.*^[84] in an observational study enrolling 1,069 T2DM patients with biopsy-proven NAFLD, documenting a significant association between history of diabetes mellitus, NASH and advanced fibrosis, even after adjusting for age, sex, body mass index, ethnicity, and presence of metabolic syndrome. Moreover, in a retrospective analysis of 235 patients with biopsy-proven NAFLD with and without T2DM, Puchakayala *et al.*^[85] documented that among T2DM patients with NAFLD, the prevalence of advanced fibrosis and ballooning were significantly greater as compared to patients with NAFLD but without T2DM. Interestingly and importantly, in the multivariate regression analysis, T2DM was associated with NASH and fibrosis in all patients with NAFLD^[85].

These data were additionally replicated in a recent meta-analysis by Younossi *et al.*^[86] including 80 observational studies for a total of nearly 49,500 individuals with T2DM (mean age: 58 years; mean body mass index: 28 kg/m²; percentage of men: 53%). In this meta-analysis, the authors found that the overall prevalence of NAFLD was approximately 55%, the global prevalence of NASH was 37%, and the prevalence of advanced fibrosis was 17%^[86].

Importantly, the coexistence of NAFLD and T2DM is also associated with a poorer cardiometabolic profile in terms of glycemic control, atherogenic dyslipidemia and hypertension^[4,87]. Coexisting NAFLD and T2DM may also increase insulin requirement in T2DM patients treated with basal bolus insulin regimen^[87].

NAFLD and risk of incident T2DM

Several epidemiological studies have documented that NAFLD, as detected by ultrasound, is associated with an increased risk of incident T2DM, even after adjustment for many metabolic confounders, such as age, sex, body mass index, smoking status, alcohol intake, physical activity, family history of diabetes, lipids and insulin resistance^[88-106]. This finding was also replicated by a 2018 meta-analysis including 19 cohort studies for a total of approximately 300,000 individuals (30% with NAFLD) and nearly 16,000 cases of incident diabetes over a median of 5 years^[107]. In fact, in this study, Mantovani *et al.*^[107] reported that patients with NAFLD had a higher risk of incident diabetes mellitus when compared to those with no liver involvement [random-effects hazard ratio (HR) 2.22, 95% confidence interval 1.84-2.60; I²=79%]. In addition, in that study, patients with more “severe” NAFLD were also more likely to develop incident diabetes mellitus^[107]. More recently, a 2020 meta-analysis of nearly 500,000 individuals reported similar results^[108].

Few studies have assessed the risk of incident T2DM in relation to the modification of NAFLD status over time^[7,97,109,110]. For instance, in a retrospective longitudinal study including approximately 13,000 Korean individuals followed for 5 years, Sung *et al.*^[109] documented that alterations in fatty liver content (on

ultrasound) over time was associated with changeable risks of incident T2DM. Recently, in an observational study including 2,726 patients in which NAFLD status change was assessed by serial abdominal ultrasonography and fatty liver index (FLI) during a follow-up of 10 years, Cho *et al.*^[110] documented that the progression and regression of NAFLD were respectively associated with positive and negative risk of incident diabetes mellitus. These findings additionally corroborate the assumption that NAFLD is a modifiable trigger factor associated with the progression to the advanced stages of diabetes mellitus^[111].

Sex as key modulator of NAFLD in patients with T2DM

Experimental data and computer modeling now indicate that female and male livers may be metabolically distinct with specific and different regulators^[112,113]. In particular, accumulating data suggest that the prevalence and severity of NAFLD tend to be greater in men as compared to women during the reproductive age. Conversely, after menopause, the prevalence of NAFLD tends to be higher in women, thereby indicating a potential protective role of the estrogens^[113]. However, most observational studies available so far, including those conducted in patients with T2DM, did not have specific statistical analyses considering sex differences or sex hormones/menopausal status as potential modifiers. In a 2020 meta-analysis of 33 cohort studies, Mantovani *et al.*^[108] did not observe an effect of sex on the relationship between NAFLD and risk of incident T2DM, but this may partly reflect the characteristics of the eligible observational studies. Along with other authors^[113], we strongly believe that future observational studies should have sex-specific analyses.

Liver complications in NAFLD patients with diabetes mellitus

Liver involvement in patients with T2DM is recognized in the form of simple steatosis, nonalcoholic steatohepatitis (NASH), advanced fibrosis, cirrhosis, hepatocellular carcinoma, glycogenic hepatopathy and hepatic arteriolosclerosis^[114]. That said, some histological analysis suggests that simple steatosis is a benign condition, while NASH with different degrees of hepatic fibrosis is closely associated with liver-related morbidity and mortality. As previously mentioned, T2DM patients have a greater prevalence of NASH and advanced fibrosis when compared to the general adult population^[114]. In addition, many observational studies have clearly demonstrated that T2DM, along with obesity and severe degrees of insulin resistance, is one of the main clinical risk factors implicated in the progression of NAFLD to NASH, advanced fibrosis or cirrhosis^[1,2,4]. Conversely, it is also reported that the presence of NAFLD may also adversely influence the prognosis of diabetes^[1,2,4]. Among various observational studies^[115-127] published so far [Table 2], the Verona Diabetes Study was one of first observational studies demonstrating that the risk of mortality from liver causes was higher in a large cohort of T2DM patients when compared to the general population^[115]. These findings were subsequently replicated in other case-control studies. For instance, in a retrospective study that used the administrative database of the Veneto region, Zoppini *et al.*^[116] observed that Italian T2DM individuals had a roughly 3-fold higher risk of dying from chronic liver diseases due to a non-virus and non-alcohol-related etiology. In another community-based cohort study involving nearly 340 T2DM patients, Adams *et al.*^[117] showed that the presence of NAFLD, as detected by imaging or biopsy, was associated with a higher risk of all-cause mortality (mainly due to cardiovascular disease, malignancy and liver-related complications) during a mean follow-up of 11 years.

An association between T2DM and liver cirrhosis is also currently known. In patients with cirrhosis, indeed, diabetes mellitus can be due to the presence of T2DM or as a direct consequence of liver insufficiency (namely hepatogenous diabetes mellitus)^[114]. In this context, several observational studies have documented an elevated prevalence of cirrhosis in patients with T2DM and NAFLD, especially if they are older or have cardiovascular complications^[114,118]. Cirrhosis is also associated with reduced hepatic mass and portosystemic shunts; two conditions able to alter insulin clearance, thereby contributing to systemic insulin resistance^[119]. In addition, cirrhosis is associated with increased levels of hypoxia-inducible factors and advanced glycation end products, which play a role in the development of T2DM^[119,120]. T2DM is an

Table 2. Principal observational studies that assessed the association between NAFLD and risk of liver-related disease and extrahepatic complications (cardiovascular diseases)

Author	Study characteristics	Complication	NAFLD diagnosis	Results	Country
De Marco <i>et al.</i> ^[115]	Population-based study: 7,148 T2DM patients (3,366 men and 3,782 women) followed for a period of 5 years	Mortality	US	The highest SMRs in the diabetic cohort were for diabetes, liver cirrhosis and cardiovascular diseases	Italy
Zoppini <i>et al.</i> ^[116]	Retrospective study: 167,621 T2DM patients, aged 30-89 years (54.6% men), followed from 2008 to 2010	Mortality	US	T2DM patients had a higher risk of dying from chronic liver diseases, in particular NAFLD	Italy
Adams <i>et al.</i> ^[117]	Community-based study: 337 T2DM patients from Olmsted County, Minnesota, followed for 11 years; 116 of them had NAFLD	Mortality	US	Overall mortality was significantly associated with a diagnosis of NAFLD, presence of ischemic heart disease and duration of diabetes	USA
Bertot <i>et al.</i> ^[119]	Prospective study: 284 patients (53% with T2DM, 15% with cirrhosis) followed up for a median period of 51 months	Death/transplantation, decompensation, HCC	Biopsy, Hepascore, NAFLD fibrosis score (NFS), APRI and FIB-4	T2DM patients had a greater risk of liver-related death/transplantation (HR 3.4, 95%CI: 1.2-9.1) decompensation (HR 4.7, 95%CI: 2.0-11.3) and HCC (HR 2.9, 95%CI: 1.2-7.3)	Australia
Sanyal <i>et al.</i> ^[121]	Prospective study: using a health care claims database from Thomas Reuters covering 18 million lives yearly and all USA census regions from 2002 to 2008, 4,406 HCC patients were identified	HCC	Radiological imaging	The incidence of HCC in the database was 0.4 per 1,000 persons. NAFLD/NASH (59%) and T2DM (36%), along with hepatitis C virus infection (22%), were the main etiologic risk factors associated with HCC	USA
Ertle <i>et al.</i> ^[122]	Cross-sectional study: 162 adults with HCC enrolled between February 2007 and March 2008	HCC	Radiological imaging	Patients with NAFLD/NASH-associated HCC exhibited a higher prevalence of T2DM, hypertension, dyslipidemia, coronary artery disease, when compared to non-NAFLD/NASH-HCC	Germany
Targher <i>et al.</i> ^[123]	Cross-sectional study: 2,893 T2DM outpatients; 1,974 had NAFLD	Coronary heart disease, myocardial infarction, angina, cerebrovascular disease and peripheral vascular disease	US	NAFLD was associated with a greater risk of prevalent CVD, independent of classical risk factors, glycemic control, medications and metabolic syndrome features	Italy
Cassidy <i>et al.</i> ^[124]	Case-control study: 19 adults with T2DM, 19 adults with NAFLD and 19 healthy controls	Cardiac structure, function and metabolism	Magnetic resonance imaging	Changes in cardiac structure were evident in adults with T2DM and NAFLD	United Kingdom
Mantovani <i>et al.</i> ^[45]	Cross-sectional study: 222 T2DM outpatients with no previous history of CVD	Left ventricular dysfunction	US	NAFLD was associated with an increased risk of mild and/or moderate left ventricular diastolic dysfunction	Italy
Mantovani <i>et al.</i> ^[125]	Cross-sectional study: 247 consecutive T2DM outpatients with no previous history of heart failure, valvular heart diseases and hepatic diseases	Cardiac calcification on echocardiography	US	NAFLD was independently associated with cardiac calcification both aortic and mitral valves	Italy

Targher <i>et al.</i> ^[126]	Cross-sectional study: 400 outpatients with T2DM	QTc interval on electrocardiograms	US	NAFLD associated with increased QTc interval in patients after adjusting for multiple established risk factors and potential confounders	Italy
Mantovani <i>et al.</i> ^[127]	Cross-sectional study: 751 hospitalized patients with T2DM	Cardiac conduction defects	US	Patients with NAFLD had a remarkably higher prevalence of any persistent heart block than those without NAFLD (31.3 vs. 16.7%, $P < 0.001$)	Italy
Mantovani <i>et al.</i> ^[56]	Cross-sectional study: 330 outpatients with T2DM who had undergone 24-h Holter monitoring for clinical reasons	Ventricular arrhythmias were defined as the presence of non-sustained ventricular tachycardia, >30 premature ventricular complexes per hour, or both	US	NAFLD was independently associated with an increased risk of prevalent ventricular arrhythmias	Italy

HCC: hepatocellular carcinoma; NAFLD: nonalcoholic fatty liver disease; T2DM: type 2 diabetes mellitus; US: ultrasound

independent risk factor for adverse outcomes in NAFLD patients with cirrhosis^[119,120]. Specifically, T2DM is associated with important complications of cirrhosis, such as renal dysfunction, ascites, bacterial infections and hepatic encephalopathy^[119,120]. Lastly, the management of patients with concurrent diabetes mellitus and liver disease has been also addressed^[119,120]. Accumulating findings suggest a beneficial effect of metformin in patients with chronic liver diseases^[119,120]. Insulin is often required in patients with advanced cirrhosis. However, the favorable impact of controlling diabetes mellitus in NAFLD patients with cirrhosis has not been clearly demonstrated yet^[119,120]. Importantly, given that NAFLD has become one of the most important indications for liver transplantation, the management of multiple metabolic co-morbidities, including T2DM and obesity, are strongly recommended in the pre- and peri-transplant period^[128].

An increased prevalence and incidence of hepatocellular carcinoma (HCC) has been observed in the last two decades worldwide. Although most cases of HCC are due to chronic infection with viral hepatitis, recent prospective studies have clearly documented that there is a close association between T2DM, NAFLD/NASH and risk of HCC^[1]. For instance, in a USA population-based longitudinal study, enrolling approximately 4,400 cases of HCC with a median follow-up of 6 years, Sanyal *et al.*^[121] documented that the most common risk factor for HCC was NAFLD (59%), followed by T2DM (36%) and HCV chronic infection (22%). Almost identical results were observed in a small cross-sectional study of 162 adults with HCC^[122]. In that study, Ertle *et al.*^[122] found that NAFLD was the most frequent etiology for HCC. Importantly, studies have also suggested that the prevalence of HCC is higher in T2DM patients with NAFLD and that the coexistence of NAFLD and T2DM markedly increases the risk of developing HCC^[129-131].

The presence of T2DM and NAFLD seems to be also associated with intrahepatic cholangiocarcinoma (ICC). In a recent meta-analysis of 6 cohort and nested case-control studies, Petrick *et al.*^[132] reported that diabetes mellitus was associated with a 53% increased risk of ICC (RR 1.53, 95% confidence interval 1.31-1.78; $I^2 = 67\%$). In another study with a total of 6,093 cholangiocarcinoma cases (ICC: $n = 4,695$; extrahepatic cholangiocarcinoma: $n = 1,396$) and 60,906 age- and sex-matched controls, the patients with ICC and extrahepatic cholangiocarcinoma were more likely to have diabetes mellitus (adjusted odds ratio 1.22, 95% confidence interval 1.07-1.39 and 1.48, 95% confidence interval 1.18-1.85, respectively) than controls^[133].

NAFLD and risk of macro- and microvascular complications in patients with diabetes mellitus

In the last decade, several observational studies documented that in patients with and without diabetes mellitus, NAFLD (as detected by imaging or liver biopsy) is associated with: (1) an increased risk of fatal

and non-fatal cardiovascular events; (2) alterations in cardiac structure and function; and (3) an increased prevalence of microvascular complications [such as chronic kidney disease (CKD) and distal symmetric polyneuropathy]^[134]. Importantly, these associations were significant even after adjustment for many established cardiovascular risk factors and diabetes-related confounders^[134].

Association between NAFLD and macrovascular complications

It is now established that the principal cause of mortality in patients with NAFLD is cardiovascular disease (CVD), followed by extrahepatic cancers and liver-related complications^[134]. In this regard, in a recent meta-analysis of 45 studies for a total of nearly 8 million individuals who were followed from 4 to 13 years, Younossi *et al.*^[135] documented that the pooled CVD-specific mortality rate among NAFLD patients (with or without diabetes mellitus) was approximately 4.8 per 1,000 person-years. Working with data from the National Vital Statistics System multiple-cause mortality data (2007-2016), Paik *et al.*^[136] further showed that CVD is one of the main causes of death among USA patients with NAFLD. In a 2016 meta-analysis of 16 observational studies, Targher *et al.*^[137] showed that patients with NAFLD had a higher risk of fatal and/or non-fatal CVD events when compared to patients with no NAFLD (random effects-odds ratio 1.64, 95% confidence interval 1.26-2.13) over a median period of nearly 7 years. In a 2020 nested cohort study of nearly 4,000 USA patients, who underwent coronary computed tomography angiography as part of the PROMISE (PROspective Multicenter Imaging Study for Evaluation of Chest Pain) study, Meyersohn *et al.*^[138] showed that hepatic steatosis (on computed tomography) was associated with major adverse cardiovascular events, even after controlling for established cardiovascular risk factors or extent of coronary artery disease (hazard ratio 1.69, 95% confidence interval 1.16-2.48).

These findings can be broadly explained by the fact that NAFLD adversely affects cardiac structure and function, leading to an increased risk of cardiomyopathy (e.g., left ventricular diastolic dysfunction and hypertrophy), cardiac valvular calcification [e.g., aortic valve sclerosis (AVS) and mitral annulus calcification (MAC)], and cardiac arrhythmias (mainly atrial fibrillation)^[134].

Restricting the discussion to the observational studies conducted in patients with T2DM [Table 2], the Valpolicella Diabetes Heart Study in 2007 documented that T2DM patients with NAFLD (on ultrasound) had an increased prevalence of coronary, cerebrovascular and peripheral vascular diseases as compared with those with no NAFLD^[123]. Interestingly, in a cross-sectional study enrolling 222 T2DM outpatients, Mantovani *et al.*^[45] documented that NAFLD (on ultrasound) was independently associated with increased risk of mild and/or moderate left ventricular diastolic dysfunction (evaluated by echocardiography). In another cross-sectional study of nearly 120 elderly T2DM patients with hypertension, Mantovani *et al.*^[139] reported that NAFLD (on ultrasound) was associated with left ventricular hypertrophy (as detected by echocardiography). In a cross-sectional study enrolling 19 adults with T2DM, 19 adults with NAFLD (on proton magnetic resonance spectroscopy [¹H-MRS]) and 19 healthy controls, Cassidy *et al.*^[124] showed that alterations in cardiac structure (evaluated by cardiac magnetic resonance) were mainly evident in T2DM patients with NAFLD. Some studies using biopsy or Fibroscan® also observed a graded relationship between functional and structural myocardial abnormalities and the severity of NAFLD in patients with and without T2DM^[134].

Regarding the heart valve calcifications, studies have demonstrated an association between NAFLD and risk of AVS and MAC in patients with and without T2DM^[125,134]. For example, in an observational study enrolling 247 consecutive T2DM outpatients, Mantovani *et al.*^[125] reported that NAFLD (on ultrasound) was independently associated with cardiac calcifications in both the aortic and mitral valves. These findings are of clinical interest, as it is established that AVS and MAC are associated with all-cause and cardiovascular mortality in T2DM patients^[140].

With regard to cardiac arrhythmias, many observational studies and some meta-analyses have reported that NAFLD (as detected by ultrasound or computed tomography) is associated with prevalent and incident atrial fibrillation in patients with and without T2DM^[134]. Atrial fibrillation is a frequent arrhythmia seen in clinical practice and it is closely related to cardiovascular morbidity and mortality^[134]. In a meta-analysis of 5 observational studies enrolling approximately 240,000 middle-aged and elderly individuals, Mantovani *et al.*^[141] documented that NAFLD was associated with higher prevalence and incidence of AF. Other studies have documented that in T2DM patients, NAFLD (on ultrasound) was associated with an increased risk of prolonged QTc, ventricular arrhythmias or other conduction defects^[56,126,127,134,142].

Collectively, these data strongly support the assertion that, as also recommended by the European and American guidelines on the management of NAFLD^[143,144], a multidisciplinary approach to NAFLD patients is necessary, based on careful assessment of cardiometabolic risk factors.

Increased risk of CKD in NAFLD patients with T2DM

Recently, several studies and some meta-analyses have demonstrated that in T2DM patients, NAFLD, as detected by liver ultrasound or biopsy, is linked to an increased risk of prevalent and incident CKD, above and beyond established cardiometabolic risk factors^[145]. For instance, regarding the association between NAFLD and prevalence of CKD, recently, in an observational study of 169 T2DM patients with NAFLD and 169 T2DM patients without NAFLD, Jia *et al.*^[146] reported that NAFLD (on ultrasound) was independently associated with an increased risk of prevalent CKD (defined as eGFR < 60 mL/min/1.73 m² and/or albuminuria). Accumulating data now suggest that the association between NAFLD and risk of prevalent CKD may be even bidirectional^[145]. For example, in an observational study enrolling approximately 2,000 Taiwanese patients with T2DM, Lee *et al.*^[73] confirmed an independent association between imaging-diagnosed NAFLD and CKD (adjusted odds ratio 1.59, 95% confidence interval 1.12-2.25). Interestingly, when the authors performed a structural equation model analysis to test the effects of NAFLD on CKD and the potential role of CKD on NAFLD, they found the existence of a bidirectional relationship between NAFLD and CKD^[73].

Accumulating data also indicate that the risk of CKD may be higher in patients with advanced forms of NAFLD (mostly liver fibrosis) as compared to patients with simple steatosis^[145]. In an observational cross-sectional study enrolling nearly 400 Italian patients with T2DM, Lombardi *et al.*^[71] reported that CKD (defined by the presence of eGFR_{CKD-EPI} < 60 mL/min/1.73 m² and/or abnormal albuminuria) was present in 36% of NAFLD patients with liver fibrosis (as detected by Fibroscan®) as compared to 21% of NAFLD patients without liver fibrosis ($P < 0.001$). Recently, Mantovani *et al.*^[65] documented that, after adjusting for established risk factors and potential confounders, LSM was significantly associated with an approximately 3-fold higher risk of prevalent CKD (adjusted odds ratio 3.28, 95% confidence interval 1.22-8.90) in a sample of nearly 140 T2DM outpatients. Interestingly, Yeung *et al.*^[62] in 2018 also reported that advanced fibrosis, as detected by Fibroscan®, but not liver steatosis on US, was independently associated with a higher risk of prevalent albuminuria in nearly 1,800 patients with T2DM (belonging to the Hong Kong Diabetes Registry). Additionally, in a cross-sectional study of 100 Indian patients with NAFLD (on US), Nampoothiri *et al.*^[147] reported that patients with impaired renal function (defined as eGFR_{Cockcroft-Gault} < 80 mL/min/1.73 m² and/or presence of proteinuria) had higher proportion of significant liver fibrosis and advanced fibrosis on Fibroscan®, when compared to those with normal renal function. Importantly, in the multivariate analyses, the authors found that T2DM and advanced fibrosis were two independent predictors of impaired renal function in patients with NAFLD. In particular, advanced fibrosis had the best diagnostic accuracy and specificity in predicting impaired renal function in these patients (diagnostic accuracy: 81%; sensitivity: 58%; specificity: 90%)^[147].

Regarding the association between NAFLD and incidence of CKD, to date, there are still few data in patients with T2DM^[148]. In 2008, the Valpolicella Heart Diabetes Study demonstrated that patients with

T2DM and NAFLD had an increased risk of incident CKD (defined as CKD stage ≥ 3 and/or overt proteinuria) as compared to those without NAFLD over a mean follow-up period of 6.5 years^[149]. Recently, in a meta-analysis involving 9 cohort studies with a total of nearly 100,000 patients with and without T2DM, Mantovani *et al.*^[150] confirmed that NAFLD was independently associated with an increased risk of incident CKD. Interestingly, when the authors performed a subgroup analysis, they found that the risk of incident CKD in patients with NAFLD was greater in patients with T2DM (random effects hazard ratio 1.56, 95% confidence interval 1.07-2.05) than in patients with no T2DM (random effects hazard ratio 1.35, 95% confidence interval 1.16-1.54)^[150].

Accumulating data also suggest that NAFLD patients with CKD tend to have a worse prognosis and an increased overall mortality as well, when compared to those with NAFLD but without CKD^[148]. For instance, in a cohort study enrolling 11,695 patients, Paik *et al.*^[151] documented that the presence of both CKD and NAFLD was associated with an increased risk for overall mortality (hazard ratio 2.34, 95% confidence interval 1.91-2.87). Interestingly, in that study, the severity of CKD was even associated with higher risk of mortality in patients with NAFLD^[151]. Specifically, the presence of NAFLD with advanced CKD stages (from stage 3B to stage 5) was associated with a nearly 5-fold (hazard ratio 4.80, 95% confidence interval 2.40-9.71) increased risk of death when compared to absence of CKD, whereas the presence of NAFLD with intermediate CKD stages (from stage 2 to stage 3A) was associated with a 2.3-fold (95% confidence interval 1.70-3.15) increased risk of death^[151]. Although further studies are needed, it is possible that the difference in overall mortality observed by Paik *et al.*^[151] might be related to the presence of specific metabolic features, including T2DM. Önnérhag *et al.*^[152] corroborated this hypothesis in an observational study involving 120 patients with biopsy-diagnosed NAFLD.

Recently, some observational studies that enrolled patients with and without T2DM have documented that *PNPLA3* rs738409 (I148M protein variant), which is the most important variant associated with NAFLD and its severe forms, is independently associated with an increased risk of CKD^[145,148]. For instance, in a recent study of 157 Italian patients with T2DM, who underwent liver ultrasound and kidney function assessment, Mantovani *et al.*^[153] reported that the association of I148M homozygosity with higher risk of CKD was independent of liver disease severity and other confounders. Interestingly, in that study, the authors also found that *PNPLA3* mRNA expression was greatest in liver and renal cortex, especially in podocytes, thereby suggesting that *PNPLA3* I148M variant might exert adverse effects on the kidney^[153].

Association between NAFLD and distal symmetric polyneuropathy in patients with diabetes mellitus

Several observational studies, although not all^[154,155], have documented a significant association between NAFLD and prevalent distal symmetric polyneuropathy in patients with T2DM^[22,39,71]. This association persisted even after adjustment for many cardiometabolic risk factors and other potential confounders. Interestingly, in a recent cross-sectional study involving approximately 400 outpatients with T2DM (mean age 68 years, 52% male) attending 5 Italian diabetes centers, who underwent liver ultrasonography, FibroScan® and evaluation of microvascular diabetic complications, Lombardi *et al.*^[71] documented that significant fibrosis (i.e., LSM $\geq 7.0/6.2$ kPa with M/XL probes) was independently associated with increased prevalence of microvascular diabetic complications, including distal symmetric polyneuropathy (3% in patients with LSM $< 7.0/6.2$ kPa vs. 14% in patients with LSM $\geq 7.0/6.2$ kPa). Contrariwise, in a retrospective study of 927 Asian patients with T2DM, Kim *et al.*^[155] did not observe a significant difference in the prevalence of diabetic peripheral neuropathy among patients with and without NAFLD.

Collectively, these data suggest that diabetic patients with NAFLD should be evaluated for the presence of distal symmetric polyneuropathy, along with other hepatic and extrahepatic complications. In addition, the issue of whether the increased risk of microvascular complications in diabetic patients with NAFLD is

restricted to patients with more severe NAFLD or applies to all patients with NAFLD is relevant given the disease burden of NAFLD. However, additional studies are needed to establish if NAFLD can increase the risk of developing distal symmetric polyneuropathy and to elucidate if improvement in NAFLD is able to prevent the development and progression of distal symmetric polyneuropathy in patients with diabetes.

Putative mechanisms linking NAFLD to vascular complications in patients with diabetes mellitus

The detailed description of the putative mechanisms linking NAFLD to vascular complications in patients with diabetes mellitus is beyond the purpose of this narrative review. Therefore, we refer the reader to other reviews for this topic^[1,2,134,142,156].

When common diseases coexist and share common risk factors, it might be difficult to separate pivotal relationships and understand the role of potential confounders. Indeed, T2DM or MetS are examples of confounding diseases linking NAFLD to cardiovascular complications.

However, there are many potential underlying mechanisms that can link NAFLD to the development and progression of vascular complications^[134]. As several studies have clearly demonstrated in the last decade, NAFLD (mainly in its more severe histological forms) can worsen hepatic and systemic insulin resistance^[156]. Insulin resistance is linked to an excessive fat accumulation in ectopic tissues, including the liver, as well as with increased circulating free fatty acids^[156]. All these factors can strongly promote endoplasmic reticulum stress and inflammation^[156]. In addition, they aggravate and maintain the insulin resistant state, thereby leading to a vicious cycle^[156]. In fact, inhibition of insulin signaling pathways associated with NAFLD can occur by various mechanisms, including inflammatory, many kinase proteins and several lipid-derived by-products^[156]. NAFLD and its more severe histological forms can also contribute to the release into the bloodstream of several proinflammatory, profibrogenic and vasoactive mediators (such as C-reactive protein, tumor necrosis factor alpha, interleukin-6, transforming growth factor-beta, factor VIII, plasminogen activator inhibitor-1 and endothelin-1). All these mediators can promote important cardiac and arrhythmic complications^[134]. Hence, it is possible that the reduction of chronic inflammation in NAFLD patients might be a potential intervention to reduce the risk of cardiac disease and arrhythmias^[134]. Accumulating experimental and clinical data also indicate that NAFLD may contribute to the activation of multiple pathways implicated in the pathophysiology of CKD^[1,2,134,142]. Impaired activation of the renin-angiotensin system (RAS) may indeed contribute to the renovascular injury by inflammation and coagulation pathways^[1,2,134,142]. Atherogenic dyslipidemia, insulin resistance, oxidative stress and pro-inflammatory factors can contribute to renal damage^[1,2,134,142]. However, in spite of the large body of evidence linking NAFLD to cardiac, arrhythmic and renal complications, it has not been conclusively established if a cause-effect relationship exists^[134].

Not only are many traditional risk factors combined between NAFLD, micro and macrovascular disease, CKD and T2DM/MetS, but novel risk factors are also emerging in each of these conditions. These novel risk factors include perturbation of the intestinal microbiota (dysbiosis) with associated inflammation, intestinal dysfunction and platelet activation.

Recently, the role of dysbiosis in NAFLD and in the development of its complications has gained scientific interest^[134]. Dysbiosis is associated with increased production of lipopolysaccharide from gram-negative bacteria, which can damage the intestinal barrier and, consequently, can increase permeability and contribute to the release of endotoxins into the systemic circulation, thereby determining a chronic inflammation and oxidative stress, mainly due to the release of pro-inflammatory cytokines^[134,157].

Altered production of short-chain fatty acids, such as acetate, propionate and butyrate, can influence hepatic gluconeogenesis and liponeogenesis^[158]. Short-term probiotic treatments should have a beneficial effect on insulin resistance by increasing butyrate production^[158].

Another consequence of dysbiosis is an increase in the uremic toxins that are associated with atherosclerosis and hypertension. There is a demonstrated relationship between trimethylamine oxide (TMAO) and atherosclerosis^[159]. Circulating levels of TMAO, an early biomarker of adipose dysfunction, are high in obese NAFLD patients^[159]. TMAO is produced from the oxidation of trimethylamine in the liver, which is derived from bacteria-dependent metabolism of dietary choline^[159]. TMAO leads to atherosclerosis acting on reverse cholesterol transport, inducing platelet aggregation, the formation of foam cells and the increased expression of scavenger receptors^[159].

Cardiovascular tissue cells, such as endothelial cells, vascular smooth cells and cardiac cells, express bile acid receptors^[160]. Gut microbiota influence the production of secondary bile acids, such as deoxycholic acid, ursodeoxycholic acid and lithocholic acid^[160]. The alteration of bile acid metabolism seems to be associated with an increased risk of CVD, because of increased LDL cholesterol levels, vasomotor tone and blood pressure^[160].

Several experimental studies also suggest that mitochondrial dysfunction may be closely associated with insulin resistance and atherosclerosis^[161], thereby indicating a potential mechanistic link between mitochondrial dysfunction, T2DM, NAFLD and CVD^[134].

Recently, Malehmir *et al.*^[162] showed that platelet number, platelet activation and platelet aggregation are increased in NASH, but not in simple steatosis, pointing to novel mechanisms that should be studied.

We suggest that future prospective and interventional studies be carried out in well-characterised cohorts of patients that can clarify mechanisms linking NAFLD to vascular complications.

CONCLUSION

The concept that NAFLD is a benign condition has changed over the last decades. At present, NAFLD is the most common chronic liver disease observed in clinical practice, especially in patients with T2DM and those with obesity, thereby becoming a relevant health care problem worldwide^[1,86,135]. In fact, NAFLD is a leading cause of liver-related and cardiovascular mortality and morbidity^[1]. Convincing evidence clearly shows that NAFLD is strongly linked to clinical and subclinical alterations in cardiac structure and function, independent of the coexistence of established cardiovascular risk factors and metabolic syndrome^[134]. These findings may partly explain the increased risk of cardiovascular death found in T2DM patients with NAFLD. Given the available data and as suggested by European and American clinical practice guidelines^[143,144], a careful assessment of cardiometabolic risk factors and regular monitoring of liver and cardiovascular complications is mandatory in patients with NAFLD, especially if they are obese or have T2DM. Some authors suggest repeating the assessments every 1 or 2 years, based on the CVD risk factors^[4]. The clinical and laboratory data that should be obtained, along with sex and age, are as follows: body weight, height, body mass index, waist circumference, cigarette smoking, alcohol consumption, blood pressure, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, fasting plasma glucose, hemoglobin A1c (HbA1c), estimated glomerular filtration rate (or serum creatinine), albuminuria, 75-g oral glucose tolerance test (in patients with impaired fasting glycaemia and/or obesity), CVD risk estimation (by using risk calculators), and carotid artery ultrasonography^[4]. In addition, seeing that the prevalence of NAFLD and significant or advanced liver fibrosis is relatively high in T2DM patients (most of whom have normal serum levels of liver enzymes)^[1], FibroScan® may be useful not only for assessing the severity of liver fibrosis, which is the strongest predictor of long-term adverse clinical outcomes in

NAFLD, but also for identifying those patients at higher risk of having CKD or other chronic vascular complications^[1,65]. Finally, despite the large body of evidence on NAFLD in T2DM patients, there are still important open issues that need to be timely resolved:

- (1) Is MAFLD definition more useful in clinical practice when compared to NAFLD definition?
- (2) Which are the appropriate screening/surveillance measures for NAFLD in individuals with T2DM?
- (3) Which is the appropriate non-invasive diagnostic strategy for NASH and advanced fibrosis in T2DM patients with NAFLD?
- (4) Is NAFLD a risk factor or only an independent predictor of non-hepatic complications in patients with T2DM, including cardiovascular disease?
- (5) What is the role of genetic factors regarding the development of advanced forms of NAFLD in patients with T2DM?

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation, performed data acquisition, as well as provided administrative, technical and material support: Mantovani A, Beatrice G, Sputia R, Dalbeni A

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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REFERENCES

1. Mantovani A, Scorletti E, Mosca A, Alisi A, Byrne CD, Targher G. Complications, morbidity and mortality of nonalcoholic fatty liver disease. *Metabolism* 2020;111S:154170.
2. Targher G, Lonardo A, Byrne CD. Nonalcoholic fatty liver disease and chronic vascular complications of diabetes mellitus. *Nat Rev Endocrinol* 2018;14:99-114.
3. Lonardo A, Mantovani A, Lugari S, Targher G. Epidemiology and pathophysiology of the association between NAFLD and metabolically healthy or metabolically unhealthy obesity. *Ann Hepatol* 2020;19:359-66.
4. Lonardo A, Nascimbeni F, Mantovani A, Targher G. Hypertension, diabetes, atherosclerosis and NASH: Cause or consequence? *J Hepatol* 2018;68:335-52.
5. Ortiz-Lopez C, Lomonaco R, Orsak B, et al. Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). *Diabetes Care* 2012;35:873-8.
6. Williams KH, Shackel NA, Gorrell MD, McLennan SV, Twigg SM. Diabetes and nonalcoholic Fatty liver disease: a pathogenic duo. *Endocr Rev* 2013;34:84-129.
7. Targher G, Marchesini G, Byrne CD. Risk of type 2 diabetes in patients with non-alcoholic fatty liver disease: Causal association or epiphenomenon? *Diabetes Metab* 2016;42:142-56.

8. Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut* 2017;66:1138-53.
9. Eslam M, Sanyal AJ, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020;158:1999-2014.e1.
10. Tilg H, Effenberger M. From NAFLD to MAFLD: when pathophysiology succeeds. *Nat Rev Gastroenterol Hepatol* 2020;17:387-8.
11. Fouad Y, Waked I, Bollipo S, Gomaa A, Ajlouni Y, Attia D. What's in a name? Renaming 'NAFLD' to 'MAFLD'. *Liver Int* 2020;40:1254-61.
12. Portillo-Sanchez P, Bril F, Maximos M, et al. High Prevalence of Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes Mellitus and Normal Plasma Aminotransferase Levels. *J Clin Endocrinol Metab* 2015;100:2231-8.
13. Kwok R, Choi KC, Wong GL, et al. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. *Gut* 2016;65:1359-68.
14. Arab JP, Barrera F, Gallego C, et al. High prevalence of undiagnosed liver cirrhosis and advanced fibrosis in type 2 diabetic patients. *Ann Hepatol* 2016;15:721-8.
15. Jung CH, Lee B, Choi DH, et al. Association of grade of non-alcoholic fatty liver disease and glycated albumin to glycated hemoglobin ratio in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2017;125:53-61.
16. Masarone M, Rosato V, Aglitti A, et al. Liver biopsy in type 2 diabetes mellitus: Steatohepatitis represents the sole feature of liver damage. *PLoS One* 2017;12:e0178473.
17. Petit JM, Pedro L, Guiu B, et al. Type 1 diabetes is not associated with an increased prevalence of hepatic steatosis. *Diabet Med* 2015;32:1648-51.
18. Wilman HR, Kelly M, Garratt S, et al. Characterisation of liver fat in the UK Biobank cohort. *PLoS One* 2017;12:e0172921.
19. Doycheva I, Cui J, Nguyen P, et al. Non-invasive screening of diabetics in primary care for NAFLD and advanced fibrosis by MRI and MRE. *Aliment Pharmacol Ther* 2016;43:83-95.
20. Ding X, Xu Y, Wang Y, et al. Nonalcoholic fatty liver disease and associated metabolic risks of hypertension in type 2 diabetes: a cross-sectional community-based study. *Int J Endocrinol* 2017;2017:5262560.
21. Al Rifai M, Silverman MG, Nasir K, et al. The association of nonalcoholic fatty liver disease, obesity, and metabolic syndrome, with systemic inflammation and subclinical atherosclerosis: the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis* 2015;239:629-33.
22. Williams KH, Burns K, Constantino M, et al. An association of large-fibre peripheral nerve dysfunction with non-invasive measures of liver fibrosis secondary to non-alcoholic fatty liver disease in diabetes. *J Diabetes Complications* 2015;29:1240-7.
23. Cusi K, Sanyal AJ, Zhang S, et al. Non-alcoholic fatty liver disease (NAFLD) prevalence and its metabolic associations in patients with type 1 diabetes and type 2 diabetes. *Diabetes Obes Metab* 2017;19:1630-4.
24. Guo K, Zhang L, Lu J, et al. Non-alcoholic fatty liver disease is associated with late but not early atherosclerotic lesions in Chinese inpatients with type 2 diabetes. *J Diabetes Complications* 2017;31:80-5.
25. Herath HMM, Kodikara I, Weeraratna TP, Liyanage G. Prevalence and associations of non-alcoholic fatty liver disease (NAFLD) in Sri Lankan patients with type 2 diabetes: A single center study. *Diabetes Metab Syndr* 2019;13:246-50.
26. Sberna AL, Bouillet B, Rouland A, et al. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO) clinical practice recommendations for the management of non-alcoholic fatty liver disease: evaluation of their application in people with Type 2 diabetes. *Diabet Med* 2018;35:368-75.
27. Krishan S. Correlation between non-alcoholic fatty liver disease (NAFLD) and dyslipidemia in type 2 diabetes. *Diabetes Metab Syndr* 2016;10:S77-81.
28. Su X, Xu J, Zheng C. The relationship between non-alcoholic fatty liver and skeletal muscle mass to visceral fat area ratio in women with type 2 diabetes. *BMC Endocr Disord* 2019;19:76.
29. Vanjiappan S, Hamide A, Ananthakrishnan R, Periyasamy SG, Mehalingam V. Nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and its association with cardiovascular disease. *Diabetes Metab Syndr* 2018;12:479-82.
30. He X, Shen Y, Ma X, et al. The association of serum FGF23 and non-alcoholic fatty liver disease is independent of vitamin D in type 2 diabetes patients. *Clin Exp Pharmacol Physiol* 2018;45:668-74.
31. Kabir MA, Uddin MZ, Siddiqui NI, et al. Prevalence of non-alcoholic fatty liver disease and its biochemical predictors in patients with type-2 diabetes mellitus. *Mymensingh Med J* 2018;27:237-44.
32. Lai LL, Wan Yusoff WNI, Vethakkan SR, Nik Mustapha NR, Mahadeva S, Chan WK. Screening for non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus using transient elastography. *J Gastroenterol Hepatol* 2019;34:1396-403.
33. Hashimoto Y, Osaka T, Fukuda T, Tanaka M, Yamazaki M, Fukui M. The relationship between hepatic steatosis and skeletal muscle mass index in men with type 2 diabetes. *Endocr J* 2016;63:877-84.
34. Zou Y, Li X, Wang C, et al. Association between non-alcoholic fatty liver disease and peripheral artery disease in patients with type 2 diabetes. *Intern Med J* 2017;47:1147-53.
35. Afolabi BI, Ibitoye BO, Ikem RT, Omisore AD, Idowu BM, Soyoye DO. The relationship between glycaemic control and non-alcoholic fatty liver disease in nigerian type 2 diabetic patients. *J Natl Med Assoc* 2018;110:256-64.
36. Moh Moh MA, Jung CH, Lee B, et al. Association of glucagon-to-insulin ratio and nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. *Diab Vasc Dis Res* 2019;16:186-95.
37. Cosma A, Cecchet D, Gaiani S, et al. Clinical and biochemical determinants of the extent of liver steatosis in type 2 diabetes mellitus. *Eur J Gastroenterol Hepatol* 2015;27:1386-91.

38. Almobarak AO, Barakat S, Suliman EA, et al. Prevalence of and predictive factors for nonalcoholic fatty liver disease in Sudanese individuals with type 2 diabetes: Is metabolic syndrome the culprit? *Arab J Gastroenterol* 2015;16:54-8.
39. Yan LH, Mu B, Guan Y, et al. Assessment of the relationship between non-alcoholic fatty liver disease and diabetic complications. *J Diabetes Investig* 2016;7:889-94.
40. Alsabaani AA, Mahfouz AA, Awadalla NJ, Musa MJ, Al Humayed SM. Non-Alcoholic fatty liver disease among type-2 diabetes mellitus patients in abha city, south western saudi arabia. *Int J Environ Res Public Health* 2018;15:2521.
41. Gutierrez-Buey G, Núñez-Córdoba JM, Llaverro-Valero M, Gargallo J, Salvador J, Escalada J. Is HOMA-IR a potential screening test for non-alcoholic fatty liver disease in adults with type 2 diabetes? *Eur J Intern Med* 2017;41:74-8.
42. Zawdie B, Tadesse S, Wolide AD, Nigatu TA, Bobasa EM. Non-alcoholic fatty liver disease and associated factors among type 2 diabetic patients in southwest ethiopia. *Ethiop J Health Sci* 2018;28:19-30.
43. Dvorak K, Hainer R, Petrtyl J, et al. The prevalence of nonalcoholic liver steatosis in patients with type 2 diabetes mellitus in the Czech Republic. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2015;159:442-8.
44. Hua X, Li M, Pan F, Xiao Y, Cui W, Hu Y. Non-alcoholic fatty liver disease is an influencing factor for the association of SHBG with metabolic syndrome in diabetes patients. *Sci Rep* 2017;7:14532.
45. Mantovani A, Pernigo M, Bergamini C, et al. Nonalcoholic fatty liver disease is independently associated with early left ventricular diastolic dysfunction in patients with type 2 diabetes. *PLoS One* 2015;10:e0135329.
46. Olusanya TO, Lesi OA, Adeyomoye AA, Fasanmade OA. Non alcoholic fatty liver disease in a Nigerian population with type II diabetes mellitus. *Pan Afr Med J* 2016;24:20.
47. Chang W, Wang Y, Sun L, Yu D, Li Y, Li G. Evaluation of left atrial function in type 2 diabetes mellitus patients with nonalcoholic fatty liver disease by two-dimensional speckle tracking echocardiography. *Echocardiography* 2019;36:1290-7.
48. Zhao CC, Wang AP, Li LX, et al. Urine uric acid excretion is associated with nonalcoholic fatty liver disease in patients with type 2 diabetes. *J Diabetes Complications* 2016;30:1074-80.
49. Zhang J, Zhang B, Cheng Y, Xu J. Low serum CTRP3 levels are associated with nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. *Cytokine* 2018;106:131-5.
50. Johansen ML, Schou M, Rasmussen J, et al. Low N-terminal pro-brain natriuretic peptide levels are associated with non-alcoholic fatty liver disease in patients with type 2 diabetes. *Diabetes Metab* 2019;45:429-35.
51. Fan N, Zhang L, Xia Z, Peng L, Wang Y, Peng Y. Sex-specific association between serum uric acid and nonalcoholic fatty liver disease in type 2 diabetic patients. *J Diabetes Res* 2016;2016:3805372.
52. García Díaz E, Guagnozzi D, Gutiérrez V, et al. Effect of incretin therapies compared to pioglitazone and gliclazide in non-alcoholic fatty liver disease in diabetic patients not controlled on metformin alone: An observational, pilot study. *Endocrinol Nutr* 2016;63:194-201.
53. Demir M, Deyneli O, Yılmaz Y. Screening for hepatic fibrosis and steatosis in Turkish patients with type 2 diabetes mellitus: A transient elastography study. *Turk J Gastroenterol* 2019;30:266-70.
54. Petit JM, Masson D, Guiu B, et al. GCKR polymorphism influences liver fat content in patients with type 2 diabetes. *Acta Diabetol* 2016;53:237-42.
55. Zhao H, Song X, Li Z, Wang X. Risk factors associated with nonalcohol fatty liver disease and fibrosis among patients with type 2 diabetes mellitus. *Medicine (Baltimore)* 2018;97:e12356.
56. Mantovani A, Rigamonti A, Bonapace S, et al. Nonalcoholic fatty liver disease is associated with ventricular arrhythmias in patients with type 2 diabetes referred for clinically indicated 24-hour holter monitoring. *Diabetes Care* 2016;39:1416-23.
57. Barchetta I, Cimini FA, De Gioannis R, et al. Procollagen-III peptide identifies adipose tissue-associated inflammation in type 2 diabetes with or without nonalcoholic liver disease. *Diabetes Metab Res Rev* 2018;34:e2998.
58. Choe EY, Lee YH, Choi YJ, et al. Waist-to-calf circumference ratio is an independent predictor of hepatic steatosis and fibrosis in patients with type 2 diabetes. *J Gastroenterol Hepatol* 2018;33:1082-91.
59. Silaghi CA, Silaghi H, Crăciun AE, et al. Age, abdominal obesity, and glycated hemoglobin are associated with carotid atherosclerosis in type 2 diabetes patients with nonalcoholic fatty liver disease. *Med Ultrason* 2015;17:300-7.
60. Lee H, Kim G, Choi YJ, et al. Association between non-alcoholic steatohepatitis and left ventricular diastolic dysfunction in type 2 diabetes mellitus. *Diabetes Metab J* 2020;44:267-76.
61. Seo DH, Lee YH, Park SW, et al. Sarcopenia is associated with non-alcoholic fatty liver disease in men with type 2 diabetes. *Diabetes Metab* 2020;46:362-9.
62. Yeung MW, Wong GL, Choi KC, et al. Advanced liver fibrosis but not steatosis is independently associated with albuminuria in Chinese patients with type 2 diabetes. *J Hepatol* 2017;147-56.
63. Bellan M, Colletta C, Barbaglia MN, et al. Severity of nonalcoholic fatty liver disease in type 2 diabetes mellitus: relationship between nongenetic factors and PNPLA3/HSD17B13 polymorphisms. *Diabetes Metab J* 2019;43:700-10.
64. Tuong TTK, Tran DK, Phu PQT, Hong TND, Dinh TC, Chu DT. Non-alcoholic fatty liver disease in patients with type 2 diabetes: evaluation of hepatic fibrosis and steatosis using fibroscan. *Diagnostics (Basel)* 2020;10:159.
65. Mantovani A, Turino T, Lando MG, et al. Screening for non-alcoholic fatty liver disease using liver stiffness measurement and its association with chronic kidney disease and cardiovascular complications in patients with type 2 diabetes. *Diabetes Metab* 2020;46:296-303.
66. Choi DH, Jung CH, Mok JO, Kim CH, Kang SK, Kim BY. Nonalcoholic fatty liver disease and abdominal fat accumulation according to vitamin d status in patients with type 2 diabetes. *J Obes Metab Syndr* 2018;27:53-60.
67. Heidari Z, Gharebaghi A. Prevalence of non alcoholic fatty liver disease and its association with diabetic nephropathy in patients with

- type 2 diabetes mellitus. *J Clin Diagn Res* 2017;11:OC04-7.
68. Mandal A, Bhattarai B, Kafle P, et al. Elevated liver enzymes in patients with type 2 diabetes mellitus and non-alcoholic fatty liver disease. *Cureus* 2018;10:e3626.
69. Motta BM, Grander C, Gögele M, et al. Microbiota, type 2 diabetes and non-alcoholic fatty liver disease: protocol of an observational study. *J Transl Med* 2019;17:408.
70. Sporea I, Mare R, Popescu A, et al. Screening for liver fibrosis and steatosis in a large cohort of patients with type 2 diabetes using vibration controlled transient elastography and controlled attenuation parameter in a single-center real-life experience. *J Clin Med* 2020;9:1032.
71. Lombardi R, Airaghi L, Targher G, et al. Liver fibrosis by FibroScan® independently of established cardiovascular risk parameters associates with macrovascular and microvascular complications in patients with type 2 diabetes. *Liver Int* 2020;40:347-54.
72. Hamid S, Subhan Butt A, Sharif F, et al. Nonalcoholic fatty liver diseases among recently diagnosed patients with diabetes mellitus and risk factors. *Euroasian J Hepatogastroenterol* 2019;9:9-13.
73. Lee YJ, Wang CP, Hung WC, et al. Common and unique factors and the bidirectional relationship between chronic kidney disease and nonalcoholic fatty liver in type 2 diabetes patients. *Diabetes Metab Syndr Obes* 2020;13:1203-14.
74. El-Ashmawy HM, Ahmed AM. Serum fetuin-B level is an independent marker for nonalcoholic fatty liver disease in patients with type 2 diabetes. *Eur J Gastroenterol Hepatol* 2019;31:859-64.
75. Mangla N, Ajmera VH, Caussy C, et al. Liver stiffness severity is associated with increased cardiovascular risk in patients with type 2 diabetes. *Clin Gastroenterol Hepatol* 2020;18:744-6.e1.
76. Byrne CD, Patel J, Scorletti E, Targher G. Tests for diagnosing and monitoring non-alcoholic fatty liver disease in adults. *BMJ* 2018;362:k2734.
77. Wang CC, Tseng TC, Hsieh TC, et al. Severity of fatty liver on ultrasound correlates with metabolic and cardiovascular risk. *Kaohsiung J Med Sci* 2012;28:151-60.
78. Chen K, Sng WK, Quah JH, et al. Clinical spectrum of non-alcoholic fatty liver disease in patients with diabetes mellitus. *PLoS One* 2020;15:e0236977.
79. Gerber L, Otgonsuren M, Mishra A, et al. Non-alcoholic fatty liver disease (NAFLD) is associated with low level of physical activity: a population-based study. *Aliment Pharmacol Ther* 2012;36:772-81.
80. Zelber-Sagi S, Salomone F, Mlynarsky L. The mediterranean dietary pattern as the diet of choice for non-alcoholic fatty liver disease: Evidence and plausible mechanisms. *Liver Int* 2017;37:936-49.
81. Eslam M, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: clinical impact. *J Hepatol* 2018;68:268-79.
82. Bazick J, Donithan M, Neuschwander-Tetri BA, et al. Clinical model for NASH and advanced fibrosis in adult patients with diabetes and NAFLD: guidelines for referral in NAFLD. *Diabetes Care* 2015;38:1347-55.
83. McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. *J Hepatol* 2015;62:1148-55.
84. Looma R, Abraham M, Unalp A, et al; Nonalcoholic Steatohepatitis Clinical Research Network. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. *Hepatology* 2012;56:943-51.
85. Puchakayala BK, Verma S, Kanwar P, Hart J, Sanivarapu RR, Mohanty SR. Histopathological differences utilizing the nonalcoholic fatty liver disease activity score criteria in diabetic (type 2 diabetes mellitus) and non-diabetic patients with nonalcoholic fatty liver disease. *World J Hepatol* 2015;7:2610-8.
86. Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. *J Hepatol* 2019;71:793-801.
87. Targher G, Byrne CD. Clinical review: nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. *J Clin Endocrinol Metab* 2013;98:483-95.
88. Okamoto M, Takeda Y, Yoda Y, Kobayashi K, Fujino MA, Yamagata Z. The association of fatty liver and diabetes risk. *J Epidemiol* 2003;13:15-21.
89. Shibata M, Kihara Y, Taguchi M, Tashiro M, Otsuki M. Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men. *Diabetes Care* 2007;30:2940-4.
90. Kim CH, Park JY, Lee KU, Kim JH, Kim HK. Fatty liver is an independent risk factor for the development of Type 2 diabetes in Korean adults. *Diabet Med* 2008;25:476-81.
91. Bae JC, Rhee EJ, Lee WY, et al. Combined effect of nonalcoholic fatty liver disease and impaired fasting glucose on the development of type 2 diabetes: a 4-year retrospective longitudinal study. *Diabetes Care* 2011;34:727-9.
92. Park SK, Seo MH, Shin HC, Ryoo JH. Clinical availability of nonalcoholic fatty liver disease as an early predictor of type 2 diabetes mellitus in Korean men: 5-year prospective cohort study. *Hepatology* 2013;57:1378-83.
93. Sung KC, Jeong WS, Wild SH, Byrne CD. Combined influence of insulin resistance, overweight/obesity, and fatty liver as risk factors for type 2 diabetes. *Diabetes Care* 2012;35:717-22.
94. Kasturiratne A, Weerasinghe S, Dassanayake AS, et al. Influence of non-alcoholic fatty liver disease on the development of diabetes mellitus. *J Gastroenterol Hepatol* 2013;28:142-7.
95. Chang Y, Jung HS, Yun KE, Cho J, Cho YK, Ryu S. Cohort study of non-alcoholic fatty liver disease, NAFLD fibrosis score, and the risk of incident diabetes in a Korean population. *Am J Gastroenterol* 2013;108:1861-8.
96. Choi JH, Rhee EJ, Bae JC, et al. Increased risk of type 2 diabetes in subjects with both elevated liver enzymes and ultrasonographically diagnosed nonalcoholic fatty liver disease: a 4-year longitudinal study. *Arch Med Res* 2013;44:115-20.

