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Review

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Hepatocellular carcinoma in transfusion dependent thalassemia patients: a review from a clinical perspective

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Abstract

Survival in patients with transfusion-dependent thalassemias (TDT) has increased, and complications such as hepatocellular carcinoma (HCC) are emerging. Risk factors include viral infection, mainly hepatitis C virus (HCV), iron overload, the presence of cirrhosis, and immune dysregulation. Median survival after HCC occurrence has been estimated at 12 months, while data regarding the incidence of HCC in this population are minimal. Implementing effective hepatitis B virus (HBV)/HCV antiviral treatment and universal HBV vaccination programs is expected to decrease the risk for hepatocarcinogenesis substantially. Significant hemosiderosis and hepatic fibrosis are common in patients with TDT despite chelation therapy and have been correlated with HCC development. Thus, iron overload should be monitored with liver iron concentration and ferritin levels, and effective chelation therapy should be applied. In addition, all TDT patients, particularly those with cirrhosis, should be under surveillance every six months with abdominal ultrasound ± alpha-fetoprotein levels, as this combination seems to provide better sensitivity for early HCC detection.

Keywords: Transfusion-dependent thalassemias, hepatocellular carcinoma, hepatitis B virus, hepatitis C virus, iron overload, liver iron concentration, liver stiffness measurement

INTRODUCTION

Beta thalassemia is caused by mutations in the hemoglobin beta-globin gene that leads to impaired production of beta-globin chains. Beta thalassemia major (TM) is the most severe form of beta-thalassemia, characterized by minimal to no beta-globin chain production. Individuals with beta-thalassemia major have profound and lifelong transfusion-dependent anemia (TDT), which leads to several organ pathological conditions, mainly due to iron overload.

However, as survival in patients with TDT has increased over time, mainly due to efficient iron chelation therapy, it seems that the complications from the heart that were common and fatal are reduced, and other previously rarer complications such as hepatocellular carcinoma (HCC) are emerging[1].

Traditionally, the risk of HCC development in these patients has been linked to hepatitis B virus (HBV) and hepatitis C virus (HCV) infections mainly transmitted by blood transfusions[2]. However, recent data highlight the risk of HCC development in HCV- and HBV-negative patients, indicating the crucial role of iron overload[3-5].

This review aims to investigate the epidemiological data and the possible mechanisms involved in developing HCC in patients with TDT, including viral hepatitis, the role of iron overload, and the immunological disturbances that characterize these patients.

HCC IN TDT EPIDEMIOLOGY

HCC is the seventh most frequently occurring cancer globally and the second most common cause of cancer mortality[6]. Its incidence rises progressively with advancing age, showing a higher prevalence among males[7]. Significant risk factors for HCC include HBV, HCV, chronic alcohol consumption, obesity, type II diabetes, and non-alcoholic steatohepatitis (NASH). In general, the prevalence and etiology of HCC present a heterogeneous geographic distribution according to the predominant risk factor of each region. Thus, most cases are associated with HBV in Western Africa, Latin America, and East Asia, while most cases are associated with HCV in North America and Western Europe[8]. However, non-alcoholic fatty liver disease (NAFLD)/NASH is rapidly increasing and is expected to become the predominant risk factor for HCC in high-income regions[9]. In the United States, the incidence rates per 100,000 persons are 13.8 in men and 4.9 in women[10].

Data concerning etiological factors and treatment outcomes of HCC appear to be lacking in patients with TDT. Moreover, data regarding the incidence of HCC in this population are minimal. The majority of HCC cases are from population-based studies from Italy and Greece[11-15]. The evaluation of the total number of patients included in the updated Italian registry revealed a cumulative incidence of HCC in patients with TDT of 1.02%[12]. Overall, the study showed 60 new cases of HCC between 2002 and December 2012, among 5855 thalassemia patients who have been followed. A Greek study evaluated 57 patients with TM and thalassemia intermedia[14]. The incidence of HCC in patients with TM has been calculated as 3.5%. Data from a prospective study with 105 adults with TDT reveal a 2% incidence of HCC during a one-year observation period[16]. Furthermore, preliminary data regarding HCC survey from 1327 thalassemic patients, aged > 30 years from 13 centers, enrolled in the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine (ICET-A), reveal a prevalence of 1.66% in TDT patients and 1.96% in non-TDT patients[17] [Table 1].

