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Guest Editor: Patrick Marcellin

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COVID-19 and the liver – Lessons learned

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Abstract

Liver involvement, indicated by elevated liver function test results, is common in hospitalized patients with coronavirus disease 2019 (COVID-19) and has been linked to disease severity and outcome. A dual pattern of elevated liver function tests can be observed especially in patients with severe or critical COVID-19, characterized by an increase in aminotransferases early in the course of this disease, followed by an increase in cholestasis-associated biochemistry markers at later stages. This dual pattern is associated with inflammatory response markers and poor outcome. Current notions on the mechanisms of liver injury in COVID-19 include direct cytopathic effects of the virus on hepatocytes and cholangiocytes, ischemic and hypoxic liver damage, drug-induced liver injury, activation of hepatic immune cells by excess cytokine production and exacerbation of pre-existing liver disease. Patients with obesity-related non-alcoholic fatty liver disease and, in particular, patients with cirrhosis are at high risk of liver injury and a fatal outcome from COVID-19. In contrast, individuals receiving stable immunosuppressive medication for autoimmune liver diseases or during long-term follow-up after liver transplantation do not have a higher case-to-infection ratio and have a fairly favourable outcome. The present review describes the epidemiology, characteristics and potential pathological mechanisms of COVID-19-related liver injury. Moreover, the influence of pre-existing liver disease on the susceptibility and severity of liver injury in COVID-19 are discussed.

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a contagious, zoonotic respiratory infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 was first reported in December 2019 in a series of patients with severe pneumonia with a fatal outcome in certain cases, following exposure to the Huanan seafood market in Wuhan, Central China,[1] and has since spread worldwide with more than 37 million cases and 1 million deaths, as of October 2020. In addition to acute respiratory tract symptoms, abnormal liver function tests were observed in 14%-69% of patients, mostly identified by transient elevation of aminotransferases.[2-4] Liver injury from COVID-19 seems to mirror disease severity, as patients with severe COVID-19 are more likely to have elevated liver function tests[3,5] and higher peaks of elevation[4,6] than those with milder disease. Whether these

Abbreviations: ACE2, angiotensin-converting enzyme 2; ACLF, acute-on-chronic liver failure; AIH, autoimmune hepatitis; ALAT, alanine aminotransferase; AP, alkaline phosphatase; ARDS, acute respiratory distress syndrome; ASAT, aspartate aminotransferase; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CTP, Child-Turcotte-Pugh; GGT, gamma-glutamyl transferase; HBV, hepatitis B virus; HCV, hepatitis C virus.; IL, interleukin; INR, international normalized ratio; LT, liver transplantation; MELD, model for end-stage liver disease; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; NSAID, non-steroidal anti-inflammatory drug; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; RNA, ribonucleic acid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2; ULN, upper limit of normal.

observations reflect direct SARS-CoV-2-mediated liver damage, secondary liver injury from systemic COVID-19 or more severe courses of COVID-19 in patients with pre-existing liver disease has not been clarified. The present review describes the epidemiology, characteristics and potential pathological mechanisms of COVID-19-related liver injury. Moreover, the influence of pre-existing liver disease on the susceptibility to, severity of and liver injury in COVID-19 are discussed.

Key points

- Liver involvement, characterized by elevated liver function tests, is common in hospitalized patients with COVID-19.
- Patients with severe or critical disease courses are more likely to have elevated liver function tests and higher peaks of elevation.
- The mechanism of elevation is probably multifactorial liver injury.
- Patients with obesity-related non-alcoholic fatty liver disease and, in particular, patients with advanced cirrhosis are at high risk of liver injury and a fatal outcome from COVID-19.
- Optimal treatment and compensation of chronic liver disease are critical to prevent severe courses of COVID-19 in these patients.

2 | ABNORMAL LIVER FUNCTION TESTS AS RISK FACTOR FOR SEVERE COVID-19

Abnormal liver function tests in patients with COVID-19 were first reported in a cohort of 99 patients at Jinyiantan Hospital in Wuhan. Nearly all patients (98%) presented with decreased albumin levels (mean 31.6 g/L, normal range 40-55 g/L). Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) levels were moderately elevated in 28% and 35% of patients respectively. One patient had severe liver damage (ALAT 7590 U/L and ASAT 1445 U/L). Slightly elevated total bilirubin levels were less common (18% of the cases).[7] Twelve single- and multicentre studies in China with a total of 2264 included patients analysed aminotransferase levels in patients with COVID 19.[2] Aminotransferase levels were above the upper limit of normal (ULN) at least once in 14%-53% of the patients. Interestingly, the proportion of patients with increased aminotransferase levels was higher in Wuhan, the epicentre of COVID-19, than outside Wuhan (21% vs 10%, $P < .0001$).[3,8] The authors suggest that this could be because of exposure to higher doses of SARS-CoV-2 with more severe courses of COVID-19 in Wuhan. A meta-analysis of 35 studies including 6686 patients evaluated elevated liver function tests in relation to the severity of COVID-19. ALAT, ASAT and total bilirubin levels were significantly higher in patients with severe COVID-19 than in those with non-severe disease (odds ratio 1.89 [$P = .0009$], 3.08 [$P < .0001$] and 1.39 [$P < .0001$] respectively).[3] In a study in 417 patients, abnormal liver tests on hospital admission were classified as hepatocellular (ALAT and/or ASAT > 3× ULN), cholestatic (alkaline phosphatase (AP) and/or gamma-glutamyl transferase (GGT) > 2× ULN) or mixed. Patients with hepatocellular- and mixed- but not cholestatic-type abnormal liver function tests upon admission had a significantly higher risk of developing severe pneumonia than those without any abnormalities (odds ratio 2.73 [$P = .02$] and 4.44 [$P < .001$]).[6] The authors concluded that liver test abnormalities upon hospital admission, in particular, elevated ALAT or ASAT, can be used to predict the severity of COVID-19. Elevation was usually (>90% of the cases) mild on admission (<2× ULN), and increased in 24% of the cases to significantly more than 3× ULN during hospitalization, again associated with the severity of COVID-19 pneumonia (odds ratio 3.19 [95% confidence interval 1.15-8.84] for hepatocellular and 11.22 [95% confidence interval 4.42-28.45] for mixed type).[6] A study at Massachusetts General Hospital in the USA followed liver function tests in 60 patients with COVID-19 for a median of 9 days during hospitalization. Aminotransferases increased to >ULN in 93% of the patients, while AP and total bilirubin levels remained normal (AP) or were mildly elevated (total bilirubin) in most patients, consistent with hepatocellular injury. Aminotransferases were >5× ULN in 17% of the patients. In particular, ASAT was higher than ALAT at admission (46 vs 30 U/L) and for most of the hospital stay ($P < .05$). Peak ASAT levels were higher in patients requiring mechanical ventilation ($P = .003$) and correlated with the length of hospital stay ($P = .03$).[4] An interesting, dual pattern of liver damage was reported in a survey including 540 hospitalized patients with severe COVID-19 from Zaragoza, Northern Spain, in which 40.9% and 47.3% of the patients presented with elevated ASAT and GGT levels at admission respectively. There was a negative correlation between initial oxygen saturation and ASAT but not GGT ($P <.001$ and $P =.944$ respectively). A longitudinal analysis showed that the progression of GGT levels was positively correlated with inflammatory markers such as C-reactive protein (CRP) and strongly increased in non-survivors but not in survivors during hospitalization ($P < .001$).[9] The authors concluded that SARS-CoV-2 may have a dual effect on the liver, characterized by elevated aminotransferases on admission followed by a marked cholestasis in patients with a fatal outcome (Figure 1). Median albumin levels were already lower on admission in non-survivors than in survivors (3 g/dL vs 3.4 g/dL, respectively, $P < .001$), and further decreased in these patients during hospitalization.[9] In conclusion, abnormal liver function tests are common in COVID-19, mainly in the form of transient increases in aminotransferases. The incidence is higher in patients with severe COVID-19 than in those with mild disease. Acute hepatitis is occasionally reported. In patients with severe COVID-19, initial liver injury is characterized by elevated aminotransferases followed by a cholestatic pattern, and a significant decrease in albumin later in the course of the disease. Studies of liver function tests in outpatients with COVID-19 are lacking.

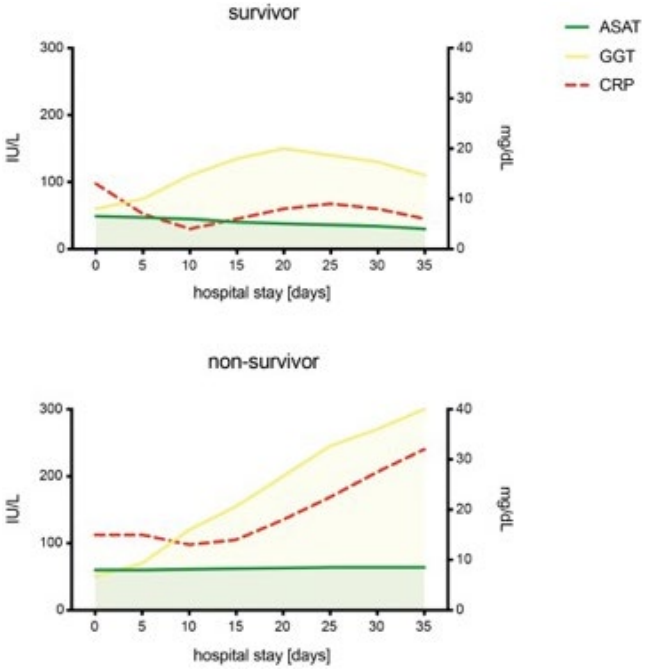

FIGURE 1 Trends of ASAT, GGT and CRP in survivors and non-survivors of COVID-19. Longitudinal variations were extrapolated from a median of 3 (1-15) laboratory tests in 540 patients (survivors n = 431, non-survivors n = 109). ASAT, aspartate aminotransferase (IU/L, left coordinate); GGT, gamma-glutamyl transferase (IU/L, left coordinate); CRP, C-reactive protein (mg/dL, right coordinate). Adapted from Ref. [9]

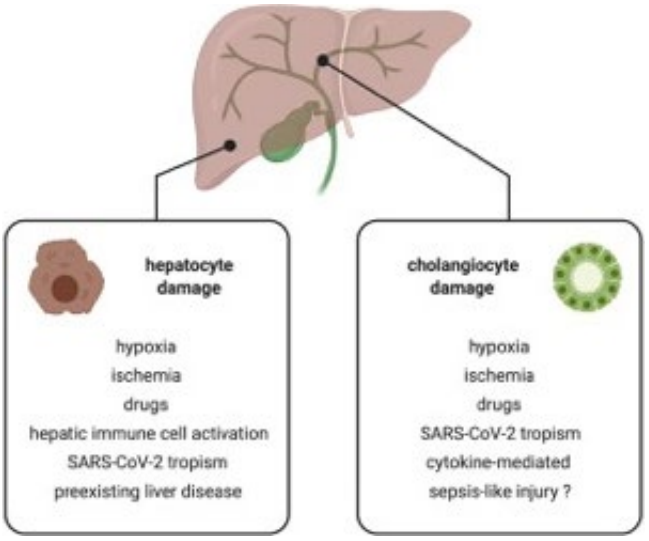

FIGURE 2 Potential mechanisms of hepatocyte and cholangiocyte injury in COVID-19. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

3 | PATHOGENESIS AND CAUSES OF HEPATIC COMPLICATIONS DURING SARS-COV-2 INFECTION

Reports on the mechanism of COVID-19-related liver injury are limited. However, several notions have been discussed (Figure 2): (i) *Direct SARS-CoV-2-induced cytopathic effects on hepatocytes and cholangiocytes*. SARS-CoV-2 expresses a surface glycoprotein, called spike, on the viral envelope, which binds to the receptor angiotensin-converting enzyme 2 (ACE2) on human host cells and, thus, mediates viral entry into the host cell cytoplasm.[10] ACE2 is expressed in a variety of human tissues such as the lungs, heart, kidneys, pancreas, blood vessels, adipose tissue and liver. Single-cell RNA sequencing revealed marked enrichment of ACE2 expression in cholangiocytes but an average expression in hepatocytes that was 20 times lower (59.7% vs 2.6% of ACE2 positive cells).[11] Hepatic immune and stromal cells were ACE2 negative. Thus, it could be hypothesized that SARS-CoV-2 infects cholangiocytes but probably not hepatocytes. However, this notion is not supported by the cell tropism profile of SARS-CoV-2. Human Huh7 hepatocellular carcinoma cells are highly susceptible to SARS-CoV-2 infection.[12] Moreover, liver and cholangiocyte organoids derived from human pluripotent stem cells are permissive to SARS-CoV-2 infection as seen by high levels of viral RNA transcription after inoculation with a SARS-CoV-2 isolate. Infected hepatocytes and cholangiocytes showed marked pro-inflammatory chemokine induction and downregulation of metabolic markers.[13] Viral genomic RNA was identified in 3 of 4 samples of a series of post-mortem examinations of the livers of patients who died from severe COVID-19.[14] One possible explanation for the unexpected tropism of SARS-CoV-2 to hepatocytes could be its exceptionally strong binding affinity to ACE2 which could facilitate virus entry despite low ACE2 expression levels.[10,12] Furthermore, as discussed below, hypoxia and pre-existing liver disease are thought to induce hepatocellular ACE2 expression and potentially increase hepatic susceptibility to SARS-CoV-2 infection.[15,16] One study observed spike structures in the cytoplasm of hepatocytes of 2 COVID-19 cases with transmission electron microscopy. These structures were defined as coronavirus particles. Affected hepatocytes exhibited potentially cytopathic lesions such as mitochondrial swelling, endoplasmatic reticulum dilatation and a decrease in glycogen granules. Furthermore, hepatocellular apoptosis and syncytialization were observed.[17] However, in contrast to the histopathological findings, these two cases did not fulfil the clinical criteria of acute liver failure. Furthermore, the observed changes may be seen during multi-organ dysfunction associated with critical illness, drug-induced liver injury and fatty liver disease, as described in.[18] It was also suggested that the spiked, 'corona-like' inclusions may have been intrahepatic cholesterol crystals or 'crown-like' structures seen in patients with fatty liver disease.[18] In conclusion, SARS-CoV-2 may target cholangiocytes and hepatocytes through ACE2 but the extent of cytopathic damage and liver injury caused by this potential infection remains to be clarified. (ii) *Complex immune dysregulation and hypoxic liver injury*. Patients with severe COVID-19 display a unique signature of immune dysregulation with two key features: overproduction of pro-inflammatory cytokines by monocytes and dysregulation of lymphocytes with lymphopenia.[19-21] SARS-CoV-2 may trigger a hyperinflammatory syndrome, called macrophage activation syndrome or secondary haemophagocytic lymphohistocytosis in a subset of patients with severe COVID-19.[20,21] This syndrome is characterized

by excessive release of cytokines ("cytokine storm"), cytopenias, disseminated intravascular coagulation and multiple organ dysfunction (including the lungs and liver). Interleukin (IL)-6 signalling plays a central role in the pathophysiology of cytokine-driven hyperinflammatory syndromes.[20] In a study from Austria, serum IL-6 levels were strongly correlated with elevated ASAT levels and peak ASAT and ALAT elevation in 96 hospitalized patients with COVID-19.[22] This correlation was stronger in patients with severe COVID-19 than in those with non-severe disease (coefficient of determination r^2 .610 vs .481, $P < .05$). Circulating cytokines can induce a transient elevation of aminotransferases (eg by activation of hepatic immune cells) without affecting liver function, a phenomenon called "bystander hepatitis", which is often observed in systemic viral infections.[23] Moreover, hyperinflammatory syndromes can induce disseminated intravascular coagulation with ischemic and hypoxic liver damage by microvascular thrombosis. Hypoxia and ischemia are probably potentiated by respiratory insufficiency with hypoxemia and haemodynamic alterations.[8,24] High levels of positive end-expiratory pressure in patients with COVID-19 who require mechanical ventilation probably further impairs hepatic perfusion by impeding venous drainage.[25] Post-mortem liver biopsies of patients with fatal COVID-19 showed microvesicular steatosis, hepatocellular degeneration, lobular focal necrosis, portal immune cell infiltration and microthrombosis with congestion of the hepatic sinuses—findings that are consistent with ischemic or hypoxic liver damage.[6,24] Preliminary autopsy results from Bergamo, Italy, suggest that partial or complete sinusoidal or portal thrombosis are common in cases of fatal COVID-19 with elevated aminotransferases, and were found in 27% and 73% of the analysed samples respectively.[26] Changes in coagulation-related biomarkers, such as elevated D-dimer levels, are consistently found in patients with COVID-19, and are more pronounced in critically ill cases.[27] Besides disseminated intravascular coagulation, SARS-CoV-2 may promote endothelial cell injury in the arteries, veins, arterioles, capillaries and venules of all major organs, which probably further impairs hepatic microcirculation and promotes thrombus formation.[28,29] In ischemic or drug-induced liver injury, ASAT levels usually peak before ALAT levels, a pattern that is often observed in patients with severe COVID-19.[4,22] In conclusion, abnormal liver function tests in COVID-19 may be a result of a severe inflammatory immune response, either as a result of "bystander hepatitis" or ischemic (hypoxic) liver damage from microvascular thrombosis, hypoxemia and altered hepatic perfusion. (iii) *Drug-induced liver injury.* The list of drugs with potentially hepatotoxic effects that are used or have been tested for the treatment of patients with COVID-19 is long, and includes antipyretic non-steroidal anti-inflammatory drugs (NSAIDs) (eg acetaminophen), traditional Chinese herbal medications (eg bitter apricot seeds), antibiotics (eg azithromycin), immune modulators (eg tocilizumab, hydroxychloroquine) and anti-viral medications (eg lopinavir/ritonavir, remdesivir). Indeed, the reported histopathological changes—in particular, microvascular steatosis and mild hepatic inflammation in these cases—are also consistent with drug-induced liver injury.[2,6,24] A study from Shenzhen, China, analysed the association of abnormal liver function tests with the use of drugs in 417 hospitalized patients with COVID-19. While antibiotics, Chinese herbal medications and NSAIDs showed a non-significant tendency towards an increased risk of abnormal liver function tests (odds ratio 2.15 [$P > .05$], 2.21 [$P > .05$] and 1.69 [$P > .05$], respectively), the risk was significantly increased by the use of lopinavir/ritonavir (odds ratio 4.44, $P < .01$).[6] Immune modulators and remdesivir were not evaluated. Although lopinavir and ritonavir have been discontinued in many centres owing to alack of efficacy,[30] drugs probably play a role in liver injury in COVID-19.

4 | COVID-19 IN PATIENTS WITH PRE-EXISTING LIVER DISEASE

A multicentre study with 2780 patients hospitalized for SARS-CoV-2 infection in 34 healthcare centres across the USA analysed the influence of pre-existing liver disease on liver function tests and mortality in COVID-19. A total of 250 (9%) of the patients had pre-existing liver disease, usually fatty liver disease or non-alcoholic steatohepatitis (42%), chronic viral hepatitis (21%), alcoholic liver disease (8%), primary sclerosing cholangitis and primary biliary cholangitis (8%) or autoimmune hepatitis (4%). Twenty-four per cent were found to have cirrhosis. The mean aminotransferases levels were elevated from baseline after the diagnosis of COVID-19 in patients with and without pre-existing liver disease, with a tendency towards increased peak aminotransferase levels in patients with pre-existing liver disease. The risk of mortality from COVID-19 was significantly increased in patients with pre-existing liver disease compared to those without (risk ratio 3, $P = .001$), especially in those with cirrhosis (risk ratio 4.6, $P < .001$).[31] A large study from Great Britain with a cohort of 20 133 hospitalized patients showed that pre-existing liver disease is not a predisposing factor for the development of COVID-19 because only 1.6% of the patients had mild and 1.8% moderate or severe pre-existing liver conditions, while chronic cardiac disease, diabetes and obesity were found in 30.9%, 28.1% and 10.5% respectively.[32] However, the increased risk of a fatal outcome of COVID-19 in patients with pre-existing liver disease was confirmed in a large survey including more than 17 million cases. Chronic liver disease resulted in a hazard ratio of 1.75 for COVID-19-related death (95% confidence interval 1.15-2.03).[33] Liver function tests were not assessed in either study. A closer look at specific patient groups is interesting:

4.1 | Obesity and non-alcoholic fatty liver disease (NAFLD)

Individuals with a poorer prognosis of COVID-19 are typically older (>60) with metabolic co-morbidities such as obesity (body mass index >30 kg/m^2) and diabetes, a profile which is similar to those at increased risk of NAFLD.[33,34] A study from 2 COVID-19 hospitals in China compared liver function tests and clinical outcome in patients with (n = 47) and without (n = 155) NAFLD.

Patients with NAFLD had a higher risk of progression to severe COVID-19 (45% vs 7%, $P < .0001$), a longer viral shedding time (17.5 ± 5.2 days vs 12.1 ± 4.4 days, $P < .0001$) and a higher likelihood of abnormal liver function tests from admission to discharge (70% vs 11.1%, $P < .0001$) compared to those without NAFLD.[35] Almost all liver injury was mild with a hepatocellular pattern. Another study from China reported a >2-fold higher prevalence of severe COVID-19 in patients with NAFLD compared to those without NAFLD but only when they were under 60 years old and even after adjustment for possibly confounding factors such as being overweight, diabetes and hypertension (odds ratio 2.67, $P = .03$). In contrast, NAFLD was not associated with the severity of COVID-19 in elderly patients (> 60 years old).[36] The authors suggest that hepatic and systemic immune responses caused by NAFLD could increase the severity of the cytokine storm in younger patients with COVID-19. In the elderly, other comorbidities such as chronic cardiac disease are more prevalent and any association with NAFLD might be masked by influence of the former.[36] Obesity is characterized by low-grade chronic inflammation with increased serum levels of pro-inflammatory cyokines such as IL-6 (which can favour macrophage activation and development of the cytokine storm in COVID-19), and a specific immune dysfunction with impaired secretion of antiviral type I interferons (which probably increases the susceptibility to respiratory viral infections such as COVID-19).[37] The adipose tissue of obese patients is thought to express high levels of ACE2 and, thus potentially functions as SARS-CoV-2 reservoir with prolonged viral shedding time.[37] SARS-CoV-2 infection and the related hyperinflammatory syndrome could act as "second hit" to a simple fatty liver and trigger "acute-on-chronic" steatohepatitis (NASH) with elevated aminotransferases.[34] Moreover, hepatic expression of ACE2 was strongly upregulated in a high-fat diet-induced NASH model in rodents,[16] possibly increasing hepatic susceptibility to SARS-CoV-2 infection in patients with NAFLD or NASH. However, the association of NAFLD and hepatic expression of SARS-CoV-2 critical entry proteins, such as ACE2 and TMPRSS2, a host cell serine protease which cleaves the SARS-CoV-2 spike protein and mediates fusion of host cellular and viral membranes, are controversial. No upregulation was found in a microarray data set comparing 12 lean and 16 obese patients without NAFLD with 9 patients with simple steatosis and 17 patients with biopsy-proven NASH.[38] In contrast, hepatic mRNA expression of ACE2 and TMPRSS2 was low in obese subjects without liver injury (n = 17) or with simple steatosis (n = 57) but significantly increased in obese patients with NASH ($P < .01$ and $P < .05$) and correlated with the NAFLD activity score (NAS) ($P = .017$ and $P = .003$, respectively).[39] Finally, obesity is related to hypercoagulation, mainly as a result of higher plasma concentrations of prothrombotic factors such as factor VII, fibrinogen and von Willebrand factor. This probably fosters microvascular thrombosis formation with ischemia-induced hypoxic liver damage.[37] In conclusion, an inherent immune activation and a tendency towards hypercoagulation are potential causes of the poorer prognosis of COVID-19 and a higher risk of abnormal liver function tests during the course of COVID-19 in patients with obesity and NAFLD.

4.2 | Cirrhosis and liver transplantation

Pre-existing cirrhosis with cirrhosis-associated immune dysfunction and immunosuppressive therapy after liver transplantation could favour liver-related complications, more severe courses of COVID-19 and higher mortality rates. A retrospective analysis of 50 patients with cirrhosis from 9 hospitals in Lombardy, Italy, showed that liver function declined in patients with cirrhosis and COVID-19 upon hospital admission compared to the last visit before SARS-CoV-2 infection. Serum ALAT (31 vs 54 IU/L, $P = .024$), ASAT (33 vs 48 IU/L, $P = .176$), bilirubin (1.3 vs 1.8 mg, $P = .026$) and international normalized ratio (INR) (1.2 vs 1.3, $P = .042$) increased and serum albumin levels (3.4 vs 2.8 g/dL, $P = .0003$) decreased, thus influencing both the Child-Turcotte-Pugh (CTP) and Model for End-Stage Liver Disease (MELD) scores. The distribution of CTP scores shifted towards class C ($P = .05$) and the proportion of patients with MELD ≥ 15 increased from 13% to 26% ($P = .037$). Acute liver injury (ALAT > 30 IU/L for men or >19 IU/L for women) developed in 45% of the patients with previously persistent normal ALAT levels, while 12% experienced a hepatic flare (ALAT ≥ 5× ULN). The 30-day cumulative probability of mortality was significantly higher in the COVID-19 cohort than in 47 patients with cirrhosis hospitalized for acute liver decompensation as a result of bacterial infection (34% vs 17%, $P = .03$).[40] A large-scale international open online reporting study coordinated by the COVID-Hep registry compared COVID-19 mortality and liver injury in chronic liver disease patients with (n = 386) and without (n = 359) cirrhosis. Overall mortality was 32% in patients with cirrhosis and 8% in those without ($P < .001$). The stage of liver disease was the most important determinant of outcome because mortality in patients with cirrhosis increased according to CTP class (A 19%, B 35% and C 51%) (Figure 3). Fifty-five per cent of patients with cirrhosis developed one or more acute-on-chronic liver failure (ACLF) criteria, defined by the Clif consortium.[41] Mortality in patients with ACLF was higher than in those without (46% vs 14%, $P < .001$). Respiratory failure was the most common cause of death (71%), even in patients with ALCF. Alcohol-related liver disease was the only aetiology that was an independent risk factor of death from COVID-19 (odds ratio 3.11, $P < .001$).[42] Another multicentre cohort study from the COVID-Hep registry compared the clinical outcomes of SARS-CoV-2 infection in patients who had undergone liver transplantation (LT) for end-stage liver disease (LT cohort, n = 151) with a matched comparison cohort (non-LT cohort, n = 627). Ninety-nine per cent of the patients in the LT cohort were taking immunosuppressive drugs when SARS-CoV-2 infection was diagnosed: tacrolimus (84%), prednisolone (44%), mycophenolate (51%), azathioprine (9%), cyclosporin (5%) and sirolimus (5%). The median time from liver transplantation was 5 years. The groups did not differ for hospitalization (82% vs 76%, $P = .106$) or the need for intensive care (31% vs 30%, $P = .837$). The percentage of patients who died in the LT cohort was lower than that in the non-LT cohort (19% vs 27%, $P = .046$). The

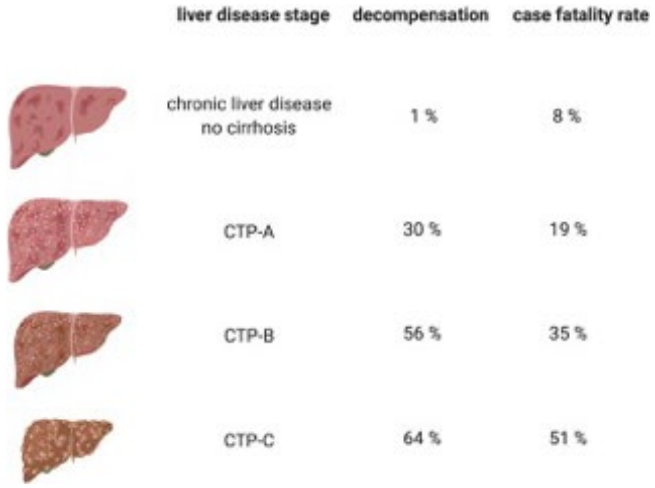

FIGURE 3 Acute hepatic decompensation and fatality rates in hospitalized patients with chronic liver disease and COVID-19. Decompensation included new or worsening ascites (28%), hepatic encephalopathy (27%), spontaneous bacterial peritonitis (3%) and variceal haemorrhage (3%). CTP, Child-Turcotte-Pugh. Adapted from Ref. [42]

main cause of death in both groups was respiratory failure (75% and 89%). The biological age (odds ratio 1.06 per 1 year increase, $P = .031$) but not the time since LT or immunosuppressive medication was associated with mortality in the LT cohort. The LT and non-LT cohorts did not differ in the frequency of mild liver injury (ALAT > 40 IU/L, 30% vs 28%, $P = .734$), moderate liver injury (ALAT > 80 IU/L, 16% vs 14%, $P = .662$) or severe liver injury (ALAT > 200 IU/L, 8% vs 4%, $P = .052$).[43] However, it is important to note that the median time from LT was 5 years, thus these results cannot be applied to patients who acquire SARS-CoV-2 infection in the perioperative period.[44] In a study from Spain, the inflammatory response after SARS-CoV-2 infection in solid organ transplant recipients (kidney, lung, liver, n = 46) and a matched control group (n = 166) was analysed. The inflammatory response in solid organ transplant recipients with COVID-19 was not stronger (according to lymphocyte count, IL-6 and CRP) than in the control group. In contrast, median IL-6 after 7 days of admission (231.4 vs 534.6 pg/ml, $P = .433$) and the incidence of acute respiratory distress syndrome (ARDS) (19.6% vs 27.1%, $P = .06$) were lower in transplant recipients than in the control group.[45] The authors suggest that immunosuppressive medication in solid organ transplant recipients might limit the inflammatory response and protect these patients from hyperinflammation and ARDS development in COVID-19. Unlike the COVID-Hep registry study,[43] this study also included patients with a shorter time after transplantation (<3 months 6.7%, 3-6 months 6.7% and >12 months 87%).[45] In conclusion, although a reporting bias may have affected the COVID-Hep registry data, the overall evidence clearly suggests that cirrhosis strongly increases the risk of COVID-19-related liver injury and mortality with a positive correlation with the stage of cirrhosis. When hepatic function is restored by LT, the risk of liver injury and mortality return to that of the general population emphasizing the close association between chronic liver disease and an adverse outcome of COVID-19.[42] Clear data on the outcome of COVID-19 and post-operative transplant engraftment (<6 months) are lacking.

4.3 | Autoimmune liver disease and chronic viral hepatitis

Studies on the influence of autoimmune liver diseases and chronic viral hepatitis without cirrhosis on COVID-19-related liver injury and outcome are limited. Reports from Northern Italy, China and Belgium suggest that the case-to-infection ratio is not higher in patients with autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC) without cirrhosis with a fairly favourable outcome for SARS-CoV-2 infection in these patients.[1,46,47] In a case series in 10 hospitalized patients with AIH and symptomatic SARS-CoV-2 infection, liver function tests remained normal throughout the hospital stay with a stable immunosuppression regimen, and improved in 2 cases with the onset of acute AIH and high-dose steroid induction therapy.[48] The authors concluded that reduction in immunosuppression during COVID-19 could be harmful, as (i) patients with AIH are at risk of relapse when immunosuppression is reduced, and (ii) immunosuppressive medication could counterbalance COVID-19-driven hyperinflammation. Little is known about the impact of viral hepatitis without associated cirrhosis on outcome and liver function tests in COVID-19. A multicentre study from China suggests that acute or chronic hepatitis B virus (HBV) infection does not affect the outcome of COVID-19, as 22 (95%) of 23 included patients with acute or chronic HBV infection (defined as positive hepatitis B surface antigen) showed a non-severe course of COVID-19.[5] A survey from Spain showed that the use of immunosuppressive drugs (eg IL-6 receptor antagonists or corticosteroids) for the treatment of patients with severe hyperinflammatory syndrome in COVID-19 and resolved chronic HBV infection does not increase the risk of HBV reactivation.[49] However, liver function tests and the outcome of COVID-19 were not assessed. Of note, the significant strain of COVID-19 on national healthcare systems around the world in 2020 has disrupted progress in the global hepatitis C virus (HCV) elimination program, which could result in more than 44 800 cases of hepatocellular carcinoma and 72 300 HCV-related deaths.[50] This analysis shows that COVID-19 extends the direct morbidity and mortality associated with exposure and infection.[50] In conclusion, autoimmune liver diseases without cirrhosis do not seem to increase the risk of COVID-19-related liver injury and mortality, even though the number of patients evaluated is small. The influence of chronic viral hepatitis on liver function tests and the severity of COVID-19 remains to be clarified.

5 | CONCLUDING REMARKS

Although evidence is limited to hospitalized patients, abnormal liver function tests in COVID-19 are common, especially in patients with severe disease. This probably reflects multifactorial mechanisms of liver injury. Initial abnormalities include elevated

aminotransferases, probably mainly because of hypoxic hepatocellular damage. The tropism of SARS-CoV-2 is broad and includes hepatocytes and cholangiocytes. Nevertheless, cases of acute hepatitis are rare. A delayed cholestatic liver biochemistry pattern can develop in patients with critical COVID-19, and its close association with inflammatory response markers supports underlying cytokine-induced molecular mechanisms. Well-controlled pre-existing chronic liver disease without cirrhosis is not associated with a risk of abnormal liver function tests or a fatal outcome, except in patients with pre-existing NAFLD, who have a higher risk of progression to severe COVID-19 and likelihood of abnormal liver function tests. Cirrhosis strongly increases the risk of COVID-19-related liver injury and mortality, with a clear positive correlation with the stage of cirrhosis. The SARS-CoV-2 infection-related risk of liver injury and mortality is similar to that of the general population following liver transplantation, although more data on its effect in the early postoperative period are needed. Optimal treatment and compensation of chronic liver diseases are highly important in this period of limited healthcare resources to prevent severe courses of COVID-19 in these patients.[51,52]

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CONFLICT OF INTEREST

The authors declare no conflict of interest with regard to this work.

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SUPPLEMENT ARTICLE

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Nucleos(t)ide analogue therapy: The role of tenofovir alafenamide

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Abstract

Chronic hepatitis B virus (HBV) infection remains an important global health problem, and may be difficult to manage in clinical practice. Nucleos(t)ide analogues (NAs) with a high barrier to resistance (entecavir [ETV], tenofovir disoproxil fumarate [TDF] and tenofovir alafenamide [TAF]) are the most frequently used HBV treatments because of their long-term effectiveness and tolerability. ETV may be less effective in patients with lamivudine-resistant strains, and TDF is associated with impaired renal function and reductions in bone mineral density. TAF, a new tenofovir prodrug, has been developed to overcome the less favourable safety profile of TDF. TAF is more stable in plasma, and higher tenofovir levels are achieved within cells at lower doses than with TDF. Several registration and real-life studies, performed up to week 144 of treatment, have shown that TAF is at least as effective as TDF, with higher rates of ALT normalization and significantly fewer kidney disturbances and changes in bone mineral density. No emergence of drug resistance has been found with TAF use. The main limitation to prescribing TAF is its price. The European Association for the Study of the Liver has suggested selecting TAF or ETV instead of TDF in patients >65 years old and in those with a risk of osteoporosis or renal abnormalities, and to prescribe TAF rather than ETV in patients previously exposed to NAs.

KEYWORDS

chronic hepatitis B, hepatitis B virus, nucleos(t)ide analogues, TAF, tenofovir

1 | INTRODUCTION

Hepatitis B virus (HBV) was discovered more than 50 years ago, and an effective HBV vaccine has been available for over 30 years.[1] Nevertheless, chronic HBV infection remains an important global health problem affecting more than 257 million people worldwide[2] and causing more than 780 000 deaths per year.[3] Although HBV treatment has progressed and improved over the years, a cure has not been achieved. Current antiviral therapies effectively reduce viral replication, but they have no or little influence on the HBV reservoir in hepatocytes.[3]

The main goal of HBV therapy is to prevent the progression of liver disease and the development of cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC) through suppression of viral replication.[1] There are two main strategies for treating chronic HBV infection: nucleos(t)ide analogues (NAs) and pegylated interferon-α.[1] There are six different types of NAs, and those with a high genetic barrier (entecavir [ETV], tenofovir disoproxil fumarate [TDF]

Abbreviations: ALT, alanine aminotransferase; BMD, bone mineral density; EASL, European association of the study of the liver; eGFR, estimated glomerular filtration rate; ETV, Entecavir; HBB, hepatocellular carcinoma; HBV, hepatitis B virus; NA, nucleos(t)ide analogues; TAF, Tenofovir alafenamide; TDF, Tenofovir disoproxil fumarate.

and recently approved tenofovir alafenamide [TAF])[1] are the recommended first-line HBV regimens because of their favourable safety profiles and high long-term antiviral effectiveness, resulting in undetectable HBV DNA levels in most patients.[1,4] These agents strongly inhibit the HBV polymerase, suppressing viral replication. ETV and TDF have been shown to be highly effective in phase III trials and real-life studies, with high rates of HBV DNA suppression (94%-99% in up to 10 years of follow-up) in both HBeAg-negative and -positive patients.[1,5] However, HBsAg loss is rare, with annual rates of <1%.[5] While there are no significant differences between ETV and TDF for the suppression of HBV DNA, ETV may be less effective in patients with lamivudine-resistant strains, a limitation that does not occur with TDF, which is associated with no drug resistance.[1,4]

This article reviews the effectiveness and safety of tenofovir alafenamide (TAF) for the treatment of patients with chronic HBV infection.

Key points

- ETV, TDF and TAF are the recommended NA treatments for HBV because of their high long-term efficacy and tolerability.
- TDF use is associated with impaired renal function and reductions in bone mineral density, and ETV may be less effective in patients with lamivudine-resistant strains.
- TAF as or more effective than TDF and ALT normalization rates are higher.
- Kidney disturbances and bone mineral density changes are much milder with TAF than with TDF.
- No emergence of HBV drug resistance has been seen with TAF after 144 weeks of treatment.