97. Yamazaki H, Tsuboya T, Tsuji K, Dohke M, Maguchi H. Independent association between improvement of nonalcoholic fatty liver disease and reduced incidence of type 2 diabetes. *Diabetes Care* 2015;38:1673-9.
98. Ming J, Xu S, Gao B, et al. Non-alcoholic fatty liver disease predicts type 2 diabetes mellitus, but not prediabetes, in Xi'an, China: a five-year cohort study. *Liver Int* 2015;35:2401-7.
99. Li WD, Fu KF, Li GM, et al. Comparison of effects of obesity and non-alcoholic fatty liver disease on incidence of type 2 diabetes mellitus. *World J Gastroenterol* 2015;21:9607-13.
100. Shah RV, Allison MA, Lima JA, et al. Liver fat, statin use, and incident diabetes: The Multi-ethnic study of atherosclerosis. *Atherosclerosis* 2015;242:211-7.
101. Fukuda T, Hamaguchi M, Kojima T, et al. The impact of non-alcoholic fatty liver disease on incident type 2 diabetes mellitus in non-overweight individuals. *Liver Int* 2016;36:275-83.
102. Chen GY, Cao HX, Li F, et al. New risk-scoring system including non-alcoholic fatty liver disease for predicting incident type 2 diabetes in East China: Shanghai Baosteel Cohort. *J Diabetes Investig* 2016;7:206-11.
103. Li Y, Wang J, Tang Y, et al. Bidirectional association between nonalcoholic fatty liver disease and type 2 diabetes in Chinese population: Evidence from the Dongfeng-Tongji cohort study. *PLoS One* 2017;12:e0174291.
104. Ma J, Hwang SJ, Pedley A, et al. Bi-directional analysis between fatty liver and cardiovascular disease risk factors. *J Hepatol* 2017;66:390-7.
105. Chen SC, Tsai SP, Jhao JY, Jiang WK, Tsao CK, Chang LY. Liver fat, hepatic enzymes, alkaline phosphatase and the risk of incident type 2 diabetes: a prospective study of 132,377 adults. *Sci Rep* 2017;7:4649.
106. Liu M, Wang J, Zeng J, Cao X, He Y. Association of NAFLD with diabetes and the impact of BMI changes: a 5-year cohort study based on 18,507 elderly. *J Clin Endocrinol Metab* 2017;102:1309-16.
107. Mantovani A, Byrne CD, Bonora E, Targher G. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. *Diabetes Care* 2018;41:372-82.
108. Mantovani A, Petracca G, Beatrice G, Tilg H, Byrne CD, Targher G. Non-alcoholic fatty liver disease and risk of incident diabetes mellitus: an updated meta-analysis of 501 022 adult individuals. *Gut* 2020;gutjnl-2020-322572.
109. Sung KC, Wild SH, Byrne CD. Resolution of fatty liver and risk of incident diabetes. *J Clin Endocrinol Metab* 2013;98:3637-43.
110. Cho HJ, Hwang S, Park JI, et al. Improvement of nonalcoholic fatty liver disease reduces the risk of type 2 diabetes mellitus. *Gut Liver* 2019;13:440-9.
111. Heo NY. Nonalcoholic fatty liver disease is a stepping stone in the path toward diabetes mellitus. *Gut Liver* 2019;13:383-4.
112. Mauvais-jarvis F, Bairey Merz N, Barnes PJ, et al. Sex and gender: modifiers of health, disease, and medicine. *Lancet* 2020;396:565-82.
113. Lonardo A, Nascimbeni F, Ballestri S, et al. Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps. *Hepatology* 2019;70:1457-69.
114. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. *JAMA* 2015;313:2263-73.
115. de Marco R, Locatelli F, Zoppini G, Verlato G, Bonora E, Muggeo M. Cause-specific mortality in type 2 diabetes. The Verona Diabetes Study. *Diabetes Care* 1999;22:756-61.
116. Zoppini G, Fedeli U, Gennaro N, Saugo M, Targher G, Bonora E. Mortality from chronic liver diseases in diabetes. *Am J Gastroenterol* 2014;109:1020-5.
117. Adams LA, Harmsen S, St Sauver JL, et al. Nonalcoholic fatty liver disease increases risk of death among patients with diabetes: a community-based cohort study. *Am J Gastroenterol* 2010;105:1567-73.
118. Elkkrief L, Rautou PE, Sarin S, Valla D, Paradis V, Moreau R. Diabetes mellitus in patients with cirrhosis: clinical implications and management. *Liver Int* 2016;36:936-48.
119. Bertot LC, Jeffery GP, de Boer B, et al. Diabetes impacts prediction of cirrhosis and prognosis by non-invasive fibrosis models in non-alcoholic fatty liver disease. *Liver Int* 2018;38:1793-802.
120. de Lédinghen V, Vergniol J, Gonzalez C, et al. Screening for liver fibrosis by using FibroScan® and FibroTest in patients with diabetes. *Dig Liver Dis* 2012;44:413-8.
121. Sanyal A, Poklepovic A, Moyneur E, Barghout V. Population-based risk factors and resource utilization for HCC: US perspective. *Curr Med Res Opin* 2010;26:2183-91.
122. Ertle J, Dechêne A, Sowa JP, et al. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *Int J Cancer* 2011;128:2436-43.
123. Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007;30:1212-8.
124. Cassidy S, Hallsworth K, Thoma C, et al. Cardiac structure and function are altered in type 2 diabetes and non-alcoholic fatty liver disease and associate with glycemic control. *Cardiovasc Diabetol* 2015;14:23.
125. Mantovani A, Pernigo M, Bergamini C, et al. Heart valve calcification in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Metabolism* 2015;64:879-87.
126. Targher G, Valbusa F, Bonapace S, et al. Association of nonalcoholic fatty liver disease with QTc interval in patients with type 2 diabetes. *Nutr Metab Cardiovasc Dis* 2014;24:663-9.
127. Mantovani A, Rigolon R, Pichiri I, et al. Nonalcoholic fatty liver disease is associated with an increased risk of heart block in hospitalized patients with type 2 diabetes mellitus. *PLoS One* 2017;12:e0185459.
128. Polyzos SA, Mantzoros CS. Making progress in nonalcoholic fatty liver disease (NAFLD) as we are transitioning from the era of NAFLD to dys-metabolism associated fatty liver disease (DAFLD). *Metabolism* 2020;111S:154318.

129. Mantovani A, Targher G. Type 2 diabetes mellitus and risk of hepatocellular carcinoma: spotlight on nonalcoholic fatty liver disease. *Ann Transl Med* 2017;5:270.
130. Wang P, Kang D, Cao W, Wang Y, Liu Z. Diabetes mellitus and risk of hepatocellular carcinoma: a systematic review and meta-analysis. *Diabetes Metab Res Rev* 2012;28:109-22.
131. Yang WS, Va P, Bray F, et al. The role of pre-existing diabetes mellitus on hepatocellular carcinoma occurrence and prognosis: a meta-analysis of prospective cohort studies. *PLoS One* 2011;6:e27326.
132. Petrick JL, Thistle JE, Zeleniuch-Jacquotte A, et al. Body mass index, diabetes and intrahepatic cholangiocarcinoma risk: the liver cancer pooling project and meta-analysis. *Am J Gastroenterol* 2018;113:1494-505.
133. Huang YJ, Wu AT, Chiou HY, et al. Interactive role of diabetes mellitus and female sex in the risk of cholangiocarcinoma: a population-based nested case-control study. *Oncotarget* 2017;8:6642-51.
134. Anstee QM, Mantovani A, Tilg H, Targher G. Risk of cardiomyopathy and cardiac arrhythmias in patients with nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2018;15:425-39.
135. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73-84.
136. Paik JM, Henry L, De Avila L, Younossi E, Racila A, Younossi ZM. Mortality related to nonalcoholic fatty liver disease is increasing in the United States. *Hepatol Commun* 2019;3:1459-71.
137. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: a meta-analysis. *J Hepatol* 2016;65:589-600.
138. Meyersohn NM, Mayrhofer T, Corey KE, et al. Association of hepatic steatosis with major adverse cardiovascular events, independent of coronary artery disease. *Clin Gastroenterol Hepatol* 2020;S1542-3565(20)30992-7.
139. Mantovani A, Zoppini G, Targher G, Golia G, Bonora E. Non-alcoholic fatty liver disease is independently associated with left ventricular hypertrophy in hypertensive type 2 diabetic individuals. *J Endocrinol Invest* 2012;35:215-8.
140. Rossi A, Targher G, Zoppini G, et al. Aortic and mitral annular calcifications are predictive of all-cause and cardiovascular mortality in patients with type 2 diabetes. *Diabetes Care* 2012;35:1781-6.
141. Mantovani A, Dauriz M, Sandri D, et al. Association between non-alcoholic fatty liver disease and risk of atrial fibrillation in adult individuals: An updated meta-analysis. *Liver Int* 2019;39:758-69.
142. Mantovani A. Nonalcoholic fatty liver disease (NAFLD) and risk of cardiac arrhythmias: a new aspect of the liver-heart axis. *J Clin Transl Hepatol* 2017;5:134-41.
143. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018;67:328-57.
144. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1388-402.
145. Byrne CD, Targher G. NAFLD as a driver of chronic kidney disease. *J Hepatol* 2020;72:785-801.
146. Jia G, Di F, Wang Q, et al. Non-Alcoholic fatty liver disease is a risk factor for the development of diabetic nephropathy in patients with type 2 diabetes mellitus. *PLoS One* 2015;10:e0142808.
147. Nampoothiri RV, Duseja A, Rath M, et al. Renal dysfunction in patients with nonalcoholic fatty liver disease is related to the presence of diabetes mellitus and severity of liver disease. *J Clin Exp Hepatol* 2019;9:22-8.
148. Mantovani A, Zusi C, Dalbeni A, Grani G, Buzzetti E. Risk of kidney dysfunction in NAFLD. *Curr Pharm Des* 2020;26:1045-61.
149. Targher G, Chonchol M, Bertolini L, et al. Increased risk of CKD among type 2 diabetics with nonalcoholic fatty liver disease. *J Am Soc Nephrol* 2008;19:1564-70.
150. Mantovani A, Zaza G, Byrne CD, et al. Nonalcoholic fatty liver disease increases risk of incident chronic kidney disease: A systematic review and meta-analysis. *Metabolism* 2018;79:64-76.
151. Paik J, Golabi P, Younoszai Z, Mishra A, Trimble G, Younossi ZM. Chronic kidney disease is independently associated with increased mortality in patients with nonalcoholic fatty liver disease. *Liver Int* 2019;39:342-52.
152. Önnérhag K, Dreja K, Nilsson PM, Lindgren S. Increased mortality in non-alcoholic fatty liver disease with chronic kidney disease is explained by metabolic comorbidities. *Clin Res Hepatol Gastroenterol* 2019;43:542-50.
153. Mantovani A, Taliento A, Zusi C, et al. PNPLA3 I148M gene variant and chronic kidney disease in type 2 diabetic patients with NAFLD: Clinical and experimental findings. *Liver Int* 2020;40:1130-41.
154. Lv WS, Sun RX, Gao YY, et al. Nonalcoholic fatty liver disease and microvascular complications in type 2 diabetes. *World J Gastroenterol* 2013;19:3134-42.
155. Kim BY, Jung CH, Mok JO, Kang SK, Kim CH. Prevalences of diabetic retinopathy and nephropathy are lower in Korean type 2 diabetic patients with non-alcoholic fatty liver disease. *J Diabetes Investig* 2014;5:170-5.
156. Asrih M, Jornayvaz FR. Metabolic syndrome and nonalcoholic fatty liver disease: is insulin resistance the link? *Mol Cell Endocrinol* 2015;418 Pt 1:55-65.
157. Miele L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009;49:1877-87.
158. Beserra BT, Fernandes R, do Rosario VA, Mocellin MC, Kuntz MG, Trindade EB. A systematic review and meta-analysis of the prebiotics and synbiotics effects on glycaemia, insulin concentrations and lipid parameters in adult patients with overweight or obesity. *Clin Nutr* 2015;34:845-58.

159. Randrianarisoa E, Lehn-Stefan A, Wang X, et al. Relationship of serum trimethylamine N-oxide (TMAO) levels with early atherosclerosis in humans. *Sci Rep* 2016;6:26745.
160. Khurana S, Raufman JP, Pallone TL. Bile acids regulate cardiovascular function. *Clin Transl Sci* 2011;4:210-8.
161. Semenkovich CF. Insulin resistance and atherosclerosis. *J Clin Invest* 2006;116:1813-22.
162. Malehmir M, Pfister D, Gallage S, et al. Platelet GPIIb is a mediator and potential interventional target for NASH and subsequent liver cancer. *Nat Med* 2019;25:641-55.

Review

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Current status of laparoscopic repeat liver resection for hepatocellular carcinoma

Zenichi Morise

Department of Surgery, Fujita Health University School of Medicine Okazaki Medical Center, Okazaki AICHI 444-0827, Japan.

Correspondence to: Prof. Zenichi Morise, Department of Surgery, Fujita Health University School of Medicine Okazaki Medical Center, Gotanda Harisakicho Okazaki, Okazaki AICHI 444-0827, Japan. E-mail: zmorise@fujita-hu.ac.jp

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Abstract

Although liver resection (LR) is often adopted to recurrent hepatocellular carcinomas, risks of complications and conversion reportedly increase in laparoscopic repeat LR (LRLR). The indication is not agreed upon even with the recent advances of laparoscopic LR. We conducted an international propensity score matching study of LRLR and open repeat LR for hepatocellular carcinoma with 1,582 patients from 42 world centers. Propensity-score matched LRLR patients have smaller blood loss and longer operation time than open repeat LR patients. Median overall survival time was 8.94 years in open and 12.55 years in LRLR; although the difference was not significant, the *P*-value was 0.0855 and the better curve of LRLR is clearly separated from that of open. In our institution, we experienced 34 LRLR and 12 cases of three times or more repeat LR until 2019. There are no significant differences in operation time, blood loss, hospital stay, conversion, and morbidity rates among first, second, and third or higher laparoscopic LR, which is different from the open situation. However, postoperative bile leakage and intraoperative bleeding causing conversion did happen in the cases with repeat extended exposure of Glissonian pedicle. LRLR is feasible for selected patients. However, the procedure is under developing stage and further accumulation of experiences and evaluation are needed.

Keywords: Laparoscopic liver resection, hepatocellular carcinoma, re-do surgery

INTRODUCTION

Hepatocellular carcinoma (HCC) can occur in multifocal and metachronous style with the neoplastic background with chronic liver diseases. Repeat liver resection (LR) is often required for the condition^[1,2].



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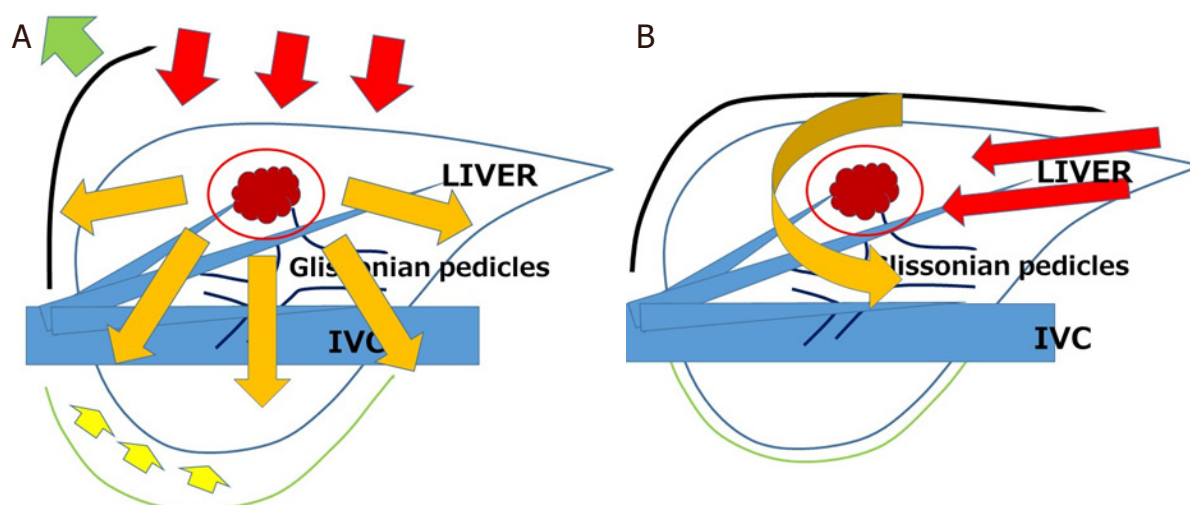


Figure 1. Open (A) and laparoscopic (B) repeat liver resections. The directions of view and manipulation in each approach are indicated with red arrows. A: in the open approach, the subphrenic rib cage is opened with a large subcostal incision and the liver is mobilized (lifted) from the retroperitoneum; B: in laparoscopic approach, the instruments intrude into the cage from the caudal direction, and the surgery is performed with minimal damage on the associated structures. Orange arrows indicate the dissection of adhesion. A: total adhesiolysis is performed in open procedure; B: direct approach to the tumor in laparoscopic procedure can facilitate small surface repeat liver resection with minimal adhesiolysis. IVC: inferior vena cava

After the beginning of laparoscopic LR (LLR) in the early 1990s^[3], the accumulated experiences plus technical/technological advancements have expanded the indication of LLR^[3-6]. However, the bulky and weighty liver protected inside the subphrenic “rib cage”, and the invisible tumors/vessels inside it, should be handled in LR. There are obstacles to overcome in LLR: restricted manipulation, poor tactile sensation, and disorientation occurring under the limited laparoscopic view^[7,8]. Increases in operation time and bowel injury were known in surgery with adhesion^[9,10], and increased morbidity and conversion in laparoscopic re-do surgeries have been reported^[10,11]. Many laparoscopic re-do surgeries^[10-14] have become usual procedures; however, the application of laparoscopic repeat LR (LRLR) is controversial. Adhesion can disturb the liver mobilization and the dissections of vessels and Glissonian pedicles. Scars/adhesions causing the deformity of the liver and its internal structures disrupt the identifications of tumors and vessels. They increase the risks of complications and conversions during LRLR.

On the other hand, LLR is reported to have benefits, such as reductions of postoperative ascites and liver failure^[15], for patients with liver cirrhosis (LC)^[16-18]. During open LR, the subphrenic “rib cage” in which the liver is protected is opened with subcostal incision, and the liver is mobilized for picking up. In LLR, directly intruding instruments to the cage perform the manipulation [Figure 1, “Caudal approach”^[19-21]] with minimum damages to surrounding structures and collateral vessels in LC patients. Similarly, direct access with minimal adhesiolysis to the working space can be enabled, especially in small surface LRLR [Figure 1]^[22-24]. It could be an advantage of LRLR over open repeat LR^[24-27].

This review describes the current status of LRLR for HCC from the result of our multi-institutional study and our own experiences.

THE PROPENSITY SCORE MATCHING STUDY FOR HCC PATIENTS

We conducted the first international propensity score matching study comparing LRLR to open repeat LR for HCC patients^[28] with 1,582 registered cases from 42 centers. LRLR was feasible for selected patients and not inferior to open procedure in both short- and long-term results of the study. The conversion rate of LLR patients in this study on an intention to treat basis was low (3.8%), which might be caused by the

patient selection. The fact that LRLR was currently adopted to patients of poor general and liver condition but with favorable factors related to tumors and surgical procedures was also shown. Notable differences between centers in the number and percentage of LRLRs were revealed. The number of LRLRs in each center ranged from 0 to 67 (median 10) and the rate among all cases was from 0% to 100% (median 57.1%). Furthermore, no correlation was found between the number and percentage ($P = 0.349$). It is thought to be because indications differ depending on each center's experience with different patient populations in terms of the prevalence of HCC, although all are high-volume centers of LR. LRLR for HCC is currently adopted only for patients with favorable characteristics depending on each center's experiences. It means that this procedure is still in its developing stage. All patients after matching, in comparison to those before matching, had better general and liver conditions, as well as tumors and surgery-related conditions. The patients after matching were favorable patients who would have been eligible for either LRLR or open repeat LR depending on the experience of each center.

The survival curve of LRLR patients after matching was clearly separated and better than that of open patients, although without significant difference (median 12.55 years vs. 8.94 years; $P = 0.086$). On the other hand, disease-free survival after matching and overall survival before matching in laparoscopic and open repeat LRs were similar. Although LRLR patients before matching were selected with poorer liver function, matched LRLR patients had better liver function and might have been able to undergo repeated treatments due to less adhesion and liver function deterioration caused by laparoscopic approach. Although resection margin should be one of the important factors for long-term results of LR theoretically, the optimal resection margin for HCC remains controversial^[29]. In our study, 6.25% of the data for resection margin could not be retrieved, and unfortunately this factor was not in the propensity-score matching analysis. However, among the patients with sufficient data, the rates of R1 resection in the original groups of open and laparoscopic repeat LR were 16.1% and 6.3%. The rate in open group is comparable to and that in LRLR is lower than previous reports^[29,30]. It is speculated that the status of resection margin in LRLR is not, at least, inferior to that in open, although this difference in our study may partially be caused by the fact that LRLR is currently adopted to patients of favorable factors related to tumors and surgical procedures.

For the short-term results, the study showed that LRLR was accompanied by less blood loss and a longer operation time. Decreased morbidity is considered as one of the advantages of LLR for HCC patients^[22-25]. However, our matched patients have favorable liver function, and, thus, the impact of LLR might be lower. The differences in hospital stay between centers/areas, possibly due to insurance systems and hospitalization practices, were large and, thus, no difference should have been observed in hospital stay.

Currently, there is no randomized-control trial for open and laparoscopic repeat resection and only four propensity-score matching studies [Table 1]^[31-33]. Besides our study, the studies include patients with other diseases than HCC with few data for long-term results. However, adding of only a few existing meta-analyses^[34,35], they all mentioned that LRLR for selected patients is feasible with, at least, comparable results to open procedure.

OUR OWN EXPERIENCES

Until 2019, we experienced 34 LRLR and 12 cases of three or more (up to five) times repeat LR [Table 2]. There are no cases with combined resection of another organ or LLR for two or more segments in repeat cases. In the comparison excluding first LLR cases with same features as well, there are no significant differences in operation time, blood loss, hospital stay, conversion, and morbidity rates among first, repeat, and three or more times repeat LLRs. This is different from the situation of open repeat LR. Open repeat LR takes generally more operation time and blood loss. This may be caused from that laparoscopic direct access to working space, after minimal adhesiolysis, can be enabled especially in small surface LRLR [Figure 1]^[22-24]. We think it could be an advantage of LRLR over open repeat LR^[24-27]. However, conversion rate and

Table 1. Summary of previous reports of LRLR (propensity score matching analyses)

Author	Year	Journal	Study design	Disease	Number (ORLR: LRLR)	Short-term outcomes	Long-term outcomes
Morise <i>et al.</i> ^[28]	2020	Br J Surg	Multicenter PSM	HCC	934:648	Blood loss: LRLR favor operation time: ORLR favor	OS no significant difference DFS no significant difference
Inoue <i>et al.</i> ^[31]	2019	J Gastrointest Surg	Single center PSM	HCC, CRLM, others	97:45	Blood loss, hospital stay, morbidity: LRLR favor	OS and DFS: not available
van der Poel <i>et al.</i> ^[32]	2019	Br J Surg	Multicenter PSM	CRLM	154:271	Blood loss, operation time, hospital stay: LRLR favor	OS and DFS: not available
Hallet <i>et al.</i> ^[33]	2017	World J Surg	Multicenter PSM	CRLM	349:27	Comparable (operation time, BT, morbidity)	OS: not available DFS: comparable

LRLR: laparoscopic repeat liver resection; ORLR: open repeat liver resection; PSM: propensity score matching analysis; HCC: hepatocellular carcinoma; OS: overall survival; DFS: disease free survival; CRLM: colorectal carcinoma liver metastasis; BT: blood transfusion rate

Table 2. Short-term outcomes of first, repeat, and three or more times repeat laparoscopic liver resection

	1st LLR <i>n</i> = 84	Repeat LLR <i>n</i> = 34	3 or more times repeat <i>n</i> = 12	
Operation time (min), mean ± SD (median)	293 ± 128 (262)	292 ± 136 (256)	299 ± 146 (274)	NS
Blood loss (mL), mean ± SD (median)	244 ± 517 (70)	331 ± 652 (50)	504 ± 864 (50)	NS
Length of hospital stay (day), mean ± SD (median)	20 ± 30 (15)	18 ± 2 (12)	26 ± 33 (14)	NS
Conversion	2/84	1/34	1/12	NS
Morbidity (Grade III or higher)	5/84	2/34	1/12	NS

Until 2019, we experienced 34 LRLR and 12 cases of three or more (up to five) times repeat LR. There are no cases with combined resection of the other organ or LLR for two or more segments in repeat cases. In the comparison excluding those cases as well, there are no significant differences in operation time, blood loss, hospital stay, conversion, and morbidity rates among first, repeat, and three or more times repeat LLRs. LRLR: laparoscopic repeat liver resection; LLR: laparoscopic liver resection; SD: standard deviation; NS: means that there is no statistically significant difference between the values of first, repeat, and three or more times repeat LLR

morbidity are higher in repeat than first surgeries, although it is not significant. Bile leakage and bleeding did happen in the cases with repeat extended exposure of Glissonian pedicle. Limited surgical field for view and manipulation with adhesion/scars around the hilar plate can lead to complicated repeat extended exposure of Glissonian pedicle. It is different from most repeat small surface resections. Evaluations of such LRLRs should be required after more accumulation of experiences.

CONCLUSION

Our propensity score study showed that neither short- nor long-term outcomes of LRLR for HCC in its current developing stage are inferior to those of open repeat LR. Although a large-scale study conducted after further establishment of the procedure and accumulation of experience is needed, LRLR is feasible for selected patients. There could be advantages of LRLR over open (especially for small surface resection), such as decreased blood loss, and less deterioration of liver function. However, repeat extended exposure of Glissonian pedicle could currently be the cause of complications, such as bile leakage and bleeding.

DECLARATIONS

Authors' contributions

The author contributed solely to the article.

Availability of data and materials

Not applicable

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None.

Conflicts of interest

The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Institutional Review Board approval was obtained from Fujita Health University (HM17-164). Data collections and analyses were performed in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

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REFERENCES

1. Morise Z, Kawabe N, Tomishige H, et al. Recent advances in the surgical treatment of hepatocellular carcinoma. *World J Gastroenterol* 2014;20:14381-92.
2. Itamoto T, Nakahara H, Amano H, et al. Repeat hepatectomy for recurrent hepatocellular carcinoma. *Surgery* 2007;141:589-97.
3. Morise Z, Wakabayashi G. First quarter century of laparoscopic liver resection. *World J Gastroenterol* 2017;23:3581-8.
4. Nguyen KT, Gamblin TC, Geller DA. World review of laparoscopic liver resection-2,804 patients. *Ann Surg* 2009;250:831-41.
5. Buell JF, Thomas MT, Rudich S, et al. Experience with more than 500 minimally invasive hepatic procedures. *Ann Surg* 2008;248:475-86.
6. Wakabayashi G, Cherqui D, Geller DA, et al. Recommendations for laparoscopic liver resection: a report from the second international consensus conference held in Morioka. *Ann Surg* 2015;261:619-29.
7. Buell JF, Thomas MJ, Doty TC, et al. An initial experience and evolution of laparoscopic hepatic resectional surgery. *Surgery* 2004;136:804-11.
8. Vibert E, Perniceni T, Levard H, et al. Laparoscopic liver resection. *Br J Surg* 2006;93:67-72.
9. Beck DE, Ferguson MA, Opelka FG, et al. Effect of previous surgery on abdominal opening time. *Dis Colon Rectum* 2000;43:1749-53.
10. Karayiannakis AJ, Polychronidis A, Perente S, Botaitis S, Simopoulos C. Laparoscopic cholecystectomy in patients with previous upper or lower abdominal surgery. *Surg Endosc* 2004;18:97-101.
11. Wiebke EA, Pruitt AL, Howard TJ, et al. Conversion of laparoscopic to open cholecystectomy. An analysis of risk factors. *Surg Endosc* 1996;10:742-5.
12. Wu JM, Lin HF, Chen KH, et al. Impact of previous abdominal surgery on laparoscopic appendectomy for acute appendicitis. *Surg Endosc* 2007;21:570-3.
13. Law WL, Lee YM, Chu KW. Previous abdominal operations do not affect the outcomes of laparoscopic colorectal surgery. *Surg Endosc* 2005;19:326-30.
14. Nunobe S, Hiki N, Fukunaga T, et al. Previous laparotomy is not a contraindication to laparoscopy-assisted gastrectomy for early gastric cancer. *World J Surg* 2008;32:1466-72.
15. Morise Z, Ciria R, Cherqui D, et al. Can we expand the indications for laparoscopic liver resection? A systematic review and meta-analysis of laparoscopic liver resection for patients with hepatocellular carcinoma and chronic liver disease. *J Hepatobiliary Pancreat Sci* 2015;22:342-52.
16. Morise Z, Sugioka A, Kawabe N, et al. Pure laparoscopic hepatectomy for hepatocellular carcinoma patients with severe liver cirrhosis. *Asian J Endosc Surg* 2011;4:143-6.
17. Wakabayashi G. Systematic reviews from the 2nd International Consensus Conference on Laparoscopic Liver Resection. *J Hepatobiliary Pancreat Sci* 2015;22:325-6.
18. Takahara T, Wakabayashi G, Beppu T, et al. Long-term and perioperative outcomes of laparoscopic versus open liver resection for hepatocellular carcinoma with propensity score matching: a multi-institutional Japanese study. *J Hepatobiliary Pancreat Sci* 2015;22:721-7.
19. Tomishige H, Morise Z, Kawabe N, et al. Caudal approach to pure laparoscopic posterior sectionectomy under the laparoscopy-specific view. *World J Gastrointest Surg* 2013;5:173-7.
20. Soubrane O, Schwarz L, Cauchy F, et al. A conceptual technique for laparoscopic right hepatectomy based on facts and oncologic principles: the caudal approach. *Ann Surg* 2015;261:1226-31.
21. Wakabayashi G, Cherqui D, Geller DA, et al. Laparoscopic hepatectomy is theoretically better than open hepatectomy: preparing for the 2nd International Consensus Conference on Laparoscopic Liver Resection. *J Hepatobiliary Pancreat Sci* 2014;21:723-31.
22. Shafae Z, Kazaryan AM, Marvin MR, et al. Is laparoscopic repeat hepatectomy feasible? A tri-institutional analysis. *J Am Coll Surg* 2011;212:171-9.
23. Hu M, Zhao G, Xu D, Liu R. Laparoscopic repeat resection of recurrent hepatocellular carcinoma. *World J Surg* 2011;35:648-55.
24. Isetani M, Morise Z, Kawabe N, et al. Pure laparoscopic hepatectomy as repeat surgery and repeat hepatectomy. *World J Gastroenterol*

- 2015;21:961-8.
25. Ahn KS, Han HS, Yoon YS, Cho JY, Kim JH. Laparoscopic liver resection in patients with a history of upper abdominal surgery. *World J Surg* 2011;35:1333-9.
 26. Ome Y, Hashida K, Yokota M, et al. The feasibility and efficacy of pure laparoscopic repeat hepatectomy. *Surg Endosc* 2018;32:3474-9.
 27. Wakabayashi T, Felli E, Memeo R, et al. Short-term outcomes of laparoscopic repeat liver resection after open liver resection: a systematic review. *Surg Endosc* 2019;33:2083-92.
 28. Morise Z, Aldrighetti L, Belli G, et al; ILLS-Tokyo Collaborator group. Laparoscopic repeat liver resection for hepatocellular carcinoma: a multicenter propensity score-based study. *Br J Surg* 2020;107:889-95.
 29. Kobayashi N, Aramaki O, Midorikawa Y, et al. Impact of marginal resection for hepatocellular carcinoma. *Surg Today* 2020; doi: 10.1007/s00595-020-02029-z.
 30. Martínez-Cecilia D, Wicherts DA, Cipriani F, et al. Impact of resection margins for colorectal liver metastases in laparoscopic and open liver resection: a propensity score analysis. *Surg Endosc* 2020; doi: 10.1007/s00464-020-07452-4.
 31. Inoue Y, Fujii K, Ishii M, et al. Laparoscopic repeat hepatic resection for the management of liver tumors. *J Gastrointest Surg* 2019;23:2314-21.
 32. van der Poel MJ, Barkhatov L, Fuks D, et al. Multicentre propensity score-matched study of laparoscopic versus open repeat liver resection for colorectal liver metastases. *Br J Surg* 2019;106:783-9.
 33. Hallet J, Sa Cunha A, Cherqui D, et al; French Colorectal Liver Metastases Working Group, Association Française de Chirurgie. Laparoscopic compared to open repeat hepatectomy for colorectal liver metastases: a multi-institutional propensity-matched analysis of short- and long-term outcomes. *World J Surg* 2017;41:3189-98.
 34. Peng L, Zhou Z, Xiao W, et al. Systematic review and meta-analysis of laparoscopic versus open repeat hepatectomy for recurrent liver cancer. *Surg Oncol* 2019;28:19-30.
 35. Peng Y, Liu F, Wei Y, Li B. Outcomes of laparoscopic repeat liver resection for recurrent liver cancer: a system review and meta-analysis. *Medicine (Baltimore)* 2019;98:e17533.

Review

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p53 functional loss, stemness and hepatocellular carcinoma

Claude Caron de Fromentel¹, Massimo Levrero^{1,2,3,4}

¹Centre de Recherche en Cancérologie de Lyon, INSERM U1052, CNRS 5286, Université Claude Bernard Lyon 1, Centre Léon Bérard, Lyon 69008, France.

²Service d'Hépatologie et de Gastroentérologie, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Lyon 69004, France.

³Dipartimento di Scienze Cliniche, Internistiche, Anestesiologiche e Cardiovascolari (SCIAC), Sapienza University, Rome 00161, Italy.

⁴Center for Life Nano Science, Istituto Italiano di Tecnologia, Sapienza University, Rome 00161, Italy.

Correspondence to: Dr. Claude Caron de Fromentel, team «Epigenetics, microenvironment and liver cancer», Centre de Recherche en Cancérologie de Lyon, INSERM U1052, 151 cours Albert Thomas, Lyon 69003, France. E-mail: claudede.fromentel@inserm.fr

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Abstract

The tumor suppressor p53 is a key player in the control of genomic integrity and homeostasis in connection with p63 and p73, the two other members of the p53 family. Loss of functional p53 leads to the proliferation and survival of mature cells and progenitor or stem cells that accumulate genetic alterations, thus favoring tumorigenesis. p53 loss of function, observed in a wide variety of human tumor types, is frequently caused by missense mutations more frequently found in the DNA binding domain, but can also be due to the expression of a plethora of viral and cellular negative regulators. Human hepatocellular carcinoma (HCC) represents a specific situation, first because the *TP53* gene mutations pattern exhibits a “hot spot” rarely found in other tumor types that is linked to Aflatoxin B1 exposure and, second, because many HCCs do not exhibit any *TP53* mutation. Here, we provide an overview of the current knowledge about the inhibition of p53 functions by the N-terminal (Δ N) truncated forms of the family, and their role in the emergence and maintenance of pre-malignant cells with stem cell characteristics and in HCC development. We focus in particular on the Nanog-IGF1R- Δ Np73 axis that is associated with stem-like features in HCC cells and that may provide an attractive new therapeutic target and help to develop new biomarkers for HCC risk stratification, as well as preventive strategies.

Keywords: p53 family, p53 functional inactivation, Δ Np73, hepatic progenitor cells, cancer stem cells, Nanog, hepatocellular carcinoma



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INTRODUCTION

Hepatocellular carcinoma (HCC) is a major public health problem, being the fourth most lethal cancer with an increasing incidence around the world^[1]. About 90% of HCC cases can be associated with four well-characterized underlying risk factors including chronic infection with hepatitis B and C viruses (HBV, HCV), ethanol consumption, and non-alcoholic fatty liver disease (NAFLD)^[2]. Although the risk of developing HCC can be reduced in patients by treatment of the underlying cause - e.g., HCV eradication by direct-acting antivirals (DAAs) and HBV suppression by nucleos(t)ide analogs (NUCs) - strategies to prevent cancer development in patients with advanced fibrosis and established cirrhosis are still lacking^[3,4]. Despite the recent improvements, treatment options for HCC remain largely unsatisfactory. Currently, curative treatment options for patients with HCC include surgical resection and loco-regional ablation, frequently associated with tumor recurrence, and orthotopic liver transplantation, a resource-intensive solution^[3,4]. However, due to its silent clinical character and the low sensitivity and specificity of currently available diagnostic biomarkers, HCC is commonly diagnosed at an advanced stage, when curative treatments are not feasible, leaving systemic drugs as the only option^[3,4]. Patients with untreated advanced HCC carry a very poor prognosis with an expected survival of 4-6 months. Treatment modalities available for patients with advanced HCC not eligible for curative treatment include the multikinase inhibitors (MTKi) sorafenib and lenvatinib as first line treatment, with an increase in survival of approximately 3 months, and MTKi regorafenib or cabozantinib as second or third line treatment that enables extension of survival by an additional 3 months^[3,4]. Immune checkpoint inhibitors (ICIs) in monotherapy achieve striking tumor responses in a few patients who have hugely improved outcomes^[3,4], but the low rate of responding patients does not allow significant improvements in the median overall survival. More recently, the combination of drugs targeting the liver microenvironment with *PD1/PD-L1* inhibitors, such as the association of bevacizumab and atezolizumab, or the combination of ICIs have shown promising results^[3-5].

In HCC, as in numerous other tumor types, the chemoresistance is thought to be due to the existence of a sub-population of poorly differentiated cancer cells, widely known as liver cancer stem cells (CSCs) or tumor initiating cells (TICs)^[6,7]. The escape mechanisms are diverse, like enzymatic inactivation or increased drug efflux^[6,7]. The microenvironment also plays an important role. By secreting growth factors and cytokines, it favors the emergence, maintenance and survival of CSCs and their resistance to chemotherapeutic drugs, resulting in the recurrence of more aggressive tumors^[8]. Chronic HBV and HCV infections and alcohol abuse induce liver inflammation, fibrosis, and cirrhosis. This pathological microenvironment exhibits alterations in cytokine secretion, extra-cellular matrix components and stiffness, angiogenesis, and liver resident immune cells. Moreover, the abnormal activation of signaling pathways, several of them strongly involved in stemness, are observed in liver fibrosis and cirrhosis^[9,10]. All these alterations could contribute to the emergence of cellular clones with characteristics of liver CSCs, as shown in other tumor types^[8] and subsequently to HCC initiation and development.

Understanding the mechanisms responsible for liver CSC maintenance and survival is therefore an important step in the search of efficient therapies for HCC. CSCs exploit signaling pathways essential for self-renewal, proliferation and differentiation that are usually used by stem cells in physiological situations. Wnt/ β -catenin, Hedgehog, Notch, and TGF β are the main pathways found activated in hepatic CSCs^[11-14]. For some of them, this activation has been associated with the expression of cell surface markers, such as EpCAM for the Wnt/ β -catenin pathway or CK19 for the TGF β pathway, and with poorly differentiated tumors, drug resistance and worse prognosis^[15-17].

In addition to these pathways, another key player of CSCs is the p53 family. In this review, we summarize the main functions of p53 and the p53 family, as well as the mechanisms that lead to their functional inactivation in tumors. We describe the alterations of p53 functions that favor the emergence of CSCs with a particular focus on liver CSCs. Finally, we highlight the close connection between the p53 family

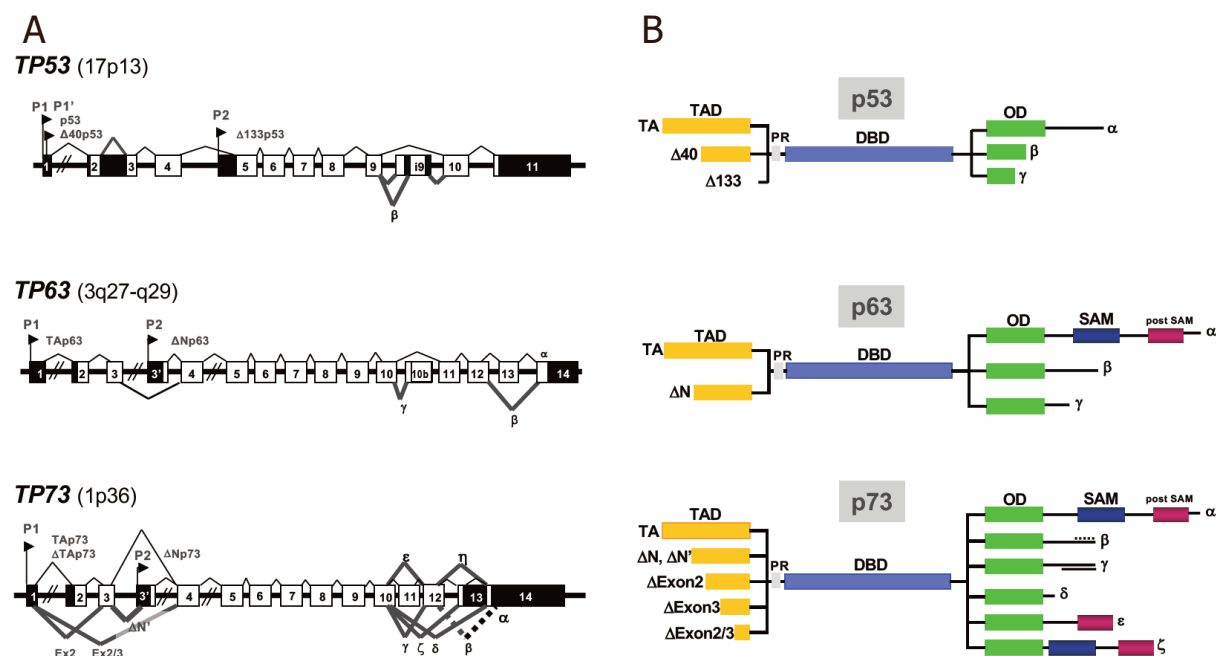


Figure 1. The p53 family - a complex expression strategy. A: schematic representation of the exon structure of *TP53*, *TP63* and *TP73* genes. The position of the P1 promoter and the internal P2 promoter relative to the exons in the 3 genes are indicated. p53 gene transcription is initiated from two distinct sites (P1 and P1'); B: p53, p63 and p73 protein domains. TAD: N-terminal transactivation domains (yellow); PR: proline-rich sequence (grey); DBD: DNA-binding domain (purple); OD: C-terminal oligomerization domain (green); SAM: sterile alpha motif (dark blue); post-SAM: post-sterile alpha motif (burgundy). The principal N-terminal truncated isoforms (Δ TA or Δ N), generated by the use of internal promoters (P2), alternative splicing of the first exons or the use of alternative translation start sites, and the COOH-terminal variants α , β , γ , δ , ϵ , ζ , generated by alternative splicing of p53, p63 and p73 are also indicated

members and key players of stemness, which could help developing new approaches to prevent the appearance of liver CSCs in the context of chronic liver diseases, inflammation in the liver, and HCC development.

THE P53 FAMILY

The p53 family encompasses three members, namely *TP53*, *TP63* and *TP73*^[18,19]. All three genes encode for multiple isoforms generated by both internal promoter usage and alternative splicing [Figure 1]. The full-length isoforms (p53, TAp63 and TAp73) share a N-terminal transactivation domain, followed by a proline-rich sequence, a central DNA-binding domain (DBD) and the C-terminal oligomerization domain involved in the formation of transcriptionally active homo-tetramers [Figure 1]. All three possess the ability to bind sequence specific cognate DNA motifs (p53RE, p53 responsive elements) and thus to transactivate a large number of direct target genes collectively referred to as p53-target genes. Due to the high sequence homology (> 70% sequence identity) in their DBDs^[18,19], p73 and p63 regulate many p53-target genes (e.g., *WAF1*, *PUMA*, *NOXA*, *BAX* and *MDM2*)^[20]. The complete repertoire of common and private target genes regulated by the p53 family members in different physiological and pathological contexts is still unknown^[20]. The C-terminus of TAp63 and TAp73 alpha isoforms also contains a sterile alpha motif and a terminal transcription inhibitory domain, not conserved in p53^[18,19,21,22]. Additional shorter isoforms (β , γ , δ , and less investigated ϵ , ζ and η) isoforms, whose specific functions are still poorly characterized, are generated by C-terminus alternative splicing^[23,24] [Figure 1]. The N-terminal truncated isoforms (Δ TA or Δ N) act as oncogenes, in part through their dominant negative effect (DNE) on TA isoforms^[18,25,26] [Figure 1].

p53 has held its position of master protein in the cancer field for 40 years. A true hub in the cell stress response, p53 is ubiquitously expressed at low levels and in a latent form in physiological conditions.