Table 1. Data regarding clinical characteristics, incidence, prevalence, and survival in TDT patients with HCC

Study	Borgna-Pignatti *et al.*[11], 2004	Borgna-Pignatti *et al.*[12], 2014	Fragatou *et al.*[14], 2010	Papadopoulos, *et al.*[15], 2020	Mancuso *et al.*[16], 2006	De Sanctis *et al.*[17], 2020
N	23	60	57 (2 patients with HCC)	42	108 (2 patients with HCC)	1022 (17 patients with HCC)
Mean/median age at diagnosis, years	45 ± 11	NA	49 (48-50)	45.5 ± 5.8	51 (39-63)	41 (36-59)
Males, *n* (%)	15 (65)	37 (60)	1 (50)	27 (64.5)	1 (50)	11 (65)
HBsAg (+), *n/N* (%)	2/22 (9)	3/60 (5)	0/2 (0)	0/48 (0)	0/2 (0)	0/17 (0)
HCV-RNA (+), *n/N* (%)	17/22 (77.3)	43/60 (71.5)	2/2 (100)	25/42 (59.5)	2/2 (100)	10/17 (59)
Mean/median level of serum ferritin (μg/l)	1926.4 (369-6300)	1041 (124-6200)	1100 (310-1890)	NA	NA	NA
Mean LIC values	-	9 mgFe/g	-	48.6 ± 29.5 μmolFe/g	NA	7.9 mgFe/g
Cirrhosis, *n/N* (%)	NA	NA	2/2 (100)	33/42 (78.5)	1/2 (50)	6/17 (35.3)
Incidence (%)	-	1.02	3.5	-	2	-
Prevalence (%)	-	-	-	-	-	1.66
Median survival, (months)	3.5 (1-26)	11.5 (1.4-107.2)	5.5 (3-8)	12 (2-96)	-	-

TDT: Transfusion-dependent thalassemias; HCC: hepatocellular carcinoma; LIC: liver iron concentration.

Based on current reports, it is clear that, compared to the general population, HCC appears at a younger age in TDT patients, suggesting the presence of multiple risk factors operating early in life for this population[3]. More precisely, the median age at diagnosis is 45 years among patients with TDT compared to 64 years among the general population[7,10,12]. Moreover, as discussed above, until 2000, HCC was relatively uncommon in TDT patients, as they died younger from cardiac problems related to iron overload[18,19]. In recent years, effective iron chelation treatment has led to a significant prolongation of survival thanks to the prevention of cardiac complications. However, many TDT patients still have liver iron overload with concomitant liver fibrosis[20]. Median survival after HCC occurrence has been estimated at 12 months (range 2-96 months) in a Greek study and was mainly dependent on the Barcelona Clinic Liver Cancer grading system[15]. In addition, data from Italy indicate a similar median survival of 11.5 months (range 1.4-107.2 months)[12].

In most countries, incidence rates of HCC among men in the general population are two- to four-fold higher than rates among women, possibly due to the higher incidence of cirrhosis, higher levels of smoking, and greater alcohol intake[7]. Interestingly, this difference seems to be less profound between men and women in TDT patients. It is well known that the effects of testosterone may increase signaling androgen receptors in men, promoting the proliferation of hepatocytes[21]. As hypogonadism has been revealed in a significant proportion of TDT patients, we may assume that lower testosterone levels could explain the almost equal occurrence of HCC between men and women[12].

HCC IN TDT - HBV INFECTION

Chronic HBV infection is the leading cause of global liver cancer incidences and deaths[22]. The incidence rate of HCC in patients with chronic HBV infection has been estimated to be 0.6% for those without cirrhosis and 3.7% for those with compensated cirrhosis[23]. Furthermore, the overall mortality rate among all cases of chronic HBV infection has been estimated as 30%-50%[24]. Besides the increased risk of HBV-related HCC in patients with cirrhosis, it is well known that HBV initiates the process of hepatic carcinogenesis by integrating into the host genome, thus leading to chromosomal instability and cell proliferation[25] [Figure 1]. The oncogenic nature of HBV *per se* is further demonstrated by the fact that the risk of developing HCC is not eliminated even in non-cirrhotic patients receiving high-barrier to resistance antiviral treatment[26].

