frontiers | Frontiers in Virology

TYPE Review
PUBLISHED 24 October 2023
DOI 10.3389/fviro.2023.1227317

OPEN ACCESS

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RECEIVED 23 May 2023
ACCEPTED 09 October 2023
PUBLISHED 24 October 2023

CITATION
Elbahrawy A, Atalla H, Mahmoud AA, Eliwa A, Alsawak A, Alboraie M, Madian A, Alashker A, Mostafa S, Alwassief A and Aly HH (2023) Prediction and surveillance of *de novo* HCC in patients with compensated advanced chronic liver disease after hepatitis C virus eradication with direct antiviral agents.
Front. Virol. 3:1227317.
doi: 10.3389/fviro.2023.1227317

Prediction and surveillance of *de novo* HCC in patients with compensated advanced chronic liver disease after hepatitis C virus eradication with direct antiviral agents

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The risk of hepatocellular carcinoma (HCC) diminishes in patients with hepatitis C virus (HCV)-related advanced chronic liver disease after virological cure. However, despite viral clearance, HCV-induced epigenetic alterations, immune dysregulations, and hepatic parenchymal injuries remain, contributing to *de novo* HCC occurrence. While HCC incidence is low (0.45 – 0.5%) in patients with advanced fibrosis (F3), the presence of liver cirrhosis and clinically significant portal hypertension increases the HCC risk. The cost-effectiveness of lifelong HCC surveillance in patients with compensated advanced chronic liver disease (cACLD) has sparked debate, raising questions about the most reliable noninvasive tests and stratification models for predicting HCC in patients with sustained virological response (SVR). Furthermore, identifying cACLD patients who may not require long-term HCC surveillance after SVR remains crucial. Several HCC risk stratification scores have been suggested for patients with cACLD, and emerging evidence supports individualized care based on personalized risk assessments. This review focuses on revising the pretreatment and posttreatment predictors of HCC, as well as the indications for HCC surveillance in cACLD patients treated with direct-acting antivirals.

KEYWORDS

HCC, cACLD, DAA, HCV, predictors, screening

1 Introduction

The introduction of direct-acting antivirals (DAAs) has decreased the incidence of hepatocellular carcinoma (HCC) in chronic hepatitis C (CHC) patients (1, 2). Achieving a virological cure after DAAs is associated with 71% reduction in the risk of developing HCC compared to treatment failure, during a mean 6.1 years of follow up (3). DAAs therapy has also demonstrated improved survival outcomes in hepatitis C virus (HCV)-related HCC patients by reducing disease progression and delaying hepatic decompensation (4).

However, the risk of HCC remains even after HCV clearance. Particularly in patients with liver cirrhosis, HCC remains the most common liver-related complication following sustained virological response (SVR) (5). Early studies conducted following DAA introduction have reported an increased risk of HCC after SVR (6, 7). The early recurrence rate of liver cancer after DAA has been ranged from 12.7%–28.8% (7, 8). A meta-analysis revealed an HCC recurrence rate of 20% person-years (PY) following DAA treatment (9). Furthermore, a three-year incidence of *de novo* HCC occurrence after SVR was found to be 5.9% (10). Notably, HCC that arises after DAA-induced viral clearance may exhibit aggressive behavior and may limit the patient's eligibility for curative treatment options (11–13).

Eradicating HCV and achieving SVR serve as a primary means to prevent HCC and reduce HCC-related mortality. D'Ambrosio et al. demonstrated that HCC is a frequent liver event in cirrhotic patients after DAA treatment, regardless of liver dysfunction or previous liver complications (5, 14). Moreover, patients with a history of HCC who experienced HCC recurrence after SVR had higher mortality rates than those without recurrence (5, 14). Recent studies have revealed that liver cancer accounts for 51.9%–90% of liver-related deaths in DAA-treated patients after SVR (5, 15, 16). These findings collectively highlight the significant impact of HCC occurrence on patient outcomes and underscore the importance of HCC surveillance despite achieving viral clearance after DAAs treatment.

Advanced liver fibrosis is widely accepted as a main underlying mechanism of HCC after SVR. However, the occurrence of HCC in noncirrhotic livers and its aggressive characteristics following DAA treatment have created a disparity in expectations regarding the underlying oncogenic mechanisms (12, 17). HCV-induced HCC involves both direct and indirect mechanisms, including the activation of hepatic fibrogenic pathways, cellular pathways and survival pathways, as well as interactions with the immune and metabolic pathways. Genomic studies have identified polymorphisms in different signaling systems associated with an increased HCC risk (18–20). Furthermore, HCV eradication may trigger a transient immunosuppression that potentially creates favorable situation for the growth of latent micronodules (21). Therefore, gaining more data about the impact of virological cure on HCV-induced oncogenic mechanisms will aid in identifying the persistent epigenetic modifications and inflammatory responses responsible for HCC after SVR, ultimately facilitating the prediction of individual susceptibility to HCC. This, in turn, will guide HCC risk stratification and enable personalized HCC screening for patients after achieving SVR.

Advanced chronic liver disease (ACLD) patients have a significantly increased incidence of HCC (1, 2, 22). The Baveno VI consensus conference in 2015 introduced the concept of compensated ACLD (cACLD), removing the borderline between cirrhosis and noncirrhotic status. This term encompasses both advanced liver fibrosis (F3) and compensated cirrhosis (F4) patients (23, 24). Prior to treatment, assessing liver stiffness (25–29) and utilizing noninvasive blood tests for liver fibrosis are valuable tools for excluding and diagnosing cACLD at baseline (30). In addition, monitoring the dynamic changes in TE and noninvasive blood tests over time appears to enhance the prognostic capability beyond single measurements in assessing cACLD after achieving SVR. Notably, evaluating these noninvasive tools may aid physicians in accurately predicting the HCC risk (low/high) in patients with cACLD following a virological cure with DAA.