In response to a wide variety of intrinsic and extrinsic stress signals, such as DNA damage, oncogenic activation or hypoxia, p53 is subjected to a number of post-translational modifications, including phosphorylation at multiple N- and C-terminal sites by Chk1/2, CK1/2, DNA-PK and other kinases. Phosphorylation usually blocks the binding of E3-Ubiquitin ligases, resulting in both stabilization and conformational shift of p53 from a latent to an activated state^[27]. Stabilization and activation are remarkably fine-tuned processes that depend not only on the type and intensity of the signal, but also on the cell type and its differentiation state^[28], leading to the transactivation of canonical p53-target genes. p53 activation translates into various cellular processes, such as cell cycle arrest^[29], DNA repair^[30], allowing cell survival, or apoptosis^[31], autophagy^[32] and ferroptosis^[33], causing the death of damaged cells. Thereby, p53 response enables the maintenance of genomic stability and prevents the emergence of pre-malignant cells. Accordingly, *tp53* knock-out mice or *tp53* knock-in mice expressing an inactivated form of p53, are prone to developing spontaneous tumors^[26]. Importantly, p53 activities do not depend on transactivation only and the interaction between p53 and other cellular proteins has also been implicated in its tumor suppression function^[34]. In addition, besides cell-cycle arrest and cell death the regulation of other cellular processes, including metabolism, cell migration (by blocking epithelia-mesenchymal transition) or induction of differentiation, contributes to the tumor suppressor function of p53^[35,36]. The repertoire of functions, pathways and genes regulated by p53, p63 and p73 has progressively widened well beyond cell fate, tumor suppression and development. p53 family members have been so far involved in reproduction, genomic repair, fidelity and recombination, metabolic processes, longevity, stem cell biology, adaptive immunity and T cell functions and changes in epigenetic marks^[26,37].

TP53 MUTATIONS AND THEIR CONSEQUENCES ON LIVER TUMORIGENESIS

Unlike *TP63* and *TP73* genes, which are rarely lost or mutated in tumors, genetic alterations of the *TP53* gene are frequently observed, but in contrast to other tumor suppressor genes, the deletion of the two alleles is a rare event^[38]. Strikingly, more than 50% of human tumors present a missense (or less frequently a nonsense or frame shift) mutation in one *TP53* allele, frequently accompanied by the deletion of the other one (loss of heterozygosity, LOH)^[39]. The location of the mutation depends on numerous parameters, such as cell type and the tumor-inducing agent. Nevertheless, some codons (175, 245, 248, 249, 273 and 282) have been found to be preferentially mutated and defined as “hot spot” codons. Mutations of these hot spots can be classified in two categories, those affecting p53 conformation (175, 245, 249, 282) and those that lie in the DNA contact domain (248, 273)^[40]. All block p53 binding to the cognate p53REs. Altogether, these six mutations account for more than 25% of the missense and nonsense mutations listed in the IARC TP53 database R20, (<https://p53.iarc.fr/>)^[41]. Figure 2A illustrates the distribution of the resulting substituted amino acids along the p53 protein (only those for which more than 100 mutations have been reported in the database are shown). Most of the mutations are located in the DBD, mostly resulting in the inability of mutant p53 (mutp53) to bind p53RE and thus to transactivate p53-target genes (Figure 3, left upper panel). The mutation at codon 249 represents a particular case among the hot spots, because it is overrepresented in HCC. Figure 2B clearly shows the high prevalence of this mutation in HCC (around 30%), whereas the five other hot spots account for only 9%. Interestingly, the arginine residue at codon 249 is almost exclusively changed to a serine residue (96%) in HCC, due to a G/C to T/A transversion. Transversions in the *TP53* gene are also found in several other types of cancer, such as those affecting the respiratory and urinary tracts, whereas G/C to A/T transitions are mainly present in colorectal carcinomas or brain tumors. Whereas transitions are expected to be due to intrinsic factors, transversions are correlated with exposure to environmental mutagens that form DNA adducts and often affect particular codons, as in the *TP53* gene^[42]. For example, *TP53* codons 157 and 158 are targets of tobacco smoke compounds in lung adenocarcinoma. Similarly, in HCC the G/C to T/A transversion at codon 249 is caused by consumption of AFB1-contaminated food^[43-46]. AFB1 exposure is mainly observed in sub-Saharan Africa and Southeast Asia, two regions in which HBV chronic infection is frequent. The combination of these two extrinsic factors has been shown to be responsible for the high incidence of HCC in these regions and to be associated

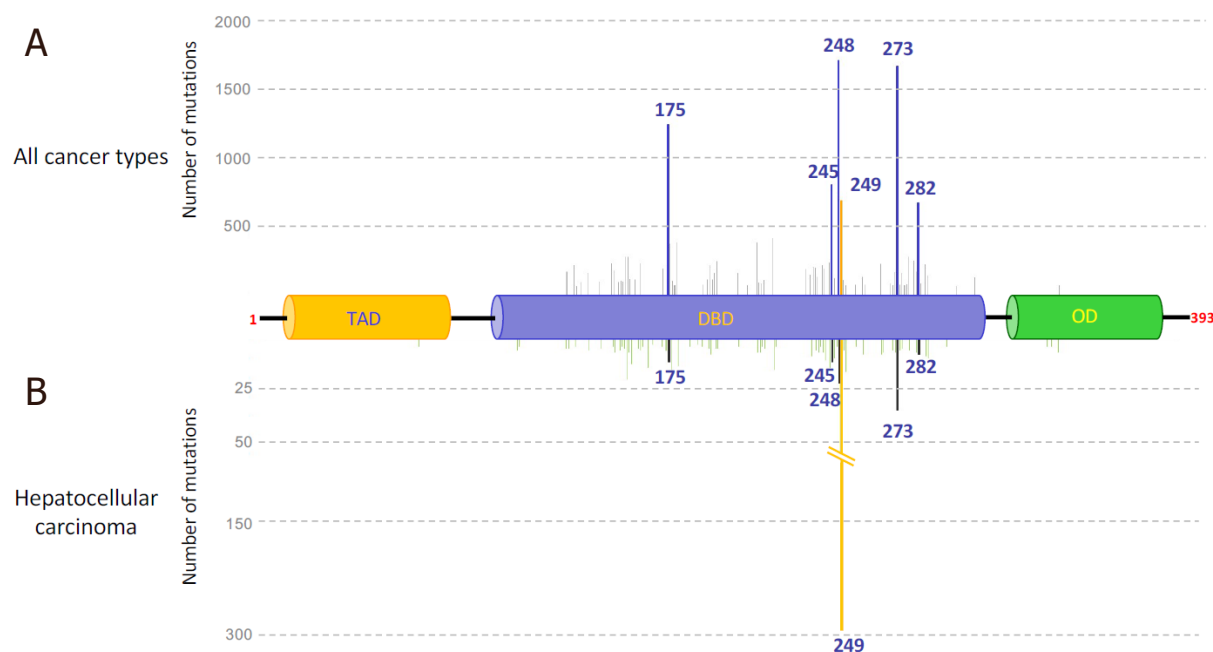


Figure 2. *TP53* missense mutations found in human cancers and liver cancer. The main mutated codons were placed on the p53 protein. A: main somatic mutations found in all cancer types (more than 100 mutations listed); B: main mutations found in HCC (more than 4 mutations listed). Source, IARC *TP53* database R20. TA: transactivation domain; DBD: DNA binding domain; OD: oligomerization domain. The position of the first and the last p53 aminoacids are noted in red. Deletions, insertions and mutations in introns have not been included in the cartoon

with the presence of the p53R249S mutant. More than 50% of HCC from these regions express a p53R249S mutant^[47-49]. It is important to underline that *TP53* is the most frequently mutated gene and is associated with a shorter survival in HBV-related HCC also independently from AFB1 exposure^[50,51].

Most p53 mutants have lost wild-type p53 (wtp53) functions (loss of function, LOF). Moreover, when expressed with wtp53 encoded from the non-mutated remaining allele, they form heterotetramers, or aggregates, and thus reduce or eliminate the ability of wtp53 to maintain genomic integrity by a dominant negative effect^[52]. In the context of response to stress of premalignant cells (*e.g.*, hepatocytes with p53R249S), the expression of mutant p53 may allow cells to survive despite the accumulation of genetic alterations, thus favoring the emergence of potential tumor cells. Many tumors harboring a missense mutation in one *TP53* allele have lost the wild-type one, suggesting that LOH provides a selective advantage in the transformation process for cells expressing at least some of the p53 mutants.

In an attempt to better understand the properties of the different p53 mutants, many functional experiments both in cells, yeast and mouse models have led to the classification of these mutants in two main categories. The first one includes LOF mutants whose expression has biological consequences similar to the homozygous deletion of the *TP53* gene. The second one includes mutants that enhance cell transformation, tumor growth and aggressiveness compared to their p53-null counterparts. This last class is expected to acquire new functions (gain of function, GOF). Numerous studies since the 1990s have shown that GOF mutp53 are involved in transcriptional activation or repression, independently of their binding to p53RE, but mediated by cooperation with other transcription factors, such as NF- κ B^[53-55]. Among the hot spot mutants, p53R175H, p53R248Q and p53R273H have been shown to be GOF mutants, whereas p53G245S is part of the LOF category with a DNE on wtp53^[53,56,57]. p53R249S has been considered a LOF mutation, mainly according to the results of *in vitro* and *in vivo* experiments performed in non-hepatic cells or tissues other than the liver^[56], whereas a GOF phenotype was observed in the liver and in the context of HCC^[58-60].

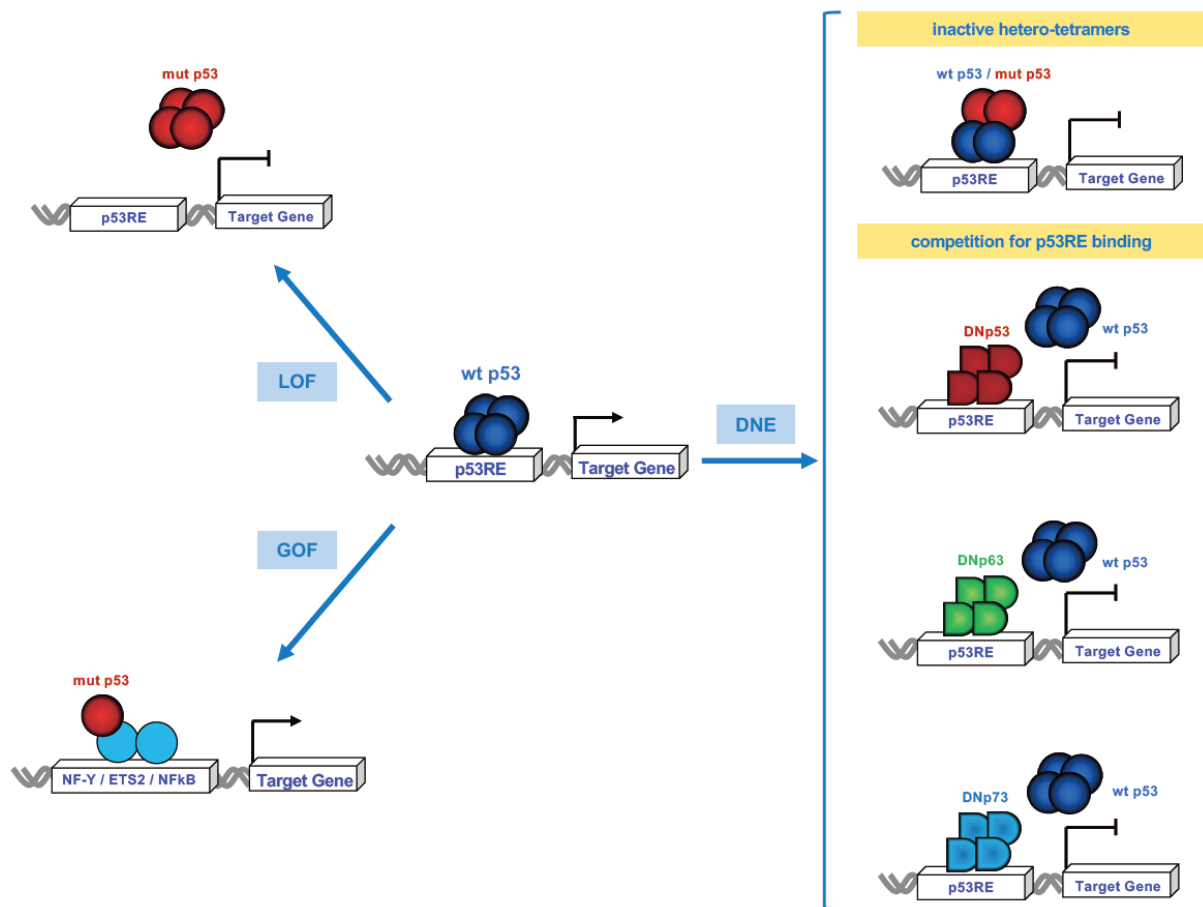


Figure 3. Wtp53 inactivation by genetic mutations and dominant negative p53 family proteins. The wtp53, once activated, works as a tetramer to bind the p53-regulatory elements (p53RE) of its direct target genes and induce transcription. At a late stage of tumor cells evolution mutant p53 forms tetramers and/or aggregates that are unable to bind to canonical target sequences to turn on its targets (LOF) (upper left panel). Some mutant p53 (p53R175H, p53R248Q, p53R273H) acquire novel gain of functions (GOFs) to activate (or repress) transcription independently of their binding to p53RE by cooperating with other transcription factors, such as NF- κ B, and drive the growth, survival and invasion of tumor cells. At earlier stages, when one allele is mutated there is reduced overall function resulting in haploinsufficiency and also the DNE of the mutant p53 on the wtp53 due to the formation of transcriptionally inactive heterotetramers (upper right panel). The N-terminal truncated isoforms (Δ TA or Δ N) of p53, p63 and p73 also exert a DNE on the wtp53. Δ N-tetramers compete with p53, TAp63 and TAp73 on the same p53RE leading to the abrogation of the wtp53 transcriptional program (middle and lower right panels)

Why p53R249S is selected in HCC compared to other AFB₁-induced *TP53* mutations remains an open question. *In vitro* mutagenesis with AFB₁ generates a transversion at codon 249, but also at hot spot codons 245 and 248^[44] and the preferential formation of AFB₁ adducts alone cannot explain the high frequency of p53R249S in HCC^[61]. The other extrinsic factor that could participate in the strong selection of this mutation is HBV. Indeed, we showed that p53R249S interacts with the viral HBx protein and may contribute to cell proliferation and survival^[58]. Together with its DNE on wtp53, one can hypothesize that this mutant could provide a selective advantage to human hepatocytes chronically exposed to AFB₁ and HBV infection. Results from knock-in mice have shown that the expression of the mouse equivalent of the human p53R249S mutant (p53R246S) under the control of the albumin promoter enhances the carcinogenic effect of AFB₁ exposure alone and the cooperative carcinogenic effect together with HBsAg expression^[59,62]. Altogether, p53R249S represents a very peculiar p53 mutant with a cell- and genotoxic-specific GOF.

Recently, Liao and colleagues reported new insights in the preferential selection of p53R249S^[60]. They found that CDK4 is able to phosphorylate p53R249S. Phosphorylated p53R249S interacts with Pin1 and

then the complex is targeted to the nucleus, where it interacts with c-Myc. This interaction stabilizes c-Myc and enhances c-Myc-driven ribosomal biogenesis and HCC cell growth. Interestingly, HBx viral protein has been shown to interplay with several of these p53R249S partners. HBx is able to increase CDK4 activity and also to interact with Pin1 and thus enhances HBV-related hepatocarcinogenesis^[63,64]. The identification of this CDK4-p53R249S-PIN1-c-Myc pathway could explain the high frequency of p53R249S mutant in HCC associated with HBV chronic infection and its low frequency in other tumor types. Moreover, the ability of p53R249S to increase c-Myc activity allows its classification among the GOF mutants, at least in the particular context of HCC.

ADDITIONAL MECHANISMS RESPONSIBLE FOR P53 FUNCTIONAL LOSS IN HEPATOCELLULAR CARCINOMA

In North America and Europe, the *TP53* mutation rate in HCC is around 25%, meaning that the majority of HCC cells express the wild-type form. This indicates that mechanisms other than genetic inactivation must ensure the abrogation of wtp53 tumor suppressor functions. Several viral (SV40 LargeT Ag, Ad12 E1B, HPV16-18 E6, HBx) and cellular proteins have been reported to be able to inactivate wtp53 by direct interaction, possibly followed by degradation^[65-68], the prototype being *MDM2*, a direct p53-target gene. *MDM2* protein is an E3 ubiquitin ligase able to bind p53 and to drive the complex to proteasomal degradation^[69]. In physiological conditions, *MDM2* is the main regulator of p53 intracellular levels, but it has been found to be overexpressed, mainly in sarcomas, but also in HCC (around 25% positivity), leading to wtp53 loss in the tumor cells^[70,71].

In addition to *MDM2*, 18 other E3 ubiquitin ligases leading to p53 proteasome-dependent degradation have been reported^[72]. Some of them are of particular interest, since their expression has been found to be altered in HCCs. *PIRH2* overexpression in human HCCs is associated with a worse prognosis^[73], while *COP1* silencing results in the inhibition of proliferation and the induction of apoptosis in human HCC cells, as well as in the suppression of tumor growth in mouse liver^[74]. Notably, *PIRH2* and *COP1*, like *MDM2*, are encoded by genes inducible by p53 and, although they act independently, all participate in the autoregulatory feedback loop that controls p53 function^[75,76]. *RING1* and *CUL4A* E3 ubiquitin ligases are also overexpressed in HCCs^[77,78] and play a role in stem cell maintenance. *RING1*, by activating the Wnt/ β -catenin pathway, promotes the transformation of hepatic progenitor cells into CSCs^[79]. The *CUL4A*/*DDB1* complex that plays a key role in HBV replication controls embryonic and hematopoietic stem cell differentiation and homeostasis^[80]. Although each of these ubiquitin ligases can, in principle, favor hepatocarcinogenesis by inducing wtp53 degradation, their contribution to HCC development is not known and the prevalent mechanism of hepatocarcinogenesis in the absence of *TP53* mutations in human HCCs may be, according to the data available so far, the overexpression of Δ Np73 (see below).

TRUNCATED MEMBERS OF THE P53 FAMILY ACT AS FUNCTIONAL INHIBITORS OF WTP53

The N-terminal truncated isoforms (Δ TA or Δ N) of p53, p63 and p73, generated by the use of internal promoters (P2), alternative splicing of the first exons or the use of alternative translation start sites, exert a DNE on TA isoforms^[18,19,81]. Two major mechanisms underlie the DNE of Δ TA/ Δ N p53, p63 and p73 [Figure 3]. The first is a direct competition of Δ N-tetramers with p53, TAp63 and TAp73 on the same p53REs and the consequent inhibition of p53/TAp73-mediated activation (*right middle and lower panels*). The second is the oligomerization with TA proteins to form transcriptionally ineffective heterocomplexes (*right upper panel*). Notably, since the oligomerization domains are only partially conserved, p73 and p63 form hetero-oligomers with each other but not, or to a very limited extent, with p53^[82].

The mechanisms responsible for the differential expression of TA and Δ N isoforms in the different tissues and pathophysiological conditions are far from being fully elucidated. Several signaling and/or oncogenic

pathways affect TA- and/or Δ N-p73 levels by affecting transcription or protein stability. TAp73 isoform transcription from the P1 promoter is primarily driven by E2F1^[83-88], but its activity can be also modulated by other factors such as C-EBP α ^[89], ZEB^[90] and Ying Yang 1 (YY1)^[91]. The regulation of the P2 promoter is much less clear. Notably, both p53 and TAp73 bind the internal P2 promoter of *TP73*, activate the transcription of P2 Δ Np73 isoforms to create a negative feedback loop between Δ Np73 and p53/TAp73 that may self-restrict their transcriptional activities^[92-95].

The ratio between Δ N- and TA isoforms has been shown to determine the net effect and, in the case of p73, to predict the effectiveness of chemotherapy^[96-98]. The TA pro-oncogenic role of dominant negative Δ TA/ Δ N p53, p63 and p73 is supported by several observations. Δ Np73 overexpression in fibroblasts increases their colony formation capacity^[99] and cooperates with RAS, c-Myc and E1A in promoting transformation and tumorigenicity^[21,100]. In melanoma xenografts Δ Np73 expression is associated with upregulation of Slug, downregulation of the actin binding protein EPLIN, activation of the IGF1R-AKT/STAT3 pathway, loss of E-cadherin and a higher ability to invade and metastasize^[101]. Notably, 83% of 12-20 month-old transgenic mice expressing Δ Np73 under the control of the albumin promoter develop HCC, confirming the oncogenic potential of Δ Np73 and its capability to drive hepatocarcinogenesis *in vivo*^[102]. In the case of Δ Np63, in addition to the mechanisms already described, its oncogenic potential in squamous cell carcinomas is related to its ability to control transcription of genes involved in skin developmental and tumorigenic pathways, such as IRF6, IKK α , and FGFR2^[103-105], and to act as a regulator of p53 tumor suppressive functions both in a cell-autonomous way and as a mediator of activation of FGF signaling pathway in a paracrine way^[26].

THE ROLE OF P53 FAMILY IN THE EMERGENCE, MAINTENANCE AND IMMATURE PHENOTYPE OF CSCS

Since their discovery, p63 and p73 have been associated with tissue development, because of the multiple developmental abnormalities observed in *tp63* and *tp73* knock-out mice^[26]. *tp63* knock-out mice are devoid of stratified and glandular epithelia and die few hours after birth, whereas *tp73* knock-out mice exhibit defects in central nervous system neurogenesis and fertility, but do not develop tumors^[18,106-109]. On the other hand, *tp53* knock-out mice exhibit only few developmental abnormalities, but are prone to the spontaneous development of a variety of neoplasms, mainly lymphomas and sarcomas by 6 months of age^[110,111]. Therefore, due to this prevailing tumor suppressor function, p53 has been extensively studied in the context of cancer. The role of TAp73 and TAp63 in DNA damage response (DDR) and cancer cell chemosensitivity^[21,96] was also described, whereas their contribution to tumor suppression was established only later through the analysis of p73 and p63 heterozygous mutation in mice^[112] and the generation of mice selectively lacking TAp73 or TA63 isoforms^[113,114]. Fifteen years ago, several groups reported p53 ability to induce the differentiation of embryonic stem (ES) cells and to negatively regulate the proliferation and survival of adult neural stem cells^[115,116]. Consequently, adult bone marrow of *tp53* knock-out mice harbors less quiescent- and more proliferative- hematopoietic stem cells^[117]. More recently, p53 has been shown to control genomic integrity during reprogramming and to dramatically reduce reprogramming efficiency in several cell types^[118,119]. p53 is also essential for the maintenance of DNA methylation homeostasis in ES cells^[120]. Based on these properties, p53 - known as the “guardian of the genome” since 1992^[121] - has also been named “guardian of reprogramming”^[122]. Notably, some p53 mutants, such as p53R175H and p53R273H, enhance reprogramming to a level that is higher than that observed in the case of p53 loss, thus supporting an additional GOF for these mutants^[119,123].

The association of *TP53* mutations with poorly differentiated human tumors has been widely reported. In breast and lung cancers, the presence of p53 mutants has been linked to stem cells and iPSCs (induced pluripotent stem cells) transcriptional signatures and downregulation of differentiation genes regulated

by the polycomb repressor complex 2^[124], a complex required for maintaining ES cell pluripotency and plasticity during embryonic development^[124-126]. A direct link between some p53 mutants, like p53R248W in osteosarcoma cells, and the acquisition of CSCs features (high proliferation rate, sphere formation, clonogenic growth, high migration and invasive ability) and increased aggressiveness has been established^[127]. Truncated forms of p53 have also been shown to favor cancer stemness. For example, the $\Delta 133p53\beta$ isoform enhances the expression of the pluripotency factors *SOX2*, *OCT3/4* and *NANOG*, promotes mammosphere formation and is associated with metastatic potential and chemoresistance in breast cancer cell lines^[128]. The N-terminal truncated isoforms of p63 and p73 are also involved in the emergence and maintenance of CSCs. $\Delta Np63$, which has been described as master regulator of normal epithelial stem cell maintenance in stratified and glandular epithelia^[129,130], promotes stem cell properties by activating signaling pathways, like the Wnt/FZD7 pathway in basal-like mammary cell lines^[130], or components of the Hedgehog (Hh) pathway (*SHH*, *PTCH1*, and *GLI2*) and the Hh-target gene *BMI1* in mammary cell lines with a luminal-like phenotype^[131]. Notably, *BMI1* plays important roles in the self-renewal of normal and CSCs^[132], as well as in the chemoresistance and survival of CSCs from several tumor types^[133]. $\Delta Np73$ also favors CSCs properties. Its overexpression in a non-tumorigenic melanoma cell line exerts a DNE on TAp73-mediated miR-885-5p expression, a negative regulator of the IGF1 receptor (IGF1R)^[134], leading to an increased expression of IGF1R and, consequently, of *CD133*, *NANOG* and *OCT4* and an enhanced ability to form tumors in xenografts^[134]. $\Delta Np73$ also forms complexes with Smad3/4 on Smad binding elements, leading to the enhanced transactivation of stemness-related TGF β -target genes^[135]. Finally, both $\Delta Np63$ and $\Delta Np73$ are able to enhance reprogramming by favoring the mesenchymal-epithelial transition (MET) required during iPSCs generation^[136,137] and in the case of $\Delta Np73$, by inhibiting wtp53^[138].

Altogether, there is enough evidence to affirm that the truncated forms of the p53 family members and some p53 mutants not only act as functional inhibitors of wtp53 but also exhibit new additional functions, some of them contributing to stemness. It is more difficult, however, to define the relative contribution of the different isoforms to the stem-related phenotype, due to their variable expression (not having discussed the C-terminal variants), the complexity of their crosstalk and their largely redundant functions.

P53 FAMILY MEMBERS IN HCC

The expression and activities of p53 family members have been studied in normal hepatocytes and HCC cells. In a small series of 16 cholangiocarcinomas (CCAs), p63 overexpression correlated with CK19 positivity and low tumor differentiation, whereas no expression was found in 37 HCCs^[139]. Similarly, in hepatocytes and HCC cell lines, TAp63 is barely expressed and the detection of $\Delta Np63$, generated from the P2 promoter, is restricted to p53-null cells^[140], because its expression is repressed by p53, as previously reported in other cell types^[141,142]. Conversely, $\Delta Np73$ expression is activated by p53 and to a lesser extent by TAp63 and TAp73^[141,143]. TAp73 and several p73 N-terminal truncated isoforms are highly expressed in HCC samples, independently of the presence of wild type or mutant p53^[144,145]. The overexpression of $\Delta Np73$ and/or a high $\Delta Np73$ /TAp73 ratio are associated with a reduced survival in patients with HCC^[146-149]. Moreover, in patients with HCC undergoing OLT, recurrence and reduced survival were correlated with an increased expression of $\Delta Np63$ and $\Delta Np73$, and a reduction of TAp63 and TAp73 expression^[150]. All these observations indicate that $\Delta Np73$ /TAp73 ratio could be a potentially relevant prognostic factor in HCC.

As already mentioned, the expression of p53 family members is highly dependent on the differentiation state of cells. Thus, $\Delta Np73$ is detected in proliferative immature HepaRG non-transformed bi-potent liver cells, but not in the differentiated state^[151], whereas p53 and TAp73 are expressed in mature hepatocytes. The presence of p53 in mature hepatocytes has been shown to play an important role in the maintenance of their cell identity when exposed to an oncogenic stress. The expression of *c-MYC* at low levels is able to induce the expression of *NANOG*, *OCT4* and *EpCAM* and to increase both sphere formation and

tumorigenicity of HCC cell lines^[152]. c-Myc-induced reprogramming and the acquisition of CSCs features are readily observed in p53-null cells but the functional inactivation of p53 is needed in cells with wtp53 alleles^[152]. HCC cells in which p53 functional inactivation is achieved by genetic alteration or expression of proteins that exert a DNE are prone to dedifferentiation if exposed to oncogenic stimuli. Similar results have been obtained in mouse models. Tschaharganeh and colleagues have shown that in the presence of high levels of YAP, an oncogene overexpressed in human HCC and CCA^[153], *tp53* deletion in mature hepatocytes facilitates their dedifferentiation *in vivo*, the emergence of progenitor tumor cells with high *NESTIN* expression and the development of tumors sharing HCC and CCA characteristics^[154]. *NESTIN* was required for the dedifferentiation and malignant expansion of p53-deficient cells and the activation of Wnt or Notch pathways in the p53-null progenitor tumors drive the development of HCC and CCA, respectively. *NESTIN* was also overexpressed in 17% of human HCCs and 40% of CCAs, associated with a reduced survival and the presence of mutations or LOH in the *TP53* gene^[154]. Altogether, these results suggest that in mature liver cells the p53-mediated inhibition of *NESTIN* restricts plasticity and tumorigenesis in response to oncogene activation.

LIVER CSCS AND THE CROSSTALK BETWEEN NANOG, IGF1R AND THE P53 FAMILY

Cancer tissues contain subpopulations of cells known as stem-like TICs (sl-TICs or CSCs) that have been identified as key drivers of tumor growth and malignant progression with drug resistance. Liver CSCs are bi-potent and give rise to two different lineage types, HCC and CCA. Indeed, combined hepatocellular-cholangiocarcinomas (cHCC-CCAs) with both hepatocytic and cholangiocytic phenotypes account for 1% to 5% of all primary liver cancers^[155], and about one third of HCCs express progenitor cell markers such as CK7 and CK19^[156], suggesting that at least a portion of HCC cells have intermediate characteristics between the bi-potent hepatic progenitor cells (HPCs) and differentiated mature hepatocytes^[157,158]. The origin of liver CSCs and liver cancer cells is still controversial^[159] and there is evidence for both their derivation from HPCs chronically activated by the persistent intrahepatic inflammation sustained by chronic HBV, HDV and HCV infections, alcohol consumption and metabolic disorders^[160] and the dedifferentiation of mature hepatocytes that acquire the expression of stem-related genes when exposed to inflammatory cytokines, lipid overload or epigenetic reprogramming^[161-163] [Figure 4A].

p53 has been shown to repress many key transcription regulators, such as Oct4, Nanog, Sox2, Zic3, Jmjd1c, Esrrb, Tcfcp2l1, Utf1, n-Myc, c-Myc, and Prdm14 in mouse ES cells^[164,165]. Among them, Nanog is of particular interest with respect to the p53 family in the context of HCC development. Nanog is overexpressed in about 30% of HCCs as shown by immunohistochemistry^[166-168], regulates the expression of genes involved in mitochondrial metabolic pathways^[169], promotes CSC properties^[169] and enhances resistance to drugs, such as sorafenib or cisplatin, as well as tumor invasion and metastasis^[169,170].

The wtp53 represses *NANOG* expression either by direct binding to the *Nanog* promoter in mouse cells^[115,164] or by transactivating miR34a-c, a direct p53-target gene that in turn represses *NANOG*^[171] in human cells^[172]. In a recent study, Liu and colleagues have shown that the enhanced mitophagy observed in HCCs increases p53 localization at the mitochondrial membrane^[173-176] and leads to its subsequent degradation. In contrast, when mitophagy is impaired, mitochondrial p53 is phosphorylated by Pink1 and translocated into the nucleus, where it represses *NANOG* expression resulting in a decrease of liver CSCs^[176,177].

Two p53 family isoforms are able to exert a DNE on p53 and TAp73 regulation of *NANOG*. $\Delta 40p53$, by titrating full-length p53, regulates the switch from pluripotency to differentiation by increasing the expression of *Nanog* and *Igf1R* in mouse ES cells^[178] and $\Delta Np73$, as already mentioned, interferes with TAp73 in the regulation of *IGF1R* expression in human melanoma cells^[134]. Furthermore, we and others demonstrated that $\Delta Np73$ transactivates *NANOG* expression independently of p53^[138,179]. The

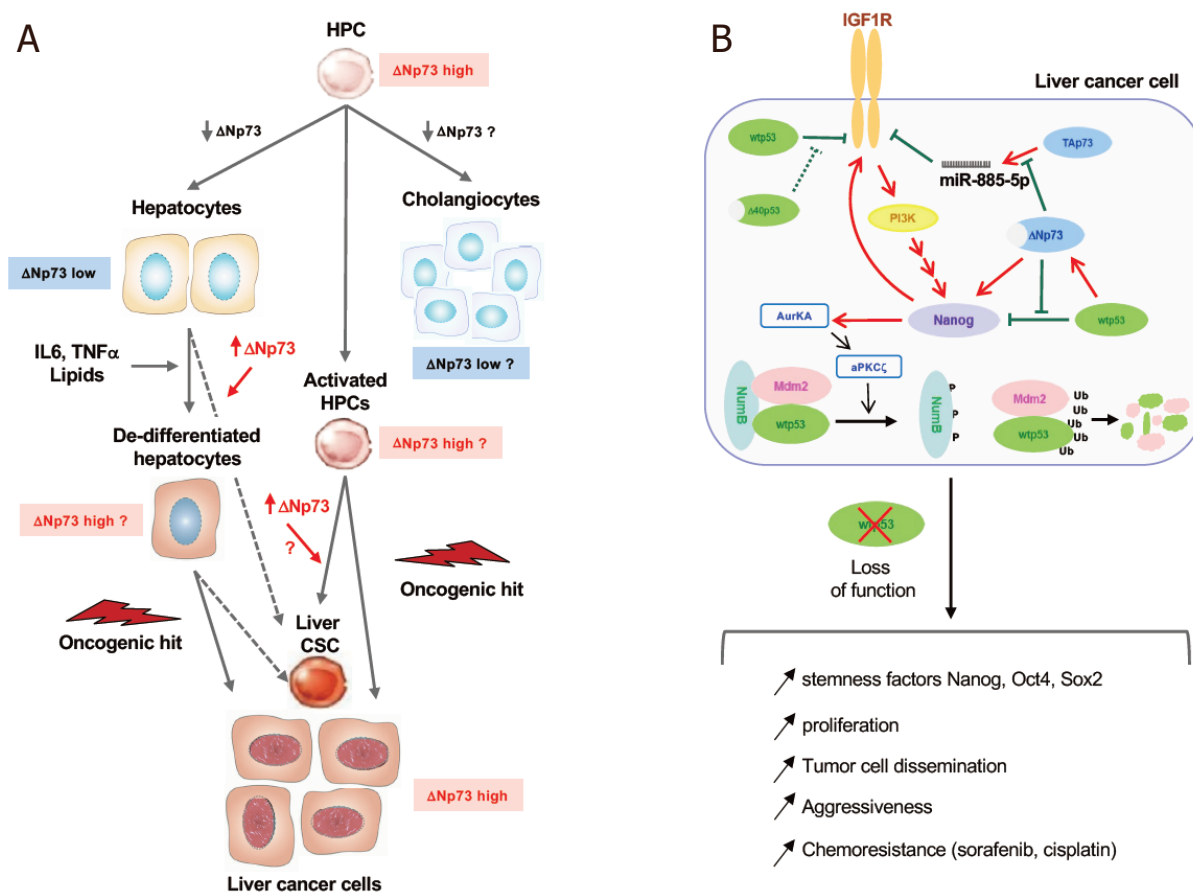


Figure 4. p53 family proteins and liver progenitor/stem cell transformation. A: the origin of cancer stem cells (CSCs) in HCC. CSCs and liver cancer cells can originate from: (1) hepatic progenitor cells (HPCs) chronically activated by the persistent intrahepatic inflammation sustained by viral infections (HBV, HDV, HCV), alcohol consumption and metabolic disorders; (2) hepatocytes undergoing dedifferentiation and expressing stem-related genes (such as Nanog). According to the currently available knowledge, ΔNp73 expression levels and its role in the different steps are highlighted; B: crosstalk between IGF1R, Nanog and p53 family in liver cancer cells. *IGF1R* repression by wtp53 and TAp73 and *NANOG* repression by wtp53 inhibit the dedifferentiation of liver cancer cells. In presence of the N-terminal truncated isoforms Δ40p53 or ΔNp73, wtp53 activity is inhibited, *IGF1R* and *NANOG* are expressed and activate a positive feedback loop. By activating AURKA-aPKCζ kinase cascade Nanog induces NumB phosphorylation and disrupts the p53-NumB interaction thus allowing Mdm2-mediated polyubiquitination and proteasome degradation of p53. The loss of p53 function, achieved either by expression of ΔN isoforms or p53 degradation, favors stemness features, increased proliferation, dissemination and chemoresistance of liver cancer cells

overexpression/accumulation of NANOG leads to p53 proteolysis. By activating the Aurora A kinase and aPKCζ, Nanog allows the phosphorylation of NumB and the dissociation of the p53-NumB complex, thus relieving NumB inhibition and freeing Mdm2 to degrade p53 and to potentiate the proliferation of CSCs^[180]. Based on all these results, the fine-tuned crosstalk between p53 and Nanog plays a key role in the emergence, maintenance and chemoresistance of CSCs [Figure 4B].

CONCLUSION

In HCC, in contrast to other tumor types, the loss of functional p53 seems to be achieved by several other mechanisms in addition to genetic mutation. p53 degradation mediated by MDM2 or other E3 ubiquitin ligases and the overexpression of N-terminal truncated isoforms of the family have a prominent place due to their important role in the emergence and regulation of liver CSCs. ΔNp73 is of particular interest because, by inhibiting p53 and TAp73 activities, it activates the expression of *NANOG* both in a p53-dependent and -independent manner and enhances *IGF1R* expression. The ΔNp73-IGF1R-NANOG axis may prove to be an attractive therapeutic target for HCC patients, as already proposed in melanoma^[134].

The characterization of how this axis is regulated in the viral and metabolic inflammatory chronic liver diseases that precede HCC development might also help to develop new biomarkers for risk-stratification and new preventive strategies.

The identification of at least three main mechanisms of wtp53 inactivation in HCCs may have therapeutic implications and lead to new strategies for treatment personalization. Besides p53-based gene therapy approaches, there are very few reports of drugs targeting the p53 family in HCC, either in preclinical or clinical settings. PRIMA-1 (p53 Reactivation and Induction of Massive Apoptosis), a small molecule capable of restoring a wild-type activity in some p53 mutants, has been shown to slow down tumor growth in immunocompromised mice xenografted with p53R249S-expressing human cells^[181]. Therefore, it might be valuable to test the potential of PRIMA-Met (an improved version of PRIMA-1) and MIRA-1 (mutant p53-dependent induction of rapid apoptosis), a small molecule targeting p53 structurally distinct from PRIMA-1^[182], to reactivate wtp53 functions in HCCs with p53 mutants, or of Nutlins to counteract MDM2-mediated degradation of wtp53^[183]. IFN α has been shown to inhibit the expression of Δ Np73 by inducing chromatin remodeling at the P2p73 promoter and increasing apoptosis of human HCC cells^[184]. These results provide a proof of concept of the possibility to target the third mechanism of wtp53 inactivation frequently engaged in HCCs, i.e., the DNE of N-terminal truncated members of the p53 family. However, IFN α has no indication in current HCC treatment algorithms and the identification of potent and selective drugs capable to target Δ Np73 expression might prove of value to increase HCC chemosensitivity and to enhance tumor and cancer stem cells apoptosis, either alone or in combination with TKIs.

DECLARATIONS

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Authors' contributions

Researched public databases for *TP53* mutations: Caron de Fromentel C

Designed and wrote the manuscript: Caron de Fromentel C, Levrero M

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* 2019;144:1941-53.
2. Llovet JM, Zucman-Rossi J, Pikarsky E, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2016;2:16018.
3. Llovet JM, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol* 2018;15:599-616.
4. Nault JC, Cheng AL, Sangro B, Llovet JM. Milestones in the pathogenesis and management of primary liver cancer. *J Hepatol* 2020;72:209-14.
5. Finn RS, Qin S, Ikeda M, et al; IMbrave150 Investigators. Atezolizumab plus Bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med* 2020;382:1894-905.
6. Cojoc M, Mäbert K, Muders MH, Dubrovskaya A. A role for cancer stem cells in therapy resistance: cellular and molecular mechanisms. *Semin Cancer Biol* 2015;31:16-27.
7. Fekir K, Dubois-Pot-Schneider H, Désert R, et al. Retrodifferentiation of human tumor hepatocytes to stem cells leads to metabolic reprogramming and chemoresistance. *Cancer Res* 2019;79:1869-83.
8. Barbato L, Bocchetti M, Di Biase A, Regad T. Cancer stem cells and targeting strategies. *Cells* 2019;8:926.
9. Philips GM, Chan IS, Swiderska M, et al. Hedgehog signaling antagonist promotes regression of both liver fibrosis and hepatocellular carcinoma in a murine model of primary liver cancer. *PLoS One* 2011;6:e23943.
10. Khosla R, Rastogi A, Ramakrishna G, et al. EpCAM+ liver cancer stem-like cells exhibiting autocrine wnt signaling potentially originate in cirrhotic patients. *Stem Cells Transl Med* 2017;6:807-18.
11. Yang W, Yan HX, Chen L, et al. Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res* 2008;68:4287-95.
12. Malfettone A, Soukupova J, Bertran E, et al. Transforming growth factor- β -induced plasticity causes a migratory stemness phenotype in hepatocellular carcinoma. *Cancer Lett* 2017;392:39-50.
13. Luo J, Wang P, Wang R, et al. The Notch pathway promotes the cancer stem cell characteristics of CD90+ cells in hepatocellular carcinoma. *Oncotarget* 2016;7:9525-37.
14. Della Corte CM, Viscardi G, Papaccio F, et al. Implication of the Hedgehog pathway in hepatocellular carcinoma. *World J Gastroenterol* 2017;23:4330-40.
15. Kawai T, Yasuchika K, Ishii T, et al. Keratin 19, a cancer stem cell marker in human hepatocellular carcinoma. *Clin Cancer Res* 2015;21:3081-91.
16. Terris B, Cavard C, Perret C. EpCAM, a new marker for cancer stem cells in hepatocellular carcinoma. *J Hepatol* 2010;52:280-1.
17. Yamashita T, Ji J, Budhu A, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology* 2009;136:1012-24.
18. Levrero M, De Laurenzi V, Costanzo A, et al. The p53/p63/p73 family of transcription factors: overlapping and distinct functions. *J Cell Sci* 2000;113:1661-70.
19. Murray-Zmijewski F, Lane DP, Bourdon JC. p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. *Cell Death Differ* 2006;13:962-72.
20. Harms K, Nozell S, Chen X. The common and distinct target genes of the p53 family transcription factors. *Cell Mol Life Sci* 2004;61:822-42.
21. Stiewe T. The p53 family in differentiation and tumorigenesis. *Nat Rev Cancer* 2007;7:165-8.
22. Sauer M, Bretz AC, Beinoraviciute-Kellner R, et al. C-terminal diversity within the p53 family accounts for differences in DNA binding and transcriptional activity. *Nucleic Acids Res* 2008;36:1900-12.
23. De Laurenzi V, Costanzo A, Barcaroli D, et al. Two new p73 splice variants, gamma and delta, with different transcriptional activity. *J Exp Med* 1998;188:1763-8.
24. De Laurenzi VD, Catani MV, Terrinoni A, et al. Additional complexity in p73: induction by mitogens in lymphoid cells and identification of two new splicing variants epsilon and zeta. *Cell Death Differ* 1999;6:389-90.
25. Courtois S, Caron de Fromentel C, Hainaut P. p53 protein variants: structural and functional similarities with p63 and p73 isoforms. *Oncogene* 2004;23:631-8.
26. Costanzo A, Pediconi N, Narcisi A, et al. TP63 and TP73 in cancer, an unresolved “family” puzzle of complexity, redundancy and hierarchy. *FEBS Lett* 2014;588:2590-9.
27. Liu Y, Tavana O, Gu W. p53 modifications: exquisite decorations of the powerful guardian. *J Mol Cell Biol* 2019;11:564-77.
28. Kastenhuber ER, Lowe SW. Putting p53 in Context. *Cell* 2017;170:1062-78.
29. El-Deiry WS. p21(WAF1) mediates cell-cycle inhibition, relevant to cancer suppression and therapy. *Cancer Res* 2016;76:5189-91.
30. Sengupta S, Harris CC. p53: traffic cop at the crossroads of DNA repair and recombination. *Nat Rev Mol Cell Biol* 2005;6:44-55.
31. Aubrey BJ, Kelly GL, Janic A, Herold MJ, Strasser A. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? *Cell Death Differ* 2018;25:104-13.
32. Crighton D, Wilkinson S, O’Prey J, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* 2006;126:121-34.