2 | TAF: A NEW TENOFOVIR PRODRUG

Tenofovir was first described in 1993 with the name (R)-RMPA. To ensure oral bioavailability of the molecule, a diester of tenofovir was formulated with fumarate resulting in the drug TDF. Following intracellular metabolism to its active form, tenofovir diphosphate, TDF inhibits reverse transcription of HBV and HIV.[6] TDF was marketed to treat HIV infection in 2001 and HBV infection in 2008. Although the high antiviral activity of TDF has been confirmed in patients with chronic HBV infection and no resistance over 10 years of use, long-term treatment is associated with impaired renal function, reductions in bone mineral density (BMD) and increases in markers of bone turnover.[5]

TAF, a new phosphonate tenofovir prodrug, was developed to improve the suboptimal safety profile of TDF. Intracellular metabolic activation of TAF occurs in peripheral blood mononuclear cells and liver cells where it is converted into tenofovir-alanine and then hydrolysed to tenofovir before being phosphorylated to obtain tenofovir diphosphate, the final active metabolite of both TAF and TDF.[7] Compared to TDF, TAF is more stable in plasma and remains mainly intact when penetrating virally infected cells, which leads to higher levels of intracellular tenofovir diphosphate at lower drug doses. Thus, systemic exposure to tenofovir is more than 90% lower with TAF than with TDF and the safety profile is considerably better.[7] TAF was found to decrease HBV DNA levels at week 4 at all doses (8, 25, 40 or 120 mg)[8] similar to TDF at 300 mg. Based on these results, the 25-mg dose was selected for clinical development of TAF as treatment of HBV infection.[8]

3 | EFFICACY OF TAF IN CHRONIC HBV INFECTION

In two identically designed double-blind, phase-III international trials, adults with chronic HBV infection and compensated liver disease were randomized 2:1 to receive 25 mg TAF or 300 mg TDF for 96 weeks, followed by an open-label TAF phase through week 144. A total of 1298 patients were enrolled, 873 HBeAg positive and 425 HBeAg negative.[9,10] The protocol was amended to extend the double-blind phase from 96 weeks to 144 weeks, followed by an open-label phase through week 384. However, before the amendment, 540 patients entered the open-label phase on week 96 (360 patients remained on TAF and 180 switched from TDF to TAF). Patients' baseline characteristics were similar between the groups: mean age 40 years old, 63% men, 78% Asian, mainly genotypes C (48%) and D (26%), mean HBV DNA 7.0 log10 IU/mL, 25% previously treated with NAs and 10% with cirrhosis.

At week 96, viral suppression was similar in HBeAg-positive patients receiving TAF or TDF (73% vs 75%, respectively, $P = .47$) and in HBeAg-negative patients (90% vs 91%, $P = .84$).[11] However, in both studies, the percentage of patients with normal alanine aminotransferase (ALT) levels at week 96 was significantly higher in patients receiving TAF than in those who received TDF (75% vs 68%, respectively, $P = .017$).[11] Patients treated with TAF had a significantly smaller mean decrease in hip and lumbar spine BMD (−0.33% vs −2.51%; $P < .001$ and −0.75% vs −2.57%; $P < .001$), respectively, and a significantly smaller median change in the estimated glomerular filtration rate (eGFR) by the Cockcroft-Gault method (−1.2 vs −4.8 mL/min; $P < .001$) than patients receiving TDF.[11]

While there were high rates of virological control in both TAF- and TDF-treated HBeAg-negative and -positive patients at week 144, at year 3, the percentage of patients with ALT normalization was greater in patients receiving TAF (71% vs 59%, $P = .052$ in HBeAg negative and 64% vs 53%, $P = .010$ in HBeAg positive). The serological response rate in HBeAg-positive participants was similar with both treatments, with HBeAg loss in 24% of patients at 3 years. Adverse events and severe events were similar for both treatments. A greater median decrease in creatinine clearance was observed with TDF, while there was only a slight decrease in the TAF group (−6 vs −1.2 mL/min; $P < .001$). Similarly, the mean decrease in hip (−2.5% vs −0.4%, $P < .001$) and spine (−2.0% vs −0.5%, $P < .001$) BMD was significantly higher in the TDF than in the TAF group.[12]

Finally, HBV DNA was undetectable in 84% of the 180 patients who switched to open-label TAF at week 96 (TDF → TAF),[13] and the ALT normalization rate was higher in TDF → TAF patients at 1 year following the switch (45% vs 29% by AASLD criteria; $P = .043$). None of the patients achieved HBsAg loss. At week 144, the median GFR had improved in the TDF → TAF group, (+4.2 [−3.3,+9] mL/min), while those remaining on TDF showed a persistent decrease in median eGFR (−0.9 [−6.6,+6.0] mL/min) $P < .001$). Hip and spine BMD significantly increased in the TAF switch group (+0.98% and + 2.04% from baseline, respectively), while values remained the same in the ongoing TDF group.

Another phase III double-blind study assessed the efficacy and safety of switching to TAF vs continued TDF treatment in chronic HBV patients with viral suppression on long-term TDF.[14] A total of 488 patients were randomized (1:1) to TAF 25 mg or TDF 300 mg for 48 weeks, and they all then received open-label TAF 25 mg until week 96. Virological suppression was similar at weeks 48 and 96 in both groups, and ALT normalization rates increased in both groups at week 96. Bone and renal safety was similar to that in the previous study.

Several real-life studies have been performed with TAF. Kaneko et al reported a similar reduction in HBV DNA levels in a study including 14 treatment-naïve patients with chronic HBV treated with TAF and 45 with TDF for 48 weeks, while eGFR was significantly decreased with TDF (−5.34 ± 7.69 mL/min/ 1.73 m2; $P < .001$).[15] Most studies have been performed in TDF-treated patients who switched to TAF. Like in registration studies, the antiviral effect was maintained for HBV DNA.[16] Real-life studies showed that decreases in eGFR and BMD were not only inhibited by switching to TAF, but even improved.[5,16,17] The results of several switch studies from TDF to TAF are shown in Table 1.

4 | EFFICACY OF TAF IN NA-EXPERIENCED PATIENTS

The two previous phase-III trials contained 386 NA-experienced patients (265 [30%] in the TAF group and 121 [28%] in the TDF group). Previous therapy was mainly ETV and lamivudine.[9,10] The virological response at weeks 96 and 144 was similar whatever the previous therapy. Several small studies in clinical practice have shown that switching from ETV to TAF is more effective and associated with higher HBV DNA suppression rates than remaining on ETV.[18] Some of these studies have also reported a significant ALT normalization rate after switching to TAF.[13,14]

5 | USE OF TAF IN SPECIAL POPULATIONS

5.1 | Elderly

No clinically relevant differences in the pharmacokinetics of TAF according to age or ethnicity have been identified.[19] The effectiveness and safety of TAF is similar in geriatric and younger patients.[20] Dose adjustment is not required in patients aged 65 years and older.[19]

5.2 | Paediatric population

The pharmacokinetics of TAF and tenofovir were evaluated in HIV-1 infected, treatment-naïve adolescents who received TAF (10 mg) given with elvitegravir, cobicistat and emtricitabine as a fixed-dose combination tablet. No clinically relevant differences in TAF or tenofovir pharmacokinetics were observed between adolescent and adult HIV-1-infected individuals. The safety and efficacy of TAF in children <12 years old or weighing <35 kg have not been established.[19]

5.3 | Women of childbearing age and family planning

Telbivudine and TDF are considered to be safe options during pregnancy, and TDF is the first choice therapy.[1] Data on TAF in pregnant or breastfeeding women are limited. However, substantial data on TDF in pregnant women have not shown any malformations or feto/neonatal toxicity. In one study in China, 26 pairs of mothers and infants were enrolled to receive TAF, while another 26 pairs received TDF. TAF concentrations were below the lower limits (0.5 ng/mL) in cord blood and breast milk samples from the TAF group, while the median tenofovir concentration was 4.98 (IQR 0.73-7.24) ng/mL and 12.83 (IQR 7.46-29.46) ng/mL in cord blood and breast milk samples from the TDF group respectively. None of the infants had congenital malformations at birth, confirming that TAF seems to be safe during the 3rd trimester of pregnancy and during breastfeeding, however, larger groups and long-term cohort studies are still need.[21] In the meantime, TAF may be considered during pregnancy if necessary, but should not be used during breastfeeding.[19]

5.4 | Patients with impaired kidney function

TAF is secreted by the kidney. No clinically relevant differences in TAF or tenofovir pharmacokinetics have been observed between healthy individuals and patients with severe renal impairment (eGFR >15 and <30 mL/min) in studies on TAF.[19] TAF dose adjustment is not required in patients with eGFR ≥15 mL/min or in those with eGFR <15 mL/min receiving haemodialysis. During haemodialysis sessions, TAF should be administered after the treatment session has been completed.[19] There are no dosing recommendations for patients with eGFR <15 mL/min who are not receiving haemodialysis.

5.5 | Patients with hepatic impairment

Total plasma concentrations of TAF and tenofovir are lower in patients with severely impaired hepatic function than in those with

TABLE 1 Results of studies focusing on treatment switch from TDF to TAF

Study	N	Population characteristics	Groups compared	HBV DNA suppression	ALT normalization	Changes in Creatinine Clearance (mL/min)	Changes in Bone mineral density
Pan et al 2017[27]	181		Baseline vs 48 weeks after switching to TAF	88% vs 89% *P = NS*	78% vs 89% *P < .001*	−4.8 vs −1.2[a] *P < .001*	0% vs −34%[d] *P < .001*
Gane et al 2018[16]	101	1 or more TDF risk factors[e]	Baseline vs 48 weeks after switching to TAF	*P = NS*	ND	+3[a] *P < .001*	Hip + 0.97%[b] *P = .002* Spine + 2.18%[b] *P < .001*
Buti et al 2019[28]	358	1 or more TDF risk factors[e]	Patients on TDF who continued on TDF vs switched to TAF for 48 weeks	97% vs 97% *P = .96*	ND	-2.7 vs + 1.86[a] *P < .0001*	+6.8% vs −31%[d] *P < .0001*
Lee et al 2019[29]	45		Baseline vs 12 weeks after switching to TAF	ND	−12.93[c] *P < .002*	*P = .6*	ND
Lim et al 2019[17]	174	Patients with HBV resistant to entecavir and/or adefovir	Patients on TDF who continued on TDF vs switched to TAF for 48 weeks	98% vs 99% *P = .99*	79% vs 92% *P = .06*	4.5% vs 8.2%[b] *P = .06*	0.08% vs 1.84%[b] *P = .01*
Kaneko et al 2019[15]	36		Baseline vs 24 weeks after switching to TAF	*P* = NS	ND	−7.32 vs + 2.89[c] *P = .02*	ND
Ahn et al 2020[30]	288	Asians with 1 or more TDF risk factors[e]	Patients on TDF who continued on TDF vs switched to TAF for 48 weeks	97% vs 97% *P = NS*	73% vs 76% *P = NS*	−2.7 vs + 2.6[a] *P < .0001*	*P < .0001*
Lampertico et al 2020[14]	488		Patients on TDF for 48 weeks switched to TAF for 48 weeks more vs TAF for 96 weeks	94% vs 95% *P = .686*	74% vs 56% *P = .051*	−0.39 vs + 0.51[a] *P = .871*	Hip 0.18% vs 1.16%[b] *P < .001* Spine 1.7% vs 2.3%[b] *P = .097*

[a]Median change mL/min.

[b]Mean % change.

[c]Mean change mL/min.

[d]Median % change in C-type collagen sequence.

[e]Age > 60 yr, osteoporosis of hip/spine, ≥Stage 2 chronic kidney disease (CKD), albuminuria (UACR > 30 mg/g), hypophosphatemia (PO4 < 2.5 mg/dL) or comorbidities associated with CKD (eg HTN, DM, obesity); ND: testing not done; NS: not significant.

normal function. When corrected for protein binding, free plasma TAF concentrations are similar in both groups.[19] The efficacy and safety of TAF in patients with decompensated chronic hepatitis B seem to be similar to that of compensated patients based on the limited data with this agent.[22]

6 | USEFULNESS OF TAF IN REAL LIFE

In certain countries, the main limitation to the prescription of TAF in patients with chronic HBV is the price of the drug, which is usually more expensive than ETV or TDF, which are both generic. To overcome the safety limitations of TDF, the European Association for the Study of the Liver (EASL) Clinical Practice Guidelines have proposed selecting TAF or ETV rather than TDF in patients >65 years old and in those with a risk of osteoporosis or renal abnormalities, and to prescribe TAF rather than ETV in patients who have received NAs.

Sixty-six per cent of 565 chronic HBV patients receiving TDF in two European centres met the EASL criteria to switch to TAF or ETV.[23] It should be noted that most of the patients in the cohort were NA experienced, and TAF should be prescribed if possible in these cases.

A study in 1037 patients in the USA found that TAF was prescribed in 38% for prevention rather than for adverse clinical changes in renal and bone function, [24] while in a Greek study the main reasons for starting TAF were renal (54%), BMD (35%) and both renal and BMD (11%) disorders/risks.[25]

All these data suggest that TAF is more often initiated indifferent countries based on cost than for its efficacy and safety, even though some studies have found TAF to be cost-effective.[26]

In summary, the initiation of TAF is important to overcome drug safety issues in patients with chronic HBV. The antiviral effectiveness of this agent is at least as potent as TDF, but it is associated with significantly lower rates of changes in renal function and BMD. Like TDF, TAF results in little or no emergence of drug resistance.

CONFLICT OF INTEREST

Maria Buti—Has received research grants from Gilead and has served as an advisor for Gilead, Bristol-Myers Squibb and Novartis. No personal conflicts of interest. Cristina Marcos-Fosch—No personal of financial conflicts of interest. Rafael Esteban—Has received research grants from Gilead and has served as an advisor for Gilead, Bristol-Myers Squibb and Novartis. No personal conflicts of interest.

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SUPPLEMENT ARTICLE

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HBV 2021: New therapeutic strategies against an old foe

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Abstract

Hepatitis B virus (HBV) affects more than 250 million people worldwide, and is one of the major aetiologies for the development of cirrhosis and hepatocellular carcinoma (HCC). In spite of universal vaccination programs, HBV infection is still a public health problem, and the limited number of available therapeutic approaches complicates the clinical management of these patients. Thus, HBV infection remains an unmet medical need that requires a continuous effort to develop new individual molecules, treatment combinations and even completely novel therapeutic strategies to achieve the goal of HBV elimination. The following review provides an overview of the current situation in chronic HBV infection, with an analysis of the scientific rationale of certain clinical interventions and, more importantly, explores the most recent developments in the field of HBV drug discovery.

KEYWORDS

antiviral agents, cccDNA, combination therapy, HBV, immune modulation

1 | INTRODUCTION

With more than 250 million patients with chronic hepatitis B virus (HBV) infection worldwide, this disease is still a clear and ever-present public health burden.[1] Indeed, phylogenetic analysis of HBV genomes suggests that certain subgenotypes originated more than 50,000 years ago.[2] We have only recently understood how such a small non-cytopathic DNA virus could be of great clinical relevance, as HBV-associated complications are the seventh highest cause of mortality worldwide. Indeed, chronic HBV infection is one of the major aetiological factors in the development of cirrhosis and hepatocellular carcinoma (HCC).[3] On the molecular level, the mechanism behind chronic HBV infection is based on the persistence of the viral genome as an episomal structure referred to as covalently closed circular DNA (cccDNA), which remains in the nucleus as a viral reservoir and template for viral replication.[4] As a by-product of viral replication, HBV DNA can be randomly integrated into the host cell genome. Although integrated HBV sequences cannot sustain viral replication, they can generate viral proteins, namely hepatitis B surface antigen (HBsAg) and the transcriptional regulator HBV x protein.[4]

Despite the implementation of universal vaccination programs, chronic HBV infection remains a major public health problem worldwide. Moreover, existing therapeutic compounds against HBV are

Abbreviations: ALT, alanine aminotransferase; ASO, antisense oligonucleotide; CAM, capsid assembly modulator; CAR, chimeric antigen receptor; cccDNA, covalently closed circular DNA; CHB, chronic HBV infection; CRISPR, clustered regularly interspaced short palindromic repeats; DAA, direct-acting antiviral; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HDV, hepatitis delta virus; HTA, host-targeting agent; NAP, nucleic acid polymer; NK, natural killer; NTCP, sodium taurocholate cotransporting polypeptide; NUC, nucleos(t)ide analogue; PD1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; Peg-IFN-α, pegylated interferon α; RIG-I, retinoic acid-inducible gene I; RISC, RNA-induced silencing complex; siRNA, small-interfering RNA; TCR, T-cell receptor; TDF, tenofovir disoproxil fumarate; TLR, Toll-like receptor; WHV, woodchuck hepatitis virus.

limited and mainly include nucleos(t)ide analogues (NUCs) (eg entecavir, tenofovir) and pegylated interferon α (Peg-IFN-α). As beneficial as they may be, these treatments do not usually achieve eradication of the virus and HBsAg loss is still rare.[5] Thus, these regimens require indefinite treatment to maintain viral suppression and prevent the virological relapse that usually occurs after treatment discontinuation.[6] Moreover, it is unrealistic to expect all patients to adhere to long-term or lifelong non-curative treatment and there is a strong patient preference for finite therapy. Drug resistance is still a concern in low-income settings that use early generation NUCs and while there is no resistance with IFN treatment, the use of this agent is rare because of problems with tolerability. The cost of lifelong therapy and monitoring is also an important economic issue in highly endemic areas. Thus, the aim of new therapeutic strategies is to achieve a "functional cure" for chronic hepatitis B (CHB), defined as sustained off-treatment loss of HBsAg, undetectable HBV DNA in serum, normalization of liver enzymes and improvement in liver histology (Figure 1). HBsAg loss is a sign of profound suppression of HBV replication and is the only existing indicator for safe treatment discontinuation. Moreover, HBsAg loss is associated with a decreased risk of developing inflammation-driven hepatic complications such as HCC.[7,8]

Thus, CHB is an unmet medical need which requires a continuous effort to develop new individual molecules, combinations therapies and completely novel therapeutic strategies to achieve the goal of HBV elimination.[9] The search for these compounds is a highly dynamic field that has grown considerably in recent years owing to the close collaboration between academic research and industry. This has led to renewed interest in the development of novel direct-acting antivirals (DAAs) and host-targeting agents (HTAs) for HBV infection. Thus, the aim of this review is to analyse the scientific rationale for potential treatments and more importantly, to describe the most recent clinical developments in the field to understand future therapies against HBV.

Key points

- HBV infection is a major public health burden with more than 250 million individuals with chronic infection worldwide.
- The clinical management of patients with HBV infection is difficult and costly, as it involves close monitoring for long periods of time.
- Improving the treatment of patients with HBV infection will require the development of new direct-acting antivirals and host-targeting agents.
- The evaluation of novel drug combinations will also be essential to achieve the goal of HBV elimination.
- Further efforts should be made to improve HBV animal models and continue the development of preclinical stage treatments.

2 | NOVEL DIRECT-ACTING ANTIVIRALS AGAINST HBV INFECTION

Based on the particularities of the HBV viral cycle, DAA-based therapeutic strategies can be classified according to the process they target. These include: 1) drugs targeting the HBV replicative cycle, in particular, inhibitors of entry, capsid assembly/disassembly, HBsAg secretion and reverse transcriptase; and 2) drugs targeting HBV gene expression, which are compounds designed to decrease the levels of viral transcripts and antigens. Both classes of drugs indirectly target the intracellular pool of cccDNA. Targeting viral expression can decrease HBsAg levels and therefore help restore antiviral immune responses. Strategies directly targeting viral cccDNA for degradation or silencing should be a priority. This review will discuss selected examples of these compounds under clinical investigation (Table 1).

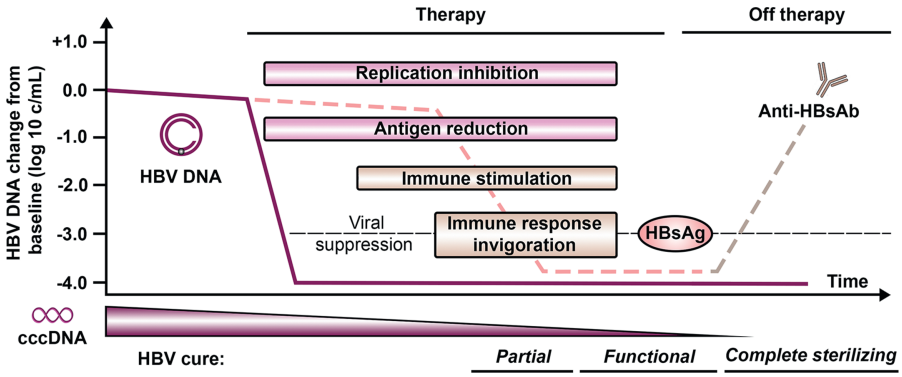

FIGURE 1 New antiviral strategies aimed to achieve HBV cure. The goal of anti-HBV therapy is to achieve a functional cure, defined as sustained off-treatment loss of HBsAg, undetectable HBV DNA, normalization of liver enzymes and improvement in liver histology. Current antiviral regimens require indefinite treatment and do not usually achieve virus eradication. Future therapies directed against the virus (inhibition of replication and antigen production) or the host (immune response stimulation and reinvigoration) and their combination may improve upon current treatments and increase the rate of patients achieving a sustained response or even allow HBV elimination. Abbreviations: cccDNA, covalently closed circular DNA; HBsAg, hepatitis B surface antigen; HBsAb, HBs antibodies; HBV, hepatitis B virus

TABLE 1 HBV antiviral compounds currently under clinical evaluation

Compound	Mechanism of action	Clinical stage	Reference/clinical trial
Entry inhibitors			
Myrcludex B (bulevirtide)	Blocks NTCP	II	[11]
CRV431	Blocks NTCP and protein folding	I	NCT03596697
Capsid assembly modulators			
ABI-H0731 (Vebicorvir)	Core binding	II	NCT04454567 [14]
JNJ-6379	Core binding	II	NCT03361956
GLS4	Core binding	II	NCT04147208
RO7049389	Core binding	II	NCT04225715
HBsAg secretion inhibitors			
REP 2139 and REP 2165	HBsAg binding	II	NCT02565719 [17]
Nucleos(t)ide analogues			
HS-10234	Polymerase inhibitor	III	NCT03903796
Viral expression inhibitors			
JNJ-3989 (ARO-HBV)	siRNA targeting HBV transcripts	II II I/II	NCT04439539 NCT04535544 NCT03365947 [20]
VIR-2218	siRNA targeting HBV transcripts	II II	NCT04507269 NCT04412863 [21]
GSK3228836 (ISIS 505358)	ASO targeting HBV transcripts	IIa	NCT04449029 [22]
RO7062931	ASO targeting HBV transcripts	I	NCT03038113 [23]
RG6346 (DCR-HBVS)	siRNA targeting HBV transcripts	I	NCT03772249
Innate immunity activators			
GS-9688 (Selgantolimod)	TLR8 agonist	II II	NCT03615066 NCT03491553 [30]
Adaptive immunity activators			
ASC22 (Envafolimab)	Anti-PD-L1 antibody	II	NCT04465890
HepTcell (FP-02.2)	Therapeutic vaccine	I, cleared for phase II	NCT02496897
TG-1050/T101	Therapeutic vaccine	II	NCT04189276 [35]
GS-4774	Therapeutic vaccine	II	[36]

Abbreviations: ASO, antisense oligonucleotide; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NTCP, sodium taurocholate cotransporting polypeptide; PD-L1, programmed cell death 1 ligand 1; siRNA, small-interfering RNA; TLR8, Toll-like receptor 8.

2.1 | Targeting the HBV replicative cycle

2.1.1 | Entry inhibitors

Because de novo infection is a central factor in the maintenance of the cccDNA pool and thus the persistence of HBV infection, targeting viral entry would be a sensible approach to prevent progression of the viral cycle.[10] Moreover, hepatitis delta virus (HDV) uses the HBV envelope and thus also uses sodium taurocholate cotransporting polypeptide (NTCP) as an entry receptor. Therefore, this approach could also help manage HBV/HDV co-infected patients. Myrcludex B (bulevirtide), a peptide containing

47 amino acids of the pre S1 domain of the HBV large surface protein, was developed to compete with HBsAg for binding to NTCP and thus inhibit virion uptake in the cell. Myrcludex B was recently evaluated for HBV/HDV co-infection, showing that the combination of Myrcludex B + Peg-IFN-α was associated with both a decline in HDV RNA titres as well as HBsAg decline/loss, which is also relevant for HBV mono-infections.[11]

CRV431 is a cyclophilin inhibitor that has been shown to prevent HBV entry in vitro by targeting NTCP.[12] The effect of CRV431 in vivo was then explored in a study using HBV transgenic mice, reporting significantly reduced hepatic HBV DNA levels and an additive inhibitory effect in combination with the prodrug tenofovir exalidex.[13] CRV431 is being evaluated in a phase I clinical trial (NCT03596697).

2.1.2 | Capsid assembly modulators

Similar to entry inhibitors, capsid assembly modulators (CAMs) could be a viable strategy to reduce HBV viral load. Depending on their chemical structure, this type of drug can induce either the production of misassembled non-capsid core polymers or morphologically normal capsids that lack HBV nucleic acid. The rationale behind their use is based on the action of these compounds on several steps of the viral cycle. Indeed, besides their capacity to alter the correct formation of new nucleocapsids (and, thus, infectious virions), they have been shown to block transport of HBV nucleocapsids to the nucleus, their disassembly and the release of viral particles, thus reducing cccDNA formation in newly infected cells. Moreover, since the core protein has been proposed as a transcriptional regulator of cccDNA, these drugs could affect HBV RNAs expression. ABI-H0731 (Vebicorvir), one of these compounds, is currently being evaluated in a phase II clinical study to assess its antiviral activity in combination with NUCs (NCT04454567). Preliminary results have confirmed the favourable safety profile of ABI-H0731. Moreover, after 24 weeks of treatment, a higher proportion of hepatitis B e antigen (HBeAg)-negative patients receiving ABI-H0731/NUC achieved undetectable HBV DNA levels compared to the placebo/NUC group.[14] The results of at least three other CAMs, JNJ-6379, GLS4 and RO7049389, have been favourable in phase I trials and are being evaluated in phase II studies (NCT03361956, NCT0414720 and, NCT04225715).[15,16]

2.1.3 | HBsAg secretion inhibitors

The most recent members of this family of drugs are nucleic acid polymers (NAPs), a class of broad-spectrum viral attachment or entry inhibitors that also prevent the release of HBsAg from HBV-infected hepatocytes. Because the immune exhaustion caused by a high viral antigen load is a key process in the progression towards CHB, this type of antiviral compound could decrease circulating levels of HBsAg and thus potentially favour clearance of the virus by the immune system. Indeed, recently published results from a phase II trial (NCT02565719) have shown that addition of the NAPs REP 2139 and REP 2165 to a regimen including tenofovir disoproxil fumarate (TDF) and Peg-IFN-α resulted in significantly increased rates of HBsAg loss and HBsAg seroconversion during therapy (60%) and a functional cure after therapy (35%).[17]

2.1.4 | Nucleos(t)ide reverse transcriptase inhibitors

Although new-generation NUCs do not eliminate HBV, they are highly efficient in suppressing viral DNA synthesis and are therefore the current backbone of treatment for CHB. There are several compounds being developed to improve available NUCs. One example is HS-10234, a 5' deoxyadenosine triphosphate analogue that is being evaluated in a phase III clinical trial to compare its efficacy and safety against TDF for CHB (NCT03903796).

2.2 | Targeting HBV gene expression

As previously mentioned, high antigen load is thought to play a role in maintaining chronic HBV, so preventing HBsAg production by both cccDNA and integrated DNA is of interest. Moreover, targeting viral expression is not limited to HBsAg because the characteristics of the HBV genome allow selection of target sequences in overlapping coding regions and thus simultaneous degradation or translation inhibition of multiple transcripts can be achieved. Most of the HBV antiviral strategies under clinical evaluation are small-interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs). At the molecular level, ASOs are distinct from siRNAs as they are not incorporated into the RNA-induced silencing complex (RISC) to silence its target, but they induce RNase H-mediated RNA cleavage by binding to target RNA.[18] Some of the molecules under evaluation include the siRNAs JNJ-3989, VIR-2218 and RG6346, and the ASOs GSK3228836 and RO7062931.

siRNAs were first tested a few years ago in CHB patients. Results showed a stronger decline in HBsAg levels in NUC-suppressed HBeAg-positive than in HBeAg-negative patients. Additional studies in chimpanzees showed that in the HBeAg-negative chronic infection phase, HBsAg may be mainly expressed from integrated viral sequences instead of cccDNA, and that integration may delete the target sequence of siRNA in the 3' end of viral transcripts.[19] Thus, siRNAs were re-designed to target the 3' end of all transcripts upstream from the integration site to be re-evaluated in clinical trials while improvements were made in delivery modes. Preliminary results of the new generation of siRNAs showed that JNJ-3989 (ARO-HBV) is well tolerated in CHB patients and induces a significant HBsAg reduction in most cases. A subset of patients also had sustained suppression of HBsAg for up to 9 months after the last treatment dose.[20] JNJ-3989 is now in phase II evaluation (NCT03365947). VIR-2218, a siRNA targeting HBV transcripts, is being evaluated in phase II studies as

monotherapy (NCT04507269) or in combination with Peg-IFN-α (NCT04412863). Preliminary results have shown VIR-2218 to be well tolerated in patients with CHB and that this agent induces marked reductions in HBsAg in both HBeAg-positive and -negative patients.[21] A third siRNA of interest is RG6346 (DCR-HBVS), which is currently in phase I clinical trials (NCT03772249).

GSK3228836 (ISIS 505358) is an ASO targeting all HBV RNAs which is being evaluated in a phase II trial (NCT04449029). Recent results from this clinical study have shown that after 4 weeks of treatment with GSK3228836 there was a significant reduction in HBsAg levels associated with alanine aminotransferase (ALT) elevation in patients. This was observed in both NUC-treated and -naïve patients. Significant reductions in HBV DNA were also reported in treatment-naïve patients.[22] In addition, preliminary results are available from a phase I clinical trial evaluating the ASO RO7062931, a locked nucleic acid targeting HBV transcripts (NCT03038113). This report showed that the compound is well tolerated with potential antiviral activity, suggested by a decrease in HBsAg levels following 4 weeks of treatment.[23]

Finally, it is worth mentioning a third category of small molecules targeting HBV antigen production via RNA destabilization (eg AB-452 and RG7834). Although these compounds are not in clinical development, drugs such as RG7834 have been shown to reduce HBsAg levels and HBV viraemia in animal models.[24]

3 | NOVEL HOST-TARGETING AGENTS AGAINST HBV INFECTION

The development of antiviral agents has mainly focused on compounds targeting viral components. The rationale behind this is that these compounds would be less likely to cross react with human molecules and thus induce less toxicity. However, because the control of HBV infection is mainly immune-mediated,[25] approaches to boost innate and/or adaptive immunity are also an area of research. Moreover, this approach is based on 1) the observation that no matter how virus specific the design of DAAs might seem, off-target and side effects may occur, 2) the fact that drug resistance often appears after extended use of DAAs and 3) the limit that the small HBV genome imposes on drug design. Therefore, HTAs appear to be an option to overcome these issues and several of them are currently under clinical evaluation (Table 1).

3.1 | Stimulating the innate immune response

Although there is still a debate about whether HBV escapes or actively suppresses the innate immune system, it is clear that it is a weak inducer of these antiviral responses. However, HBV replication can be suppressed by reactivating innate signalling pathways in hepatocytes, such as during co-infection with HDV, in which a reduction in HBV is observed. These antiviral responses are not limited to HBV-infected hepatocytes, as cytokines produced in non-parenchymal cells (eg IFN-γ, IL-1β) also play a role in controlling infection. Indeed, this is the rationale for IFN-α because it not only presents direct antiviral action but also boosts natural killer (NK) and T-cell responses. Therefore, direct activation of innate immunity in hepatocytes via retinoic acid-inducible gene I (RIG-I) or in neighbouring cells via Toll-like receptor (TLR) signalling has been explored as possible immunostimulatory therapy against CHB.

For example, Inarigivir, a RIG-I agonist was reported to inhibit HBV replication via induction of IFN-α in hepatocytes, however, results were not confirmed in the clinical evaluation. Despite an initial assessment concluding that Inarigivir was well tolerated following 12 weeks of administration, a second longer clinical trial reported severe toxicity in several patients and the development of organ failure and death in one.[26] Similarly, results with the TLR7 agonist GS-9620 were highly promising in the woodchuck hepatitis virus (WHV) and chimpanzee models,[27] however, the clinical evaluation was disappointing, with no significant decreases in HBsAg despite target engagement, demonstrated by increased ISG15 expression.[28]

More recent results with GS-9688 (Selgantolimod), a TLR8 agonist that favours production of IL-12, IFN-γ and stimulation of T-cell function, have been encouraging in the WHV model.[29] GS-9688 is under evaluation in a phase II trial to determine its safety, tolerability and antiviral activity in untreated patients (NCT03615066). Preliminary results have shown that GS-9688 is well tolerated after 24 weeks of treatment, with HBsAg loss and HBsAg decline more apparent in the GS-9688-treated group.[30]

3.2 | Stimulating the adaptive immune response

An interesting observation has shown that patients with CHB who received a bone marrow transplant from donors with resolved HBV infection may become HBsAg negative. This highlights the efficacy of HBV-specific immunity via the action of memory B and T cells. A similar situation is observed in patients with controlled HBV infection, showing coordinated activation of humoral and cellular immunity against HBV. However, this is not observed in most patients, as T cells progressively become dysfunctional and lose their proliferative and cytotoxic activity owing to continued exposure to HBV antigens (*T-cell exhaustion*).[31] Thus, activating HBV-specific responses could be another option in antiviral regimens, which could be achieved with checkpoint inhibitors or therapeutic vaccines.

Checkpoint inhibitors reinvigorate pre-existing antiviral immunity by preventing the action of signalling pathways that limit the duration and amplitude of immune responses. This type of negative regulatory mechanism is induced to reduce tissue damage. One strategy, for example, is to prevent the inhibitory signals generated from the interaction between programmed cell death 1 ligand 1 (PD-L1) and its receptor programmed cell death 1 (PD1). This PD-L1/PD1 interaction regulates the activity of T cells in peripheral tissues, playing a key role during inflammatory responses directed to control infection.[32] ASC22

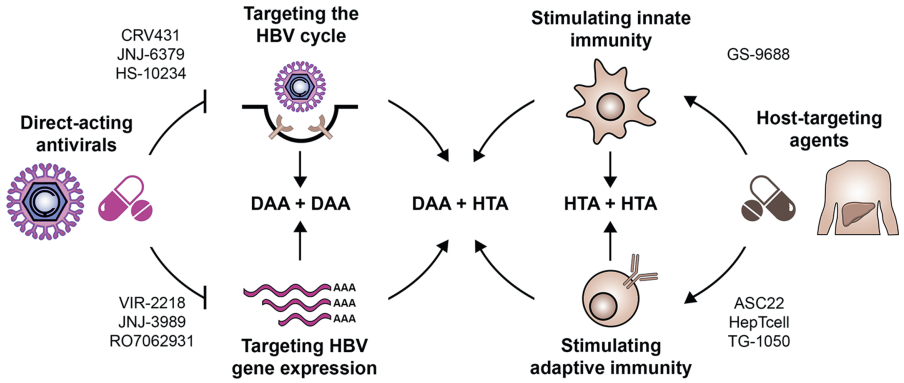

FIGURE 2 Combination of drugs with different mechanisms of action as a strategy against HBV infection. DAAs are divided into 1) drugs targeting the HBV replicative cycle and 2) drugs targeting HBV gene expression. HTAs are divided into 1) drugs that stimulate the innate response and 2) drugs that stimulate the adaptive immune response. Selected examples from table 1 are displayed alongside each category. The development of new compounds allows the potential combination of DAAs, HTAs or both, as a means of increasing the likelihood of HBV elimination. Abbreviations: HBV, hepatitis B virus; DAA, direct-acting antiviral; HTA, host-targeting agent

(Envafolimab) is an anti-PD-L1 antibody currently in phase II clinical trial to evaluate its safety and efficacy in CHB patients (NCT04465890).

Unlike checkpoint inhibitors, therapeutic vaccines boost immunity by priming new antiviral responses. This type of intervention mainly relies on the induction of effective CD4 and CD8 T-cell immunity and to a lesser extent on B cells and antibody responses. It is interesting to note that intrahepatic presentation of HBV antigens to T cells has been reported to result in their inappropriate activation.[33] Thus, it is preferable for these antigens to be present in other organs such as lymph nodes to undergo processing by professional antigen-presenting cells (eg dendritic cells). In this context, HepTcell (FP-02.2), a peptide-based immunotherapeutic, has recently completed a phase I trial and been cleared to initiate phase II evaluation (NCT02496897). TG-1050 is a replication-defective adenovirus serotype 5 expressing multiple HBV-specific antigens which has been shown to induce a significant reduction in circulating viral parameters in a mouse model. A phase I trial has confirmed a good safety profile with this agent which has been shown to induce HBV-specific cellular immune responses.[34,35] T101, a therapeutic vaccine based on the TG-1050 technology, is currently undergoing evaluation in a phase II trial (NCT04189276).

Finally, GS-4774, a therapeutic T-cell vaccine containing epitopes derived from HBs, x and core protein, has been shown to induce IFN-γ and IL-2 by CD8 T cells in TDF-treated patients. Although the use of GS-4774 was not significantly associated with a decrease in HBsAg levels, its strong immune stimulatory effect could be useful in combination with other antiviral agents or immune modulators to boost the immune response against HBV.[36]

4 | OPENING THE POSSIBILITY FOR NEW DRUG COMBINATIONS

Based on the knowledge of the mechanisms of HBV persistence, it is now clear that elimination of HBV will probably require combination therapies. Moreover, these therapies will probably include the combined effect of DAAs with HTAs. These approaches must obtain complete suppression of virus production, de novo infection and circulating HBsAg levels, while boosting the immune system to increase and maintain HBV-specific adaptive responses. This has been previously evaluated by a combination of NUCs and IFN, with practically no improvement compared to monotherapy. However, the new therapeutic agents under development such as those mentioned, represent interesting options to explore the efficacy of novel combination strategies.

