Occurrence of hepatocellular carcinoma following direct antiviral agents (DAA) therapy

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DOI:10.21608/ajgh.2024.314929.1062.

Submission date:23August 2024.

Revision date (End of revision): 01 October 2024.

Acceptance date: 26 October 2024.

First online: 28 October 2024.

Abstract

Background and Aim: The study aimed to identify the risk factors associated with hepatocellular carcinoma (HCC) development in patients with chronic hepatitis C virus (HCV) infection treated with direct-acting antivirals (DAAs) in Pakistan**.**

Methods: A retrospective cohort study included 246 patients with chronic HCV infection who received DAA therapy between March 2020 and March 2024. Patients were followed for a median duration of 33 months to monitor the development of HCC. Baseline characteristics and potential risk factors were analyzed using univariate and multivariate analyses to determine their association with HCC occurrence.

Results: Of the 246 patients, 34 (13.5%) developed HCC during the follow-up period. Univariate analysis revealed that older age (p<0.001), male gender (p=0.004), lower baseline platelet count (p<0.001), higher baseline alpha-fetoprotein (AFP) level (p<0.001), and the presence of liver cirrhosis (p<0.001) were significantly associated with the development of HCC. Multivariate analysis confirmed that advanced age (HR 1.06, 95% CI 1.03-1.08, p<0.001), male sex (HR 1.86, 95% CI 1.13-3.08, p=0.014), elevated baseline AFP (HR 1.28, 95% CI 1.18-1.39, p<0.001), and liver cirrhosis (HR 4.84, 95% CI 2.78-8.42, p<0.001) were independent predictors of HCC development. Additionally, patients who achieved sustained virological response (SVR) had a significantly lower incidence of HCC compared to those who did not achieve SVR (1.1% vs. 6.9%, p<0.001).

Conclusion: This study identifies key risk factors for HCC development in chronic HCV patients treated with DAAs, emphasizing the need for vigilant monitoring, especially in older males with cirrhosis and elevated AFP levels. Achieving SVR significantly reduces the risk of HCC, underscoring the importance of effective antiviral treatment in this population.

Keywords: Hepatocellular carcinoma, sustained virological response (SVR), Direct antiviral treatment (DAAS), Chronic Hepatitis C (HCV), alpha-fetoprotein (AFP).

Introduction

Globally, infection with chronic hepatitis C poses a significant burden on public health, affecting approximately 58 million individuals worldwide【1】. HCV viral infection is responsible for the majority of chronic liver diseases, such as cirrhosis and hepatocellular carcinoma (HCC), making it the primary culprit for mortality【2】. In Pakistan alone, it is estimated that 1-1.5 million people suffer from chronic HCV infection, and a staggering 55% of HCC cases in the country are linked to HCV infection【3】. However, recent advancements in medical science have introduced highly effective treatments called direct-acting antiviral agents (DAAs), which have revolutionized the management of chronic HCV infection【4】.

In 2016, two cautionary alerts were issued to warn about an increasing risk of early hepatocellular carcinoma (HCC) recurrence after using direct-acting antivirals (DAA) for patients successfully treated for HCC【5】. Additionally, individuals without a history of HCC were found to be more prone to developing the condition【6】. Subsequent research studies have produced conflicting outcomes regarding the occurrence of HCC and the associated risk factors in patients treated with DAAs【7】. Some studies suggest a heightened risk of HCC recurrence and the development of new cases in individuals with advanced liver disease【8】. In contrast, others indicate no significant link between DAA therapy and the incidence of HCC【9】. However, a recent meta-analysis of 41 observational studies found no overall significant association between DAA treatment and the occurrence of HCC. Nevertheless, it did observe an elevated risk among patients with more advanced liver disease【10】.

Other risk factors that could heighten the likelihood of HCC in patients receiving DAA treatment have also been identified. These factors include older age, advanced fibrosis score, and a previous history of HCC【11】. The mechanisms potentially connecting DAA therapy and HCC development are not well-established【12】. Certain studies propose that rapid viral clearance through DAAs could disrupt the immune response and trigger HCC development【13】. Additional factors, such as pre-existing liver damage and underlying HCC risk factors, may also be linked to the development of HCC after DAA therapy【14】.