33. Jiang L, Kon N, Li T, et al. Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 2015;520:57-62.
34. Ho T, Tan BX, Lane D. How the other half lives: what p53 does when it is not being a transcription factor. *Int J Mol Sci* 2019;21:13.
35. Valente LJ, Gray DH, Michalak EM, et al. p53 efficiently suppresses tumor development in the complete absence of its cell-cycle inhibitory and proapoptotic effectors p21, Puma, and Noxa. *Cell Rep* 2013;3:1339-45.
36. Pitolli C, Wang Y, Candi E, et al. p53-mediated tumor suppression: DNA-damage response and alternative mechanisms. *Cancers (Basel)* 2019;11:1983.
37. Barili V, Fiscicaro P, Montanini B, et al. Targeting p53 and histone methyltransferases restores exhausted CD8+ T cells in HCV infection. *Nat Commun* 2020;11:604.
38. Soussi T, Wiman KG. TP53: an oncogene in disguise. *Cell Death Differ* 2015;22:1239-49.
39. Parikh N, Hilsenbeck S, Creighton CJ, et al. Effects of TP53 mutational status on gene expression patterns across 10 human cancer types. *J Pathol* 2014;232:522-33.
40. Seemann S, Maurici D, Olivier M, Caron de Fromentel C, Hainaut P. The tumor suppressor gene TP53: implications for cancer management and therapy. *Crit Rev Clin Lab Sci* 2004;41:551-83.
41. Bouaoun L, Sonkin D, Ardin M, et al. TP53 variations in human cancers: new lessons from the IARC TP53 database and genomics data. *Hum Mutat* 2016;37:865-76.
42. Puisieux A, Lim S, Groopman J, Ozturk M. Selective targeting of p53 gene mutational hotspots in human cancers by etiologically defined carcinogens. *Cancer Res* 1991;51:6185-9.
43. Pfeifer GP, Denissenko MF, Olivier M, et al. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene* 2002;21:7435-51.
44. Aguilar F, Hussain SP, Cerutti P. Aflatoxin B1 induces the transversion of G-->T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. *Proc Natl Acad Sci U S A* 1993;90:8586-90.
45. Olivier M, Hussain SP, Caron de Fromentel C, Hainaut P, Harris CC. TP53 mutation spectra and load: a tool for generating hypotheses on the etiology of cancer. *IARC Sci Publ* 2004:247-70.
46. Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* 1991;350:429-31.
47. Gouas DA, Villar S, Ortiz-Cuaran S, et al. TP53 R249S mutation, genetic variations in HBx and risk of hepatocellular carcinoma in The Gambia. *Carcinogenesis* 2012;33:1219-24.
48. Marchio A, Amougou Atsama M, Béré A, et al. Droplet digital PCR detects high rate of TP53 R249S mutants in cell-free DNA of middle African patients with hepatocellular carcinoma. *Clin Exp Med* 2018;18:421-31.
49. Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. *Oncogene* 2007;26:2166-76.
50. Amaddeo G, Cao Q, Ladeiro Y, et al. Integration of tumour and viral genomic characterizations in HBV-related hepatocellular carcinomas. *Gut* 2015;64:820-9.
51. Levrero M, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. *J Hepatol* 2016;64:S84-101.
52. Brosh R, Rotter V. When mutants gain new powers: news from the mutant p53 field. *Nat Rev Cancer* 2009;9:701-13.
53. Hanel W, Marchenko N, Xu S, et al. Two hot spot mutant p53 mouse models display differential gain of function in tumorigenesis. *Cell Death Differ* 2013;20:898-909.
54. Aschauer L, Muller PA. Novel targets and interaction partners of mutant p53 gain-of-function. *Biochem Soc Trans* 2016;44:460-6.
55. Dell'Orso S, Fontemaggi G, Stambolsky P, et al. ChIP-on-chip analysis of in vivo mutant p53 binding to selected gene promoters. *OMICS* 2011;15:305-12.
56. Lee MK, Teoh WW, Phang BH, et al. Cell-type, dose, and mutation-type specificity dictate mutant p53 functions in vivo. *Cancer Cell* 2012;22:751-64.
57. Sabapathy K, Lane DP. Therapeutic targeting of p53: all mutants are equal, but some mutants are more equal than others. *Nat Rev Clin Oncol* 2018;15:13-30.
58. Gouas DA, Shi H, Hautefeuille AH, et al. Effects of the TP53 p.R249S mutant on proliferation and clonogenic properties in human hepatocellular carcinoma cell lines: interaction with hepatitis B virus X protein. *Carcinogenesis* 2010;31:1475-82.
59. Ghebranious N, Sell S. The mouse equivalent of the human p53ser249 mutation p53ser246 enhances aflatoxin hepatocarcinogenesis in hepatitis B surface antigen transgenic and p53 heterozygous null mice. *Hepatology* 1998;27:967-73.
60. Liao P, Zeng SX, Zhou X, et al. Mutant p53 gains its function via c-Myc activation upon CDK4 phosphorylation at serine 249 and consequent PIN1 binding. *Mol Cell* 2017;68:1134-46.e6.
61. Besaratinia A, Kim SI, Hainaut P, Pfeifer GP. In vitro recapitulating of TP53 mutagenesis in hepatocellular carcinoma associated with dietary aflatoxin B1 exposure. *Gastroenterology* 2009;137:1127-37, 1137.e1-5.
62. Sell S. Mouse models to study the interaction of risk factors for human liver cancer. *Cancer Res* 2003;63:7553-62.
63. Gearhart TL, Bouchard MJ. The hepatitis B virus X protein modulates hepatocyte proliferation pathways to stimulate viral replication. *J Virol* 2010;84:2675-86.
64. Pang R, Lee TK, Poon RT, et al. Pin1 interacts with a specific serine-proline motif of hepatitis B virus X-protein to enhance hepatocarcinogenesis. *Gastroenterology* 2007;132:1088-103.
65. Truant R, Antunovic J, Greenblatt J, Prives C, Cromlish JA. Direct interaction of the hepatitis B virus HBx protein with p53 leads to inhibition by HBx of p53 response element-directed transactivation. *J Virol* 1995;69:1851-9.
66. Wang XW, Forrester K, Yeh H, et al. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and

- association with transcription factor ERCC3. *Proc Natl Acad Sci U S A* 1994;91:2230-4.
67. Levine AJ. The common mechanisms of transformation by the small DNA tumor viruses: The inactivation of tumor suppressor gene products: p53. *Virology* 2009;384:285-93.
68. Sanz G, Singh M, Peugot S, Selivanova G. Inhibition of p53 inhibitors: progress, challenges and perspectives. *J Mol Cell Biol* 2019;11:586-99.
69. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. *Nature* 1997;387:299-303.
70. Cordon-Cardo C, Latres E, Drobnjak M, et al. Molecular abnormalities of mdm2 and p53 genes in adult soft tissue sarcomas. *Cancer Res* 1994;54:794-9.
71. Endo K, Ueda T, Ohta T, Terada T. Protein expression of MDM2 and its clinicopathological relationships in human hepatocellular carcinoma. *Liver* 2000;20:209-15.
72. Bang S, Kaur S, Kurokawa M. Regulation of the p53 family proteins by the ubiquitin proteasomal pathway. *Int J Mol Sci* 2019;21:261.
73. Huang X, Qian X, Cheng C, et al. Expression of Pirh2, a p27(Kip1) ubiquitin ligase, in hepatocellular carcinoma: correlation with p27(Kip1) and cell proliferation. *Hum Pathol* 2011;42:507-15.
74. Lee YH, Andersen JB, Song HT, et al. Definition of ubiquitination modulator COP1 as a novel therapeutic target in human hepatocellular carcinoma. *Cancer Res* 2010;70:8264-9.
75. Leng RP, Lin Y, Ma W, et al. Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. *Cell* 2003;112:779-91.
76. Dornan D, Wertz I, Shimizu H, et al. The ubiquitin ligase COP1 is a critical negative regulator of p53. *Nature* 2004;429:86-92.
77. Shen J, Li P, Shao X, et al. The E3 ligase RING1 targets p53 for degradation and promotes cancer cell proliferation and survival. *Cancer Res* 2018;78:359-71.
78. Pan Y, Wang B, Yang X, et al. CUL4A facilitates hepatocarcinogenesis by promoting cell cycle progression and epithelial-mesenchymal transition. *Sci Rep* 2015;5:17006.
79. Zhu K, Li J, Li J, et al. Ring1 promotes the transformation of hepatic progenitor cells into cancer stem cells through the Wnt/ β -catenin signaling pathway. *J Cell Biochem* 2019;Online ahead of print.
80. Gao J, Buckley SM, Cimmino L, et al. The CUL4-DDB1 ubiquitin ligase complex controls adult and embryonic stem cell differentiation and homeostasis. *Elife* 2015;4:e07539.
81. Surget S, Khoury MP, Bourdon JC. Uncovering the role of p53 splice variants in human malignancy: a clinical perspective. *Onco Targets Ther* 2013;7:57-68.
82. Davison TS, Vagner C, Kaghad M, et al. p73 and p63 are homotetramers capable of weak heterotypic interactions with each other but not with p53. *J Biol Chem* 1999;274:18709-14.
83. Irwin M, Marin MC, Phillips AC, et al. Role for the p53 homologue p73 in E2F-1-induced apoptosis. *Nature* 2000;407:645-8.
84. Seelan RS, Irwin M, van der Stoop P, et al. The human p73 promoter: characterization and identification of functional E2F binding sites. *Neoplasia* 2002;4:195-203.
85. Stiewe T, Pützer BM. Role of the p53-homologue p73 in E2F1-induced apoptosis. *Nat Genet* 2000;26:464-9.
86. Costanzo A, Merlo P, Pediconi N, et al. DNA damage-dependent acetylation of p73 dictates the selective activation of apoptotic target genes. *Molecular Cell* 2002;9:175-86.
87. Pediconi N, Ianari A, Costanzo A, et al. Differential regulation of E2F1 apoptotic target genes in response to DNA damage. *Nat Cell Biol* 2003;5:552-8.
88. Pediconi N, Guerrieri F, Vossio S, et al. hSirT1-dependent regulation of the PCAF-E2F1-p73 apoptotic pathway in response to DNA damage. *Mol Cell Biol* 2009;29:1989-98.
89. Marabese M, Vikhanskaya F, Rainelli C, Sakai T, Broggin M. DNA damage induces transcriptional activation of p73 by removing C-EBPalpha repression on E2F1. *Nucleic Acids Res* 2003;31:6624-32.
90. Fontemaggi G, Gurtner A, Strano S, et al. The transcriptional repressor ZEB regulates p73 expression at the crossroad between proliferation and differentiation. *Mol Cell Biol* 2001;21:8461-70.
91. Wu S, Murai S, Kataoka K, Miyagishi M. Yin Yang 1 induces transcriptional activity of p73 through cooperation with E2F1. *Biochem Biophys Res Commun* 2008;365:75-81.
92. Grob TJ, Novak U, Maisse C, et al. Human delta Np73 regulates a dominant negative feedback loop for TAp73 and p53. *Cell Death Differ* 2001;8:1213-23.
93. Vossio S, Palescandolo E, Pediconi N, et al. DN-p73 is activated after DNA damage in a p53-dependent manner to regulate p53-induced cell cycle arrest. *Oncogene* 2002;21:3796-803.
94. Kartasheva NN, Contente A, Lenz-Stöppler C, Roth J, Dobbelsstein M. p53 induces the expression of its antagonist p73 Delta N, establishing an autoregulatory feedback loop. *Oncogene* 2002;21:4715-27.
95. Nakagawa T, Takahashi M, Ozaki T, et al. Autoinhibitory regulation of p73 by Delta Np73 to modulate cell survival and death through a p73-specific target element within the Delta Np73 promoter. *Mol Cell Biol* 2002;22:2575-85.
96. Lunghi P, Costanzo A, Mazzera L, et al. The p53 family protein p73 provides new insights into cancer chemosensitivity and targeting. *Clin Cancer Res* 2009;15:6495-502.
97. Irwin MS, Kondo K, Marin MC, et al. Chemosensitivity linked to p73 function. *Cancer Cell* 2003;3:403-10.
98. Leong CO, Vidnovic N, DeYoung MP, Sgroi D, Ellisen LW. The p63/p73 network mediates chemosensitivity to cisplatin in a biologically defined subset of primary breast cancers. *J Clin Invest* 2007;117:1370-80.
99. Ishimoto O, Kawahara C, Enjo K, et al. Possible oncogenic potential of DeltaNp73: a newly identified isoform of human p73. *Cancer Res* 2002;62:636-41.

100. Petrenko O, Zaika A, Moll UM. deltaNp73 facilitates cell immortalization and cooperates with oncogenic Ras in cellular transformation in vivo. *Mol Cell Biol* 2003;23:5540-55.
101. Beitzinger M, Hofmann L, Oswald C, et al. p73 poses a barrier to malignant transformation by limiting anchorage-independent growth. *EMBO J* 2008;27:792-803.
102. Tannapfel A, John K, Mise N, et al. Autonomous growth and hepatocarcinogenesis in transgenic mice expressing the p53 family inhibitor DNp73. *Carcinogenesis* 2008;29:211-8.
103. Botti E, Spallone G, Moretti F, et al. Developmental factor IRF6 exhibits tumor suppressor activity in squamous cell carcinomas. *Proc Natl Acad Sci U S A* 2011;108:13710-5.
104. Marinari B, Moretti F, Botti E, et al. The tumor suppressor activity of IKKalpha in stratified epithelia is exerted in part via the TGF-beta antiproliferative pathway. *Proc Natl Acad Sci U S A* 2008;105:17091-6.
105. Ramsey MR, Wilson C, Ory B, et al. FGFR2 signaling underlies p63 oncogenic function in squamous cell carcinoma. *J Clin Invest* 2013;123:3525-38.
106. Koster MI, Kim S, Mills AA, DeMayo FJ, Roop DR. p63 is the molecular switch for initiation of an epithelial stratification program. *Genes Dev* 2004;18:126-31.
107. De Laurenzi V, Raschella G, Barcaroli D, et al. Induction of neuronal differentiation by p73 in a neuroblastoma cell line. *J Biol Chem* 2000;275:15226-31.
108. Yang A, Walker N, Bronson R, et al. p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours. *Nature* 2000;404:99-103.
109. Yang A, Schweitzer R, Sun D, et al. p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. *Nature* 1999;398:714-8.
110. Donehower LA, Harvey M, Slagle BL, et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* 1992;356:215-21.
111. Jacks T, Remington L, Williams BO, et al. Tumor spectrum analysis in p53-mutant mice. *Current Biology* 1994;4:1-7.
112. Flores ER, Sengupta S, Miller JB, et al. Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. *Cancer Cell* 2005;7:363-73.
113. Guo X, Keyes WM, Papazoglu C, et al. TAp63 induces senescence and suppresses tumorigenesis in vivo. *Nat Cell Biol* 2009;11:1451-7.
114. Tomasini R, Tsuchihara K, Wilhelm M, et al. TAp73 knockout shows genomic instability with infertility and tumor suppressor functions. *Genes Dev* 2008;22:2677-91.
115. Lin T, Chao C, Saito S, et al. p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. *Nat Cell Biol* 2005;7:165-71.
116. Meletis K, Wirta V, Hede SM, et al. p53 suppresses the self-renewal of adult neural stem cells. *Development* 2006;133:363-9.
117. Liu Y, Elf SE, Miyata Y, et al. p53 regulates hematopoietic stem cell quiescence. *Cell Stem Cell* 2009;4:37-48.
118. Kim J, Lengner CJ, Kirak O, et al. Reprogramming of postnatal neurons into induced pluripotent stem cells by defined factors. *Stem Cells* 2011;29:992-1000.
119. Yi L, Lu C, Hu W, Sun Y, Levine AJ. Multiple roles of p53-related pathways in somatic cell reprogramming and stem cell differentiation. *Cancer Res* 2012;72:5635-45.
120. Tovy A, Spiro A, McCarthy R, et al. p53 is essential for DNA methylation homeostasis in naïve embryonic stem cells, and its loss promotes clonal heterogeneity. *Genes Dev* 2017;31:959-72.
121. Lane DP. Cancer. p53, guardian of the genome. *Nature* 1992;358:15-6.
122. Menendez S, Camus S, Izpisua Belmonte JC. p53: guardian of reprogramming. *Cell Cycle* 2010;9:3887-91.
123. Sarig R, Rivlin N, Brosh R, et al. Mutant p53 facilitates somatic cell reprogramming and augments the malignant potential of reprogrammed cells. *J Exp Med* 2010;207:2127-40.
124. Mizuno H, Spike BT, Wahl GM, Levine AJ. Inactivation of p53 in breast cancers correlates with stem cell transcriptional signatures. *Proc Natl Acad Sci U S A* 2010;107:22745-50.
125. Lee TI, Jenner RG, Boyer LA, et al. Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* 2006;125:301-13.
126. Boyer LA, Plath K, Zeitlinger J, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* 2006;441:349-53.
127. Di Fiore R, Marcatti M, Drago-Ferrante R, et al. Mutant p53 gain of function can be at the root of dedifferentiation of human osteosarcoma MG63 cells into 3AB-OS cancer stem cells. *Bone* 2014;60:198-212.
128. Arsic N, Gadea G, Lagerqvist EL, et al. The p53 isoform $\Delta 133p53\beta$ promotes cancer stem cell potential. *Stem Cell Reports* 2015;4:531-40.
129. Senoo M, Pinto F, Crum CP, McKeon F. p63 is essential for the proliferative potential of stem cells in stratified epithelia. *Cell* 2007;129:523-36.
130. Chakrabarti R, Wei Y, Hwang J, et al. $\Delta Np63$ promotes stem cell activity in mammary gland development and basal-like breast cancer by enhancing Fzd7 expression and Wnt signalling. *Nat Cell Biol* 2014;16:1004-15, 1-13.
131. Memmi EM, Sanarico AG, Giacobbe A, et al. p63 Sustains self-renewal of mammary cancer stem cells through regulation of Sonic Hedgehog signaling. *Proc Natl Acad Sci U S A* 2015;112:3499-504.
132. Liu S, Dontu G, Mantle ID, et al. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res* 2006;66:6063-71.

133. Siddique HR, Saleem M. Role of BMI1, a stem cell factor, in cancer recurrence and chemoresistance: preclinical and clinical evidences. *Stem Cells* 2012;30:372-8.
134. Meier C, Hardstock P, Joost S, Alla V, Pützer BM. p73 and IGF1R regulate emergence of aggressive cancer stem-like features via miR-885-5p Control. *Cancer Res* 2016;76:197-205.
135. Niemantsverdriet M, Nagle P, Chiu RK, et al. Δ Np73 enhances promoter activity of TGF- β induced genes. *PLoS One* 2012;7:e50815.
136. Alexandrova EM, Petrenko O, Nemajero A, et al. Δ Np63 regulates select routes of reprogramming via multiple mechanisms. *Cell Death Differ* 2013;20:1698-708.
137. Martin-Lopez M, Maeso-Alonso L, Fuertes-Alvarez S, et al. p73 is required for appropriate BMP-induced mesenchymal-to-epithelial transition during somatic cell reprogramming. *Cell Death Dis* 2017;8:e3034.
138. Lin Y, Cheng Z, Yang Z, Zheng J, Lin T. Δ Np73 improves generation efficiency of human induced pluripotent stem cells. *BMC Cell Biol* 2012;13:9.
139. Ramalho FS, Ramalho LN, Della Porta L, Zucoloto S. Comparative immunohistochemical expression of p63 in human cholangiocarcinoma and hepatocellular carcinoma. *J Gastroenterol Hepatol* 2006;21:1276-80.
140. Petitjean A, Cavard C, Shi H, et al. The expression of TA and Δ Np63 are regulated by different mechanisms in liver cells. *Oncogene* 2005;24:512-9.
141. Lanza M, Marinari B, Papoutsaki M, et al. Cross-talks in the p53 family: Δ Np63 is an anti-apoptotic target for Δ Np73 α and p53 gain-of-function mutants. *Cell Cycle* 2006;5:1996-2004.
142. Ruptier C, De Gasp  ris A, Ansieau S, et al. TP63 P2 promoter functional analysis identifies β -catenin as a key regulator of Δ Np63 expression. *Oncogene* 2011;30:4656-65.
143. Petitjean A, Ruptier C, Tribollet V, et al. Properties of the six isoforms of p63: p53-like regulation in response to genotoxic stress and cross talk with Δ Np73. *Carcinogenesis* 2008;29:273-81.
144. Stiewe T, Tuve S, Peter M, et al. Quantitative TP73 transcript analysis in hepatocellular carcinomas. *Clin Cancer Res* 2004;10:626-33.
145. Castillo J, Go  n S, Latasa MU, et al. Amphiregulin induces the alternative splicing of p73 into its oncogenic isoform Δ Ex2p73 in human hepatocellular tumors. *Gastroenterology* 2009;137:1805-15.e1-4.
146. M  ller M, Schilling T, Sayan AE, et al. TAp73/ Δ Np73 influences apoptotic response, chemosensitivity and prognosis in hepatocellular carcinoma. *Cell Death Differ* 2005;12:1564-77.
147. Schuster A, Schilling T, De Laurenzi V, et al. Δ Np73 β is oncogenic in hepatocellular carcinoma by blocking apoptosis signaling via death receptors and mitochondria. *Cell Cycle* 2010;9:2629-39.
148. Zhao L, Fu L, Xu Z, et al. The anticancer effects of cinobufagin on hepatocellular carcinoma Huh-7 cells are associated with activation of the p73 signaling pathway. *Mol Med Rep* 2019;19:4119-28.
149. Yoon MK, Ha JH, Lee MS, Chi SW. Structure and apoptotic function of p73. *BMB Rep* 2015;48:81-90.
150. Gonz  lez R, De la Rosa   J, Rufini A, et al. Role of p63 and p73 isoforms on the cell death in patients with hepatocellular carcinoma submitted to orthotopic liver transplantation. *PLoS One* 2017;12:e0174326.
151. Gifu P, Pez F, Bian L, et al. Δ Np73 isoforms are involved in the immature phenotype of liver cancer cells. *J Hepatol* 2017;66:S646.
152. Akita H, Marquardt JU, Durkin ME, et al. MYC activates stem-like cell potential in hepatocarcinoma by a p53-dependent mechanism. *Cancer Res* 2014;74:5903-13.
153. Li H, Wolfe A, Septer S, et al. Deregulation of Hippo kinase signalling in human hepatic malignancies. *Liver Int* 2012;32:38-47.
154. Tschaharganeh DF, Xue W, Calvisi DF, et al. p53-dependent Nestin regulation links tumor suppression to cellular plasticity in liver cancer. *Cell* 2014;158:579-92.
155. Brunt E, Aishima S, Clavien PA, et al. cHCC-CCA: consensus terminology for primary liver carcinomas with both hepatocytic and cholangiocytic differentiation. *Hepatology* 2018;68:113-26.
156. Machida K. Existence of cancer stem cells in hepatocellular carcinoma: myth or reality? *Hepatol Int* 2017;11:143-7.
157. Mishra L, Banker T, Murray J, et al. Liver stem cells and hepatocellular carcinoma. *Hepatology* 2009;49:318-29.
158. Yamashita T, Wang XW. Cancer stem cells in the development of liver cancer. *J Clin Invest* 2013;123:1911-8.
159. Wu Y, Zhang J, Zhang X, et al. Cancer stem cells: a potential breakthrough in HCC-targeted therapy. *Front Pharmacol* 2020;11:198.
160. Li XF, Chen C, Xiang DM, et al. Chronic inflammation-elicited liver progenitor cell conversion to liver cancer stem cell with clinical significance. *Hepatology* 2017;66:1934-51.
161. Dubois-Pot-Schneider H, Fekir K, Coulouarn C, et al. Inflammatory cytokines promote the retrodifferentiation of tumor-derived hepatocyte-like cells to progenitor cells. *Hepatology* 2014;60:2077-90.
162. Belloni L, Di Cocco S, Guerrieri F, et al. Targeting a phospho-STAT3-miRNAs pathway improves vesicular hepatic steatosis in an in vitro and in vivo model. *Sci Rep* 2018;8:13638.
163. Pediconi N, Salerno D, Lupacchini L, et al. EZH2, JMJD3, and UTX epigenetically regulate hepatic plasticity inducing retro-differentiation and proliferation of liver cells. *Cell Death Dis* 2019;10:518.
164. Li M, He Y, Dubois W, et al. Distinct regulatory mechanisms and functions for p53-activated and p53-repressed DNA damage response genes in embryonic stem cells. *Mol Cell* 2012;46:30-42.
165. Young RA. Control of the embryonic stem cell state. *Cell* 2011;144:940-54.
166. Yin X, Li YW, Zhang BH, et al. Coexpression of stemness factors Oct4 and Nanog predict liver resection. *Ann Surg Oncol* 2012;19:2877-87.
167. Zhao X, Sun B, Sun D, et al. Slug promotes hepatocellular cancer cell progression by increasing sox2 and nanog expression. *Oncol Rep* 2015;33:149-56.

168. Liang C, Zhang K, Ge H, et al. Prognostic and clinicopathological value of Nanog in hepatocellular carcinoma: a meta-analysis. *Clin Chim Acta* 2018;477:24-31.
169. Chen CL, Uthaya Kumar DB, Punj V, et al. NANOG metabolically reprograms tumor-initiating stem-like cells through tumorigenic changes in oxidative phosphorylation and fatty acid metabolism. *Cell Metab* 2016;23:206-19.
170. Shan J, Shen J, Liu L, et al. Nanog regulates self-renewal of cancer stem cells through the insulin-like growth factor pathway in human hepatocellular carcinoma. *Hepatology* 2012;56:1004-14.
171. Choi YJ, Lin CP, Ho JJ, et al. miR-34 miRNAs provide a barrier for somatic cell reprogramming. *Nat Cell Biol* 2011;13:1353-60.
172. He L, He X, Lim LP, et al. A microRNA component of the p53 tumour suppressor network. *Nature* 2007;447:1130-4.
173. Marchenko ND, Zaika A, Moll UM. Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. *J Biol Chem* 2000;275:16202-12.
174. Blandino G, Valenti F, Sacconi A, Di Agostino S. Wild type- and mutant p53 proteins in mitochondrial dysfunction: emerging insights in cancer disease. *Semin Cell Dev Biol* 2020;98:105-17.
175. Comel A, Sorrentino G, Capaci V, Del Sal G. The cytoplasmic side of p53's oncosuppressive activities. *FEBS Lett* 2014;588:2600-9.
176. Liu K, Lee J, Kim JY, et al. Mitophagy controls the activities of tumor suppressor p53 to regulate hepatic cancer stem cells. *Mol Cell* 2017;68:281-92.e5.
177. Liu K, Lee J, Ou JJ. Autophagy and mitophagy in hepatocarcinogenesis. *Mol Cell Oncol* 2018;5:e1405142.
178. Ungewitter E, Scrabble H. Delta40p53 controls the switch from pluripotency to differentiation by regulating IGF signaling in ESCs. *Genes Dev* 2010;24:2408-19.
179. Voeltzel T, Flores-Violante M, Zylbersztejn F, et al. A new signaling cascade linking BMP4, BMPR1A, ΔNp73 and NANOG impacts on stem-like human cell properties and patient outcome. *Cell Death Dis* 2018;9:1011.
180. Siddique HR, Feldman DE, Chen CL, et al. NUMB phosphorylation destabilizes p53 and promotes self-renewal of tumor-initiating cells by a NANOG-dependent mechanism in liver cancer. *Hepatology* 2015;62:1466-79.
181. Shi H, Lambert JM, Hautefeuille A, et al. In vitro and in vivo cytotoxic effects of PRIMA-1 on hepatocellular carcinoma cells expressing mutant p53ser249. *Carcinogenesis* 2008;29:1428-34.
182. Bykov VJN, Eriksson SE, Bianchi J, Wiman KG. Targeting mutant p53 for efficient cancer therapy. *Nat Rev Cancer* 2018;18:89-102.
183. Hientz K, Mohr A, Bhakta-Guha D, Efferth T. The role of p53 in cancer drug resistance and targeted chemotherapy. *Oncotarget* 2017;8:8921-46.
184. Testoni B, Schinzari V, Guerrieri F, et al. p53-paralog DNp73 oncogene is repressed by IFNα/STAT2 through the recruitment of the Ezh2 polycomb group transcriptional repressor. *Oncogene* 2011;30:2670-8.

Review

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Current status of laparoscopic repeat liver resection for recurrent hepatocellular carcinoma

Daisuke Hokuto, Takeo Nomi, Takahiro Yoshikawa, Yasuko Matsuo, Naoki Kamitani, Masayuki Sho

Department of Surgery, Nara Medical University, Nara 634-8522, Japan.

Correspondence to: Dr. Takeo Nomi, Department of Surgery, Nara Medical University, 840 Shijo-cho, Kashihara-shi, Nara 634-8522, Japan. E-mail: t.nomi45@gmail.com

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Abstract

Repeat liver resection (RLR) is an effective treatment approach for recurrent hepatocellular carcinoma (HCC) and can provide acceptable long-term outcomes in select patients. Recent randomized controlled trials comparing RLR with radiofrequency ablation revealed that the latter approach was associated with a higher rate of early recurrence compared with RLR. With recent advances in laparoscopic liver resection (LLR), RLR has been increasingly performed using laparoscopy. Several propensity score-matched studies reported that laparoscopic RLR achieved lower blood loss and shorter hospital stays compared to open RLR. However, laparoscopic RLR requires more advanced techniques because of adhesions formed after the previous liver resection, changes in anatomical landmarks, and deformity of the remnant liver. The recently described difficulty classification of laparoscopic RLR is based on five factors including type of previous liver resection (open or laparoscopic), number of previous liver resections, surgical procedure used in previous liver resections, tumor location in previous liver resections, and difficulty score of LLR for recurrent HCC. We reviewed the available literature to summarize available evidence suggesting that laparoscopic RLR might be considered a more minimally invasive surgical treatment approach for recurrent HCC as long as the indication for laparoscopic RLR is carefully determined.

Keywords: Hepatocellular carcinoma, repeat liver resection, laparoscopic repeat liver resection

INTRODUCTION

Repeat liver resection (RLR) is an effective treatment approach for recurrent hepatocellular carcinoma (HCC) and can provide acceptable long-term outcomes for select patients^[1-3]. However, RLR is considered



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a difficult procedure due to adhesions associated with previous liver resection and changes in anatomical recognition. In fact, several studies have reported high morbidity rates after RLR^[4-6]. With advances in laparoscopic liver resection (LLR), RLR has been increasingly performed laparoscopically^[7]. LLR is a less invasive procedure that is associated with better short-term outcomes compared with open liver resection (OLR)^[8,9]. Similarly, the superiority of laparoscopic RLR over open RLR in short-term outcomes has been reported^[10-12]. However, none of the studies were randomized controlled trials and laparoscopic RLR might be performed in select patients. Laparoscopic RLR was discussed at the first European Guidelines Meeting on Laparoscopic Liver Surgery (Southampton 2017) and considered an appropriate option^[13]. However, most laparoscopic liver surgeons suggest that repeat liver resection significantly increases the difficulty of LLR^[14]. Currently, the indications for laparoscopic RLR vary between centers. Considered a difficult procedure, RLR poses a range of challenges depending on the location of recurrent tumor and previous liver resections. A recent report has introduced a difficulty classification for laparoscopic RLR^[15]. Specifically, the level of difficulty was determined on the basis of the type of previous liver resection (open or laparoscopic), number of previous liver resections, surgical procedure and tumor location in previous liver resections, and difficulty score of LLR for recurrent HCC^[16]. RLR after LLR has been increasing in parallel with the increasing number of LLR for HCC. In colorectal surgery, the laparoscopic approach has been reported to reduce adhesion formation^[17]. Similarly, RLR after LLR is associated with less adhesion formation compared with RLR performed after OLR. Therefore, RLR in the era of LLR has distanced characteristics. Conversely, percutaneous radiofrequency ablation (RFA), a technique developed in the last two decades^[18,19], has been demonstrated to be useful in small HCCs^[20]. In general, patients who previously undergo hepatectomy for HCC are examined regularly by imaging studies, which facilitate the frequent detection of small-diameter recurrent HCCs. Within this framework, the role of liver resection for recurrent HCC might be changing. In this review, we describe the current status of laparoscopic RLR for HCC in the era of LLR and RFA.

SIGNIFICANCE OF REPEAT LIVER RESECTION FOR RECURRENT HEPATOCELLULAR CARCINOMA

Relatively good survival outcomes of RLR for HCC have been reported since the 1990s^[21-24]. These earlier studies reported that 5-year survival rate after RLR was around 50%, which was comparable to the prognosis of primary liver resection and was better than the prognosis of transarterial chemoembolization^[25,26]. However, RLR was performed in select patients with relatively better liver function and tumor factors compared to those undergoing transarterial chemoembolization. Moreover, disease-free survival rates were significantly lower with RLR compared to initial liver resection^[2,3]. Studies investigating prognostic factors after RLR for HCC reported that portal vein invasion at the time of first liver resection, portal vein invasion in RLR, multiple HCCs at the time of first liver resection, and disease-free interval of less than one year were independent prognostic factors after RLR^[1,3,27]. The 5-year survival rate was 86.0% in patients without prognostic factors^[1]. These results provide clear evidence that RLR for recurrent HCC is a useful treatment option for select patients.

In the last decade, several studies investigating long-term outcomes in patients undergoing three or more RLRs reported that the 5-year survival and disease-free survival rates after the third RLR were 40.0%-68.2% and 12.8%-33.8%, respectively^[6,28,29]. These results were comparable to those observed after the second RLR. Regarding short-term outcomes, Mise *et al.*^[6] reported significantly longer surgical duration and higher postoperative morbidity with three or more RLRs, whereas Yamashita *et al.*^[28] reported no significant differences. These results indicated that three or more RLRs might be considered an acceptable treatment approach in patients whose liver function and tumor factors were within surgical indications.

ADVANCES IN LAPAROSCOPIC REPEAT LIVER RESECTION

Progress in surgical procedures and devices has enabled the expansion of surgical indications for LLR^[30-32]. However, laparoscopic RLR demands a more advanced technique due to adhesion formation following prior liver resection, changes in anatomical landmarks, and deformity of the remnant liver. In 2009, Belli *et al.*^[33] reported 12 patients who underwent laparoscopic RLR for recurrent HCC. The authors concluded that laparoscopic RLR was feasible and that the degree of adhesion was mild in patients undergoing LLR as initial liver resection. In 2011, Hu *et al.*^[34] reported six patients who underwent laparoscopic RLR for recurrent HCC. No intra- or postoperative complications were observed; however, the authors noted that the patients were carefully selected. Another report in 2011 was a tri-institutional analysis of 76 patients undergoing laparoscopic RLR^[35]. The study cohort comprised 63, 3, and 10 patients with metastatic liver tumors, HCC and benign tumors, respectively. Seven patients (9.2%) were converted to open surgery, and there were no perioperative deaths. The patients who underwent OLR as initial liver resection experienced higher intraoperative blood loss compared to those who underwent LLR. In 2016, a review by Goh *et al.*^[36], which included 103 patients who underwent laparoscopic RLR for recurrent HCC, reported that only 2 patients (1.9%) were converted to open surgery. These retrospective analyses have provided evidence for the feasibility and safety of LLR in select patients.

In 2018, Noda *et al.*^[37], and Ome *et al.*^[38], reported their findings on the comparison between laparoscopic RLR and open RLR in their institutions indicating that blood loss was less and hospital stay shorter with laparoscopic RLR; there were no differences in operative duration and postoperative complications. Thereafter, similar results have been reported from several single centers^[7,12,39]. In 2019, a multicenter propensity score-matched study compared laparoscopic and open RLR for colorectal liver metastasis^[40]. After matching, 105 pairs were extracted from the initial cohort of 271 patients who underwent laparoscopic RLR and 154 patients who underwent open RLR. Laparoscopic RLR was associated with a significantly shorter operative duration (200 min *vs.* 256 min), less intraoperative blood loss (200 mL *vs.* 300 mL), and shorter postoperative hospital stay (5 days *vs.* 6 days), whereas postoperative morbidity and mortality rates were similar between the groups. Similar results of laparoscopic RLR for HCC were reported in another multicenter propensity score based study^[11]. Table 1 summarizes the comparison between laparoscopic RLR and open RLR of these reports.

Although no randomized controlled trials to date have compared laparoscopic RLR and open RLR, evidence from previous studies indicate that laparoscopic RLR is feasible and safe as long as the indications are within the capabilities of institutions and surgeons.

DIFFICULTY CLASSIFICATION OF LAPAROSCOPIC REPEAT LIVER RESECTION

Several factors affect the level of difficulty in laparoscopic LLR. Previous OLR was reported to increase difficulty in laparoscopic RLR^[33,35]. In 2014, Ban *et al.*^[16] described the first difficulty scoring system for LLR. The difficulty score was based on tumor location, extent of liver resection, tumor size, tumor proximity to major vessels and liver function. The difficulty of LLR was classified into low, intermediate and high levels. This score can be effortlessly utilized for risk assessment in patients undergoing laparoscopic RLR. Recently, Kinoshita *et al.*^[15] reported on difficulty classification of laparoscopic RLR. They reviewed 60 cases of laparoscopic RLR in their institution and analyzed the factors accounting for prolonged operative duration or severe adhesion. As a result, an intermediate or high LLR difficulty score, two or more previous liver resections, a history of previous major liver resection, and tumor location near the resected surface of previous liver resection were identified. These five factors were reported to be correlated with operative duration in laparoscopic RLR. The authors then classified the patients undergoing RLR into low-risk (score, 0-1), intermediate-risk (score, 2-3), and high-risk (score, 4-5) categories and found that the risk was significantly correlated with operative duration^[15].

Table 1. Outcome of treatment after recurrent hepatocellular carcinoma

	Laparoscopic repeat liver resection (laparoscopic RLR)	Open repeat liver resection (open RLR)	P value
Morise <i>et al.</i> ^[11] (2020)			
Blood loss (mL)	268	497	0.001
Duration of operation (min)	272	232	0.007
90-day morbidity, beyond Clavien-dindo II (%)	15.1	13.0	0.611
90-day Mortality (%)	0.42	0.84	0.623
Postoperative hospital stay (days)	10.4	9.6	0.327
van der Poel <i>et al.</i> ^[40] (2019)			
Blood loss (mL)	200 (50-450)	300 (100-600)	0.077
Duration of operation (min)	200 (123-273)	256 (199-320)	< 0.001
90-day morbidity, beyond Clavien-dindo II (%)	5.7	5.7	0.319
90-day Mortality (%)	1.9	0	0.5
Postoperative hospital stay (days)	5 (3-8)	6 (5-8)	0.028
Onoe <i>et al.</i> ^[39] (2020)			
Blood loss(mL)	100 (0-1050)	435 (30-1920)	0.001
Duration of operation (min)	276 (125-589)	292 (96-972)	0.861
90-day morbidity, beyond Clavien-dindo III (%)	6.75	14.3	0.297
90-day Mortality (%)	0	0	1
Postoperative hospital stay (days)	10 (4-50)	14.5 (10-76)	0.002
Goh <i>et al.</i> ^[12] (2019)			
Blood loss (mL)	200 (100-425)	250 (125-475)	0.345
Duration of operation (min)	315 (181-395)	125 (99-184)	< 0.001
30-day morbidity, beyond Clavien-dindo III (%)	0	5	0.48
30-day Mortality (%)	0	10	0.56
Postoperative hospital stay (days)	4 (3-5)	7.5 (6-10)	0.001
Ome <i>et al.</i> ^[38] (2018)			
Blood loss (mL)	30 (0-1012)	652 (20-12046)	< 0.001
Duration of operation (min)	217 (43-356)	222 (84-923)	0.56
30-day morbidity, beyond Clavien-dindo III (%)	6.1	10.8	0.393
30-day Mortality (%)	3	0	0.471
Postoperative hospital stay (days)	6.5 (3-47)	9.0 (5-78)	< 0.001
Noda <i>et al.</i> ^[37] (2018)			
Blood loss (mL)	159	502	0.004
Duration of operation (min)	225	237	0.601
30-day morbidity, beyond Clavien-dindo III (%)	0	14.5	0.009
30-day Mortality (%)	0	0	1
Postoperative hospital stay (days)	14.2	19.2	0.028

LONG-TERM OUTCOMES AFTER LAPAROSCOPIC REPEAT LIVER RESECTION FOR RECURRENT HEPATOCELLULAR CARCINOMA

A global systematic review and meta-analysis in 2013 reported that the long-term outcomes were comparable between LLR and OLR for initial HCC^[41]. Subsequently, propensity score-matched studies comparing LLR and OLR for initial HCC revealed that overall survival (OS) and recurrence-free survival (RFS) were not different between LLR and OLR, while short-term outcomes were significantly better with LLR^[42-44]. Similar results were obtained in a study limited to patients undergoing major hepatectomy^[45]. However, no randomized controlled trials have compared LLR and OLR for initial HCC. LLR might be performed in select patients. Moreover, Stiles *et al.*^[46] reported that unplanned conversion to OLR from LLR for HCC was associated with inferior OS compared to non-converted cases. Therefore, the long-term prognosis of LLR might be comparable to OLR as long as the indication for LLR is carefully evaluated. Regarding RLR, few studies have compared long-term survival outcomes between laparoscopic RLR and open RLR. A recent multicenter, propensity score-based study by Morise *et al.*^[11], which included 42 surgery centers around the world, reported comparable median survival times between laparoscopic RLR and open RLR (12.55 years *vs.* 8.94 years; $P = 0.086$), although intraoperative blood loss was significantly

greater with open RLR (268 mL *vs.* 497 mL; $P = 0.001$) and operative duration was significantly longer with laparoscopic LLR (272 min *vs.* 232 min; $P = 0.007$). These findings suggest acceptable long-term outcomes with laparoscopic RLR for recurrent HCC.

LAPAROSCOPIC REPEAT LIVER RESECTION AND RADIOFREQUENCY ABLATION FOR RECURRENT HEPATOCELLULAR CARCINOMA

Percutaneous RFA, a technique developed in the last two decades has been reported to be an effective and safe treatment for small HCCs^[18-20]. Nowadays, RFA is widely used as non-surgical treatment for HCCs because the therapeutic effect was reported to be more effective than microwave coagulation therapy or percutaneous ethanol injection therapy^[47,48]. In general, recurrent HCCs are detected at a small diameter because of routine screening after initial liver resection. Therefore, RFA for recurrent HCCs could be an effective treatment.

However, a very recent randomized controlled trial comparing RLR with RFA for recurrent HCC revealed that RFA was associated with a higher rate of early recurrence compared with RLR (40.3% *vs.* 23.3%, $P = 0.04$)^[49]. On the other hand, the trial results indicated that RLR had a higher complication rate than RFA (22.4% *vs.* 7.3%, $P = 0.001$). These findings highlight that RLR remains an important treatment approach for recurrent HCC even in the era of RFA. As mentioned previously, laparoscopic RLR can be performed safely, with less blood loss and shorter postoperative hospital stay compared to open RLR in select cases. Therefore, laparoscopic RLR for recurrent HCC might be considered a standard treatment with reduced surgical invasiveness as long as the indication for RLR is carefully considered.

CONCLUSION

RLR for recurrent HCC remains an important treatment strategy even in the era of RFA. Laparoscopic RLR could be considered a more minimally invasive surgical treatment than open RLR for patients with recurrent HCC.

DECLARATIONS

Authors' contributions

Wrote this article: Hokuto D

Supervised this article: Nomi T, Sho M

Treated the patients included in this article: Yoshikawa T, Matsuo Y, Kamitani N

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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REFERENCES

1. Minagawa M, Makuuchi M, Takayama T, Kokudo N. Selection criteria for repeat hepatectomy in patients with recurrent hepatocellular carcinoma. *Ann Surg* 2003;238:703-10.
2. Nakajima Y, Ko S, Kanamura T, et al. Repeat liver resection for hepatocellular carcinoma. No competing interests declared. *Journal of the American College of Surgeons* 2001;192:339-44.
3. Shimada M, Takenaka K, Taguchi K, et al. Prognostic factors after repeat hepatectomy for recurrent hepatocellular carcinoma. *Ann Surg* 1998;227:80-5.
4. Sadamori H, Yagi T, Shinoura S, et al. Risk factors for major morbidity after liver resection for hepatocellular carcinoma. *Br J Surg* 2013;100:122-9.
5. Yoshioka R, Saiura A, Koga R, Seki M, Kishi Y, Yamamoto J. Predictive factors for bile leakage after hepatectomy: analysis of 505 consecutive patients. *World J Surg* 2011;35:1898-903.
6. Mise Y, Hasegawa K, Shindoh J, et al. The feasibility of third or more repeat hepatectomy for recurrent hepatocellular carcinoma. *Ann Surg* 2015;262:347-57.
7. Wakabayashi T, Felli E, Memeo R, et al. Short-term outcomes of laparoscopic repeat liver resection after open liver resection: a systematic review. *Surg Endosc* 2019;33:2083-92.
8. Fretland ÅA, Dagenborg VJ, Bjørnelv GMW, et al. Laparoscopic versus open resection for colorectal liver metastases: the OSLO-COMET randomized controlled trial. *Ann Surg* 2018;267:199-207.
9. Ciria R, Cherqui D, Geller DA, Briceno J, Wakabayashi G. Comparative short-term benefits of laparoscopic liver resection: 9000 cases and climbing. *Ann Surg* 2016;263:761-77.
10. Liang Y, Lin C, Zhang B, et al. Perioperative outcomes comparing laparoscopic with open repeat liver resection for post-hepatectomy recurrent liver cancer: a systematic review and meta-analysis. *Int J Surg* 2020;79:17-28.
11. Morise Z, Aldrighetti L, Belli G, et al; ILLS-Tokyo collaborator group. Laparoscopic repeat liver resection for hepatocellular carcinoma: a multicentre propensity score-based study. *Br J Surg* 2020;107:889-95.
12. Goh BKP, Syn N, Teo JY, et al. Perioperative outcomes of laparoscopic repeat liver resection for recurrent HCC: comparison with open repeat liver resection for recurrent HCC and laparoscopic resection for primary HCC. *World J Surg* 2019;43:878-85.
13. Abu Hilal M, Aldrighetti L, Dagher I, et al. The southampton consensus guidelines for laparoscopic liver surgery: from indication to implementation. *Ann Surg* 2018;268:11-8.
14. Halls MC, Cherqui D, Taylor MA, Primrose JN, Abu Hilal M; Collaborators of The Difficulty of Laparoscopic Liver Surgery Survey. Are the current difficulty scores for laparoscopic liver surgery telling the whole story? An international survey and recommendations for the future. *HPB (Oxford)* 2018;20:231-6.
15. Kinoshita M, Kanazawa A, Kodai S, et al. Difficulty classifications of laparoscopic repeated liver resection in patients with recurrent hepatocellular carcinoma. *Asian J Endosc Surg* 2020;13:366-74.
16. Ban D, Tanabe M, Ito H, et al. A novel difficulty scoring system for laparoscopic liver resection. *J Hepatobiliary Pancreat Sci* 2014;21:745-53.
17. Dowson HM, Bong JJ, Lovell DP, Worthington TR, Karanjia ND, Rockall TA. Reduced adhesion formation following laparoscopic versus open colorectal surgery. *Br J Surg* 2008;95:909-14.
18. Tiong L, Maddern GJ. Systematic review and meta-analysis of survival and disease recurrence after radiofrequency ablation for hepatocellular carcinoma. *Br J Surg* 2011;98:1210-24.
19. Lau WY, Lai EC. The current role of radiofrequency ablation in the management of hepatocellular carcinoma: a systematic review. *Ann Surg* 2009;249:20-5.
20. Kang TW, Kim JM, Rhim H, et al. Small hepatocellular carcinoma: radiofrequency ablation versus nonanatomic resection--propensity score analyses of long-term outcomes. *Radiology* 2015;275:908-19.
21. Matsuda Y, Ito T, Oguchi Y, Nakajima K, Izukura T. Rationale of surgical management for recurrent hepatocellular carcinoma. *Ann Surg* 1993;217:28-34.
22. Shimada M, Matsumata T, Taketomi A, Yamamoto K, Itasaka H, Sugimachi K. Repeat hepatectomy for recurrent hepatocellular carcinoma. *Surgery* 1994;115:703-6.
23. Suenaga M, Sugiura H, Kokuba Y, Uehara S, Kurumiya T. Repeated hepatic resection for recurrent hepatocellular carcinoma in eighteen cases. *Surgery* 1994;115:452-7.
24. Hu RH, Lee PH, Yu SC, et al. Surgical resection for recurrent hepatocellular carcinoma: prognosis and analysis of risk factors. *Surgery* 1996;120:23-9.
25. Takayasu K, Wakao F, Moriyama N, et al. Postresection recurrence of hepatocellular carcinoma treated by arterial embolization: analysis of prognostic factors. *Hepatology* 1992;16:906-11.
26. Okazaki M, Yamasaki S, Ono H, Higashihara H, et al. Chemoembolotherapy for recurrent hepatocellular carcinoma in the residual liver after hepatectomy. *Hepatogastroenterology* 1993;40:320-3.
27. Itamoto T, Nakahara H, Amano H, et al. Repeat hepatectomy for recurrent hepatocellular carcinoma. *Surgery* 2007;141:589-97.
28. Yamashita Y, Shirabe K, Tsujita E, et al. Third or more repeat hepatectomy for recurrent hepatocellular carcinoma. *Surgery*

- 2013;154:1038-45.
29. Wu CC, Cheng SB, Yeh DC, Wang J, P'eng FK. Second and third hepatectomies for recurrent hepatocellular carcinoma are justified. *Br J Surg* 2009;96:1049-57.
30. Kaneko H, Takagi S, Shiba T. Laparoscopic partial hepatectomy and left lateral segmentectomy: Technique and results of a clinical series. *Surgery* 1996;120:468-75.
31. Nitta H, Sasaki A, Fujita T, et al. Laparoscopy-assisted major liver resections employing a hanging technique: the original procedure. *Ann Surg* 2010;251:450-3.
32. Buell JF, Thomas MT, Rudich S, et al. Experience with more than 500 minimally invasive hepatic procedures. *Ann Surg* 2008;248:475-86.
33. Belli G, Cioffi L, Fantini C, et al. Laparoscopic redo surgery for recurrent hepatocellular carcinoma in cirrhotic patients: feasibility, safety, and results. *Surg Endosc* 2009;23:1807-11.
34. Hu M, Zhao G, Xu D, Liu R. Laparoscopic repeat resection of recurrent hepatocellular carcinoma. *World J Surg* 2011;35:648-55.
35. Shafae Z, Kazaryan AM, Marvin MR, et al. Is laparoscopic repeat hepatectomy feasible? A tri-institutional analysis. *J Am Coll Surg* 2011;212:171-9.
36. Goh BK, Teo JY, Chan CY, Lee SY, Cheow PC, Chung AY. Review of 103 Cases of Laparoscopic Repeat Liver Resection for Recurrent Hepatocellular Carcinoma. *J Laparoendosc Adv Surg Tech A* 2016;26:876-81.
37. Noda T, Eguchi H, Wada H, et al. Short-term surgical outcomes of minimally invasive repeat hepatectomy for recurrent liver cancer. *Surg Endosc* 2018;32:46-52.
38. Ome Y, Hashida K, Yokota M, et al. The feasibility and efficacy of pure laparoscopic repeat hepatectomy. *Surg Endosc* 2018;32:3474-9.
39. Onoe T, Yamaguchi M, Irei T, et al. Feasibility and efficacy of repeat laparoscopic liver resection for recurrent hepatocellular carcinoma. *Surg Endosc* 2020;34:4574-81.
40. van der Poel MJ, Barkhatov L, Fuks D, et al. Multicentre propensity score-matched study of laparoscopic versus open repeat liver resection for colorectal liver metastases. *Br J Surg* 2019;106:783-9.
41. Yin Z, Fan X, Ye H, Yin D, Wang J. Short- and long-term outcomes after laparoscopic and open hepatectomy for hepatocellular carcinoma: a global systematic review and meta-analysis. *Ann Surg Oncol* 2013;20:1203-15.
42. Lee JJ, Conneely JB, Smoot RL, et al. Laparoscopic versus open liver resection for hepatocellular carcinoma at a North-American Centre: a 2-to-1 matched pair analysis. *HPB (Oxford)* 2015;17:304-10.
43. Han HS, Shehta A, Ahn S, Yoon YS, Cho JY, Choi Y. Laparoscopic versus open liver resection for hepatocellular carcinoma: Case-matched study with propensity score matching. *J Hepatol* 2015;63:643-50.
44. Sposito C, Battiston C, Facciorusso A, et al. Propensity score analysis of outcomes following laparoscopic or open liver resection for hepatocellular carcinoma. *Br J Surg* 2016;103:871-80.
45. Komatsu S, Brustia R, Goumard C, Perdigao F, Soubrane O, Scatton O. Laparoscopic versus open major hepatectomy for hepatocellular carcinoma: a matched pair analysis. *Surg Endosc* 2016;30:1965-74.
46. Stiles ZE, Glazer ES, Deneve JL, Shibata D, Behrman SW, Dickson PV. Long-Term Implications of Unplanned Conversion During Laparoscopic Liver Resection for Hepatocellular Carcinoma. *Ann Surg Oncol* 2019;26:282-9.
47. Ohmoto K, Yoshioka N, Tomiyama Y, et al. Comparison of therapeutic effects between radiofrequency ablation and percutaneous microwave coagulation therapy for small hepatocellular carcinomas. *J Gastroenterol Hepatol* 2009;24:223-7.
48. Seki T, Wakabayashi M, Nakagawa T, et al. Percutaneous microwave coagulation therapy for patients with small hepatocellular carcinoma: comparison with percutaneous ethanol injection therapy. *Cancer* 1999;85:1694-702.
49. Xia Y, Li J, Liu G, et al. Long-term Effects of Repeat Hepatectomy vs Percutaneous Radiofrequency Ablation Among Patients With Recurrent Hepatocellular Carcinoma: A Randomized Clinical Trial. *JAMA Oncol* 2020;6:255-63.