Worldwide, 0.3%-5.7% of thalassemia patients are hepatitis B surface antigen (HBsAg) positive[19]. In the Italian updated registry data, the HBsAg positivity reached 5% among 60 TDT patients with HCC. In contrast, no HBsAg positivity was revealed in a Greek study with 42 TDT patients with HCC[12,15]. Thus, HBV infection does not play a significant role in HCC development in these patients, at least in Western countries. However, since the prevalence of HBV infection presents a large heterogeneous geographical distribution, its role is likely to remain important in high endemicity areas such as Asia and Africa. The most effective method to control HBV transmission is universal vaccination. Successful outcomes of nationwide vaccination programs implemented by Taiwan in 1984 proved that HBV infection and HCC incidence rates could be substantially reduced by a rigorous vaccination program[27]. Thus, HBV-related HCC, including in high-endemic areas, is expected to decrease in the coming years, even in TDT patients[28].

HBV treatment improves survival and quality of life by preventing liver disease progression and HCC. The indications for treatment in TDT patients do not differ from those recommended in the general population and are primarily based on the combination of three criteria; HBV-DNA levels, serum alanine aminotransferase levels, and severity of liver disease[29].

HCC IN TDT - HCV INFECTION

Based on previously published data, HCV infection has been proposed as the leading risk factor for liver fibrosis in TDT patients[30]. TDT patients transfused before the universal blood donors screening, which was introduced in 1992 after the discovery of HCV, were at higher risk of blood-borne HCV infection. Although the prevalence of HCV infection among these patients has dramatically decreased during the last twenty years, it remains higher than the general population. It is still a significant problem in underdeveloped countries. Anti-HCV positive was present in 87% of thalassemic patients based on an Italian registry, 39.6%-74.4% in two Greek studies, and 18%-70% in studies from the Middle East[12,15,31,32]. However, whether HCV plays a direct role or merely an indirect one through cirrhosis in the pathogenesis of HCC remains uncertain[33]. It seems that oxidative stress production enhanced by the HCV core protein would partly contribute to the development of HCC, even rarely in the absence of liver cirrhosis[34] [Figure 1]. In a recent study from Greece, which reported all cases of malignant neoplastic disorders occurring in 3652 thalassemic patients diagnosed between 1985 and 2018, a strong positive association between HCV and HCC was found[13]. Moreover, it has been identified that the most frequent HCV genotype in TDT patients is 1b, which seems to play an essential role in HCC development according to a recent meta-analysis[35,36].

The new interferon-free regimens with direct-acting antiviral (DAA) agents have changed the treatment landscape of HCV infection. The indication for HCV treatment in this population is similar to that of the general population[37]. In a real-life study, DAAs achieved a sustained virological response rate of > 90% without any additional adverse events or drug-drug interactions with iron-chelating drugs[38]. However, it is