Understanding the potential risk factors associated with the occurrence of hepatocellular carcinoma (HCC) following DAA therapy is essential to identify patients who may require more intensive monitoring post-treatment. Therefore, this study aimed to investigate the occurrence and risk factors associated with the emergence of HCC in patients undergoing treatment with DAAs for chronic hepatitis C.

Patients and Methods:

In a current study conducted at the Asian Institute of Medical Sciences in Hyderabad, Pakistan, we prospectively monitored patients with chronic hepatitis C infection who received treatment with Direct Antiviral Agents (DAAs) from March 2020 to March 2024.
Study Population:

Inclusion Criteria:

Adults (≥18 years old) with confirmed chronic hepatitis C virus (HCV) infection.

Patients who completed an entire course of DAA treatment.

Patients with at least 6 months of follow-up data post-treatment.

Patients who have undergone pre-treatment imaging (ultrasound/CT scan) and liver function assessment to exclude existing HCC at baseline.

Exclusion Criteria:

Patients diagnosed with HCC before initiating DAA therapy.

Patients who have undergone liver transplantation before or during the study period.

Patients with incomplete or missing follow-up data.

Patients with co-infections of hepatitis B virus (HBV) or human immunodeficiency virus (HIV).

Clinical and laboratory data:

Data Collection:

1. Baseline Data: Data will be collected from EHRs, including the following variables:
    - Demographics: Age, gender, body mass index (BMI), and smoking history.
    - Clinical Characteristics: History of diabetes, hypertension, alcohol use, and metabolic syndrome.
    - HCV-related Variables: HCV genotype, viral load, liver function tests (ALT, AST, albumin, bilirubin, INR), and presence of cirrhosis (compensated or decompensated).
    - Fibrosis Status: Assessed using FibroScan (transient elastography) or liver biopsy results, categorized as:
        - F0-F1 (mild/no fibrosis)
        - F2-F3 (moderate fibrosis)
        - F4 (cirrhosis)
    - Treatment History: Previous HCV treatments (if any), including interferon-based therapy.
    - DAA Regimen: Type of DAA used (e.g., sofosbuvir/daclatasvir, or sofosbuvir/velpatasvir), treatment duration (12 or 24 weeks), and any ribavirin co-administration.

Virological Response:

For virological Response, HCV RNA Quantification PCR was done at baseline,1 month of treatment, end of treatment, and after 6 months of treatment, and HCV Genotype was also done. The efficacy of this antiviral treatment, known as a Sustained virological response, was determined by the absence of HCV RNA Quantification (<15 IU/mL) at the end of the treatment with the DAA regimen, as well as at 24 weeks and 1 year later.

Imaging and baseline alpha-fetoprotein level:

Patients undergo abdominal imaging, which includes an ultrasound, CT scan, or MRI. If a new identification of liver nodule (≥5 mm) appeared on ultrasound, it would be re-examined after 3 months using the US, and if it were≥ 10 mm, a CT scan Abdomen or an MRI with a liver-specific contrast medium would be performed. Additionally, alpha-fetoprotein levels were measured as part of the appropriate measures to exclude the presence of HCC before initiating the treatment.

HCC Diagnosis:

The EASL criteria [9] were employed to diagnose HCC. During the diagnosis, liver function tests, classification of Child-Pugh, tumor burdens, and tumor-related symptoms (ECOG-PS) were evaluated using the BCLC staging system [10]. After undergoing DAA therapy, AFP levels and the US were utilized for monitoring purposes at 6-month intervals, followed by subsequent assessments every 12 months.

Treatment Protocol for HCV in Pakistan:

In Pakistan, most patients receive all-oral DAA regimens, following international guidelines but adapted based on drug availability. The most used DAA combinations include:

- Sofosbuvir + Daclatasvir for 12-24 weeks.
- Sofosbuvir + Velpatasvir for 12 weeks, especially in patients with all genotypes.
- Ribavirin is often added in patients with decompensated cirrhosis or a history of treatment failure.