This review focuses on a comprehensive analysis of the literature on predictors of *de novo* HCC occurrence and the indications for HCC surveillance in cACLD patients after achieving a DAA-mediated virological cure.

2 Incidence of *de novo* HCC after SVR

The use of pegylated interferon (IFN) plus ribavirin resulted in a significant reduction in HCV-related complications among responders versus nonresponders. Despite this reduction, HCC still occur after SVR (31). Advanced liver fibrosis (≥F3) was found to have a higher risk of *de novo* HCC than earlier stages of fibrosis, regardless of the response to therapy. Morgan et al.'s meta-analysis revealed that 17.8% of HCV patients with cACLD (F3–F4) and no SVR developed HCC, with an incidence rate of 3.3% PY. However, among HCV patients with cACLD (F3–F4) and SVR after IFN-therapy, 4.2% developed HCC, with an incidence rate of 1.05% PY (32).

According to a large retrospective study, the HCC incidence in cirrhotic patients after virological cure was lower in patients treated with DAA (2.12% PY) and IFN (2.28% PY) than in untreated patients (4.53% PY) (33).

Recently, a total HCC incidence of 1.46% PY was reported in DAA-treated patients after SVR (34). The HCC incidence was 2.31% PY and 0.45% PY in cirrhotic patients and in patients with advanced fibrosis (F3), respectively. Similar HCC incidences of 2.10% PY among patients with cirrhosis and 0.50% PY among patients with F3 fibrosis, were reported in a large metanalysis (35).

3 Persistence of HCV-induced oncogenic changes after SVR

HCV-induced epigenetic alterations (36), immune system dysregulations, metabolic disorders, host genetic mutations, and hepatic parenchymal injuries (Figure 1) may persist after DAA treatment and correlate with HCC occurrence after SVR.

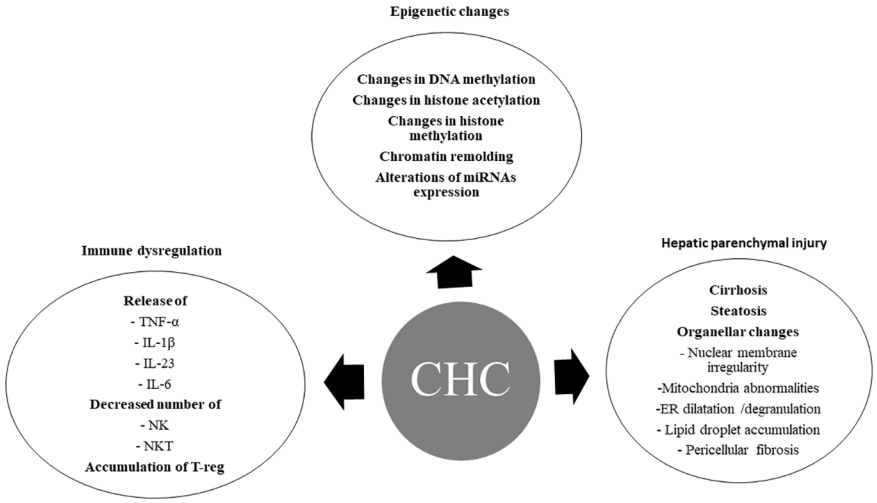

FIGURE 1
Chronic hepatitis C-induced oncogenic changes. CHC, chronic hepatitis C; DNA, Deoxyribonucleic acid; miRNAs, micro ribonucleic acids; TNF-α, tumor necrosis factor -alpha; IL-1β, interleukin-1 beta; IL-23, interleukin – 23; IL-6, interleukin -6; NK, natural killer cells; NKT, natural killer T cells; T-reg, regulatory T cells; ER, endoplasmic reticulum.

3.1 Persistence of HCV-induced epigenetic changes

Hepatitis C-infected cells and HCV-induced inflammatory and/or fibrotic responses in the liver microenvironment can produce hepatic epigenetic changes. Increasing evidences indicated that CHC infection induces changes in DNA methylation, histone modification and chromatin remodeling, and miRNAs expression. These epigenetic changes may play a pivotal role in hepatocarcinogenesis (37) (Figure 1).

In vitro and *in vivo* studies have demonstrated the oncogenic behavior of HCV proteins (NS3, NS5A, and the core protein) (38–41). Indeed, DNA methyltransferase 1 (DNMT1) and DNA methyltransferase 3B (DNMT3B) levels were significantly increased in HCV core protein expressing HepG2 cells and in Huh-7 cells (42–45). Furthermore, the correlation between CHC infection and aberrant methylation of multiple genes has been established in HCV-positive HCC (42, 44, 46–49). Impaired expression of these genes contributes to hepatocarcinogenesis by promoting cell proliferation, invasion, and immune evasion (42, 44, 46–49).