Review

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Diabetes and NAFLD: a high-risk cohort with definite therapeutic potential

Lucia Brodosi^{1,2}, Alessandra Musio^{1,2}, Francesca Alessandra Barbanti^{1,2}, Dorina Mita^{1,2}, Giulio Marchesini¹, Maria Letizia Petroni^{1,2}

¹Department of Medical and Surgical Sciences, "Alma Mater" University, Bologna 40138, Italy.

²Azienda Ospedaliero-Universitaria di Bologna, Bologna 40138, Italy.

Correspondence to: Dr. Lucia Brodosi, Azienda Ospedaliero-Universitaria di Bologna, via Albertoni 15, Bologna 40138, Italy.
E-mail: lucia.brodosi2@unibo.it

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Abstract

Despite the fact that non-alcoholic fatty liver disease (NAFLD) and its severe clinical forms [non-alcoholic steatohepatitis (NASH) and NASH-cirrhosis] are highly prevalent in the general population, there are no licensed drugs for NAFLD, and lifestyle intervention remains the only treatment accepted by international guidelines. This is despite massive investments in research by pharmaceutical companies. In the presence of type 2 diabetes, novel anti-diabetic drugs offer an opportunity to reduce the burden of NAFLD, by adequate control of glucose and lipid metabolism, also reducing the risk of NASH progression, advanced fibrosis, and finally hepatocellular carcinoma. We extensively reviewed the literature, based either on registration studies, ad hoc randomized studies or real-world data, to define the effectiveness of anti-diabetic drugs in the treatment of NAFLD and prevention of hepatocellular carcinoma (HCC). Metformin provides the best evidence for decreased risk of HCC, pioglitazone was associated with decreased progression to fibrosis, glucagon-like peptide-1 receptor agonists offer a possible opportunity to reduce NAFLD progression coupled with a definite protection for cardiovascular outcomes, and sodium-glucose cotransporter-2 inhibitors are likely to reduce lipid burden, simultaneously reducing the risk of progressive renal and heart failure. For the latter two drug classes, the effects on NAFLD might largely explained by decreased body weight, in keeping with the beneficial effects of intensive lifestyle intervention.

Keywords: Metformin, pioglitazone, incretins, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT-2 inhibitors, insulin, cirrhosis



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INTRODUCTION

The clinical and economic burden associated with non-alcoholic fatty liver disease (NAFLD) is becoming of paramount importance for national health systems globally. Most recent data indicate that approximately 25% of adults may be classified as NAFLD^[1], one in 4 to 5 patients with NAFLD have non-alcoholic steatohepatitis (NASH)^[1], and 1.5% have advanced fibrosis^[2], the hallmark of disease progression to cirrhosis^[3].

The hepatic disease is part of a multifaceted involvement of other tissues and organs, primarily the cardiovascular system and the kidney^[4], within the frame of the metabolic syndrome^[5], that adds to the liver in driving long-term outcomes^[6]. For this reason, there is a compelling need to adjust treatment to minimize cardiovascular risk in all patients with NAFLD, as suggested by national^[7] and international^[8] guidelines.

Despite much research and investment by pharmaceutical companies, no drugs have so far been approved for treatment by regulatory authorities, and adherence to healthier lifestyle remains the only accepted treatment strategy^[9]. Several drugs failed the agreed treatment outcomes (reduced fibrosis without worsening of NASH or reduced necroinflammation, no worsening of fibrosis^[10]) for approval during phase 2 or phase 3 randomized controlled studies (RCTs)^[11]; only obeticholic acid fulfilled the targets in a phase 3 study^[12], but the Food and Drug Administration required additional studies considering the low benefit/risk ratio^[13].

Individuals with Type 2 diabetes mellitus (T2DM) constitute a large cohort of NAFLD cases. The prevalence of NAFLD in T2DM is as high as 60%^[14], and T2DM increases the risk of disease progression to cirrhosis as well as the occurrence of hepatocellular carcinoma (HCC)^[15-17]. The relationship between T2DM and NAFLD appears to be bidirectional, with T2DM increasing the risk of NAFLD and NAFLD favoring the development of altered glucose regulation and T2DM^[18]. Initially considered the hepatic manifestation of metabolic syndrome^[19], and consequently as a likely effect of diabetes^[20], it has also been suggested that liver fat accumulation and NAFLD might indeed be the metabolic driver of T2DM^[21]. This evidence makes the development of T2DM an additional outcome of NAFLD treatment and prompts the need for strict control of glucose metabolism in NAFLD cases.

In the past 15 years the treatment of T2DM has completely changed. Second-generation sulfonylureas and glinides, very effective oral drugs long considered the standard of treatment before prescribing insulin injection, have been moved as third-line treatment and limited to rare settings in most recent international guidelines, because of poor durability and a high risk of hypoglycemia and coronary artery disease^[22-24]. Very effective and safer drugs dipeptidylpeptidase-4 inhibitors (DPP-4Is), glucagon-like peptide-1 receptor agonists [(GLP-1Ras) and sodium-glucose transporter-2 inhibitors (SGLT-2Is)] were added to the classical armamentarium (metformin, acarbose, sulfonylureas and glinides, insulin) with definite advantages on the impending risk of hypoglycemia, cardiovascular disease and heart failure^[22]. Their efficacy and safety has been demonstrated in registration studies as well as in large cardiovascular outcome trials (CVOTs) required by regulatory agencies such as the FDA and European Medicines Agency^[25]. The effects on liver fat accumulation have been tested *vs.* sulfonylureas/glinides, or are under investigation. Also, pioglitazone, an insulin-sensitizer of limited use following a series of warning data on class safety, initially involving rosiglitazone^[26], has shown positive effects on cardiovascular outcomes^[27]. In patients with NAFLD, irrespective of the presence of T2DM, it was associated a reduced risk of advanced fibrosis^[28], and its use is now recommended by national and international guidelines^[8].

The present review is aimed at defining the role of novel anti-diabetic drugs for the treatment of NAFLD in patients with T2DM, with particular reference to the prevention of HCC. Data were retrieved from ad

Table 1. Metabolic and clinical effects of anti-diabetic drugs

Drug class	Metabolic control	Hypo-glycemia	Cardiovascular system	Heart failure	Specific beneficial/adverse effects
Metformin	+	±	Uncertain protection	Null	Cancer protection Acidosis, anemia
α-Glucosidase inhibitors	+	-	Null	Null	Modest weight loss GI discomfort
Pioglitazone	++	±	Protective	Increased risk	Weight gain, non-osteoporotic fractures
Sulfonylureas/Glinides	+++	+++	Increased risk	Increased risk	Alcohol interaction Weight gain, low durability
DPP-4 inhibitors	++	-	Null	Null	High durability Flu-like symptoms, runny nose
GLP-1 receptor agonists	+++	-	Protective	Null	Weight loss Nausea, vomiting
SGLT-2 inhibitors	+++	-	Protective	Protective	Weight loss, renal protection Genito-urinary infections
Insulin (basal or basal-bolus)	++++	++++	Protective*	Protective*	Weight gain, highly negative impact on quality of life

*Protection exerted by improved metabolic control; Null: no evidence of specific protection

hoc RCTs, as well as from the re-analysis of large registration or CVOT trials. More recently, several large epidemiological surveys of real-world data became available, and their support to define the best treatment to prevent liver disease progression is also reported.

DATA SEARCH AND ANALYSIS

We searched PubMed and www.clinicaltrials.gov for studies on novel anti-diabetic drug use in patients with NAFLD or NASH. In PubMed we used the string [liver steatosis (MeSH Terms)] OR [NAFLD (MeSH Terms)] OR [HCC (Text Word)] OR [carcinoma (Text Word)] AND [adult onset diabetes mellitus (MeSH Terms)] AND [treatment (Text Word)] filtered by “humans”. The string retrieved 694 references published in the period 1988-2020. On www.clinicaltrials.gov we used the string “NAFLD OR NASH” as “condition or disease” field, while the names of the classes and later the names of the individual molecules were entered in the “other terms” field. On the left bar, in “Study Phase” we selected “Phase 2, 3, and 4”. Only studies with more than 10 participants were considered. Later, the references of all retrieved studies and review articles were scrutinized for missing references, and duplicate studies were removed. Data of the available evidence is summarized in [Table 1](#).

RESULTS

Metformin

Metformin has long been considered the first-line drug for the treatment of T2DM and it is still indicated for all individuals who can tolerate its use without gastrointestinal discomfort. Despite its insulin-sensitizing activity, potentially reducing lipid burden, metformin is no longer specifically indicated for NAFLD, following a few studies and a review article where it failed to reduce histological severity of NAFLD^[29]. However, metformin is now living a second life, as it were, considering its HCC-preventive action^[30-32], coupled with reduced all-site cancer risk^[33]. Continuation of metformin was also shown to improve overall survival in NASH-cirrhosis with Child-Pugh class A and B^[34]; these beneficial effects justify the statement of international guidelines suggesting the use of background metformin for all T2DM patients with NAFLD^[8].

Pioglitazone

Pioglitazone is an anti-diabetic drug that activates peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear receptor, mostly expressed in the adipose tissue, and to a lesser extent in other organs, including the liver. The activation of the PPARγ quells the production of liver collagen by hepatic stellate cells,

promotes the differentiation of adipocytes, decreases leptin and IL-6 concentration, increases adiponectin levels, and above all, reduces insulin resistance, the driver of NAFLD^[35]. Pioglitazone has been tested in NAFLD at the target dose of 30-45 mg/day in several RCTs with histologic outcomes, showing a reduction of necroinflammation (NAFLD activity score - NAS)^[36-40], as well as improvement in fibrosis in a systematic meta-analysis^[41]. Pioglitazone also reduces the risk of cardiovascular and cerebrovascular outcomes^[42,43], as well as of HCC (odds ratio, OR = 0.83, 95%CI: 0.72-0.95)^[44]. This makes pioglitazone the treatment of choice of NASH, independent of the presence of T2DM. Notably, treatment discontinuation is followed by NASH recurrence^[45]. Pioglitazone treatment is associated with moderate weight gain, and the risks of non-osteoporotic fractures and, particularly, of heart failure are also increased; for these reasons the drug should not be used in elderly patients^[46]. Adverse events are probably rare at lower doses (15 mg/day), but the effects on the liver are also unknown. At present, the use of pioglitazone is off-label outside T2DM and informed consent is needed before treatment in individuals without diabetes.

Dipeptidyl-peptidase-4 inhibitors

DPP-4Is (sitagliptin, vildagliptin, saxagliptin and alogliptin) decrease blood glucose by preventing the rapid degradation in incretins, thus increasing glucose-dependent insulin release^[47]. This class of antidiabetic drugs has progressively entered the market in the past 15 years, showing a moderate effect on glucose control, and no risk of hypoglycemia or adverse cardiovascular outcomes. A meta-analysis by Carbone *et al.*^[48] on the effects of incretin treatment in patients with NASH and T2DM including 66 participants treated with sitagliptin for between 16 and 36 weeks found a significant mean reduction of alanine aminotransferase (ALT) in the two sitagliptin-treated cohorts (mean 17.7 U/L; 95%CI: 12.4-23.1; $P < 0.001$). In a small cohort with T2DM and NASH, the administration of sitagliptin 100 mg/day for one year determined a significant improvement in hepatocyte ballooning ($P = 0.014$) and total NAS ($P = 0.04$), as well as a decrease in ALT and aspartate aminotransferase (AST), an index more closely correlated with chronic liver damage^[49]. Similar data on liver enzymes were reported in 44 patients treated for six months with DPP-4Is^[50], whereas the improvement of NAS was confirmed in 40 NASH patients^[51], randomized to lifestyle changes *vs.* lifestyle changes associated with sitagliptin (NAS: -1.9 ± 1.4 *vs.* -0.7 ± 1.1 ; $P = 0.006$).

On the contrary, no differences in aminotransferases, liver fat content or liver stiffness were reported in a 24-week RCT including patients with pre-diabetes or early diabetes^[52,53], treated with sitagliptin (100 mg per day), as well as in two studies in which sitagliptin was tested against placebo for 12 weeks (no differences in serum liver enzymes, hepatic fat content, fibrosis). No differences were reported in surrogate biomarkers of fibrosis, namely NAFLD fibrosis score [NFS], Fibrosis-4 score [FIB-4], aminotransferase-to-platelet ratio index [APRI]^[51,53]. In summary, the use of DPP-4Is in T2DM with NAFLD appears to be safe, but without any systematic advantage on progressive liver disease. There are no specific studies on their possible effects on the risk of HCC in T2DM.

Glucagon-like peptide-1 receptor agonists

GLP-1RAs (exenatide, lixisenatide, liraglutide, dulaglutide, semaglutide) are potent injectable anti-diabetic drugs, mimicking the effects of endogenous incretins on insulin release, gastrointestinal motility, and the central nervous system (reduced appetite and food intake^[47]). CVOTs demonstrated that GLP-1RAs, as a class but with some differences between rapid- (exenatide b.i.d. and lixisenatide) and long-acting drugs, reduce the risk of major cardiovascular events in T2DM^[23], and lead to a systematic weight loss^[54]. In patients with NAFLD and T2DM, liraglutide was initially reported to reduce liver inflammation (AST, ALT) and liver fibrosis scores (APRI index). These favorable effects might possibly derive from or be enhanced by the concomitant weight and HbA1c reduction^[55]. Eguchi *et al.*^[56] also found a reduction in NAS and Brunt's classification grade after a 96-week treatment with liraglutide in ten patients with biopsy-proven NASH/NAFLD.

A beneficial role of liraglutide has been convincingly demonstrated in the pilot LEAN (Liraglutide Efficacy and Action in NASH) study^[57], a 48-week RCT in which liraglutide was tested *vs.* placebo in 52 patients with biopsy-confirmed NASH. The study included patients with stage 3 fibrosis (38% in liraglutide *vs.* 8% in placebo) and cirrhosis (8% *vs.* 15%, respectively), and 35% of the liraglutide group had T2DM (*vs.* 31% in placebo). Liraglutide led to histologic NASH resolution in 35% of cases, compared with 8% of placebo-treated patients [relative risk (RR) 4.5; 95%CI: 1.1-18.9; $P = 0.017$]. Specifically, liraglutide led to the resolution of NASH in 3 out of 8 patients with T2DM (38%) (RR = 4.7, 95%CI: 0.3-75, $P = 0.020$), and only 9% of patients in the liraglutide group *vs.* 36% in the placebo group had fibrosis progression during treatment.

Less convincing data support a similar role for dulaglutide. A post-hoc analysis of the phase 3 AWARD studies [Assessment of Weekly Administration of LY2189265 (Dulaglutide) in Diabetes], involving 760 patients with T2DM and high likelihood of NAFLD/NASH based on elevated ALT values and exclusion of other hepatic diseases, showed a significantly greater reduction of ALT after 6-month treatment with dulaglutide 1.5 mg once a week (-2.1 IU/L; 95%CI: -3.9 to -0.3 ; $P = 0.022$). Similar changes were observed when the results were adjusted for body weight (-8.7 IU/L; 95%CI: -10.1 to -7.3)^[58].

Exenatide also reduced ALT and AST levels in people with T2DM and elevated baseline ALT levels^[59] in a case series of eight patients with NASH treated for 28 weeks. Some patients also experienced an improvement in histological features, including fibrosis^[60]. Furthermore, the previously mentioned meta-analysis by Carbone *et al.*^[48] showed a significant mean ALT reduction in both the liraglutide and exenatide treated cohorts (mean 12.2 U/L; 95%CI: 4.9 - 19.4 ; $P < 0.001$). Finally, exenatide effectively reduced hepatic triglyceride content compared to reference treatment ($+12.5 \pm 9.6\%$, $P = 0.007$, when assigned to 44 obese subjects with T2DM^[61], again in a weight loss-dependent manner; $r = 0.47$, $P = 0.03$). Cuthbertson *et al.*^[62] reported a 42% median reduction of intracellular fat content ($P < 0.0001$), measured by magnetic resonance spectroscopy (MRS), independently of weight loss, after six months of exenatide or liraglutide.

GLP-1RAs have also been investigated in combination with lifestyle interventions or other drugs. Fan *et al.*^[63] found a significant reduction in ALT, AST, and gamma-glutamyl transpeptidase, and an increase in the AST/ALT ratio in a cohort of 49 patients affected by both T2DM and NAFLD and treated by the combination of exenatide and lifestyle interventions. The MRS-assessed hepatic content was significantly higher in individuals receiving the combination of exenatide and pioglitazone for 12 months (12.1 ± 1.7 to $4.7 \pm 1.3\%$), however, compared with pioglitazone alone (11.0 ± 3.1 to $6.5 \pm 1.9\%$)^[64].

A phase 2 study of semaglutide, a longer-acting, weekly dosing GLP-1 analogue, has recently been completed. A preliminary release reports that after 72 weeks of therapy with the highest dosage tested (0.4 mg), 33 of 56 patients (59%) with fibrosis stages F2 to F3 met the primary end-point of NASH resolution and no worsening in liver fibrosis, *vs.* 10 of 58 patients (17%) in the control arm^[65]. Semaglutide is very effective on body weight; a phase 3-4 trial in obesity reported a mean weight loss of 14.9% with semaglutide 2.4 mg/week for 68 weeks, increasing to 17.4% at follow up^[66]. An oral formulation of semaglutide is also being tested in pre-registration studies^[67].

Concern on the use of GLP-1RA in NASH cirrhosis was recently raised by the observation that liraglutide. While providing optimal control of blood glucose, HbA1c, and body weight in patients, it blunted the effect of beta-blockers on heart rate, possibly indicating a raised bleeding risk after starting GLP-1RA^[68]. The researchers proposed a mechanistic molecular explanation of how a GLP-1RA might prevent beta-adrenergic receptor blockade^[69]. For this reason, the treatment of T2DM with GLP-1RA in subjects at risk of bleeding requires additional studies.

Sodium-glucose co-transporter-2 inhibitors (Gliflozins)

Empagliflozin, dapagliflozin, canagliflozin, ertugliflozin, and many other SGLT-2Is under development block renal exchange of glucose in the proximal tubule, being responsible for the reuptake of 90% of the pre-urinary glucose^[70]. They entered the market in the last decade; registration and CVOT trials showed that gliflozins reduce cardiovascular events and, particularly, heart failure^[71], prevent the deterioration of renal function^[72], and induce a moderate weight loss^[73]. The risk of genitourinary tract infections are the principal adverse events associated with gliflozin use^[74].

Their effects of SGLT-2Is on liver fat have not been systematically studied, but a few data have recently become available, based either on RCTs or epidemiological studies. In a RCT involving 84 patients, dapagliflozin significantly reduced hepatic fat content measured by magnetic resonance imaging (dapagliflozin, from 17.3% to 15.1%, $P < 0.05$; placebo from 15.1% to 14.5%, P not significant), as well as liver enzymes (AST, ALT, GGT) when compared to placebo^[75]. Similar results on liver fat were reported in a prospective RCT with empagliflozin involving 50 patients (mean difference between patients treated with and without empagliflozin, -4%; $P < 0.0001$)^[76], and in another RCT in 20 patients treated with canagliflozin (from $17.6\% \pm 7.5\%$ to $12.0\% \pm 4.6\%$ after 6 months and $12.1\% \pm 6.1\%$ after 12 months; $P < 0.005$ for both)^[77].

In real-world studies, a larger reduction in liver enzymes is commonly observed during treatment with SGLT-2Is when compared with other antidiabetic drugs^[78-81], such as sulfonylureas^[80] or DPP-4Is^[81]. In a large observational study involving 3,667 patients with T2DM, after a mean follow-up of 4.8 months, ALT levels (independently of weight and HbA_{1c}) were lower in the group treated with canagliflozin and dapagliflozin, compared with those treated with liraglutide and sitagliptin^[82].

Very few data are available on SGLT-2Is and histological changes in NAFLD patients. In a prospective open-label study involving five patients who underwent serial liver biopsies, all patients treated with canagliflozin had an improvement in liver steatosis and NAS at 24 weeks, together with a decrease in fibrosis stage in two of them^[83]. The authors also confirmed these results in nine patients after 24 weeks of canagliflozin treatment, with reduced lobular inflammation, ballooning, and fibrosis stage in 33%, 22%, and 33% of patients, respectively^[83].

A significant proportion of the beneficial effects of gliflozins might be derived by reduced body weight. A network meta-analysis of 29 RCTs confirmed that gliflozin treatment was significantly associated with a higher probability to achieve significant weight loss ($\geq 5\%$) vs. placebo^[84]. In a recent study, canagliflozin was also reported to reduce the risk of prostate, lung, and pancreatic cancers, without deleterious effects on HCC^[85].

CONCLUSION

Progress in pharmacotherapy of T2DM has opened interesting areas of research and treatment for patients with NAFLD. The use of old drugs should be systematically abandoned in favor of safer and effective treatments, also addressing the associated cardiovascular and cancer risks, as well as the impending risk of hypoglycemia that may be particularly harmful for frail patients with NASH and non-NASH cirrhosis. A decalogue summarizing the novel evidence is reported in Table 2. Needless to say that the use of novel drugs must be accompanied by intense lifestyle interventions, the only effective strategy to reduce the burden of NAFLD in the long term, as well as by adherence to international guidelines, supporting a change from treatment-to-target to treatment-to-cure, while being respectful of patients' frailty and economic resources^[86].

Insulin treatment remains the most effective therapy to control glucose metabolism in very advanced stages; the risk of hypoglycemia and insulin-associated lipogenesis and weight gain - as well as difficulties

Table 2. A decalogue for safe and effective NAFLD treatment in patients with T2DM

1.	Implement a systematic, intensive, continuing lifestyle intervention (healthy diet and habitual physical activity) aimed at maintaining or slowly achieving a near-normal body weight. Physical activity is particularly needed to prevent sarcopenia
2.	Carefully assess NAFLD stage by surrogate biomarkers, as well as T2DM comorbidities (cardiovascular and renal involvement). In patients with cirrhosis determine Child-Pugh class and MELD score
3.	Define treatment targets on the basis of patients' frailty and disease severity. Although HbA1c below 6.5% (48 mmol/mol) may be the desired target in subjects without comorbidities, in individual cases values up to 8% (64 mmol/mol) may be acceptable. Consider that HbA1c may be unreliable in the presence of recent hemorrhage, and random glucose monitoring may be advisable
4.	Background metformin (2 g/day) treatment should always be used and maintained also in compensated cirrhosis, although at reduced doses (1-1.5 g/day), as long as compatible with gastrointestinal symptoms and renal function
5.	Sulfonylureas and glinides should not be used, except as third-line therapy; they both increase the risk of hypoglycemia, and sulfonylureas are also associated with increased cardiovascular risk
6.	Add pioglitazone (30-45 mg/day) in patients not at risk of heart failure or ascites. Further intensify lifestyle intervention to prevent weight gain
7.	Add DPP4-Is to improve glucose control to near-normal glucose targets in patients without comorbidities
8.	Add GLP-1RAs in patients at high risk of cardiovascular disease, including patients with previous cardiovascular events. Caution should be used in subjects with cirrhosis at risk of bleeding
9.	SGLT2-Is should be preferred in patients at risk of heart failure, as well as in patients with progressive decline of glomerular filtration rate. Consider the risk of genitourinary infection, particularly in women and in elderly men with prostate problems
10.	Avoid insulin use as long as possible, to reduce the risk of hypoglycemia and the impact on quality of life. Late insulin use may be needed in most advanced stages; whenever possible use basal or basal-bolus regimens. Combination of basal insulin with GLP-1RAs may be a likely option in selected cases

to lose weight for subjects with obesity - suggests that efforts should be aimed at limiting insulin use. The use of oral DPP-4Is and, later, of weekly-injectable GLP-1RAs or SGLT-2Is in comparison to basal insulin is under investigation^[87,88]. There is evidence that early initiation of GLP-1RAs may achieve similar or even better results than treatment with basal insulin^[89,90] and the improved ease of treatment is associated with better quality of life in advanced disease states. Fewer data are available for SGLT-2Is, but also this drug class appears to be non-inferior to add-on basal insulin as to effectiveness and safety^[91,92].

In conclusion, we are living a totally new era in the pharmacologic treatment of type 2 diabetes and patients with NAFLD are likely to take the greatest advantage from novel agents. The beneficial effects of GLP-1RAs and SGLT-2Is on metabolic outcomes extend well beyond the area of diabetes, namely to obesity, cardiovascular risk, heart failure and renal disease^[93,94], and might soon be available for NAFLD patients outside of T2DM^[95,96].

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and interpretation: Brodosi L, Musio A, Marchesini G, Petroni ML

Performed data acquisition, as well as provided technical, and material support: Barbanti FA, Mita D

Drafted the manuscript: Brodosi L, Marchesini G

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest in relation to the material presented here.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73-84.
2. Petta S, Di Marco V, Pipitone RM, et al. Prevalence and severity of nonalcoholic fatty liver disease by transient elastography: genetic and metabolic risk factors in a general population. *Liver Int* 2018;38:2060-8.
3. Taylor RS, Taylor RJ, Bayliss S, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Gastroenterology* 2020;158:1611-25.e12.
4. Loria P, Marchesini G, Nascimbeni F, et al. Cardiovascular risk, lipidemic phenotype and steatosis. A comparative analysis of cirrhotic and non-cirrhotic liver disease due to varying etiology. *Atherosclerosis* 2014;232:99-109.
5. Marchesini G, Babini M. Nonalcoholic fatty liver disease and the metabolic syndrome. *Minerva Cardioangiol* 2006;54:229-39.
6. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006;44:865-73.
7. Italian Association for the Study of the Liver (AISF). AISF position paper on nonalcoholic fatty liver disease (NAFLD): updates and future directions. *Dig Liver Dis* 2017;49:471-83.
8. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1388-402.
9. Sanyal AJ. Past, present and future perspectives in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2019;16:377-86.
10. Sanyal AJ, Brunt EM, Kleiner DE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology* 2011;54:344-53.
11. Rinella ME, Noureddin M. STELLAR 3 and STELLAR 4: lessons from the fall of Icarus. *J Hepatol* 2020;73:9-11.
12. Younossi ZM, Ratzin V, Loomba R, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 2019;394:2184-96.
13. Intercept release. Complete Response Letter (CRL) from the FDA regarding our new drug application for obeticholic acid (OCA) for the treatment of liver fibrosis due to NASH. Available from: <https://ir.interceptpharma.com/news-releases/news-release-details/intercept-receives-complete-response-letter-fda-obeticholic-acid>;2020. [Last accessed on 22 Oct 2020]
14. Portillo-Sanchez P, Bril F, Maximos M, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *J Clin Endocrinol Metab* 2015;100:2231-8.
15. Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. *Metabolism* 2016;65:1096-108.
16. Hossain N, Afendy A, Stepanova M, et al. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009;7:1224-9, 29.e1-2.
17. Jarvis H, Craig D, Barker R, et al. Metabolic risk factors and incident advanced liver disease in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of population-based observational studies. *PLoS Med* 2020;17:e1003100.
18. Ballestri S, Zona S, Targher G, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2016;31:936-44.
19. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003;37:917-23.
20. Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001;50:1844-50.
21. Taylor R. Pathogenesis of type 2 diabetes: tracing the reverse route from cure to cause. *Diabetologia* 2008;51:1781-9.
22. American Diabetes Association. 9. Pharmacologic Approaches to Glycemic Treatment: *Standards of Medical Care in Diabetes-2020*. *Diabetes Care* 2020;43:S98-110.
23. Kristensen SL, Rørth R, Jhund PS, et al. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet Diab Endocrinol* 2019;7:776-85.
24. Zelniker TA, Wiviott SD, Raz I, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* 2019;393:31-9.
25. U.S. Department of Health and Human Services FaDA, Center for Drug Evaluation and Research. Guidance for Industry. Diabetes mellitus - evaluating cardiovascular risk in new antidiabetic therapies to treat type 2 diabetes. 2008. Available from: <http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm071627.pdf>. [Last accessed on 22 Oct 2020]
26. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med*

- 2007;356:2457-71.
27. Lincoff AM, Wolski K, Nicholls SJ, Nissen SE. Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of randomized trials. *JAMA* 2007;298:1180-8.
 28. Musso G, Cassader M, Paschetta E, Gambino R. Pioglitazone for advanced fibrosis in nonalcoholic steatohepatitis: New evidence, new challenges. *Hepatology* 2017;65:1058-61.
 29. Donadon V, Balbi M, Mas MD, Casarin P, Zanette G. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients with chronic liver disease. *Liver Int* 2010;30:750-8.
 30. Zhang ZJ, Zheng ZJ, Shi R, Su Q, Jiang Q, Kip KE. Metformin for liver cancer prevention in patients with type 2 diabetes: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2012;97:2347-53.
 31. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Anti-diabetic medications and the risk of hepatocellular cancer: a systematic review and meta-analysis. *Am J Gastroenterol* 2013;108:881-91; quiz 892.
 32. Yu H, Zhong X, Gao P, et al. The potential effect of metformin on cancer: an umbrella review. *Front Endocrinol (Lausanne)* 2019;10:617.
 33. Zhang X, Harmsen WS, Mettler TA, et al. Continuation of metformin use after a diagnosis of cirrhosis significantly improves survival of patients with diabetes. *Hepatology* 2014;60:2008-16.
 34. Hauner H. The mode of action of thiazolidinediones. *Diabetes Metab Res Rev* 2002;18:S10-5.
 35. Hauner H. The mode of action of thiazolidinediones. *Diabetes Metab Res Rev* 2002;18:S10-5.
 36. Belfort R, Harrison SA, Brown K, Darland C, Finch J, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006;355:2297-307.
 37. Belfort R, Harrison SA, Brown K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006;355:2297-307.
 38. Chalasani NP, Sanyal AJ, Kowdley KV, et al. Pioglitazone versus vitamin E versus placebo for the treatment of non-diabetic patients with non-alcoholic steatohepatitis: PIVENS trial design. *Contemp Clin Trials* 2009;30:88-96.
 39. Promrat K, Lutchman G, Uwaifo GI, et al. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology* 2004;39:188-96.
 40. Sanyal AJ, Chalasani N, Kowdley KV, et al; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010;362:1675-85.
 41. Musso G, Cassader M, Paschetta E, Gambino R. Thiazolidinediones and advanced liver fibrosis in nonalcoholic steatohepatitis: a meta-analysis. *JAMA Intern Med* 2017;177:633-40.
 42. Dormandy JA, Charbonnel B, Eckland DJ, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005;366:1279-89.
 43. Kernan WN, Viscoli CM, Furie KL, et al; IRIS Trial Investigators. Pioglitazone after ischemic stroke or transient ischemic attack. *N Engl J Med* 2016;374:1321-31.
 44. Chang CH, Lin JW, Wu LC, Lai MS, Chuang LM, Chan KA. Association of thiazolidinediones with liver cancer and colorectal cancer in type 2 diabetes mellitus. *Hepatology* 2012;55:1462-72.
 45. Bril F, Lomonaco R, Kalavalapalli S, Lai J, Cusi K. 223-OR: Pioglitazone discontinuation in patients with nonalcoholic steatohepatitis (NASH) is associated with disease recurrence. *Diabetes* 2019;68:223-OR.
 46. Motola D, Piccinni C, Biagi C, et al. Cardiovascular, ocular and bone adverse reactions associated with thiazolidinediones: a disproportionality analysis of the US FDA adverse event reporting system database. *Drug Saf* 2012;35:315-23.
 47. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006;368:1696-705.
 48. Carbone LJ, Angus PW, Yeomans ND. Incretin-based therapies for the treatment of non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2016;31:23-31.
 49. Yilmaz Y, Yonal O, Deyneli O, Celikel CA, Kalayci C, Duman DG. Effects of sitagliptin in diabetic patients with nonalcoholic steatohepatitis. *Acta Gastroenterol Belg* 2012;75:240-4.
 50. Kanazawa I, Tanaka K, Sugimoto T. DPP-4 inhibitors improve liver dysfunction in type 2 diabetes mellitus. *Med Sci Monit* 2014;20:1662-7.
 51. Alam S, Ghosh J, Mustafa G, Kamal M, Ahmad N. Effect of sitagliptin on hepatic histological activity and fibrosis of nonalcoholic steatohepatitis patients: a 1-year randomized control trial. *Hepat Med* 2018;10:23-31.
 52. Cui J, Philo L, Nguyen P, et al. Sitagliptin vs. placebo for non-alcoholic fatty liver disease: a randomized controlled trial. *J Hepatol* 2016;65:369-76.
 53. Smits MM, Tonneijck L, Muskiet MH, et al. Twelve week liraglutide or sitagliptin does not affect hepatic fat in type 2 diabetes: a randomised placebo-controlled trial. *Diabetologia* 2016;59:2588-93.
 54. Brown E, Cuthbertson DJ, Wilding JP. Newer GLP-1 receptor agonists and obesity-diabetes. *Peptides* 2018;100:61-7.
 55. Armstrong MJ, Houlihan DD, Rowe IA, et al. Safety and efficacy of liraglutide in patients with type 2 diabetes and elevated liver enzymes: individual patient data meta-analysis of the LEAD program. *Aliment Pharmacol Ther* 2013;37:234-42.
 56. Eguchi Y, Kitajima Y, Hyogo H, et al; Japan Study Group for NAFLD (JSG-NAFLD). Pilot study of liraglutide effects in non-alcoholic steatohepatitis and non-alcoholic fatty liver disease with glucose intolerance in Japanese patients (LEAN-J). *Hepat Res* 2015;45:269-78.
 57. Armstrong MJ, Gaunt P, Aithal GP, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *The Lancet* 2016;387:679-90.

58. Cusi K, Sattar N, García-Pérez LE, et al. Dulaglutide decreases plasma aminotransferases in people with Type 2 diabetes in a pattern consistent with liver fat reduction: a post hoc analysis of the AWARD programme. *Diabet Med* 2018;35:1434-9.
59. Buse JB, Klonoff DC, Nielsen LL, et al. Metabolic effects of two years of exenatide treatment on diabetes, obesity, and hepatic biomarkers in patients with type 2 diabetes: an interim analysis of data from the open-label, uncontrolled extension of three double-blind, placebo-controlled trials. *Clin Ther* 2007;29:139-53.
60. Kenny PR, Brady DE, Torres DM, Ragozzino L, Chalasani N, Harrison SA. Exenatide in the treatment of diabetic patients with non-alcoholic steatohepatitis: a case series. *Am J Gastroenterol* 2010;105:2707-9.
61. Dutour A, Abdesselam I, Ancel P, et al. Exenatide decreases liver fat content and epicardial adipose tissue in patients with obesity and type 2 diabetes: a prospective randomized clinical trial using magnetic resonance imaging and spectroscopy. *Diabetes Obes Metab* 2016;18:882-91.
62. Cuthbertson DJ, Irwin A, Gardner CJ, et al. Improved glycaemia correlates with liver fat reduction in obese, type 2 diabetes, patients given glucagon-like peptide-1 (GLP-1) receptor agonists. *PLoS One* 2012;7:e50117.
63. Fan H, Pan Q, Xu Y, Yang X. Exenatide improves type 2 diabetes concomitant with non-alcoholic fatty liver disease. *Arq Bras Endocrinol Metabol* 2013;57:702-8.
64. Sathyanarayana P, Jogi M, Muthupillai R, Krishnamurthy R, Samson SL, Bajaj M. Effects of combined exenatide and pioglitazone therapy on hepatic fat content in type 2 diabetes. *Obesity (Silver Spring)* 2011;19:2310-5.
65. NOVO Nordisk. Semaglutide in NASH phase 2 trial successfully completed. Financial report for the period 1 January 2020 to 31 March 2020. Available from: https://www.novonordisk.com/content/dam/Denmark/HQ/investors/irmaterial/quarterly_financial_reports/2020/Financial%20report%20for%20Q1%202020.pdf;2020. [Last accessed on 22 Oct 2020]
66. NOVO Nordisk. Semaglutide 2.4 mg demonstrates superior and sustained weight loss versus placebo and in addition a 17.4% weight loss after 68 weeks in STEP 4 trial. In: editor^editors, editor. GlobeNewswire. Available from: <https://ml-eu.globenewswire.com/Resource/Download/4951d1a2-3bd1-47ea-840a-a1234109c018;2020>. [Last accessed on 22 Oct 2020]
67. Zinman B, Aroda VR, Buse JB, et al; PIONEER 8 Investigators. Efficacy, safety, and tolerability of oral semaglutide versus placebo added to insulin with or without metformin in patients with type 2 diabetes: the PIONEER 8 trial. *Diabetes Care* 2019;42:2262-71.
68. Vukotic R, Raimondi F, Brodosi L, et al. The effect of liraglutide on β -blockade for preventing variceal bleeding: a case series. *Ann Intern Med* 2020;173:404-5.
69. Drucker DJ. The cardiovascular biology of glucagon-like peptide-1. *Cell Metab* 2016;24:15-30.
70. Simes BC, MacGregor GG. Sodium-glucose cotransporter-2 (SGLT2) inhibitors: a clinician's guide. *Diabetes Metab Syndr Obes* 2019;12:2125-36.
71. Wu JHY, Foote C, Blomster J, et al. Effects of sodium-glucose cotransporter-2 inhibitors on cardiovascular events, death, and major safety outcomes in adults with type 2 diabetes: a systematic review and meta-analysis. *Lancet Diab & Endocrinol* 2016;4:411-9.
72. Kluger AY, Tecson KM, Lee AY, et al. Class effects of SGLT2 inhibitors on cardiorenal outcomes. *Cardiovasc Diabetol* 2019;18:99.
73. Cai X, Yang W, Gao X, et al. The association between the dosage of SGLT2 inhibitor and weight reduction in type 2 diabetes patients: a meta-analysis. *Obesity (Silver Spring)* 2018;26:70-80.
74. Raschi E, Parisotto M, Forcisi E, et al. Adverse events with sodium-glucose co-transporter-2 inhibitors: a global analysis of international spontaneous reporting systems. *Nutr Metab Cardiovasc Dis* 2017;27:1098-107.
75. Eriksson JW, Lundkvist P, Jansson PA, et al. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. *Diabetologia* 2018;61:1923-34.
76. Kuchay MS, Krishan S, Mishra SK, et al. Effect of empagliflozin on liver fat in patients with type 2 diabetes and nonalcoholic fatty liver disease: a randomized controlled trial (E-LIFT Trial). *Diabetes Care* 2018;41:1801-8.
77. Inoue M, Hayashi A, Taguchi T, et al. Effects of canagliflozin on body composition and hepatic fat content in type 2 diabetes patients with non-alcoholic fatty liver disease. *J Diabetes Investig* 2019;10:1004-11.
78. Kurinami N, Sugiyama S, Yoshida A, et al. Dapagliflozin significantly reduced liver fat accumulation associated with a decrease in abdominal subcutaneous fat in patients with inadequately controlled type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2018;142:254-63.
79. Shimizu M, Suzuki K, Kato K, et al. Evaluation of the effects of dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, on hepatic steatosis and fibrosis using transient elastography in patients with type 2 diabetes and non-alcoholic fatty liver disease. *Diabetes Obes Metab* 2019;21:285-92.
80. Sattar N, Fitchett D, Hantel S, George JT, Zinman B. Empagliflozin is associated with improvements in liver enzymes potentially consistent with reductions in liver fat: results from randomised trials including the EMPA-REG OUTCOME® trial. *Diabetologia* 2018;61:2155-63.
81. Choi DH, Jung CH, Mok JO, Kim CH, Kang SK, Kim BY. Effect of dapagliflozin on alanine aminotransferase improvement in type 2 diabetes mellitus with non-alcoholic fatty liver disease. *Endocrinol Metab (Seoul)* 2018;33:387-94.
82. Bajaj HS, Brown RE, Bhullar L, Sohi N, Kalra S, Aronson R. SGLT2 inhibitors and incretin agents: associations with alanine aminotransferase activity in type 2 diabetes. *Diabetes Metab* 2018;44:493-9.
83. Akuta N, Watanabe C, Kawamura Y, et al. Effects of a sodium-glucose cotransporter 2 inhibitor in nonalcoholic fatty liver disease complicated by diabetes mellitus: preliminary prospective study based on serial liver biopsies. *Hepatol Commun* 2017;1:46-52.
84. Wang H, Yang J, Chen X, Qiu F, Li J. Effects of sodium-glucose cotransporter 2 inhibitor monotherapy on weight changes in patients with type 2 diabetes mellitus: a bayesian network meta-analysis. *Clin Ther* 2019;41:322-34.e11.
85. Hung MH, Chen YL, Chen LJ, et al. Canagliflozin inhibits growth of hepatocellular carcinoma via blocking glucose-influx-induced β -catenin activation. *Cell Death Dis* 2019;10:420.

86. Del Prato S. Heterogeneity of diabetes: heralding the era of precision medicine. *Lancet Diab Endocrinol* 2019;7:659-61.
87. Tricco AC, Antony J, Soobiah C, et al. Safety, effectiveness, and cost of dipeptidyl peptidase-4 inhibitors versus intermediate acting insulin for type 2 diabetes: protocol for a systematic review and network meta-analysis. *Syst Rev* 2013;2:47.
88. Liu ST, Su KQ, Zhang LH, Liu MH, Zhao WX. Hypoglycemic agents for non-alcoholic fatty liver disease with type 2 diabetes mellitus: a protocol for systematic review and network meta-analysis. *Medicine (Baltimore)* 2020;99:e21568.
89. MacIsaac RJ. Dulaglutide and insulin: how can the AWARD studies help guide clinical practice? *Diabetes Ther* 2020;11:1627-38.
90. Abdul-Ghani M, Migahid O, Megahed A, DeFronzo RA, Al-Ozairi E, Jayyousi A. Combination therapy with pioglitazone/exenatide improves beta-cell function and produces superior glycaemic control compared with basal/bolus insulin in poorly controlled type 2 diabetes: a 3-year follow-up of the Qatar study. *Diabetes Obes Metab* 2020; doi: 10.1111/dom.14153.
91. Vilsbøll T, Ekholm E, Johnsson E, Dronamraju N, Jabbour S, Lind M. Dapagliflozin plus saxagliptin add-on therapy compared with insulin in patients with type 2 diabetes poorly controlled by metformin with or without sulfonylurea therapy: a randomized clinical trial. *Diabetes Care* 2019;42:1464-72.
92. Zaccardi F, Dhalwani NN, Dales J, et al. Comparison of glucose-lowering agents after dual therapy failure in type 2 diabetes: a systematic review and network meta-analysis of randomized controlled trials. *Diabetes Obes Metab* 2018;20:985-97.
93. Cherney DZI, Dekkers CCJ, Barbour SJ, et al. Effects of the SGLT2 inhibitor dapagliflozin on proteinuria in non-diabetic patients with chronic kidney disease (DIAMOND): a randomised, double-blind, crossover trial. *Lancet Diab Endocrinol* 2020;8:582-93.
94. Papazafiropoulou AK, Melidonis A, Antonopoulos S. Effects of glucagon-like peptide-1 receptor agonists and sodium-glucose cotransporter 2 inhibitors on cardiorenal and metabolic outcomes in people without diabetes. *Curr Pharm Des* 2020; doi: 10.2174/1381612826666200909142126.
95. Vincent RK, Williams DM, Evans M. A look to the future in non-alcoholic fatty liver disease: are glucagon-like peptide-1 analogues or sodium-glucose co-transporter-2 inhibitors the answer? *Diabetes Obes Metab* 2020; doi: 10.1111/dom.14196.
96. Brodosi L, Marchignoli F, Petroni ML, Marchesini G. NASH: a glance at the landscape of pharmacological treatment. *Ann Hepatol* 2016;15:673-81.

Review

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Sex disparity in hepatocellular carcinoma owing to NAFLD and non-NAFLD etiology: epidemiological findings and pathobiological mechanisms

Amedeo Lonardo¹, Stefano Ballestri², Pierce K. H. Chow^{3,4}, Ayako Suzuki^{5,6,7}

¹Operating Unit of Metabolic Syndrome, Azienda Ospedaliero-Universitaria di Modena, Ospedale Civile di Baggiovara, Modena 41126, Italy.

²Operating Unit Internal Medicine, Ospedale di Pavullo, Azienda Unità Sanitaria Locale, Modena 41126, Italy.

³Division of Surgery and Surgical Oncology, National Cancer Center Singapore and Singapore General Hospital, Council 169610, Singapore.

⁴Surgery Academic Clinical Program and Office of Education, Duke-NUS Medical School, Singapore 169857, Singapore.

⁵Division of Gastroenterology, Duke University Health System, Durham, NC 27710, USA.

⁶Division of Gastroenterology, Durham VA Medical Center, Durham, NC 27705, USA

⁷Programme in Cardiovascular and Metabolic Disorders, Duke-NUS Medical School, Singapore 169857, Singapore.

Correspondence to: Prof. Amedeo Lonardo, Department of Internal Medicine, Ospedale Civile di Baggiovara, 1135 Via Giardini, Modena 41126, Italy. E-mail: a.lonardo@libero.it

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Abstract

Nonalcoholic fatty liver disease (NAFLD) exhibits sexual dimorphism, with men being more exposed than women to the risk of simple steatosis, nonalcoholic steatohepatitis fibrosis, and hepatocellular carcinoma (HCC), while the protection conferred to women seemingly disappears with aging and reproductive senescence (i.e., menopause). HCC, the most common primary liver cancer, which carries an ominous prognosis, may result from various genetic and non-genetic risk factors. NAFLD is now projected to become the most common cause of HCC. HCC also exhibits a definite sexual dimorphism in as much as it has a worldwide high male-to-female ratio. In this review article, we focus on sex differences in the epidemiological features of HCC. Moreover, we discuss sex differences in the clinical outcome and molecular pathobiology of NAFLD-HCC. By highlighting the research gaps to be filled, the aim of this review is to prompt future research of sex differences in HCC and facilitate developing personalized cancer prevention strategies, detection, and treatments to achieve better patient outcomes in NAFLD-HCC, considering sex differences in HCC pathobiology.



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Keywords: Liver cancer, pathobiology, personalized medicine, sex differences

BACKGROUND

Hepatocellular carcinoma (HCC), the most common primary liver cancer (PLC), carries an ominous prognosis, and is the fourth most common cause of mortality owing to cancer^[1,2]. The chief modifiers of HCC risk include geographic variability, demographics and severity of liver disease^[2]. Cirrhosis, irrespective of aetiology, increases the risk of HCC^[1]. On a global basis, the proportion of HCCs attributed to nonalcoholic fatty liver disease (NAFLD) is increasing owing to trajectories of declining HCV infection and escalating NAFLD^[1,3,4]. Additional risk factors for the development of HCC are infection with HBV, alcoholic liver disease, aflatoxin, and genetic haemochromatosis^[5].

Spanning a wide range of liver histology changes, NAFLD faithfully recapitulates the whole spectrum of alcoholic liver disease though it is observed in the nonalcoholic patient^[6] and in the absence of other competing causes of (steatogenic) liver disease^[7]. Similar to HCC, NAFLD accounts for a substantial clinical burden and exacts a heavy toll of healthcare-related expenses^[8].

Sex disparities in various human diseases, from initial manifestations to disease outcome, are often encountered in clinical practice. In fact, sex and gender act as powerful modifiers of the top ten causes of mortality and morbidity, including heart disease, cancer, chronic lung disease, Alzheimer's disease, influenza and pneumonia, chronic kidney and chronic liver diseases^[9]. Clear sex disparity exists in HCC, which is twice as common in men as in women^[1]. NAFLD also exhibits multifaceted sexual dimorphism^[10,11]. It occurs more often in men than in women of fertile age and is heavily affected by reproductive status^[12]. Understanding these sex differences is the key to deciphering the pathophysiology of the disease as well as in guiding personalized care^[12].

On this background of evidence, we aimed at illustrating our current knowledge of sex differences in HCC and clarifying gaps to be filled in future research, while placing special focus on NAFLD-related HCC.

METHODS

The PubMed database was extensively searched for articles published as of the 31st of July 2020. The keywords used in our search include, but are not limited to: HCC, liver cancer, sex differences, gender differences, epidemiology, natural course, pathogenesis, risk factors, immune response, genetics, and sex hormones. Additional terms were used to search for articles reporting sex differences and/or the effect of sex hormones in specific mechanisms pertaining to carcinogenesis. Among the retrieved publications, only those that were deemed to be relevant based on consensus among the authors were retained.

EPIDEMIOLOGICAL MODIFIERS OF HCC RISK

Irrespective of its aetiology, HCC affects men more commonly than women owing to complex and multifactorial reasons. This section reviews risk factors of HCC in general and discusses interactions between sex and risk factors.

Geographic area and ethnicity

Eastern Asia, Southeast Asia, and sub-Saharan Africa exhibit a high incidence and prevalence of HCC; Mongolia, China, Japan, Papua New Guinea, and Egypt are top-ranked countries^[1]. By contrast, countries with a low incidence and prevalence include India, Russia, northern countries of South America, Argentina, European countries (except for southern countries), USA, and Australia with the rest of the world exhibiting intermediate rates of incidence and prevalence^[1].