The evaluation of these drug combinations will need to have a solid scientific basis with careful monitoring of potential drug-drug interactions and to be initially performed in patients without advanced liver disease.[6] We will now discuss some of these potential combinations (Figure 2).

4.1 | DAA combinations with or without inhibition of HBV expression

The goal of combining multiple DAAs is to target the HBV replicative cycle to reduce the pool of cccDNA by inducing more potent inhibition of viral genome replication and decreasing the rate of intracellular cccDNA recycling and/or of new rounds of hepatocyte infection. The combination of DAAs with different mechanisms of action would also prevent the development of drug resistance. Combining an entry inhibitor with a NUC could also be an option to reduce the cccDNA pool maintained by de novo infection. Similarly, the combination of NUCs with a potent CAM could provide stronger suppression of viral replication, leading to a decrease in intracellular recycling of cccDNA and its impaired formation in de novo-infected hepatocytes, thus reducing the pool of intrahepatic cccDNA. Whether this type of approach can achieve a functional cure or high rate of virological control after cessation of therapy needs to be evaluated in clinical trials.[37]

The idea of combining drugs that target the HBV replicative cycle with compounds targeting HBV expression is based on the hypothesis that reducing not only viral replication but also expression

of viral proteins and antigens to much lower levels than those obtained with NUC monotherapy could increase HBV-specific immune reconstitution in patients with CHB. Clinical trials are underway to evaluate this approach using siRNA JNJ-3989 in combination with the CAM JNJ-6379 and NUCs for the treatment of CHB patients (NCT04439539),[38] or in combination with NUCs in HBV/HDV co-infected individuals (NCT04535544).

4.2 | DAA combinations with immunotherapy

It has been suggested that combination strategies including NUCs and new immunological therapies could be promising for the management of CHB patients. This based on the observation that NUC treatment not only reduces the production of new virions but also because it resolves hepatic inflammation, thus increasing the accessibility and functionality of HBV-specific immune cells. This approach will be evaluated with HepTcell as an add-on therapy to entecavir or tenofovir in CHB patients (NCT02496897). Similarly, GS-9688 will be evaluated in patients receiving a variety of NUCs (NCT03491553).

Because of the limited efficacy of therapeutic vaccines up to now, it is thought that they may need to be given in combination with other immune therapies. This approach has been explored in preclinical models (ie WHV-infected woodchucks) which have shown that a combination of therapeutic vaccines and checkpoint inhibitors might have a beneficial effect against HBV infection.[39] Although there are no clinical investigations of this option as yet,[40] a combination of immunotherapeutic agents against CHB is an important field that warrants further investigation.

Finally, the combination of siRNAs with a therapeutic vaccine was recently evaluated in an animal model, which showed that reducing the HBV antigen load is highly relevant to overcome immune tolerance and achieve a cure for HBV in mice.[41]

5 | CONCLUSION AND PERSPECTIVES

With only 10 years until the 2030 deadline for the elimination of viral hepatitis,[9] we can see how much progress has been made in the development of new therapeutic agents against CHB. However, there are several challenges that must be addressed if this goal is to be met. In particular, the scientific community will need to focus on the development of better animal models for the study of HBV infection and antiviral drug discovery.[42] These models could help overcome the challenges of HBV cccDNA targeting, evaluating immune stimulation and preclinical testing of drug combinations.

Although this review has focused on drugs under clinical evaluation, we must highlight the investigations that are at early stages of development. Examples of promising strategies in this category are chimeric antigen receptor (CAR) T cells, HBV T-cell receptor (TCR)-designed CD8 T cells and soluble TCRs, to redirect HBV-specific T cells to infected hepatocytes and gene editing approaches to directly target cccDNA by clustered regularly interspaced short palindromic repeat- (CRISPR)/Cas9-based approaches.[43,44]

In summary, HBV eradication will require a thorough understanding of HBV biology, the specificities of the liver microenvironment and their interactions with the immune system. The design of future therapeutic approaches against HBV will need to take these factors into account, as they will probably pave the way for the next generation of antiviral agents and their combinations.

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CONFLICT OF INTEREST

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SUPPLEMENT ARTICLE

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Hepatitis B cure: How and when

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Abstract

Background: First- and second- generation new treatments are being evaluated to provide a cure for hepatitis B. The life cycle of HBV includes several well- categorized steps that are targets for new treatments. A cure remains a major challenge even if it is measured by HBsAg seroclearance alone. The notion of a functional cure of hepatitis B has been accepted, while a partial functional cure has been more tentatively defined as a decline in HBsAg concentrations to lower levels after finite treatment.

Methods: More profound suppression of hepatitis B replication through the addition of capsid inhibitors with nucleoside analogues could improve patient prognosis and a sustained treatment response. Several strategies are being evaluated to achieve a cure: (a) deepening inhibition of HBV replication or (b) a reduction in HBsAg presentation for HBsAg seroclearance.

Results: Fortunately, there are signs of important progress in the treatment of hepatitis B including improved on- treatment reductions or seroclearance of HBsAg in phase 2 studies that was not achieved with chain terminators and inhibitors of initiation of DNA synthesis. Progress in immunomodulatory therapy has lagged behind that of antiviral therapy.

Conclusions: Increasing the multilayered impaired and dysfunctional immune response in hepatitis B is perhaps more likely and feasible after a reduction in host antigen burden. Other potential experimental strategies include CRISPR- Cas9 genome- editing nucleases to specifically target and cleave cccDNA or novel monoclonal antibodies.

Key points

- The life cycle of HBV involves several well-categorized steps that are targets for new investigational treatments.
- A cure remains a major challenge, even if it is solely measured by HBsAg seroclearance.
- Several strategies are being evaluated to achieve cure:
  a. Deepening inhibition of HBV replication or
  b. A reduction in HBsAg presentation for HBsAg seroclearance.
- The right combinations and sequential strategies will require careful empirical research.

1 | INTRODUCTION

First- and second-generation new investigational treatments are being evaluated to provide a cure for hepatitis B. The life cycle of HBV includes several well-categorized steps that are targets for new treatments, including viral entry, viral uncoating, nuclear HBV DNA importation, cccDNA transcription, nucleocapsid assembly, HBV RNA reverse transcription and viral assembly and secretion from host hepatocytes. Detailed reports of these innovative, potentially curative treatments in phases 1 and 2 clinical trials will be presented

at the 2021 Paris Hepatology Congress and are published elsewhere in this issue. This brief commentary provides an overview of the current status of curative therapies. A cure for hepatitis B for most treated patients will be difficult and confirmed data are limited.

A cure is still a major challenge, even if it is measured by HBsAg seroclearance alone. Indeed, the random integration of the HBV genome and continued production of HBsAg is a problem.

HBsAg in serum is derived from a large excess of subviral particles, as well as from mature infectious virions containing the outer glycosylated envelope. Partially double-stranded relaxed circular DNA (rcDNA) is transported to the hepatocyte nucleus during replication and converted into a covalently closed circular minichromosome (cccDNA). The stable episomal cccDNA minichromosome is the transcriptional template for HBV mRNAs, and pregnomic RNA (pgRNA) to initiate viral replication. cccDNA is thought to be synthesised from rcDNA derived from incoming virions but replenished from intracellular nucleocapsids via an intracellular cccDNA shuttle amplification pathway, and is therefore maintained as a stable minichromosome in the nucleus of hepatocytes.[1] The conversion of rcDNA into cccDNA requires disassembly of the HBV capsid. HBV replication occurs within viral cores (capsids). cccDNA is not rapidly degraded by current nucleoside analogue therapy, as only minus-strand and plus-strand DNA synthesis is targeted.

Transcription of HBsAg occurs from integrated viral genomes in both HBeAg-positive and -negative patients.[2] Functional HBV genomic integrations drive S-gene transcription, and then high HBsAg protein antigen concentrations may drive antigen-specific immune dysfunction and T- and B-cell exhaustion. There are ongoing studies examining long-reading frames and transcription from HBV integration, which are randomly scattered throughout human chromosomes.[3] HBx encoded by the X gene functions as a regulatory protein. HBx enhances cccDNA transcriptional activation and is an attractive viral target to potentially silence cccDNA.[4,5] The protein is highly conserved.[6]

2 | FUNCTIONAL CURE OF HEPATITIS B

The concept of a functional cure of hepatitis B has been accepted. A functional cure is defined as sustained loss of HBsAg, with or without acquisition of anti-HBs, and undetectable HBV DNA 6 months after completing treatment. Finite treatment rather than continued long-term treatment is implied.[7] It has also been accepted that a finite cure of hepatitis B is not a complete cure or eradication of HBV infection from the host. A complete sterilizing cure is not considered to be possible for most patients at present because this would require eradication of all hepatocytes harbouring both episomal cccDNA and integrated viral genomes from the host. Nevertheless, antiviral treatment may be discontinued in the presence of a finite cure with loss of HBsAg from serum, using a test with a sensitivity of at least 0.05 U/L. Seroclearance of HBsAg improves the prognosis of hepatitis B, but to guarantee improved outcome and survival, HBsAg loss should ideally occur relatively early in the course of the disease, at a comparatively young age, and before the onset of advanced fibrosis to minimize the risk of subsequent hepatic failure and hepatocellular carcinoma (HCC). It may be possible to define molecular characteristics accompanying HBsAg seroclearance to predict a benign outcome. In our current state of knowledge, the advantages of HBsAg loss can be inferred from either spontaneous loss of HBsAg or treatment-induced HBsAg seroclearance.[8] Maintained DNA suppression reduces the risk of HCC. However, HBsAg loss versus suppression of HBV DNA further reduces the risk, despite the probable persistence of integrated viral genomes.[9]

A partial functional cure has been tentatively defined as a decline in HBsAg concentrations to lower levels after finite treatment. These patients remain HBsAg positive with low concentrations of serum HBV DNA and normal serum aminotransferases. This low replicative state is recognized in chronic hepatitis B. HBsAg-positive individuals with low replication and without evidence of hepatic inflammation or fibrosis are not normally treated with anti-viral agents. The outcome is considered to be relatively favourable. However, it is not clear whether regulatory authorities will accept a partial functional cure as an endpoint because of the risk of reactivation and the necessity of continued long-term monitoring before confirming the benefits this type of cure. There is also a question of whether more profound suppression of hepatitis B replication—which could be achieved with a combination of capsid inhibitors and nucleoside analogues—would improve the prognosis and a sustained treatment response. Further trials are needed to respond to this questions.

3 | NUCLEOSIDE ANALOGUE OR PEGYLATED INTERFERON THERAPY

Anti-viral nucleoside analogues can change the natural history of hepatitis B (and reduce the risk of cirrhosis, hepatic decompensation and hepatocellular carcinoma) but are given as maintenance suppressive therapies. Nucleoside analogues act as chain terminators to block reverse transcription of pgRNA to rcDNA, and minus- and plus-strand synthesis. Spontaneous loss of HBsAg is rare and only occurs in a small fraction of patients, perhaps 1%-2% per year.[10] Thus, HBsAg remains detectable for a substantial period during long-term nucleoside analogue therapy, although there may be a gradual decrease in cccDNA concentrations.[11]

PEG IFN exerts pleotropic antiviral molecular and immunological effects that are still not well understood. PEG IFN decreases cccDNA transcription via epigenetic modification in experimental systems. Interferon alpha and lymphotoxin beta receptor agonists lead to upregulation of APOBEC3A cytidine deaminases in infected cells, to degrade cccDNA.[12]

Treatment with PEG IFN or nucleoside analogues may result in HBsAg loss in approximately 2.5%-10% of patients after 1 year of treatment. A response to interferon alpha requires an immunological primed state that is still poorly defined and identified except for the presence of clearly elevated serum aminotransferases.[13] It is not clear whether HBeAg loss or prolonged HBV DNA suppression

below the level of detection (<20 IU/mL) represents a reduction in hepatocytes harbouring HBV cccDNA minichromosomes or a reduction, inactivation or silencing of cccDNA.

4 | NUCLEOSIDE ANALOGUES AND PEG IFN

Add-on or switch therapies may increase treatment responses to achieve HBsAg seroclearance, but the probability of HBsAg loss remains fairly unpredictable in both HBeAg-positive and -negative patients. Although the addition of or switching to PEG IFN provides a synergy that could still be exploited with new investigational agents,[14] PEG IFN is being phased out as a primary treatment of hepatitis B. The compound is still used for testing proof of principle in the experimental steps towards a cure in clinical trials, as well as an adjunct or additive to other therapies.

5 | CESSATION OF NUCLEOSIDE ANALOGUES AFTER LONG-TERM SUPPRESSION

An increasing number of studies have examined discontinuing treatment after long-term nucleoside analogue maintenance suppressive therapy.[15] There are two aims to this approach: to achieve cessation of treatment but maintain suppression, or to trigger HBsAg loss. Paradoxically, HBsAg loss can be achieved in a proportion of patients following cessation of treatment. This strategy is generally only successful for the discontinuation of nucleoside analogues in anti-HBe-positive patients. The intensity of the immune response has been determined by an elevation of serum aminotransferase, but overt serum ALT flares are only an indirect "proxy" measurement of a poorly understood and complex immune response.[16] The outcome after discontinuation of nucleoside analogue therapy is unpredictable, difficult to control and not yet sufficiently well-defined to accurately predict the results and safety of cessation, or to predict sustained HBsAg loss. Guidelines and criteria for cessation have been set by APASL, EASL and AASLD,[17] and cessation after HBsAg loss is only specified in the latter. Severe exacerbations are detrimental to the liver, and thus nucleoside analogue therapy should not be discontinued in patients with advanced fibrosis or cirrhosis.

Recent studies of nucleotide analogue cessation have varied in design and ethnic composition. The baseline criteria in the studies are heterogeneous and investigators have a not included patients with cirrhosis. The timing of the onset of biochemical and virological flares differs between tenofovir and entecavir. Off-treatment 48-week follow-up has shown rates of HBsAg loss varying from 4% to 10%.[18,19] Although lower concentrations of HBsAg favour HBsAg loss, predictors of HBsAg vary from <1000 U/L to <10 U/L. The positive predictive value of HBsAg loss in patients with defined, low concentrations of HBsAg have ranged from 27% to 63%. Conversely, high negative predictive values (98%-100%) have been reported with HBsAg concentrations above these concentrations when therapy is discontinued.

6 | CURE OF HEPATITIS B

There are two major strategies to achieve a cure: (a) deepening inhibition of HBV replication to achieve a cure or (b) a reduction in HBsAg presentation for ultimate HBsAg seroclearance. Fortunately, important progress has been made in the treatment of hepatitis B infection. Indeed, we are beginning to see significantly improved on-treatment reductions or seroclearance of HBsAg in phase 2 studies that is not achieved by chain terminators and inhibitors of initiation of DNA synthesis. Both HBeAg-positive and -negative patients, and naive and nucleoside analogue-suppressed patients are being enrolled in phase 2 clinical trials. Several compounds will be discussed in detail at this symposium.

7 | HBsAg SEROCLEARANCE STRATEGIES

Although HBsAg derived from integrated viral genomes is a relatively inaccessible source of HBsAg, which might prove difficult to reduce, newer compounds interfering with translation or HBsAg assembly could directly reduce HBsAg in serum. Thus, RNA interference and nucleic acid polymers reduce HBsAg concentrations and seem to (although this must be confirmed) reduce HBsAg protein assembly directed to subviral particles as well as complete HBV virions. Hepatocyte cytolysis or apoptosis may be required to obtain sustained declines in HBsAg concentrations or HBsAg seroclearance.

Although no immunomodulatory trial has been found to be effective (other than interferon alpha treatment), an immunomodulatory strategy may be needed for immunological control. The sensitivity of detection of HBsAg will require standardization, as HBsAg can be detected by ultrasensitive assays in a proportion of patients who appear to have cleared HBsAg when defined by a standard assay sensitivity of 0.05 U/L.

Specific, directed strategies to promote HBsAg loss, to specifically decrease HBsAg translation by RNA interference or interference with intracellular chaperoning and assembly of HBsAg are being investigated in current trials. Although these compounds inhibit sub-viral particle production and perhaps virion assembly, their effect on HBV replication and cccDNA remains less well characterized. A reduction in HBsAg presentation may result in enhancement and recovery of dysfunctional T- and B-cell responses. An effect of RNA interfering agents upon natural killer or other immunoreactive cells or toll-like receptor agonism has not been excluded. A rapid 2-4 $\log_{10}$ reduction in HBsAg may also enhance the efficacy of immunomodulatory therapies.

RNA interference can be achieved by antisense oligonucleotides, locked nucleic acids or small interfering RNAs (siRNAs). Several RNA interfering compounds are under development and result in a decrease in HBsAg of up to 4 $\log_{10}$ during treatment. siRNAs target HBV

transcripts and cause their destruction by the RISC/Ago2 complex. RNA interference is being studied in combination with capsid assembly modulators in both naïve and nucleoside analogue-treated patients. Forty-eight weeks of JNJ 6379 (a capsid assembly modulator) plus JNJ 3989 (a siRNA) plus a nucleoside analogue is being evaluated in the REEF series. Some heterogeneity in the decline in HBsAg has been reported in phase 1 studies. The outcome of ongoing phase 2 studies will indicate the percentage of individuals who achieve log declines in HBsAg on treatment, and sustained off-treatment HBsAg seroclearance, or conversely, the proportion of patients who maintain lower post-treatment levels of HBsAg and the clinical significance of these reductions. To date, a ≥1.0 log10 reduction in HBsAg at nadir was achieved in 98% of nucleoside analogue-experienced or naïve and HBeAg-positive or -negative patients receiving three subcutaneous JNJ-3989 doses (days 1, 27 and 57) of 100, 200, 300 or 400 mg. A subset of patients had sustained suppression of HBsAg approximately 9 months after the last RNAi dose (mean 1.74). Sustained suppression of other viral parameters was also seen. [20] Although some decreases in pgRNA and HBcrAg were reported, the mechanism and significance of these results require further studies.

GSK3228836 (previously called ISIS 50535A), a second-generation ASO, was combined with nucleoside analogue treatment in a phase 2 study.[21] ASO (300 mg) or placebo was administered subcutaneously on Days 1, 4, 8, 11, 15 and 22. The primary assessment of the effect on HBV was on Day 29. Dosing over 28 days in nucleoside analogue-naive and suppressed and HBeAg-positive and -negative patients was completed.

The mean HBsAg $\log_{10}$ IU/mL change from baseline was −2.514 in patients receiving nucleoside analogues. The most common adverse events for the 300 mg of ASO were erythema, pain, pruritus, swelling and/or bruising at injection sites.

Interestingly, HBsAg reductions were frequently followed by serum aminotransferase flares. Longer phase II studies are ongoing. Post-ASO ALT flares with peaks ranging from 1.7 to 15 x ULN occurred in the HBsAg < LLoQ patients. ALT flares were asymptomatic and self-resolved.

Other routes to decrease particulate HBsAg are also being evaluated. Nucleic acid polymers (NAPs) and STOPS (S antigen traffic inhibiting oligonucleotide polymers) are a class of amphipathic phosphorothioate oligonucleotides.[22] NAPS and STOPs may selectively inhibit HBsAg particle assembly and secretion of subviral particle HBsAg. The efficacy of NAPs (REP-2139) has been tested in combination with tenofovir and PEG IFN, and the mechanisms of action were recently elucidated. The host target for the NAP (and the entire class?) was identified. The endoplasmic reticulum Golgi intermediate compartment-endoplasmic reticulum (ER/ERGIC) resident HSP40 chaperone DNAJB12—a member of a diverse family of HSP40 chaperones—guides the assembly of subviral particles. Serum ALT flares usually occur and anti-HBs develops.[23,24] Further evidence of the safety and efficacy of this approach is being studied in a larger population.

ALG-010133 is a HBsAg transport inhibiting oligonucleotide polymer (STOP) (or NAP) currently under development. The compound has been evaluated in HBV cell models[25] and the efficacy and safety of ALG-010133 is being assessed in phase 2 trials.

Small molecule substrates of sodium taurocholate cotransporting peptide and NTCP inhibitors, for example, myrcludex B (bulevertide), block the entry of HBV (and, hence, HDV) and there is a decline in HBsAg. Inhibition of HBV entry reduces the spreading cycle. Reductions in HBsAg have been observed in a certain proportion of patients. However, long-term daily subcutaneous maintenance therapy is required and the effect on integrated viral genomes is uncertain.[26]

8 | INHIBITORS OF HBV REPLICATION

Deepening inhibition or obtaining a shutdown of HBV replication could be achieved with a combination of nucleoside analogues and capsid inhibitors. Two major classes of capsid inhibitors have been developed: Class 1 (or class A) causes aberrant core protein aggregates, and class 2 (class N) (most compounds in trials) results in "normal" but empty capsids. Interference with capsid assembly and inhibition of pgRNA encapsidation (the so-called primary mechanism) is the most important instrument of inhibition. An additive check on HBV replication has been shown on ultra-sensitive HBV DNA tests[27] by the turnover of cccDNA and by the monitoring of emergence of signature mutations.[28] A reduction in pgRNA encapsidation is shown by a reduction in particles containing HBV RNA. A secondary mechanism that prevents capsid disassembly is possible—inhibiting de novo formation and replenishment of cccDNA in vitro.[29]

Twenty-eight-day dosing with capsid inhibitors was shown to have a clear effect on log reductions of HBV DNA but no effect on HBsAg, for example, with NVR3-778, JNJ 6379, RO7049389 or ABI-HO731 (vebicorvir).[30] There is no clear effect of capsid inhibitors on HBsAg—in particular derived from integrated viral genomes.

More prolonged administration of up to 48 weeks is being evaluated in phase II studies. The combination of a nucleoside analogue and a capsid inhibitor, for example, JNJ-56136379 in the JADE study (CAM-N, ie a normal capsid structure agent) results in a relatively limited decline (0.5_{10} log) of HBsAg concentrations in HBeAg-positive nucleoside-naïve patients after 24 weeks of treatment.[31]

A lower reduction in HBsAg has been observed in HBeAg-positive nucleoside analogue suppressed, HBeAg-negative naive or nucleoside analogue suppressed patients. The reductions in PgRNA from baseline are most significant in HBeAg-positive nucleoside analogue naive patients. HBV RNA concentrations are low at baseline in HBeAg-negative cohorts. Somewhat similar findings were observed in recent phase 2 extension studies of ABI-H0731.[32,33] The population-dependent reductions in HBsAg may be mainly because of the reduction in HBsAg from Dane particles. This suggests that the "primary" mechanism of action could predominate. Next-generation core inhibitors, for example, H2158 and H3733 may have more profound effects on cccDNA.

The combination of subcutaneous injections of JNJ-73763989 and orally administered JNJ-56136379, the capsid assembly

modulator, is being studied to combine ribonucleic acid interference with a capsid inhibitor. Results are expected from the REEF studies in 2021 (ClinicalTrials.gov Identifier: NCT03982186).

9 | AUGMENTATION AND RESTORATION OF BOTH T- AND B-CELL HOST IMMUNITY

Progress in immunomodulatory therapy has lagged behind antiviral therapy. Multiple immunomodulatory agents including toll-like receptor agonists, immune check point inhibitors, therapeutic vaccines, immunological engineered cells to enhance T- and B-cell recognition and cytokine stimulation as well as pathogen receptor agonists have begun—with disappointing results to date. Data are limited to in vitro and woodchuck efficacy. Target engagement has been shown using agonists of compounds such as selgantolimod (GS 9688, an oral TLR8 agonist). Immune cell subsets indicate activation, and dose-dependent cytokine responses have been observed, and (in relatively small studies) 5% of patients lost HBsAg after 24 weeks of treatment in virally suppressed patients. [34]

An increase in the multilayered impaired and dysfunctional immune response in hepatitis B is perhaps more likely and feasible after a reduction in host antigen burden. Studies in male C57BL/6 mice that persistently replicate HBV either from a transgene or infection with an adeno-associated virus have provided an important proof of concept. siRNAs were used to knock down HBsAg expression in mouse hepatocytes. Mice were then immunized with adjuvanted HBV S and core antigen, followed by a modified vaccinia virus Ankara vector to induce antigen-specific T- and B-cell responses. siRNA administration reduced levels of HBsAg and vaccination-induced production of neutralizing antibodies as well as increasing the number and functionality of HBV-specific, CD8T cells in mice with low levels of HBsAg, eliminating HBV.[35] These data suggest that increased understanding of how to provoke innate and adaptive immunity and restore T-cell function could provide a scaffold to eliminate HBV in a larger groups of patients.

10 | CONCLUSIONS

There has been progress in achieving a cure and considerable efforts are being made to improve hepatitis B cure rates. New compounds are promising, but finding the effective combinations and sequential treatments will require careful empirical research. Other potential, but still experimental, approaches include CRISPR-Cas9 genome editing nucleases to specifically target and cleave cccDNA. Future programs will examine the epigenetic silencing or genetic editing of cccDNA and X gene transcription to provide a targeted strategy.

New biomarkers including HBV RNA and HBcrAg reflect the transcriptional activity of cccDNA and will be used to understand the check points of HBV replication. Tools to identify a reduction in the pool of infected hepatocytes are still lacking. Biopsy studies to confirm the measurement of intrahepatic cccDNA are technically and ethically difficult. The role of immune modulators after reducing HBsAg antigen concentrations—or even amplification by PEG IFN—requires further study.[36] Numerous unsolved problems remain, including an urgent need to differentiate drug-related liver injury from immunological (and potentially beneficial responses) following serum aminotransferase elevations.

Hepatitis B remains a major public health problem. The disease is common in endemic regions and in low-income countries of sub-Saharan Africa, Asia and the America's. Complex combination treatments are potential cures and will help achieve sustainable goals to overcome the public health threat of viral hepatitis. However, the cost of these cures may result in further healthcare inequalities for this disease.

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SUPPLEMENT ARTICLE

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New therapies for hepatitis delta virus infection

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Abstract

Hepatitis delta virus (HDV) infection is a defective virus requiring hepatitis B virus (HBV) for its complete replication cycle. HDV is a small hepatotropic RNA virus and around 15 to 25 million people worldwide are living with chronic hepatitis delta (CHD) infection. However, the prevalence of HDV may be underestimated, and screening is frequently insufficient. HDV infection remains endemic in several regions including Central and West Africa, the Mediterranean basin, the Middle East, Eastern Europe, Northern Asia, certain areas of Southeast Asia and the Amazon basin of South America. The best preventive strategy to decrease HDV infection is to improve coverage of the prophylactic HBV vaccine. HDV infection may occur by HBV-HDV co-infection or superinfection, and the latter is usually more severe. CHD is associated with a higher risk of cirrhosis and hepatocellular carcinoma (HCC) compared to HBV mono-infection. Pegylated interferon alpha (PEG-IFNα) therapy is limited by moderate effectiveness (around 20%) and its adverse effects. The entry inhibitor, bulevirtide (BLV, Hepcludex®), which was recently approved in Europe at a dose of 2 mg in sub-cutaneous injection per day, is indicated for the treatment of CHD in adult patients with compensated liver disease and positive HDV viremia. BLV can be administrated in monotherapy or in combination with PEG-IFNα. Nucleos(t)ide analogues can be used in combination for underlying HBV infection. The optimal treatment duration has not yet been determined and treatment should be continued if a clinical benefit is observed. There are other promising therapies such as IFN lambda (IFNλ) (immunomodulator), lonafarnib (prenylation inhibitor) and nucleic acid polymers (Inhibitors of HBsAg release). In this review, we will present an update on CHD and future promising treatments.

KEYWORDS

direct-acting antivirals, entry inhibitor, interferons, prenylation inhibitors

Abbreviations: ADAR 1, adenosine deaminase acting on RNA 1; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BLV, bulevirtide; CHB, chronic hepatitis B; ETV, entecavir; HBsAg, Hepatitis B surface antigens; HBV, hepatitis B virus; HBx, viral protein X; HCC, hepatocellular carcinoma; HDV RNP, Hepatitis delta virus ribonucleoprotein; HDV, Hepatitis delta virus; hNTCP, human sodium taurocholate cotransporting polypeptide; HSPGs, heparan sulphate proteoglycans; IFNα, interferon alpha; L-HBsAg, large hepatitis B virus surface antigen; L-HDAg, large hepatitis delta antigen; LNF, lonafarnib; M-HBsAg, medium hepatitis B virus surface antigen; NA, nucleoside analogue; NAPs, nucleic acid polymers; ORF, open reading frames; PEG-IFN, pegylated interferon; S-HBsAg, small hepatitis B virus surface antigen; S-HDAg, small hepatitis delta antigen; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TFV, tenofovir.

1 | INTRODUCTION

Hepatitis delta virus (HDV) is a small enveloped RNA virus first identified by Pr. Rizzetto in 1977.[1] HDV infection can induce severe chronic hepatitis leading to cirrhosis and hepatocellular carcinoma (HCC). The HDV replication cycle requires co-infection with the hepatitis B virus (HBV) since HDV requires hepatitis B surface antigen (HBsAg) and uses it as its own envelope protein to became infectious..[2] Patients with HBV infection should be systematically screened for HDV infection because of the high risk of co-infection.

A prophylactic hepatitis B vaccine is available and has been on the list of compulsory vaccines in France since 2018.[3] This vaccine protects against HBV infection as well as HDV infection. However, in some countries vaccination campaigns are not effective and new infections still occur. Significant advances have been made in the treatment of HDV with promising new therapies. This review presents the most recent aspects of chronic hepatitis delta (CHD) as well as the most recently approved therapy and drugs under development for CHD.

Key points

- Around 15 to 25 million individuals are living with chronic hepatitis delta (CHD) infection worldwide with a high prevalence in several countries.
- Hepatitis delta virus (HDV) requires the presence of Hepatitis B virus (HBV) for HDV virion assembly.
- The best preventive strategy is to improve implementation of the prophylactic hepatitis B vaccine.
- Patients with CHD infection are at a high risk of developing decompensated cirrhosis and hepatocellular carcinoma (HCC).
- Nucleos(t)ide analogues approved for HBV chronic infection have no efficacy on HDV.
- The entry inhibitor, bulevirtide (Hepcludex®), has been approved in Europe. Prenylation inhibitors, lambda interferon and nucleic acid polymers are under development.

2 | EPIDEMIOLOGY

Around 15 to 25 million individuals are living with CHD. Patients with CHD represent around 5% individuals with chronic hepatitis B (CHB) infection.[4,5] An estimated 257 million people are living with CHB, defined as HBsAg positivity.[6] The number of patients with CHD is probably underestimated because of the lack of systematic screening and the limited availability of diagnostic tests. Chen et al published a systematic review and meta-analysis on the prevalence of HDV infection in the global population.[7] They evaluated 182 studies from 61 countries and regions worldwide and observed that the global prevalence of HDV is about 0.98% (95% CI 0.61 to 1.42) with 14.57% (95% CI 12.93-16.27) in patients with HBV infection. The estimated prevalence in populations without risk factors such as intravenous drug users or HDV sexual risk factors, is 10.58% (95% CI 9.14 to 12.11).[7] Thus, two times higher than previous estimations.

According to Stockdale et al and in collaboration with the World Health Organisation (WHO), HDV infection is endemic in Mongolia, Central and West Africa (Mauritania), Central and North Asia, Vietnam, Pakistan, Taiwan, Japan, China, Middle East (all countries), Eastern Europe (Mediterranean regions and Turkey), South America (Amazon basin and Brazil), Greenland and the Pacific Islands (Nauru and Kiribati).[5,8]

HDV prevalence in France is around 4% of patients with CHB, with detectable antibodies against HDV.[9] These patients are mainly from medium and highly prevalence countries.

3 | VIROLOGY

Member of the *Deltavirus* genus, HDV is considerated as a satelitte virus of HBV. HDV is a small hepatotropic enveloped RNA virus (measuring ≈ 36 nm in diameter) which specifically targets liver hepatocytes (Figure 1).[1,10] HDV genome is a negative single-stranded RNA and contains 60 to 70% of complementary sequence which allows to circularize into circular RNA. HDV exhibits a great genetic variability with eight different genotypes with at least two to four subgenotypes.[11]

HDV RNA (≈1,7 kilo-base) is associated with multiple copies of the two different forms of HDV-encoding hepatitis delta antigen (HDAg), the Small (S-) and de Large (L-HDAg), and all these elements compose the HDV nucleocapsid (Figure 1).[12]

HDV is defined as a defective virus depending on HBV for its full replication cycle. Indeed, HDV does not encode for envelope protein but hijacks HBsAg to compose its lipidic envelope. The main step of HDV replication is summarized in Figure 2. Mechanisms of HDV entry into the hepatocyte are similar to those of HBV's. First, HDV particles are concentrated at the cell surface by heparan sulphate proteoglycans (HSPGs). Then, the pre-S1 domain within the L-HBsAg of HDV infectious particles induces the endocytosis process interacting with high specificity with the human sodium taurocholate cotransporting polypeptide receptor (hNTCP, SLC10A1).[13,14] Viral entry is important for the viral multiplication. Thus, blocking HDV entry is one of the targets for new drugs to prevent HDV and HBV infections and as discussed futher on section 6.1.

After entry into the hepatocyte, the HBV ribonucleo-complex (HDV RNP) is transported into the nucleus and HDV genomic RNA replication occurs by host cellular machinery following the double-rolling circle mechanism.[12,15]

The particularity of the HDV genome is that HDV genomic RNA encodes for the unique S-HDAg. Another HDAg, the L-HDAg is generated from HDV antigenomic RNA (derived from HDV genomic RNA by sequence complementarity) by the cellular adenosine deaminase 1 (ADAR 1) editing which switches the codon stop and extends the unique open reading frame (ORF) by 19 additional amino acids.[16,17]

Formation of new virions requires the assembly of all components of the HDV virion. As mentioned before, HDV does not encode

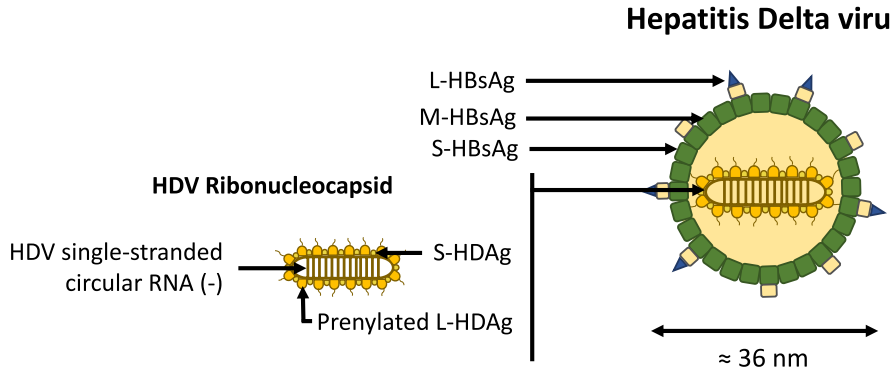

FIGURE 1 Hepatitis delta virus (HDV) viral structure. HDV is a small virus measuring approximately 36 nm in diameter using the three HBV antigens (HBsAg), L-, M and L-HBsAg to form its lipidic viral envelope. This viral envelope contains the HDV ribonucleocapsid composed of the HDV genome (HDV single-stranded circular RNA with negative polarity) and the two different hepatitis delta antigens (HDAg), the S- and L-HDAg. L- and S-HDAg have similar sequences with 19 additional amino acids for L-HDAg. Compared to S-HDAg, L-HDAg is prenylated allowing interaction with HBsAg for viral structure formation. HBV, Hepatitis B virus; HDV, Hepatitis delta virus; L-HBsAg, large hepatitis B virus surface antigen; L-HDAg, large hepatitis delta antigen; M-HBsAg, medium hepatitis B virus surface antigen; S-HBsAg, small hepatitis B virus surface antigen; S-HDAg, small hepatitis delta antigen

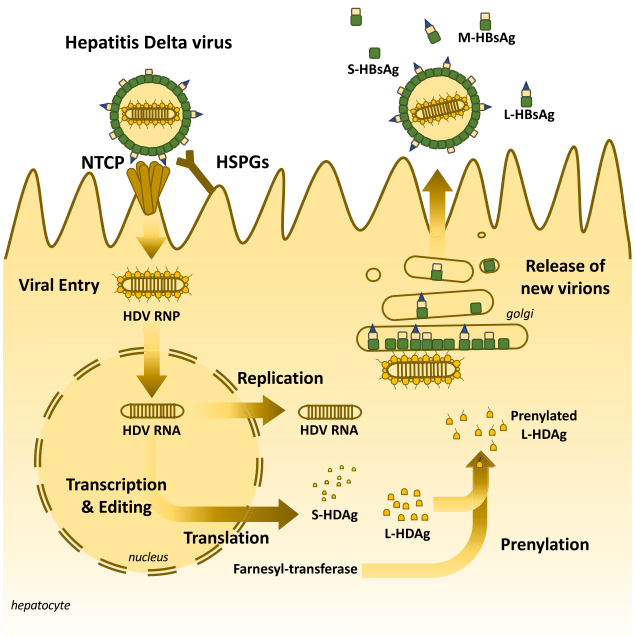

FIGURE 2 Hepatitis delta virus replication cycle. HDV is an hepatotropic virus which infects hepatocytes by attachment to HSPGs and highly specific interaction with NTCP at the surface of hepatocytes. HDV RNP joins the nucleus where the HDV genome is replicated and transcripted. S-HDAg is synthetized directly from HDV genomic RNA and L-HDAg from HDV antigenomic RNA after ADAR-1 editing. L-HDAg is prenylated by the host farnesyltransferase and neosythesized HBV RNP interacts with HBsAg inducing the release of new virions. ADAR-1, adenosine deaminase acting on RNA 1; HBsAg, hepatitis B surface antigens; HBV, Hepatitis B virus; HDV RNP , hepatitis delta virus ribonucleoprotein; HDV, Hepatitis delta virus; HDV RNP, Hepatitis delta virus Ribonucleoprotein; HSPGs, heparan sulphate proteoglycans; L-HBsAg, large hepatitis B virus surface antigen; L-HDAg, large hepatitis delta antigen; M-HBsAg, medium hepatitis B virus surface antigen; NTCP, human sodium taurocholate cotransporting polypeptide receptor; S-HBsAg, small hepatitis B virus surface antigen; S-HDAg, small hepatitis delta antigen

for viral envelope proteins and hijacks the HBV surface antigen. Thus, assembly of the lipidic envelope requires the interaction of L-HDAg with HBsAg to form the HDV envelope, an interaction which requires cysteine discussed in the section 6.2[2,11] L-HDAg prenylation involves the host farnesyltransferase.[2,18] L-HDAg prenylation allows the HDV RNP to anchor to HBsAg and then the formation and release of neo-synthetized virions. To prevent L-HDAg interaction with HBsAg, an inactivation of L-HDAg prenylation by the farnesyltransferase inhibitor, lonafarnib (LFN) occurs, as discussed below.