Chronic hepatitis C infection is associated with changes in histone acetylation (50) and histone methylation (51, 52). *In vivo* CHC infection induced specific changes in H3K27ac, which correlated with the expression of HCV messenger ribonucleic acids and HCV proteins (36). The HCV core protein increased the levels of DNMT1 and histone deacetylases 1 (HDAC1) and stimulated their binding to the secreted frizzled related protein 1 (SFRP1) promoter. Silencing of SRFP1 led to activation of the Wnt/β-catenin signaling and may contribute to hepatocarcinogenesis (50). Similarly, *in vitro* CHC infection induces significant enrichment of the transcriptional active chromatin markers H3K9ac and H3K4me, and of the transcriptional silent chromatin marker H3K9me3, changes that may be associated with hepatocarcinogenesis (53).

Chronic hepatitis C infection induces changes in cellular miRNAs expression (54–57). The HCV core protein can stimulate tumor migration and invasion by DNMT1/methylation-mediated inhibition of miR-124 expression, and thus preventing miR-124-induced silencing of set and mynd domain containing 3 (SMYD3) gene (56). Moreover, HCV core protein can affect H3K27me3 through a miR-124/enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) pathway (58).

While *in vitro* results showed the reversion of epigenetic markers levels to a level comparable to uninfected cells after INF-based HCV clearance (53), *in vivo* experiment support the persistence of epigenetic changes after DAA- and IFN-based HCV clearance (36). The persistence of these epigenetic changes has been implicated in HCC occurrence after a DAA viral clearance (36), (Figure 2). Indeed, HCV infection induced H3K27ac modifications was associated with HCC risk after viral clearance (36). The functional knockout of Sphingosine kinase 1 gene and sex-determining region Y-box-9 (SOX-9) gene can inhibit HCC growth (36). While the expression levels of both genes increased during chronic hepatitis C, they remained unaltered after the DAA viral clearance (36). Notably, dysregulation of Wnt/β-catenin signaling plays a paramount role in HCC development; it can be activated by directly binding to HCV-NS5A protein; and promotes proliferation, angiogenesis, and epithelial–mesenchymal transition (59). Of note, Wnt/β-catenin signaling can be switched on in spite of DAA-induced HCV clearance (60), possibly due to the dual effect of DAA treatment; eradicating HCV while activating Wnt/β-catenin signaling (60). The exact mechanism of this phenomenon remains unidentified (60). In cirrhosis, exosomes are crucial modifiers of the tumor microenvironment, and miR-122 downregulation is related with HCC occurrence. In fact, liver-specific miR-122 levels exhibited a significant drop after DAA therapy (61) and remained low among SVR achieved patients

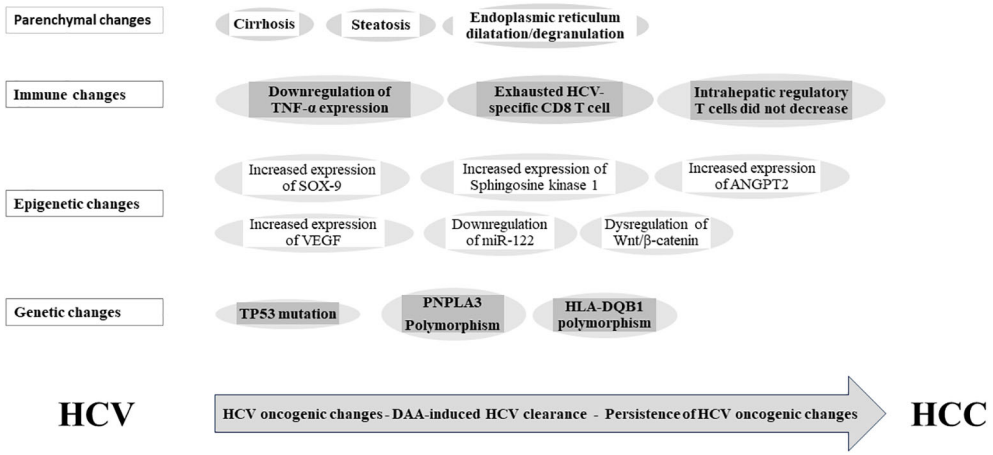

FIGURE 2
Persistence of hepatitis C-induced oncogenic changes after viral clearance. SOX-9, SYR-box transcriptional factor 9; VEGF, vascular endothelial growth factor; ANGPT2, Angiopoietin-2; miR-122, miRNA-122; TP53, tumor protein P53; PNPLA3, Patatin-like phospholipase domain-containing protein 3; HLA-DQB1, Major histocompatibility complex, class II, DQ beta1; HCV, hepatitis C virus; DAA, direct-acting antiviral; HCC, hepatocellular carcinoma.

(62). In cirrhotic tissue, Angiopoietin-2 (ANGPT2) expression before DAA treatment significantly relates to circulating vascular endothelial growth factor (VEGF) and independently relates to HCC occurrence after DAA. Notably, VEGF and ANGPT2 increased following DAA treatment and up to 3-month follow-up (63, 64). Interestingly, the epigenetic signature induced by CHC and persist after DAA treatment is reverted *in vitro* by the epidermal growth factor receptor (EGFR) inhibitor, Erlotinib (53).

3.2 Persistence of immune dysregulation

Inflammation is a major constituent of HCV-induced HCC. The inflammatory cells predispose to HCC occurrence by reactive oxygen species (ROS) and reactive nitrogen species production, and lipid peroxidation. Numerous inflammatory cytokines secreted during chronic HCV infection, such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), interleukin-23 (IL-23), and interleukin-6 (IL-6), are associated with HCC occurrence (65–67). The decreased members of innate immunity, particularly natural killer and natural killer T (NKT) cells, and the increased number of regulatory T (T-reg) cells may correlate with HCC occurrence in chronic HCV infection (68), (Figure 1).