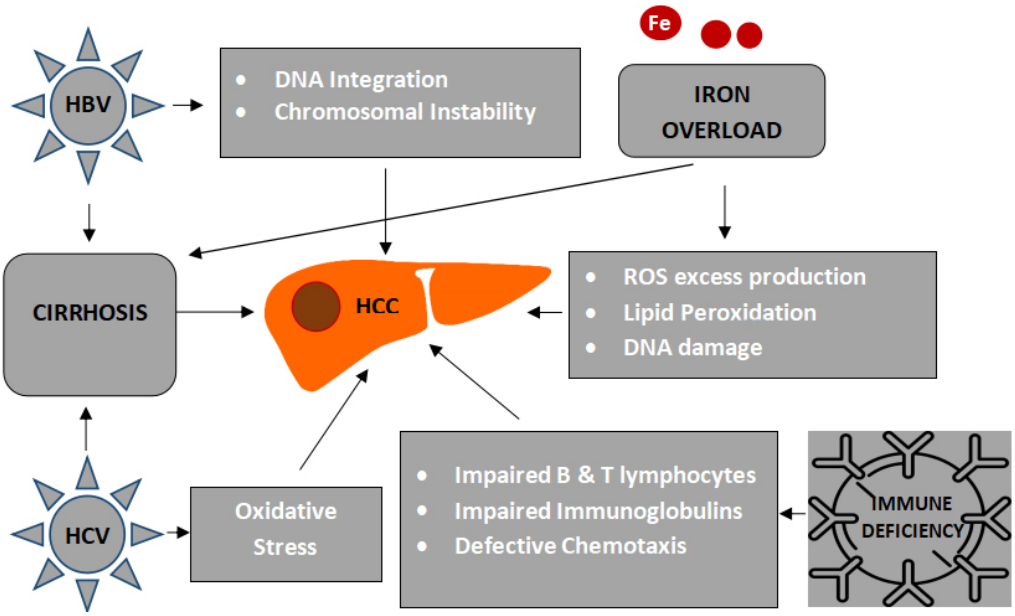

Figure 1. Hepatocarcinogenesis in patients with transfusion-dependent thalassemias (TDT). HCC: Hepatocellular carcinoma; HBV: hepatitis B virus; HCV: hepatitis C virus; ROS: reactive oxygen species; DNA: deoxyribonucleic acid.

well established that, in patients treated with DAAs, the absolute risk of HCC remains in patients with cirrhosis or other concomitant etiological factors[39].

HCC IN TDT - CIRRHOSIS

It is well known that cirrhosis is the dominant risk factor for the development of HCC. In the Western world, up to 90% of HCC arises in the presence of cirrhosis[40]. It was estimated that up to one third of patients with cirrhosis would develop HCC during their lifetime[41]. The annual incidence of HCC depends on the etiology of cirrhosis. Thus, it has been estimated as 3.23% for HBV, 4.81% for HCV, and 1.2% for genetic hemochromatosis[42].

Significant hemosiderosis and hepatic fibrosis were common in patients with TM despite chelation therapy[43]. Worldwide available data indicate that the prevalence of cirrhosis among patients with thalassemia ranges 11.1%-78.5%[14-17].

The co-existence of specific risk factors such as iron overload and HCV or HBV infection are additive factors that increase the risk of cirrhosis in this group of patients. However, the HCC development in patients with TDT is not inextricably linked to the presence of cirrhosis[16].

Since HCC incidence is higher in patients with more advanced cirrhosis, surveillance for early HCC detection with abdominal ultrasound (US) every six months is a reasonable and cost-effective route to reduce mortality in these patients[44]. Moreover, recently published data indicate that alpha-fetoprotein levels (with 20 ng/mL as a cut-off) in combination with US is better in detecting patients with HCC, providing a sensitivity of more than 95%[45].

HCC IN TDT - IRON OVERLOAD

It is well known that hereditary hemochromatosis (HH) is associated with a 20-200-fold increased risk for HCC[46]. The overall standardized incidence ratio of HCC in patients with HH was estimated as 1.7 (95% confidence interval: 1.5-2.0) in a Swedish study[47].

While iron is essential for normal human physiology, its excess is toxic as there is no physiological pathway for removal from the body. In addition, free iron accelerates the Fenton reaction that generates noxious reactive oxygen species (ROS), which severely damage cells and tissues and promote fibrogenesis[48].

Iron overload is implicated in the development of HCC in other hereditary disorders such as TDT. Kountouras *et al.*[49] investigated the role of iron overload and HCV in the severity of liver disease in a cohort of 211 adult Greek patients with beta-thalassemia major. Based on the findings from 109 patients with liver biopsy, they demonstrated that advanced fibrosis was present with even minimal hemosiderosis. Moreover, the presence of fibrosis was independent of ferritin values or HCV history. Consistent with other reports, iron seems to have a leading role in liver disease initiation and progression[50]. Liver stiffness measurement by transient elastography, as hepatic fibrosis assessment, is closely related to the degree of hepatic siderosis in TDT patients, indicating that chelation therapy is mandatory to prevent liver disease progression[51,52].