Multi-ethnic populations display a clear ethnic gradient. For example, in the United States, Asians/Pacific Islanders have been reported to have the highest incidence rate per 100,000 (11.7), followed by Hispanics (9.5), Blacks (7.5), while Whites had the lowest (4.2)^[13].

Sex

With few exceptions, the male to female (M:F) ratio of the incidence of HCC ranges between 2 to 3 in the most of the countries, irrespective of whether they are high-rate areas or not, and are maximal in middle European countries (M:F ratio up to 5)^[13,14]. In contrast, in Costa Rica, Colombia, Ecuador and Uganda, the M:F ratio of the incidence of HCC is smaller, ranging from 1.3 to 1.6^[13,14].

The biological grounds underlying this sex disparity in the prevalence of HCC are incompletely defined and probably related to multiple behavioural, hormone-metabolic risk factors, and cancer biology. Sex differences in HCC pathogenesis are discussed below under sex disparity in HCC pathobiology.

The difference in the M:F ratio of the incidence of HCC among different countries is intriguing, suggesting potential race/ethnicity-sex interplay in HCC. At this point, sufficient data do not exist to delineate whether the difference is explained by a biological interplay and/or an interplay of gender attributes and culture/ethnicity.

Age

The overall incidence of HCC consistently peaks at 70 years in various countries worldwide, such as France, Italy, Japan, and USA (whites) and this is approximately 5-15 years before the peak occurrence of cholangiocarcinoma, the second most common PLC after HCC^[15]. However, other authors report that the mean ages of diagnosis with HCC are 55-59 years in China and 63-65 years in Europe and North America^[14]. In Qidong, China, where the HCC burden is among the world's highest, the age-specific incidence rates increase up to the age of 45 among men and then plateau; while increasing to the age of 60 and then plateauing among women^[14]. A surveillance, epidemiology, and end results (SEER) Analysis (from 1988 to 2010) including 39,345 patients with HCC (Men 76%, women 34%) showed that men are diagnosed 4-7 years earlier than women across the race/ethnic groups^[16]. These findings suggest that sex and age interact in the occurrence of HCC, implying that consideration of this interaction (as opposed to treating age and sex as independent variables) will be essential in future research.

Severity of liver histology

While cirrhosis is an almost essential pre-requisite for the development of HCC in those with HCV infection, infection with HBV exerts a more direct carcinogenic effect on the liver^[1]. Similar to HBV infection and to alcoholic liver disease, NAFLD-HCC may occur in non-cirrhotic livers^[14,15]. A Japanese descriptive study reported that men with nonalcoholic steatohepatitis (NASH) developed HCC at earlier liver fibrosis stages than women^[17]. The study was too small to confirm the sex difference but provides an intriguing hypothesis pertaining to carcinogenesis. Further larger studies are warranted to investigate this.

Viral hepatitis

With the exceptions of Japan and Egypt (where HCV infection is the chief risk factor of HCC), in most high-risk countries, chronic HBV infection and aflatoxin B1 are the major risk factors for the development of HCC, whereas HCV infection, excessive alcohol consumption, and common metabolic disorders (diabetes, obesity and metabolic syndrome) prevail in low-rate areas^[15]. Chronic drinking of alcohol > 80 g/day for over 10 years increases the risk of HCC by a factor of 5; and alcohol consumption enhances the risk of HCC in those with either chronic hepatitis C or NAFLD^[10,18]. However, given that these data often combine both sexes, research needs to be conducted urgently to clarify the sex-specific thresholds of alcohol consumption that are associated with a raised HCC risk.

Family history

HCC commonly exhibits familial clustering, and family history of disease is a risk factor for the development of HCC. Interestingly, family history of HCC was identified as a favourable prognostic factor for improved survival particularly in those individuals whose tumours can be radically cured, even in the stage-stratified analysis^[19]. In the study, female sex and younger age, non-diabetics, and lifetime non-drinkers were more common among individuals with first-degree family histories of HCC than among those without such histories^[19]. The exact mechanisms underlying the above associations remain uncertain.

Genetic risk determinants of NAFLD and inherited metabolic liver diseases

Genetic variants associated with an increased risk of NAFLD, advanced NAFLD, and NAFLD-HCC appear to contribute to the risk of HCC in the general population. A recent study conducted using Danish and UK databases demonstrated that a genetic risk score using three genetic variants [i.e., patatin-like phospholipase domain-containing protein 3 (PNPLA3) p.I148M, transmembrane 6, superfamily member 2 (TM6SF2) p.E167K, and hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) rs72613567], is associated with an up to 12-fold higher risk of cirrhosis and up to a 29-fold higher risk of HCC in individuals from the general population from these countries^[20].

Certain inheritable metabolic disorders such as hemochromatosis, α -1 antitrypsin deficiency, tyrosinemia, glycogen storage diseases and several porphyrias also increase HCC risk, although they account for a negligible HCC risk globally^[21,22].

Other risk factors of HCC

Smoking and co-infection with HIV also contribute to the development of HCC^[1]. Certain environmental factors or occupational factors, such as vinyl chloride, polycyclic aromatic hydrocarbons, aflatoxins, and aristolochic acid, a common ingredient of traditional herbal medicine, have been suggested to play a role in the development of HCC^[23,24]. How these factors and underlying mechanisms intersect with sex and sex hormones in the development of HCC has not been fully elucidated.

Interaction between sex/gender and metabolic risk factors

Sexual dimorphisms in metabolism are well-known (recently reviewed elsewhere^[9,10,12]) and likely account for sex differences in HCC risk. A few other risk factors have been suggested for sex/gender-interaction, which are also discussed in this section.

Obesity has been associated with a higher risk of HCC incidence in men than women, especially in non-Asians^[25]. A recent study conducted in an Asian population found a different relationship between BMI and HCC risk according to sex, following a U-shaped and a linear curve in men and women, respectively^[26]. Studies reported a stronger risk association between pre-diabetes/diabetes and HCC in men than women^[26-28].

NAFLD has a definite sexual dimorphism; men are more prone than women to the risk of uncomplicated steatosis, NASH fibrosis, and HCC. However, aging and menopause are associated with the disappearance of protection in women^[12,29,30].

Prospective studies indicate that regular alcohol intake, although within safe thresholds, is a risk factor for the progression to HCC among individuals with NAFLD^[31]. Moreover, among those with HCC, alcohol use is more frequent in men than in women^[26,32].

Additionally, men are more prone to acquire HBV and HCV infection, develop chronic hepatitis, cirrhosis and HCC than women^[14].

CLINICAL SEX DIFFERENCES IN HCC

Recent large epidemiological studies conducted in the USA have shown that, compared to men, women with HCC presented with older age, higher frequency of NAFLD, non-cirrhotic HCC, less-advanced tumour stage (by size, local/vascular invasion, metastasis) and lower frequency of alcoholic liver disease^[32-34].

Studies regarding sex differences in survival rates have yielded conflicting results so far. Two recent large multi-centre US studies enrolling 5,327 patients with HCC (22.6% women) and 1,110 (23.5% women), respectively, reported higher overall survival rates among women after adjusting for confounding factors^[33,34]. Consistently, one of these found that female sex was independently associated with early tumour detection [odds ratio (OR) 1.46] and response to first HCC treatment (OR 1.72)^[33]. Conversely, other US studies found no sex-related difference in HCC prognosis^[32,35], while Asian studies did^[36-39]. Another US study showed age, sex, and ethnicity intersection in survival rates; women had higher survival rates from HCC than men before age 55, while after 65 years or among Hispanics, there was no such a survival difference between sexes^[38]. Another US study also suggested a similar interplay of age and sex in HCC survival and further possible age-, sex-, and race/ethnicity-interaction in HCC survival^[16]. Table 1 provides a synthetic overview of sex differences in risk factors, presentation, and outcome of HCC owing to NAFLD and non-NAFLD aetiologies. Future studies with proper consideration of these interactions are warranted to reconcile some inconsistency in the literature.

SEX DISPARITY IN HCC PATHOBIOLOGY

Chronic persistent injury induces wound-healing responses through the release of pro-inflammatory cytokines (e.g., IL-6, TNF-alpha) and increased oxidative stress in the liver^[40]. The wound-healing process, together with persistent liver damage, promotes fibrogenesis and tissue DNA damages and facilitates hepatic carcinogenesis^[41]. The liver tissue concentration of 8-OHdG, a marker of oxidative DNA damage, has been associated with epigenetic inactivation of tumour suppressor genes (i.e., methylated tumour suppressor genes)^[42,43]. An increased number of methylated tumour suppressor genes was, in its turn, associated with a shorter time-to-HCC development in patients with chronic hepatitis C^[44], demonstrating a mechanistic link of oxidative DNA damage, epigenetic alteration of tumour suppressor genes, and development of HCC. In NAFLD patients, chronic metabolic stress to hepatocytes aggravates oxidative stress, induces cellular protein/DNA damage, and promotes premature senescence of hepatocytes, contributing to an increased risk of HCC among obese individuals^[12,45], even in the absence of cirrhosis.

Sex differences are well documented in cancer mechanisms^[46]. Several well studied mechanisms accounting for sex differences are summarized in Table 2^[47-56]. Compared to females, males are more susceptible to oxidative stress due to a higher NADPH oxidase activity, a lower NFR-2, and lower anti-oxidants^[51,52,57], and have a higher induction of IL-6 by hepatic Kupffer cells under liver injury^[54]. In contrast, higher physiological oestrogens protect females from HCC development via the anti-oxidative effects of estrogenic^[51,57], anti-fibrotic effects^[12], and inhibitory effects on IL-6 production by hepatic Kupffer cells^[54]. The protective effects of oestrogen are lost after menopause, which may explain the fact that the male predominance observed in HCC incidence decreases with advancing age^[58]. Gut microbiota also exhibits sex differences^[56,59-61]. In an experimental mouse model, higher hepatic hydrophobic bile acids were observed in males, which was causally associated with a decreased expression of tumour-suppressive microRNA in the liver and increased incidence of HCC^[61]. Importantly, similar sex differences in bile acid profile exist in humans^[56], suggesting that sex-differences in gut microbiota and bile acid profile may contribute to male dominance in the development of HCC in man.

Despite the fact that evidence supports oestrogen exerting protective effects on the development of HCC, whether oestrogens have protective effects on the progression of HCC and patients' survival remains

Table 1. Sex differences in risk factors, presentation, and outcome of HCC owing to NAFLD and non-NAFLD aetiologies

Authors	Study characteristics	Sex differences		
		Risk factors	Tumour features	Outcome
Yang <i>et al.</i> ^[16]	Retrospective cohort from a national registry, US, 39345 HCC patients, 9557 (24%) F, diagnosed between 1988-2010	F older age (67 years <i>vs.</i> 61 years)	F more liver-limited (32% <i>vs.</i> 26%) and less metastatic (14% <i>vs.</i> 16%) disease	F better overall survival (11 months <i>vs.</i> 10 months; HR 0.93) independent of age, race, disease stage, or treatment. The protective effect of sex was greatest in patients aged 18-44 years (14 months <i>vs.</i> 10 months; HR 0.75) and it was lost after 65 years. No survival sex difference among Hispanics
Ladenheim <i>et al.</i> ^[35]	Retrospective cohort, US, 1,886 HCC patients, 437 (23.2%) F, diagnosed between 1998-2015	F older age (64 years <i>vs.</i> 60 years); M more HCV+ (43% <i>vs.</i> 37%), alcohol use (63% <i>vs.</i> 35%) and smoking (58% <i>vs.</i> 31%)	F less likely to present with tumours > 5 cm (30% <i>vs.</i> 40%) and more likely to be diagnosed by routine screening (66% <i>vs.</i> 58%)	No significant difference in median survival (30.7 months <i>vs.</i> 33.1 months)
Wu <i>et al.</i> ^[32]	Retrospective cohort single centre, Hawaii (US), 1,206 HCC patients, 307 (25%) F, diagnosed between 1993-2017	F older age (66.0 years <i>vs.</i> 62 years), more NAFLD/NASH (22% <i>vs.</i> 7%) M more HCV+ (43% <i>vs.</i> 37%), alcohol (53% <i>vs.</i> 12%) and smoking (68% <i>vs.</i> 38%)	F smaller mean size at diagnosis (5 cm <i>vs.</i> 6 cm), less vascular invasion (7.5% <i>vs.</i> 12%). F more likely to undergo HCC surveillance but less to undergo liver transplant	Similar overall survival. Mortality predictors at MVA: NAFLD/NASH for both M and F, age and smoking for M. Transplant predictive of survival for M
Lai <i>et al.</i> ^[39]	Retrospective cohort single centre, Asia (Taiwan), 516 consecutive HCC patients, 118 (22.9%) F, who received surgical resection between 2000-2007, F-up > 10 years	F more HCV + (37% <i>vs.</i> 23%); lower HBV + (59% <i>vs.</i> 73%)	F less micro-vascular invasion (25% <i>vs.</i> 36%)	Similar overall survival. F better recurrence-free survival and distant metastasis-free survival in patients with alpha-fetoprotein M 35 ng/mL, independent of other clinical variables
Rich <i>et al.</i> ^[33]	Retrospective cohort single centre, US, 1,110 HCC patients, 258 (23.5%) F, diagnosed between 2008-2017	F older age (63 years <i>vs.</i> 59 years), more NAFLD (27% <i>vs.</i> 8%) M more alcohol alone (17% <i>vs.</i> 5%) or with HCV+ (33% <i>vs.</i> 15%)	F <i>vs.</i> M earlier- BCLC stage tumours (53% <i>vs.</i> 44%) but similar liver function	F < 65 years had better overall survival than M (18.3 months <i>vs.</i> 11.2 months). However, older F and M had similar overall survival (15.5 months <i>vs.</i> 15.7 months) at UVA F sex associated with lower mortality (HR 0.82), early tumour detection (OR 1.46) and response to first HCC treatment (OR 1.72) at MVA
Phipps <i>et al.</i> ^[34]	Retrospective cohort multi-centric, US, 5,327 HCC patients, 1,203 (22.6%) F, diagnosed between 2000-2014	F more NAFLD (23% <i>vs.</i> 12%) and less alcoholic liver disease (5% <i>vs.</i> 15%)	Non-cirrhotic HCC higher among F (17% <i>vs.</i> 10%). F less-advanced HCC by tumour, node, metastasis staging and a higher proportion within Milan criteria (39% <i>vs.</i> 35%)	F greater overall survival (2.5 ± 2.9 years <i>vs.</i> 2.2 ± 2.7 years)

AFP: alpha-fetoprotein; BCLC: barcelona clinic liver cancer; F: female; F-up: follow-up; HR: hazard ratio; M: male; MVA: multivariate analysis; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; OR: odds ratio; US: United States; UVA: univariate analysis

uncertain. A few epidemiological studies implicated a potentially favourable effect of oestrogen on HCC survival, by demonstrating a beneficial association of exogenous oestrogen use with overall survival among women with HCC^[62] and the better overall survival rates of women compared to men, which, however, disappears in advanced age^[16]. However, possible beneficial effects of oestrogen on HCC survival have not been tested in experiments, and the mechanisms, if any, through which oestrogens affect HCC survival, remain uncertain.

HCC tumour tissue expresses oestrogen receptors, although the clinical and pathological significance of these remain controversial^[63,64]. The positive expression rates of oestrogen receptors among HCC cases also

Table 2. Sex differences relevant to the pathomechanisms of HCC in NAFLD

Authors	Study characteristics	Sex differences		
		Risk factors	Outcome	Impact on HCC
Pre-hepatic factors increasing hepatic metabolic stress				
Lemieux <i>et al.</i> ^[47]	Total body fat and abdominal adipose tissue were evaluated in 89 men and 75 women using CT	Visceral adiposity	After adjusting for total body fat mass, men had significantly higher values of visceral adipose tissue volume and areas, measured by CT, than women. An increase in total fat mass was associated with a significantly greater increase in visceral adipose tissue volume in men than in women	Higher FFA release in males induces inflammation, insulin resistance, and lipotoxicity and fosters a tumour-promoting environment in the liver and may contribute to an increased recurrence of HCC
Laughlin <i>et al.</i> ^[48]	A cross-sectional study to measure serum leptin, adiponectin and sex hormone levels in 1510 community dwelling men and postmenopausal women aged 50-92 years	Visceral adipokines	Serum adiponectin and leptin levels were higher in women than in men. In both sexes, adiponectin concentrations were lower, and leptin levels higher, with increasing BMI and waist girth	A higher adiponectine level may protect women from developing HCC via the activation of AMPK and p38 α ^[49]
Lönnqvist <i>et al.</i> ^[50]	BMI and age matched obese subjects (22 male and 23 female) undergoing elective surgery were evaluated for visceral fat lipolysis	Visceral fat lipolysis	Catecholamine-induced rate of FFA mobilization from visceral fat to the portal venous system is higher in obese men than in obese women, probably due to a larger fat-cell volume but also to a decrease in the function of α_2 -adrenoceptors, an increase in the function of β_3 -adrenoceptors, and an increased ability of cyclic AMP to activate hormone-sensitive lipase	Higher FFA release in males induces inflammation, insulin resistance, and lipotoxicity and fosters a tumour-promoting environment in the liver and may contribute to an increase recurrence of HCC
Oxidative stress/Senescence				
Augustine <i>et al.</i> ^[51]	Compared Nqo1 mRNA and protein expression and activity in males and females before and after applying known inducers using SD and August Copenhagen x Irish (ACI) rat strains	NAD(P)H: quinone oxidoreductase 1 (Nqo1)	ACI rats showed minimal differences in Nqo1. In SD rats, Nqo1 mRNA, protein, and activity levels were significantly higher in females than in males. Female SD rats showed greater induction than male	Higher Nqo1 may lead to greater protection against oxidative stress and thus decreased susceptibility to carcinogens
Kratschmar <i>et al.</i> ^[52]	The interaction among corticosteroid, 11b-HSD1, and NFR-2 was evaluated using transfected HEK-293 cells and hepatic H4IIE cells. The hepatic expression levels of 11b-HSD1 and NFR-2 target genes were also compared between male and female Han Wistar rats	NFR-2	The study using the cell lines demonstrated that glucocorticoids, activated by 11b-HSD1 and acting through GR, suppress the Nrf2-dependent antioxidant response. This research also demonstrated that the hepatic expression of 11b-HSD1 was higher in male rats vs. female rats while the Nrf-2 target genes (HMOX1, NQO1 and ABCC3) were lower in male vs. female rats, confirming the above-demonstrated pathway	Higher activity of 11b-HSD1 and/or corticosteroid may lead to suppressed antioxidant response, which may lead to higher oxidative DNA damage
DNA damage/repair				
Hofer <i>et al.</i> ^[53]	DNA SSB and ALS were measured in blood samples from 99 subjects (age: 19-31 years) living in Stockholm, Sweden. Oxidative DNA damage was also analyzed using the DNA repair glycosylase FPG as well as HPLC-ECD for specific analysis of 8-oxodG	Oxidative DNA damage	Males had higher levels of SSB + ALS than females, although no difference was seen for oxidative lesions. There was no correlation between FPG sites and 8-oxodG. In females, there was a positive correlation between FPG levels and BMI and a negative correlation between SSB + ALS and fruit intake	Men are associated with a higher risk of oxidative DNA damage
Immune response				

Naugler <i>et al.</i> ^[54]	In mice administered with DEN, HCC incidence, and its relationship with hepatic IL-6 induction, Toll-like receptor adaptor protein MyD88, and oestrogen were evaluated in male and female mice	IL-6	A higher HCC incidence was observed in male vs. female DEN-induced hepatocarcinogenesis model. The higher incidence of HCC in males was associated with higher MyD88-dependent induction of IL-6 in male hepatic Kupffer cells under liver injury. Estradiol inhibited IL-6 production by hepatic Kupffer cells	Higher induction of IL-6 in Kupffer cells under liver injury partly explains higher incidence of HCC in males while estrogens protect females from HCC, in part, via reducing IL-6
Fibrosis Yasuda <i>et al.</i> ^[55]	Using the DEN model, hepatic fibrosis was compared between male and female rats	Stellate cell activation/fibrogenesis	In male rats the induction of fibrotic response was significantly stronger than in female rats. Estradiol reduced hepatic fibrogenesis in male rats while concomitant administration of a neutralizing antibody against rat estradiol enhanced fibrogenesis. Oophorectomy in the female rats had a fibrogenic effect	Higher oestrogen protects premenopausal women from advanced hepatic fibrosis, a major risk factor of HCC
Tumor suppressor genes Xie <i>et al.</i> ^[56]	The mechanistic link between microbiota and hepatocellular carcinogenesis using a STZ-HFD induced NASH-HCC murine model and compared results for both sexes	Bile acid/microbiota	STZ-HFD feeding induced a higher incidence of HCC in male mice, which was associated with increased intrahepatic retention of hydrophobic BAs and decreased hepatic expression of tumor-suppressive microRNAs. Metagenomic analysis showed differences in gut microbiota involved in BA metabolism between male and female mice. Treating STZ-HFD male mice with 2% cholestyramine led to significant improvement of hepatic BA retention, tumor-suppressive microRNA expressions, microbial gut communities, and prevention of HCC	Sex differences in microbiota lead to higher intrahepatic retention of hydrophobic BAs, decreased tumor suppressor microRNA in the liver, and an increased incidence of HCC in male mice

ALS: alkali labile sites; AMPK: AMP-activated protein kinase; BA: bile acids; CT: computed tomography; DEN: diethylnitrosamine; FFA: free fatty acids; FPG: DNA repair glycosylase; IL-6: Interleukin-6; NASH-HCC: nonalcoholic steatohepatitis-hepatocellular carcinoma; SSB: single strand breaks; SD: sprague dawley; STZ-HFD: streptozotocin-high fat diet

significantly varies in the literature, probably due to the differences in the methodologies and populations of the studies (e.g., ethnicity, sex), as well as stage and aetiologies of disease^[64]. HCC tumour tissues express both oestrogen receptor alpha (ER α) and beta (ER β), and also a variant form of ER α (vER α), which lacks exon 5 in the hormone-binding domain^[65]. Compared to patients with ER-negative HCC, patients with ER-positive HCC have a shorter survival rate after curative resection^[66]. Several randomized controlled trials were conducted to ascertain whether blockage of oestrogen signalling in HCC by the anti-oestrogen tamoxifen would improve the survival of patients with HCC. However, the results were consistently negative^[67,68]. The presence of the liver vER receptor in the tumour is a strong negative predictor of survival in inoperable HCC patients and is a marker of clinical aggressiveness compared to wild-type ER α ^[69,70]. HCC positive for the vER receptor is unresponsive to tamoxifen but responds to megestrol^[71].

Sex differences in HCC biology have extensively been explored in recent years in functional signatures of differentially expressed genes^[71,72], expression quantitative trait loci (eQTL)^[73], and cancer-driver genes^[73]. These studies strongly suggest that HCC in males and females are biologically distinct and may respond differently to treatments. This is in agreement with epidemiological data summarized in Table 1. However, no randomized controlled trials have demonstrated sex differences in HCC treatment response or clinical outcomes. The consideration of sex and women's reproductive history in clinical studies designs and

analyses is warranted in future trials to better understand whether and how these factors may modify treatment response to specific therapeutic targets and influence clinical outcomes.

CONCLUSIONS AND RESEARCH AGENDA

A robust line of research has shown multifaceted sexual dimorphisms in the NAFLD domain^[10-12]. It is important to note that the observed sex differences in NAFLD are not linear throughout the course of the disease but rather mechanism-specific. Further studies will eventually contribute to more effectively reducing the NAFLD-HCC incidence by delineating sex differences in individual pathways and therefore allowing the development of a personalized approach in preventing NAFLD progression. Similarly, sex differences in HCC epidemiology have not been fully characterized with proper consideration of women's reproductive status/history. Therefore, we recommend that sex/gender and reproductive history should be considered in future clinical and epidemiological HCC studies. Further mechanistic understanding, together with the epidemiological characterization of sex differences and the impact of reproductive history will predictably help clinicians by allowing more accurate risk stratification and personalized therapeutic approaches in the future. A scoring system combining genetic and non-genetic HCC risk factors while considering biological disparities by sex and reproductive status may improve our future care, although sufficient data to develop such a scoring system is pending future research.

DECLARATIONS

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Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Lonardo A, Suzuki A

Wrote the section on the epidemiology: Ballestri S, Lonardo A

Contributed to the section on pathobiology: Chow PKH, Suzuki A

Edited the final draft: Lonardo A, Ballestri S, Chow PKH, Suzuki A

Prepared the revised version of the manuscript based on Reviewers' suggestions and comments: Lonardo A, Suzuki A

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REFERENCES

1. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019;16:589-604.
2. Lok AS, Seeff LB, Morgan TR, et al. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009;136:138-48.
3. Pennisi G, Celsa C, Giammanco A, Spatola F, Petta S. The burden of hepatocellular carcinoma in non-alcoholic fatty liver disease: screening issue and future perspectives. *Int J Mol Sci* 2019;20:5613.
4. Estes C, Anstee QM, Arias-Loste MT, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. *J Hepatol* 2018;69:896-904.
5. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004;127:S35-50.
6. Diehl AM, Goodman Z, Ishak KG. Alcohollike liver disease in nonalcoholics. A clinical and histologic comparison with alcohol-induced liver injury. *Gastroenterology* 1988;95:1056-62.
7. Italian Association for the Study of the Liver (AISF). AISF position paper on nonalcoholic fatty liver disease (NAFLD): updates and future directions. *Dig Liver Dis* 2017;49:471-83.
8. Younossi ZM, Henry L, Bush H, Mishra A. Clinical and economic burden of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Clin Liver Dis* 2018;22:1-10.
9. Mauvais-Jarvis F, Bairey Merz N, Barnes PJ, et al. Sex and gender: modifiers of health, disease, and medicine. *Lancet* 2020;396:565-82.
10. Lonardo A, Nascimbeni F, Ballestri S, et al. Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps. *Hepatology* 2019;70:1457-69.
11. Balakrishnan M, Patel P, Dunn-Valadez S, et al. Women have a lower risk of nonalcoholic fatty liver disease but a higher risk of progression vs men: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2020; doi: 10.1016/j.cgh.2020.04.067.
12. Lonardo A, Suzuki A. Sexual dimorphism of NAFLD in adults. Focus on clinical aspects and implications for practice and translational research. *J Clin Med* 2020;9:1278.
13. McGlynn KA, Petricka JL, London WT. Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability. *Clin Liver Dis* 2015;19:223-38.
14. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012;142:1264-73.e1.
15. Petrick JL, Braunlin M, Laversanne M, Valery P, Bray F, McGlynn KA. International trends in liver cancer incidence, overall and by histologic subtype, 1978-2007. *Int J Cancer* 2016;139:1534-45.
16. Yang D, Hanna DL, Usher J, et al. Impact of sex on the survival of patients with hepatocellular carcinoma: a surveillance, epidemiology, and end results analysis. *Cancer* 2014;120:3707-16.
17. Yasui K, Hashimoto E, Komorizono Y, et al. Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2011;9:428-33; quiz e450.
18. Morgan TR, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. *Gastroenterology* 2004;127:S87-96.
19. An J, Chang S, Kim HI, Song GW, Shim JH. The clinical behavior and survival of patients with hepatocellular carcinoma and a family history of the disease. *Cancer Med* 2019;8:6624-33.
20. Gellert-Kristensen H, Richardson TG, Davey Smith G, Nordestgaard BG, Tybjaerg-Hansen A, Stender S. Combined effect of PNPLA3, TM6SF2, and HSD17B13 variants on risk of cirrhosis and hepatocellular carcinoma in the general population. *Hepatology* 2020; doi: 10.1002/hep.31238.
21. Dragani TA. Risk of HCC: genetic heterogeneity and complex genetics. *J Hepatol* 2010;52:252-7.
22. Jang HJ, Yang HR, Ko JS, Moon JS, Chang JY, Seo JK. Development of hepatocellular carcinoma in patients with glycogen storage disease: a single center retrospective study. *J Korean Med Sci* 2020;35:e5.
23. Santella RM, Wu HC. Environmental exposures and hepatocellular carcinoma. *J Clin Transl Hepatol* 2013;1:138-43.
24. Ng AWT, Poon SL, Huang MN, et al. Aristolochic acids and their derivatives are widely implicated in liver cancers in Taiwan and throughout Asia. *Sci Transl Med* 2017;9:eaan6446.
25. Yao KF, Ma M, Ding GY, et al. Meta-analysis reveals gender difference in the association of liver cancer incidence and excess BMI. *Oncotarget* 2017;8:72959-71.
26. Chen CL, Kuo MJ, Yen AM, et al. Gender difference in the association between metabolic factors and hepatocellular carcinoma. *JNCI Cancer Spectr* 2020;4:pkaa036.
27. Borena W, Strohmaier S, Lukanova A, et al. Metabolic risk factors and primary liver cancer in a prospective study of 578,700 adults. *Int J Cancer* 2012;131:193-200.
28. El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006;4:369-80.
29. Non-alcoholic Fatty Liver Disease Study Group, Lonardo A, Bellentani S, et al. Epidemiological modifiers of non-alcoholic fatty liver disease: Focus on high-risk groups. *Dig Liver Dis* 2015;47:997-1006.
30. Ballestri S, Nascimbeni F, Baldelli E, Marrazzo A, Romagnoli D, Lonardo A. NAFLD as a sexual dimorphic disease: role of gender and reproductive status in the development and progression of nonalcoholic fatty liver disease and inherent cardiovascular risk. *Adv Ther* 2017;34:1291-326.
31. Petroni ML, Brodosi L, Marchignoli F, Musio A, Marchesini G. Moderate alcohol intake in non-alcoholic fatty liver disease: to drink or not to drink? *Nutrients* 2019;11:E3048.

32. Wu EM, Wong LL, Hernandez BY, et al. Gender differences in hepatocellular cancer: disparities in nonalcoholic fatty liver disease/steatohepatitis and liver transplantation. *Hepatoma Res* 2018;4:66.
33. Rich NE, Murphy CC, Yopp AC, Tiro J, Marrero JA, Singal AG. Sex disparities in presentation and prognosis of 1110 patients with hepatocellular carcinoma. *Aliment Pharmacol Ther* 2020;52:701-9.
34. Phipps M, Livanos A, Guo A, et al. Gender matters: characteristics of hepatocellular carcinoma in women from a large, multicenter study in the United States. *Am J Gastroenterol* 2020;115:1486-95.
35. Ladenheim MR, Kim NG, Nguyen P, et al. Sex differences in disease presentation, treatment and clinical outcomes of patients with hepatocellular carcinoma: a single-centre cohort study. *BMJ Open Gastroenterol* 2016;3:e000107.
36. Lam CM, Yong JL, Chan AO, et al. Better survival in female patients with hepatocellular carcinoma: oral contraceptive pills related? *J Clin Gastroenterol* 2005;39:533-9.
37. Dohmen K, Shigematsu H, Irie K, et al. Longer survival in female than male with hepatocellular carcinoma. *J Gastroenterol Hepatol* 2003;18:267-72.
38. Tangkijvanich P, Mahachai V, Suwangool P, et al. Gender difference in clinicopathologic features and survival of patients with hepatocellular carcinoma. *World J Gastroenterol* 2004;10:1547-50.
39. Lai MW, Chu YD, Lin CL, et al. Is there a sex difference in postoperative prognosis of hepatocellular carcinoma? *BMC Cancer* 2019;19:250.
40. Li S, Hong M, Tan HY, Wang N, Feng Y. Insights into the Role and Interdependence of Oxidative Stress and Inflammation in Liver Diseases. *Oxid Med Cell Longev* 2016;2016:423406.
41. Yang SF, Chang CW, Wei RJ, Shiue YL, Wang SN, Yeh YT. Involvement of DNA damage response pathways in hepatocellular carcinoma. *Biomed Res Int* 2014;2014:153867.
42. Nishida N, Kudo M. Oxidative stress and epigenetic instability in human hepatocarcinogenesis. *Dig Dis* 2013;31:447-53.
43. O'Hagan HM, Wang W, Sen S, et al. Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. *Cancer Cell* 2011;20:606-19.
44. Nishida N, Kudo M, Nagasaka T, Ikai I, Goel A. Characteristic patterns of altered DNA methylation predict emergence of human hepatocellular carcinoma. *Hepatology* 2012;56:994-1003.
45. Makarova-Rusher OV, Altekruse SF, McNeel TS, et al. Population attributable fractions of risk factors for hepatocellular carcinoma in the United States. *Cancer* 2016;122:1757-65.
46. Rubin JB, Lagas JS, Broestl L, et al. Sex differences in cancer mechanisms. *Biol Sex Differ* 2020;11:17.
47. Lemieux S, Prud'homme D, Bouchard C, Tremblay A, Després JP. Sex differences in the relation of visceral adipose tissue accumulation to total body fatness. *Am J Clin Nutr* 1993;58:463-7.
48. Laughlin GA, Barrett-Connor E, May S. Sex-specific association of the androgen to oestrogen ratio with adipocytokine levels in older adults: the Rancho Bernardo Study. *Clin Endocrinol (Oxf)* 2006;65:506-13.
49. Manieri E, Herrera-Melle L, Mora A, et al. Adiponectin accounts for gender differences in hepatocellular carcinoma incidence. *J Exp Med* 2019;216:1108-19.
50. Lönnqvist F, Nordfors L, Jansson M, Thörne A, Schalling M, Arner P. Leptin secretion from adipose tissue in women. Relationship to plasma levels and gene expression. *J Clin Invest* 1997;99:2398-404.
51. Augustine LM, Fisher CD, Lickteig AJ, et al. Gender divergent expression of Nqo1 in Sprague Dawley and August Copenhagen x Irish rats. *J Biochem Mol Toxicol* 2008;22:93-100.
52. Kratschmar DV, Calabrese D, Walsh J, et al. Suppression of the Nrf2-dependent antioxidant response by glucocorticoids and 11beta-HSD1-mediated glucocorticoid activation in hepatic cells. *PLoS One* 2012;7:e36774.
53. Hofer T, Karlsson HL, Möller L. DNA oxidative damage and strand breaks in young healthy individuals: a gender difference and the role of life style factors. *Free Radic Res* 2006;40:707-14.
54. Naugler WE, Sakurai T, Kim S, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007;317:121-4.
55. Yasuda M, Shimizu I, Shiba M, Ito S. Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. *Hepatology* 1999;29:719-27.
56. Xie G, Wang X, Zhao A, et al. Sex-dependent effects on gut microbiota regulate hepatic carcinogenic outcomes. *Sci Rep* 2017;7:45232.
57. Tenkorang MA, Snyder B, Cunningham RL. Sex-related differences in oxidative stress and neurodegeneration. *Steroids* 2018;133:21-27.
58. Liu P, Xie SH, Hu S, et al. Age-specific sex difference in the incidence of hepatocellular carcinoma in the United States. *Oncotarget* 2017;8:68131-7.
59. Santos-Marcos JA, Rangel-Zuniga OA, Jimenez-Lucena R, et al. Influence of gender and menopausal status on gut microbiota. *Maturitas* 2018;116:43-53.
60. Santos-Marcos JA, Haro C, Vega-Rojas A, et al. Sex differences in the gut microbiota as potential determinants of gender predisposition to disease. *Mol Nutr Food Res* 2019;63:e1800870.
61. Kim YS, Unno T, Kim BY, et al. Sex differences in gut microbiota. *World J Mens Health* 2020;38:48-60.
62. Hassan MM, Botrus G, Abdel-Wahab R, et al. Estrogen replacement reduces risk and increases survival times of women with hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2017;15:1791-9.
63. Sukocheva OA. Estrogen, estrogen receptors, and hepatocellular carcinoma: are we there yet? *World J Gastroenterol* 2018;24:1-4.
64. Kalra M, Mayes J, Assefa S, et al. Role of sex steroid receptors in pathobiology of hepatocellular carcinoma. *World J Gastroenterol* 2008;14:5945-61.

65. Villa E, Camellini L, Dugani A, et al. Variant estrogen receptor messenger RNA species detected in human primary hepatocellular carcinoma. *Cancer Res* 1995;55:498-500.
66. Jonas S, Bechstein WO, Heinze T, et al. Female sex hormone receptor status in advanced hepatocellular carcinoma and outcome after surgical resection. *Surgery* 1997;121:456-61.
67. Chow PK, Tai BC, Tan CK, et al. High-dose tamoxifen in the treatment of inoperable hepatocellular carcinoma: a multicenter randomized controlled trial. *Hepatology* 2002;36:1221-6.
68. Barbare JC, Bouche O, Bonnetain F, et al. Randomized controlled trial of tamoxifen in advanced hepatocellular carcinoma. *J Clin Oncol* 2005;23:4338-46.
69. Villa E, Dugani A, Fantoni E, et al. Type of estrogen receptor determines response to antiestrogen therapy. *Cancer Res* 1996;56:3883-5.
70. Wu Y, Yao N, Feng Y, et al. Identification and characterization of sexual dimorphism-linked gene expression profile in hepatocellular carcinoma. *Oncol Rep* 2019;42:937-52.
71. Villa E, Moles A, Ferretti I, et al. Natural history of inoperable hepatocellular carcinoma: estrogen receptors' status in the tumour is the strongest prognostic factor for survival. *Hepatology* 2000;32:233-8.
72. Natri HM, Wilson MA, Buetow KH. Distinct molecular etiologies of male and female hepatocellular carcinoma. *BMC Cancer* 2019;19:951.
73. Li CH, Haider S, Shiah YJ, et al. Sex differences in cancer driver genes and biomarkers. *Cancer Res* 2018;78:5527-37.

Review

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Nonalcoholic fatty liver disease in lean subjects: is it all metabolic-associated fatty liver disease?

Mariana Verdelho Machado^{1,2}

¹Faculdade de Medicina, Universidade de Lisboa, Lisbon 1649-035, Portugal.

²Gastroenterology Department, Hospital de Vila Franca de Xira, Lisbon 1649-035, Portugal.

Correspondence to: Prof. Mariana Verdelho Machado, Gastroenterology Department, Faculdade de Medicina, Universidade de Lisboa, Avenida Professor Egas Moniz, Lisbon 1649-035, Portugal. E-mail: mverdelhomachado@gmail.com

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Abstract

The epidemiology of nonalcoholic fatty liver disease goes hand-in-hand with the obesity pandemic. The pathogenesis of fatty liver has shifted from an hepatocentric view to an adipocentric view, in which the overloaded adipose tissue spills out lipids that spread to ectopic tissues and organs such as the liver, elicits inflammation, and changes its adipokines profile promoting insulin resistance and the metabolic syndrome. Up to 40% of nonalcoholic fatty liver disease (NAFLD) patients are not obese and up to 20% are actually lean. Furthermore roughly 10% of lean subjects have NAFLD. In fact, adiposopathy can occur in patients with normal weight, and it is associated with expansion of metabolically active visceral fat and a qualitatively different adipose tissue that becomes overwhelmed after challenged by a mildly positive energy balance. This defines the concept of personal fat threshold that when exceeded results in metabolic dysfunction. Overweight/obese persons have higher probability of exceeding that threshold, explaining why adiposopathy/metabolic syndrome/NAFLD is more frequent in the obese. In this article, the epidemiology, pathogenesis, and management of patients with lean NAFLD are reviewed with an emphasis on reconciling the concepts of NAFLD in its relationship with adiposity and of NAFLD in lean individuals.

Keywords: Lean nonalcoholic fatty liver disease, metabolically obese normal weight, visceral adipose tissue



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INTRODUCTION

Fatty liver disease is the most common liver disease worldwide^[1]. The realization that liver steatosis may progress to cirrhosis is credited to the Austrian pathologist Carl von Rokitansky in mid-nineteenth century. Rokitansky also acknowledged that alcohol might not explain all types of fatty liver^[2]. The link with obesity was well-established one century after, with the description of liver biopsies from 20 obese men by Zelman^[3]. The designation “nonalcoholic steatohepatitis” (NASH) was first proposed by Ludwig 40 years ago^[4], and the more general “nonalcoholic fatty liver disease (NAFLD)” roughly 20 years ago^[5,6]. In 2020, an international group of experts changed the designation to “metabolic dysfunction-associated fatty liver disease” (MAFLD), focusing on the positive association with the metabolic syndrome (MS) rather than the negative association with alcohol, while acknowledging the possible concomitance of metabolic and alcohol-associated liver disease, in the same patient^[7].

NAFLD goes hand-in-hand with obesity and its associated co-morbidities, particularly the MS and its components^[8]. The prevalence of both NAFLD and obesity are rising. Data from the Fifth National Health and Nutrition Examination Survey (NHANES), in the US, showed that in the late 1980s, the prevalence of obesity was 22% and of NAFLD 20%, whereas currently, it is 39% and 32%, respectively^[9]. In fact, obesity increases the risk for NAFLD by 10-fold^[9].

Not all patients with NAFLD are overweight or obese. In those patients, liver steatosis has been called “lean-NAFLD”. Lean-NAFLD was first recognized in studies from Asia^[10], but it has gained relevance in Western populations as well^[11].

New diagnostic criteria for MAFLD in lean persons were proposed^[7], in which alcohol consumption is not an exclusion criteria, but requires, evidence of hepatic steatosis and the presence of at least 2 of the following metabolic abnormalities:

- (1) Waist circumference (WC) ≥ 102 and 88 cm in Caucasian men and women, respectively (or ≥ 90 and 80 cm in Asian men and women, respectively);
- (2) Blood pressure $\geq 130/85$ mmHg or specific drug treatment;
- (3) Plasma triglycerides ≥ 150 mg/dL or specific drug treatment;
- (4) Plasma high-density lipoprotein (HDL)-cholesterol < 40 mg/dL for men and < 50 mg/dL for women or specific drug treatment;
- (5) Prediabetes (*i.e.*, fasting glucose levels 100-125 mg/dL, or 2-h post-load glucose levels 140-199 mg/dL or HbA1c 5.7%-6.4%);
- (6) Homeostasis model assessment (HOMA) of insulin resistance score ≥ 2.5 ;
- (7) Plasma high-sensitivity C-reactive protein level > 2 mg/L.

Research on lean-NAFLD is flourishing; however, many gaps in knowledge still subsist: Does lean-NAFLD always associate with metabolic dysfunction and hence can be classified as MAFLD? Does lean-NAFLD follow the same fate as obesity-associated MAFLD? How should lean-NAFLD patients be managed? This article is a comprehensive review of the pathogenesis and clinical relevance of lean-NAFLD that aims to help clinicians in their daily practice.

EPIDEMIOLOGY

Roughly 20% of patients with hepatic steatosis present with normal body mass index (BMI) and 40% are not obese^[12,13]. As such, lean-NAFLD represents a significant burden on the daily practice of hepatologists.

Epidemiological studies on lean-NAFLD present very heterogeneous data, which result from: (1) different populations with different adipose tissue compartmentalization; (2) different definitions of lean (some

studies consider normal weight, others non-obese, while studies in rural populations in Asia include a high proportion of patients underweight^[14]; and (3) different tools to diagnose liver steatosis. Most studies rely on ultrasound to diagnose NAFLD, which has very low sensitivity for mild steatosis; others take advantage of non-invasive scores such as fatty liver index, some quantify liver triglycerides content through magnetic resonance spectroscopy, and several rely on liver biopsies. Those different diagnostic tools have known variations in accuracies for detecting liver steatosis^[15].

Notably, epidemiological studies were performed before the new designation of MAFLD; as such, alcohol intake was excluded, and patients may not fulfill the diagnostic criteria of MAFLD. Because of this, the designation “lean NAFLD” is used in this review.

The definition of normal weight using BMI varies depending on the racial background of the individual. Caucasians are considered as having normal weight when BMI is 18.5-25 kg/m² with overweight being 25-30 kg/m² and obese being > 30 kg/m²^[2,16]. However, lower BMI cutoffs are applied to Asians because a specific BMI reflects a higher percentage of body fat and higher health risk compared to Caucasians^[17]. Accordingly, in Asians, normal weight is considered when BMI is < 23 kg/m², with overweight being 23-25 kg/m² and obese being > 25 kg/m²^[2,16].

Although studies have shown a wide range of prevalence of NAFLD in lean individuals (from as low as 5% to as high as 26%), two recent meta-analysis showed that roughly 10% of lean adults have NAFLD. The prevalence rises to 16% in non-obese adults^[12,18]. Interestingly, the prevalence of lean-NAFLD gradually increased from 5.6% (95%CI: 3.6-8.8) in studies before 2000 to 12.6% (95%CI: 8.8-17.9) in studies after 2011^[18]. The prevalence of NAFLD is around 4 times lower in the lean population compared to the overweight/obese population^[11,19-24]. Asians seem to have a higher prevalence of lean-NAFLD, and African Americans lower^[12,25], which might be explained, at least to some extent, with different compartmentalization of fat depots and intrinsic differences in adipose tissue structure/function in individuals with different racial backgrounds^[26].

Regarding the association with metabolic features, lean patients with NAFLD seem to have an intermediate phenotype between healthy subjects and obese patients with MAFLD^[12,18,27,28], regarding glucose intolerance and insulin resistance (IR), type-2 diabetes mellitus (T2DM), hypertension, and hyperuricemia. The lipid profile, however, appears to be similar between lean and obese patients with NAFLD, with similar levels of total cholesterol and triglycerides, although lean patients tend to present higher levels of HDL cholesterol^[20,27,29,30].

On the other hand, a risk factor for NAFLD in lean subjects seems to be - besides older age - the presence of MS components (which seems to have an even stronger impact than in obese)^[31]. In particular, hypertriglyceridemia associates with a 2-fold increased risk for hepatic steatosis in lean^[10,27,32]. Furthermore, baseline hypertriglyceridemia or T2DM were predictors of incident lean-NAFLD in longitudinal studies^[33-35].

Even though they are in the normal weight range, history of weight gain over 10 kg since early adulthood increased the risk for NAFLD 2.5-fold in lean individuals^[21]. Furthermore, patients with lean-NAFLD tend to present higher BMI as compared to lean subjects without NAFLD^[30]. Interestingly, a Japanese study showed a linear increase on the prevalence of NAFLD with increasing BMI, starting at 18 kg/m² until 28 kg/m²^[2,21,36]. Similarly, two longitudinal community-based studies in non-obese subjects, from South Korea and Sri-Lanka, showed that body weight changes were indicators for the development or regression of ultrasound-defined NAFLD^[33,35]. Apparently, the lower the body fat (excluding underweight), the lower is the susceptibility for developing NAFLD.

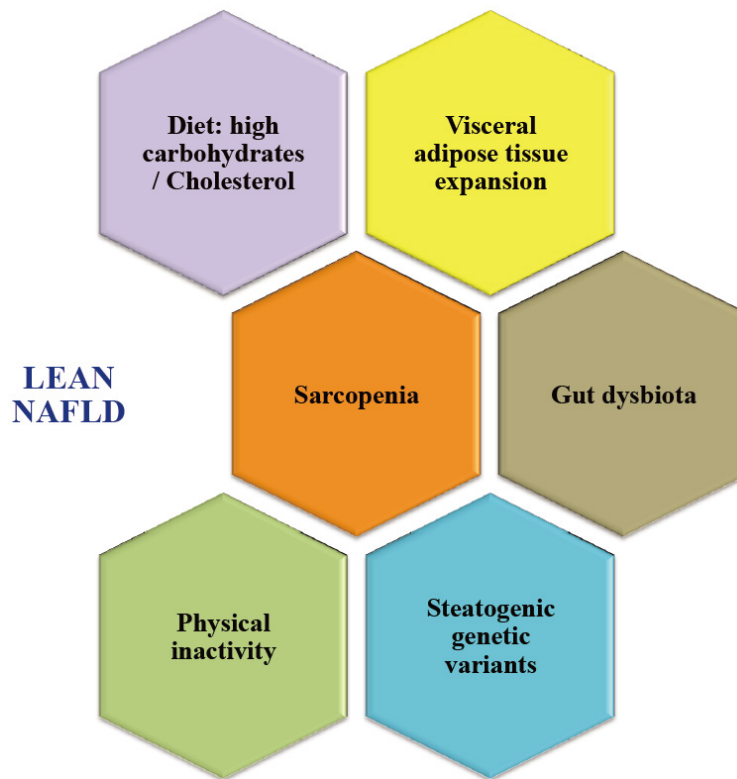


Figure 1. Pathogenesis of lean-NAFLD. NAFLD: nonalcoholic fatty liver disease

More important than BMI, a poor surrogate for adiposity, is central obesity which seems to predispose individuals to lean-NAFLD. In fact, lean patients with NAFLD tend to have higher WC as compared to lean subjects without NAFLD^[32]. When comparing lean NAFLD with obese NAFLD, expectedly lean subjects present with lower WC. However, lean patients with NAFLD present similar waist-to-hip (WTH) ratio^[22] but higher visceral adiposity indexes as compared to obese patients^[20]. This can be explained by the poor accuracy of WC for visceral adipose tissue (VAT). In fact, WC is determined by both visceral and abdominal subcutaneous fat^[37].

Lastly, in lean individuals, higher ferritin and hemoglobin levels seem to be associated with NAFLD, which suggest iron overload and oxidative stress as a cofactor to lipotoxicity in this subset of patients^[32,38].