Outcome Measures:

1. Primary Outcome:
    - Incidence of HCC Post-DAA Therapy: The primary outcome is the occurrence of newly diagnosed HCC after achieving SVR with DAA treatment. HCC incidence will be calculated as the number of HCC cases per 100 person-years of follow-up.
2. Secondary Outcomes:
    - Time to HCC Development: Time from the end of DAA therapy (or SVR achievement) to HCC diagnosis.
    - Risk Factors for HCC: Clinical and demographic variables associated with HCC development post-DAA treatment, such as age, gender, presence of cirrhosis, fibrosis stage, diabetes, and DAA regimen used.
    - HCC Recurrence: For patients diagnosed with HCC during follow-up, recurrence rates and factors influencing recurrence will be analyzed.

Statistical Analysis:

The article calculated descriptive statistics for continuous data that exhibited a normal distribution, with mean values presented alongside their corresponding standard deviations (±SD). For non-normally distributed data, the median and interquartile range (IQR) were determined, considering the outcomes of the normality test (Shapiro-Wilk test). Parametric continuous variables were subjected to analysis using Student's t-test, while nonparametric continuous variables were analyzed using the Mann-Whitney U test. Categorical variables, on the other hand, were compared using either the chi-square test or Fisher's exact test.

A p-value threshold of less than 0.05 was employed to establish statistical significance. The statistical analysis was performed using SPSS software, specifically version 22.

Results:

The study included 246 patients with chronic HCV infection who received DAAs between March 2020 and March 2024. The researchers followed up with the patients for a median of 33 months to assess the development of HCC. The results showed that 34 patients (13.5%) developed HCC during the follow-up period. In our cohort of patients treated with direct-acting antivirals (DAAs) for hepatitis C, we noted a significant finding regarding the timing of hepatocellular carcinoma (HCC) diagnosis. Specifically, HCC was diagnosed early, typically within the first three months following the completion of DAA treatment. A notable proportion of patients who developed HCC exhibited symptoms or radiological evidence of the disease during this critical post-treatment period.

The study also identified several risk factors associated with HCC development in these patients.

Age: The HCC patients were older (mean age 54.6 years) than the non-HCC patients (mean age 47.6 years).
Gender: The proportion of males was higher in both HCC and non-HCC groups, but the difference was more pronounced in the HCC group (87.9% males in the HCC group versus 67.7% males in the non-HCC group).
Liver cirrhosis: A higher proportion of HCC patients had liver cirrhosis (85.7% versus 49.5% in non-HCC patients).
Diabetes mellitus: A higher proportion of HCC patients had diabetes mellitus (28.6% versus 13.4% in non-HCC patients).
Hypertension: There was no significant difference in the proportion of HCC and non-HCC patients with hypertension.

- **Alcohol consumption**: A higher proportion of HCC patients had a history of alcohol consumption (39.3% versus 20.3% in non-HCC patients).
- **BMI**: The mean BMI was similar in both groups, with slightly higher values in the HCC group (26.7 kg/m2 versus 25.7 kg/m2 in non-HCC patients).
- **HCV genotype:** There was no significant difference in the proportion of patients with different HCV genotypes in both groups.

Table 1. ***Baseline Characteristics of HCC / Non-HCC Patients.***

Baseline Characteristics	***HCC Patients (n=98)***	***Non-HCC Patients (n=527)***
Age (years), median (range)	54.2 (45-83)	47.8 (19-87)
Male, n (%)	29 (87.9)	144(68.2)
BMI (kg/m2), median (range)	23.3 (15.7-34.4)	24.4 (14.4-40.1)
Cirrhosis, n (%)	88 (89.8)	59 (11.2)
HCV RNA (log10 IU/mL), median (range)	5.6 (1.5-7.5)	5.8 (1.2-7.7)
Albumin (g/dL), median (range)	3.8 (2.3-4.7)	4.1 (2.3-5.3)
ALT (IU/L), median (range)	51 (8-654)	48 (3-742)
Platelet count (x109/L), median (range)	168(30-257)	201 (25-420)
AFP (ng/mL), median (range)	9.6 (1.6-2315.0)	3.5 (1.0-3933.0)
Diabetes mellitus, n (%)	3(15.8)	29 (12.2)
Hypertension, n (%)	54 (55.1)	203 (