The downregulation of IFN-stimulated genes was rapid after a DAA-induced virological cure (69). The incomplete restoration of innate immune surveillance following DAA-induced HCV eradication has been related to the rapid decrease or normalization of immunosurveillance, potentially leading to early HCC development post-DAA treatment (21, 70). Notably, serum TNF-α expression was persistently downregulated and significantly related to the HCC risk after SVR (71). Moreover, the exhausted phenotype of the HCV-specific CD8 T cell response is not restored by DAA-induced SVR (72–74). Similarly, the intrahepatic regulatory CD4 T cells and CD4(+) CD25(+) FoxP3(+) T-reg cells did not decrease after DAA-induced virological cure (75, 76). This failure of immune function restoration after DAA-induced virological cure may be implicated in HCC occurrence after achieving SVR (77), (Figure 2).

3.3 Persistence of liver injury

The persistence of advanced liver fibrosis and liver injury after DAA treatment are associated with HCC development (78), (Figure 1). Emerging data regarding cirrhosis regression after DAA-induced HCV cure are primarily derived from transient elastography (TE)-based liver stiffness measurement (LSM) (79–84). However, true liver fibrosis regression cannot be solely equated with regressed liver stiffness (85). The hepatic venous pressure gradient (HVPG) stands as the most accurate predictor for liver-related outcomes in cACLD patients (86). Indeed, the disappearance of clinically significant portal hypertension (CSPH) is a rare event after SVR. TE measurements after SVR do not correlate with the HVPG measurements (87, 88), suggesting that viral clearance does not indicate regression of liver cirrhosis (85) and indicating a persistent risk of clinical progression and occurrence of liver-related events after SVR. Liver fibrosis progression after SVR may be triggered by obesity, diabetes mellitus, and alcohol consumption (89). Recently, it was suggested that the persistence of abnormal endoplasmic reticulum after a virological cure is associated with *de novo* HCC occurrence (90). Collectively, these data proposed that overlapping liver injury associated with viral and nonviral causes may lead to the persistence of HCC risk after SVR (Figure 2).

4 Pretreatment predictors of *de novo* HCC after SVR

Pretreatment risk stratification for HCC is useful for identifying patients who require long-term surveillance following DAA treatment (Table 1). The prognostic importance of baseline

TABLE 1 Prediction of hepatocellular carcinoma in cACLD after DAAs-induced HCV clearance.

	Low risk	High risk
Pretreatment predictors of HCC	Bassline M2BPGi < 1 Bassline FIB-4 < 1.5	Diabetes mellitus Alcohol intake Hepatic steatosis Bassline M2BPGi ≥ 4 Bassline Fib-4 ≥ 3.25 LSM ≥ 20 kPa Non-characterized hepatic nodule HCV genotype 3 plus liver cirrhosis
Predictors of HCC at SVR	FIB-4 remained <1.50 FIB-4 improved to <1.50 Platelet count ≥120,000/μl	Diabetes mellitus Continuous alcohol intake Hepatic Steatosis EOT-AFP is elevated Elevated AFP FIB-4 ≥ 3.25 PLT count ≤120,000/μl
Predictors of HCC after SVR 1-year post-SVR 3-years post-SVR 5-years post-SVR	FIB-4 remained at <1.50 FIB-4 improved to <1.50 Platelet count ≥120,000/μl	Diabetes mellitus Steatosis EOT-AFP is elevated FIB-4 ≥3.25 Platelet count ≤120,000/μl
	FIB-4 remained at <1.50 FIB-4 improved to <1.50 Platelet count ≥120,000/μl	Diabetes mellitus Platelet count ≤120,000/μl
	FIB-4 remained at <1.50 FIB-4 improved to <1.50	—

DAAs, direct-acting antivirals; HCV, hepatitis C virus; cACLD, compensated advanced chronic liver disease; HCC, hepatocellular carcinoma; M2BPGi, Mac-2 binding protein glycosylation isomer; Fib-4, fibrosis-4; LSM, liver stiffness measurement; SVR, sustained virological response; EOT-AFP, end of treatment alfa foetoprotein; AFP, alfa foetoprotein.

demographic characteristics and noninvasive tests in predicting HCC risk after DAA treatment has been reported (91).

4.1 Host-related risk factors

4.1.1 Age and male sex

Age has been recognized as an independent risk factor (92) and a valuable marker for *de novo* HCC following SVR (93). The rates of HCC occurrence in patients aged ≥73 and <73 years were 2.2% and 0.5%, 3.9% and 0.7%, 6.1% and 2.1%, and 7.6% and 3.3% at one, two, three, and four years, respectively. Notably, patients aged 75–84 years carried a higher risk of HCC even after DAA-induced virological cure, irrespective of the existence of baseline cirrhosis (94). Early development of HCC after SVR is independently associated with male sex (95), as evidenced by a higher 3-year estimated cumulative HCC occurrence rate in males (9%) than in females (2%) (96).

4.1.2 Continuous alcohol intake

Alcohol can increase HCC risk via the formation of active carcinogen molecules from pro-carcinogens (97). Kanwal et al. (1) recorded a higher annual occurrence rate of HCC in alcohol consumed-patients (1.01% PY) compared to those without alcohol use (0.72% PY) after achieving SVR post-DAA treatment (1). A recent multicenter retrospective study (98) linked baseline alcohol intake to an elevated risk of HCC development after a virological cure.