PATHOPHYSIOLOGY

Hepatic steatosis, in lean individuals, can be induced by different mechanisms [Figure 1]:

- (1) The visceral adipose tissue spills over fatty acids that reach the liver, and promotes an inflammatory milieu, producing adipokines that are diabetogenic and steatogenic^[39];
- (2) The liver itself is prone to steatosis, for example, as a consequence of genetic traits that modulate the metabolism and export of lipids^[40];
- (3) Malnutrition and malabsorptive diseases induce hepatic steatosis^[41,42];
- (4) The bowel induces hepatic steatosis through direct and indirect effects of a steatogenic and pro-inflammatory intestinal microbiota^[43];
- (5) External factors induce hepatic steatosis such as drugs (for example, amiodarone, methotrexate, and tamoxifen);
- (6) Other liver diseases may predispose to steatohepatitis (for example, hepatitis C particularly genotype 3, Wilson's disease, and inborn errors of metabolism)^[44].

NAFLD is in intimate association with obesity and is characterized by features of adiposopathy^[45]. However, adiposopathy may exist even with normal BMI, associating with an expansion of VAT, the so-called metabolically obese normal weight (MONW)^[46,47]. Adiposopathy may also occur in association with a lipodystrophic phenotype, which can be genetic (rare genetic lipodystrophy disorders have an estimated prevalence of one in a million^[48]) or acquired [for example, an adverse effect of highly active antiretroviral therapy in patients infected with human immunodeficiency virus (HIV)^[39]].

Metabolically obese normal weight

BMI is an imperfect measurement of obesity/adiposity because it does not take into consideration body composition (for example, differences in adipose and muscle mass), or the different patterns of fat distribution^[49]. MONW refers to subjects with normal weight according to BMI, but with associated cardiometabolic abnormalities such as IR, altered lipid profile, and/or hypertension^[50]. The estimated prevalence of MONW is around 20% in normal weight subjects^[51]. The prevalence seems to gradually increase from Caucasians to African American, Hispanics, and Asians^[50]. The prevalence of MONW increases with age and with increasing BMI, even in the normal range^[50]. Importantly, MONW is associated with a 3-fold increase in all-cause mortality, T2DM, and cardiovascular events, resembling obese or overweight subjects with associated metabolic dysfunction^[48]. MONW might explain a high fraction of patients with lean-NAFLD^[39]. The unsolved question is why normal weight patients develop metabolic features of overweight/obese patients. There seems to be a “personal fat threshold” independent of BMI, that when exceeded results in metabolic dysfunction^[52]. In fact, MONW subjects may have normal body fat percentage, but different compartmentalization of fat, with central obesity, and particularly with increased visceral fat. Indeed, epidemiological studies demonstrate a strong association between the MONW phenotype and visceral adiposity^[53].

VAT, that is intra-abdominal adipose tissue, accounts for 7%-15% of total body fat^[39], and expands when subcutaneous adipose tissue (SAT) surpasses its capability to adapt to a positive caloric balance^[54]. VAT is morphologically and metabolically distinct from SAT. Adipocytes in VAT respond to energy surplus with cell hypertrophy, with enlarged adipocytes presenting decreased ability to further store lipids, enhanced lipolytic response, and blunted insulin-inhibition of lipoprotein lipase, demonstrating adipose tissue IR^[55]. Unlike abdominal SAT, VAT is drained by the portal vein which allows exposure of the liver directly to high concentrations of free fatty acids (FFA) and glycerol from oversized visceral adipocytes^[54]. In fact, while in lean healthy subjects the VAT contributes to only 5-10% of the FFA that reach the portal vein, in subjects with expanded VAT, its contribution can increase to 50%^[56]. FFAs that reach the liver contribute to the accumulation of ectopic fat in the liver and promote hepatic IR^[57]. In the liver, FFAs activate nuclear receptors such as peroxisome proliferator activated receptor (PPAR)- α and hepatic nuclear factor, which profoundly modulate hepatic lipid metabolism, promoting the synthesis and export of triglycerides, and explaining the strong association between hypertriglyceridemia and NAFLD, particularly in lean-NAFLD^[54].

The oversized VAT is prone to cell death and inflammation with macrophage infiltration^[58], which are responsible for local deregulation of adipokine secretion - with increased production of tumor necrosis factor (TNF)- α and interleukin-6, and decreased production of the insulin-sensitizer and anti-inflammatory adiponectin - and a systemic metabolic inflammatory state, further exacerbating IR^[59].

Epidemiological studies showed that VAT was an independent risk factor for NAFLD, whereas BMI was not^[60-65]. Furthermore, there seems to be a dose-response association between VAT and prevalence of NAFLD^[62]. In contrast, between SAT and NAFLD, a negative association was even described^[61]. Similarly, longitudinal studies showed that VAT expansion was associated with incident NAFLD, whereas SAT expansion was associated with NAFLD regression^[66].

Studies on lean-NAFLD showed similar associations between hepatic steatosis and both central obesity and visceral fat^[20,29,67-69]. Interestingly, lean-NAFLD patients present lower levels of adiponectin than their lean healthy counterparts. Furthermore, even though lean-NAFLD patients tend to have lower levels of leptin compared to overweight/obese NAFLD patients, which translates to lower body fat, they have similar levels of adiponectin, suggesting similar adipose tissue malfunction and adiposopathy^[28,68,70].

The differentiation between upper and lower body fat has unraveled interesting new concepts on the metabolic consequences of body fat distribution^[71]. SAT can be divided into upper body fat, which refers to abdominal subcutaneous fat, and lower body fat, which refers to gluteofemoral subcutaneous fat. Data from the Third NHANES, in the US, showed that normal weight subjects with upper body obesity (defined by an increased WTH ratio) had the highest all-cause mortality rate, more than doubling both normal weight and overweight/obese subjects without upper body obesity, and being matched only by overweight/obese that also had upper body obesity^[72]. Unlike abdominal/upper body fat, femoral/lower body subcutaneous fat seems to be an independent protective factor for cardiometabolic diseases and mortality^[71,73,74]. In fact, abdominal SC fat depots are physiologically different from femoral SC fat depots. Abdominal SAT demonstrates a rapid diet-derived fat uptake and high lipid turnover responsive to adrenergic activation. Femoral SAT, on the contrary, has a low lipid turnover and is more efficient in storing fat, mounting a hyperplastic response (recruiting new adipocytes from precursors rather than enlarging the adipocytes as occurs in abdominal SAT) to a caloric challenge^[75], and being less susceptible to inflammation^[71]. Upper and lower body fat are under different epigenetic control of genes involved in adipocyte differentiation, with higher expression of TBX5 and HOXA5 in upper SAT and higher expression of HOTAIR in lower SAT^[76]. Noteworthy, polymorphisms in genes that regulate adipocyte differentiation such as CCDC92, DNAH10, and L3MBTL3, associate with lower body fat, and paradoxically with IR, T2DM, and coronary heart disease. This suggests that subjects with predisposed limited storage capacity of peripheral adipose tissue, are more susceptible to IR and to cardiometabolic diseases^[77].

Concordant with a role of decreased lower body fat in the susceptibility to NAFLD in lean individuals, WC and WTH ratio are better predictors for incident lean-NAFLD as compared to BMI^[34,78].

Sarcopenia

Sarcopenia, that is, loss of skeletal muscle mass and function, seems to confer susceptibility to NAFLD^[79]. Sarcopenia may help explain the occurrence of hepatic steatosis in patients with BMI within the normal range, since BMI cannot differentiate between fat deprived nor muscle mass deprived subjects.

Data from the Korean National Health and Nutrition Examination Survey (KNHANES) showed that sarcopenia associates with NAFLD and liver fibrosis, independent of obesity and IR^[79]. Other studies and recent meta-analysis corroborated that data^[80-85]. Furthermore, skeletal muscle mass seems to be lower in lean *versus* obese patients with NAFLD^[86].

The relationship between sarcopenia and metabolic dysfunction goes both ways. IR and chronic inflammation promotes myosteatosis^[87], which exacerbates muscle catabolism. On the other hand, sarcopenia (and disturbed myokines secretion) promotes IR and hepatic steatosis^[88].

IR and T2DM promote muscle depletion and dysfunction through several mechanisms: (1) FFAs released by an insulin resistant adipose tissue inhibit the growth hormone/insulin growth factor-1 axis, decreasing its protective effect on muscle regeneration^[89]; (2) myosteatosis associates with a blunted activation of muscle peroxisome proliferator-activated receptor gamma coactivator-1 α and an increase in oxidative stress resulting in mitochondrial dysfunction^[90]; and (3) an increase in gluconeogenesis drives proteolysis. Obesity and NAFLD associate with a small grade inflammatory state, with an increase in proinflammatory

cytokines such as TNF- α and interleukin-6 that stimulate proteolysis/muscle catabolism further contributing to sarcopenia^[91].

On the other hand, myosteatosis induces IR through FFA modulation of p38 mitogen-activated protein kinase^[92]. The muscle is the primary organ responsible for insulin-mediated glucose disposal, hence a decrease in muscle mass translates in impaired glucose metabolism, with postprandial hyperglycemia^[93]. Sarcopenia also decreases the tolerance to exercise, further decreasing energy expenditure, exacerbating energy surplus, and promoting weight gain and IR^[94].

Myokines also have an important role in the pathogenesis of NAFLD. Irisin is an exercise-inducible myokine that promotes transdifferentiation of white into brown adipose tissue, with upregulation of uncoupling proteins, resulting in an increase in heat production, energy expenditure, and weight loss^[95]. In the liver, irisin has direct anti-steatogenic effects through activation of PPAR- α and upregulation of fibroblast growth factor (FGF)-21^[88]. Accordingly, a study in obese patients showed a negative correlation between irisin and hepatic steatosis severity^[96]. Myostatin is a myokine whose expression is downregulated by exercise^[97]. Myosin has a negative impact on sarcopenia, since it promotes proteolysis and inhibits muscle regeneration and function^[98]. Myostatin also has metabolic actions, promoting adipose tissue expansion through direct effects on the adipose tissue and indirectly through downregulation of irisin^[99,100]. Lastly, myostatin has fibrogenic properties through direct action on hepatic stellate cells^[101]. Interestingly, myostatin levels correlate with liver steatosis in lean subjects^[69]. Increased myostatin levels are also seen in end-stage liver disease^[102], and associate with mortality in patients with liver cirrhosis^[103].

Sarcopenia and expansion of VAT act synergistically in the pathogenesis and progression of NAFLD. In patients with NAFLD, a decrease in muscle mass and increase in VAT are associated with worsening of steatosis and, more importantly, progression of liver fibrosis^[104].

Genetics

In 2008, a genome wide association study unraveled a polymorphism in patatin-like phospholipase domain-containing protein 3 (PNPLA3), rs738409 C>G, encoding the substitution of isoleucine for methionine at position 148 (I148M), which associates with increased risk for hepatic steatosis disconnected from obesity or the MS^[105,106]. Subsequent studies found that polymorphism is also associated with steatohepatitis, advanced fibrosis, and progression to hepatocellular carcinoma^[107-110].

PNPLA3 (adiponutrin) is a membrane-bound protein highly expressed in the liver and adipose tissue, that regulates remodeling of lipid droplets on hepatocytes and hepatic stellate cells. Diet can modulate PNPLA3 expression. Carbohydrates upregulate^[111,112], whereas unsaturated fatty acids suppress PNPLA3 expression^[113]. The rs738409 variant results in the accumulation of a dysfunctional adiponutrin on lipid droplets^[114].

Regarding the role of PNPLA3 rs738409 on lean-NAFLD, studies consistently found an increased G allele frequency on lean-NAFLD patients as compared to lean healthy subjects, mirroring studies on MAFLD^[28]. Some studies also found an increased G allele or GG phenotype in lean vs. obese NAFLD patients^[22,35,115,116], though not all studies reproduced those findings^[28,34,86,117]. An Italian study, albeit did not find a difference between G allele frequencies in lean or obese NAFLD, found that this variant is associated with disease severity (presence of steatohepatitis and significant fibrosis) only in lean patients^[118]. Two meta-analyses failed to find a difference in G allele between lean and obese patients with NAFLD^[12,18], which indicates that variants in PNPLA3 might not be a major factor in the development of lean-NAFLD. Of note, Asian studies showed higher differences in the PNPLA3 variant in lean-NAFLD as opposed to overweight/obese NAFLD^[119], suggesting interplay between racial background and PNPLA3 phenotype in the development of lean-NAFLD.

Transmembrane-6 superfamily-2 (TM6SF2) is another gene that regulates lipid metabolism and has been implicated on the pathogenesis of NAFLD. TM6SF2 modulates intestinal cholesterol absorption and clearance, hepatic cholesterol biosynthesis and secretion, as well as the flux of triglycerides from lipid droplets to the synthesis and secretion of very low-density lipoproteins (VLDL) from the liver^[40,120]. A variant of TM6SF2, rs58542926 C>T, possesses a substitution of glutamate for lysine at position 167 (E167K), and is a loss-of-function variant that increases the risk of NAFLD and hepatocellular carcinoma, albeit decreasing circulating lipids and protecting from cardiovascular disease^[121-125].

Regarding the association between TM6SF2 rs58542926 and lean-NAFLD, two studies found a higher prevalence of the T allele in lean compared to overweight/obese patients with NAFLD^[118,126]. However, others could not reproduce those findings^[28,117].

Cholesteryl ester transfer protein mediates the exchange of triglycerides from triglycerides-rich lipoproteins to HDL, allowing reverse cholesterol transport from peripheral tissues back to the liver. Two variants, rs12447924 and rs12597002, are associated with NAFLD in lean individuals, but not obese individuals, in an Australian cohort^[127].

SREBP-2 regulates cholesterol biosynthesis, uptake, and excretion. The SREBP-2 polymorphism rs133291 is associated with increased risk of NAFLD and steatohepatitis in non-obese, non-diabetic patients without the MS, once again helping to explain a dissociation between NAFLD and metabolic disturbances and obesity^[128].

Lastly, a variant in phosphatidylethanolamine N-methyltransferase (PEMT), rs7946 C>T was associated with lean-NAFLD and unhealthy lipid profile with an increase in low-density lipoproteins (LDL) and a decrease in HDL^[129,130]. PEMT catalyzes *de novo* synthesis of choline, which is required for the export of hepatic triglycerides as VLDL^[131]. Accordingly, patients with lean-NAFLD have low hepatic PEMT expression^[132].

It would be interesting to evaluate whether lean patients with NAFLD have an increased risk of carrying at least one of the above different risk alleles as compared to overweight/obese NAFLD.

Microbiota

Microbiota may have a strong role on the development of NAFLD in lean subjects. When comparing the microbiota from lean and overweight/obese patients, significant differences have been consistently reported^[43,126,133].

A multicenter study recruited patients from Italy and Australia, and found that lean-NAFLD, compared to non-lean NAFLD and controls, had an altered fecal microbiota profile. Lean patients had enrichment of the genera *Erysipelotrichaceae* UCG-003, *Ruminococcus*, *Clostridium sensu stricto* 1, *Romboutsia*, and *Ruminococcaceae* UCG-008, and an impoverishment of *Ruminiclostridium* and *Streptococcus*, as compared to non-lean patients^[126]. This microbiota profile of lean patients seems to promote the formation of bile acids, and hence to associate with increased levels of secondary bile-acids, as well as FGF-19. This increase in bile acids and FGF-19 could explain a metabolic adaptation to an obesogenic diet, promoting a peripheral increase in energy expenditure. On the other hand, as compared to healthy subjects, lean-NAFLD patients showed impoverishment of species believed to be protective against hepatic steatosis such as *Marvinbryantia* and *Christensenellaceae* R7 group, and enrichment of *Dorea* species that have previously been associated with NASH^[126].

Other studies found impoverishment of potentially protective bacteria such as *Faecalibacterium* and *Lactobacillus*^[43]. *Faecalibacterium prausnitzii* are butyrate-producing bacteria with known immune-

modulation and anti-inflammatory properties that have already been implicated in the pathogenesis of NAFLD^[134].

A Japanese study also found that lean-NAFLD patients more frequently carry an HLA haplotype (HLA-B*54:01) that modulates gut microbiota towards an impoverishment of bacteria potentially protective against progression to cirrhosis, such as the phylum Verrucomicrobia and the genus *Akkermansia*^[134,135].

Another way gut dysbiosis can promote hepatic steatosis is through the enrichment of bacteria, such as *Weissella confusa* that can produce ethanol through the fermentation of glucose and fructose^[136,137]. A first pass effect of ethanol in the liver would abrogate systemic metabolic effects in patients harboring ethanol-producing microorganisms in the gut.

Studies regarding the use of probiotics on NAFLD do not specifically evaluate lean patients, are small sized, use different combinations of probiotic agents, and do not evaluate hard outcomes such as liver biopsy. However, a recent meta-analysis suggests a benefit of probiotics in the treatment of patients with NAFLD, inducing an improvement in liver enzymes^[138].

Lifestyle

A South Korean study evaluated a cohort of patients from the KNHANES, and found a high carbohydrate energy ratio and lower than moderate-level physical activity to be predictors of lean-NAFLD^[139]. Carbohydrates are known to downregulate PNPLA3, which may partially explain its steatogenic effect^[111,112]. A caveat of this finding is that the type of carbohydrates consumed in Asia, where rice is the major carbohydrate, is likely not the same as in Western populations, hence it is difficult to extrapolate these findings to non-Asians^[139]. High fructose intake has also been associated with lean-NAFLD^[140]. Lean patients that develop NAFLD consume more than double added sugar than lean subjects without NAFLD. Of note, most of the added sugar, in lean-NAFLD patients, comes from soft drinks and juices. Indeed, the prevalence of excessive soft drink consumers is 4 times as higher in lean-NAFLD patients than healthy lean subjects^[140].

Lean-NAFLD patients tend to eat high-cholesterol diets. Both European and Asian cohorts showed lean-NAFLD patients to consume more cholesterol and less polyunsaturated fatty acids, as compared to healthy lean subjects, even without an excessive caloric intake^[68,141]. Cholesterol has steatogenic effects, since it promotes fatty acids synthesis and *de novo* lipogenesis through activation of SREBP-1c and liver X-receptor- α ^[68].

Even though lean-NAFLD patients by definition have normal weight, they tend to adopt an intermediate obesogenic lifestyle. In fact, lean subjects that gain weight, over 10 kg, in their adulthood have a 2.5 fold increased risk of developing NAFLD, even when maintaining a normal BMI^[21]. Probably in patients that develop NAFLD, those 10 extra kilograms surpass their “personal fat threshold”^[52] inducing adiposopathy and its associated comorbidities.

Malabsorption

NAFLD is the most frequent hepatic manifestation of small bowel diseases with malabsorption such as inflammatory bowel disease (IBD) and celiac disease. We must consider these diagnoses in patients with lean-NAFLD, since 3% of patients with NAFLD have underlying celiac disease, which is 3 times the overall prevalence of celiac disease^[142-145]. If we consider lean-NAFLD, the prevalence of celiac disease raises to up to 15%^[144].

Overall, the reported prevalence of hepatic steatosis in patients with IBD is roughly 40%^[146]. Also celiac disease patients, even when in a gluten-free diet, present 3 times higher prevalence of hepatic steatosis

as compared to healthy matched controls^[147,148]. Interestingly, the risk of hepatic steatosis in non-obese celiac disease patients was almost 6 times higher than in weight-matched controls^[147]. It seems that the more severe the malabsorption the higher the risk of hepatic steatosis. Indeed, patients with IBD that are underweight have 4 times higher prevalence of hepatic steatosis (up to 85%) than the ones with normal weight. Hepatomegaly, severity of steatosis, and elevation of aminotransferases are also more severe^[41].

One important mechanistic link between bowel diseases and NAFLD is gut dysbiosis and increased intestinal permeability that may promote steatosis and inflammation in the liver^[149]. Furthermore, malnutrition might lead to an impaired function of liver mitochondria and a loss of peroxisomes, which are important to maintain the normal liver function^[150].

Lastly, intestinal failure/parenteral nutrition-associated fatty liver disease is a different entity, in which liver histology is characterized by steatosis (often microsteatosis) in association with cholestasis. This form of fatty liver disease is associated with rapid and frequent progression to steatohepatitis and end-stage liver failure. Mechanistically, it associates with choline deficiency, which impairs lipid metabolism resulting in reduced synthesis of phospholipids and reduced export of hepatic lipids^[42].

Hepatic steatosis as a manifestation of other liver diseases

NAFLD is more frequent in overweight/obese subjects as compared to lean subjects. Hence, other liver diseases that increase liver fat should be actively excluded in lean subjects who present with hepatic steatosis. Chronic viral hepatitis (particularly hepatitis C virus genotype 3^[151]) and Wilson's disease can present with liver steatosis. Celiac disease should be systematically excluded since the prevalence of celiac disease in lean-NAFLD can reach up to 15%^[144]. Cystic fibrosis should also be investigated, particularly in young patients with history of recurrent infections.

Inborn errors of metabolism should be suspected in young patients with atypical facial appearance, low stature, severe metabolic derangements (such as severe hyper- or hypolipidemia, hypoglycemia, and metabolic acidosis), and other organs involvement, with particular attention for muscle abnormalities, kidney disease (particularly tubulopathy), cardiovascular, and neurological symptoms^[44].

Lysosomal acid lipase (LAL)-deficiency is a rare autosomal recessive lysosomal disease that can present in the adulthood. It manifests by hepatosplenomegaly, dyslipidemia, and hepatic steatosis^[152]. Its clinical course is characterized by early onset atherosclerosis with severe cardiovascular disease, and rapidly progressive liver disease. Indeed, liver cirrhosis and end-stage liver disease may develop within 3 years of the clinical onset. Liver biopsy presents mixed macro- and microvesicular steatosis, and the presence of birefringent cholesteryl ester crystals is pathognomonic. Diagnosis can be made with determination of LAL activity in dry blood sample. Albeit a rare disease (estimated prevalence of 1:40000 to 1:300000), an enzyme replacement therapy (sebelipase-alpha) is available. As such, it is worthwhile to investigate LAL-deficiency in young lean-NAFLD patients with severe hypercholesterolemia (LDL higher than 160 mg/dL or 130 mg/dL in patients taking lipid-lowering drugs; and HDL lower than 40 mg/dL in men and 50 mg/dL in women), with cryptogenic cirrhosis, or with liver biopsy that shows microvesicular steatosis^[152]. In fact, one third of patients with microvesicular steatosis or cryptogenic cirrhosis may have LAL-deficiency^[153].

Glycogen storage diseases might be misdiagnosed as lean-NAFLD because glycogen and fat accumulation in the liver may be undistinguishable by ultrasonography^[154]. Some types of glycogen storage diseases may present a milder phenotype that can manifest only in adulthood, particularly types I, III, VI and IX. Besides symptoms related to hypoglycemia, these diseases may present with hepatosplenomegaly, that can progress to liver cirrhosis, hyperlipidemia (particularly hypertriglyceridemia), recurrent infections, myopathy, and kidney affection with tubular dysfunction and Fanconi-Bickel syndrome^[44].

Hypobetalipoproteinemia is a common autosomal dominant disease that is characterized by defective transport of triglycerides from the liver, causing intrahepatic accumulation. Mutations in ApoB and microsomal triglyceride transfer protein can induce hepatic steatosis^[155]. Hypobetalipoproteinemia associates with hypotriglyceridemia, absence of cardiometabolic dysfunction/IR^[156], severe forms of NAFLD, and even hepatocellular carcinoma^[157].

CLINICAL FEATURES AND PROGNOSIS

Patients with lean-NAFLD may present the whole spectrum of liver disease, from isolated steatosis to liver cirrhosis, hepatocellular carcinoma, and end-stage liver disease. Most epidemiological studies suggest that liver histology might be less severe in lean patients as compared to overweight/obese patients with NAFLD/MAFLD^[12,117,118,158-160]. This finding, however, may be biased as clinicians might have a lower threshold to perform liver biopsy in lean patients with hepatic steatosis, in whom it may be clinically relevant to exclude other diseases. As such, liver biopsy may be performed in patients with milder forms of liver injury. In a community-based Hong Kong cohort, non-obese NAFLD presented lower liver stiffness (4.6 kPa vs. 5.6 kPa) despite similar intrahepatic triglycerides content. However, the percentage of advanced fibrosis, defined by liver stiffness > 9.6 kPa, was similar^[22]. Indian studies also showed similar prevalence of steatohepatitis and advanced fibrosis in lean and overweight/obese NAFLD patients^[30,161]. An Austrian study of 466 liver biopsies, on the contrary, found worse liver injury with more severe fibrosis and more prevalent cirrhosis in lean patients^[162].

Central obesity/VAT expansion was the most transversal risk factor for advanced fibrosis in different epidemiological studies on lean-NAFLD^[118]. In fact, each 10-cm² increase in VAT is associated with a 20% increased risk for significant fibrosis^[63]. Other risk factors seem to be T2DM, hypertriglyceridemia, hypertension^[118], and higher levels of hemoglobin (suggesting mild iron overload and hence increased oxidative stress)^[163,164].

Lean subjects with NAFLD have a 3-fold increased risk for incident T2DM, even in the absence of MS. The risk for *de novo* T2DM seems similar to overweight/obese NAFLD patients^[23,24,165].

An analysis of the Third NHANES, showed that compared to healthy lean subjects, lean patients with ultrasound-diagnosed NAFLD had a 50% increase in all-cause mortality and over a two-fold increase in cardiovascular mortality^[166].

Three long-term follow-up studies evaluated the outcome of patients with biopsy-confirmed lean-NAFLD^[117,160], one of them was presented as a poster^[158]. A recent meta-analysis including the 2 published studies suggested that lean-NAFLD patients had 28% and 78% lower odds of overall mortality and liver-related mortality, respectively. However, the cardiovascular death rate was similar, compared to obese patients with MAFLD^[12]. The Asian published study followed 307 NAFLD patients for up to 5 years and did not find a difference in clinical events between lean and non-lean. The majority of the events were cardiovascular. Only 6 deaths occurred, all of them in obese patients. Furthermore, relevant liver events were limited to 2 cases of hepatocellular carcinoma and one of liver failure, again all obese patients^[117]. The European published study followed 646 patients for up to 20 years^[160]. They found no difference on overall mortality in lean *versus* non-lean NAFLD patients, but lean patients presented an increased risk for severe liver disease compared to overweight and similar to risk compared to obese patients. Of note, 58% of lean patients who developed severe liver disease had no or mild fibrosis (F0-2) at baseline^[160]. The unpublished study is an international cohort of 1,900 patients and found that even though lean-NAFLD patients had milder histology at baseline, they had lower liver-transplant free survival than non-lean patients.

MANAGEMENT

The main treatment in MAFLD is lifestyle modification in order to lose weight targeting normal weight^[167]. Lean-NAFLD patients already have normal weight, should weight loss be pursued? What would be the target weight loss?

Epidemiological studies taught us that even in the normal weight range, the lower the BMI, the lower the risk for NAFLD^[21,36]. Also, lean patients with NAFLD frequently present with VAT expansion^[20]. The concept that everyone has their own personal fat threshold^[52] suggests that weight loss decreasing adiposity below the fat threshold might be beneficial metabolically and to the liver. In fact, in a small study with 20 lean-NAFLD patients, any weight loss was associated with steatosis improvement, and being above 5% corporal weight was associated with improvement in NAFLD activity score^[168].

Exercise should be highly encouraged. Exercise, particularly aerobic exercise, seems to preferentially target VAT over SAT^[169]. Also a significant decrease in VAT can occur even without significant weight loss^[170]. Patients should be advised to perform aerobic exercise below current recommendations for overweight/obese, with at least 150 min per week of moderate intensity exercise (for example, brisk walking, light jogging, or stationary ergometer usage)^[171].

Cardiometabolic risk factors should be aggressively treated. Of note, incretin-based therapy may be more effective in overweight/obese because it associates with weight loss^[47]. On the contrary, thiazolidinediones promote adipocyte differentiation and fat cell hyperplasia in adipocytes in subcutaneous compartments, and may be more effective in MONW patients^[58].

Lastly, modulation of gut microbiota in the treatment of lean-NAFLD should be further explored, giving the known role of gut dysbiota in its pathogenesis.

CONCLUSION

Lean-NAFLD is a frequent condition in the daily practice of hepatologists. Efforts should be made to differentiate between lean MAFLD and other conditions that are also associated with hepatic steatosis, such as other liver diseases (for example, chronic viral hepatitis and Wilson's disease), small bowel diseases with malabsorption (for example, celiac disease and Crohn's disease), and inborn errors of metabolism (for example, LAL-deficiency).

Patients with lean MAFLD have a disorder of the adipose tissue, despite their normal weight. Each individual might have his own personal fat threshold that when exceeded results in adipose tissue malfunction, cardiometabolic disturbances, and ectopic fat accumulation in the liver. The personal fat threshold is dependent on the different compartmentalization of the adipose tissue (visceral vs. subcutaneous and upper vs. lower body fat) as well as intrinsic properties of the adipose tissue that allow it to deal with fat challenges (hypertrophic vs. hyperplastic responses to energy surplus).

Patients with lean-NAFLD/MAFLD can have the whole spectrum of liver disease from isolated steatosis to liver cirrhosis and end-stage liver disease. Although studies evaluating the prognosis of lean NAFLD/MAFLD are scarce, lean patients probably do not have a more benign prognosis as overweight/obese patients.

Management of patients with lean NAFLD/MAFLD should follow the same principles as for non-lean patients. Lifestyle modifications should be advised in order to address visceral fat. Weight loss might be beneficial even in patients with normal BMI, for whom their personal fat threshold falls below the upper normal BMI cutoff.

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The author contributed solely to the article.

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All authors declared that there are no conflicts of interest.

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REFERENCES

1. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018;15:11-20.
2. Best CH, Hartroft WS, et al. Liver damage produced by feeding alcohol or sugar and its prevention by choline. *Br Med J* 1949;2:1002-6, pl.
3. Zelman S. The liver in obesity. *AMA Arch Intern Med* 1952;90:141-56.
4. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: mayo clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980;55:434-8.
5. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002;346:1221-31.
6. Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. *Gastroenterology* 2002;122:1649-57.
7. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol* 2020;73:202-9.
8. Machado MV, Cortez-Pinto H. Management of fatty liver disease with the metabolic syndrome. *Expert Rev Gastroenterol Hepatol* 2014;8:487-500.
9. Younossi ZM, Stepanova M, Younossi Y, et al. Epidemiology of chronic liver diseases in the USA in the past three decades. *Gut* 2020;69:564-8.
10. Chen CH, Huang MH, Yang JC, et al. Prevalence and risk factors of nonalcoholic fatty liver disease in an adult population of taiwan: metabolic significance of nonalcoholic fatty liver disease in nonobese adults. *J Clin Gastroenterol* 2006;40:745-52.
11. Younossi ZM, Stepanova M, Negro F, et al. Nonalcoholic fatty liver disease in lean individuals in the United States. *Medicine (Baltimore)* 2012;91:319-27.
12. Young S, Tariq R, Provenza J, et al. Prevalence and profile of nonalcoholic fatty liver disease in lean adults: systematic review and meta-analysis. *Hepatol Commun* 2020;4:953-72.
13. Ye Q, Zou B, Yeo YH, et al. Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020;5:739-52.
14. Das K, Das K, Mukherjee PS, et al. Nonobese population in a developing country has a high prevalence of nonalcoholic fatty liver and significant liver disease. *Hepatology* 2010;51:1593-602.
15. Machado MV, Cortez-Pinto H. Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal. *J Hepatol* 2013;58:1007-19.
16. Weir CB, Jan A. BMI classification percentile and cut off points. StatPearls. Treasure Island (FL): StatPearls Publishing; 2020.
17. Consultation WHOE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004;363:157-63.
18. Shi Y, Wang Q, Sun Y, et al. The prevalence of lean/nonobese nonalcoholic fatty liver disease: a systematic review and meta-analysis. *J Clin Gastroenterol* 2020;54:378-87.

19. Bellentani S, Saccoccio G, Masutti F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000;132:112-7.
20. Feng RN, Du SS, Wang C, et al. Lean-non-alcoholic fatty liver disease increases risk for metabolic disorders in a normal weight Chinese population. *World J Gastroenterol* 2014;20:17932-40.
21. Nishioji K, Sumida Y, Kamaguchi M, et al. Prevalence of and risk factors for non-alcoholic fatty liver disease in a non-obese Japanese population, 2011-2012. *J Gastroenterol* 2015;50:95-108.
22. Wei JL, Leung JC, Loong TC, et al. Prevalence and severity of nonalcoholic fatty liver disease in non-obese patients: a population study using proton-magnetic resonance spectroscopy. *Am J Gastroenterol* 2015;110:1306-14; quiz 15.
23. Kim SS, Cho HJ, Kim HJ, et al. Nonalcoholic fatty liver disease as a sentinel marker for the development of diabetes mellitus in non-obese subjects. *Dig Liver Dis* 2018;50:370-7.
24. Sinn DH, Kang D, Cho SJ, et al. Lean non-alcoholic fatty liver disease and development of diabetes: a cohort study. *Eur J Endocrinol* 2019;181:185-92.
25. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;40:1387-95.
26. Alberti KG, Zimmet P, Shaw J, Group IDFETFC. The metabolic syndrome--a new worldwide definition. *Lancet* 2005;366:1059-62.
27. Sookoian S, Pirola CJ. Systematic review with meta-analysis: risk factors for non-alcoholic fatty liver disease suggest a shared altered metabolic and cardiovascular profile between lean and obese patients. *Aliment Pharmacol Ther* 2017;46:85-95.
28. Feldman A, Eder SK, Felder TK, et al. Clinical and metabolic characterization of lean caucasian subjects with non-alcoholic fatty liver. *Am J Gastroenterol* 2017;112:102-10.
29. Margariti E, Deutsch M, Manolakopoulos S, Papatheodoridis GV. Non-alcoholic fatty liver disease may develop in individuals with normal body mass index. *Ann Gastroenterol* 2012;25:45-51.
30. Kumar R, Rastogi A, Sharma MK, et al. Clinicopathological characteristics and metabolic profiles of non-alcoholic fatty liver disease in Indian patients with normal body mass index: do they differ from obese or overweight non-alcoholic fatty liver disease? *Indian J Endocrinol Metab* 2013;17:665-71.
31. Kwon YM, Oh SW, Hwang SS, Lee C, Kwon H, Chung GE. Association of nonalcoholic fatty liver disease with components of metabolic syndrome according to body mass index in Korean adults. *Am J Gastroenterol* 2012;107:1852-8.
32. Xu C, Yu C, Ma H, Xu L, Miao M, Li Y. Prevalence and risk factors for the development of nonalcoholic fatty liver disease in a nonobese Chinese population: the Zhejiang Zhenhai Study. *Am J Gastroenterol* 2013;108:1299-304.
33. Kim NH, Kim JH, Kim YJ, et al. Clinical and metabolic factors associated with development and regression of nonalcoholic fatty liver disease in nonobese subjects. *Liver Int* 2014;34:604-11.
34. Wong VW, Wong GL, Yeung DK, et al. Incidence of non-alcoholic fatty liver disease in Hong Kong: a population study with paired proton-magnetic resonance spectroscopy. *J Hepatol* 2015;62:182-9.
35. Niriella MA, Kasturiratne A, Pathmeswaran A, et al. Lean non-alcoholic fatty liver disease (lean NAFLD): characteristics, metabolic outcomes and risk factors from a 7-year prospective, community cohort study from Sri Lanka. *Hepatol Int* 2019;13:314-22.
36. Li L, Liu DW, Yan HY, Wang ZY, Zhao SH, Wang B. Obesity is an independent risk factor for non-alcoholic fatty liver disease: evidence from a meta-analysis of 21 cohort studies. *Obes Rev* 2016;17:510-9.
37. Kumar R, Mohan S. Non-alcoholic fatty liver disease in lean subjects: characteristics and implications. *J Clin Transl Hepatol* 2017;5:216-23.
38. Bernhardt P, Kratzer W, Schmidberger J, Graeter T, Gruener B, Group ES. Laboratory parameters in lean NAFLD: comparison of subjects with lean NAFLD with obese subjects without hepatic steatosis. *BMC Res Notes* 2018;11:101.
39. Younes R, Bugianesi E. NASH in Lean Individuals. *Semin Liver Dis* 2019;39:86-95.
40. Trepo E, Valenti L. Update on NAFLD genetics: from new variants to the clinic. *J Hepatol* 2020;72:1196-209.
41. Adams LC, Lubbe F, Bressen K, Wagner M, Hamm B, Makowski MR. Non-alcoholic fatty liver disease in underweight patients with inflammatory bowel disease: a case-control study. *PLoS One* 2018;13:e0206450.
42. Buchman AL, Naini BV, Spilker B. The differentiation of intestinal-failure-associated liver disease from nonalcoholic fatty liver and nonalcoholic steatohepatitis. *Semin Liver Dis* 2017;37:33-44.
43. Duarte SMB, Stefano JT, Miele L, et al. Gut microbiome composition in lean patients with NASH is associated with liver damage independent of caloric intake: a prospective pilot study. *Nutr Metab Cardiovasc Dis* 2018;28:369-84.
44. Yildiz Y, Sivri HS. Inborn errors of metabolism in the differential diagnosis of fatty liver disease. *Turk J Gastroenterol* 2020;31:3-16.
45. Machado MV, Diehl AM. Pathogenesis of nonalcoholic steatohepatitis. *Gastroenterology* 2016;150:1769-77.
46. Ruderman NB, Schneider SH, Berchtold P. The "metabolically-obese" normal-weight individual. *Am J Clin Nutr* 1981;34:1617-21.
47. Stefan N, Schick F, Haring HU. Causes, characteristics, and consequences of metabolically unhealthy normal weight in humans. *Cell Metab* 2017;26:292-300.
48. Kramer CK, Zinman B, Retnakaran R. Are metabolically healthy overweight and obesity benign conditions? A systematic review and meta-analysis. *Ann Intern Med* 2013;159:758-69.
49. Rothman KJ. BMI-related errors in the measurement of obesity. *Int J Obes (Lond)* 2008;32 Suppl 3:S56-9.
50. Gujral UP, Vittinghoff E, Mongraw-Chaffin M, et al. Cardiometabolic abnormalities among normal-weight persons from five racial/Ethnic Groups in the United States: a cross-sectional analysis of two cohort studies. *Ann Intern Med* 2017;166:628-36.
51. Wang B, Zhuang R, Luo X, et al. Prevalence of metabolically healthy obese and metabolically obese but normal weight in adults worldwide: a meta-analysis. *Horm Metab Res* 2015;47:839-45.

52. Taylor R, Holman RR. Normal weight individuals who develop type 2 diabetes: the personal fat threshold. *Clin Sci (Lond)* 2015;128:405-10.
53. Du T, Yu X, Zhang J, Sun X. Lipid accumulation product and visceral adiposity index are effective markers for identifying the metabolically obese normal-weight phenotype. *Acta Diabetol* 2015;52:855-63.
54. Neeland IJ, Ross R, Despres JP, et al. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. *Lancet Diabetes Endocrinol* 2019;7:715-25.
55. Zierath JR, Livingston JN, Thorne A, et al. Regional difference in insulin inhibition of non-esterified fatty acid release from human adipocytes: relation to insulin receptor phosphorylation and intracellular signalling through the insulin receptor substrate-1 pathway. *Diabetologia* 1998;41:1343-54.
56. Nielsen S, Guo Z, Johnson CM, Hensrud DD, Jensen MD. Splanchnic lipolysis in human obesity. *J Clin Invest* 2004;113:1582-8.
57. Feng R, Luo C, Li C, et al. Free fatty acids profile among lean, overweight and obese non-alcoholic fatty liver disease patients: a case-control study. *Lipids Health Dis* 2017;16:165.
58. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. *Physiol Rev* 2013;93:359-404.
59. Wensveen FM, Valentis S, Sestan M, Turk Wensveen T, Polic B. The “Big Bang” in obese fat: events initiating obesity-induced adipose tissue inflammation. *Eur J Immunol* 2015;45:2446-56.
60. Hamaguchi M, Kojima T, Itoh Y, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol* 2007;102:2708-15.
61. Park BJ, Kim YJ, Kim DH, et al. Visceral adipose tissue area is an independent risk factor for hepatic steatosis. *J Gastroenterol Hepatol* 2008;23:900-7.
62. Chung GE, Kim D, Kwark MS, et al. Visceral adipose tissue area as an independent risk factor for elevated liver enzyme in nonalcoholic fatty liver disease. *Medicine (Baltimore)* 2015;94:e573.
63. Yu SJ, Kim W, Kim D, et al. Visceral obesity predicts significant fibrosis in patients with nonalcoholic fatty liver disease. *Medicine (Baltimore)* 2015;94:e2159.
64. Choe EY, Lee YH, Choi YJ, et al. Waist-to-calf circumference ratio is an independent predictor of hepatic steatosis and fibrosis in patients with type 2 diabetes. *J Gastroenterol Hepatol* 2018;33:1082-91.
65. Kure T, Mawatari S, Imamura Y, et al. Nonalcoholic fatty liver disease is associated with both subcutaneous and visceral adiposity: a cross-sectional study. *Medicine (Baltimore)* 2019;98:e17879.
66. Kim D, Chung GE, Kwak MS, et al. Body fat distribution and risk of incident and regressed nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2016;14:132-8.e4.
67. Kim HJ, Kim HJ, Lee KE, et al. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. *Arch Intern Med* 2004;164:2169-75.
68. Yasutake K, Nakamuta M, Shima Y, et al. Nutritional investigation of non-obese patients with non-alcoholic fatty liver disease: the significance of dietary cholesterol. *Scand J Gastroenterol* 2009;44:471-7.
69. Shida T, Oshida N, Suzuki H, et al. Clinical and anthropometric characteristics of non-obese non-alcoholic fatty liver disease subjects in Japan. *Hepatology Res* 2020; doi: 10.1111/hepr.13543.
70. Nakamuta M, Kohjima M, Higuchi N, et al. The significance of differences in fatty acid metabolism between obese and non-obese patients with non-alcoholic fatty liver disease. *Int J Mol Med* 2008;22:663-7.
71. Karpe F, Pinnick KE. Biology of upper-body and lower-body adipose tissue--link to whole-body phenotypes. *Nat Rev Endocrinol* 2015;11:90-100.
72. Sahakyan KR, Somers VK, Rodriguez-Escudero JP, et al. Normal-weight central obesity: implications for total and cardiovascular mortality. *Ann Intern Med* 2015;163:827-35.
73. Snijder MB, Zimmet PZ, Visser M, Dekker JM, Seidell JC, Shaw JE. Independent and opposite associations of waist and hip circumferences with diabetes, hypertension and dyslipidemia: the AusDiab Study. *Int J Obes Relat Metab Disord* 2004;28:402-9.
74. Yusuf S, Hawken S, Ounpuu S, et al. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet* 2005;366:1640-9.
75. Tchoukalova YD, Votruba SB, Tchkonja T, Giorgadze N, Kirkland JL, Jensen MD. Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. *Proc Natl Acad Sci U S A* 2010;107:18226-31.
76. Pinnick KE, Nicholson G, Manolopoulos KN, et al. Distinct developmental profile of lower-body adipose tissue defines resistance against obesity-associated metabolic complications. *Diabetes* 2014;63:3785-97.
77. Lotta LA, Gulati P, Day FR, et al. Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. *Nat Genet* 2017;49:17-26.
78. Zheng RD, Chen ZR, Chen JN, Lu YH, Chen J. Role of body mass index, waist-to-height and waist-to-hip ratio in prediction of nonalcoholic fatty liver disease. *Gastroenterol Res Pract* 2012;2012:362147.
79. Lee YH, Kim SU, Song K, et al. Sarcopenia is associated with significant liver fibrosis independently of obesity and insulin resistance in nonalcoholic fatty liver disease: nationwide surveys (KNHANES 2008-2011). *Hepatology* 2016;63:776-86.
80. Hong HC, Hwang SY, Choi HY, et al. Relationship between sarcopenia and nonalcoholic fatty liver disease: the Korean sarcopenic obesity study. *Hepatology* 2014;59:1772-8.
81. Issa D, Alkhouri N, Tsien C, et al. Presence of sarcopenia (muscle wasting) in patients with nonalcoholic steatohepatitis. *Hepatology* 2014;60:428-9.
82. Koo BK, Kim D, Joo SK, et al. Sarcopenia is an independent risk factor for non-alcoholic steatohepatitis and significant fibrosis. *J*

- Hepatol* 2017;66:123-31.
83. Petta S, Ciminnisi S, Di Marco V, et al. Sarcopenia is associated with severe liver fibrosis in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2017;45:510-8.
 84. Yu R, Shi Q, Liu L, Chen L. Relationship of sarcopenia with steatohepatitis and advanced liver fibrosis in non-alcoholic fatty liver disease: a meta-analysis. *BMC Gastroenterol* 2018;18:51.
 85. Cai C, Song X, Chen Y, Chen X, Yu C. Relationship between relative skeletal muscle mass and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Hepatol Int* 2020;14:115-26.
 86. Tobari M, Hashimoto E, Tanai M, et al. Characteristics of non-alcoholic steatohepatitis among lean patients in Japan: Not uncommon and not always benign. *J Gastroenterol Hepatol* 2019;34:1404-10.
 87. Machado MV, Ferreira DM, Castro RE, et al. Liver and muscle in morbid obesity: the interplay of fatty liver and insulin resistance. *PLoS One* 2012;7:e31738.
 88. Bhanji RA, Narayanan P, Allen AM, Malhi H, Watt KD. Sarcopenia in hiding: the risk and consequence of underestimating muscle dysfunction in nonalcoholic steatohepatitis. *Hepatology* 2017;66:2055-65.
 89. Kalyani RR, Corriere M, Ferrucci L. Age-related and disease-related muscle loss: the effect of diabetes, obesity, and other diseases. *Lancet Diabetes Endocrinol* 2014;2:819-29.
 90. Hernandez-Alvarez MI, Thabit H, Burns N, et al. Subjects with early-onset type 2 diabetes show defective activation of the skeletal muscle PGC-1 α /Mitofusin-2 regulatory pathway in response to physical activity. *Diabetes Care* 2010;33:645-51.
 91. Phillips T, Leeuwenburgh C. Muscle fiber specific apoptosis and TNF- α signaling in sarcopenia are attenuated by life-long calorie restriction. *FASEB J* 2005;19:668-70.
 92. Rubio-Ruiz ME, Guarner-Lans V, Perez-Torres I, Soto ME. Mechanisms underlying metabolic syndrome-related sarcopenia and possible therapeutic measures. *Int J Mol Sci* 2019;20:647.
 93. DeFronzo RA. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 2009;58:773-95.
 94. Kim TN, Yang SJ, Yoo HJ, et al. Prevalence of sarcopenia and sarcopenic obesity in Korean adults: the Korean sarcopenic obesity study. *Int J Obes (Lond)* 2009;33:885-92.
 95. Bostrom P, Wu J, Jedrychowski MP, et al. A PGC1- α -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012;481:463-8.
 96. Zhang HJ, Zhang XF, Ma ZM, et al. Irisin is inversely associated with intrahepatic triglyceride contents in obese adults. *J Hepatol* 2013;59:557-62.
 97. Huh JY. The role of exercise-induced myokines in regulating metabolism. *Arch Pharm Res* 2018;41:14-29.
 98. Han HQ, Zhou X, Mitch WE, Goldberg AL. Myostatin/activin pathway antagonism: molecular basis and therapeutic potential. *Int J Biochem Cell Biol* 2013;45:2333-47.
 99. Li F, Li Y, Duan Y, Hu CA, Tang Y, Yin Y. Myokines and adipokines: Involvement in the crosstalk between skeletal muscle and adipose tissue. *Cytokine Growth Factor Rev* 2017;33:73-82.
 100. Konopka AR, Wolff CA, Suer MK, Harber MP. Relationship between intermuscular adipose tissue infiltration and myostatin before and after aerobic exercise training. *Am J Physiol Regul Integr Comp Physiol* 2018;315:R461-8.
 101. Delogu W, Caligiuri A, Provenzano A, et al. Myostatin regulates the fibrogenic phenotype of hepatic stellate cells via c-jun N-terminal kinase activation. *Dig Liver Dis* 2019;51:1400-8.
 102. Garcia PS, Cabbabe A, Kambadur R, Nicholas G, Csete M. Brief-reports: elevated myostatin levels in patients with liver disease: a potential contributor to skeletal muscle wasting. *Anesth Analg* 2010;111:707-9.
 103. Nishikawa H, Enomoto H, Ishii A, et al. Elevated serum myostatin level is associated with worse survival in patients with liver cirrhosis. *J Cachexia Sarcopenia Muscle* 2017;8:915-25.
 104. Shida T, Oshida N, Oh S, Okada K, Shoda J. Progressive reduction in skeletal muscle mass to visceral fat area ratio is associated with a worsening of the hepatic conditions of non-alcoholic fatty liver disease. *Diabetes Metab Syndr Obes* 2019;12:495-503.
 105. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40:1461-5.
 106. Speliotes EK, Butler JL, Palmer CD, et al. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology* 2010;52:904-12.
 107. Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ, Nash CRN. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. *Hepatology* 2010;52:894-903.
 108. Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010;51:1209-17.
 109. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011;53:1883-94.
 110. Liu YL, Patman GL, Leathart JB, et al. Carriage of the PNPLA3 rs738409 C>G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014;61:75-81.
 111. Dubuquoy C, Robichon C, Lasnier F, et al. Distinct regulation of adiponutrin/PNPLA3 gene expression by the transcription factors ChREBP and SREBP1c in mouse and human hepatocytes. *J Hepatol* 2011;55:145-53.
 112. Huang Y, He S, Li JZ, et al. A feed-forward loop amplifies nutritional regulation of PNPLA3. *Proc Natl Acad Sci U S A* 2010;107:7892-7.
 113. Hao L, Ito K, Huang KH, Sae-tan S, Lambert JD, Ross AC. Shifts in dietary carbohydrate-lipid exposure regulate expression of the

- non-alcoholic fatty liver disease-associated gene PNPLA3/adiponutrin in mouse liver and HepG2 human liver cells. *Metabolism* 2014;63:1352-62.
114. BasuRay S, Smagris E, Cohen JC, Hobbs HH. The PNPLA3 variant associated with fatty liver disease (I148M) accumulates on lipid droplets by evading ubiquitylation. *Hepatology* 2017;66:1111-24.
115. Nishioji K, Mochizuki N, Kobayashi M, et al. The impact of PNPLA3 rs738409 genetic polymorphism and weight gain ≥ 10 kg after age 20 on non-alcoholic fatty liver disease in non-obese Japanese individuals. *PLoS One* 2015;10:e0140427.
116. Honda Y, Yoneda M, Kessoku T, et al. Characteristics of non-obese non-alcoholic fatty liver disease: Effect of genetic and environmental factors. *Hepatol Res* 2016;46:1011-8.
117. Leung JC, Loong TC, Wei JL, et al. Histological severity and clinical outcomes of nonalcoholic fatty liver disease in nonobese patients. *Hepatology* 2017;65:54-64.
118. Fracanzani AL, Petta S, Lombardi R, et al. Liver and cardiovascular damage in patients with lean nonalcoholic fatty liver disease, and association with visceral obesity. *Clin Gastroenterol Hepatol* 2017;15:1604-11.e1.
119. Younossi ZM. Non-alcoholic fatty liver disease - a global public health perspective. *J Hepatol* 2019;70:531-44.
120. Fan Y, Lu H, Guo Y, et al. Hepatic transmembrane 6 superfamily member 2 regulates cholesterol metabolism in mice. *Gastroenterology* 2016;150:1208-18.
121. Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014;46:352-6.
122. Liu YL, Reeves HL, Burt AD, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014;5:4309.
123. Goffredo M, Caprio S, Feldstein AE, et al. Role of TM6SF2 rs58542926 in the pathogenesis of nonalcoholic pediatric fatty liver disease: a multiethnic study. *Hepatology* 2016;63:117-25.
124. Chen X, Zhou P, De L, Li B, Su S. The roles of transmembrane 6 superfamily member 2 rs58542926 polymorphism in chronic liver disease: a meta-analysis of 24,147 subjects. *Mol Genet Genomic Med* 2019;7:e824.
125. Dongiovanni P, Petta S, Maglio C, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology* 2015;61:506-14.
126. Chen F, Esmaili S, Rogers GB, et al. Lean NAFLD: a distinct entity shaped by differential metabolic adaptation. *Hepatology* 2020;71:1213-27.
127. Adams LA, Marsh JA, Ayonrinde OT, et al. Cholesteryl ester transfer protein gene polymorphisms increase the risk of fatty liver in females independent of adiposity. *J Gastroenterol Hepatol* 2012;27:1520-7.
128. Musso G, Cassader M, Bo S, De Michieli F, Gambino R. Sterol regulatory element-binding factor 2 (SREBF-2) predicts 7-year NAFLD incidence and severity of liver disease and lipoprotein and glucose dysmetabolism. *Diabetes* 2013;62:1109-20.
129. Bale G, Vishnubhotla RV, Mitnala S, et al. Whole-exome sequencing identifies a variant in phosphatidylethanolamine N-methyltransferase gene to be associated with lean-non-alcoholic fatty liver disease. *J Clin Exp Hepatol* 2019;9:561-8.
130. Dong H, Wang J, Li C, et al. The phosphatidylethanolamine N-methyltransferase gene V175M single nucleotide polymorphism confers the susceptibility to NASH in Japanese population. *J Hepatol* 2007;46:915-20.
131. Song J, da Costa KA, Fischer LM, et al. Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). *FASEB J* 2005;19:1266-71.
132. Nakatsuka A, Matsuyama M, Yamaguchi S, et al. Insufficiency of phosphatidylethanolamine N-methyltransferase is risk for lean non-alcoholic steatohepatitis. *Sci Rep* 2016;6:21721.
133. Yun Y, Kim HN, Lee EJ, et al. Fecal and blood microbiota profiles and presence of nonalcoholic fatty liver disease in obese versus lean subjects. *PLoS One* 2019;14:e0213692.
134. Machado MV, Cortez-Pinto H. Diet, microbiota, obesity, and NAFLD: a dangerous quartet. *Int J Mol Sci* 2016;17:481.
135. Ponziani FR, Bhoori S, Castelli C, et al. Hepatocellular carcinoma is associated with gut microbiota profile and inflammation in nonalcoholic fatty liver disease. *Hepatology* 2019;69:107-20.
136. Elshaghabee FMF, Bockelmann W, Meske D, et al. Ethanol production by selected intestinal microorganisms and lactic acid bacteria growing under different nutritional conditions. *Front Microbiol* 2016;7:47.
137. Elshaghabee FMF, Ghadimi D, Habermann D, et al. Effect of oral administration of *Weissella confusa* on fecal and plasma ethanol concentrations, lipids and glucose metabolism in wistar rats fed high fructose and fat diet. *Hepat Med* 2020;12:93-106.
138. Koutnikova H, Genser B, Monteiro-Sepulveda M, et al. Impact of bacterial probiotics on obesity, diabetes and non-alcoholic fatty liver disease related variables: a systematic review and meta-analysis of randomised controlled trials. *BMJ Open* 2019;9:e017995.
139. Kwak JH, Jun DW, Lee SM, et al. Lifestyle predictors of obese and non-obese patients with nonalcoholic fatty liver disease: a cross-sectional study. *Clin Nutr* 2018;37:1550-7.
140. Assy N, Nasser G, Kamayse I, et al. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. *Can J Gastroenterol* 2008;22:811-6.
141. Musso G, Gambino R, De Michieli F, et al. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology* 2003;37:909-16.
142. Bardella MT, Valenti L, Pagliari C, et al. Searching for coeliac disease in patients with non-alcoholic fatty liver disease. *Dig Liver Dis* 2004;36:333-6.
143. Emami MH, Hashemi M, Kouhestani S, Taheri H, Karimi S. Should we look for celiac disease among all patients with liver function test abnormalities? *Int J Prev Med* 2012;3:167-72.

