

Review

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

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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 interferone 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
Thrombocytopenia
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.

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Authors' contributions

The author contributed solely to the article.

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The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

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