As discussed above, iron overload in TDT patients leads to denaturation of ferritin, producing an excess of ROS into the cytoplasm of the hepatocytes, thus provoking DNA damage and genomic instability[53] [Figure 1]. Free iron overproduction in hepatic tissue could be responsible for carcinogenesis, overcoming the protective effect of activation of tumor suppressor genes such as *p53*, which regulates cell cycle arrest, apoptosis, and senescence in response to cellular stress[54,55]. In addition, free intracellular ferrous ions react with hydrogen peroxide and activate the lipoxygenase that induces the peroxidation of polyunsaturated fatty acids in cell membranes[56,57]. This mechanism, called ferroptosis, provides evidence about the role of iron in HCC even in the absence of liver cirrhosis[57,58].

There is sufficient evidence that iron chelation therapy improves or stabilizes liver fibrosis independently of liver iron concentration or HCV prevalence[59]. However, it is uncertain whether iron chelation treatment may reduce the risk of HCC, although there is evidence that it may reduce the risk of developing HCC or even suppresses HCC growth in experimental studies[60,61]. Thus, monitoring of iron overload in patients with TDT is crucial.

Nevertheless, iron plays a significant role in the pathogenesis of liver disease and HCC in TDT patients, and effective chelation therapy is mandatory.

Iron overload measurements include liver iron concentration (LIC) using magnetic resonance imaging, which accurately reflects the total body iron stores and ferritin levels that may predict changes in the total body iron levels[62]. Annual targets of LIC 2-5 mg/g dry weight and ferritin levels < 1000 ng/mL appear to be a reasonable strategy in TDT patients[63].

HCC IN TDT - OTHER OR COMBINED RISK FACTORS

Immune deficiency

Transfusion-related immunomodulation was reported several years ago and has been linked with several adverse effects in oncology[64]. Some of the reported immune abnormalities include quantitative and functional defects involving impaired B-lymphocyte function, defective chemotaxis and phagocytosis, and

impaired immunoglobulin production, all of which are essential mechanisms in anticancer immune surveillance[65,66] [Figure 1]. It also has been suggested that iron homeostasis is an important determinant of valid T cell-mediated immune response, as either iron overload or iron deficiency induces immunologic aberrancies[67].

"Second-hit" mechanisms

The iron-related oxidative damage can be further enhanced by several factors such as HCV infection and NAFLD, promoting liver injury and fibrosis synergistically as a "second hit" phenomenon[53,68,69].

In a landmark study, Angelucci *et al.*[53] described that the combination of iron overload and HCV substantially increases the progression of liver fibrosis.

Dysmetabolic iron overload syndrome is now a frequent finding in the general population, as it is detected in about one-third of patients with NAFLD[70,71]. Moreover, hepatocellular iron accumulation was associated with a higher risk of fibrosis than the absence of siderosis in patients with NAFLD, and, once again, the mechanism involves increased oxidative stress[71].

CONCLUSIONS

HCC development in patients with TDT is a complex phenomenon. Several factors such as HBV and HCV infections, iron overload, and immune dysfunction are of paramount importance. Moreover, NAFLD represents a newly emerging risk factor. The efficient antiviral treatment for HCV and HBV infections, the preventive anti-HBV immunization, and, most importantly, the monitoring and treatment of iron overload are expected to reduce HCC in these patients in the future. In all patients, particularly those with cirrhosis, surveillance programs for HCC are mandatory for early detection and application of more effective and curative treatment.

DECLARATIONS

Authors' contributions

Analyzed the data: Papadopoulos N
Wrote the paper: Papadopoulos N, Koskinas J
Designed and directed the project: Koskinas J

Availability of data and materials

Not applicable.

Financial support and sponsorship

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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.