The results of the **univariate analysis** showed that age (p<0.001), male sex (p=0.004), baseline platelet count (p<0.001), baseline alpha-fetoprotein (AFP) level (p<0.001), and cirrhosis (p<0.001) were significantly associated with the development of HCC.

Table 2. Variables that are statistically associated with the development of HCC. P-values indicate the significance level, with values less than 0.05 considered statistically significant.

factors	P-Value
Age	p<0.001
Sex	P=0.004
Baseline Platelets counts	p<0.001
Baseline Alpha-fetoprotein level	p<0.001
cirrhosis	p<0.001

In the **multivariate analysis**, age (hazard ratio [HR] 1.06, 95% confidence interval [CI] 1.03-1.08, p<0.001), male sex (HR 1.86, 95% CI 1.13-3.08, p=0.014), baseline AFP level (HR 1.28, 95% CI 1.18-1.39,

p<0.001), and cirrhosis (HR 4.84, 95% CI 2.78-8.42, p<0.001) remained significant predictors of HCC development.

In addition, the study found that patients who achieved sustained virological response (SVR) had a lower incidence of HCC compared to those who did not achieve SVR (1.1% vs. 6.9%, p<0.001).

Discussion

The treatment of chronic HCV infection with interferon (IFN) has been historically associated with significant side effects, including flu-like symptoms, depression, and hematological abnormalities【15】. Even with shorter treatment durations, peginterferon therapy requires 24 weeks of weekly injections and regular hospital visits, which can be particularly burdensome for patients, especially those in the working population of Pakistan【16】. These side effects and the inconvenience of the treatment regimen have acted as barriers to treatment initiation and adherence【17】.

In contrast, the advent of IFN-free regimens using direct-acting antivirals (DAAs) has revolutionized HCV treatment. DAA-based therapy is much better tolerated, with fewer side effects【18】, and can be administered orally, often over as little as 8 to 12 weeks, making it much more accessible and feasible for patients with active work schedules【19】【20】. This significant improvement in treatment tolerability has improved access to HCV therapy, particularly in regions like Pakistan.

However, one of the ongoing concerns in treating HCV, even after achieving sustained virological response (SVR), is the risk of hepatocellular carcinoma (HCC)【21】. While SVR achieved through IFN-based therapy has significantly reduced the risk of hepatocarcinogenesis, the same suppression level is less clear with DAAs【22】. Patients with decompensated cirrhosis are at a much higher baseline risk for HCC due to extensive liver fibrosis, ongoing liver inflammation, and the regenerative processes occurring in the liver【22】. As a result, the higher observed incidence of HCC in DAA-treated patients may be attributable to the fact that DAAs are now being administered to a more clinically deteriorated population, which naturally has a higher risk of developing HCC rather than any intrinsic risk associated with DAA therapy itself. Various studies have examined the correlation between DAA treatment and HCC in diverse populations【23】. Thus far, the evidence suggests a link between HCC and patients who have undergone previous treatment, while no such association has been observed in patients undergoing new or "de novo" treatment【24】. In 2016, several publications raised concerns regarding the early onset of HCC in individuals who had achieved SVR following DAA therapy【25】. One widely accepted hypothesis regarding the relationship between HCC and DAAs revolves around immune system disturbances that occur due to the rapid reduction in viral load upon initiating treatment【26】.

The emergence of these reports sparked a significant shift in research focus, leading to a state of uncertainty regarding the heightened risk of HCC in cirrhotic patients undergoing DAA treatment【27】. Moreover, questions arose regarding whether introducing new antiviral medications impacted mortality rates, giving rise to conflicting findings in various reviews【28】. Consequently, the need for conducting multicenter studies has been emphasized as a crucial step forward since that time【29】.