4.1.3 Diabetes mellitus

Degasperi et al. (96) recognized diabetes mellitus (DM) as a potent independent risk factor for *de novo* HCC. Notably, early HCC occurrence was independently related to the existence of DM (95). Moreover, the 3-year estimated cumulative rate of HCC occurrence after DAA treatment was 16% in DM patients compared to 4% in patients without DM (96).

4.1.4 Host genetics

Whole-exome sequencing was performed on HCC specimens that developed in HCV patients after treatment. The analysis detected higher incidence of TP53 mutations in HCV-SVR-DAA tumors than in HCV-SVR-IFN tumors (99). Host genetic variations can contribute to HCC development after HCV eradication. Recent studies have identified PNPLA3 and HLA-DQB1 polymorphisms as independent risk factors for HCC occurrence following SVR (100) (Figure 2). These findings hold the potential for refining and customizing HCC risk stratification models and surveillance guidelines after SVR.

4.2 Virus-related risk factors

4.2.1 HCV genotype

HCV genotype 3 patients exhibit a more progressive liver disease than those with other genotypes (101). An elevated occurrence of *de novo* HCC has been identified in HCV genotype 3 patients after DAA treatment, particularly among those with pre-existing liver cirrhosis (102).

4.2.2 HCV/HIV coinfection

HCV-infected patients with advanced liver fibrosis, coinfected with human immunodeficiency virus (HIV), appears to have a lower risk of HCC after SVR. The occurrence rates of HCC were recorded as 0.3% PY (103) and 1.2% PY (104) after median follow-up periods of 31.6 and 43 months, respectively. The specific underlying causes that result in the reduced HCC risk among HCV/HIV-coinfected patients remain unknown (104).

4.3 Liver-related risk factors

4.3.1 Hepatic steatosis

In CHC patients, liver steatosis is independently associated with the progression to advanced fibrosis, and both liver steatosis and

advanced fibrosis are significant risk factors for HCC in CHC (105). Liver steatosis exhibit higher occurrence rate of HCC (5.23% PY) in comparison with advanced fibrosis (3.51% PY). When categorized based on their baseline fibrosis and steatosis status, patients with liver steatosis without advanced fibrosis have higher rates of HCC incidence compared to those with advanced fibrosis but without steatosis (106).

4.3.2 Noncharacterized hepatic nodule

DAA treatment has been associated with an early elevated HCC incidence in patients with noncharacterized hepatic nodules (107). The presence of noncharacterized hepatic nodules before DAA treatment is accompanied by an approximate 30% cumulative risk of *de novo* HCC within the first year following treatment (34). Similarly, the presence of noncharacterized hepatic nodules prior to DAA treatment is accompanied by a threefold increased risk of HCC (98). Notably, in patients with Child-Pugh class A, the incidence of HCC in those with noncharacterized hepatic nodules before DAA therapy was 7.24% PY compared to 2.77% PY in those without noncharacterized nodules (98). These noncharacterized hepatic nodules are presumed to represent low/high-grade dysplastic nodules, macro regenerative nodules (98), or microscopically undetectable tumors (108). The temporal relation between initiating DAA therapy and HCC development, along with the existence of noncharacterized hepatic nodules at baseline, indicates that antiviral treatment may trigger a mechanism that promotes the growth and clinical recognition of HCC shortly after treatment (98). This association emphasizes the importance of rigorous surveillance when noncharacterized hepatic nodules are present prior to initiating DAA therapy (10).

4.4 Noninvasive tests

Various baseline noninvasive tests are available for anticipating HCC in cACLD patients after a DAA-induced virological cure.

4.4.1 Alpha-fetoprotein

An elevated pretreatment alpha-fetoprotein (AFP) level before DAA therapy strongly correlates with hepatocarcinogenesis (109). This elevated AFP level may indicate liver regeneration, ongoing inflammation, liver fibrosis, or microscopic HCC. The significant decrease in AFP levels from pretreatment to the end of treatment (EOT), along with the normalization of liver enzymes and viral suppression, suggests that the elevated pretreatment AFP level may reflect the degree of liver inflammation (110). However, a nonreduction in the elevated pretreatment AFP level during treatment is independently related to HCC occurrence (110). In addition, if this level remains elevated after achieving a virological cure, it may suggest advanced liver fibrosis or microscopic HCC.

4.4.2 Mac-2 binding protein (M2BP) glycosylation isomer (M2BPGi)

Liver fibrosis is a major risk factor for developing hepatic carcinogenesis. The M2BPGi level is highly associated with the liver fibrosis stage (111–116). Yamasaki et al. found that the average serum levels of M2BPGi were 1.3, 2.2, 3.3, and 5.2 in patients with fibrosis stages of F0–F1, F2, F3, and F4, respectively, as determined by liver histopathology (117). M2BPGi cutoff values of less than 1.51, 2.48, and 3.50 can exclude the existence of ≥F2, ≥F3, and F4, with negative predictive values of 95.9%, 95.1%, and 99.6%, respectively. However, M2BPGi cutoff values of more than 2.08, 2.87, and 4.35 can propose the existence of ≥F2, ≥F3, and F4, with positive predictive values of 94.2%, 83.1%, and 85.3%, respectively. Several studies have reported that an elevated serum M2BPGi levels more than 1.70–2.00 is associated with increased risk of HCC occurrence or recurrence (118–124). Indeed, the risk of HCC steadily increasing when the serum M2BPGi levels are ranked as <1.00, 1.00–4.00, and ≥4.00 (117).