REFERENCES

1. Camaschella C, Nai A. Ineffective erythropoiesis and regulation of iron status in iron loading anaemias. *Br J Haematol* 2016;172:512-23. DOI PubMed
2. Vichinsky E, Neumayr L, Trimble S, et al; CDC Thalassemia Investigators. Transfusion complications in thalassemia patients: a report from the Centers for Disease Control and Prevention (CME). *Transfusion* 2014;54:972-81; quiz 971. DOI PubMed PMC
3. Marsella M, Ricchi P. Thalassemia and hepatocellular carcinoma: links and risks. *J Blood Med* 2019;10:323-34. DOI PubMed PMC
4. Mancuso A. Evidence-based medicine and management of hepatocellular carcinoma in Thalassemia. *BMC Gastroenterol* 2020;20:409. DOI PubMed PMC
5. Mangia A, Bellini D, Cillo U, et al. Hepatocellular carcinoma in adult thalassemia patients: an expert opinion based on current evidence. *BMC Gastroenterol* 2020;20:251. DOI PubMed PMC
6. 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. DOI PubMed
7. McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of hepatocellular carcinoma. *Hepatology* 2021;73 Suppl 1:4-13. DOI PubMed PMC
8. Singal AG, Lampertico P, Nahon P. Epidemiology and surveillance for hepatocellular carcinoma: new trends. *J Hepatol* 2020;72:250-61. DOI PubMed PMC
9. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2021;7:6. DOI PubMed
10. Cancer stat facts: liver and intrahepatic bile duct cancer. Available from: https://seer.cancer.gov/statfacts/html/livibd.html [Last accessed on 25 Nov 2021].
11. Borgna-Pignatti C, Vergine G, Lombardo T, et al. Hepatocellular carcinoma in the thalassaemia syndromes. *Br J Haematol* 2004;124:114-7. DOI PubMed
12. Borgna-Pignatti C, Garani MC, Forni GL, et al. Hepatocellular carcinoma in thalassaemia: an update of the Italian Registry. *Br J Haematol* 2014;167:121-6. DOI PubMed
13. Kourakli A, Diamantidis MD, Skafidas M, et al. Hepatitis C virus infection, but not hepatic iron overload is the dominant risk factor for the manifestation of hepatocellular carcinoma among Greek thalassemic patients. *Blood* 2018;132:2347. DOI
14. Fragatou S, Tsourveloudis I, Manesis G. Incidence of hepatocellular carcinoma in a thalassemia unit. *Hemoglobin* 2010;34:221-6. DOI PubMed
15. Papadopoulos N, Kountouras D, Malagari K, Tampaki M, Theochari M, Koskinas J. Characteristics and prognosis of hepatocellular carcinoma in multi-transfused patients with β-thalassemia. Experience of a single tertiary center. *Mediterr J Hematol Infect Dis* 2020;12:e2020013. DOI PubMed PMC
16. Mancuso A, Sciarrino E, Renda MC, Maggio A. A prospective study of hepatocellular carcinoma incidence in thalassemia. *Hemoglobin* 2006;30:119-24. DOI PubMed
17. De Sanctis V, Soliman AT, Daar S, et al. A concise review on the frequency, major risk factors and surveillance of hepatocellular carcinoma (HCC) in β-thalassemias: past, present and future perspectives and the ICET-A experience. *Mediterr J Hematol Infect Dis* 2020;12:e2020006. DOI PubMed PMC
18. Mancuso A. Hepatocellular carcinoma in thalassemia: a critical review. *World J Hepatol* 2010;2:171-4. DOI PubMed PMC
19. Finianos A, Matar CF, Taher A. Hepatocellular carcinoma in β-thalassemia patients: review of the literature with molecular insight into liver carcinogenesis. *Int J Mol Sci* 2018;19:4070. DOI PubMed PMC
20. Aydinok Y, Porter JB, Piga A, et al. Prevalence and distribution of iron overload in patients with transfusion-dependent anemias differs across geographic regions: results from the CORDELIA study. *Eur J Haematol* 2015;95:244-53. DOI PubMed
21. Pok S, Barn VA, Wong HJ, et al. Testosterone regulation of cyclin E kinase: a key factor in determining gender differences in hepatocarcinogenesis. *J Gastroenterol Hepatol* 2016;31:1210-9. DOI PubMed
22. Akinyemiju T, Abera S, Ahmed M, 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. DOI PubMed PMC
23. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol* 2008;48:335-52. DOI PubMed
24. Huang YT, Jen CL, Yang HI, et al. Lifetime risk and sex difference of hepatocellular carcinoma among patients with chronic hepatitis B and C. *J Clin Oncol* 2011;29:3643-50. DOI PubMed PMC
25. Ghouri YA, Mian I, Rowe JH. Review of hepatocellular carcinoma: epidemiology, etiology, and carcinogenesis. *J Carcinog* 2017;16:1. DOI PubMed PMC
26. Papatheodoridis G, Dalekos G, Sypsa V, 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. DOI PubMed
27. Chien YC, Jan CF, Kuo HS, Chen CJ. Nationwide hepatitis B vaccination program in Taiwan: effectiveness in the 20 years after it was launched. *Epidemiol Rev* 2006;28:126-35. DOI PubMed
28. Franco E, Bagnato B, Marino MG, Meleleo C, Serino L, Zaratti L. Hepatitis B: epidemiology and prevention in developing countries. *World J Hepatol* 2012;4:74-80. DOI PubMed PMC