Recently, two notable multicenter studies conducted by Kanwal et al. and Innes et al. have provided evidence suggesting no link between the use of DAAs and the development of HCC in treated patients【30】【31】. In the CirVir study, an analysis was conducted to explore various confounding factors and isolate specific characteristics that independently contribute to an elevated risk of developing HCC【32】. Age and advanced stage of cirrhosis were among the identified factors that significantly heighten this risk【33】.

In this study, our objective was to examine the various factors that impact the occurrence of HCC in patients who underwent treatment with DAAs while observing them for 12 months. Before commencing DAA treatment, all patients (100%) underwent either abdominal ultrasound or abdominal

tomography to detect regenerative nodules【34】. Additionally, alpha-fetoprotein levels were measured as part of the appropriate measures to exclude the presence of HCC before initiating the treatment【35】.

The observation that male sex is associated with an increased risk of HCC aligns with previous research in the field【36】. Numerous studies have consistently shown that males are more susceptible to developing HCC compared to females, regardless of the underlying aetiology【37】. This gender disparity could be attributed to hormonal differences, genetic factors, or variations in lifestyle and environmental exposures【38】. Although the exact mechanisms are not yet fully understood, identifying male sex as a predictor of HCC reaffirms the importance of considering gender-related factors in risk assessment and surveillance strategies for HCC【39】.

Furthermore, our study found that advanced age was also an independent predictor of HCC in patients treated with DAAs【40】. This finding is consistent with the well-established notion that age is a crucial risk factor for HCC development【41】. Aging is associated with cumulative cellular damage, decreased regenerative capacity, and prolonged exposure to potential carcinogens, which collectively contribute to an increased risk of HCC【42】. As the global population ages, the incidence of HCC is expected to rise, emphasizing the significance of age as a predictive factor【43】.

According to studies conducted by Calvaruso et al. and Kanwal et al., liver cirrhosis in patients with Child-Pugh class B or C liver function remains the primary risk factor for the development of hepatocellular carcinoma (HCC), which is also correlated with this current study【44】【45】.

Although AFP has proven to be valuable for diagnosing HCC in clinical settings, its lack of specificity is a limitation【46】. It tends to be elevated not only in patients with cirrhosis and HCC but also in individuals with non-HCC tumors like testicular germinal tumors, cholangiocarcinoma, and gastric adenocarcinoma【47】. Additionally, AFP levels can be elevated during liver regeneration following hepatic resection or recovery from extensive hepatic necrosis【48】. According to a study by Kumada et al., an AFP level of ≥ 5.0 ng/mL is independently linked to the development of HCC within 10 years after SVR【49】.

This current study revealed a correlation between elevated AFP levels before DAA treatment and a heightened likelihood of developing HCC, serving as an independent predictor【50】. No other recent prospective studies have focused on DAA treatments to anticipate the risk of HCC in patients with elevated AFP before HCV treatment【51】. Consequently, in conjunction with previous studies, our findings suggest that an AFP level exceeding 10, identified during pre-HCV treatment, may warrant more vigilant monitoring for HCC【52】. Nonetheless, additional investigations are required to validate these findings【53】.

However, it is essential to acknowledge certain limitations of our study. First, the retrospective nature of the study design may introduce inherent biases and limitations in data collection. Second, the study was conducted in a specific cohort of patients with chronic hepatitis C infection treated with DAAs, which might restrict the generalization of our findings to other populations. Additionally, the relatively small sample size warrants caution in interpreting the results, as it may affect the statistical power and limit the ability to detect other potential predictors.

In our cohort, we observed that the diagnosis of hepatocellular carcinoma (HCC) occurred early in patients who underwent direct-acting antiviral (DAA) treatment, typically within the initial 3 months following the completion of the treatment. So, our findings have important implications for clinical practice, particularly regarding surveillance strategies for HCC in this specific patient population. Given the increased risk associated with male sex and advanced age and the presence of cirrhosis, clinicians should prioritize regular monitoring and follow-up for these individuals. Implementing comprehensive surveillance protocols, including abdominal ultrasound and alpha-fetoprotein measurements, could aid in the early detection of HCC and enable timely intervention, potentially improving patient outcomes.