4 | NATURAL HISTORY AND DIAGNOSIS

HDV infection can occur in two ways: HBV-HDV co-infection and HDV superinfection. Co-infection occurs when HBV and HDV are transmitted simultaneously. Superinfection occurs when the individual has already been infected with HBV and is superinfected with HDV at another time.[19] Acute liver disease can progress to severe or even fulminant hepatitis in both cases. In adults with HBV-HDV co-infection, spontaneous viral elimination usually occurs (>90%). However, in adults with HDV superinfection, chronicity usually occurs (80%).

Patients with HBV infection who are anti-HDV positive must be screened by PCR for detection of HDV viral RNA in serum. HDV RNA viral load monitoring must be an integral part of the management of the infected patients during natural history but also to monitor treatment. We recommend to use a test with high sensibility/specificity in detection and quantification of HDV viral load, regardless of the genotype. The evaluation of the stages of the disease and liver damage is essential. The evaluation of fibrosis is important and can be determined by non-invasive or invasive biomarkers. Non-invasive biomarkers detection can be easily and rapidly performed. However, in certain cases, the results of non-invasive tests are insufficient and a liver biopsy may be required to evaluate the stage of fibrosis (Metavir score F1 to F4) and the degree of necro-inflammatory activity (A0 to A3).[20]

5 | ANTIVIRAL AND IMMUNOMODULATOR THERAPY

5.1 | Interferon alpha therapy (IFNα)

Since 1994, interferon alpha (IFNα) treatment has been proposed for CHD with the regression of fibrosis in patients with advanced fibrosis.[21,22] Pegylated-interferon alpha 2a (PEG-IFN) was then used with around 20% to 25% efficacy and numerous adverse effects limiting patient tolerance.[23-26] However, these adverse effects generally disappear after the end of treatment, and most frequently include a flu-like syndrome (fever, arthralgia, headache, chills) that is usually moderate and well-controlled with paracetamol. Other possible effects are asthenia, weight loss, hair loss, sleep disturbances, irritability and psychiatric disorders.

IFN has two mechanisms of action with antiviral and immunomodulator effects. The duration of treatment is usually 48 weeks with the goal of achieving undetectable HDV-RNA by PCR, 24 weeks after the end of the treatment. However, many relapses occur, thus long-term monitoring is necessary after the end of treatment. Except for HBsAg seroconversion, there are no virological markers associated with HDV elimination and studies are needed to identify novel markers.[27]

The ideal goal of long-term HDV eradication is through HBsAg, seroclearance but this is a rare event.[28] Indeed, if the antiviral effect of PEG-IFN is sufficient and prolonged with effective immune response by clearance of infected hepatocytes, HBsAg seroconversion (HBsAg negative; anti-HBs positivity) may occur and chronic hepatitis as well as the risk of reactivation disappear.

5.2 | Pegylated interferon alpha 2a and Nucleos(t)ide Analogue Combination therapy

Two large studies, the Hep-NET/International Delta Hepatitis Intervention Trial (HIDIT-1 and −2) investigated the combination of HBV nucleos(t)ide analogues such as adefovir (ADV) or tenofovir (TFV) with PEG-IFN in patients with CHD infection and compensated liver disease.[24,25] In the first study, HIDIT-1, 90 patients were included and treated with or without ADV 10 mg plus PEG-IFNα 180 μg for 48 weeks. After 48 weeks on-treatment, a decrease in HDV RNA levels was observed in the combination therapy group. Approximately 24% of these patients achieved HDV RNA negativity 24 weeks after treatment.[24] Many relapse were observed several years after the end of treatment.(ref : eidrich B. Yurdaydin C. Kabacam G. et al. Late HDV RNA relapse after peginterferon alpha-based therapy of chronic hepatitis delta.Hepatology. 2014; 60: 87-97) HIDIT-2 investigated the combination of PEG-IFNα and TFV at concentrations of 180 μg and 300 mg, respectively, for 96 weeks. No significant changes in HDV viral load were observed at the end of treatment.[27] These two trials suggest that the strategy of combining interferon with nucleos(t)ide analogues is not effective in patients with CHD, and other therapies need to be developed.

6 | HEPATITIS DELTA VIRUS THERAPIES

As mentioned above it is essential to develop new therapies against HDV infection because patients with CHD progress more rapidly to end-stage liver disease and HCC. There are many treatments under development to cure HDV.[29] Recently approved therapies and new results are reported and discussed in the next part of this review.

6.1 | Approved therapy: Bulevirtide (BLV), Inhibitor of HBsAg-NTCP interaction

The first step of viral replication involves the concentration of viral particles on the cell surface for an interaction between viral surface proteins and cell receptors. This step is crucial to initiate the intracellular replication cycle, allowing the virus to enter into the target cells. Targeting this viral entry by blocking the interaction of viral surface proteins with a targeted cell receptor is an attractive therapeutic strategy to prevent infections.

As mentioned above, HDV hijacks HBsAg as its own envelope protein and uses the same HBV receptor, NTCP. MYR GMBH (recently acquired by Gilead) has developed the molecule, BLV (Hepcludex®), an acetylated fragment of 47-amino acids derived from the N-terminal domain of the HBV pre-S1 HBsAg, which acts on the first step of the viral cycle by inhibiting HDV entry into hepatocytes.[30-34] Thus, BLV works by competing for the attachment of the HBsAg surface antigen to the NTCP receptor, thus blocking HBsAg-NTCP interaction (Figure 3).

The efficacy of BLV was investigated in MYR203 therapeutic trial, in 60 patients randomized into four arms 15 per arm and treated for during 48 weeks with PEG-IFNα or BLV as mono- or in combination. BLV was administrated at different concentrations (2 or 5 mg per day) by subcunaneous injections.[35]

Combination therapy with PEG-IFNα plus BLV 2 mg per day showed the best results with a decrease in HDV RNA of 4.81 log at the end of the therapy and 4.04 log, 24 weeks after the end of the treatment. Combination therapy with PEG-IFNα plus BLV 2 mg per day was associated with undetectable HDV RNA in 50% of cases, with normalization of ALAT in 47% and decrease by 1 log in HBsAg levels in 40% of the cases.[35]

Combination therapy associating PEG-IFNα plus BLV was well tolerated in patients with CHD, with no serious adverse events. Some mild adverse events were described with PEG-IFNα. The main reported adverse events from BLV were related to increases in total bile acids because the NTCP receptor, which is the target of BLV, is also a hepatocyte transporter of bile salts. Thus, total bile acid levels should be monitored during therapy. Moreover, an in vitro study suggests that BLV is associated with inhibition of uptake of transporters OATP1B1, OATP1B3 and cytochrome P450 3A (CYP3A) activity. Further studies are needed to better understand these observations.[36]

Another study has reported the effectiveness and safety of 48 weeks of BLV 10 mg per day in three patients with CHD compensated cirrhosis.[37]

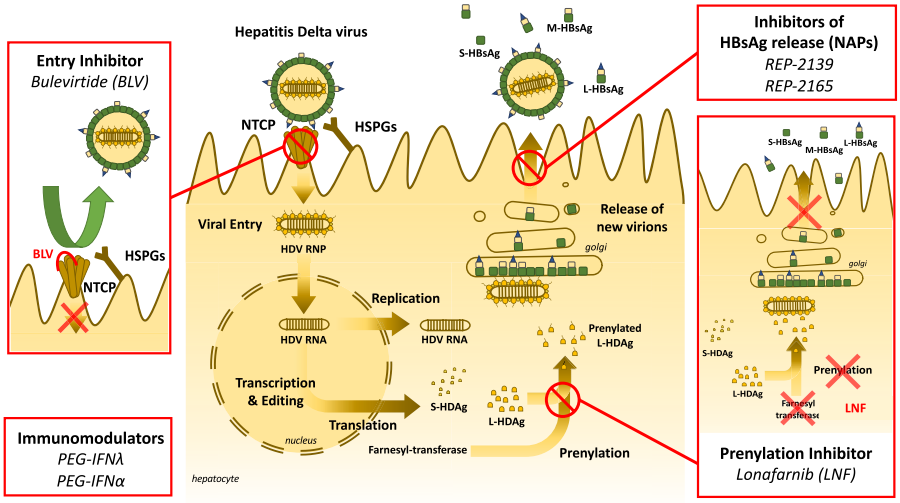

FIGURE 3 Therapeutics targets for HDV infection: Entry inhibitor, Prenylation inhibitor, inhibitors of HBsAg release and immunomodulators. Bulevertide (entry inhibitor) interacts with NTCP blocking HDV entry into hepatocytes. Lonafarnib (Prenylation inhibitor) inhibits L-HDAg prenylation blocking farnesyltransferase enzyme. Nucleic Acid Polymers (NAPs) inhibits the release of HBsAg. Interferons (immunomodulators) stimulate immunity and have antiviral property. BLV, bulevertide; HBsAg, hepatitis B surface antigens; HBV, hepatitis B virus, HDV, hepatitis delta virus; HDV RNP, hepatitis delta virus ribonucleoprotein; HSPGs, heparan sulphate proteoglycans; L-HBsAg, large hepatitis B virus surface antigen; L-HDAg, large hepatitis delta antigen; LNF, lonafarnib; M-HBsAg, medium hepatitis B virus surface antigen; NAPs, Nucleic Acid Polymers; NTCP, human sodium taurocholate cotransporting polypeptide receptor; S-HBsAg, small hepatitis B virus surface antigen; S-HDAg, small hepatitis delta antigen

BLV (Hepcludex®) was approved in 2020 in Europe. The EMA (European Medicines Agency) approved BLV (Hepcludex®) at a dose of 2 mg sub-cutaneous per day for the treatment of chronic HDV infection in adult patients with compensated liver disease and positive HDV viremia. The optimal treatment duration has not been determined and treatment should be continued if a clinical benefit is associated with BLV administration. If the treatment is associated with HBsAg seroconversion for at least 6 months or in case of the loss of virological and biochemistry responses, treatment discontinuation can be considered. MYR301 and MYR204 studies are ongoing to better understand BLV effects.(1) There are several questions for future drug development: (i) what is the clinical long-term benefit of BLV ? (ii) What is the optimal dose: 2 or 10 mg? (iii) Which patients will benefit from the combination with PEG-IFN (predictors of response)? What is the ideal duration of therapy (maintenance therapy)?

6.2 | Lonafarnib (LNF), farnesyl transferase inhibitor

HDV proteins must interact with HBV surface proteins to initiate the formation of infectious HDV particles. This interaction involves L-HDAg and HBsAg. L-HDAg contains a prenylation CXXX box motif at its last four amino acids that is required for post-translational modification by the cellular farnesyltransferase. This enzyme renders L-HDAg more lipophilic by addition of a 15-carbon prenyl lipid-farnesyl-moiety to the cysteine present in the prenylation CXXX box motif. The L-HDAg prenylation makes it possible to anchor to the HBsAg during virion assembly for formation of the infectious HDV particle.[38] These steps are crucial for HDV to infect other hepatocytes and to promote its multiplication. Lonafarnib (LNF) from Eiger BioPharmaceuticals, Inc is an oral inhibitor preventing L-HDAg prenylation and HDV virion formation (Figure 3B).

The efficacy, tolerability and safety of LNF were investigated in a therapeutic trial, LOWR HDV-1 to -4 (lonfarnib with and without ritonavir).[39-42]

The best antiviral response and optimal efficacy was obtained with 50mg LFN and 100 mg RTV bitherapy for 6 months with a decrease in HDV RNA in 90% of cases and normalization of transaminases in 100% of CHD patients. However, this combination does not affect the HBsAg quantification.

Some adverse events were observed with high concentrations of LNF (>75 mg 2 per day) in association with RTV in particular digestive disturbances, anorexia, nausea, diarrhea and weight loss.

6.3 | Pegylated Interferon Lambda 1a (PEG-IFNλ)

In 2006, the potential antiviral activity of type III interferons such as lambda interferon was confirmed against certain viral infections.[43] This antiviral activity was shown against HBV in LIRA-B, a randomized study with a decline in HBV viral load.[44] PEG-IFNλ safety, tolerability and efficacy was investigated in 33 patients with CHD with two different concentrations for 48 weeks.[45] The best results were obtained with 180 μg and this treatment was associated with a 2.3 log decrease in HDV RNA 6 months after the end of treatment.

Ongoing trials are evaluating as PEG-IFNλ can be used for monotherapy or in combination therapy. More studies are needed with different combinations.

6.4 | Nucleic acid polymers (NAPs)

Recently, safety and efficacy results of 48-week treatments with two different "HBsAg-targeting" nucleic acid polymers (NAPs) REP-2139-Mg or REP-2165-Mg, combined with tenofovir-disoproxil-fumarate (TDF) and PEG-IFN, were reported.[46] In this open-labelled, randomized, controlled, phase-2 study involving 40 patients with CHB, REP 2139-Mg or REP 2165-Mg in association with PEG-IFN and TDF, provided important efficacy with around half of the patients achieving HBsAg loss/HBsAg seroconversion. These impressive results need to be confirmed in larger studies.[47]

7 | CONCLUSION AND EXPERT OPINION

HDV is a defective virus that requires the presence of HBV for successful replication. CHD is the most severe form of chronic viral hepatitis, with a high risk of morbidity and mortality caused by end-stage liver disease acceleration of fibrosis progression, decompensation of cirrhosis and HCC. HDV is still endemic in many developing countries. The best preventive strategy to decrease HDV infection is to improve coverage with the HBV vaccine. The revolution of the cure of hepatitis C virus infection with direct-acting antivirals, with excellent efficacy and favourable safety, has increased hope for a cure to HBV and HDV.[48-50] A cure of HBV will also lead to a cure of HDV.[49-51]

It is essential to improve knowledge of the HDV replication cycle to identify targets for future drugs because each step is a potential target for HDV cure. Ideally, the aim of treatment for HDV and HBV infection is to obtain a serological response with HBsAg loss and HBsAg seroconversion (functional cure) which is associated with an excellent prognosis (reduced risk of HCC). There are several endpoints (listed in Figure 4) with different type of responses: biochemical (ALT normalization), virological (HDV RNA decrease >2 log or achieving an HDV RNA undetectable by sensitive PCR), an histological response (fibrosis regression, reduction in necroinflamfmation), and a clinical response (reduction in HCC, cirrhosis decompensation, importing survival). A decrease in HBsAg may also restore the immune response. Improved understanding of HBsAg quantification and decrease as well as improved characterization of specific HBsAg epitopes will be important.[52] Other endpoints and markers should be further investigated such as HDV RNA decline or HBcrAg (Hepatitis B core-related antigen).[53]

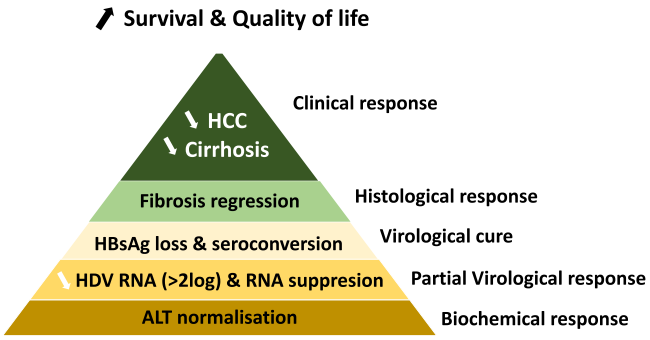

FIGURE 4 Endpoints for clinical trials for HDV drug development

For many years the only available treatment for CHD was PEG-IFNα for 48 weeks. The efficacy of this treatment was limited (around 20%) and tolerability was poor. BLV (Hepcludex®) an entry inhibitor, was recently approved in Europe. HDV, like HBV, infects hepatocytes via a highly specific interaction with the human sodium taurocholate cotransporting polypeptide (NTCP) receptor. BLV is well tolerated with an antiviral efficacy that increases with the duration of treatment. Thus, BLV may be suitable for prolonged administration with follow-up for potential adverse events.

Several drugs are under development. The viral response with lonafarnib appears to be profound and early, with antiviral efficacy in some cases especially after 8 and 12 weeks of treatment. Twelve weeks of treatment could also be evaluated in studies to assess the potential synergy with a combination of two antiviral agents.

It should be noted that the best results have been obtained when these new compounds are combined with PEG-IFNs. Thus, interferons may be continued until more effective and well-tolerated immune modulators become available. For the underlying HBV infection, combination therapy with nucleos(t)ide analogues could be considered to control HBV replication and avoid HBV reactivation during the treatment of CHD. Finally, different pathways and combinations should be investigated to help obtain a functional cure. Different mechanisms of action are being studied, such as long-term nucleoside analogue treatment, IFNs, entry inhibitors, or by targeting viral translation with siRNA or inhibiting HBsAg release by nucleic acid polymers by neutralizing HBsAg via specific antibodies.

CONFLICT OF INTEREST

Tarik Asselah has acted as a speaker and/or advisor board and/or investigator for Abbvie, Eiger Biopharmaceutical, Janssen, Gilead, Myr Pharmaceutical, Roche, and Merck. Nathalie Boyer has acted as a speaker and investigator for Janssen, Gilead, Roche and Merck. Corinne Castelnau, Myr Pharmaceutical, Roche and Merck. Patrick Marcellin has acted as a speaker and investigator for Eiger Biopharmaceutical, Janssen, Gilead, Myr Pharmaceutical, Roche, and Merck. Dimitri Loureiro, Issam Tout, Stéphanie Narguet, Zeina Louis, Nathalie Pons-Kerjean, Nathalie Giuly and Abdel Mansouri declare no competing interests.

AUTHOR CONTRIBUTIONS

DL and TA designed, supervised and prepared the manuscript. All the authors contributed to the drafting of the review, the critical revision of the manuscript and its final approval. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENT ARTICLE

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Is hepatitis delta underestimated?

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Abstract

Hepatitis D virus may be underestimated because it is a significant problem in HBsAg-positive patients, especially those who inject drugs, have HIV or HCV co-infections and/or live in certain endemic regions. In the past few decades, the prevalence of HDV was expected to have decreased as a result of improvements in public healthcare policies and universal HBV vaccination programs. However, HDV has continued to spread in low-income countries, with local outbreaks and migration to less endemic areas, so that its prevalence has remained stable or even increased in certain regions. As a result, research has been focused on the epidemiology of HDV. Contradicting data from three large recent meta-analyses have reported that the prevalence of HDV may be between 0.16% and 1.00% in the global general population, and 4.5% and 14.6% in HBsAg-positive patients, with an estimated 12 to 70 million HDV patients worldwide. The exact prevalence and estimated number of HDV patients is still a subject of debate for several reasons, including the unreliable assessment of the infection and a lack of real-world screening. HDV infection is associated with an increased risk of progression to cirrhosis and the development of HCC compared to patients with HBV mono-infection, a risk which is even higher in patients with HIV co-infection. Morbidity and mortality from HDV-related cirrhosis should not be overlooked. In conclusion, hepatitis D virus is probably underestimated and certainly underdiagnosed, and screening for HDV should be performed in all HBsAg-positive patients in clinical practice.

KEYWORDS

cirrhosis, HBV co-infection, HDV epidemiology, hepatitis D, hepatocellular carcinoma, prevalence

Key Points

- Infection with hepatitis D virus (HDV) represents a major health problem in HBsAg-positive patients and especially those who inject drugs as well as those who have HIV and HCV co-infections, and/or live in several endemic regions.
- Contradicting data from three large recent meta-analyses have suggested that the number of HDV patients is probably higher than previously estimated, although the exact number is still a subject of debate.
- HDV infection is associated with a significantly increased risk of hepatocellular carcinoma compared to HBV mono-infection, which is even higher in the presence of HIV co-infection.
- Hepatitis D virus is underdiagnosed, therefore HDV screening should be performed in all HBsAg-positive patients in clinical practice.

Abbreviations: anti-HDV, antibodies to HDV; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HDV, hepatitis D virus; IVDUs, iv drug users.

1 | INTRODUCTION

If hepatitis delta virus (HDV) has been underestimated until now, it is time to change this. Recent progress in the virology and immunology of this disease, as well as emerging drug developments have redirected the attention of the hepatology community to focus on HDV. Indeed, the latest global epidemiological data show that the prevalence of HDV is probably much higher than previously thought.[1]

HDV and hepatitis B virus (HBV) co-infection or usually HDV superinfection in patients with chronic HBV infection is known to result in the most severe forms of viral hepatitis and patients infected with both HDV and HBV often develop severe acute hepatitis which usually rapidly progresses to the advanced stages of chronic liver disease. However, because of the declining prevalence of this disease in the past few decades, diagnosis has been neglected, and treatment options for this disease are limited. HDV infection is mainly found in patients with HBV infection in low-income countries, while in western countries it is usually found in high-risk groups with HBV infection including intravenous drug users (IVDUs), people with high-risk sexual behaviours (HRSB) and immigrants from endemic countries.[2] However, even in these settings, epidemiological studies suggest that screening for HDV is insufficient. As a result, findings from the latest meta-analyses discussed below are conflicting, which may be caused in part by the quality of studies from real-world cohorts.

Effective treatment for HDV has been suboptimal until now, as the only therapeutic option, interferon therapy, provides limited results. Results of clinical trials for new treatment approaches are encouraging, in particular for specific entry inhibitors and prenylation inhibitors, while other options such as interferon lambda polymers and novel HBV treatments targeting Toll-like ligands and check-point inhibitors may also be beneficial.[3] In any case, these findings may represent an important breakthrough in the history of HDV infection[1] and in the battle against this disease as its burden continues to rise.

This article reviews the recent changes in the epidemiology of HDV, the latest data on the burden and global distribution of the disease as well as updated evidence on the clinical risks and implications for HDV-infected patients.

2 | CHANGES IN HDV EPIDEMIOLOGY

In 1977, Mario Rizzetto and his colleagues identified a small distinct antigen by immunofluorescence on liver biopsies of patients with HBV and severe liver damage. This was later attributed to a novel human pathogen and called HDV.[4] Since then, a number of studies have tried to define the epidemiological map of HDV infection.

Although the prevalence of HDV infection has been considered to vary widely with distinct geographical regions, the reason for these marked differences in prevalence compared to neighbouring regions has not been clarified. Initial epidemiological surveys in the 1980s showed that highly endemic regions were usually low-income countries in central Africa and South America, with rates of HDV infection of up to 60% in HBsAg-positive patients and more than 80% in the islands of the Western Pacific, such as Kiribati and Nauru.[5]

The Mediterranean area was considered to be a zone of intermediate endemicity with a prevalence of 25% and 30% in Italy and Turkey respectively. Indeed, Italy was used as a paradigm to study the pattern of spreading of HDV infection. It was then discovered that epidemic trends in high-risk individuals in combination with the level of endemicity in the general population were responsible for the increased rates of HDV. Thus, the high risk of contamination in IVDUs as well as institutionalized individuals and frequently transfused patients was as a result of shared materials, while transmission caused by close contact in overcrowded housing units was the cause in the general population of Southern Italy and some studies from Taiwan.[6]

Regions known for a low prevalence of HBV including Northern Europe and North America were traditionally considered to be low endemic areas for HDV. In these regions, HDV infection was almost exclusively restricted to IVDUs with occasional reports of fulminant hepatitis usually caused by acute HBV-HDV co-infection, perhaps on pre-existing chronic HCV infection.[7] However, a low prevalence of HDV has also been found in regions that are endemic for HBV, such as Japan, Korea and Indonesia. The reason for these prevalence rates has not been clarified, and it is surprising that despite a low overall prevalence of HDV in Japan there were specific regions such as the Mikayo islands and Okinawa with a high prevalence of HDV and more than 20% in HBsAg-positive patients. These hyperendemic areas have been called “endemic pockets” of HDV infection and throughout the 1980’s several small-scale studies have revealed similar disparate and uneven rates of prevalence in small regions around the world. These might be as a result of diverse socio-economic limitations, differences in the virulence of HDV genotypes or perhaps a distinct genetic susceptibility of HBV-infected patients to the HDV superinfection, but the real causes have not been clarified. Possible differences in the transmission routes of HDV and HBV may also contribute to occasional deviant rates of prevalence for these two infections. While parenteral transmission has clearly been shown for HDV in animal studies, evidence of transmission in men who have sex with men and mother-to-child vertical transmission has not been confirmed.[6,8]

Nevertheless, there is no doubt about the significant influence of the prevalence of HBV on the epidemiological trends of HDV infection. This strong association between HBV and HDV contributed to the declining HDV rates at the end of 1980s and throughout the 1990s especially in European countries. Significant improvements in public health, modifications in sexual behaviours as a result of infection with the human immunodeficiency virus (HIV), the introduction of universal HBV vaccination at an early age and the widespread availability of single-use syringes resulted in a decrease in the incidence of HBV and HDV rates as well. Data from Italy during this period showed that the seroprevalence of HDV was more than three times lower in 1997 than in 1983 with similar trends in Turkey and other Eastern European countries.[8] On the basis of the decreasing rates at the end of 1990s, the complete eradication of HDV infection was expected sooner rather than later.

In retrospect, it was at this point that HDV was underestimated. Certain important signs should have suggested that it was too early to believe that HDV was no longer a significant public health problem. First, despite the improvements in hygiene and socioeconomic conditions in the developed countries with the most advanced research such as Europe and the United States, unfortunately, the rest of the world was not following the same pattern and the risk of infection never significantly decreased, especially in the developing countries. Although epidemiological data from these regions are scarce and potentially unreliable, some recent cohorts have shown HDV rates of more than 10% in HBsAg-positive patients, with 70% in HBsAg-positive patients in certain hyperendemic areas such as Nigeria, Gabon, India, western Brazil and, especially, Mongolia.

Furthermore, even in western countries, increased immigration from HDV endemic countries resulted in new cases of HDV. As a result, in the last two decades, the prevalence of HDV has not continued to decline in Europe. In a recent report from Italy, the overall prevalence of HDV has remained at 10% in HBsAg-positive patients with similar rates in London and Berlin which seem to be associated with the increasing number of immigrants from endemic regions such as Eastern Europe, Africa, Middle East and Turkey. The consequences of these migrations can also explain the presence of HDV genotypes 5-7, which were previously only found in Africa. Greece is another similar example which shows that more than half of the burden of HDV is found in immigrants rather than in native inhabitants. Similarly, Spain has also had a large number of immigrants from the highly endemic regions of North Africa and South America, and has shown an increase in the prevalence of HDV in the last 5 years, despite a decrease in the rates of infection in the previous two decades.[8]

Thus, the existing burden of HDV in Europe seems to be composed of the residual reservoir of previously infected native population as well as recent immigrants. Most of the first group are patients with advanced liver disease as a result of their age and the long duration of infection, while the latter group includes younger patients with active or sometimes indolent hepatitis and at high risk of progressing to more severe states in the future.

Finally, local outbreaks in areas such as Venezuela, Ecuador and Greenland resembling those described in the 1980s in Japan (Okinawa), central Africa and the Amazon basin may be subject for alarm. There is a fear that the unexplained outbreaks in these hotspots could spread uncontrolled throughout populations as groups continue to migrate around the globe. Thus, the threat of HDV infection should not be underestimated and the demand for adequate public health measures needed to confine its spread should not be ignored.

3 | LATEST DATA ON THE GLOBAL BURDEN OF HDV INFECTION

Despite the many but scattered regional and national epidemiological observations, the global prevalence of HDV infection is still a subject of debate and was declared uncertain by the 2017 World Health Organization (WHO) Global Hepatitis Report.[9] Data from 1980s suggested that 5% of chronic HBsAg carriers (15-20 million individuals) were co-infected with HDV worldwide, but until very recently there had been no systematic assessment. In the past year, three meta-analyses have been published to evaluate the global prevalence of HDV infection but results are conflicting (Table 1).

First, in the systematic review and meta-analysis by Chen et al,[10] an estimated 70 million individuals or more are infected with HDV worldwide, suggesting for the first time that the burden of HDV might be markedly higher than previous estimations. The authors analysed 182 studies from 61 countries published from 1977 to 2016 and reported an overall estimated HDV prevalence in the global general population of 0.98% (0.00%-8.03%) (Table 2). The rate is highest in Mongolia, and China had the highest disease burden mainly because of its large population. The global prevalence of HDV in all HBV chronic carriers reached 14.6%, which is nearly three times higher than previous estimations. HDV prevalences were reported to be 10.58% in subjects without risk factors, 37.57% in IVDU HBsAg-positive cases and 17.01% in HBsAg-positive patients with HRSB. In an updated analysis published as a short letter by the same authors, in 2017-2018, the reported HDV prevalences in the general population and HBsAg-positive carriers were 1.00% and 7.06%, respectively, compared to 1.00% and 10.07% between 1977 and 2017. This difference was not statistically significant.[11] Despite all the potential limitations of these studies and even with the most conservative calculations showing that only 20%-50% of the estimated HDV seroprevalence by Chen et al represents chronic HDV infection,[12] this first large meta-analysis of the decade suggests that HDV infection is an important global public health problem.

Miao et al[13] published a large systematic review and meta-analysis including 634 studies published between 1982 and 2019 from 48 and 83 countries, for the estimation of HDV prevalence in the general population and HBsAg-positive patients respectively (Table 2). The pooled global HDV prevalence was 0.80% in the general population and 13.02% in HBsAg-positive cases, which corresponds to 50-60 million cases of HDV. Recently, Miao et al revised these rates slightly and reported a prevalence of HDV in the general population of 0.70% corresponding to 50 million HDV cases worldwide, with 13.02% in HBsAg-positive cases corresponding to 32-61 million cases of HDV depending on the existing HBsAg-positive cases (250-500 million) worldwide.[14] Although these numbers are lower than those reported by Chen et al, they again show that the global prevalence of HDV infection is higher than previously estimated. The largest disease burden was also found to be in China, followed by India and Nigeria. The risk factors of increased HDV prevalence were IVDU and co-infection with HIV or HCV. This emphasizes the hidden risk of HDV in patients with parenterally transmitted virus co-infections other than HBV caused by common routes of transmission. This is also in line with a recent breakthrough study suggesting that non-HBV viruses might facilitate HDV entry into hepatocytes.[15] Furthermore, this meta-analysis showed no difference in the prevalence of HDV infection between men and women, in contrast to

TABLE 1 Basic methodological characteristics of three large meta-analyses on global hepatitis D virus (HDV) prevalence

	Chen, 2019[10]	Miao, 2020[13,14]	Stockdale, 2020[16]
Databases searched	PubMed, Embase, Cochrane Library, China knowledge Integrated databases	Embase, Medline, Ovid, Cochrane, China knowledge Integrated database	PubMed, Embase, Scopus and grey literature
Language of Studies included	English, Chinese	English, Chinese	All languages
Time period of publication of studies included	01/01/1977 - 31/12/2016	01/01/1982 - 01/02/2019	01/01/1998 - 28/01/2019
Inclusion Criteria	Available data on HDV seroprevalence, patient selection methods, geographical and clinical setting included in the analysis	Studies with data on prevalence and outcome of HDV. The prevalence of HDV was defined by the detection of HDV antibodies (anti-HDV IgG and/ or anti-HDV IgM), supplemented by the additional detection of HDAg and HDV RNA.	Studies that examined geographic and clinical setting of participants with HBsAg and applied a systematic selection method to anti-HDV testing, where all/random selection of eligible participants was tested.
Exclusion criteria	Data on infants of children	Studies with fewer than 100 subjects from general population or 20 HBsAg carriers	
Patient groups where HDV prevalence was assessed	General population from 1977 to 1996 and 1997 to 2016, Mixed population (HBsAg carriers without risk factors). HBsAg carriers and IVDU, HBsAg carriers with HRSB	General population, HBsAg carriers; Blood donors, IVDUs, people with HRSB, HIV and/or HCV, frequent blood transfusion, Mixed patients, Liver disease patients, Asymptomatic HBsAg carriers	General HBsAg-positive populations, comprising people tested in community surveys, antenatal clinics or occupational settings, students and blood donors (unless repeat or remunerated); ii) HBsAg population visiting hepatology clinics, regardless of disease status; and iii) selected population groups, comprising IVDUs, haemodialysis recipients, MSM, CSW and people with HCV or HIV
Records identified in literature search	2717	3518	2104
Studies included in meta-analysis	182	634	282
Number of subjects included	40 127 988 general population subjects from 61 countries (one study from France: 39 911 011 subjects); 101 363 HBsAg-positive cases from 51 countries	332 155 general population subjects from 48 countries 271 629 HBsAg-positive cases from 83 countries	24 025 000 general population subjects from 50 countries; 120 293 HBsAg-positive cases from 95 countries
Global HDV prevalence	0.98% in general population; 14.6% in HBsAg-positive cases	0.80% in general population; 13.0% in HBsAg-positive cases	0.16% in general population; 4.5% in HBsAg-positive cases of general population; 16.4% in HBsAg-positive cases of Hepatology clinics
Estimated global number of HDV cases	Approximately 72 million	48-60 million; revised to 32-61 million	12 (8.7-18.7) million

Abbreviations: CSW, commercial sex workers; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; HRSB, high-risk sexual behaviour; IVDU, intravenous drug users; MSM, men who have sex with men.

data from previous studies.[8] In any case and in agreement with the conclusions of Chen et al,[10] the authors once again suggested that the burden of HDV infection might be underestimated, and that HDV screening may have been neglected in middle- and low-income countries as well as in high-risk groups and should be carefully performed in all at-risk individuals worldwide.

TABLE 2 Estimated hepatitis D virus prevalence per country according to three large recent meta-analyses

	Chen, 2019[10]	Miao, 2020[13]	Stockdale, 2020[16]
Albania	0.45%	0.53%	
Argentina		0.48%	0.40%
Australia	4.75%		0.01%
Bangladesh		0.00%	0.30%
Belarus			0.10%
Benin	3.74%	1.77%	1.70%
Botswana			0.10%
Brazil	2.09%	1.13%	0.05%
Burkina Faso		0.56%	0.20%
Cameroon		0.80%	1.00%
Canada		0.00%	
Central Africa Republic	0.45%	0.83%	0.90%
China	1.22%	0.69%	0.40%
Colombia		1.94%	
Cote d'Ivoire			1.20%
Djibouti		1.68%	
Egypt	0.60%		0.10%
Ethiopia	0.00%	0.43%	0.30%
Former Republic of Yugoslavia	0.00%		
France	0.00%	0.03%	0.01%
Gabon		1.87%	2.00%
Gambia			0.10%
Germany			0.02%
Ghana		0.68%	0.50%
Greece	0.00%	0.56%	
Greenland	1.88%		
Guinea-Bissau			3.90%
India		0.35%	
Indonesia	0.11%	0.17%	0.02%
Iran		0.08%	0.10%
Iraq	0.33%		0.20%
Italy	0.73%	0.08%	0.02%
Japan		0.65%	0.70%
Kosovo		0.08%	0.10%
Lebanon			0.02%
Malawi			0.20%
Mali	2.40%		0.80%
Mauritania		2.59%	2.90%
Micronesia	4.40%		
Moldova	8.03%	1.40%	1.30%
Mongolia	4.01%	8.31%	4.00%
Mozambique	0.00%		
Nauru	5.04%	4.01%	
Niger	2.43%	5.04%	
Nigeria		2.09%	1.60%
Pakistan		1.31%	0.60%
Peru	0.00%		0.10%
Romania	0.39%	0.00%	0.40%
Saudi Arabia		0.30%	0.10%
Senegal		1.42%	0.20%
Serbia		0.00%	
Somalia		3.19%	
South Africa		0.00%	0.10%
Sultanate of Oman	0.05%		
Tanzania	0.00%		0.20%
Thailand		0.67%	0.02%
Togo			1.10%
Tunisia	0.03%	15.33%	0.20%
Turkey		0.10%	0.10%
Uganda		3.07%	0.20%
United Kingdom	0.00%		0.02%
United States	0.92%	0.20%	0.02%
Venezuela	0.24%	1.72%	
Vietnam	0.14%	0.24%	1.40%
Yemen		0.25%	0.10%

Finally, the most recent meta-analysis on this topic was published by Stockdale et al,[16] who were already well known for their meta-analysis on the prevalence of HDV infection in sub-Saharan Africa[17] as well as for a letter disputing the meta-analysis by Chen et al.[10] These authors searched all available and relatively recent publications in all languages and included 282 studies published between 1998 and 2019[16] with approximately 100 more studies than Chen et al[10] but less than half of the studies included by Miao et al.[13] Their findings in the general population showed that the global pooled prevalence of HDV was 4.5% [95% confidence interval (CI): 3.6%-5.7%] in HBsAg-positive patients and 0.16% (95% CI: 0.11%-0.25%) overall (Table 2). Based on the 2017 WHO report on the estimated prevalence of HBsAg in the general population (approximately 250 million HBsAg-positive cases worldwide),[9] these authors estimated that there are approximately 12 million HDV cases worldwide. The prevalence of HDV in HBsAg-positive patients followed in hepatology clinics was reported to be much higher (16.4%) in this meta-analysis, while HDV was found to be associated with 18% of cases with cirrhosis and 20% of the cases with HCC. As in previous reports, the prevalence of HDV in HBsAg-positive cases was higher in IVDU, followed by those with HRSB and HIV or HCV co-infections.