4.4.3 FIB-4 index

A baseline FIB-4 index ≥3.25 is strongly related to HCC risk (1, 125). The persistent elevation of FIB-4 ≥3.25 before treatment is linked to an increased HCC risk (5.07% PY) in HCV-related ACLD following DAA treatment. Conversely, a persistently lower FIB-4 level (<3.25) before treatment is linked to the lowest HCC risk (1.0% PY) (126). Notably, changes in the FIB-4 score before treatment and after achieving SVR are correlated with modifications in HCC risk. A reduction in FIB-4 from >3.25 to <3.25 after SVR is linked to a decline in HCC risk, while an elevation from <3.25 to ≥3.25 is linked to a raised HCC risk (126).

4.4.4 Liver stiffness

Patients with cACLD are of particular interest, as a significant proportion of them (50%–60%) have CSPH (127). Among the cACLD patients it is crucial to identify those with low HCC risk. LSM has emerged as a useful tool for stratifying cACLD patients who have attained SVR based on their HCC risk (128, 129). Populations with a high pretreatment LSM are at an elevated potential of developing *de novo* HCC (7, 96). The failure to reduce pretreatment LSM by <30% after treatment is independently linked to HCC development (130). Notably, the five-year occurrence rate of HCC increased with higher pretreatment LSM values (9.7% for LSM ≥15 kPa and 11.4% for LSM ≥20 kPa). Different pretreatment LSM cutoff values (14.3 kPa, 20 kPa (34), and ≥30 kPa (96)) have been suggested as the best predictors of *de novo* HCC following SVR. Collectively, these varying cutoff values indicate that increased LSM at the bassline implies increased attributed risk. Thus, rigorous inclusion of patients with pretreatment LSM ≥20 kPa in HCC surveillance programs is recommended (34, 131).

4.5 DAA-related risk

It was found that early HCC incidence is linked to sofosbuvir (SOF)-based therapy without ribavirin, whereas ribavirin usage seems to exert an immunomodulating protective effect on HCC occurrence (95, 132). While SOF increased the cell proliferation in OR-6 and Huh 7.5.1 cells, the cell proliferation was not significantly

influenced after treatment of OR-6 and Huh 7.5.1 cells with different doses of interferon-α and ribavirin (133). Next generation sequencing (NGS) studies revealed that PHOSPHO2-KLHL23, TSNAX-DISC1, TRIM39, RPP21 were upregulated by SOF in OR-6 cells. Indeed, the SOF-increased cell proliferation is improved after siRNA mediated knockdown of PHOSPHO2-KLHL23, TSNAXDISC1, TRIM39 and RPP21 in OR-6 and Huh 7.5.1 cell cultures. Tsai et al. concluded that SOF-induced gene expression in mouse liver cells increased HCC proliferation and migration, which may be related to HCC occurrence (133). However, Ogawa et al. (134) concluded that DAA–SOF treatment was not linked to HCC occurrence within five years after treatment.

5 Post treatment predictors of *de novo* HCC after SVR

Post treatment DM, alcohol intake, and hepatic steatosis are linked to elevated HCC risk. The sustained elevation of pretreatment AFP and FIB-4 values, alongside with the failure of liver stiffness regression after virological cure, strongly relates to HCC occurrence. The dynamic changes in noninvasive liver fibrosis markers following SVR have been correlated with HCC risk (91) (Table 1).

DM was found as a risk factor for HCC in DAA-treated populations after achieving SVR (135) and maintained a significant risk factor for HCC following four years of SVR (136). Although the HCC–DM interrelationship is incompletely understood, hyperinsulinemia and oxidative stress are considered the main factors facilitating DM progression to cancer (137). Hyperinsulinemia is directly involved in carcinogenesis, as it promotes cancer initiation and progression, and is indirectly involved via its stimulation of insulin-like growth factor 1 (IGF-1). In patients with diabetes, increased oxidative stress is linked to DNA damage, mutation in oncogenes, and development of cancer (138, 139).

Alcohol's effects on hepatic fibrogenesis persist post-SVR in alcohol-consuming individuals (1), as evidenced by the increased annual rate of HCC reported among DAA-treated populations who consumed alcohol post-SVR (1). Similarly, while hepatic steatosis is linked to increased HCC risk after DAA (116), liver steatosis is identified as a major predictor of HCC up to two years following SVR, irrespective of fibrosis stage (116).

EOT-AFP (140, 141) and SVR24-AFP levels (142, 143) were identified as predictors of HCC occurrence in DAA treated patients. An increased one-year cumulative HCC occurrence rate correlated with an elevated EOT-AFP level (144). FIB-4 ≥3.25 estimated at SVR or at any time post-SVR was linked to HCC risk, while FIB-4 ≤1.45 was not linked to HCC risk post-SVR in IFN-treated patients (145). Patients treated with DAA whose FIB-4 elevated post-SVR to at least 3.25 had a higher HCC occurrence rate than those who remained persistently below 3.25 (2.29% vs. 1.02%) (93, 126). In addition, patients whose FIB-4 decreased to <1.50 or persisted at <1.50 during follow-up post-SVR had extremely low rates of hepatocarcinogenesis (146). Finally, many studies have confirmed that repeated measurements of FIB-4 is an accurate and cheap method to differentiate patients who may require intensive HCC surveillance after a DAA-induced virological cure (147, 148).

The long-term HCC risk profile in ACLD patients, after DAA-mediated HCV eradication, suggested the reliable predictability of portal hypertension indicators (149). Indeed, posttreatment platelet count ≤ 120,000/μl is independently linked to *de novo* HCC occurrence, after age adjustment (149).