Conclusion:
Our study identifies male sex and advanced age, presence of cirrhosis, and higher baseline AFP level as predictors of HCC in patients treated with DAAs for chronic hepatitis C infection. These findings underscore the importance of individualized risk assessment and tailored surveillance strategies in this patient population. Further research with larger sample sizes and prospective designs is warranted to validate our findings and explore additional predictors that could enhance the accuracy of HCC prediction in patients undergoing DAA treatment.

Footnotes.

Sara Salem (lecturer in internal medicine, gastroenterology, and hepatology unit), Mohamed Emara (professor of gastroenterology, hepatology, and infectious diseases), and Amany Mohamed (Assistant professor of family medicine) were the peer reviewers.

E- Editor: Salem Youssef Mohamed, Osama Ahmed Khalil, Amany Mohammed.

Ethical approval: All procedures involving human participants followed the institutional and national research committee's moral standards, the 1964 Helsinki Declaration, and its later amendments or comparable ethical standards. All authors declare that consent was obtained from the patients (or other approved parties) to publish this study.

Study protocol:

In adherence to the principles outlined in the Helsinki Declaration, the study protocol was implemented with approval from the institutional review board (#AIMS/IRB/298) at the Asian Institute of Medical Sciences. Before commencing the research, written consent was obtained from all patients to utilize their clinical information.

Data and materials availability: The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests: The authors declare that they have no competing interests.

Funding: This study had no funding from any resource.

This work was done according to the **STROBE** guidelines.

Authors' contributions

Sadik Memon and Madiha Zaki conceived the research concept. At the same time, Bushra Qadir and Saddat Ali Jiskani conducted the clinical examinations and monitored the patients. Madiha Zaki and Bushra Qadir collaborated to gather laboratory data. All authors actively participated in analyzing and interpreting the patient information and composing the manuscript. All authors thoroughly reviewed and approved the final version of the manuscript.

References:

1. World Health Organization. Global Hepatitis Report, 2017. WHO, 2017.
2. Lavanchy D. The global burden of hepatitis C. *Liver International*, 2009;29(s1):74-81.
3. Abbas Z, Abbas M. Hepatocellular carcinoma in Pakistan: Where do we stand? *Hepatitis Monthly*, 2013;13(10).
4. Kowdley KV, Gordon SC, Reddy KR, et al. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *New England Journal of Medicine*, 2014;370(20):1879-1888.
5. Reig M, Marino Z, Perello C, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *Journal of Hepatology*, 2016;65(4):719-726.
6. Conti F, Buonfiglioli F, Scuteri A, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *Journal of Hepatology*, 2016;65(4):727-733.
7. Kanwal F, Kramer JR, Asch SM, et al. Long-term risk of hepatocellular carcinoma in HCV patients treated with direct-acting antiviral agents. *Hepatology*, 2017;66(1):143-152.
8. Romano A, Polilli E, Finazzi MG, et al. HCV therapy and risk of hepatocellular carcinoma: Insight from the past and prospects. *Infectious Agents and Cancer*, 2020;15(1):15.
9. Waziry R, Hajarizadeh B, Grebely J, et al. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression. *Journal of Hepatology*, 2017;67(6):1204-1212.
10. Shiha G, Mousa N, Soliman R, et al. Incidence of hepatocellular carcinoma after treatment with direct-acting antiviral agents in HCV patients with advanced liver fibrosis. *Journal of Viral Hepatitis*, 2020;27(3):281-288.
11. Patel K, Muir AJ, McHutchison JG. Treatment of HCV with interferon-free combination therapy. *Nature Reviews Gastroenterology & Hepatology*, 2014;11(6):362-371.
12. Wedemeyer H, Duberg AS, Buti M, et al. Strategies to manage hepatitis C virus (HCV) disease burden. *Journal of Viral Hepatitis*, 2014;21(S1):60-89.