There may be several reasons for the marked differences in the estimated HDV global prevalence and HDV cases among the meta-analyses by Chen et al,[10] Miao et al[13] and Stockdale et al.[16] First,

these differences may be a result of differences in: selection criteria including the chronological periods of the published studies included in each meta-analysis; assumptions about global or regional HBV prevalence rates or national or regional HDV prevalence rates based on data from specific subgroups; on different methods of selecting weighted samples or of the diagnosis of HDV infection; approaches in the use of clinical or hospital-based cohorts, proportions of included cohorts with high-risk groups and even methodologies used to estimate HDV prevalence rates in the general population. Nevertheless, all three meta-analyses clearly show that HDV infection is common in HBsAg-positive patients with a significant geographical heterogeneity and that the global burden of HDV infection should not be disregarded. Future studies should focus on filling in the gaps of epidemiological data which have been identified in certain regions.[12,16] It has also been shown that the unbiased detection of HDV seroprevalence requires large study samples to identify a reliable subgroup of HBV-infected patients, which is especially challenging in areas of low HBsAg prevalence.

4 | CLINICAL IMPLICATIONS OF THE HDV INFECTION

Despite the findings on the global health burden of HDV, the Global Health Sector Strategy for Viral Hepatitis 2015-2021 did not present any specific approach for HDV-infected patients in the attempt to eradicate viral hepatitis by 2030.[18] Current guidelines from the European Association for the Study of Liver Diseases[19] and the Asian-Pacific Association for the Study of the Liver[20] recommend screening for HDV in all patients with chronic HBV infection. However, these recommendations do not seem to be followed in clinical practice because several studies have shown that HDV testing is only performed in a minority of chronic HBV cases.[21] The same problem is also found in the United States where the recommendations are less strict. In particular, the American Association for the Study of Liver Diseases[22] only recommends HDV antibody testing in patients at high risk of HDV infection including patients with HIV infection, IVDUs and HRSB or immigrants from highly endemic regions as well as in chronic HBV patients with low-serum HBV DNA levels but with unexplained elevated liver aminotransferases. However, data from one real-world cohort assessing veterans in the United States showed that fewer than 10% of chronic HBV patients were tested for anti-HDV,[23] suggesting that HDV screening is clearly underperformed in this setting.

The importance of this issue is not just the epidemiological inconsistencies, but also the undiagnosed patients with advanced liver disease. HDV-related chronic hepatitis is more frequently associated with severe necro-inflammation and faster progression to more advanced stages of liver fibrosis and cirrhosis. Chronic HDV and HBV infection may also be associated with a higher risk of portal hypertension, HCC and all-cause mortality compared to patients with chronic HBV mono-infection.[24-26]

Because of the severe clinical course of chronic HDV infection, the global burden of advanced liver disease and HCC from HDV is greater than the infection rates per se. Stockdale et al estimated that 1 in 5 and in 6 cases of cirrhosis and HCC, respectively, are related to HDV infection worldwide, although data from Southeast Asia and the Western Pacific were inadequate with a significant heterogeneity in these results.[16] These authors also mentioned that the lack of available data makes evaluation of HDV-attributed mortality impossible.

Available data on HCC were published in a meta-analysis this year by Alfaiete et al. The authors showed that HDV-HBV-infected patients are at a significantly higher risk of HCC than HBV mono-infected patients (pooled odds ratio 1.28), and the risk seems even higher in patients co-infected with HIV and/or HCV.[27] The molecular pathways leading to the development of HCC in these patients have not yet been clarified. Although HBV is well known to have direct oncogenic properties, data on HDV are scarce and therefore have not yet been clarified. While recent data have evaluated the effect of HDV on altered DNA methylation that could trigger carcinogenesis[28] and oncogenic gene activation that could promote genetic instability,[29] further research is needed to clarify these complex mechanisms. On the other hand, the increased risk of HCC in patients with HDV/HBV/HIV triple infection may also be as a result of the associated immune dysregulation in these patients which could facilitate the development of liver cancer, even under antiretroviral treatment.[30]

5 | CONCLUSIONS

Recent results show that HDV infection should not be underestimated and especially should not go undiagnosed. Thus, adequate screening should be part of routine clinical practice for all chronic HBV patients around the world. Public health policies should also improve promotion of preventive measures against the spread of viral hepatitis, as well as the implementation of universal HBV vaccination wherever it has not been sufficiently performed. Finally, while upcoming breakthroughs are anticipated in the field of antiviral therapies, ongoing and future studies including HDV patients should focus on meticulous and reliable study design to remedy the shortcomings in natural history and epidemiology.

CONFLICT OF INTEREST

M Papatheodoridi has no conflict of interest; G Papatheodoridis has participated as principal investigator in clinical trials sponsored by Eiger BioPharmaceuticals.

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SUPPLEMENT ARTICLE

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Is elimination of HCV realistic by 2030: France

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Abstract

The World Health Organization (WHO) has proposed a plan for the elimination of viral hepatitis with a goal of reducing new hepatitis infections by 30% and 90% in 2020 and 2030, and associated mortality by 10% and 65% respectively. Actions and targets to reach these goals include improving hepatitis B virus (HBV) vaccination programs, the prevention of mother-to-child transmission of HBV, improving the safety of blood products and injections, risk reduction policies and optimizing the diagnosis and treatment of hepatitis. The goal of eliminating hepatitis C virus (HCV) by 2030 is based on three main actions: increased screening, strengthening access to care and the prevention of infections and re-infections. But, can this goal be reached? The answer to this question is yes in some countries, perhaps in others and no in most countries. Success will be limited by a "diagnosis burn-out" with 5 times more new viral infections than diagnoses in 2016 and a "treatment burn-out" with cure rates that are 5 times lower than the number of new infections. Nevertheless, France, like 10 other countries, is on track to achieve the WHO elimination plan by 2030. In France, the prioritization of oral antivirals in 2013-2014 which was extended to high-risk populations in 2015 (HIV-infected patients) and 2016 (men who have sex with men, dialyzed or kidney transplant recipients), then in 2017 to universal treatment with full coverage by French national healthcare (10 to 15 000 treatments per year) has resulted in half of the 120 000 patients needed to be treated by 2022 have been treated. Renewed efforts should make it possible to reach the target announced by the French Minister of Health in May 2018 by 2025.

KEYWORDS

Direct-acting antivirals, elimination, hepatitis C virus, World Health Organization

1 | INTRODUCTION

Viral hepatitis was the seventh leading cause of death in the world in 2013 and represents the second cause of mortality from infectious disease worldwide, exceeding HIV, tuberculosis or malaria.[1] Chronic hepatitis C virus (HCV) infection, with 71 million infected individuals,[2] is a systemic disease with hepatic (cirrhosis, hepatocellular carcinoma) and extrahepatic (cryoglobulinemic vasculitis, fatigue, diabetes, arteriosclerosis, neuro-cognitive disturbances) effects.[3] HCV infection is responsible for significant hepatitis-related mortality (one third) compared to those in whom HCV is cured or uninfected[4,5] and a continuous increase in mortality from viral

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immune deficiency virus; PWID, persons who inject drugs; WHO, World Health Organization.

hepatitis is expected until at least 2030.[1] This led the World Health Organization (WHO) to propose a plan for the elimination of viral hepatitis with the reduction of new hepatitis infections by 30% and 90% in 2020 and 2030, and associated mortality by 10% and 65% respectively (Figure 1, panel A).[2] Interventions and targets are improving HBV vaccination, prevention of mother-to-child transmission of hepatitis B virus, improving blood safety and injections, developing risk reduction policies and the diagnosis and treatment of hepatitis (Figure 1, panel B). The goal of eliminating hepatitis C by 2030 is focused on three main interventions: increased outreach screening, strengthening access to care and prevention of infections and re-infections. The question is, however, can this plan be achieved? The answer is yes in some countries, perhaps in others and not in most countries. There is indeed a "diagnosis burn-out" with 5 times more new viral infections than diagnoses in 2016 (in 10 of the 91 countries studied) as well as a "treatment burn-out" with cure rates for HCV infection that are 5 times lower than the number of new infections (in 23 of the 91 countries tested). Thus, these figures suggest that the possibility of eliminating hepatitis C virus is unlikely.[6] It may be possible to eliminate HCV in France, which is one of the countries that is on track for the WHO elimination plan by 2030 (Figure 2).[7] This plan requires 4 main points: 1- knowledge of the epidemiology of HCV; 2- improved screening for chronic infection; 3- access to healthcare and 4- an economic model authorizing the treatment of all infected patients.

Key points

- The goal of the World Health Organization's plan for the elimination of viral hepatitis is to reduce new hepatitis infections by 30% and 90% in 2020 and 2030, and associated mortality by 10% and 65% respectively.
- Interventions and targets include improving HBV vaccination, prevention of mother-to-child transmission of HBV, improving the safety of blood products and injections, risk reduction policies and the diagnosis and treatment of hepatitis.
- Successful elimination is uncertain owing to the higher rate of new viral infections than diagnoses, and of new infections than treatments.
- France, like 10 other countries, is on track to reach the goals of the WHO elimination plan by 2030 as a result of the prioritization of oral antivirals followed by universal treatment and coverage.
- With 10 to 15.000 treatment courses per year, the target of 120,000 patients treated by 2022 is at the halfway mark, but increased efforts announced by the French Minister of Health in May 2018 should make it possible to reach elimination goals by 2025.

2 | EPIDEMIOLOGY OF HEPATITIS C VIRUS IN FRANCE

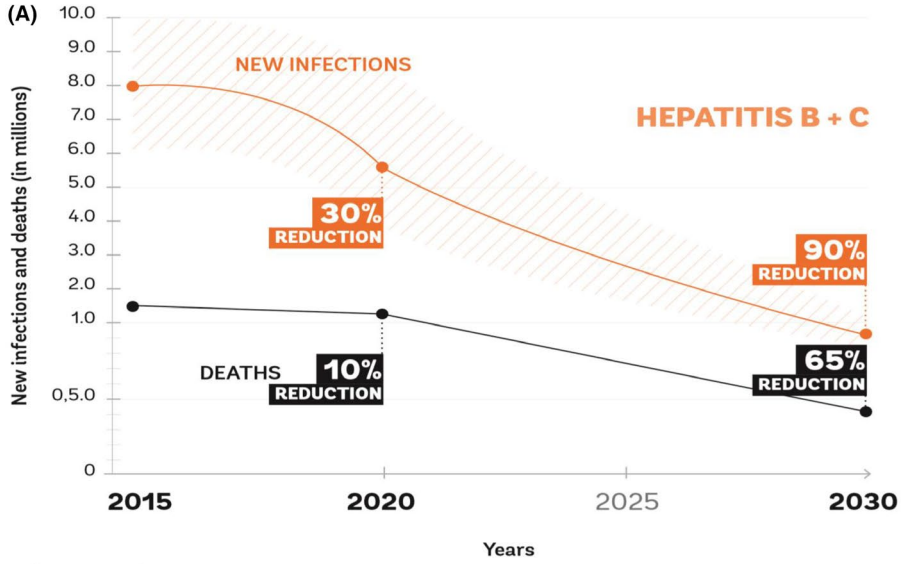
2.1 | General considerations

Very few countries have unbiased population-based data on the prevalence of HCV at the country level since reporting of acute (most often undiagnosed) and chronic HCV infection is rarely mandatory. Information is usually sparse, provided by prevalence studies in certain populations such as blood donors, persons who inject drugs (PWIDs) or healthcare workers, which cannot provide accurate estimates of the disease burden of HCV. In 2015, the Polaris Observatory was created to monitor and forecast the disease burden for hepatitis C (and B).[7] Data from 100 countries representing more than 85% of the world's population were used to estimate the WHO regional prevalence rates, which were then applied to countries with missing data to estimate the global prevalence of HCV. Experts from 59 countries validated the data used in these models.[7] The extensive study performed by the Polaris team led to estimates in the prevalence of HCV viraemia and genotype distribution. All of these results were endorsed by WHO in its global report 2017.[2]

Besides the wide variations in different geographical areas caused by blood safety measures on one hand and risk reduction policies in patients who inject drug (PWID) on the other hand, the methodologies to evaluate the incidence and prevalence of HCV have certain limitations. In particular, in the absence of universal screening and mandatory declarations of HCV infection, all the results are open to discussion for two main reasons. First, the existence of "hidden populations" creates the risk of underestimation. For example, certain precarious high-risk populations or underdiagnosed groups of patients may not be included, such as those with psychiatric illnesses, who are not usually included in incidence and prevalence rates, while they are 4 to 10 times more frequently infected than the general population. Second, there is a risk of overestimation when models and calculations are based on high-risk populations such as drug users, migrants or prisoners. In addition, figures must be evaluated in dynamic models and regularly updated since they may decrease or increase according to various epidemiological situations, for example, the marked decline in prevalence and incidence in Western countries, in contrast to HCV epidemics associated with the opioid overdose outbreak in USA. Although these data are scarce, they exist. These same models could be used to follow-up measures implemented in each country to reach the global hepatitis strategy.

2.2 | HCV epidemiology in France

In France, two studies using the same methodology and based on data from French national health insurance have clearly shown that 1.2% of the general insured population in 1994[8] had HCV infection. Ten years later an evaluation using the same methodology showed that this figure had decreased to 0.8% (in 2004).[9] Later figures were



Objectives by 2030:

1. 90% aware of HCV infection by 2030;
2. 80% of people treated;
3. 1.4 million deaths (in 2015) to under 500,000 deaths (by 2030);
4. 6-10 million infections (in 2015) to 900,000 infections (by 2030)

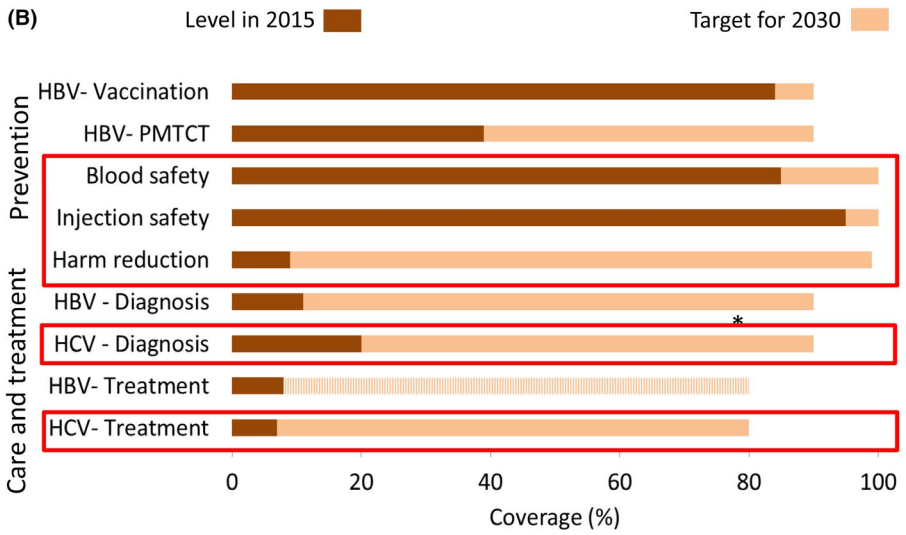


FIGURE 1 Panel A. The objectives of the World health Organization for the hepatitis elimination plan by 2030. Panel B. The interventions and targets and tools of the World health Organization for the hepatitis elimination plan by 2030, including prevention tools and access to care

not based on the same methodology, and were estimates from at-risk populations (drug users, prisoners and migrants but also the general population) suggesting a decrease from 0.8% to 0.55% in 2011 and to less than 0.4% of the general population in 2015. The most recent data (2016) based on the "Santé Publique France" (the French Center for Disease Control) barometer (Barotest) shows that the estimated prevalence of chronic HCV in the general population aged 18 to 75 was 0.3% (95% CI: 0.13-0.70).[10] An estimated 80.6% of infected persons were aware of their status. Thus, approximately 135,000 individuals are infected with chronic HCV in France. This decline in the past 2 decades illustrates both a reduction in prevalence and incidence related to improvement in the safety of blood products after 1992, risk reduction policies in PWID with needle/syringe exchange programs, the death of infected patients as well as an increase in the sustained virological response rate after treatment.[11] For example, the incidence of chronic hepatitis C among drug users, the population at the highest risk, decreased from 7.9% person/year in 2004 to 4.4% person/year in 2011.

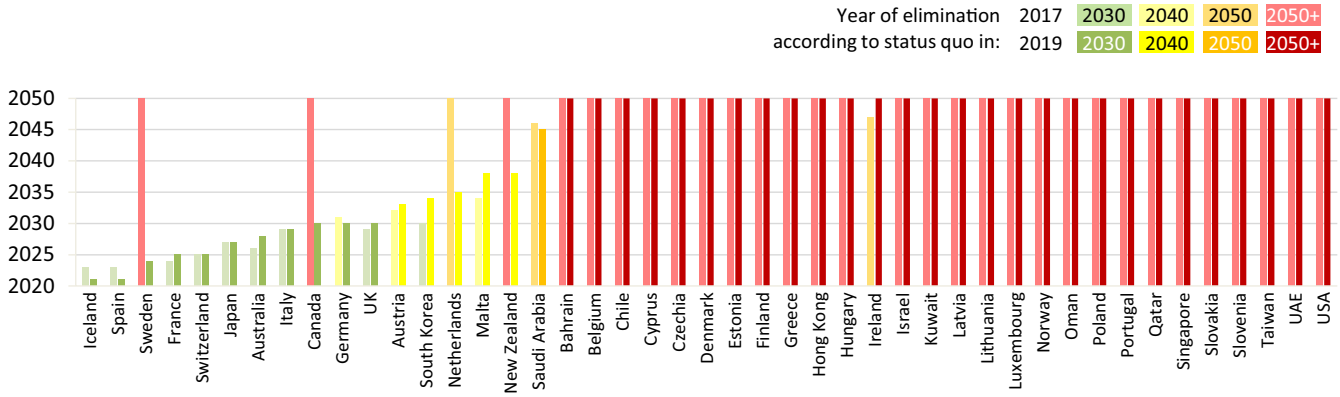


FIGURE 2 The progress towards the WHO's 2030 HCV elimination targets in high-income countries from the Polaris observatory (reference 7, actualized according to the last EASL presentation by Razavi H et al) (Razavi H, et al EASL/ILC2020. #THU365: Global timing of HCV elimination in high-income countries: An updated analysis). In summary, 11/45 high-income countries are on track to meet the2030 HCV elimination target, 5 by 2040 and 1 by 2050. None of these has restrictions on treatment by fibrosis. 28/45 high-income countries are off track by ≥20 years and 11 still have treatment restrictions by fibrosis score

3 | IMPROVED SCREENING FOR HCV CHRONIC INFECTION

Numerous strategies have been considered to improve screening, for example, reflex testing, which corresponds to systematic viraemia in all anti-HCV-positive subjects. Scientific guidelines suggest that this approach significantly improves genomic testing as well as access to treatment. In France, a universal and combined screening option for HCV, HBV and HIV would involve testing a significant proportion of the general population between 15 and 75 years old since one third of this population has never been screened for any of the 3 viruses and 85% have been tested for at least 1 of these viruses.[10]

Santé Publique France experimented with screening for viral infections in the general population with a home self-administered blood test on a blotting paper in 2016.[10] The kit was accepted by 73.4% of participants and returned by 50% of them, for an overall participation rate of 37%, which was identical for men and women. Almost 99% of the blots received could be tested for the 3 infections. These results show a good feasibility/acceptability of screening for HIV and hepatitis B and C at home with this kit which could be an interesting alternative to existing options. Universal screening of the 3 viruses has not yet been approved by French health authorities because it is not cost-effective.

Screening activities must be reorganized to improve screening and access to care. Mobile teams have been created for effective diagnosis and immediate treatment in high-risk populations.[12] This has been evaluated in local initiatives with rapid blood tests accompanied by Fibroscan (non-invasive evaluation of fibrosis) and have been shown to improve access to treatment and a cure in difficult populations. It is clear that these efforts must be accompanied by harm- and risk-reduction measures in drug users (mobile risk-reduction service, injection room and syringe exchange) to limit infection and re-infection in this population.

4 | ACCESS TO HEALTHCARE

Measures to improve the treatment of hepatitis C have been rapidly and extensively implemented in France.[13] Treatment has been made widely available in France, first with interferon and then pegylated interferon combined with ribavirin (an estimated 120 000 courses of treatment before the development of direct antivirals, providing recovery in more than 60% of the population).[13] Between 2007 and 2015, 72 277 patients started at least one antiviral treatment. The yearly number of patients initiating treatment decreased from approximately 13 300 until 2010 to approximately 10 000, then it increased with the introduction of first-generation protease inhibitors (12 500 in 2012 before decreasing to 8400 in 2013) while waiting for second-generation antivirals in 2014 (11 600 subjects treated). Since oral antivirals became available in 2014, more than 60 000 subjects have been treated and 95% of them have been cured.

When oral antiviral treatment became available, France first adopted a policy of prioritizing treatment because of the high costs of these drugs. Liver transplant patients were authorized to receive oral antiviral treatment after December 2013 and patients with extensive fibrosis or cirrhosis and/or with cryoglobulinemic vasculitis in 2014. In 2015 (HIV-infected) and 2016 high-risk populations (men who have sex with men, dialysed or kidney transplant patients) could be treated. Finally, universal access was authorized in April 2017 with full reimbursement of all treatments (there is 100% coverage by French national health insurance for "long-term diseases") and the goal to eliminate hepatitis C by 2025 in France was announced by the French Minister of Health in May 2018.

From January 2014 to December 2017during the 4 years that national health insurance has covered these treatments, 58 943 patients have started treatment with oral antivirals (11 500 in 2014, 13 904 in 2015, 14 291 in 2016 and 19 248 in 2017 with universal access). Patients' median age decreased from 56 to 54 years old and the proportion of men receiving treatment decreased from 65% to

57% with universal access to direct antivirals. This resulted in a significant increase in the number of patients who initiated treatment between 2016 and 2017 (+35%)[13] with more treatment in younger patients and women. The target of 120,000 patients treated by 2022 is at the halfway mark, but increased mobilization should make it possible to reach the goal of elimination by 2025.

The number of treatments can be expected to increase with universal access to oral antivirals and price reductions.

5 | ECONOMIC CONSIDERATION

The cost of a virological cure has significantly decreased in 5 years from 75 000 to approximately 18 500 euros along with a reduction in the duration of treatment from 24 to 8–12 weeks. Effective treatment of the infected population is only possible if it is fully reimbursed. Several studies have shown the cost/effectiveness of these treatments despite their price, which can be significantly reduced by the use of generics (the cost of such a cure is now between 120 and 400 dollars).

In conclusion, chronic HCV infection is the only chronic viral infection that can be cured. With the recent policies including prioritization of treatment to the most severe patients as a first step, then populations at risk and finally universal access without restriction and with complete coverage, France appears to be one of the countries than can remain on track for the elimination of this disease by 2030. Other countries, in particular Iceland, also have policies that should make it possible to reach the WHO HCV elimination targets by 2030.[14,15]

The goal of eliminating HCV in France by 2025 can only be achieved if the diagnosis of chronic infection is reinforced (with rapid serological and virological diagnostic tests), through diagnostic outreach programs and therapeutic care with access to direct oral antivirals, and by using non-invasive tests to evaluate fibrosis. The elimination of HCV infection should not overshadow the importance of co-morbidities related to alcohol and the metabolic syndrome[16,17] which contribute to the worsening of liver disease and which should be included in a model of multidisciplinary management of patients infected with HCV, who often have other comorbidities.

CONFLICT OF INTEREST

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SUPPLEMENT ARTICLE

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Is elimination of hepatitis C virus realistic by 2030: Eastern Europe

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Abstract

The WHO elimination goals (diagnosis of 90% of the cases of hepatitis C virus (HCV), treatment coverage in 80% and a 65% reduction in deaths from HCV) are set to be reached by 2030. Although these elimination programmes are extremely important in the Eastern European countries (Russia, Ukraine, Belarus and Moldova) with a high prevalence of HCV, limited economic resources prevent their development and implementation. Regardless of the decrease in the incidence HCV in all Eastern European countries, low diagnosis and treatment access, especially in high-risk populations, will not allow to achieve HCV elimination or even to control the infection by 2030.

KEYWORDS

Eastern Europe, elimination, HCV

Key points

- Nearly a half of all patients with hepatitis C virus (HCV) infection in Europe live in Eastern European countries (Russia, Ukraine, Belarus and Moldova).
- People who inject drugs will be the main source of new cases of HCV in Eastern European countries in the next 5-10 years as a result of the poor efficacy of harm reduction programmes and poor access to HCV treatment.
- Despite differences in the populations, economies and healthcare systems of the Eastern European countries, the barriers to elimination are similar (lack of awareness of HCV, underestimation of the economic burden, limited funds and resources for testing and universal treatment access).
- In 2020, none of the Eastern European countries was on track to reach WHO elimination goals by 2030.

1 | INTRODUCTION

The estimated prevalence of hepatitis C virus (HCV) in Europe is 1.7% representing over 13 million cases,[1] with nearly the half of them living in Eastern European (EE) countries. The prevalence of HCV infection varies (2%-5%) in EE countries as does the population densities, economies, healthcare systems and public awareness of the disease. Thus, it is logical to evaluate the perspectives of HCV elimination in each country separately, focusing on the major factors that may negatively or positively influence the planning and implementation of the HCV elimination programme.

2 | RUSSIA

Russia is the largest country in EE with a population of more than 146 million people. The prevalence of HCV is high (4;1%), and Russia has the highest estimated number of cases of HCV infection (approx.

Abbreviations: CHC, chronic hepatitis C; DAAs, direct-acting antivirals; HCV, hepatitis C virus; IDU, intravenous drug use; PWID, people who inject drugs.

5 million).[1-3] However, the real number of patients is unknown, although official statistics reported 591 830 registered patients with chronic hepatitis C (CHC) by the end of 2016.[4] While the incidence of HCV infection is high, it has gradually declined in the last 10 years. Thus, the incidence of acute hepatitis decreased from 2.1/100 000 in 2010 to 1.0/100 000 in 2019.[5]

In 2019, 45 400 new CHC cases were registered and the incidence rate was 30.9/100 000, which is substantially lower than 10 years ago, 40.2/100 000. Nevertheless, the incidence was highly variable depending on the region.[5] This high geographical variability in the prevalence of HCV was confirmed in a study of 4764 blood samples from 5 Russian regions obtained from the healthy population. Anti-HCV antibodies were found in 2.6% (126/4764) of samples, and the prevalence of HCV varied from 1.3% to 3.3% in different regions. However, HCV-RNA was only found in 1.1% (50/4764) of samples, and there was no HCV-RNA found in children (0-14 years old), but an increase in HCV-RNA positivity in older age groups.[6] This long-term trend and a cohort phenomenon of a persistent annual decrease in the incidence of CHC were also found in a 20- to 29-year-old cohort, in which the incidence gradually decreased from 64/100 000 in 2011 to 38.3/100 000 in 2016.[4] According to this analysis, nearly half of all CHC cases are found in the 30- to 49-year-old age group, which is a decade younger than in Western European countries. The most prevalent HCV genotypes in Russia are genotype 1b (48.9%-58%) and 3a (34%-39.6%), while genotypes 2 (7;8%) and 1a (3.7%) are rare.[4,6] The distribution of HCV genotypes is also influenced by a cohort phenomenon. While HCV genotype 3a is extremely rare in older groups of patients, nearly half of the group of younger patients (30-39) was infected with this genotype[6] and many of them were infected as a result of intravenous drug use (IDU). According to recent national statistics, the prevalence of drug abusers was high (1293.35/100 000 in 2019) and 44.1% of them still use drugs intravenously.[7] Therefore, IDU is still one of the major modes of transmission of HCV in Russia (Table 1). According to a modelling study, 100% of new HCV cases associated with IDU from 2018 to 2030 could be prevented if the additional HCV transmission risk caused by IDU was removed.[8] This suggests the need for extensive syringe exchange programmes, opioid substitution programmes and extensive treatment of high risk groups, with a special focus on people who inject drugs (PWID). However, in Russia, there are no specific programmes for the treatment of HCV in PWID, no opioid substitution programmes and only 20 centres for syringe exchange.[8]

Considering the estimated number of cases of HCV infection in Russia as well as the prevalence of cirrhosis and the associated mortality, one would expect the economic burden of HCV to be high. However, according to a national report chronic HCV infection is only ranked 14 among all infectious diseases, with an economic loss of 1.7 billion Russian rubles (USD 22.5 million) during 2019, which is much lower than for HIV, tuberculosis and many other infectious diseases.[5]

Although all known direct-acting antivirals (DAAs) combinations except SOF/VEL/VOX have been approved in Russia, access to treatment is restricted by the stage of liver disease (F3/F4) and the financial resources of the region where the patient lives. Infectious disease specialists or gastroenterologist/hepatologists usually prescribe treatment, and reimbursement is provided by the regional registry of hepatitis C patients and only in specialized centres. In 2019, 6.2 billion rubles (USD 83 million) was spent for reimbursement of HCV treatment, for the treatment of approximately 15 600 patients.[9] There are no DAAs generics approved in Russia, but under Russian law, individual citizens can import non-registered medicines for their personal use. As a result of restricted access to the reimbursement of HCV treatment, increasing numbers of Russians are treating their HCV infection with generic drugs produced in India, China or Egypt at prices that are 10 times lower than the drugs approved in the country. Although the efficacy of these generics is expected to be the same as the original drugs,[10] it is not possible to estimate the number of patients who treat themselves with these drugs because there are no statistical data.

The elimination of HCV by 2030 is not possible in Russia because of the high prevalence/incidence of HCV, as well as limited access to

TABLE 1 Key factors for the development of elimination programmes in Eastern European countries

	Eastern European countries			
	Belarus	**Moldova**	**Russia**	**Ukraine**
HCV prevalence, % /estimated number of patients[2,3]	2%-3%/250 000	4%/142 000	4,1%/4,5—5 000 000	5%/2 100 000
HCV prevalence in children, % (95% CI) /estimated number of cases (95% CI)[13]	0,41% (0,01-0,42)/7900 (120-8200)	0,44% (0,01-0,46)/3600 (50-3700)	0,37% (0,26-0,39)/118 000 (80 500-123 000)	0,54% (0,01-0,56)/46 500 (700-48 100)
HCV prevalence in PWID, % (95% CI)/ estimated number of PWID with active HCV infection (95% CI)[12]	43,7 (32,3-55,1)/18 000 (7000-31 500)	37,5 (25,5-49,7)/4500 (2500-7000)	51,6 (44,2-58,9)/969 500 (463 000-1 570 500)	40,4 (36,3-44,6) 129 000 (54 000-222 000)
Estimated number of compensated cirrhosis patients/mortality per 100 000[30]	231 686/17,5	137 489/55,6	3 913 270/24,3	1 289 123/31,7
Average number of HCV treatment courses reimbursed per year	6000	4500	15 600	2668

Abbreviations: HCV, hepatitis C virus; PWID, people who inject drugs.

treatment (the number of patients who are reimbursed for treatment is 2.5 times lower than the number of new cases every year). Decision makers do not recognize HCV infection as a significant problem because of its low economic impact compared to other infectious (HIV and tuberculosis) or non-infectious diseases, which are on the top of a list of the most common causes of death in Russia. However, if this situation is not managed, the number of patients with HCV in Russia could double by 2030. Universal access to treatment should be the first step in preventing this scenario, but this is only possible if DAAs generics are approved and can be prescribed by all doctors. Also, universal diagnosis of HCV in high-risk groups such as IDUs is needed and treatment should be provided to all infected individuals as soon as possible to break the chain of transmission of HCV and markedly decrease its incidence, thus helping to control future infections. Although the 2019 national report stated that a programme for the prophylaxis and treatment of HCV should be created to reach the WHO target of eliminating HCV as a major public health threat by 2030,[5] the start of this programme has still not been announced.

3 | UKRAINE

Ukraine is the second largest country in EE with a population of 42.7 million as well as a high estimated prevalence of HCV (5%) and a high number of patients with HCV infection (2.1 million).[2] Recent data reported that 470 new cases of acute hepatitis C were registered in 2017 and 5714 patients with CHC, and the incidence of acute hepatitis C/CHC was 1.1/13.42 per 100 000 inhabitants.[11] The incidence of CHC varies considerably in the different regions of the country, ranging from 3.0/100 000 to 28.37/100 000. These data are based on extensive testing of the population for HCV infection. Indeed, from 2013 to 2016, 4 976 448 individuals were tested and 205 449 (4.13%) were found to be HCV positive. Like other EE countries, the most prevalent HCV genotypes in the Ukraine are genotypes 1b (42.1%) and 3 (28.8%). It is interesting to note that the highest percentage of mixed genotypes (25.1%) was also found in Ukraine compared to Russia (0.6%) or to other EE countries (0%).[3] IDU is an important source of transmission of infection because at least 40% of PWID are HCV positive and there are an estimated 129 000 PWID with HCV.[12] A large international modelling study[13] showed that Ukraine had the highest prevalence of HCV in children among the EE countries (Table 1). However, local data showed that between 2013 and 2017 there was a decrease in the incidence of acute hepatitis C from 0.24 to 0.11 and in CHC from 0.62 to 0.55 per 100 000 children (0-17 years old) with a total of 600 cases registered by 2017.[6] Illicit drug use has increased in the last 5 years in Ukraine in teenagers from 12% to 18%,[14,15] which may be one explanation for the high prevalence of HCV in children. According to the modelling study, the estimated prevalence of HCV infection in teenagers (aged 12-18) was the highest of all the EE countries, 1.06% with up to 30 000 cases.[13] The prevalence of CHC was found to be much lower than estimated. According to statistical forms, 51 848 patients with CHC were registered in Ukraine by 2017.[11] This publication showed that the proportion of chronic viral hepatitis in the category "chronic hepatitis" increased persistently from 2013 to 2017 from 17.79% to 24.54%, and that this phenomenon could be explained by the increase in incidence as well as by better diagnostics. The prevalence of chronic HCV infection was 123.7 per 100 000 in 2017, with an average prevalence during the 5-year period of 112.7. There was a high variability in the different regions of the country from 44.86 to 314.22 per 100 000.[11] Only 15.5% of the patients were older than 55, therefore most chronic hepatitis patients are younger than in other EE countries.

All DAAs combinations except SOF/VEL/VOX are approved in Ukraine and, like in other EE countries, the access to treatment is mainly determined by the stage of liver disease and the cost of drugs. Between 2013 and 2017, a total of 13 340 courses of treatment were reimbursed from central or regional budgets as well as from different non-governmental sources of funding. However, at the beginning of 2018, 24 786 patients were still on the waiting list for treatment and 5873 needed immediate treatment because of the stage of disease.[11] In 2017, Gilead expanded their HIV and HCV licensing programme to include Ukraine and Belarus, granting access to the generics of sofosbuvir, SOF/LED and SOF/VEL. The generics were approved, and a recent publication stated that the project using generic drugs to treat hepatitis C in Ukraine, run by Médecins Sans Frontières, had been transferred to local authorities in July 2020 with hopes that it would be implemented across the country.[16]

Elimination of HCV in Ukraine by 2030 will not be possible because of the high prevalence/incidence of HCV, especially in teenagers as well as the limited access to treatment. However, a national strategy for the elimination of viral hepatitis was developed with the help of the CDA Foundation and World Hepatitis Alliance, which is now under review. There are several reasons to expect this national strategy to be successful. First, DAAs generics were approved, which is essential for programmes in low-income countries with a high prevalence of HCV, and also, the opioid substitution and syringe exchange programmes for PWID are well established, which is essential to begin universal testing and treatment to reduce the HCV transmission rate in this group of patients. However, all of these measures require both funds and human resources.

4 | BELARUS

The Republic of Belarus has a population of 9.4 million and a high prevalence of HCV infection similar to other EE countries.[17] In 2019, 3420 cases of HCV infection were registered (incidence 36.09/100 000), including 72 cases of acute hepatitis C (0.8/100 000), 2889—CHC (30.5/100 000) and 159 (1.7/100 000) patients who were anti-HCV positive, but in whom the HCV-RNA test was not yet performed. There is a long-term trend towards a decrease in the incidence of acute and CHC in Belarus. The incidence of acute hepatitis was the lowest among EE countries 0.7/100 000 in 2017 and in the last 10 years it has never been higher than 1.1/100 000. A similar trend can be noted in CHC, with a marked decline between 2008 and 2017

from 71.5 to 45.9/100 000 with an average decrease of 3.2%.[18] According to one international review, the estimated number of patients with HCV is around 250 000;[2] however, the most recent local publication reported that 33 830 patients with chronic HCV were officially registered at the end of 2018, and the estimated number of patients is 136 500.[19]

The distribution of HCV genotypes in Belarus is similar to that in Russia and Ukraine. The results of a study of 887 patients with HCV infection showed that HCV genotype 1b was found in 59.8% of patients, genotype 3a in 27.7%, genotype 1a in 7.1% and genotype 2 in 3.3%.[20] Like in other EE countries, the distribution of genotypes is dependent upon a cohort phenomenon (patients with HCV genotype 1b are older than those with genotype 3a) and also they differ in the mode of transmission of HCV. The main possible mode of transmission indicated by patients with HCV genotype 1b was a previous medical intervention or it was unknown, while it was IDU or non-medical events such as tattoos or piercing in patients with genotypes 3a or 1a.[20] IDU is expected to be the main mode of transmission of HCV in the next 5-10 years because the prevalence of drug abuse increased from 63/100 000 in 2005 to 100,7/100 000 in 2018, when drug dependence was diagnosed in 9593 persons.[17] However, modelling studies have shown that the estimated number of PWID with HCV viraemic infection was 18 000, which only represents 43.7% of the total number of PWID, which is possibly 4 times higher than the official registers.[12] Although harm reduction programmes have been developed in Belarus, the number of distributed syringes (27 per PWID per year) is lower than in Ukraine or Moldova and only 2 of 100 PWID are provided with opioid substitution therapy.[21] Analysis of the efficacy of the methadone substitution programme showed that by 2019 only 728 patients were on methadone substitution therapy in Belarus. In Minsk, the largest city in the country, 478 patients were enrolled into the programme between 2009 and 2019 and 339 dropped out, with an average number of active participants per year of only 149. During this period, the number of HIV-positive patients among these programme participants increased up to 26.3%, and all of them were receiving anti-HIV treatment. However, 100% were also HCV positive, but none of them was treated for viral hepatitis.[22]

All known DAAs combinations, except SOF/VEL/VOX, have been approved in Belarus, but access to reimbursement is restricted, like in other EE countries.[19] However, unlike Russia, where access to the treatment is highly dependent upon the region the patient lives in, the indications for and access to treatment in Belarus are regulated by order of the Ministry of Health (June 1, 2017), which determines which groups of patients have priority for reimbursement of treatment. Thus, reimbursement is provided to patients with advanced liver fibrosis (F3/F4), extrahepatic manifestations, post-transplant patients, advanced CKD/dialysis, HBV and HIV co-infection, women who plan pregnancy and medical workers. Major DAAs generics (SOF, DAC and SOF/LED) have been approved and are locally produced in Belarus, providing a better cost/efficacy ratio for antivirals and easier access to treatment. Their efficacy has been confirmed in local studies,[23] which have shown results similar to up-to-date real-world data. Treatment guidelines are regularly updated, with the last update published in 2019.[19] In 2018, 2000 treatment courses (SOF/DAC and SOF/LED) were reimbursed by the government and this figure was multiplied by three times for 2019 (Table 1).[19] It should also be mentioned that in 2018 more than 3000 treatment courses were bought by patients themselves through pharmacies. Like in Russia, individual citizens in Belarus can import non-registered drugs for their personal use, thus some patients may have obtained less expensive Indian or Chinese generics than the generics at local pharmacies. However, it is not possible to estimate the number of patients, as there is no statistical data. Therefore, from 9000 to 10 000 treatment courses were administered in Belarus in 2019, and at least 6000 of them were reimbursed.