A reduction in LSM of less than 30% in cACLD patients after SVR is an independent risk factor for HCC (96). The estimated three-year incidence of *de novo* HCC was 20% and 5% in patients with LSM > 30 kPa and ≤ 30 kPa, respectively.

Collectively, these data indicate that patients with cACLD should be closely monitored after SVR for *de novo* HCC occurrence in the presence of severe portal hypertension, elevated AFP, and other risk factors for ongoing liver injury (e.g., DM, alcohol drinking, and steatosis).

6 HCC surveillance after SVR

The recent introduction of DAA for treating HCV has significantly influenced the disease outcome course, in particular the occurrence of *de novo* HCC (1, 150). HCC surveillance primarily aims to early detection of HCC to enable curative management, and increasing overall survival (Table 2). Following a DAA-induced virological cure, society guidelines recommend HCC surveillance in cirrhotic (F4) patients, often with biannual abdominal ultrasonography with or without AFP. However, there has been considerable discussion regarding the cost-effectiveness and potential risks of lifelong HCC surveillance in patients with advanced fibrosis (F3) following SVR.

6.1 Cost-effectiveness of HCC surveillance

Although a higher frequency of ultrasound scans during the two years before HCC diagnosis increases the chance of receiving curative treatment (151), surveillance is deemed cost-effective and advantageous to an individual if it increases life expectancy at a cost

TABLE 2 Surveillance of hepatocellular carcinoma in cACLD after hepatitis C virological cure.

	Indication
Patient who should underwent surveillance	Liver cirrhosis on histopathology Pretreatment LSM ≥20 kPa Posttreatment FIB-4 ≥3.2
Patients who should not underwent surveillance	Pretreatment LSM of < 10 Posttreatment FIB-4 < 1.5
Controversial indications of HCC surveillance	F3 fibrosis on histopathology cACLD pretreatment LSM 10 - 20 kPa cACLD posttreatment Fib-4 1.5 - 3.2

HCC, hepatocellular carcinoma; cACLD, compensated advanced chronic liver disease; LSM, liver stiffness measurement; FIB-4, fibrosis-4.

of less than $50,000 per additional year (152). Lin et al. assessed the cost-effectiveness of HCC surveillance in cirrhotic patients and found that biannual AFP and annual abdominal ultrasonography were effective, with a cost-effectiveness ratio of less than $50,000 per quality-adjusted life-year (QALY) (153). Moreover, a recent meta-analysis revealed the beneficial effect of HCC surveillance programs for cirrhotic patients, revealing a lower risk of mortality in the continued surveillance versus nonsurveillance arms. Surveillance was also associated with a greater ability to detect early-stage tumors and receive curative treatment (154). In contrast, biannual HCC surveillance after virological cure was not cost-effective for patients with F3 fibrosis (155).

6.2 Potential harms of HCC surveillance

Physicians must consider both the clear advantages and possible drawbacks of HCC surveillance. These may include financial, psychological, and physical costs, as well as the efficiency of biannual abdominal ultrasound. Patients may suffer physical harm due to false positive or inconclusive surveillance results. Financial harms include the price of diagnostic testing and screening, as well as travel expenses and lost workdays. Being constantly informed of one's chance of contracting fatal cancer and having to wait to learn the outcome every six months can be emotionally exhausting and result in fear, anxiety, and despair (156). The low efficiency of biannual abdominal ultrasound was recently highlighted, as 10,376 abdominal ultrasound scans were performed to allow curative treatment for only 49 patients (151). Taken together, although the benefits of HCC surveillance significantly outweighing the risks (157, 158), additional research is required to accurately quantify the financial and psychological burdens of this surveillance (156).

6.3 Individualization of HCC surveillance

Patients with compensated cirrhosis (F4) and advanced liver fibrosis (F3) were enrolled in the cACLD group. Long-term monitoring is necessary for patients with liver cirrhosis who receive DAA treatment due to their high risk of developing HCC after SVR (159). Notably, patients with cirrhosis were found to benefit financially from biannual HCC surveillance following SVR, whereas those with F3 fibrosis were not (155), demonstrating that more specified stratification models are required to estimate the risk magnitude and benefits of long-term surveillance in the latter.

Numerous HCC risk categorization scores for cACLD patients who obtained SVR after DAA treatment have been reported (160, 161). Liu and colleagues developed a recently simplified risk analysis approach for *de novo* HCC due to LSM and the FIB-4 index (162). Patients with pretreatment LSM 12 kPa and SVR-FIB-4 3.7 had the lowest likelihood of developing *de novo* HCC after SVR; those who did not meet one or both of these criteria were classified as intermediate-risk (3.6% PY) or high-risk (5% PY), respectively. The low-risk group's HCC incidence was below the cost-effectiveness cutoff for HCC surveillance (163). Semmler et al. developed an algorithm for correctly categorizing patients with cACLD based on their risk of *de novo* HCC after SVR. This algorithm was based on posttreatment age, albumin, LSM, and, optionally, AFP and alcohol use. In both the derivation and validation cohorts, around two-thirds of patients with cACLD were found to have an HCC risk of less than 1% PY, and falling below the cost-effectiveness criterion for HCC surveillance (129).

Although these data open the door for a more individualized surveillance approach for patients with cACLD after a virological cure, no HCC risk score has been demonstrated to be significantly better than others or adequately validated for incorporation into existing standard practice. However, a number of models are currently undergoing late-stage clinical validation and could become widely available soon (164).