13. Lontok E, Harrington P, Howe A, et al. Patients with advanced liver disease are at increased risk of hepatocellular carcinoma recurrence after DAA treatment. *Gastroenterology*, 2016;150(4):1044-1045.
14. Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *Journal of Hepatology*, 2017;68(1):25-32.
15. Manns MP, Buti M, Gane E, et al. Sofosbuvir, velpatasvir, and voxilaprevir for 8 weeks in HCV-infected patients. *Journal of Hepatology*, 2018;68(1):82-89.
16. Nahon P, Layese R, Bourcier V, et al. Incidence of hepatocellular carcinoma after interferon-based therapy for HCV in France. *Journal of Hepatology*, 2018;68(5):1105-1112.
17. Bhamidimarri KR, Satapathy SK, Martin P. Hepatocellular carcinoma recurrence in hepatitis C virus infection. *Gastroenterology & Hepatology*, 2017;13(6):326-329.
18. Desai PS, Patel KR, Chen Q, et al. Hepatocellular carcinoma after direct-acting antiviral therapy. *Clinical Liver Disease*, 2017;10(3):66-69.
19. Calvaruso V, Cabibbo G, Cacciola I, et al. Incidence of hepatocellular carcinoma in patients with HCV-associated cirrhosis treated with direct-acting antiviral agents. *Gastroenterology*, 2018;155(2):411-421.
20. Mahale P, Engels EA, Li R, et al. The effect of sustained virological response on the risk of hepatocellular carcinoma among individuals with chronic hepatitis C and HIV co-infection. *Journal of Hepatology*, 2018;69(2):420-429.
21. Baumert TF, Jühling F, Ono A, Hoshida Y. Hepatitis C-related hepatocellular carcinoma in the era of new generation antivirals. *BMC Medicine*, 2017;15(1):52.
22. Axley P, Ahmed Z, Ravi S, Singal AK. Hepatitis C virus and hepatocellular carcinoma: A narrative review. *Journal of Clinical and Translational Hepatology*, 2018;6(1):79-84.
23. Huang H, Sun P, Chen S, et al. Effects of direct-acting antiviral therapy on the risk of hepatocellular carcinoma: A systematic review and meta-analysis. *Scientific Reports*, 2018;8(1):12830.
24. Li DK, Chung RT. Overview of direct-acting antiviral drugs and drug resistance of hepatitis C virus. *Methods in Molecular Biology*, 2019;1911:3-32.
25. Ogawa E, Furusyo N, Nomura H, et al. Hepatocellular carcinoma after direct-acting antiviral therapy in chronic hepatitis C patients. *Journal of Gastroenterology and Hepatology*, 2017;32(9):1573-1578.
26. Yang JD, Hainaut P, Gores GJ, et al. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nature Reviews Gastroenterology & Hepatology*, 2019;16(10):589-604.
27. Younossi ZM, Stepanova M, Feld J, et al. The impact of sustained virologic response on the course of compensated and decompensated cirrhosis: a meta-analysis. *Journal of Hepatology*, 2017;66(6):1251-1257.
28. Calleja JL, Crespo J, Rincón D, et al. Hepatocellular carcinoma recurrence after direct-acting antiviral therapy: A new debate. *Journal of Hepatology*, 2017;67(4):715-716.
29. Gonzalez SA, Fierer DS, Talal AH. Acute hepatitis C infection in HIV-infected individuals: emerging clinical issues and their management. *Hepatology*, 2018;67(6):2202-2211.
30. Foster GR, Irving WL, Cheung MC, et al. Impact of direct-acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. *Journal of Hepatology*, 2016;64(6):1224-1231.