Belarus is the only country in EE which has a good chance of if not eliminating than controlling HCV infection by 2030. Unlike other EE countries, it has a centralized state reimbursement system with a clear indication for priority treatment, and it can provide treatment yearly to at least twice the number of patients as the number of new cases registered per year. Local production of DAAs generics is the most important reason that the number of patients treated every year can be increased, as the prices of these drugs allow the government to reimburse more treatments. Second, when a certain number of treatment courses are provided per year (15 000-20 000), the healthcare system requires more doctors/nurses to prescribe and distribute drugs and, thus, actively looks for new patients in risk groups or certain cohorts. In EE countries, the modernization of the healthcare system has often caused a decrease in the number of beds in hospitals and in medical workers. However, the highest number of doctors in EE countries is found in Belarus (58.5/10 000),[17] which makes it much easier to organize the treatment and distribution of DAAs than in other EE countries. One of the major difficulties of preparing and implementing national programmes for the elimination of HCV (once they have been developed and approved) will be the treatment of PWID. Although syringe-exchange programmes and opioid substitution programmes exist, they are not effective, while the prevalence of drug abuse is increasing, access to treatment for PWID is low and there is a lack of special programmes for HCV screening in this population. All of these factors could significantly reduce the effect of increased access to treatment to other groups of patients, by rapidly spreading HCV through the PWID population.

5 | MOLDOVA

The Republic of Moldova has a population of 2.68 million with a high prevalence of HCV infection that is similar to that in other EE countries, and an estimated 142 000 patients.[2] The incidence of acute hepatitis C has gradually decreased in the last 20 years from 3.72 in 2000 to 1.26/100 000 in 2017, but it is still the highest of all the EE countries. The incidence of CHC also decreased from 46.7 in 2011 to 34.6/100 000 in 2016, however, the officially registered number of patients (13 432) is much lower than estimated.[24] The distribution of HCV genotypes is different from other EE countries because there is

a majority of genotype 1b (95.5%), with a low per cent of genotypes 3a and 2.[25] This may be as a result of the modes of transmission of HCV in acute hepatitis patients. It is unknown in 70%, because of medical interventions in 12% and surprisingly only 4.2% are as a result of IDU, which is much lower than in other EE countries. By the end of 2018, the number of officially registered people who used drugs in Moldova was 11 805 and 3664 (31%) of them were PWID, [26] which is a lower proportion than in other EE countries. However, it still represents one of the major modes of transmission of HCV in the population. Nosocomial transmission of HCV is still significant in Moldova as seen by the high seroprevalence of HCV in healthcare workers (4.4%), dentists (7.8%) and in haemodialysis patients (43.2%).[27]

Although the major DAAs combinations have been approved in Moldova, access to reimbursement is restricted to generics (SOF, DAC and SOF/LED), which are provided by a national programme. Four national programmes for the diagnosis and treatment of viral hepatitis have been implemented since 1997 in Moldova. They included different measures to decrease transmission, vaccination programmes as well as improving diagnosis and treatment for patients and high-risk groups. The goal of the most recent national programme (2017-2021) was to reduce the incidence and prevalence of acute and chronic viral hepatitis B, C and D and cirrhosis caused these viruses by 50% by 2021.[24] In a recent report, a total of 15 754 patients received antiviral treatment with DAAs in the national programme before June 2020.[28] SOF/LED was used in 60.1% of treated patients, while the others received SOF/DAC. An SVR was achieved by more than 99% in all groups of patients except in those with HCV genotype 3 (SVR 93.6%). Patients had universal access to treatment independently of the stage of liver disease (26.2% F4, 16.5% F3, 19.1% F2, 26.6% F1 and 116% F0).[28]

Moldova has had extensive experience in the implementation of national programmes against viral hepatitis for more than 20 years, with success in decreasing the incidence of acute hepatitis, reducing the prevalence of chronic hepatitis especially related to HBV, but also in the incidence of CHC. The estimated economic loss prevented by these national programmes from 1997 to 2015 was 822.4 million lei (USD 48 million), while the cost to the National Budget was only 84.6 million lei (USD 4.9 million).[29] Although the number of patients with HCV treated every year exceeds the number of new cases registered per year, this is not enough to reach the WHO target for elimination of HCV by 2030. Moldova has a high rate of migration (at least 10% of population), thus, the real number of new cases of HCV is difficult to evaluate. Owing to the high total number of untreated patients in the population, the number of patients treated every year must be increased by at least three-fold to achieve the WHO goal by 2030. A significant nosocomial HCV transmission rate and a high proportion of PWID will also make it difficult to decrease the incidence of new CHC cases in the next 5-7 years.

In conclusion, it is obvious that none of the EE countries will be able to achieve elimination of HCV by 2030. Despite differences in economies, populations and healthcare systems, the difficulties preventing the elimination of HCV are common to all EE countries. Many decision makers are still not convinced that the economic burden of HCV is significant enough in their countries because HCV infection is not highly ranked on the national lists of diseases with a high disability and mortality rate. The prevalence of HCV is high in all EE countries, therefore, even in countries with national programmes the aim is not to eliminate, but to control HCV infection by the end of the programme. Lack of funds and human resources for the decentralization of access to treatment is common to all EE countries, partly owing to the modernization of the healthcare system in the last 20 years, which has greatly reduced the total number of doctors and nurses. Not all countries have approved locally manufactured DAAs generics, which is essential for universal access to treatment. PWID will be the main source of HCV infection in the next 5-10 years in all EE countries and although most countries have harm reduction programmes, their efficacy is poor. Special programmes for extensive testing and treatment of HCV in PWID are badly needed because the number of PWID is increasing in all these countries and nearly half of them are HCV positive.

CONFLICT OF INTEREST

All authors have nothing to declare.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

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SUPPLEMENT ARTICLE

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Is elimination of HCV in 2030 realistic in Central Europe

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Abstract

According to the recent data presented by Central-European HCV experts, the estimated prevalence of HCV is between 0.2% and 1.7% in certain countries in this region. There are no financial limitations to access to treatment in most countries. Patients in these countries have access to at least one pangenotypic regimen. The most common barriers to the elimination of HCV in Central Europe are a lack of established national screening programmes and limited political commitment to the elimination of HCV. Covid-19 has significantly affected the number of patients who have been diagnosed and treated, thus, delaying the potential elimination of HCV. These data suggest that the elimination of HCV elimination projected by WHO before 2030 will not be possible in the Central Europe.

KEYWORDS

epidemiology, hepatitis C virus, liver, therapy

Key Points

- According to the recent estimations, the prevalence of HCV is between 0.2% and 1.7% in certain Central European countries.
- There are no financial limitations to access to treatment including pangenotypic regimens.

Abbreviations: COVID-19, coronavirus diseases 2019; DAA, direct-acting antivirals; G, genotype; GLE/PIB, glecaprevirem/pibrentasvir; HCV, Hepatitis C virus; NHIF, National Health Insurance Fund; PWID, people who inject drugs; SOV/VEL, sofosbuvir/velpatasvir; SOV/VEL/VOX, sofosbuvir/velpatasvir /voxilaprevir; WHO, World Health Organization.

- The major barrier to HCV elimination in central Europe is lack of national screening programmes and lack of political commitment to make the elimination of HCV a priority.
- The Covid-19 pandemic has significantly affected the number of treated patients.
- The elimination of HCV before 2030 is not possible in central Europe.

1 | INTRODUCTION

The availability of highly effective and safe direct-acting antivirals (DAA) markedly changed the treatment of Hepatitis C virus (HCV) infection. In addition, this therapeutic revolution, whose scale is unprecedented in the history of medicine, has stimulated epidemiological research in the simulation and prediction of hepatitis C and its consequences with projects on the global, regional and national levels in different regions of the world.[1-3] New therapeutic regimens have resulted in the elimination of waiting lists for treatment, the cure of patients in whom previous interferon-based therapy had failed and in those with advanced liver disease. Unfortunately, a large group of patients with HCV infection remain undiagnosed and are at risk of developing cirrhosis and/or hepatocellular carcinoma.[3] In 2016, the WHO announced a plan to eliminate viral hepatitis as a public health threat by 2030.[4] Estimations suggest that this target will be difficult to achieve in most countries.[5] A recent analysis of expert data from eight Central European countries (Bulgaria, Croatia, Czech Republic, Hungary, Latvia, Lithuania, Poland and Slovakia) showed that while HCV could be eliminated in some countries, it would not be possible in the whole region.[6] Unfortunately, this did not include the effect of the COVID-19 pandemic, so conclusions must be re-evaluated.

2 | EPIDEMIOLOGICAL SITUATION

As shown in Table 1, the prevalence of HCV in Central Europe is between 0.2% in Slovakia and 1.7% in Latvia, with an estimated total number of 410,000 infected individuals in these countries. The most frequently diagnosed infection is genotype (G) 1b, followed by either G1a in the southern (Bulgaria, Croatia, Czech Republic and Hungary) or G3 in the northern countries (Latvia, Lithuania, Poland and Slovakia) of the region. Patients with HCV infection are eligible for reimbursement of treatment in all countries (Table 1).

3 | TREATMENT OPPORTUNITIES

Despite certain limitations related to the stage of the disease and a lack of access to treatment in people who inject drugs (PWID) in Slovakia, there are no longer any restrictions that cause waiting lists for treatment. Based on data between 2015 and 2019, the number of treated patients have been stable or is increasing except in Poland and Hungary, which already reached the highest volume of treatment in 2017 or 2018 and has since begun decreasing as a result of a low rate of diagnosis (Table 1). Unfortunately, owing to the outbreak of COVID-19, the number of treated patients was reduced in some countries by more than 50% (Poland and Slovakia). The only country that seems to be unaffected is the Czech Republic. Patients in Central Europe have access to at least one basic pangenotypic regimen with glecaprevirem/pibrentasvir (GLE/PIB) or sofosbuvir/velpatasvir (SOV/VEL), and also have some access to SOV/VEL combined with voxilaprevir SOV/VEL/VOX. However, the use of pangenotypic regimens in 2019 varied from 15% in Hungary to 88% in the Czech Republic (Table 1).

4 | BARRIERS OF HCV ELIMINATION

Data show that the most common barrier to elimination of HCV in these countries is a lack of political commitment to make HCV a priority, as the countries in this region have adequate coverage of HCV therapy but the budget needs to be approved every year. There are still certain restrictions to access to treatment for uninsured patients (Bulgaria, Hungary and Slovakia), active alcohol and drug users (Croatia, Czech Republic and Poland), as DAA failures (Latvia). Access to pangenotypic regimens is reduced by complicated procedures for the reimbursement of these therapeutic options in Hungary. All of the Central European companies are limited by poor linkage to care because of the number of out-patient visits needed for reimbursed protocols as well as insufficient healthcare staff. Since none of the reporting countries has established a national screening programme, the WHO 2030 target would seem to be impossible to achieve. However, according to expert opinions from Croatia, Latvia and Lithuania, the WHO 2030 target can still be reached. Representatives from Hungary and Slovakia feel that the goal could be reached under certain conditions, whereas Bulgaria, Czech Republic and Poland are pessimistic about reaching the WHO 2030 target. A scoring system was created to evaluate the factors affecting the elimination of HCV by the year 2030 (Table 2). The highest score (31) of all the factors that affect the elimination of HCV was financial coverage of treatment, which corresponds to no limitations for the reimbursement of treatment. The lowest score (11) was the presence of national screening programmes, which **should be considered?** the most relevant barrier.

5 | EFFECT OF COVID-19

The global consequences of COVID-19 have recently been calculated in relation to the elimination of HCV and showed that a "1-year-delay"

TABLE 1 Characteristics of HCV infections in selected Central European countries [Flisiak-CEH]; data provided by national experts

	Bulgaria	Croatia	Czech Rep.	Hungary	Latvia	Lithuania	Poland	Slovakia
HCV RNA (+) prevalence—n, %	80 000 1.1%	20 000 0.6%	40 000 0.5%	30 000 0.3%	40 000 1.7%	25 500 0.9%	150 000 0.4%	10 000 0.2%
Genotypes prevalence in 2019								
1a	26%	30%	20%	5%	5%	11%	5%	16%
1b	59%	25%	41%	86%	52%	52%	75%	51%
2	1%	2%	0	0	2%	5%	0	0
3	14%	39%	37%	3%	37%	22%	13%	31%
4	0	4%	1%	0	0	0	6%	2%
Other	0	0	1%	6%	4%	10%	1%	0
Number of treated in particular years								
2016	720	179	622	916	486	966	8000	450
2017	1325	342	620	928	1173	998	11 700	350
2018	1230	440	648	2446	1632	1164	7100	400
2019	1000	468	1360	1332	3000	1816	8500	400
2020 (expected)	1000	400	2500	1000	2250	1100	3500	150
Regimens administered in 2019								
GLE/PIB	40%	52%	57%	8%	27%	82%	40%	60%
SOF/LDV	16%	7%	2%	18%	0	0	7%	17%
SOF/VEL	32%	29%	26%	5%	10%	0	25%	7%
SOF/VEL/VOX	0	2%	5%	2%	0	0	0	2%
GZR/EBR	12%	10%	7%	40%	31%	18%	28%	14%
OBV/PTV/r ± DSV	0	0	3%	27%	28%	0	0	0
SOF + RBV±PegIFN	0	0	0	0	0	0	0	0
Other	0	0	0	0	4%	0	0	0
Effect of COVID-19 on HCV elimination								
Did COVID-19 affected HCV elimination?	yes	yes	no	Yes	Yes	yes	yes	yes
HCV treated in 2020, compared to 2019	50%-100%	=100%	>100%	50%-100%	50%-100%	50%-100%	<50%	<50%

TABLE 2 Score for particular factors affecting HCV elimination, from 0 (minimal) to 4 (maximal)

	Bulgaria	Croatia	Czech Rep.	Hungary	Latvia	Lithuania	Poland	Slovakia	Sum
Political will	1	3	3	3	2	2	1	1	16
Financial coverage of therapy	4	4	4	4	4	4	4	3	31
No treatment restrictions	4	3	3	3	3	3	3	1	23
Medical staff capacity	3	3	3	3	2	3	3	3	23
National screening programme	2	2	0	1	2	1	1	2	11
Linkage to care programmes	2	3	3	2	3	2	1	1	17

scenario could result in 44 800 excess hepatocellular carcinoma cases and 72 300 excess liver-related deaths.[7] Data from national experts show that the total number of treated patients, which was almost 18 000 in 2019, are expected to decrease to fewer than 12 000 in 2020. The most significant decline is expected in Poland and Slovakia, while the Czech Republic is the only country with an increase (Table 1). The specific situation in each country is presented below.

5.1 | Bulgaria

In Bulgaria, a prescription for anti-HCV therapy is managed by the National Health Insurance Fund (NHIF) and can only be prescribed by gastroenterologists working in 13 gastroenterology clinics. In 2020, there was lockdown in the country for 2 months—March and April—and all screening activities and new prescriptions for HCV

infection were stopped during that time. Patients on therapy were monitored. All activities were gradually resumed in the following months. According to NHIF, 288 patients were treated until April and by the end of August there are 580 patients being treated or on treatment. The number of treated HCV patients for 2020 is expected to be nearly the same as in 2019. Screening activities for HCV are mainly performed in high-risk groups (PWUD and prisoners) by scientific organizations. The new triple regime SOV/VEL/VOX is also reimbursed by the NHIF since March. No additional funding has been provided from the state so far for people without health insurance.

5.2 | Czech Republic

In the Czech Republic, anti-HCV therapy is provided in 22 specific centres by gastroenterologists and infectious disease specialists. Patient recruitment was lower in March and April as a result of a decrease in screening in low-threshold centres managing PWIDs. However, patient screening and recruitment resumed in May 2020 with an increase in the number of patients starting treatment, and is expected to double the number of patients treated in 2020 compared to 2019.

5.3 | Croatia

Although they are preoccupied by COVID-19 cases, the largest ID centres in Croatia (Zagreb, Split) are trying to maintain outpatient viral hepatitis departments active as non-COVID departments. However, the number of newly diagnosed patients decreased by 30% from March to September 2020, and in July there were no "first-time" patients at all. The Croatian Insurance Fund approves all treatments requested by specialists, so there are no patients waiting for therapy. However, the total number of treated patients is decreasing in Croatia in 2020. The biggest problems are limited screening because of the number of cases of COVID-19 in primary care, OST centres, the lack of outreach programmes and the closing of voluntary testing centres. Although a national action plan is being drafted, it has still not been endorsed by the government. The 2030 WHO goals will be challenged.

5.4 | Hungary

The care of patients with HCV infection is managed by infectious diseases specialists, gastroenterologists and specialists in tropical medicine. Although the budget for treatment and healthcare personnel is nearly sufficient, low rates of screening, referral and linkage to care are insufficient to achieve elimination, a situation that has worsened with COVID-19. Although there is no ban on the treatment of HCV, state-based providers have been basically restricted to providing emergency care for almost 3 months, reducing HCV-related activities to a minimum. Most patients and providers were restrained from seeking/providing services for HCV during that period. Moreover screening activities were significantly reduced, and the initiation of new treatments was down by 50% during April, May and mid-June 2020. Although the restrictions were withdrawn in mid-June, the total number of treated patients will probably be reduced by 30%-40% compared to 2019. This is only one third of the patients who need to be treated annually to reach the WHO 2030 target in Hungary.

5.5 | Latvia

Treatment of HCV infection is managed by infectious disease specialists in Latvia. There are 5 specialized centres where meetings are organized and treatment is prescribed. After July 2020, approval from the National Health Center is not acquired for most treatment schedules, which accelerates access to treatment. However, there was a quarantine in Latvia for 3 months because of Covid-19 infection and out-patient visits were severely limited. Thus, the number of treated patients decreased, and are expected to have decreased by ~25% compared to last year. At the same time there is financial support for HCV treatment and screening programmes are planned in psychiatric hospitals, social care centres and shelters.

5.6 | Lithuania

The Lithuanian government issued a quarantine for 3 months from March 16 to June 16 as a result of COVID-19 infection. During that period, most consultations with physicians were perfumed remotely and the number declined significantly. In the first half of 2020, the number of newly treated HCV patients fell by 47.5% compared to the same period in 2019. After the end of quarantine, the number of HCV patients treated began to increase again. The total number of patients treated in 2020 is expected to be around 60% of that in 2019. The planned National HCV Screening Program has been postponed indefinitely as a result of COVID 19 infection, therefore the previously optimistic expert forecast on the implementation of the WHO 2030 target in Lithuania might be more pessimistic and delay the date for the elimination of HCV.

5.7 | Poland

HCV therapy has always been managed by infectious disease specialists. Patient recruitment in January and February was similar to that observed in the same months of 2019, but it then decreased in March owing to the participation of infectious disease departments in the management of COVID-19. By the end of March 2020, the Minister of Health finally assigned specific staff members from infectious diseases departments to work exclusively for COVID-19. Physicians and nurses from these departments were not allowed to manage patients with other diseases in public and private healthcare facilities. Thus, the volume of patients treated in May 2020

decreased to 25% of that in May 2019. The total number of patients treated in 2020 is expected to be about 40% of 2019.

5.8 | Slovakia

Management of chronic HCV infection is provided in 19 centres (8 infectious disease centres and 11 hepatology centres). Patients who began DAA treatment for chronic hepatitis before the COVID-19 pandemic continued to be treated until completion and underwent all indicated laboratory tests. Screening for HCV infections and recruitment of new HCV patients for treatment were stopped from mid-March to June 2020. During this period infectious disease specialists were managing the COVID-19 pandemic and hepatologists only evaluated acute patients. Other patients were managed by phone or e-mail. At the end of June 2020, almost no patients had been treated for chronic HCV infection. Recruitment of new HCV patients for DAA treatment began again in June 2020. The elimination of chronic HCV infection is expected to be delayed in Slovakia. Thirty to fifty per cent of HCV patients are expected to be treated in 2020 compared to 2019.

6 | CONCLUSION

According to our data, the elimination of HCV, which was previously considered to be possible in certain Central-European countries by 2030, will not be possible by this date throughout the region. The number of treated patients in 2020 decreased by one third because of COVID-19, thus a significant reduction in the prevalence of HCV cannot be achieved by 2030 because of a breakdown in the healthcare system.

CONFLICT OF INTERESTS

None.

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SUPPLEMENT ARTICLE

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Real-world evidence in hepatocellular carcinoma

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Abstract

Real-world evidence includes all health-related information, such as electronic health records, insurance claims, pharmacy records and wearables that are obtained outside of clinical trials. These data can provide critical insights into the natural history of disease and evaluate the safety and effectiveness of treatment regimens used in clinical practice. Real-world data have been applied to varying degrees by global regulatory agencies to inform and expedite many phases of drug development and help refine the use of therapeutic regimens after marketing, especially in populations that are under-represented in registration trials. For the management of hepatocellular carcinoma, early detection provides the best chance for curative therapies, whose success has been evaluated in numerous cohorts. The availability of novel systemic therapies, including kinase inhibitors and immunotherapies, has provided new treatment options and improved survival in patients with advanced stage hepatocellular carcinoma. Real-world longitudinal observational studies can help understand the long-term safety and effectiveness of these agents.

KEYWORDS

hepatocellular carcinoma, real-world data, real-world evidence

1 | SOURCES AND ROLES OF REAL-WORLD DATA

Real-world data (RWD) may be obtained from a variety of sources, such as registries or observational studies, pragmatic trials (ie trials designed to more closely reflect usual clinical practice vs a traditional clinical trial), insurance claims, prescriptions, electronic health records and hospital chargemaster data.[1] Other newer sources of data may include those obtained from social media or wearables such as smart watches (Figure 1).

Real-world evidence (RWE) has regularly filled the gap that exists between evidence generated from clinical trials and the use of approved medications in usual clinical practice.[2-4] Real-world data have been used to inform multiple phases of drug development, including preclinical development, identification of unmet needs, development of product profiles and clinical trial designs by informing patient characteristics and comorbid conditions, frequently used concomitant medications and treatment paradigms, and the feasibility of inclusion and exclusion criteria planned for the clinical study, as well as by providing detailed information on the natural history of disease.[1] Perhaps RWE's most widely recognized contributions are in the post-authorization space by supporting label expansion for approved medicinal products and to fulfil post-authorization requirements including long-term safety. Real-world evidence has also played a critical role in improving the understanding of treatment effectiveness and safety in expanded patient populations that were under-represented in registration trials.[5]

A more complete picture of a patient's journey may be obtained from disease-specific registries. These longitudinal observational studies differ from traditional clinical trials, which have narrowly selected patient populations to answer specific clinical questions and support ultimate approval with regulatory agencies.[6] Observational

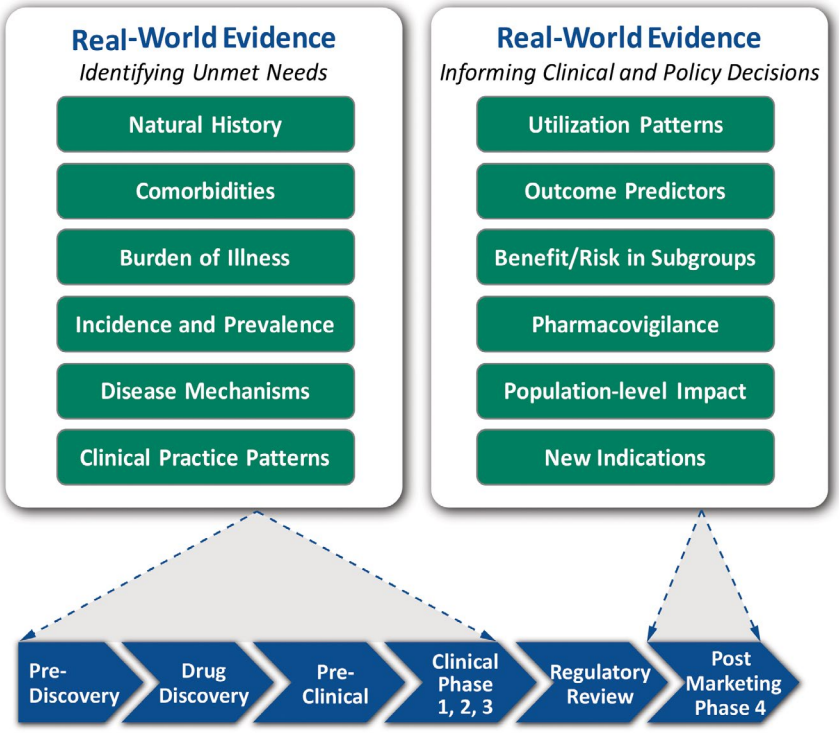
studies collect data from patients treated in usual clinical practice and may include either a retrospective look-back period and/or a prospective longitudinal period. These studies can inform clinicians about the optimal use of medicinal products in the real world, such as in specific populations for which information may be lacking from clinical trials, such as those with certain comorbid diseases, severe disease, different races or older age, as well as provide long-term safety data.[6]

Key points

- Real-world evidence can be used to fill gaps between data generated from traditional clinical trials and the use of approved medicines in clinical practice.
- There are many sources of real-world data, ranging from electronic health records and claims data to observational longitudinal cohort studies. The latter can be used for the assessment of long-term safety of approved medicines, populations that were under-represented in clinical trials and the natural history of disease in a real-world setting.
- Real-world evidence continues to play an important role in understanding disease progression in hepatocellular carcinoma, and the safety and effectiveness of approved therapies and treatment paradigms.

2 | REGULATORY VIEW OF REAL-WORLD DATA AND REAL-WORLD EVIDENCE

The 21st Century Cures Act of 2016 was designed to accelerate the development of new drugs and more quickly and efficiently make these therapies available to patients in the United States (US).[7] Pursuant to this act, the Food and Drug Administration (FDA) released a framework in 2018 for the evaluation of the use of real-world evidence for supporting either a new indication of a previously approved drug or to meet post-market regulatory requirements.[8] This framework defines real-world data (RWD) as 'data relating to patient health status and/or the delivery of health care routinely collected' outside of clinical trials, and real-world evidence (RWE) as the 'clinical evidence regarding the usage and potential benefits or risks of a medicinal product derived from the analysis of RWD'.[7] Thus, RWD may contribute information directly related to the safety and effectiveness of a medicinal product or contribute to the design and efficiency of a planned traditional clinical study (eg feasibility, inclusion/exclusion criteria, selection of geographical regions, etc).[1] Of note, the FDA regularly uses RWE to monitor the safety of medicinal products approved in the US via the Sentinel System. The full system was officially launched in 2016 and consists of administrative claims data and electronic health record data.[9] The Sentinel System



Adapted from Galson and Simon 2016[1]

FIGURE 1 Real-world evidence informs the drug development process from the early discovery phases through post-market surveillance. Adapted from Galson and Simon (2016)[1]

has been used to assess post-market safety, patterns of medication use including use in specific subpopulations and to determine the impact of medical countermeasures in public health emergencies.[9]

In Europe, a Heads of Medicines Agencies/European Medicines Agency (EMA) Joint Task Force on Big Data was established in 2017. Subsequent published reports have addressed both RWD and, in a final report released on January 2020, made recommendations for the use and implementation of big data.[10,11] As described by the Joint Task Force, 'big data' includes RWD sources such as electronic health records, registry data and claims data, among others. The EMA has conducted numerous studies using RWD. Real-world data have also been accepted by other regulatory authorities, such as Health Canada and Japan's Pharmaceuticals and Medicinal Devices Agency (PDMA), to support the approval of new applications or line extensions. Moreover, Health Canada released guiding principles for regulatory decision-making related to RWE.[12,13] According to the study by Bolislis et al, in most instances, the EMA, FDA, Health Canada and the PDMA used RWD as a control or historical control group or as supportive data to validate findings, and these data were generally utilized to support the development of products for rare diseases, where there was an unmet medical need or where a traditional randomized controlled trial was not feasible.[12]

3 | REAL-WORLD EVIDENCE IN HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is increasingly responsible for a significant number of deaths and is currently a fourth leading cause of cancer-related deaths worldwide.[14] Unfortunately, the incidence of HCC is rising in areas such as the US, partly because of the high number of patients with advanced hepatitis C virus (HCV) infection, and also to an increasing number of patients with NAFLD.[15-17] Despite advances in therapies for HCC in recent years, studies are generally limited to phase 2 and 3 trials with strict inclusion and exclusion criteria, thus lacking generalizability to usual clinical practice. A summary of evidence from traditional registry clinical trials used in the approval of new therapies, as well as observational cohort studies used for the surveillance of disease progression are described below.

4 | INCIDENCE AND SURVEILLANCE

Multiple HCC cohort studies have been performed in a variety of geographical regions throughout the world. One large European study using electronic health records data to determine new diagnoses of advanced liver disease included primary care data from the European Medical Information Framework Network, specifically from the United Kingdom, the Netherlands, Italy and Spain, which included over 18 million adults, 136 703 with a diagnosis of nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH). These RWD were used to identify the most frequently observed comorbidities observed in the NAFLD/NASH group compared to matched controls (diabetes, hypertension and obesity), as well as a baseline diagnosis of diabetes as a strong predictor of a diagnosis of HCC or cirrhosis.[18]

Another study, the Hepatocellular Carcinoma Early Detection Strategy study, is a multicentre National Cancer Institute Early Detection Research Network initiative to establish a large biorepository and database on patients who are considered at risk for the development of HCC. As of 2018, this database includes 1482 participants with cirrhosis and without HCC at enrolment and should provide a valuable opportunity to examine the incidence of HCC in this population as well as to study potential biomarkers.[19]

Projections and determining the odds of survival are important in the management and treatment of patients with HCC. Cohort studies in both Denmark and the US examining the progression of HCC over a 12-year and 6-year period, respectively, both showed an increased incidence of HCC over time.[20,21] However, it cannot be determined whether this was related to an increase in the prevalence of liver diseases, or an awareness of HCC screening in clinical practice. The increased incidence of HCC has escalated the importance of examining the likelihood of survival and potential mitigating factors. Surveillance of liver cancer has been shown to lead to earlier detection and a better chance of receiving curative treatment.[22] A cohort study in Italy from 1999 to 2010 using 320 HCC patients with a new diagnosis of HCC showed that patients with Barcelona Clinic Liver Cancer (BCLC) stage D at baseline had a 1-year survival of less than 5%.[23] These findings confirm the importance of early detection and subsequent treatment of HCC globally.

One area of interest in patients with HCC is the impact of racial/ethnic minorities and socio-economic status on mortality. As reviewed by Rich et al, HCC disproportionately affects disadvantaged populations in the US, including racial and ethnic minorities, with African Americans having lower odds of detection of HCC at an early stage and overall survival than Caucasians. Others noted that HCC is often clustered geographically in areas with low socio-economic status. These disparities affect the prevention, early detection and outcomes of HCC.[24-27]

5 | LOCOREGIONAL THERAPIES AND SURGICAL RESECTION FOR EARLY STAGE HCC

Clinical guidelines recommend the use of locoregional therapies and surgical resection for the management of early or moderately advanced HCC.[28,29] Numerous real-world cohort studies have examined the impact of locoregional therapies and/or surgical resection on early stage HCC, and several examples are discussed below.[30-32] In a retrospective Australian study of patients with BCLC-0/A, those treated with curative intent had better overall survival and recurrence-free survival than patients receiving transarterial chemoembolization (TACE).[30] A study from Thailand showed that approximately one third of patients across all stages of HCC had first-line treatment that deviated from recommended treatment guidelines,

which regularly influenced their survival.[31] A study by the Liver Cancer Study Group of Japan showed that use of surgical resection in patients with HCC was associated with significantly lower risks of both death and recurrence in patients with early or moderately advanced HCC.[33] While resection can be beneficial to some patients, survival is influenced by disease severity.[34] A study in Germany showed that the overall survival of patients undergoing resection was 34 ± 23 months with the 1-, 3- and 5-year overall survival rates decreasing from 82.9% to 41.8% and 13.7% respectively.

6 | SYSTEMIC TREATMENT FOR HCC

Until recently, therapeutic options were limited and the prognosis for patients with advanced HCC was poor. Sorafenib, an oral multikinase inhibitor approved by the FDA in 2007, was the first agent for the treatment of inoperable HCC that demonstrated a modestly better survival than with placebo.[35] Subsequently, numerous other systemic and immunotherapeutic agents, such as regorafenib, lenvatinib, ramucirumab, nivolumab, pembrolizumab, and combinations such as nivolumab/ipilimumab and atezolizumab/bevacizumab, have been approved based on compelling phase 2 and phase 3 studies and provide additional potential benefits for patients with advanced HCC (Table 1).[36-41]

Evidence for approval of these therapies was mainly obtained from traditional clinical trials and thus restricted to stringent inclusion and exclusion criteria limiting the overall generalizability of the study population to patients presenting with HCC in clinical practice. Real-world evidence from registries and cohort studies can provide additional confidence in the effectiveness and safety of these medications in expanded patient populations and represents a natural evolution in research in HCC management.

Numerous real-world studies have shown the efficacy of sorafenib.[42,43] A prospective multicentre clinical study from 2009 to 2014 examined overall survival with sorafenib treatment in 13 centres in Japan.[42] Results from this study showed that sorafenib could be administered as a long-term treatment for patients with advanced HCC.[42] The utility of sorafenib has been shown across patients with HCC, including the elderly. An international observational study examined 5598 patients from 2007 to 2018 to test the influence of age on overall survival. Sorafenib was shown to be effective in an elderly population (≥75 years of age).[43] A combination of TACE and sorafenib led to an improvement in survival rates with a reduced mortality of 26%.[44]

Regorafenib is an oral multikinase inhibitor that blocks the activity of protein kinases involved in angiogenesis, oncogenesis, metastasis and tumour immunity.[45,46] A randomized, double-blind, parallel-group phase 3 clinical trial was conducted in 21 countries in adults with HCC who tolerated sorafenib and progressed.[36] A total of 567 patients began treatment (374 receiving regorafenib; 193 placebo) resulting in 10.6 and 7.8 months median survival respectively. This treatment strategy has been used in patients in whom disease progresses during sorafenib treatment and has been shown to provide benefits to survival in HCC patients.[36] The use of regorafenib meets a previously unmet need for treatment options in patients with HCC by prolonging overall survival, progression-free survival and time to progression.[47]

Nivolumab is an immunotherapy that inhibits programmed death receptor-1 (PD-1) used as a second-line systemic treatment in HCC patients who have been treated with, or are intolerant to, sorafenib. Nivolumab treatment resulted in durable responses at all dose levels with a 6-month OS rate of 72%[48] Nivolumab was originally tested in patients with advanced HCC with or without prior exposure and was found to result in a prolonged tumour response. An observational study confirmed the safety and efficacy of nivolumab across various lines of therapy.[49] The use of immune checkpoint inhibitors (ICI) in advanced HCC has been shown to be a comparable to that in HCC patients with Child-Pugh A cirrhosis.[50]

Lenvatinib has been shown to be an effective second-line therapy in a number of real-world settings.[37,51-53] Atezolizumab-bevacizumab,

TABLE 1 Systemic therapies for the treatment of hepatocellular carcinoma

Name	Approval date in the US	Class	Line of therapy
Sorafenib	2007	Kinase inhibitor	First
Regorafenib	2017	Kinase inhibitor	Second
Nivolumab	2017	PD-1 blocking antibody	Second
Pembrolizumab	2018	PD-1 blocking antibody	Second
Lenvatinib	2018	Kinase inhibitor	First
Ramucirumab	2019	VEGFR2 antagonist	Second
Atezolizumab/Bevacizumab	2020	PD-L1 blocking antibody/ Vascular endothelial growth factor inhibitor	First
Nivolumab/Ipilimumab	2020	PD-1 blocking antibody/ CTLA-4-blocking antibody	Second

Abbreviations: CTLA-4, Human cytotoxic T-lymphocyte antigen 4; PD-1, programmed death receptor-1; US = United States; VEGFR2, Human vascular endothelial growth factor receptor 2.

a drug combination recently approved by the FDA, has been shown to markedly improve overall survival and progression-free survival compared to sorafenib, and real-world studies are expected.[40,54]

7 | DIRECT-ACTING ANTIVIRALS AND THE RISK OF HCC RECURRENCE

It is well established that patients with cirrhosis who are cured of hepatitis C have a continued risk of developing HCC and that ongoing surveillance for the development of HCC is warranted.[55] Unexpectedly, reports of an increased risk of recurrent HCC after successful direct-acting antiviral (DAA) therapy in those with a complete tumour response to treatment raised concerns of the association of DAA therapy with an increased risk of early HCC recurrence. A report by Reig et al (2016) concluded that patients treated with DAAs had an unexpected, increased risk of early HCC recurrence, which sparked numerous questions.[56] Additional studies have been published both supporting and refuting findings from the Reig study.[57-59] Singal and colleagues conducted a large retrospective study in North America that evaluated the impact of DAA therapy in nearly 800 patients after a complete response to therapy for HCC.[60] There was no difference in early recurrence or in the pattern of recurrence between those treated with DAAs and those without DAA therapy for hepatitis C.[60] The EMA required all marketing authorization holders of DAAs to perform a prospective study of DAA treatment among patients with previously treated HCC. Thus, the DAA-PASS international, observational study, a substudy of TARGET-HCC described below, was designed to investigate the impact of exposure to DAAs on early recurrence of HCC in adult HCV-infected participants following successful HCC treatment (NCT03707080).