144. Bakhshipour A, Kaykhaei MA, Moulaei N, Mashhadi MA. Prevalence of coeliac disease in patients with non-alcoholic fatty liver disease. *Arab J Gastroenterol* 2013;14:113-5.
145. Rahimi AR, Daryani NE, Ghofrani H, et al. The prevalence of celiac disease among patients with non-alcoholic fatty liver disease in Iran. *Turk J Gastroenterol* 2011;22:300-4.
146. Bargiggia S, Maconi G, Elli M, et al. Sonographic prevalence of liver steatosis and biliary tract stones in patients with inflammatory bowel disease: study of 511 subjects at a single center. *J Clin Gastroenterol* 2003;36:417-20.
147. Tovoli F, Negrini G, Fari R, et al. Increased risk of nonalcoholic fatty liver disease in patients with coeliac disease on a gluten-free diet: beyond traditional metabolic factors. *Aliment Pharmacol Ther* 2018;48:538-46.
148. Reilly NR, Lebowitz B, Hultcrantz R, Green PH, Ludvigsson JF. Increased risk of non-alcoholic fatty liver disease after diagnosis of celiac disease. *J Hepatol* 2015;62:1405-11.
149. Abenavoli L, Milic N, De Lorenzo A, Luzzo F. A pathogenetic link between non-alcoholic fatty liver disease and celiac disease. *Endocrine* 2013;43:65-7.
150. van Zutphen T, Ciapaitis J, Bloks VW, et al. Malnutrition-associated liver steatosis and ATP depletion is caused by peroxisomal and mitochondrial dysfunction. *J Hepatol* 2016;65:1198-208.
151. Machado MV, Cortez-Pinto H. Insulin resistance and steatosis in chronic hepatitis C. *Ann Hepatol* 2009;8 Suppl 1:S67-75.
152. HH AK. Lysosomal acid lipase deficiency: a form of non-obese fatty liver disease (NOFLD). *Expert Rev Gastroenterol Hepatol* 2017;11:911-24.
153. Reynolds TM, Mewies C, Hamilton J, Wierzbicki AS, group PPC. Identification of rare diseases by screening a population selected on the basis of routine pathology results-the PATHFINDER project: lysosomal acid lipase/cholesteryl ester storage disease substudy. *J Clin Pathol* 2018;71:608-13.
154. Khoury J, Zohar Y, Shehadeh N, Saadi T. Glycogenic hepatopathy. *Hepatobiliary Pancreat Dis Int* 2018;17:113-8.
155. Welty FK. Hypobetalipoproteinemia and abetalipoproteinemia: liver disease and cardiovascular disease. *Curr Opin Lipidol* 2020;31:49-55.
156. Noto D, Arca M, Tarugi P, Cefalu AB, Barbagallo CM, Averna MR. Association between familial hypobetalipoproteinemia and the risk of diabetes. Is this the other side of the cholesterol-diabetes connection? A systematic review of literature. *Acta Diabetol* 2017;54:111-22.
157. Pelusi S, Baselli G, Pietrelli A, et al. Rare pathogenic variants predispose to hepatocellular carcinoma in nonalcoholic fatty liver disease. *Sci Rep* 2019;9:3682.
158. Dela Cruz ACD, Bugianesi E, George J, et al. Characteristics and long-term prognosis of lean patients with nonalcoholic fatty liver disease: international follow up study. *Gastroenterology* 2014;146:A379.
159. Shao C, Ye J, Li F, Feng S, Wang W, Zhong B. Different predictors of steatosis and fibrosis severity among lean, overweight and obese patients with nonalcoholic fatty liver disease. *Dig Liver Dis* 2019;51:1392-9.
160. Hagstrom H, Nasr P, Ekstedt M, et al. Risk for development of severe liver disease in lean patients with nonalcoholic fatty liver disease: a long-term follow-up study. *Hepatol Commun* 2018;2:48-57.
161. Alam S, Gupta UD, Alam M, Kabir J, Chowdhury ZR, Alam AK. Clinical, anthropometric, biochemical, and histological characteristics of nonobese nonalcoholic fatty liver disease patients of Bangladesh. *Indian J Gastroenterol* 2014;33:452-7.
162. Denkmayr L, Feldman A, Stechemesser L, et al. Lean patients with non-alcoholic fatty liver disease have a severe histological phenotype similar to obese patients. *J Clin Med* 2018;7.
163. Akyuz U, Yesil A, Yilmaz Y. Characterization of lean patients with nonalcoholic fatty liver disease: potential role of high hemoglobin levels. *Scand J Gastroenterol* 2015;50:341-6.
164. Yilmaz Y, Senates E, Ayyildiz T, et al. Characterization of nonalcoholic fatty liver disease unrelated to the metabolic syndrome. *Eur J Clin Invest* 2012;42:411-8.
165. Fukuda T, Hamaguchi M, Kojima T, et al. The impact of non-alcoholic fatty liver disease on incident type 2 diabetes mellitus in non-overweight individuals. *Liver Int* 2016;36:275-83.
166. Golabi P, Paik J, Fukui N, Locklear CT, de Avilla L, Younossi ZM. Patients with lean nonalcoholic fatty liver disease are metabolically abnormal and have a higher risk for mortality. *Clin Diabetes* 2019;37:65-72.
167. European Association for the Study of the L, European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1388-402.
168. Alam S, Jahid Hasan M, Khan MAS, Alam M, Hasan N. Effect of weight reduction on histological activity and fibrosis of lean nonalcoholic steatohepatitis patient. *J Transl Int Med* 2019;7:106-14.
169. Albu JB, Heilbronn LK, Kelley DE, et al. Metabolic changes following a 1-year diet and exercise intervention in patients with type 2 diabetes. *Diabetes* 2010;59:627-33.
170. Ohkawara K, Tanaka K, Miyachi M, Ishikawa-Takata K, Tabata I. A dose-response relation between aerobic exercise and visceral fat reduction: systematic review of clinical trials. *Int J Obes (Lond)* 2007;31:1786-97.
171. Ismail I, Keating SE, Baker MK, Johnson NA. A systematic review and meta-analysis of the effect of aerobic vs. resistance exercise training on visceral fat. *Obes Rev* 2012;13:68-91.

Review

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Genetic risk factors associated with NAFLD

Dong Yun Kim^{1,2}, Jun Yong Park^{3,4}

¹Department of Medicine, Yonsei University Graduate School of Medicine, Seoul 03722, Korea.

²Department of Medicine, Physician-Scientist Program, Yonsei University Graduate School of Medicine, Seoul 03722, Korea.

³Division of Gastroenterology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul 03722, Korea.

⁴Yonsei Liver Center, Severance Hospital, Seoul 03722, Korea.

Correspondence to: Dr. Jun Yong Park, Division of Gastroenterology, Department of Internal Medicine, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea. E-mail: drpjy@yuhs.ac

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is estimated to affect 25% of the worldwide population, and is the leading cause of chronic liver disease in developed countries. Genetic research on NAFLD has included heritability studies, candidate gene studies, familial aggregation studies, and genome-wide association studies (GWAS). Next-generation sequencing approaches, such as whole-genome sequencing and whole-exon sequencing, are emerging as the post-GWAS era of genetic research. However, GWAS remains more practical for elucidating the genetic factors related to NAFLD, which is affected by thousands of common genetic variants and does not follow Mendelian inheritance. In the present review, we summarize the current knowledge regarding five GWAS-identified genetic loci that are associated with NAFLD. We also discuss the relationships between NAFLD-predisposing polymorphisms and cardiovascular disease, and potential applications for these identified genetic loci.

Keywords: Genome-wide association study, non-alcoholic fatty liver disease, PNPLA3, TM6SF2, GCKR, MBOAT7, HSD17B13

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is estimated to affect a quarter of the global population, and is the leading cause of chronic liver diseases in developed countries^[1]. NAFLD etiologies are complex and the factors driving NAFLD progression are not completely understood, although they likely include



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Table 1. List of five established variants associated with NAFLD

Region ¹	Variant	Reported gene	Effect of the variant	MAF ²				NAFLD spectrum			
				European	Latino	East Asian	African	Simple Steatosis	NASH	Fibrosis	HCC
chr22:43928847	rs738409	<i>PNPLA3</i>	I148M	0.2281	0.5493	0.3816	0.1357	+ [11,22,25,26]	+ [22]	+ [22]	+ [23]
chr19:19268740	rs58542926	<i>TM6SF2</i>	E167K	0.07387	0.03248	0.06969	0.03248	+ [27]	+ [31,32]	+ [30]	+ [30]
chr19:54173068	rs641738	<i>MBOAT7</i>	Linked to 3'-UTR	0.4322	0.3300	0.2382	0.3338	+ [33,35]	+ [35]	+ [35]	+ [33]
chr2:27508073	rs1260326	<i>GCKR</i>	P446L	0.5914	0.6666	0.5099	0.8670	+ [8,36,38]	+ [38]	+ [37]	
chr4:87310241	rs72613567	<i>HSD17B13</i>	Alternate splicing P260S	0.2658	0.09614	0.3266	0.06386	+ [45]	+ [44,45]	+ [45,47]	

¹The region of variant was annotated using the UCSC Genome Browser (GRCh38); ²MAF data was from the genome aggregation database browser (gnomAD, <https://gnomad.broadinstitute.org/>). MAF: minor allele frequency; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; HCC: hepatocellular carcinoma

environmental factors (e.g., diet), insulin resistance, increased visceral adiposity, and genetics. Genetic research on NAFLD has included heritability studies^[1,2], candidate gene studies^[3], familial aggregation studies^[4,5], and genome-wide association studies (GWAS)^[6-8]. NAFLD heritability was initially evaluated through a candidate gene study. Such studies are designed to examine the association of a phenotype with single-nucleotide polymorphisms (SNPs) in selected genes; however, they have weak statistical power^[9].

Following candidate gene studies, GWAS have become the default methodology for testing the associations between diseases (phenotypes of interest) and millions of SNPs throughout the genome. Over the recent years, GWAS have dramatically improved our understanding of the genetic factors related to NAFLD susceptibility, progression, and outcomes^[10]. GWAS have led to the identification of several variants that are significantly associated with NAFLD. For example, one well-known genetic risk factor for NAFLD is a coding variant in patatin-like phospholipase domain containing protein 3 (*PNPLA3*), an I-to-M substitution at position 148 (chr22:43928847, rs738409 C>G)^[11]. This rs738409 variant has been repeatedly found to be associated with NAFLD or elevated hepatic fat content^[7,8,12]. Additionally, NAFLD susceptibility is significantly associated with four other genes: transmembrane 6 superfamily member 2 (*TM6SF2*), membrane-bound O-acyltransferase domain containing 7 (*MBOAT7*), glucokinase regulator (*GCKR*), and hydroxysteroid 17 β -dehydrogenase (*HSD17B13*)^[9].

This was followed by next-generation sequencing, such as whole-genome sequencing and whole-exon sequencing, emerging as post-GWAS era advancements in genetic research. However, unlike monogenic diseases, heritability in complex diseases like NAFLD is affected by thousands of common genetic variants and thus does not follow Mendelian inheritance. GWAS have been used to uncover thousands of genetic variants that influence the risks for complex human traits and diseases, and are thus more appropriate for elucidating the genetic factors related to NAFLD.

In the present review, we describe the five GWAS-identified risk variants that exhibit the most well-established associations with NAFLD [Table 1]. We also identify and discuss genetic associations between NAFLD and cardiovascular diseases, and suggest potential applications of genomic data for precision medicine.

PAPTATIN-LIKE PHOSPHOLIPASE DOMAIN CONTAINING PROTEIN 3

PNPLA3 p.I148M (chr22:43928847, rs738409 C>G) was the first NAFLD-related variant identified using GWAS^[7], and has exhibited a robust and well-replicated association with NAFLD in several studies^[13-15]. *PNPLA3* is highly expressed in the liver and adipose tissues. Its expression is regulated by insulin through a signaling pathway that includes *LXR* and *SREBP-1c*^[16], and is thus increased with feeding in animal

studies^[17]. The PNPLA3 protein hydrolyzes triglycerides and retinyl esters^[18]. The variant rs738409 C>G causes an isoleucine-to-methionine substitution at amino acid position 148 in *PNPLA3*, which results in impaired retinyl ester release and reduced hydrolase activity, causing fat accumulation within hepatocytes, including hepatic stellate cells^[19].

Studies of *PNPLA3* have transformed our knowledge of hepatic steatosis, revealing that lipid remodeling in intracellular lipid droplets is a common pathway underlying NAFLD progression, regardless of the environmental triggers. While the wild-type *PNPLA3* protein is rapidly degraded, the variant protein has no lipase activity, thereby leading to triglyceride accumulation in the liver^[19,20]. This can induce liver damage and inflammation, and can block the release of several extracellular proteins that protect against liver fibrosis, including matrix metalloproteinases and tissue inhibitor of metalloproteinases^[21]. The G allele of rs738409 is significantly associated with NAFLD activity score (NAS, $P = 0.004$), steatosis ($P = 0.03$), lobular inflammation ($P = 0.005$), portal inflammation ($P = 2.5 \times 10^{-4}$), and fibrosis ($P = 7.7 \times 10^{-6}$)^[22]. Moreover, homozygosity of this variant is reportedly linked to a 10-fold increased risk of developing NAFLD-associated HCC in the European population^[23]. Overall, these findings indicate that the G allele of rs738409 increases susceptibility to the whole spectrum of NAFLD - from steatosis to NASH (an inflammation-associated form of NAFLD), fibrosis, and HCC.

The *PNPLA3* gene could also be responsible for the different prevalence rates of NAFLD between ethnic groups. Different populations showed diverse odds ratios (ORs) for the variant rs738409 C>G, ranging from 2.08 to 18.23 [combined OR: 3.41 (2.57-4.52), $P < 0.00001$]^[7,8,11,12]. According to the genome aggregation database browser (gnomAD, <https://gnomad.broadinstitute.org/>), the G allele of rs738409 has a frequency of 27.1% in the general population, but occurs at a lower frequency in persons of African ethnicity (26.1%), and at a higher frequency in persons of Latino ethnicity (54.9%), which may have an impact on NAFLD risk in Latino populations^[7,12]. Accordingly, compared to other ethnic groups, persons of Latino ethnicity are reportedly more likely to progress to more severe forms of NAFLD^[24]. The effect of *PNPLA3* on NAFLD has also been described in East Asian cohorts. In two Japanese cohorts, the G risk allele of the rs738409 variant is significantly associated with NAFLD [OR: 1.66 (1.43-1.94), $P = 1.4 \times 10^{-10}$ and OR: 2.05, $P = 6.8 \times 10^{-14}$]^[11,25]. Similarly, among Korean NAFLD patients, rs738409 in *PNPLA3* is significantly associated with NAFLD [OR: 1.537 (1.383-1.709), $P = 1.74 \times 10^{-15}$]^[26]. Therefore, the rs738409 variant C>G in *PNPLA3* is strongly related to NAFLD progression in both Latino and East Asian cohorts.

TRANSMEMBRANE 6 SUPERFAMILY 2

Transmembrane 6 superfamily member 2 (TM6SF2) is a protein that localizes to the endoplasmic reticulum-Golgi apparatus of hepatocytes, and is involved in the increased hepatocytic secretion of triglyceride-rich lipoproteins *via* the pathway of very-low-density lipoprotein secretion. The *TM6SF2* polymorphism rs58542926 C>T (chr19:19268740, C>T) involves a C-to-T substitution at nucleotide 499, encoding a glutamate-to-lysine change at codon 167 (E167K). The variant rs58542926 leads to reduced *TM6SF2* expression, and is thus associated with increased hepatic lipid content. In a multi-ancestry study, the rs58542926 polymorphism was related to increased serum liver enzyme [alanine transaminase (ALT)] levels and a decreased serum lipid profile (total cholesterol and triglycerides)^[27]. Interestingly, rs58542926 has also been linked to a decreased risk of cardiovascular events based on the decreased circulating lipoprotein levels^[28,29]. The relationship between *TM6SF2* and serum liver enzyme (ALT) has also been identified in other large cohorts ($n > 80,000$)^[27]. Moreover, the variant rs58542926 has been associated with increased liver fibrosis ($P = 5.57 \times 10^{-5}$), independently of *PNPLA3* I148M^[30].

The G risk allele of rs58542926 occurs with a lower frequency (0.06969, gnomAD) in East Asia; thus, we explored the association of rs58542926 with NAFLD in East Asian studies. A Korean study reported that the co-existence of the risk alleles rs738409 and rs58542926 was associated with an increased risk of NASH

[OR: 2.03 (1.50-2.73), $P < 0.001$] and significant fibrosis [OR: 1.61 (1.19-2.17), $P < 0.002$], after adjustment for confounding variables^[31]. Similar findings were reported in a Chinese cohort^[32]. These results indicate that the rs58542926 variant in *TM6SF2* is associated with NAFLD, even in East Asia, where the allele frequency is low.

MEMBRANE-BOUND O-ACYLTRANSFERASE DOMAIN CONTAINING 7

Membrane-bound O-acyltransferase domain containing 7 (MBOAT7) is a protein involved in phosphatidylinositol remodeling with arachidonic acid in the Lands cycle. MBOAT7 is mainly expressed in the liver, including in hepatic sinusoidal cells, hepatic stellate cells, and hepatocytes. In several studies, the T allele of rs641738 in *MBOAT7* (chr19:54173068, rs641738 C>T) has been reported to increase the risk of developing the whole spectrum of NAFLD. Each T allele was associated with an increased risk of the development of hepatic steatosis [OR: 1.42 (1.07-1.91), $P = 0.015$], NASH [OR: 1.18 (1.00-1.40), $P = 0.05$], significant fibrosis [OR: 1.30 (1.06-1.70), $P = 0.012$], and HCC without advanced fibrosis [OR: 2.10 (1.33-3.31)]^[33-35].

GLUCOKINASE REGULATOR

Glucokinase regulator (GCKR) controls *de novo* lipogenesis by regulating the glucose influx into hepatocytes, which boosts the lipogenic pathway by providing further substrate for liver biosynthesis. Several variants in the *GCKR* gene are reportedly associated with NAFLD^[8,36]. The rs1260326 variant (chr2:27508073, C>T) encoding P446L has been considered as a causal variant for this association. In NAFLD patients, the T allele of rs1260326 is significantly associated with the hepatic fibrosis stage as compared to the F1 stage [OR: 2.06 (1.02-1.14), $P = 0.0008$]^[37]. The rs780094 variant in *GCKR* has also been significantly associated with computed tomography-proven and biopsy-proven NAFLD in a genome-wide association study (OR: 1.45, $P = 2.59 \times 10^{-8}$)^[8], and in a meta-analysis of five studies comprising of 2,091 NAFLD cases and 3,003 controls [OR: 1.25 (1.14-1.36), $P < 0.00001$]^[38].

The T risk allele of rs1260326 is associated with higher *GCKR* expression^[39]. Unlike the wild-type *GCKR* protein, the *GCKR* P446L protein is not sustained by fructose-6-phosphate, resulting in enhanced hepatic uptake of glucose, glucokinase activity^[40], and *de novo* lipogenesis^[41]. Interestingly, the risk allele of rs1260326 is also associated with decreased serum glucose levels and reduced T2DM risk^[42,43].

17β-HYDROXYSTEROID DEHYDROGENASE TYPE 13

Several recent GWAS have identified a protective variant against NAFLD: rs72613567:TA in hydroxysteroid 17β dehydrogenase 13 (*HSD17B13*)^[44,45]. This variant seems to be associated with decreased serum aspartate aminotransferase (AST, $P = 6.2 \times 10^{-10}$) and serum ALT ($P = 4.2 \times 10^{-12}$), and ameliorated progressive NASH among persons of European descent ($n = 46,544$). Furthermore, this splice variant (chr4:87310241, rs72613567: TA) in *HSD17B13* protects against chronic liver diseases, including both NAFLD and alcoholic liver disease, and this finding has been replicated in two studies^[45,46].

The protective effect of *HSD17B13* is mediated by reduced activity of the enzyme, which is involved in the conversion of retinol to retinoic acid^[47]. Retinoic acid reportedly suppresses fibrosis in NAFLD. This means that the protective effect of *HSD17B13* is not due to changes in hepatic fat accumulation, but rather caused by the enzymatic activity of lipid droplet-associated retinol dehydrogenase activity.

ASSOCIATION BETWEEN NAFLD-PREDISPOSING POLYMORPHISMS AND CARDIOVASCULAR DISEASE

Several studies report that cardiovascular disease (CVD) is the most common cause of mortality among NAFLD patients^[48-50]. This can be explained by the fact that NAFLD and CVD share common pathological

pathways, such as inflammation, endothelial dysfunction, and oxidative stress^[51,52]. Therefore, we explored the relationship between CVD and two of the most well-validated NAFLD-associated loci: *PNPLA3* and *TM6SF2*.

A meta-analysis study, including 60,801 coronary heart disease (CHD) cases and 123,504 controls, determined that the rs738409 variant in *PNPLA3* showed a protective effect against CVD [OR: 0.92 (0.87-0.97), $P = 0.002$]^[53]. In another study, the G allele of rs738409 was inversely related to CHD in 576 patients who underwent elective coronary angiography ($P = 0.02$)^[54]. However, this trend of association between the rs738409 G risk allele and CHD has been inconsistent. Another study found no association between the G risk allele of rs738409 and CHD [OR: 0.98 (0.95-1.02), $P = 0.79$]^[55]. Interestingly, in a study including 1,103 premature CHD patients and 1,469 healthy controls, the presence of the G allele of rs738409 was associated with increased risk of premature CHD development among T2DM patients [OR 1.20 (1.011-1.421), $P = 0.042$]^[56]. In addition to CHD, the G risk allele of rs738409 was reportedly linked to a greater risk of increased thickness of the carotid artery intima-media in 162 patients with biopsy-proven NAFLD [OR: 2.94 (1.12-7.70), $P = 0.02$], and this finding was validated in 267 patients with biopsy-proven or clinical NAFLD^[57].

The rs58542926 variant in *TM6SF2*, which is associated with fatty liver, is also reportedly protective against CVD by lowering serum lipid levels (total cholesterol, LDL-cholesterol, and triglycerides)^[58]. This means that the T allele of rs58542926 in *TM6SF2* confers protection against CVD at the expense of a higher risk of NAFLD. A meta-analysis confirmed that the rs58542926 T allele is associated with a tendency of decreased CVD risk [OR: 0.951 (0.92-0.98), $P = 0.005$]^[53]. Moreover, in a smaller cross-sectional study, this allele was related to a decreased risk of carotid artery plaques [OR: 0.49 (0.25-0.94)]^[29].

Despite these studies, the shared genetic causality between CVD and NAFLD remains unclear. The four genes that are most commonly reported to be associated with NAFLD - *PNPLA3*, *TM6SF2*, *MBOAT7*, and *GCKR* - have been analyzed by weighted fixed-effects statistical modeling, revealing no association of NAFLD with CVD [OR: 1.00 (0.99-1.91), $P = 0.93$]^[59]. These findings indicate that more complex relationships may exist between NAFLD and CVD. Exploring the roles of NAFLD-associated variants in CVD risk demonstrates that biologically relevant insights can be obtained by identifying individual pleiotropic loci that affect different outcomes. Clearly, more research is needed.

APPLICATIONS FOR PRECISION MEDICINE

There are several other genetic variants associated with NAFLD [Supplementary Table 1]. These associations with SNPs from GWAS are generally reported as P values and/or effect sizes. However, these metrics do not fully reflect the SNP's ability to differentiate between the control and the phenotype of interest. To apply genetic information for disease prediction, it is important to not only focus on the statistical power of a variant but also on the measurement of area under the ROC curve, which summarizes the true and false positive rates for a binary outcome^[60].

Another emerging metric is the development of polygenic risk scores (PRSs), which reflect the risk accumulation based on multiple SNPs, and can be calculated as a weighted sum of the disease risk alleles carried by an individual^[61]. PRS use would be a sensible approach in the study of NAFLD, as both common and rare SNPs are related to NAFLD risk, irrespective of clinical risk factors^[62]. However, there is little evidence of the clinical application of PRSs. Similarly, Krawczyk *et al.*^[63] reported that the summed number of risk alleles (0-5) for three genes (*PNPLA3*, *TM6SF2*, and *MBOAT7*) was significantly correlated with the individual risks of increased hepatic triglyceride content and elevated serum liver enzyme levels (AST and ALT). This study further suggests that the historical concept of genetic risk score (GRS) - which includes the contemporary concept of a continuous spectrum of NAFLD risk - could also be useful for predicting

Personalized Medicine

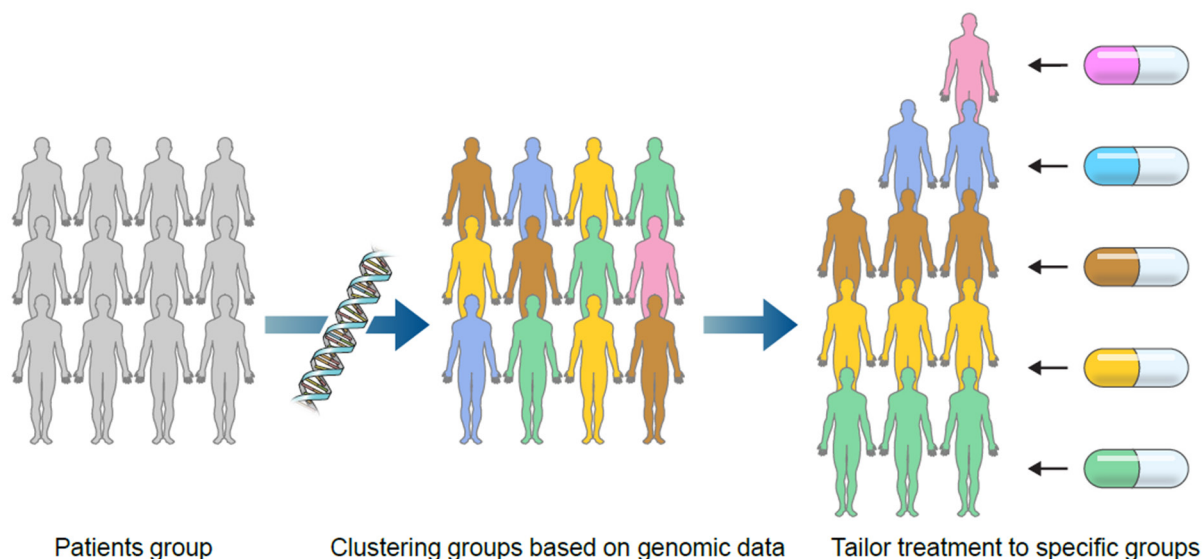


Figure 1. Toward personalized medicine based on genetic information. Personalized medicine makes it possible to tailor a treatment as individualized as the disease. This approach can be applied to solve tackling diseases that have far eluded effective treatments

NAFLD development and progression. Several NAFLD risk scoring models that incorporate both genetic and clinical information have also been proposed^[64]. For example, Hyysalo *et al.*^[65] developed a model for predicting NASH, which combines clinical variables and genetic information, based on European cohorts with biopsy-proven NAFLD. In another study, NAFLD-HCC was identified based on genotype information, age, sex, obesity, T2DM, and severe fibrosis, showing an AUROC of 0.96 ± 0.04 (89% specificity and 96% sensitivity)^[33].

Additional research is needed before PRSs, GRs, or prediction models using both clinical variables and genetic information can be effectively applied for NAFLD investigation in clinical practice. This knowledge of genetic loci is potentially useful for risk stratification in patients with NAFLD. Moreover, considering that there are presently no approved drugs for NAFLD treatment^[66], there is an urgent need for more research and development of therapeutic targeting of the products of these genes in NAFLD patients with specific genetic variants that could provide insight into personalized treatments for NAFLD [Figure 1].

TRANSLATIONAL IMPLICATIONS AND CHALLENGES

As discussed above, several attempts have been made to predict NAFLD and/or NASH using genetic information alone or in combination with clinical information. The results of these efforts can be applied to the development of a new scoring model with better diagnostic performance compared to the previous models. Notably, a prediction model developed by combining serum metabolites, serum biochemical parameters, and genotype information was reported to discriminate NASH from NAFL with a good diagnostic performance^[67]. Such a model would be appealing for clinical translation, considering that the current gold standard for NASH diagnosis is liver biopsy, which is an invasive method. However, there are several challenges hindering the clinical translation of genetic information in NAFLD.

Firstly, most studies performed to evaluate the diagnostic accuracy of predictive models for NAFLD and/or NASH risk have been based on a cross-sectional design. Although these results can be useful, it is not an optimal study design for investigating models based on genetic information. Indeed, unlike classical factors such as biochemical results (AST, ALT), genetic variants have the strength of being stable over time. Thus,

if an ideal prediction model based on genetic information is properly established, it could be possible to stratify NAFLD before it develops or progresses, thus enabling intervention at an early stage or early age. However, to properly apply this concept, results should be accumulated from multiple longitudinal studies.

Secondly, another important issue to consider when utilizing genetic information is the interaction between environment and gene. For example, with regards to rs738409 in the *PNPLA3* gene, it has been reported that the variant's effect is especially amplified in the setting of obesity^[68]. This suggests that adiposity (environment) can influence how specific genetic information influences the full spectrum of NAFLD. Considering this interaction between gene and environment, a prediction model based exclusively on genetic information may not exhibit sufficient predictive power, while a model with integration of relevant NAFLD-associated clinical factors would be more likely to reach significant predictive power.

Overall, prediction models that use both genetic information and relevant clinical factors derived from longitudinal studies can achieve sufficient predictive power for NAFLD risk stratification at the individual level.

CONCLUSION

The identification of genes associated with NAFLD development and progression is expected to provide important insights into its pathophysiology, as well as to guide disease risk stratification and further new opportunities for timely therapeutic intervention. Several genetic variants have been implicated in NAFLD development and progression, and here we focused on the five genes whose associations with NAFLD have been most extensively replicated. These genetic risk variants can improve the accuracy of NAFLD diagnosis, and may also be useful for the identification of high-risk NAFLD patients who have unfavorable prognoses. An understanding of these NAFLD-associated genetic risk factors will help identify individuals at risk, and potentially guide the provision of appropriate treatments based on an individual's risk and likelihood of disease progression.

DECLARATIONS

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Authors' contributions

Wrote and reviewed the manuscript: Kim DY, Park JY

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

1. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018;15:11.
2. Eslam M, George J. Genetic and epigenetic mechanisms of NASH. *Hepatol Int* 2016;10:394-406.
3. Daly AK, Ballestri S, Carulli L, Loria P, Day CP. Genetic determinants of susceptibility and severity in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2011;5:253-63.
4. Abdelmalek MF, Liu C, Shuster J, Nelson DR, Asal NR. Familial aggregation of insulin resistance in first-degree relatives of patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2006;4:1162-9.
5. Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001;96:2957-61.
6. Chalasani N, Guo X, Loomba R, et al. Genome-wide association study identifies variants associated with histologic features of nonalcoholic fatty liver disease. *Gastroenterology* 2010;139:1567-76.e6.
7. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40:1461-5.
8. Speliotes EK, Yerges-Armstrong LM, Wu J, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet* 2011;7:e1001324.
9. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003;33:177-82.
10. Eslam M, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: clinical impact. *J Hepatol* 2018;68:268-79.
11. Kawaguchi T, Sumida Y, Umemura A, et al. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PLoS One* 2012;7:e38322.
12. Xu R, Tao A, Zhang S, Deng Y, Chen G. Association between patatin-like phospholipase domain containing 3 gene (PNPLA3) polymorphisms and nonalcoholic fatty liver disease: a HuGE review and meta-analysis. *Sci Rep* 2015;5:9284.
13. Dongiovanni P, Romeo S, Valenti L. Genetic factors in the pathogenesis of nonalcoholic fatty liver and steatohepatitis. *Biomed Res Int* 2015;2015:460190.
14. Dongiovanni P, Donati B, Fares R, et al. PNPLA3 I148M polymorphism and progressive liver disease. *World J Gastroenterol* 2013;19:6969.
15. Singal AG, Manjunath H, Yopp AC, et al. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014;109:325.
16. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science* 2011;332:1519-23.
17. Huang Y, He S, Li JZ, et al. A feed-forward loop amplifies nutritional regulation of PNPLA3. *Proc Natl Acad Sci U S A* 2010;107:7892-7.
18. Huang Y, Cohen JC, Hobbs HH. Expression and characterization of a PNPLA3 protein isoform (I148M) associated with nonalcoholic fatty liver disease. *J Biol Chem* 2011;286:37085-93.
19. BasuRay S, Smagris E, Cohen JC, Hobbs HH. The PNPLA3 variant associated with fatty liver disease (I148M) accumulates on lipid droplets by evading ubiquitylation. *Hepatology* 2017;66:1111-24.
20. Luukkonen PK, Nick A, Hölttä-Vuori M, et al. Human PNPLA3-I148M variant increases hepatic retention of polyunsaturated fatty acids. *JCI Insight* 2019;4:e127902.
21. Pingitore P, Dongiovanni P, Motta BM, et al. PNPLA3 overexpression results in reduction of proteins predisposing to fibrosis. *Hum Mol Genet* 2016;25:5212-22.
22. Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ, CRN N. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. *Hepatology* 2010;52:894-903.
23. Liu YL, Patman G, Leathart J, et al. Carriage of the PNPLA3 rs738409 C>G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014;61:75-81.
24. Carrion AF, Ghanta R, Carrasquillo O, Martin P. Chronic liver disease in the Hispanic population of the United States. *Clin Gastroenterol Hepatol* 2011;9:834-41.
25. Kitamoto T, Kitamoto A, Yoneda M, et al. Genome-wide scan revealed that polymorphisms in the PNPLA3, SAMM50, and PARVB genes are associated with development and progression of nonalcoholic fatty liver disease in Japan. *Hum Genet* 2013;132:783-92.
26. Chung GE, Lee Y, Yim JY, et al. Genetic polymorphisms of PNPLA3 and SAMM50 are associated with nonalcoholic fatty liver disease in a Korean population. *Gut Liver* 2018;12:316-23.
27. Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014;46:352-6.
28. Holmen OL, Zhang H, Fan Y, et al. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing

- total cholesterol and myocardial infarction risk. *Nat Genet* 2014;46:345-51.
29. Dongiovanni P, Petta S, Maglio C, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology* 2015;61:506-14.
 30. Liu YL, Reeves HL, Burt AD, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014;5:1-6.
 31. Koo BK, Joo SK, Kim D, et al. Additive effects of PNPLA3 and TM6SF2 on the histological severity of non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2018;33:1277-85.
 32. Wang X, Liu Z, Wang K, et al. Additive effects of the risk alleles of PNPLA3 and TM6SF2 on non-alcoholic fatty liver disease (NAFLD) in a Chinese population. *Front Genet* 2016;7:140.
 33. Donati B, Dongiovanni P, Romeo S, et al. MBOAT7 rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals. *Sci Rep* 2017;7:1-10.
 34. Buch S, Stickel F, Trépo E, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. *Nat Genet* 2015;47:1443-8.
 35. Mancina RM, Dongiovanni P, Petta S, et al. The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. *Gastroenterology* 2016;150:1219-30.e6.
 36. Valenti L, Alisi A, Nobili V. Unraveling the genetics of fatty liver in obese children: additive effect of P446L GCKR and I148M PNPLA3 polymorphisms. *Hepatology* 2012;55:661-3.
 37. Petta S, Miele L, Bugianesi E, et al. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. *PLoS One* 2014;9:e87523.
 38. Zain SM, Mohamed Z, Mohamed R. A common variant in the glucokinase regulatory gene rs780094 and risk of nonalcoholic fatty liver disease: a meta-analysis. *J Gastroenterol Hepatol* 2015;30:21-7.
 39. Rodríguez ML, Silva LF, Vangipurapu J, et al. Functional variant in the GCKR gene affects lactate levels differentially in the fasting state and during hyperglycemia. *Sci Rep* 2018;8:1-8.
 40. Beer NL, Tribble ND, McCulloch LJ, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. *Hum Mol Genet* 2009;18:4081-8.
 41. Anstee QM, Day CP. The genetics of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013;10:645-55.
 42. Sparsø T, Andersen G, Nielsen T, et al. The GCKR rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinaemia, and reduced risk of type 2 diabetes. *Diabetologia* 2008;51:70-5.
 43. Onuma H, Tabara Y, Kawamoto R, et al. The GCKR rs780094 polymorphism is associated with susceptibility of type 2 diabetes, reduced fasting plasma glucose levels, increased triglycerides levels and lower HOMA-IR in Japanese population. *J Hum Genet* 2010;55:600-4.
 44. Abul-Husn NS, Cheng X, Li AH, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. *N Engl J Med* 2018;378:1096-106.
 45. Pirola CJ, Garaycoechea M, Flichman D, et al. Splice variant rs72613567 prevents worst histologic outcomes in patients with nonalcoholic fatty liver disease. *J Lipid Res* 2019;60:176-85.
 46. Ma Y, Belyaeva OV, Brown PM, et al. HSD17B13 is a hepatic retinol dehydrogenase associated with histological features of non-alcoholic fatty liver disease. *Hepatology* 2019;69:1504.
 47. Ma Y, Belyaeva OV, Brown PM, et al. 17-beta hydroxysteroid dehydrogenase 13 is a hepatic retinol dehydrogenase associated with histological features of nonalcoholic fatty liver disease. *Hepatology* 2019;69:1504-19.
 48. Haddad TM, Hamdeh S, Kanmanthareddy A, Alla VM. Nonalcoholic fatty liver disease and the risk of clinical cardiovascular events: a systematic review and meta-analysis. *Diabetes Metab Syndr* 2017;11:S209-16.
 49. Younossi ZM, Stepanova M, Rafiq N, et al. Nonalcoholic steatofibrosis independently predicts mortality in nonalcoholic fatty liver disease. *Hepatol Commun* 2017;1:421-8.
 50. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: a meta-analysis. *J Hepatol* 2016;65:589-600.
 51. Liu H, Lu HY. Nonalcoholic fatty liver disease and cardiovascular disease. *World J Gastroenterol* 2014;20:8407.
 52. Francque SM, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications. *J Hepatol* 2016;65:425-43.
 53. Simons N, Isaacs A, Koek GH, Kuč S, Schaper NC, Brouwers MCGJ. PNPLA3, TM6SF2, and MBOAT7 genotypes and coronary artery disease. *Gastroenterology* 2017;152:912-3.
 54. Rüschenbaum S, Schwarzkopf K, Friedrich-Rust M, et al. Patatin-like phospholipase domain containing 3 variants differentially impact metabolic traits in individuals at high risk for cardiovascular events. *Hepatol Commun* 2018;2:798-806.
 55. Lauridsen BK, Stender S, Kristensen TS, et al. Liver fat content, non-alcoholic fatty liver disease, and ischaemic heart disease: Mendelian randomization and meta-analysis of 279 013 individuals. *Eur Heart J* 2017;39:385-93.
 56. Posadas-Sánchez R, López-Urbe ÁR, Posadas-Romero C, et al. Association of the I148M/PNPLA3 (rs738409) polymorphism with premature coronary artery disease, fatty liver, and insulin resistance in type 2 diabetic patients and healthy controls. The GEA study. *Immunobiology* 2017;222:960-6.
 57. Petta S, Valenti L, Marchesini G, et al. PNPLA3 GG genotype and carotid atherosclerosis in patients with non-alcoholic fatty liver disease. *PLoS One* 2013;8:e74089.
 58. Pirola CJ, Sookoian S. The dual and opposite role of the TM6SF2-rs58542926 variant in protecting against cardiovascular disease and conferring risk for nonalcoholic fatty liver: a meta-analysis. *Hepatology* 2015;62:1742-56.

59. Brouwers MC, Simons N, Stehouwer CD, Koek GH, Schaper NC, Isaacs A. Relationship between nonalcoholic fatty liver disease susceptibility genes and coronary artery disease. *Hepatol Commun* 2019;3:587-96.
60. Kraft P, Wacholder S, Cornelis MC, et al. Beyond odds ratios - communicating disease risk based on genetic profiles. *Nat Rev Genet* 2009;10:264-9.
61. Chatterjee N, Shi J, García-Closas M. Developing and evaluating polygenic risk prediction models for stratified disease prevention. *Nat Rev Genet* 2016;17:392.
62. Dongiovanni P, Stender S, Pietrelli A, et al. Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. *J Intern Med* 2018;283:356-70.
63. Krawczyk M, Liebe R, Lammert F. Toward genetic prediction of nonalcoholic fatty liver disease trajectories: PNPLA3 and beyond. *Gastroenterology* 2020;158:1865-80.e1.
64. Vespasiani-Gentilucci U, Gallo P, Dell'Unto C, Volpentesta M, Antonelli-Incalzi R, Picardi A. Promoting genetics in non-alcoholic fatty liver disease: combined risk score through polymorphisms and clinical variables. *World J Gastroenterol* 2018;24:4835.
65. Hyysalo J, Männistö VT, Zhou Y, et al. A population-based study on the prevalence of NASH using scores validated against liver histology. *J Hepatol* 2014;60:839-46.
66. Kim DY, Park JY. Overview of emerging treatment of non-alcoholic fatty liver disease: more than one drug needed? *Hepatobiliary Surg Nutr* 2019;8:522.
67. Zhou Y, Orešič M, Leivonen M, et al. Noninvasive detection of nonalcoholic steatohepatitis using clinical markers and circulating levels of lipids and metabolites. *Clin Gastroenterol Hepatol* 2016;14:1463-72.e6.
68. Romeo S, Sentinelli F, Dash S, et al. Morbid obesity exposes the association between PNPLA3 I148M (rs738409) and indices of hepatic injury in individuals of European descent. *Int J Obes* 2010;34:190-4.

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Manuscript Type	Definition	Abstract	Keywords	Main Text Structure
Original Article	An Original Article describes detailed results from novel research. All findings are extensively discussed.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
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Authors' full names should be listed. The initials of middle names can be provided. Institutional addresses and email addresses for all authors should be listed. At least one author should be designated as corresponding author. In addition, corresponding authors are suggested to provide their Open Researcher and Contributor ID upon submission. Please note that any change to authorship is not allowed after manuscript acceptance.

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Methods should contain sufficient details to allow others to fully replicate the study. New methods and protocols should be described in detail while well-established methods can be briefly described or appropriately cited. Experimental participants selected, the drugs and chemicals used, the statistical methods taken, and the computer software used should be identified precisely. Statistical terms, abbreviations, and all symbols used should be defined clearly. Protocol documents for clinical trials, observational studies, and other non-laboratory investigations may be uploaded as supplementary materials.

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Conference paper	Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. <i>Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming</i> ; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. pp. 182-91.
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