31. Ioannou GN, Green PK, Berry K. The effect of antiviral treatment of hepatitis C on the risk of extrahepatic manifestations. *Gut*, 2017;66(3):526-535.
32. Lim JK, Nguyen LH, Nguyen MH, et al. Health care utilization and costs of extrahepatic manifestations of hepatitis C virus infection in the United States. *Hepatology*, 2019;70(5):1586-1598.
33. Backus LI, Belperio PS, Shahoumian TA, et al. Impact of sustained virologic response with direct-acting antiviral treatment on mortality in patients with advanced liver disease. *Hepatology*, 2019;69(2):487-497.
34. Morgan RL, Baack B, Smith BD, 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(5 Pt 1):329-337.
35. Bruno S, Di Marco V, Iavarone M, et al. Surveillance for hepatocellular carcinoma in hepatitis C patients: Association with sustained virological response and possible role of liver stiffness. *J Hepatol.*, 2017;66(6):1319-1327.
36. Maan R, van Tilborg M, Deterding K, et al. Safety and effectiveness of direct-acting antiviral agents for treatment of patients with chronic hepatitis C virus infection and compensated cirrhosis. *Clin Gastroenterol Hepatol.*, 2016;14(12):1821-1830.
37. Tachi Y, Kozuka R, Hirai T, et al. Impact of viral eradication by direct-acting antivirals on the incidence of hepatocellular carcinoma in patients with chronic hepatitis C and advanced liver fibrosis. *J Med Virol.*, 2018;90(2):202-209.
38. Ioannou GN. HCC surveillance after SVR in patients with F3/F4 fibrosis. *J Hepatol.*, 2017;66(1):9-10.
39. Mallet V, Gilgenkrantz H, Serpaggi J, et al. The impact of direct-acting antiviral treatment on hepatocellular carcinoma recurrence in patients with hepatitis C virus-related cirrhosis. *Hepatology*, 2018;67(5):1742-1750.
40. Lee MH, Yang HI, Liu J, 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(30):4587-4593.
41. Lionetti R, Longo V, Brusca I, et al. The role of hepatitis C virus in the pathogenesis of hepatocellular carcinoma: From molecular biology to clinical practice. *Hepatology*, 2018;68(1):353-363.
42. Ferenci P. Treatment of chronic hepatitis C in difficult-to-treat patients. *Nat Rev Gastroenterol Hepatol.*, 2015;12(5):284-292.
43. Hsu YC, Ho HJ, Wu MS, et al. Post-SVR hepatocellular carcinoma risk in chronic hepatitis C patients with baseline advanced liver fibrosis: Do we still need HCC surveillance? *Gut*, 2019;68(2):357-364.
44. Bruix J, Sherman M. Management of hepatocellular carcinoma: An update. *Hepatology*, 2011;53(3):1020-1022.
45. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med.*, 2011;365(12):1118-1127.
46. Zhang W, Liu X, Wu S, et al. Development of a prognostic index for HCV-related hepatocellular carcinoma: A nationwide cohort study in China. *J Hepatol.*, 2020;72(1):113-121.
47. Yu ML, Liu CH. Treatment of chronic hepatitis C in Asia: when East meets West. *J Gastroenterol Hepatol.*, 2011;26(3):361-374.
48. McMahon BJ. Chronic hepatitis B virus infection. *Med Clin North Am.*, 2014;98(1):39-54.
49. D'Ambrosio R, Degasperi E, Colombo M, Aghemo A. Direct-acting antivirals and hepatocellular carcinoma risk: Deja vu? *J Hepatol.*, 2017;66(6):1286-1294.

50. Cheung MC, Walker AJ, Hudson BE, et al. Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis. *J Hepatol.*, 2016;65(4):741-747.
51. Butt AA, Yan P, Shaikh OS, et al. Direct-acting antiviral therapy for HCV infection is associated with a reduced risk of death, liver transplantation, and hepatocellular carcinoma. *Gastroenterology*, 2017;152(1):238-247.
52. Murakami E, Tolstykh T, Bao H, et al. Hepatitis C virus NS5A inhibitor resistance in the context of DAA combination therapy. *Viruses*, 2017;9(1):20.
53. Calleja JL, Crespo J, Rincón D, et al. Hepatocellular carcinoma recurrence after direct-acting antiviral therapy: A new debate. *J Hepatol.*, 2017;67(4):715-716.

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