8 | TARGET-HCC

TARGET-HCC is an ongoing, longitudinal observational cohort of adult patients with a diagnosis of HCC who are receiving standard care at academic and community sites across the US and Europe.[61] TARGET-HCC was designed to better understand the natural course of the disease, the utilization of available therapies, interventions, concomitant medications and outcomes in patients managed for HCC in usual clinical practice. Patients are enrolled at a variety of site types such as those with specialties in gastroenterology/hepatology, hepatobiliary/transplant surgery and oncology. Clinical data are obtained directly from the electronic medical record, thus allowing a detailed review and centralized abstraction of data from a complete record including clinical narratives, laboratory assessments, concomitant medications, therapies, procedures, imaging and pathology reports. Patient-reported outcome measures and health-related quality of life questionnaires are assessed throughout the study, and blood samples are obtained for future analysis. Key disease stage indicators assessed include BCLC tumour staging and Milan criteria; cirrhosis status, defined by biopsy and/or clinical criteria; and Child-Pugh status, which is derived from clinical data abstracted from records.[61-64]

Over 1800 patients have been enrolled in TARGET-HCC with a wide range of disease severities and patient characteristics from 67 sites in the US and Europe.[61] Patients are mostly Caucasian men with a median age of 64. The most common aetiology of liver disease is HCV infection, followed by NAFLD/NASH, alcohol-related liver disease and hepatitis B, and most patients have cirrhosis including decompensated cirrhosis in over 70%. At diagnosis, most patients with available tumour staging were BCLC stage A and over half were within the Milan criteria. Most patients received locoregional

TABLE 2 Initial therapy for HCC according to BCLC staging at time of diagnosis for patients enrolled in TARGET-HCC (from Cabrera et al)[61]

Summary	BCLC 0 (N = 146)	BCLC A (N = 774)	BCLC B (N = 187)	BCLC C (N = 91)	BCLC D (N = 67)	All patients (N = 1421)
Total Subjects	126	696	166	70	46	1246
Locoregional Therapy	105 (83.3%)	547 (78.6%)	144 (86.7%)	27 (38.6%)	37 (80.4%)	955 (76.6%)
Ablation	53 (42.1%)	144 (20.7%)	17 (10.2%)	1 (1.4%)	8 (17.4%)	246 (19.7%)
Embolization	52 (41.3%)	406 (58.3%)	127 (76.5%)	24 (34.3%)	29 (63.0%)	708 (56.8%)
TACE	38 (30.2%)	292 (42.0%)	89 (53.6%)	9 (12.9%)	23 (50.0%)	503 (40.4%)
Radioembolization	13 (10.3%)	106 (15.2%)	38 (22.9%)	17 (24.3%)	5 (10.9%)	195 (15.7%)
Other	1 (0.8%)	8 (1.1%)	0 (0.0%)	0 (0.0%)	1 (2.2%)	12 (1.0%)
Surgery	18 (14.3%)	111 (15.9%)	8 (4.8%)	4 (5.7%)	1 (2.2%)	175 (14.0%)
Transplant	0 (0.0%)	2 (0.3%)	1 (0.6%)	0 (0.0%)	1 (2.2%)	4 (0.3%)
Resection	18 (14.3%)	109 (15.7%)	7 (4.2%)	4 (5.7%)	0 (0.0%)	171 (13.7%)
Radiation	1 (0.8%)	27 (3.9%)	0 (0.0%)	7 (10.0%)	3 (6.5%)	39 (3.1%)
Systemic	6 (4.8%)	18 (2.6%)	16 (9.6%)	32 (45.7%)	5 (10.9%)	91 (7.3%)
Not Available	20	78	21	21	21	175

Note: Initial HCC therapies include any treatments taken on the first date of treatment for each patient.

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; TACE, Transarterial chemoembolization.

therapies as the initial treatment, while fewer had surgery or systemic therapies, probably because of earlier stage disease in these patients, who were mostly recruited from hepatology sites (Table 2).[61] Patients enrolled in TARGET-HCC are being longitudinally followed to evaluate disease progression or regression in relation to serial locoregional and systemic therapies, as well as long-term outcomes.

9 | CONCLUSIONS

The use of RWE contributes valuable information to numerous areas including the assessment of the applicability of current therapies to broad populations with characteristics that may have been underrepresented in registration trials, the optimization of treatment effectiveness in subpopulations and the long-term safety of regimens used in clinical practice. As new therapies and treatment modalities become available, HCC registries with carefully curated data can be used to provide a rapid assessment of the safety and effectiveness of these new therapeutic regimens and to continuously evaluate the impact of shifting treatment paradigms on long-term outcomes.

DISCLOSURES

Drs. Mospan and Morris are employees of Target RWE. Dr Fried is Chief Medical Officer for TARGET RWE and receives personal fees and is a stockholder in the company.

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SUPPLEMENT ARTICLE

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Hepatitis E, what is the real issue?

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Abstract

Hepatitis E virus (HEV) infection is a worldwide disease and the primary cause of acute viral hepatitis with an estimated 3.3 million symptomatic cases every year and 44,000 related deaths. It is a waterborne infection in the developing countries. In these countries, HEV genotypes 1 and 2 cause large outbreaks and affect young subjects resulting in significant mortality in pregnant women and patients with cirrhosis. In developed countries, HEV genotypes 3 and 4 are responsible for autochthonous, sporadic hepatitis and transmission is zoonotic. Parenteral transmission by the transfusion of blood products has been identified as a potential new mode of transmission. HEV can also cause neurological disorders and chronic infections in immunocompromised patients. The progression of acute hepatitis E is usually asymptomatic and resolves spontaneously. Diagnosis is based on both anti-HEV IgM antibodies in serum and viral RNA detection in blood or stools by PCR in immunocompetent patients, while only PCR is validated in immunocompromised individuals. Ribavirin is the only validated treatment in chronic infection. A vaccine has been developed in China.

KEYWORDS

acute viral hepatitis, chronic hepatitis, hepatitis E virus, neurologic symptoms, ribavirin, zoonosis

1 | BACKGROUND AND VIROLOGY

The hepatitis E virus (HEV) belongs to the *Hepeviridae* family. There are two types of infectious particles. The unenveloped virions, first identified by Balayan, are found in the faeces while quasi-enveloped virions circulate in the blood.[1] HEV is composed of a positive-strain RNA genome. There are three open reading frames (ORFs). ORF1 encodes functional domains involved in replication of viral genome. ORF2 and ORF3 encode the capsid protein and a protein involved in releasing new virions respectively.[2] Strains which infect humans belong to the *Orthohepevirus* genus, comprising four species (A-D). While *Orthohepevirus* A is the main species that infects humans, strains belonging to *Orthohepevirus* C have also been recently identified in humans in contact with rats.[3] Recent analysis of genomic and subgenomic sequences has led to recognition of 8 genotypes and 31 subtypes.[4] Among the eight genotypes of *Orthohepevirus* A, only 1 (Asia and Africa) and 2 (Mexico and Africa) infect only humans whereas 3 and 4 cause zoonotic infections and are endemic in pigs, wild boar, rabbits and were recently described in dogs.[5] Genotypes 5 and 6 have only been reported in wild boar, and genotype 7 in camels and humans who consumed camel meat or milk. Strains belonging to genotype 3 have mainly been identified in North America, Europe, South America and Japan. Genotype 4 has mainly been identified in China, Taiwan, Japan and Vietnam, but a few cases have also been reported in Europe (Figure 1).[6]

Abbreviations: GBS, Guillain-Barré syndrome; HEV, hepatitis E virus; HIV, human immunodeficiency virus; ORF, open reading frame; PTS, parsonage turner syndrome.

2 | EPIDEMIOLOGY

Hepatitis E is an underestimated cause of acute hepatitis worldwide with approximately 3.3 million symptomatic cases every year leading to 44,000 related deaths[7] and the most common cause of acute viral hepatitis in several European countries. If the incidence rate is still high in hyperendemic regions, it is also emerging in low endemic regions such as Oceania and Western Europe.[8] In developing countries, HEV infection is characterized by large waterborne epidemics. Transmission is caused by ingestion of water contaminated by human feces. There is also a risk of maternal-fetal transmission of HEV, causing neonatal infections.[9] In these countries, symptomatic HEV infection usually affects men 15 to 30 years old. The mortality rate among adults in an epidemic area is 0.2% to 4%.[2] Patients with chronic liver disease and pregnant women have much higher mortality rates, up to 70% and 25% respectively.[10] There is an increased risk of maternal complications, mainly during the third quarter, with a higher risk of fulminant hepatitis and obstetric complications, probably linked to a particular placenta tropism and pathogenesis of HEV genotype 1.[11]

In developed countries HEV is a zoonosis and transmission is caused by ingestion of contaminated meat (mainly pork, with HEV genotype 3 or 4).[12] HEV RNA was recently identified in goat and sheep milk and could represent a source of infection to consumers.[13] Parenteral transmission by blood transfusion is also a potential mode of contamination. In a recent study from a population of blood donors in England, 1 in 2,848 had a positive HEV viraemia. Sixty-two contaminated blood products were transfused, leading to HEV infection in recipients in 42% of cases.[14] In France, the prevalence of positive HEV viraemia in blood donors is estimated to be 1/800.[15] A French study reported 23 cases of transfusion-transmitted infections between 2006 and 2016, including 14 with chronic hepatitis in immunosuppressed patients.[16]

In developed countries, acute hepatitis E usually affects middle aged and elderly men (sex ratio of 4/1, median age 55) often with excessive alcohol consumption.[2,12] Patients with chronic liver disease are at risk of decompensation and death,[17] and maternal complications have not yet been described.

Key points

- Hepatitis E virus infection is usually a zoonotic infection in the developed countries, transmitted by ingestion of contaminated food.
- It is a cause of severe decompensation in patients with cirrhosis.
- Chronic hepatitis can develop in immunocompromised patients.
- Neurological manifestations, including neuralgic amyotrophy and Guillain-Barré syndrome, are now well recognized.
- The diagnosis of acute hepatitis is based on both anti-HEV-IgM and HEV RNA testing in immunocompetent patients.

3 | ACUTE HEPATITIS IN DEVELOPED COUNTRIES

The period of incubation is 2 to 5 weeks. Over 90% of cases are asymptomatic. This percentage is based on the latest data in blood donors (asymptomatic) which showed HEV viraemia in 1/600 to 1/2,500 in highly endemic European countries and in 1/2,300 to 1/14,500 in European countries with intermediate to low endemicity.[18] Acute hepatitis E is virulent when it is symptomatic, as shown by the high percentage of hospitalized patients (74.5%). Jaundice is present in approximately 43% of cases.[19] The symptoms are non-specific and common to other viral hepatitis: asthenia, diarrhoea,

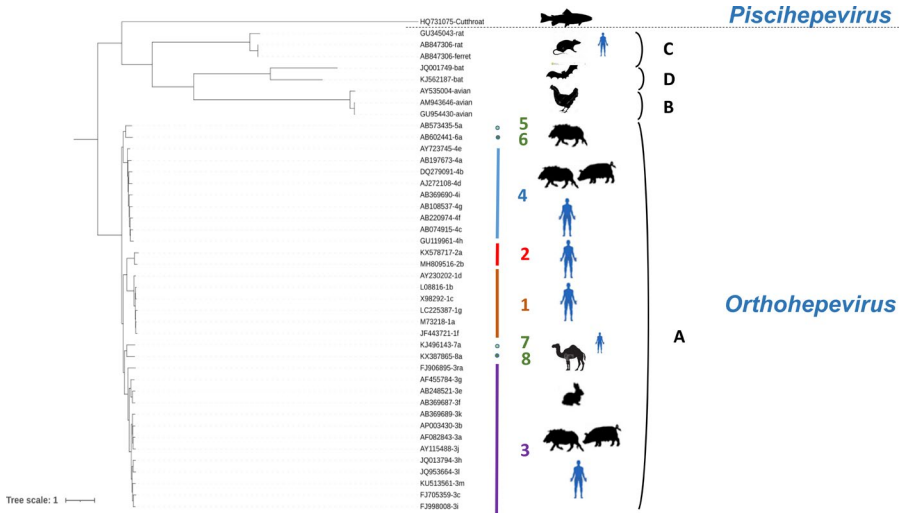

FIGURE 1 Hepeviridae phylogenetic tree

nausea, fever, arthralgia, vomiting and abdominal pain. ALT levels are usually very high (1000-3000 IU/L), but the increase may be more moderate depending on the time of diagnosis.[12] HEV is a self-limiting infection that lasts for 4 to 6 weeks. There are severe forms in patients with cirrhosis and the elderly.[17] There are no chronic forms in immunocompetent patients. Cholestatic jaundice can last from several weeks to several months. There is no cytolysis rebound after normalization of liver function tests.

Acute hepatitis E remains the principal differential diagnosis of drug-induced liver Injury (DILI) and 8% of patients with suspected DILI were found to be seropositive with anti-HEV IgM in a Spanish cohort.[20]

4 | NEUROLOGICAL INJURY

HEV tropism is not restricted to the liver and HEV can potentially complete the full viral cycle in the neuronal-derived tissues.[21] Neurological symptoms have been described worldwide and with all genotypes. In Europe, a prospective French study described neurological symptoms in 16.5% of symptomatic cases.[19] Neurological injury includes neuralgic amyotrophy (Parsonage-Turner syndrome, PTS), Guillain-Barré syndrome (GBS), meningoradiculitis and mononeuropathis multiplex. In these patients, neurological symptoms are predominant, cytolysis can be moderate, and jaundice absent. The prevalence of HEV infection in patients with neuralgic amyotrophy is 10%.[22] In these cases, motor weakness and sensory disturbances are more often bilateral, asymmetric and are not confined to the brachial plexus compared to non-HEV-infected patients[23,24] The prevalence of HEV infection in patients with GBS is 5%.[25]

The mechanisms by which HEV causes PTS or GBS are unknown. The first hypothesis is an immune-mediated response induced by the virus and the second is direct viral toxicity. The risk of sequelae is important in these two entities. Meningoradiculitis is probably related to a direct viral effect (the virus is found in the cerebrospinal fluid with lymphocytic meningitis) and patients usually heal without sequelae. There is an increased awareness of a new entity of neurological injury: small fibre neuropathy which could be responsible for neuropathic pain in patients infected with HEV.[19]

5 | OTHER EXTRAHEPATIC MANIFESTATIONS

A broad range of HEV extrahepatic manifestations have been described. Renal complications with both membranoproliferative and membranous glomerulonephritis have been reported.[26] In a systematic review in 73 patients, pancreatitis has been shown to be associated with viral hepatitis and HEV was involved in 29%.[27] Rheumatic manifestations and haematological complications such as aplastic or haemolytic anaemia, cryoglobulinemia and thrombocytopenia have also been described.[28]

6 | CHRONIC HEPATITIS

Zoonotic HEV may cause chronic hepatitis in immunocompromised patients such as solid organ transplants, patients undergoing chemotherapy for haematological malignancies,[29] in patients with HIV and in patients with rheumatoid arthritis. A series of 94 patients with persistent HEV infection (viraemia of more than 12 weeks) from England and Wales showed that all patients were immunocompromised: 70.2% were transplant recipients, 17% had an underlying haematological malignancy and 6% had advanced HIV infection. Seventeen per cent of the 65 patients treated by ribavirin, had a virological relapse.[30]

In chronic hepatitis, liver enzymes are usually moderately elevated and patients are often asymptomatic. The incidence of infection with genotype 3 HEV after organ transplantation was 3.2/100 person-years in the southwest of France.[31] Sixty per cent of these patients with acute hepatitis E will develop chronic hepatitis.[32] Without treatment, progression to cirrhosis can be rapid.

Three cases of re-infection in transplant patients who were immunized prior to transplantation with IgG 0.3, 2.1 and 6.2 WHO units/mL, have been described. Therefore, low levels of anti-HEV IgG (<7 WHO units/mL) before transplantation do not seem to protect organ transplant recipients.[33]

Chronic hepatitis E has also been described in patients with haematological malignancies.[29] Transaminases are moderately high, at about 500 IU/L. These patients may experience viral clearance over time and the return of immunity. This may induce a rebound in cytolysis and severe acute hepatitis.[30]

Chronic hepatitis only occurs in patients infected with HIV when there is a very low CD4 count, always <250/mm^3.[34] There is a risk of progression to cirrhosis in these cases. There is a risk of severe or fulminant hepatitis in subjects with CD4 counts >250 cells/mm^3, which is the same as in the general population.[35] The management of HIV treatment can be complicated during acute hepatitis E infection. A European cohort study also reported chronic HEV infection in patients with rheumatoid arthritis.[36]

7 | DIAGNOSIS

Direct diagnosis is based on the detection of viral RNA in the serum and/or faeces. Detection is performed by amplification of the genome in the conserved region overlapping ORF3/ORF2.[37] The genotype can be determined to study the movement of different viral strains. An indirect diagnosis is based on the detection of anti-HEV antibodies. IgM, markers of acute infection, appears early and last at least 16 weeks.[38] The sensitivity of tests in immunocompetent patients is excellent (>98%). Thus, the diagnosis in immunocompetent patients can be based on serology. However, RNA detection is essential in immunocompromised subjects. IgG appears shortly afterwards and last for years. EASL guidelines recommend using both anti-HEV-IgM and HEV RNA testing in immunocompetent patients with acute hepatitis E. HEV RNA testing

is mandatory in immunocompromised patients and serology is optional.[39]

8 | WHO SHOULD WE TEST?

European guidelines recommend HEV serology in any patient with an unexplained ALT elevation. Moreover, screening should be performed in patients admitted for GBS or Neuralgic Amyotrophy regardless of ALT rate. Immunosuppressed patients should be screened for HEV annually and when liver function testing is abnormal.[39]

9 | TREATMENT AND PREVENTION

In most cases, the infection is self-limiting, and does not require treatment. Like in any viral acute hepatitis, monitoring of liver function tests is recommended to detect the progression to severe acute hepatitis.

Reduction of immunosuppression in solid organ transplants, including reducing the doses of tacrolimus and corticosteroids, induces viral clearance in 30% of cases. The standard treatment is ribavirin for three months in patients who have not achieved viral clearance. A sustained virological response (cure) is achieved in more than 70% of cases. Patients who relapse can be retreated with ribavirin for 6 months. A large-scale retrospective study performed in patients after organ transplantation reported a SVR rate of 81.2% after a first course of ribavirin, which increased to 89.8% when some patients were offered a second course. An increased lymphocyte count at the initiation of therapy was a predictive factor for SVR, while poor haematological tolerance to ribavirin requiring its dose reduction (28%) and blood transfusion (15.7%) was associated with more relapses after ribavirin cessation. Pretreatment mutations in the HEV polymerase and de novo mutations under ribavirin did not negatively influence HEV clearance. HEV RNA polymerase mutations do not play a role in HEV clearance.[40]

Chronic or persistent hepatitis in patients with HIV or receiving chemotherapy for haematological malignancies can be treated with ribavirin in the same manner.[29]

In developing countries, prevention is based on providing clean drinking water and improving sanitary structures.

In developed countries where transmission is essentially because of the ingestion of contaminated food, prevention can be based on the usual recommendations for zoonotic disease transmission. Products with the highest risk of HEV are undercooked pork products (fresh or dried liver sausage, dry liver, figatelli and liver dumplings) and raw or undercooked products made from wild boar or deer (meat and offal). These products should be avoided, especially by the elderly, patients with cirrhosis, and immunocompromised patients.

Blood donations are already screened in Ireland, the UK, the Netherlands and Switzerland.

In France, HEV is screened for in plasma donations used in the preparation of fresh frozen plasma treated by solvent detergent since January 2013. It is not yet tracked systematically in all blood donations. A vaccine was recently developed and recognized by the Chinese health authorities: HEV 239 recombinant vaccine (Hecolin; Innovax Biotech Xiamen, Xiamen, China). Its routine use began following a randomized placebo-controlled phase III study between 2007 and 2009 in China.[41] More than 100,000 people were vaccinated in a series of three injections (0, 1, 6 months). At 4.5 years, 53 cases of acute hepatitis E genotype 1 were found in the placebo group compared to seven in the vaccine group. The efficacy of the vaccine was 86.8%, with good tolerance. This vaccine was approved by the Chinese authorities in healthy adults aged 16-65 years old and pregnant women and has been authorized for sale since October 2012. Long-term persistence of protective immunity has not been evaluated. Moreover, its efficacy in immunocompromised patients, pregnant women and patients with chronic liver disease must be determined.

10 | CONCLUSION

HEV infection is the primary cause of acute viral hepatitis worldwide. Patients with symptomatic acute hepatitis, biochemical evidence of hepatitis or decompensated chronic liver disease should therefore be tested. Patients with acute neurological symptoms including neuralgic amyotrophy and GBS should also be tested for HEV regardless of liver test abnormalities. Serology can be sufficient for a diagnosis in immunocompetent patients, but detection of the virus in blood and/or stools by molecular biology techniques is the gold standard and is required for immunocompromised patients. There are chronic forms in immunocompromised patients with low transaminases and there is a risk of progression to cirrhosis in these patients. They should be treated with ribavirin. In the developed countries, prevention is based on avoiding undercooked pork and by systematic screening of blood donors in some countries. A vaccine is available in China.

CONFLICT OF INTEREST

The authors do not have any disclosures to report.

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SUPPLEMENT ARTICLE

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HCC advances in diagnosis and prognosis: Digital and Imaging

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Abstract

Hepatocellular carcinoma (HCC) is a major cause of cancer-related death worldwide. Understanding of the pathogenesis of HCC has significantly improved in the past few years due to advances in genetics, molecular biology and pathology. Several subtypes have been identified with different backgrounds and outcomes, leading to possible changes in disease management and challenging the role of imaging. Indeed, despite its pivotal role in the diagnostic workup, prognosis, and the decision-making process in patients with HCC, these recent developments are progressively redefining the role of imaging. First and most important, liver imaging is shifting from a purely qualitative to a quantitative paradigm, integrating quantitative imaging and radiomics in a digital era. Second, to improve patient management, imaging has gradually moved beyond tumor-centered assessment to include a broader evaluation of the liver and its function. This review describes and discusses these advances in the imaging for the diagnosis and prognosis of HCC.

KEYWORDS

diagnosis, hepatocellular carcinoma, prognosis, quantitative imaging

Key points

- Liver imaging is shifting from a purely qualitative to a quantitative paradigm, integrating quantitative imaging and radiomics.
- The goal of quantitative imaging techniques is to construct reproducible, non-invasive image-based signatures reflecting the underlying pathophysiology.
- Quantitative imaging of primary liver tumors allows the assessment of the background liver, which until now has been determined by clinical and biological measurements in most staging systems.

1 | INTRODUCTION

Much of the surveillance, diagnosis, staging and follow-up of patients with hepatocellular carcinoma (HCC) is performed non-invasively by imaging.[1] This is especially true in the presence of cirrhosis. The efficacy of imaging is dependent upon constant technical and technological progress to obtain improved image quality, resolution, and safety. Progress has also been made as a result of the collective efforts of the medical imaging community to optimize, refine, expand and validate knowledge. Historically, most academic medical imaging studies have focused on 'qualitative' or 'morphological' features. This has been a powerful approach and has led to the development of many clinically relevant and standardized imaging tools for tumor detection, characterization, follow-up and the assessment of response that have now become routine (eg, Response Evaluation Criteria In Solid Tumours (RECIST) and Liver Imaging Reporting and Data System (LI-RADS)).[2]

Over the past decade, the development of precision medicine and quantitative numerical data has opened new avenues and possibilities in

medical imaging.[3,4] Quantitative imaging corresponds to *"the extraction of quantifiable features from medical images for the assessment of normal or the severity, degree of change, or status of a disease, injury, or chronic condition relative to normal."*.[5] Quantitative imaging allows validation of accurate image-derived parameters with anatomically and physiologically relevant meaning. Its aim is to address needs that are unmet with conventional morphological imaging. Combined with conventional qualitative imaging and clinical data, this approach could help identify numerous biomarkers to construct predictive models that could optimize qualitative approaches for the diagnosis, prognosis, treatment selection and monitoring of response to treatment. Quantitative imaging is not limited to research and has already been shown to be of value in patients with HCC. This review presents recent advances in quantitative imaging in HCC, in particular texture analysis and radiomics, and discusses their application in association with conventional qualitative imaging.

I | BRIEF OVERVIEW OF QUANTITATIVE ANALYSIS: TEXTURE ANALYSIS AND RADIOMICS

Among quantitative imaging techniques, texture analysis and radiomics are the focus of intense interest[6] and extensive research with more than 3000 citations in PubMed in the last 3 years. Texture analysis refers to the computerized analysis and quantification of local spatial variations in image brightness that are related to properties such as coarseness and regularity of voxel densities and intensities.[7] Radiomics, which was first the subject of a publication in 2012, was originally defined as the high-throughput extraction of imaging features from radiographic images.[8] This definition was later adapted to integrate standard digital images into the radiomics process and to convert them into mineable, higher dimensional data to improve support in decision-making, especially for cancer patients. The goal of all of these quantitative imaging techniques is to construct reproducible, non-invasive image-based signatures reflecting the underlying pathophysiology.[9] One of the strengths of quantitative imaging for HCC is its ability to evaluate the phenotype of tumors by analyzing both the intra- and peri-tumoral regions, especially because HCCs are heterogeneous tumors, even in the same patient.[10] Another advantage of quantitative imaging of primary liver tumors is the importance of assessing the background liver which until now, has been determined by clinical and biological measurements in most staging systems.

II | EXAMPLES OF ADVANCES AS A RESULT OF QUANTITATIVE MODELS FOR HEPATOCELLULAR CARCINOMA

3.1 | Diagnosis of HCC: toward LI-RADS refinement

Although conventional radiological features are central to the assessment of the liver in patients at risk of HCC, their relevance may be limited due to ambiguous or inconsistent terminology. To improve the standardization of and consensus in the diagnosis of HCC on imaging, as well as to improve communication with referring clinicians, a comprehensive system for interpreting and reporting liver imaging was developed as an image-based semi-quantitative scoring algorithm: the Liver Imaging Reporting and Data System (LI-RADS). As a result of its dynamic design, LI-RADS has gradually been upgraded to integrate the characteristics of different imaging modalities, including computed tomography (CT), magnetic resonance imaging (MRI), and contrast-enhanced ultrasound (CEUS), as well as new multidisciplinary scientific evidence.[2] Interestingly, referenced report databases have also been created for the LI-RADS, which is especially important in this period of large-scale data analysis. Once LI-RADS conformed to the American Association for the Study of Liver diseases (AASL) recommendations, the worldwide application of this tool became a cornerstone in the evaluation of HCC.[11,12]

However, although up to 38% of LR-3 classifications (intermediate probability of malignancy) and 13% of LR-2 classifications (probably benign) result in a final pathological diagnosis of HCC, only the LR-1 (definitely benign observation) and LR-5 (definitely HCC) categories provide a definite diagnosis of the absence or presence of HCC. Thus, the management of patients with an intermediate LI-RADS score remains a challenge, and may result in continued surveillance or biopsy of the lesion. This lack of specificity requires improved, more accurate imaging to guide clinical decisions.[13]

In a multicenter retrospective cohort including patients with cirrhosis, Mokrane et al used a two-step synergic approach, comparing conventional interpretations by experts and radiomics analysis of triphasic-CT scans. These authors showed that a single-feature radiomics signature quantifying changes between the arterial and portal venous phases provided the most valuable contribution to characterization of visually indeterminate liver nodules according to the LI-RADS classification. This approach could reduce the rate of patients with cirrhosis requiring liver biopsy or a wait-and-see strategy and suggests that combining conventional interpretation of images and the use of a radiomics system could provide a valid response to a precise clinical question.[14]

Moreover, very few preliminary studies combining automatic detection of HCC and classification by LI-RADS have been performed. For example, in a retrospective study of 174 patients with 231 lesions, a proof-of-concept to automate the segmentation and application of the LI-RADS score in MRI examinations was found to be feasible using a deep convolutional neural network, suggesting that automatic algorithms could improve the workflow efficacy of the diagnosis of liver lesions.[15]

These preliminary results are encouraging for the development of detection and classification systems for the surveillance of patients with chronic liver disease.

3.2 | Keeping up with recent improvements in pathological correlations: radio-pathology integration

Integrated radiopathological diagnostics, defined as the seamless collaboration between these two disciplines, is of increasing importance in the management of liver tumors.[16] A high degree of histological heterogeneity can be observed in HCC with different, easily defined, subtypes such as the pseudoglandular HCC, scirrhous HCC, spindle cell HCC, steatohepatitic HCC. A new subtype was recently described, which is the macrotrabecular variant of HCC, with an aggressive phenotype, frequent satellite nodules and both macro and micro-vascular invasion.[17] Because of the clinical relevance of this aggressive phenotype, two studies evaluated whether preoperative imaging could help identify the macrotrabecular histological subtype.[18,19] These two studies, performed on pre-operative MRI in 152 and 476 patients with HCC treated with surgical resection, showed that specific MRI features such as tumor heterogeneity and arterial phase hypoenhancement could be suggestive of this subtype. To date, no radiomics-based study has been performed to characterize subtypes of HCC, but this could become an interesting field of research.

With the emerging importance of targeted therapies, immunoprofiling of HCC has become promising for the prediction of the response to therapy. However, this requires a biopsy which is invasive and associated with a risk of sampling bias. A preliminary study has assessed the value of qualitative and quantitative MRI with radiomics features for the prediction of immunological characteristics of HCC. Although this study requires future validation, it helps confirm the future role of imaging in personalised treatment.[20]

3.3 | Preoperative workup

3.3.1 | Microvascular invasion

Microvascular invasion (MVI) is a well-known and important prognostic factor of HCC after surgical resection or liver transplant, and its presence is a major risk factor for early recurrence after curative treatment. Preoperative identification of MVI is rare because it requires a histopathological diagnosis of peritumoral tissue. It is therefore highly important to identify effective pre-operative imaging biomarkers to confirm the presence of MVI. In a retrospective study including 197 patients with surgically resected HCC and preoperative gadoxetic acid-enhanced MRI, Lee et al showed that a combination of at least two qualitative features (including arterial peritumoral enhancement, non-smooth tumor margin and peritumoral hepatobiliary phase hypo-intensity) predicted MVI with a specificity of 92%. Moreover, early recurrence rates were higher in patients with positive MRI findings.[21] Nevertheless, unlike macrovascular invasion, which is more easily detected on preoperative cross-sectional imaging, the assessment of MVI with classical qualitative imaging is associated with significant interobserver variability, even with more experienced radiologists.[22]

Xu et al showed that the performance of a CT- based computational approach, integrating large-scale clinicoradiological and radiomic features and using 3D segmentation of both tumors and peritumoral regions, was good for the prediction of MVI and the Edmondson-Steiner grade of the lesion, with an AUROC of 0.889 in the test set. The combined radiographic-radiomics model was shown to be independently associated with disease-specific recurrence and mortality.[23]

In an MRI-based study, preoperative gadoxetic acid-enhanced MRI with a 3D intratumoural and peritumoural radiomics model traced at the hepatobiliary phase, had an AUC of 0.83 for the detection of MVI, with a sensitivity of 90% and a specificity of 75% in the validation cohort. The radiomics model was shown to be better than the radiologist's reading.[24]

3.3.2 | Liver assessment

Both liver status and portal hypertension must be evaluated for the preoperative strategy of HCC, in particular, major liver resection. Clinically-significant portal hypertension is associated with a 22%-50% increase in perioperative morbidity and is considered to be a major factor when considering HCC resection.[25] Even if the most frequent non-invasive technique is liver stiffness measurement (LSM) using transient elastography and derived scores (ie LSM-spleen-size-to-platelet ratio score (LSPS)), imaging plays a pivotal role in the non-invasive evaluation of portal hypertension. The diagnostic performance of CT-derived quantification of liver surface nodularity (LSN), a well known qualitative radiological feature of cirrhosis, was found to be good for the detection of clinically-significant portal hypertension. Its performance as also found to be similar to LSM in patients with HCC with a two-step algorithm combining LSN and LSPS, resulting in accurate detection of clinically-significant portal hypertension in more than 75% of patients.[26] Finally, this simple quantitative biomarker was shown to be a practical tool for the assessment of preoperative risk in patients with resectable HCC, with a better performance than the usual liver function tests such as the MELD score, APRI and FIB-4 score.[27] As for radiomics, LSN measurement is retrospectively performed on routine cross-sectional images, making it easier to transfer these results from research to clinical reality.

3.4 | Prognosis and the assessment of treatment response

A wide range of imaging-guided locoregional treatments are available for the management of HCC, including transcatheter arterial chemoembolization (TACE), which selectively delivers high-concentrations of chemotherapy agents to HCC, usually as a stand-alone therapy. The performance of most scoring systems in predicting survival, such as the Barcelona Clinic Liver Cancer staging system or the Okuda criteria, is mostly based on clinical and laboratory parameters.[28] This limited reliance on imaging is striking in TACE, which is still considered

to be the "best" treatment of unresectable intermediate stage HCC. In particular, 'intermediate stage HCC' includes a large and heterogeneous population with variable median overall survival ranging from 13 to 43 months. At present, the best hepatology scores for the prediction of patient response and outcome after TACE are still based on the size and number of tumors alone.[29] Indeed, like any interventional procedure, TACE is limited by a lack of standardization, which influences the accuracy and reproducibility of prediction models. As a result, and because of the relative failure of semantic imaging predictors to accurately stratify patients, efforts are now focused on building individualized models that integrate new quantitative measurements which will record the underlying pathophysiology of tumors. Song et al performed a well-designed study that proposes a combined clinicoradiological MR-based model integrating radiomics features. This promising model was shown to be associated with recurrence-free survival.[30] Another avenue of research is evaluating advanced quantitative methods such as intravoxel incoherent motion MRI focusing on the metabolic function of tumoral tissue.[31] Finally, like in most medical fields, machine learning is now being evaluated in predicting the response to TACE. One published study attempted to evaluate the performance of a CT-based neural network with encouraging results.[32]

Similarly, transarterial radioembolization (TARE) using yttrium-90 is an option for intermediate- and advanced-stage HCC. For the moment, optimal imaging criteria to assess tumor response have not been established. A retrospective study evaluated quantitative changes in tumor vascularization pre- and post- TARE on CT, and assessed increases in the areas of tumoral necrosis, suggesting that quantitative imaging could play a role as a predictive imaging feature of response to treatment.[33]

Nevertheless, in addition to the lack of multicenter validation and the difficulties in harmonizing radiomic features, these new quantitative techniques are also limited by the heterogeneity of the measurements of performance.[34] The evaluation of outcomes must be standardized to pool results and for validation in meta-analyses.

Unlike locoregional treatments, the direct efficacy of systemic therapies is easier to determine because subsequent therapies have significantly less effect on outcomes in case of treatment failure. Mulé et al published a proof of concept study assessing the ability of a radiomics-based model to predict overall survival in patients with advanced HCC treated by sorafenib.[35] The model was shown to outperform conventional visual predictors. Nevertheless, treatment of advanced HCC is constantly evolving thus requiring updates in these newly developed quantitative imaging models. Thus, the promising results of the combination of atezolizumab and bevacizumab will be the next target of quantitative imaging models, especially with the difficulties encountered in evaluating the response to immunotherapies.

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SUPPLEMENT ARTICLE

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Natural history of NASH

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the fastest growing cause of chronic liver disease worldwide. Although only a small proportion of NAFLD patients will progress to end-stage liver disease and death, the clinical burden of NAFLD is substantial due the sheer number of individuals affected worldwide. In fact, recent estimates suggest that 25% of the world have NAFLD, which is now one of the leading causes of cirrhosis and indications for liver transplantation. Although liver-related mortality is common, the most common cause of death in patients with NAFLD is related to cardiovascular diseases, followed by extra-hepatic cancers. There is a significant interindividual variability in the susceptibility to liver disease. The severity of metabolic alterations is the main risk factor for progressive NAFLD, but the qualitative components of diet, physical activity and genetic factors also play an important role. In particular, common variants in patatin-like phospholipase domain-containing 3 (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2), membrane bound O-acyl transferase 7 (MBOAT7) and glucokinase regulator (GCKR) have been shown to contribute to the full spectrum of NAFLD. In those at risk of a potentially progressive form of NAFLD or non-alcoholic steatohepatitis or in those with hepatic fibrosis, additional assessment must be made.