6.4 Duration of post-SVR surveillance

As no rule specifies a certain age beyond which surveillance should end, Mueller et al., 2022 conducted a comparative cost-effectiveness study determining the age at which the advantages of HCC surveillance outweigh the costs by modeling the life course of people who have been cured of HCV (165). They came to the conclusion that abdominal ultrasound-based biennial screening for HCC is cost-effective in patients with cirrhosis up to age 70 and in those with severe (F3) fibrosis up to age 60.

6.5 Patients with ACLD who are ineligible for HCC surveillance

HCC surveillance is not advised for people with Child-Pugh class C cirrhosis who are ineligible for transplantation, as HCC surveillance will not offer longer life expectancy with the increased mortality risk from liver failure in those patients. Similarly, evidence suggesting that patients without cirrhosis or advanced fibrosis (F3) deserve a universal surveillance approach is insufficient (166). In addition, those with advanced (F3) fibrosis and extremely low estimated risk for HCC based on the recent stratification algorithms could have a more prolonged interval or even discontinue surveillance, particularly when resources are limited. Ciancio et al. (2023) did a recent study in Italy over a span of 2 years and involving 1000 cACLD patients demonstrated that patients with baseline LSM between 9.5 and 14.5 kPa, FIB-4 below 3.25, and APRI below 1.5, who have achieved SVR posttreatment with DAAs, could be considered eligible for suspension from HCC surveillance programs due to the negligible risk (0.09% PY) for HCC occurrence (148).

6.6 Patient adherence to surveillance

Despite clear advantages, such as early tumor diagnosis, only 20% of cirrhotic patients receive HCC surveillance (148, 151, 167). The underutilization of HCC surveillance can be attributed to some screening process errors, including the failure of the provider to

recognize liver disease, identify the silent progression to cirrhosis, the failure to order HCC screening tests, and the failure of the patient to follow screening advice (167).

Based on 2015 survey (167), it was discovered that 65% of the physicians claimed to order annual HCC screening, whereas only 15% reported to request biannual screening. Furthermore, there are significant differences in the rate of HCC screening utilization between individuals followed in gastroenterology clinics (73.7%) and those in population-based cohorts (8.8%) (168). Notably, interventions such public health outreach, reminder and recall systems, and patient-provider education are thought to be beneficial for improving the rates of HCC screening utilization (168).

7 Interventions reducing HCC risk after SVR

7.1 Metformin

Metformin could reduce HCC incidence in patients who achieved a virological cure after INF-based therapy (169). The five-year cumulative HCC incidence was 10.9% in DM-non-metformin users and 2.6% in DM-metformin users, compared to 3.0% in patients without DM. In this study, DM-non-metformin use was significantly associated with HCC risk (169). Interestingly, metformin was found to revert Wnt/β-catenin signaling after DAA-induced virological cure through PKA/GSK-3β-mediated β-catenin degradation (60). These data indicate that metformin may stop the growth of HCC by blocking Wnt/β-catenin signaling and may be recommended to reduce the risk of HCC after SVR.

7.2 Zinc

Hosui et al. (170), showed that zinc supplementation reduced the long-term cumulative incidence of *de novo* HCC in DAA-treated patients after SVR. Zinc administration is required for the activation of many liver enzymes, such as superoxide dismutase (SOD), which has strong antioxidant activity. SOD deactivation increases ROS formation, subsequently inducing DNA damage, and protein modification, which are characteristics of hepatocarcinogenesis. The cellular loss of zinc in malignant hepatoma cells compared with the higher zinc levels in normal hepatocytes establishes the consistency of the zinc role in HCC (171). Furthermore, zinc supplementation directly inhibits fibrosis progression, which may decrease the risk of hepatocarcinogenesis (172). Similarly, zinc deficiency delays extracellular ATP clearance, which promote inflammation and the risk of hepatocarcinogenesis (173). Overall, these data indicate that zinc therapy may reduce the risk of HCC in cACLD patients after a virological cure.

8 Conclusion

cACLD encompasses a diverse range of patients with varying risks of HCC after HCV eradication. Although individualized risk stratification of cACLD patients appears reasonable and appealing, no validated HCC risk score has been established for tailoring HCC surveillance after achieving SVR. However, certain factors such as old age, DM, ongoing alcohol intake, and hepatic steatosis indicate a high risk for HCC and require close monitoring. In addition, the existence of noncharacterized hepatic nodules before DAA treatment indicates the need for short-term, meticulous HCC surveillance. Specifically, pretreatment values of FIB-4 index ≥3.25, M2BPGi >1.70–2.00, and LSM ≥20 kPa warrant rigorous HCC surveillance. Prolonged HCC surveillance is recommended when the pretreatment values of AFP, FIB-4 index, and LSM remain elevated after SVR. Conversely, pretreatment values of FIB-4 <1.5, M2BPGi <1, and LSM <12 kPa suggest a lower HCC risk, allowing for the suspension of HCC surveillance after virological cure.

A more clarification of the impact of DAA-induced HCV eradication on the persistence of HCV oncogenic effects may facilitate the prediction of individual susceptibility to HCC, leading to more accurate risk categorization for patients with cACLD.

Author contributions

HHA and AElb conceptualized the review. HA, AM, AEli, AAls, ME, AM, AAla, SM, AAlw, wrote all drafts. HHA and AElb reviewed and edited the manuscript. HHA provided supervision. All authors contributed to the article and approved the submitted version.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Grant in aid for scientific research: 23K06575. This study was funded by the Japanese Agency for Medical Research and Development (23fk0310503j0102, 23fk0310513j0702, and 23fk0310507j0902).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors HHA and MA declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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