29. 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. DOI
30. Di Marco V, Capra M, Gagliardotto F, et al. Liver disease in chelated transfusion-dependent thalassemics: the role of iron overload and chronic hepatitis C. *Haematologica* 2008;93:1243-6. DOI PubMed
31. Triantos C, Kourakli A, Kalafateli M, et al. Hepatitis C in patients with β-thalassemia major. A single-centre experience. *Ann Hematol* 2013;92:739-46. DOI PubMed
32. Alavian SM, Tabatabaei SV, Kamran BL. Epidemiology of HCV infection among thalassemia patients in eastern Mediterranean countries: a quantitative review of literature. *Iran Red Crescent Med J* 2010;12:365-76. DOI
33. Koike K. Pathogenesis of HCV-associated HCC: dual-pass carcinogenesis through activation of oxidative stress and intracellular signaling. *Hepatol Res* 2007;37 Suppl 2:S115-20. DOI PubMed
34. Koike K. Hepatitis C virus contributes to hepatocarcinogenesis by modulating metabolic and intracellular signaling pathways. *J Gastroenterol Hepatol* 2007;22 Suppl 1:S108-11. DOI PubMed
35. Origa R, Ponti ML, Filosa A, et al; Italy for THAlassemia and hepatitis C Advance - Società Italiana Talassemie ed Emoglobinopatie (ITHACA-SITE). Treatment of hepatitis C virus infection with direct-acting antiviral drugs is safe and effective in patients with hemoglobinopathies. *Am J Hematol* 2017;92:1349-55. DOI PubMed
36. 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. DOI PubMed
37. Association for the Study of the Liver, Electronic address: easloffice@easloffice.eu, Clinical Practice Guidelines Panel: Chair:, EASL Governing Board representative, Panel members. EASL recommendations on treatment of hepatitis C: Final update of the series. *J Hepatol* 2020;73:1170-218. DOI
38. Sinakos E, Kountouras D, Koskinas J, et al. Treatment of chronic hepatitis C with direct-acting antivirals in patients with β-thalassaemia major and advanced liver disease. *Br J Haematol* 2017;178:130-6. DOI PubMed
39. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, El-Serag HB. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. *Gastroenterology* 2017;153:996-1005.e1. DOI PubMed
40. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018;391:1301-14. DOI PubMed
41. Ioannou GN, Splan MF, Weiss NS, McDonald GB, Beretta L, Lee SP. Incidence and predictors of hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2007;5:938-45, 945.e1. DOI PubMed
42. Tarao K, Nozaki A, Ikeda T, 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. DOI PubMed PMC
43. Li CK, Chik KW, Lam CW, et al. Liver disease in transfusion dependent thalassaemia major. *Arch Dis Child* 2002;86:344-7. DOI PubMed PMC
44. European Association for the Study of the Liver, Electronic address: easloffice@easloffice.eu., European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182-236. DOI
45. Colli A, Nadarevic T, Miletic D, et al. Abdominal ultrasound and alpha-foetoprotein for the diagnosis of hepatocellular carcinoma in adults with chronic liver disease. *Cochrane Database Syst Rev* 2021;4:CD013346. DOI PubMed PMC
46. Bradbear RA, Bain C, Siskind V, et al. Cohort study of internal malignancy in genetic hemochromatosis and other chronic nonalcoholic liver diseases. *J Natl Cancer Inst* 1985;75:81-4. PubMed
47. Elmberg M, Hultcrantz R, Ekbom A, et al. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. *Gastroenterology* 2003;125:1733-41. DOI PubMed
48. Sharp P, Srai SK. Molecular mechanisms involved in intestinal iron absorption. *World J Gastroenterol* 2007;13:4716-24. DOI PubMed PMC
49. Kountouras D, Tsagarakis NJ, Fatourou E, et al. Liver disease in adult transfusion-dependent beta-thalassaemic patients: investigating the role of iron overload and chronic HCV infection. *Liver Int* 2013;33:420-7. DOI PubMed
50. Siagris D, Giannakoulas N, Christofidou M, et al. Virological, immunological and histological aspects in adult beta-thalassemic patients with chronic hepatitis C virus infection. *Liver Int* 2004;24:204-9. DOI PubMed
51. Maira D, Cassinerio E, Marcon A, et al. Progression of liver fibrosis can be controlled by adequate chelation in transfusion-dependent thalassemia (TDT). *Ann Hematol* 2017;96:1931-6. DOI PubMed
52. Delicou S, Maragkos K, Tambaki M, Kountouras D, Koskinas J. Transient elastography (TE) is a useful tool for assessing the response of liver iron chelation in sickle cell disease patients. *Mediterr J Hematol Infect Dis* 2018;10:e2018049. DOI PubMed PMC
53. Angelucci E, Muretto P, Nicolucci A, et al. Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. *Blood* 2002;100:17-21. DOI PubMed
54. Kim E, Viatour P. Hepatocellular carcinoma: old friends and new tricks. *Exp Mol Med* 2020;52:1898-907. DOI PubMed PMC
55. Fargion S, Valenti L, Fracanzani AL. Role of iron in hepatocellular carcinoma. *Clin Liver Dis (Hoboken)* 2014;3:108-10. DOI PubMed PMC
56. Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012;149:1060-72. DOI PubMed PMC
57. Li J, Cao F, Yin HL, et al. Ferroptosis: past, present and future. *Cell Death Dis* 2020;11:88. DOI PubMed PMC
58. Kew MC. Hepatic iron overload and hepatocellular carcinoma. *Cancer Lett* 2009;286:38-43. DOI PubMed
59. Deugnier Y, Turlin B, Ropert M, et al. Improvement in liver pathology of patients with β-thalassemia treated with deferasirox for at least 3 years. *Gastroenterology* 2011;141:1202-11, 1211.e1-3. DOI PubMed
60. Kato J, Miyanishi K, Kobune M, et al. Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular

carcinoma from chronic hepatitis C. *J Gastroenterol* 2007;42:830-6. DOI PubMed

61. Ba Q, Hao M, Huang H, et al. Iron deprivation suppresses hepatocellular carcinoma growth in experimental studies. *Clin Cancer Res* 2011;17:7625-33. DOI PubMed
62. St Pierre TG, Clark PR, Chua-anusorn W, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood* 2005;105:855-61. DOI PubMed
63. Porter J, Viprakasit V, Kattamis A. Chapter 3: Iron overload and chelation. Guidelines for the management of transfusion dependent thalassaemia (TDT). Nicosia (CY): Thalassaemia International Federation; 2014.
64. Cata JP, Wang H, Gottumukkala V, Reuben J, Sessler DI. Inflammatory response, immunosuppression, and cancer recurrence after perioperative blood transfusions. *Br J Anaesth* 2013;110:690-701. DOI PubMed PMC
65. Moukhadder 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. DOI PubMed
66. Farmakis D, Giakoumis A, Polymeropoulos E, Aessopos A. Pathogenetic aspects of immune deficiency associated with beta-thalassemia. *Med Sci Monit* 2003;9:RA19-22. PubMed
67. Shen L, Zhou Y, He H, et al. Crosstalk between macrophages, T Cells, and iron metabolism in tumor microenvironment. *Oxid Med Cell Longev* 2021;2021:8865791. DOI PubMed PMC
68. 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. DOI PubMed
69. Sekine S, Ito K, Watanabe H, et al. Mitochondrial iron accumulation exacerbates hepatic toxicity caused by hepatitis C virus core protein. *Toxicol Appl Pharmacol* 2015;282:237-43. DOI PubMed
70. Dongiovanni P, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. *J Hepatol* 2011;55:920-32. DOI PubMed
71. Valenti L, Fracanzani AL, Bugianesi E, et al. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2010;138:905-12. DOI PubMed