KEYWORDS

cirrhosis, fibrosis of the liver, genetics, hepatocellular carcinoma, natural history, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis

1 | INTRODUCTION

The acronym non-alcoholic fatty liver disease (NAFLD) defines a spectrum of clinico-pathological liver diseases that ranges from non-alcoholic fatty liver (NAFL or simple steatosis) to non-alcoholic steatohepatitis (NASH) and cirrhosis with its complications.[1] NAFLD clusters with obesity and T2DM and is commonly considered to be the hepatic manifestation of the metabolic syndrome (MS), reflecting shared pathogenic factors. The pathogenesis of the transition from simple steatosis to progressive disease is still not completely understood, and is probably multifactorial.[1] NAFLD is rapidly becoming the leading cause of liver disease worldwide and one of the top causes of cirrhosis and hepatocellular carcinoma (HCC). Currently, 25% of the general population is thought to have NAFLD

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; CVD, Cardiovascular disease; GCKR, Glucokinase regulator; GGT, gammaglutamiltransferase; HCC, hepatocellular carcinoma; MBOAT7, Membrane bound O-acyl transferase 7; MS, metabolic syndrome; NAFL, non-alcoholic fatty liver; NAFLD, Non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PNPLA3, Patatin-like phospholipase domain-containing 3; RCTs, randomized controlled trials; SEERD, Surveillance, Epidemiology and End Results Database; SRTR, Scientific Registry of Transplant Recipients; T2DM, type 2 diabetes mellitus; TM6SF2, Transmembrane 6 superfamily member 2; VLDL, very low-density lipoproteins.

worldwide, with the highest prevalence found in the Middle East and South America (31.79% and 30.45% respectively) and the lowest in Africa (13.48%).[2] The prevalence of NAFLD is higher (57.80%) in patients with T2DM.[3] Furthermore, the prevalence of NAFLD in the morbidly obese can be as high as 95%.[4]

The true prevalence of NASH is uncertain because the diagnosis of NASH is based on histology. Estimated prevalence rates of NASH in the general population range from 1.5% to 6.45%.[2] The highest rates of NASH are found in diabetic patients (65.26%, 15.05% with advanced fibrosis ≥F3),[3] and in morbidly obese subjects (20%-50%, 10% with advanced fibrosis).[4] Recent global modelling analyses based on changes in adult obesity and T2DM, suggest that the prevalence of NAFLD is set to grow exponentially over the next decade.[5]

Key points

- NAFLD can progress to advanced liver disease, hepatocellular carcinoma, liver transplantation and death.
- NAFLD is associated with an increased mortality rate, with the three top causes of death being cardiovascular disease, extra-hepatic cancer and liver disease.
- Prediction models for NAFLD forecast a 30% increase in total NAFLD cases in the next decade.
- Worsening of liver damage depends on both environmental and genetic factors, with fluctuant phases of fibrosis progression and regression.
- Advanced fibrosis bears a seven times higher risk for developing hepatocellular carcinoma, which has become the fastest growing indication for liver transplantation in the USA.

2 | NATURAL HISTORY OF NAFLD

A diagnosis of NAFLD is associated with an increased rate of mortality, with the three top causes of death being cardiovascular disease, extra-hepatic cancer and liver disease.[6] On the other hand, liver-related mortality predominates in patients with NASH and advanced fibrosis. Data on the natural history of NAFLD are mainly from tertiary centres including histological cohorts with mortality data or repeated liver biopsies performed during clinical follow-up.[7] Other evidence shows that most patients with cryptogenic cirrhosis have the metabolic profile of patients with NASH and a high recurrence of NASH post-liver transplantation.[8] More recent data from placebo arms of clinical trials of NASH with sequential protocol biopsies provide a much more dynamic picture. These results show that 20%-30% of patients with NAFLD will have NASH and that 10%-15% of these can progress to cirrhosis. Overall, the histological worsening of liver damage is not a smooth transition from simple fatty liver to NASH and fibrosis, but rather the composite result of episodes of inflammation leading to the progression of fibrosis alternating with periods of regression. This pattern is especially evident in the placebo arms of randomized controlled trials (RCTs), where the primary endpoint of the regression of NASH with no worsening of fibrosis or the regression of fibrosis regression with no worsening of NASH, is achieved in more than 20% of subjects.[9] The exact reasons for these fluctuating patterns in the progression and regression of fibrosis are not completely understood, but lifestyle changes probably play a major role.

3 | HEPATIC COMPLICATIONS OF NAFLD

The severity of liver fibrosis is the main prognostic factor in patients with NAFLD.[10] Compared to NAFLD patients without fibrosis, those with fibrosis are at an increased risk of all-cause mortality, while the risk of liver-related mortality increases exponentially with each increase in the stage of fibrosis. The estimated mortality rate ration for stage 1 is 1.41 (95% confidence interval (CI) 0.17-11.95); stage 2, 9.57 (95% CI 1.67-54.93); stage 3, 16.69 (95% CI 2.92-95.36); and for stage 4 (cirrhosis), 42.30 (95% CI 3.51-510.34).[10] A meta-analysis of early studies has shown that the progression of one stage of fibrosis takes an average of 14 years in patients with steatosis and 7 years in those with NASH.[11] However, the progression of fibrosis progression varies widely; a significant proportion of patients without histological NASH can progress rapidly, especially those with visceral obesity, T2DM, older age and Hispanic ethnicity. NASH without liver fibrosis does not seem to result in an increased risk of mortality, but it is probably associated with a faster progression of liver fibrosis[12] because of the role of necroinflammatory changes in the development of fibrosis. Liver-specific mortality in those with NAFLD has also been reported to be 0.77 per 1000 person-years, but this rate is almost 10 times higher in patients who develop NASH, with a reported rate of 11.77 per 1000 person-years.[13,14] Liver disease becomes the leading cause of death in patients with cirrhosis. Furthermore, the risk of HCC related to NAFLD has increased substantially. In fact, the estimated incidence of HCC in patients with NAFLD is 0.44 per 1000 person-years.[15] Patients with NAFLD stage 3 and 4 fibrosis have an almost seven times higher risk of developing HCC than those without significant liver disease.[16] The presence of the metabolic syndrome, especially obesity and T2DM, may hasten the development of HCC. The Surveillance, Epidemiology and End Results Database (SEERD) study suggests that although NAFLD is among the top 3 causes of HCC, the mortality in patients with NAFLD HCC is higher 1-year after diagnosis because of a higher rate of diagnosis outside of surveillance programs.[15] NAFLD/NASH is also rapidly becoming a major indication for liver transplantation in the USA. A recent analysis of the US Scientific Registry of Transplant Recipients (SRTR) from 2012 to 2016 found that NASH was the fastest growing indication for liver transplantation in listed patients, positioning NASH to become the most common cause of liver transplantation in the near future.[17] Another analysis of SRTR suggests that NASH-related HCC is the fasting growing indication for HCC listing for liver transplantation in the USA.[18] Because of the lack of systematic screening or failure to screen for HCC in these individuals, it is possible that most

patients with NASH-related HCC do not get listed for liver transplantation or die while waiting for an organ.[16]

Prediction models for NAFLD in Asia and Europe show that there could be an increase of up to 30% in total NAFLD cases between 2016 and 2030[5] in relation to the increase in obesity and T2DM. Modelling shows a slow growth in total cases and greater increase in advanced cases.

The prevalence of NASH will increase by 15%-56%, while liver-related mortality and advanced liver disease will more than double. In the European countries, the greatest increase in NASH and HCC cases is expected in Germany while France is projected to have the most cases of compensated and decompensated cirrhosis by 2030. China is expected to have the greatest increase in NAFLD cases, with an estimated 29% increase from 243.7 million in 2016 to 314.6 million in 2030. The USA will experience the highest rate of decompensated cirrhosis with an estimated 56% increase from 17.3 million cases in 2016 to 27.0 million cases in 2030.[5]

4 | EXTRAHEPATIC COMPLICATIONS IN NAFLD

Cardiovascular disease and extra-hepatic cancer predominate in subjects with lower stages of fibrosis and NAFLD can also increase the risk of morbidity and mortality related to T2DM and to cardiovascular disease (CVD).[6] A systematic review and a meta-analysis of 21 prospective, population-based studies in different ethnic groups found that ultrasound-diagnosed NAFLD and increased liver function tests (alanine aminotransferase [ALT] and gammaglutamiltransferase [GGT]) were associated with an increased risk of incident T2DM.[19] In subjects with T2DM, the presence of NAFLD further increases the risk of incident CVD and the presence of complications of T2DM.[20] NAFLD is associated with an increased prevalence of CVD, as well as incident non-fatal CVD events and CVD mortality. Among liver enzymes, GGT rather than ALT levels are most closely associated with incidental CVD events, even when they are within the normal range. In a systematic review and a meta-analysis of 10 studies in different ethnic groups, 1 U/L higher GGT (on a log scale) was associated with a 20% increase in the risk of CVD, a 54% increase in the risk of stroke, and a 34% increase in the risk of CVD and stroke combined.[21] In most cases, the association between NAFLD and mortality from CVD was independent of classical CVD risk factors and, in a few cases, of the diagnosis of MS. In biopsy-proven NAFLD, the presence of hepatic fat accumulation was associated with increased carotid artery intima-media thickness and the presence of carotid plaques,[22] with significant carotid atherosclerosis occurring approximately 5-10 years earlier in subjects with NAFLD, independently of T2DM and endothelial dysfunction. Cardiac involvement in NAFLD is not limited to coronary artery disease. Fatty liver is also associated with increased intrapericardial and extrapericardial fat and a reduced phosphocreatine/adenosine triphosphate ratio, a recognized in vivo marker of myocardial energy metabolism, even in subjects without risk factors for cardiovascular diasease.[23] Subjects with high liver fat have lower insulin-stimulated myocardial glucose uptake and lower coronary flow reserve compared to the low liver fat group,[24] suggesting that liver fat content is an independent indicator of myocardial insulin resistance and reduced coronary functional capacity.

5 | ENVIRONMENTAL AND GENETIC RISK FACTORS FOR DISEASE PROGRESSION

The severity of metabolic abnormalities, insulin resistance, and in particular the presence of T2DM, represent the major risk factors for the development of advanced liver disease, and the progression of fibrosis in prospective studies in patients with NAFLD[1] (Figure 1). Variations in body weight and associated metabolic abnormalities are the main clinical predictors for the progression of liver disease during follow-up. A key mediator of the progression of liver disease induced by metabolic risk factors can be represented by the severity of hepatic fat accumulation, which has been linked to short- and long-term progression of fibrosis independently of several confounders.[25] Industrial fructose intake has been associated with a higher risk of both the development and progression of NAFLD, probably by stimulating de novo lipogenesis,[26] as well as an increase in the ratio of dietary saturated/unsaturated fat intake. On the other hand, the role of red meat consumption has not been clearly established.

Accumulating evidence shows that hepatic fat and NAFLD are strongly inheritable conditions.[27] Multi-ethnic cohort studies show that there is a strong interethnic variability in the susceptibility to the development of NAFLD, which is higher in Hispanics, intermediate in Europeans and lower in African-Americans, independent of weight, T2DM and socioeconomic factors. The risk of progressive NAFLD is higher in first-degree relatives of patients with NAFLD cirrhosis compared to the general population, independent of several confounders. In the past few years, the most important common genetic determinants of hepatic fat variability and the susceptibility to develop NAFLD have been identified with the advent of genome-wide association studies. The major determinant is the rs738409 C > G encoding for the I148 M protein variant of patatin-like phospholipase domain-containing 3 (PNPLA3), which accounts for a large fraction of the increased risk of this condition in Hispanics. The I148 M variant

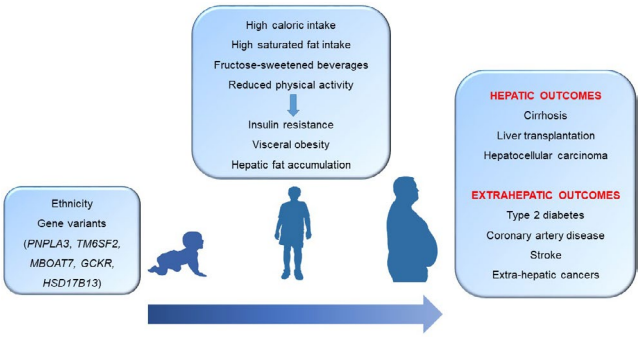

FIGURE 1 Environmental and genetic factors implied in the development of NAFLD and its complications, according to different ages of life

increases susceptibility to the whole spectrum of NAFLD-related liver damage, from simple steatosis, to NASH, fibrosis and cirrhosis, thus representing a general modifier in the progression of liver disease. Furthermore, the I148 M variant increases the risk of progression to HCC independently from the effect on fibrosis. In Europeans, homozygosity for the mutation is enriched almost nine-fold in patients who develop NAFLD-HCC compared to the general population, while an absence of this variant can exclude the risk of HCC with a high specificity in the general population; polygenic risk scores can help to gain insight into the causal relationship between NAFLD and HCC and to improve HCC risk stratification.[27,28] Carriage of this variant influences the risk of liver disease especially during developmental ages, interacting with dietary factors such as the intake of fructose-enriched drinks, and a lack of physical activity.[29] Other common genetic mutations regulating hepatocellular lipid contribute to the risk of NAFLD. The rs58542926 C > T encoding for the E167K variant in transmembrane 6 superfamily member 2 (TM6SF2) favours hepatic fat accumulation by decreasing lipid secretion in very low-density lipoproteins (VLDL), also leading to increased susceptibility to liver damage. At the same time, this genetic factor protects from CVD by reducing circulating lipids.[27] Variants in glucokinase regulator (GCKR) and in membrane bound O-acyl transferase 7 (MBOAT7) also contribute to the risk, by increasing de novo lipogenesis and altering the remodelling of phospholipids respectively. All these factors result in fat accumulation and a higher risk of liver disease.[27,28] Conversely, the most recent HSD17B13 variant T > TA confers protection from liver damage in NAFLD.[30] The impact of the genetic variants on hepatic fat content, the risk of NAFLD and that of cirrhosis increases exponentially with an increasing body mass index (BMI), indicating the presence of a synergy among these components of the disease. It is important note to that in individuals at a high genetic risk, a healthy dietary pattern modelled on the Mediterranean diet as well as regular physical activity can reduce the risk of NAFLD.[31]

6 | CONCLUSIONS

Non-alcoholic fatty liver disease is not a benign disease because it can progress to advanced liver disease, hepatocellular carcinoma, liver transplantation and death. The prevalence and incidence of NAFLD is increasing globally. Although the number of patients with disease progression is small, the global disease burden is substantial. Further studies are needed to develop interventions to reverse the course of NAFLD, especially as we increase our understanding of NAFLD.

CONFLICT OF INTEREST

The authors declare no conflict of interest with the present article.

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SUPPLEMENT ARTICLE

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Hepatocellular Carcinomas: Towards a pathomolecular approach

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Abstract

Molecular analysis of primary liver malignancies has provided a refinement of the pathological diagnosis of this entity and the identification of an increasing number of tumor subtypes of hepatocellular proliferation, either malignant (hepatocellular carcinomas) or benign (hepatocellular adenomas). Besides the diagnosis, a combined pathomolecular approach can also provide further insights into patient prognosis, and help select patients who can benefit from targeted therapies. Hepatocellular carcinomas define a heterogeneous group of malignant hepatocellular proliferation at various levels: macroscopic, histological and molecular. While most carcinomas occur in patients with chronic liver diseases and advanced fibrosis in the background liver, some arise from the malignant transformation of a pre-existing hepatocellular adenoma. *TERT* promoter mutations are the most frequent genomic alterations observed in the process of malignancy, and they occur early in the process of liver carcinogenesis. Overall, a more active biopsy strategy should be considered a key step in the management of patients with HCC.

KEYWORDS

dysplastic nodules, hepatocellular adenoma, hepatocellular carcinoma, molecular classification, tumor biopsy

1 | INTRODUCTION

Hepatocellular carcinomas (HCC) account for 75%-85% of primary malignant liver tumors in adults, are the sixth most common cancer, and the fourth leading cause of cancer-related deaths worldwide. In most cases, HCC develops in patients with chronic liver diseases showing advanced fibrosis and cirrhosis, which is consistent with a multistep process of carcinogenesis through the progressive malignant transformation of preneoplastic lesions (i.e dysplastic cirrhotic nodules). This progression is also associated with a growing accumulation of genetic and epigenetic abnormalities in the liver cells from regenerative to malignant nodules.[1] The main risk factors of HCC include chronic viral infections (Hepatitis B and C), excessive alcohol consumption and metabolic syndrome, which is a new leading risk factor. It is important to note that in the latter setting, HCC may develop in the absence of advanced liver fibrosis in up to 45% of cases, suggesting the involvement of specific mechanisms that are probably related to the pathogenesis of the underlying disease rather than fibrosis alone.[2,3]

The diagnosis of HCC is based on dynamic imaging showing a specific vascular pattern with a wash-in/wash-out profile in the arterial and portal phases, respectively. Although the diagnostic performance of imaging is not a subject of debate (especially for nodules > 2 cm), a more exhaustive tumor characterization providing an accurate evaluation of the prognosis and potential response to treatments is needed in an era of precision medicine. Several robust subgroups of HCC have now been recognized with the use of complementary molecular techniques (such as transcriptomics and

exome sequencing), leading to a comprehensive molecular landscape of HCC, and the correlations between molecular and pathological features.[4-7] Thus, the role of the pathologist and a more active biopsy strategy should be considered to be key steps in the management of patients with HCC.

Key points

- Hepatocellular carcinomas define a heterogeneous entity
- Different molecular subgroups of HCC are recognized with prognostic value
- Some of the molecular subgroups may be identified through histopathological analysis
- Hepatocellular carcinoma may derive from malignant transformation of preneoplastic lesions
- Specific subtypes of hepatocellular adenoma are risk factors for malignant transformation

2 | HCC: A HETEROGENEOUS GROUP OF TUMORS

HCC heterogeneity may be assessed at various levels. First, while three main macroscopic patterns are described (ie, nodular, infiltrative or diffuse), this definition is limited because the categorization of a tumor within one single growth pattern can be difficult. Second, on the microscopic level, the diagnosis of HCC is based on the resemblance between tumor cells and normal hepatocytes. Therefore, the microscopic evaluation involves an assessment of the cytological characteristics of tumor cells and an evaluation of their architectural pattern. Tumor proliferation may present varying degrees of hepatocellular differentiation within a single tumor, especially larger ones. The three main classic architectural patterns of growth of HCC are trabecular, compact/solid, and acinar/pseudo glandular. Interestingly, these growth patterns are closely linked to the molecular subtypes.[8] Third, at the molecular level, different subclasses of HCC have been described, mostly linked to the clinical context (including etiological factors) and prognosis (including tumor recurrence and survival).[4,5,7,9,10] Schematically, HCCs are divided into two major subgroups, one associated with chromosomal stability associated with a better prognosis, and the other associated with chromosomal instability and a poorer prognosis. In addition, a pathomolecular classification has recently been proposed, based on the G1-G6 classification and pathologic features specifically associated with the molecular patterns.[8] Finally, transcriptomic analyses have identified two major sub-classes of HCC: tumors with a high proliferation which are more frequently activated for TGFß, are more aggressive and with frequent progenitor phenotypes. In contrast, less proliferative HCC are more differentiated and frequently mutated for CTNNB1 with ß-catenin activation.[4]

According to the most recent WHO classification, HCC is classified into eight morphological subtypes.[11] One of these, *the steatohepatitic variant (SH-HCC)*, which was initially described in HCV transplanted patients and then in patients with an alcoholic or metabolic clinical context, is characterized by the morphological hallmarks of NASH, including steatosis, ballooning malignant hepatocytes, Mallory-Denk bodies within tumor cells, inflammatory infiltrates and pericellular fibrosis (Figure 1A).[12-14] This variant was assigned to the G4 transcriptomic subgroup characterized by a lack of Wnt/β-catenin pathway activation and low GS expression.[8,15] While no significant changes in genes involved in lipid metabolism were observed, activation of the IL6/AKT/STAT pathway was frequent in this subgroup, which is consistent with the involvement of this pathway in the transition from NAFL to NASH.[8] Immunophenotypically, ballooned tumor hepatocytes are negative for Cytokeratin 8/18, except for Mallory-Denk bodies, which are also labeled by ubiquitin. Additionally, SH-HCCs are diffusely stained with sonic hedgehog ligand, while a minority of them express progenitor markers including SALL4, EpCAM and CK19.[15] Whether the prognosis of SH-HCC is better or worse than conventional HCC is difficult to conclude because available data are derived from resected or transplanted patients. Nevertheless, almost none of these patients had any statistical differences in overall survival or disease-free survival.[13,14] This clinical picture is supported by the less aggressive histological phenotype with a lack of satellite nodules and microvascular invasion, which SH-HCC seems to display.[8]

Macrotrabecular-massive HCC (MTM-HCC), observed in 10%-20% of HCC, is defined by a predominant (>50% of the total tumoral area) macrotrabecular (>6 cells thick) architectural proliferation (Figure 1B).[8] It is more frequently observed in patients with HBV infection, associated with high alpha-fetoprotein serum levels and exhibits features of a poorer prognosis, including vascular invasion and satellite nodules.[8,9,16] MTM-HCC are clustered with the G3 transcriptomic subgroup, which is linked to cell cycle activation and chromosomal instability. *TP53* mutations and/or *FGF19* amplifications are common hallmarks. Immunophenotypically, MTM-HCC is characterized by high expression of Endothelial-Specific Molecule 1 (ESM1) and Carbonic Anhydrase IX (CAIX).[17]

The CTNNB1-mutated HCC is generally well-differentiated, characterized by trabecular and pseudo glandular architectural patterns, intratumoral cholestasis and lack of immune infiltrates (Figure 1C). These tumors, usually related to HCV infection and obesity in non-HBV patients, are clustered with the G5-G6 transcriptomic subgroups and display the expression of genes involved in hepatocellular differentiation and function as well as in bile uptake.[18] Immunophentotypically, this subtype displays a strong and diffuse glutamine synthetase positivity as well as nuclear ß-catenin accumulation in tumor hepatocytes (Figure 1D).[19] Using the microarray technology, several studies have shown that a subset of adult HCC displays phenotypical traits of progenitor cells. These tumors retain stem cell markers and express CK19, a marker of biliary lineage. Interestingly, poorer survival was found in this subgroup.[20,21]

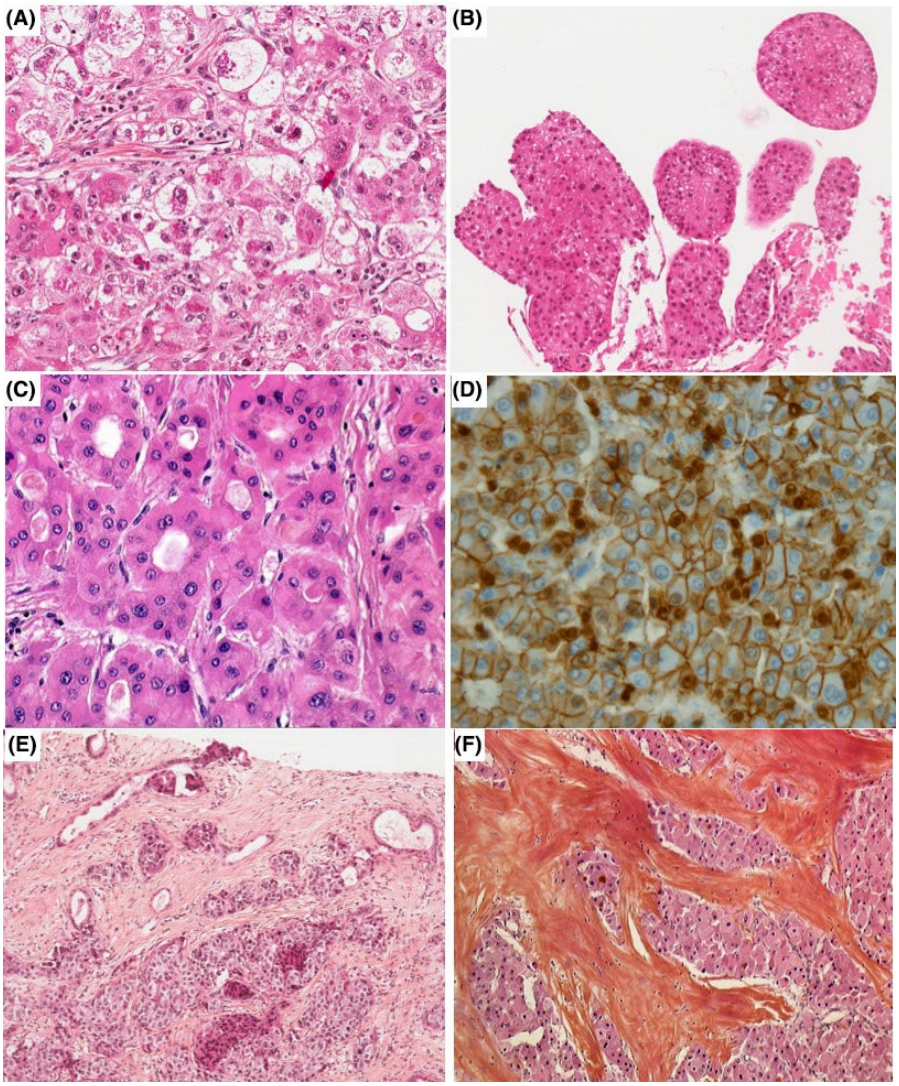

FIGURE 1 Hepatocellular carcinomas subtypes. A, Steato-hepatitic variant: The tumor is predominantly composed of large ballooned cells with Mallory-Denk bodies, some inflammation and pericellular fibrosis; B, macrotrabecular-massive pattern: Biopsy specimen showing tumor trabeculae are composed of > 6 cell plates, outlined by endothelial cells; C-D, Micro-trabecular & pseudoglandular pattern: Hepatocytes display gland-like organization around dilated canaliculi between tumor cells with or without bilirubinostasis (C); β-catenin immunostaining showing tumoral hepatocytes with aberrant nuclear positivity (D); E, Hepatocholangiocarcinoma: Tumor biopsy showing hepatocellular differentiation (massive proliferation) with several malignant glandular structures embedded in the fibrous stroma; F, Fibrolamellar hepatocellular carcinoma: The tumor is composed of large eosinophilic tumor cells organized in cords between dense acellular fibrous bands

HCC may also be recognized as an individualized component within more complex primary liver malignancies, i.e combined hepatocholangiocarcinoma (cHCC-CC), which display clear features of both HCC and cholangiocarcinoma (Figure 1E).[11,22] Although rare, their recognition seems to increase, with further molecular evidence supporting their clonal status derived from hepatic progenitor cells.[23-25]

Finally *the Fibrolamellar carcinoma (FLC) variant* is a specific entity, first described in 1980, characterized by DNAJB1-PRKACA gene fusion leading to PKA activation with large eosinophilic neoplastic cells embedded in a dense fibrous stroma (Figure 1F).[26] Classically, FLC is mainly observed in younger patients in an absence of chronic liver diseases. Another subtype of HCC characterized by BAP1 (gene encoding BRCA1 associated protein-1) inactivating HCC, together with PKA activation, was found to have FLC-like pathological features but belonged to the G1 transcriptomic subtype.[27]

HCC grading relies on the Edmondson & Steiner system which subdivides HCC into four grades, from I to IV, on the basis of histological and cytological resemblance to the normal liver. In fact, most of HCC present as grade II or III. Therefore, and as for other carcinomas, there is a general tendency to summarize the grading to a 3-scale system with well-, moderately- and poorly-differentiated HCC. Tumor grade can predict patient survival and disease-free survival after resection as well as after liver transplantation, with the poorest grade driving the prognosis. It is important to note that grading from

needle biopsies correlates well with the grading from the respective resection specimen.[28]

Several staging systems have been proposed for HCC, including prognostic factors related to tumor stage, liver function and general health status. Among tumor-related prognostic factors, the presence of microscopic vascular invasion can only be accurately identified by pathological analysis of the resected specimen. The development of surrogate markers of microvascular invasion from biopsy samples would be helpful to choose the most appropriate therapeutic option. While several potential markers have been proposed, none of them have been validated in independent series (Figure 2).[29,30]

3 | PREMALIGNANT HEPATOCELLULAR LESIONS AND SMALL HCC

There is a consensus that HCC is a result of cumulative genetic and epigenetic events that can differ depending on the etiology of the background chronic liver disease. Although recurrent genetic abnormalities have been reported in fully developed HCC, the early molecular events are less well known. It is important to note that *TERT* promoter mutations, which have been reported to be the most frequent mutations in liver carcinogenesis, with increasing rates from dysplastic nodules (<20%) to HCC (around 60%), appear to be a prerequisite for malignant transformation, followed by additional mutations in a panel of genes (TP53, ARID, β-catenin,...) for cancer progression.[31]

Morphologically, nodules < 2cm that develops on cirrhosis are classified into preneoplastic nodules (including macroregenerative, low- and high-grade dysplastic nodules) and neoplastic nodules (small HCC).[32] Small HCCs are morphologically further subdivided into vaguely nodular (early HCC) and well-circumscribed HCC (small progressed HCC). These two patterns have different prognoses with a better prognosis in the vaguely nodular form than in well-circumscribed nodules, which have already acquired an ability to invade vessels and metastasize. A differential diagnosis between dysplastic nodules and small HCC is based on a large set of cytological and architectural criteria including the presence of "clonal foci", increased numbers of unpaired arteries, focal loss of the associated reticulin framework and stromal invasion.[33] Additional immunohistochemical markers such as Glypican 3, HSP 70, Glutamine synthetase and Arginase-1 have been shown to be useful, improving the pathological performance for the discrimination of HCC from precancerous lesions, especially high-grade dysplastic nodules.[34,35]

4 | MALIGNANT TRANSFORMATION OF HEPATOCELLULAR ADENOMA INTO HCC

Whereas most HCC occur in patients with advanced liver fibrosis in the background liver, some may develop in the absence of recognized risk factors of chronic liver diseases, following malignant transformation of pre-existing hepatocellular adenoma (HCA). HCA is a rare, benign liver cell neoplasm strongly associated with oral contraceptive (OC) use and androgen steroid therapy. Like HCC, HCA represents a heterogeneous group of tumors, with a risk of malignant transformation to HCC of between 4% and 10%.[36,37] Comprehensive molecular studies have provided further insight into the understanding of HCA, with a pathomolecular classification defining 5 main subtypes according to phenotype and molecular features [HNF1α-mutated steatotic (H-HCA), inflammatory (I-HCA), β-catenin-mutated HCA (b-HCA), mixed (I/b-HCA), and sonic-hedgehog HCA (sh-HCA)], and identified genetic alterations associated with their malignant potential.[37]

Several risk factors of malignant transformation of HCA have been identified, including male gender, metabolic syndrome, tumor size and HCA subtyping. Indeed, the highest risk of malignant transformation into HCC is found with b-HCA, reaching 40% in some series. It should be noted that b-HCAs are a heterogeneous group of tumors with various levels of activation of the WNT signaling pathway, as a result of mutations or deletions of CTNNB1 involving exons 3, 7 and 8.[38] Of the three subtypes, the one with exon 3 abnormalities (except S45) has the highest risk for malignant transformation into HCC.[38] This subtype is mostly found in men and frequently shows significant cell atypias, pseudo-glandular formations, and pigment accumulation (bile, lipofuschins). Immunophenotypically, this subtype displays nuclear β-catenin staining in some tumoral cells (usually focal) with diffuse, homogeneous and strong GS staining. Because I-HCA may

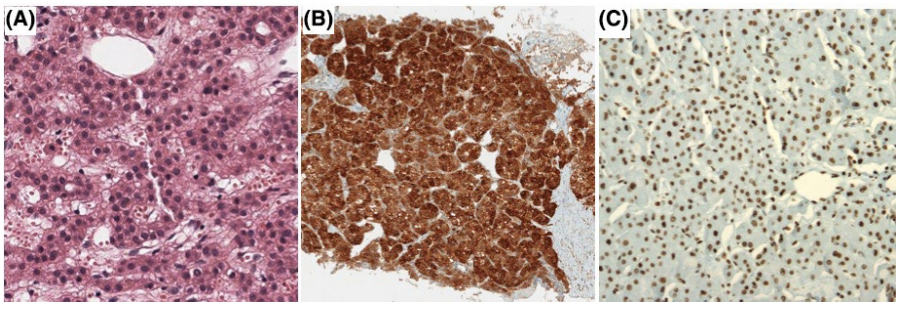

FIGURE 2 Hepatocellular carcinoma with vascular invasion: input of immunophenotyping on tumor biopsy. A, tumor biopsy showing moderately-differentiated hepatocellular carcinoma (Hematein & eosin); B, PIVKA immunostaining showing strong and diffuse positivity in tumoral hepatocytes; C, Histone H4 immunostaining showing diffuse nuclear positivity of tumoral hepatocytes

also exhibit marked WNT signaling pathway activation, they also have a higher risk of malignancy. In contrast to the molecular steps described in HCC that develop on cirrhosis, the *CTNNB1* exon 3 mutation is the earliest genetic alteration, while mutations in the *TERT* promotor seem to be involved in the final transitional step from HCA to HCC.[39] Malignant transformation has recently been reported in the H-HCA subtype, with a greater frequency in women with multiple lesions.[40] It should be noted that the mean size of the H-HCA with malignant transformation was 8.9 cm, suggesting that tumor size probably plays a role in malignant progression.[40]

Because HCA can progress to malignancy, borderline hepatocellular lesions can be expected. These HCAs do not have a definite pathomolecular subtype, but are examples of an uncertain diagnosis between HCA and HCC. Various terms have been proposed, including "atypical hepatocellular neoplasm", "hepatocellular neoplasm of uncertain malignant potential," and "well-differentiated hepatocellular neoplasm with atypical or borderline features". Although it is difficult, recognition of these nodules is based on a spectrum of morphological criteria suggesting HCC but insufficient for a definite diagnosis, as well as negative classical immunomarkers of HCC such as glypican 3 and heat shock protein 70. Molecular analysis of *TERT* promoter mutations can be useful as a marker of malignancy. For instance, *TERT* promoter mutations have been reported in 17% of borderline lesions compared to 50%-60% of HCC.

Because of the higher risk of malignant transformation of HCA, resection is recommended (i) in men, (ii) with proven β-catenin exon 3 mutations (except S45) irrespective of tumor size (iii) in large HCA (≥ 5cm), after a period of 6 months observation with lifestyle changes, including removal of oral contraceptives in women and (iv) in borderline lesions.[41,42]

In conclusion, the management of patients with HCC is entering an era of precision medicine with the development of molecular techniques for the identification of prognostic tumor subgroups. The application of these techniques in clinics with a comprehensive pathomolecular approach based on tumor biopsy will pave the way for a stratified therapeutic strategy.

CONFLICT OF INTEREST

The authors do not have any disclosuresto report.

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SUPPLEMENT ARTICLE

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Current management of NAFLD/NASH

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Abstract

NAFLD is the most common cause of liver disease worldwide, and its prevalence is significantly increasing. Studies have shown that it is associated with comorbidities such as diabetes, metabolic syndrome and obesity. Early diagnosis and management are highly important and could modify the prognosis of the disease. Evaluating the possibility of multiple aetiologies and recognizing the additional causes of liver disease should be a part of the patient's initial assessment. There are no approved drug treatments as yet, so the main management strategies should involve lifestyle changes such as physical activity and dietary re-education.

KEYWORDS

diet, lifestyle, NAFLD, NASH, physical activity

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) includes a broad spectrum of histological changes ranging from simple accumulation of fat in the liver—hepatic steatosis—to the presence of necroinflammation, fibrosis and cirrhosis, including an increased risk of developing hepatocellular carcinoma (HCC).[1] The presence of steatosis is associated with a low risk of progression to cirrhosis; however, cardiovascular complications are a significant concern in these individuals.[2] Nevertheless, non-alcoholic steatohepatitis (NASH), characterized by a pattern of intricate parenchymatous involvement, can progress to cirrhosis in 15%-20% of these patients.[1]

The progressive, overall increase in the prevalence of NAFLD is mainly related to a sedentary lifestyle and dietary habits. Although the real prevalence of NAFLD is still unknown, it is estimated to be present in about 20%-30% of the general population in Western countries and 5%-18% in Asia.[1] In addition, the estimated prevalence of steatohepatitis is 2%-3%, and even higher in patients with type 2 diabetes mellitus (T2DM), with metabolic syndrome (MetS) and obesity (body mass index >30 kg/m^2).[2] NAFLD is strongly linked to obesity, with a prevalence of 80% in obese individuals compared to only 16% in individuals with an average body mass index (BMI) and without metabolic risk factors.[1] In obese patients with comorbidities, bariatric surgery has been found to be an alternative management strategy, and several studies have reported a regression of steatohepatitis after the procedure.[3] Thus, with the increased prevalence of this disease and its potential for progression as well as the risk of cardiovascular events and complications related to liver disease, it is essential to define the best strategy for the diagnosis, follow-up and intervention in these patients. Initially, the diagnosis of other possible causes of liver diseases must be considered, such as alcohol use, viral hepatitis, autoimmune diseases, medications, hemochromatosis and less common aetiologies such as Wilson's disease.

Abbreviations: BMI, body mass index; HCC, hepatocellular carcinoma; IHTGs, intrahepatic triglycerides; MAFLD, metabolic associated fatty liver disease; MetS, Metabolic Syndrome; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; T2DM, Type 2 diabetes mellitus.

2 | ALCOHOL USE

A systematic analysis for the Global Burden of Disease Study from 1999 to 2016 was recently published and concluded that drinking alcohol (ETHO) substantially impacts health and is a leading risk factor for global disease burden. Additionally, it was found that the risk of all-cause mortality, including cancers, rises with increasing levels of consumption, and the level of consumption that would minimize health loss would be zero.[4] However, in real life, many individuals drink alcohol, and the impact of alcohol use in NAFLD patients is a subject of debate.

Owing to the recent consensus suggesting changing the name from NAFLD to MAFLD, alcohol is no longer an exclusion criterion for diagnosing this entity, as it was for the diagnosis of NAFLD.[5] The new nomenclature was extensively discussed among the experts who participated in this consensus, and many issues were considered. First, it is undeniable that the evaluation of alcohol consumption is challenging in daily clinical practice. The difficulties evaluating alcohol intake include the choice of the adequate questionnaire/interview to precisely define alcohol use, accurate serum biomarkers, how to overcome the underestimation of alcohol consumption by patients and the lack of standardization of expressions such as the definition of 'social' and 'binge' drinking. Currently, excess alcohol drinking may be defined by the ingestion of more than 20 mg of alcohol for women and 30 g for men, which would be equivalent to three standard drinks per day for men and two for women.[6]

There is still some controversy on the benefit of drinking small amounts of alcohol. Dunn et al studied 331 adults who drank moderate amounts alcohol, excluding those who drank >20 g/ETHO/day, binge drinkers and non-drinkers with previous alcohol consumption. This group was compared with lifelong non-drinkers, and the odds of having NASH were evaluated in both groups. Surprisingly, modest drinkers had a lower risk of having NASH and a lower risk of liver fibrosis. However, this was a cross-sectional study and thus did not evaluate long-term outcomes.[7] Kwon et al evaluated the impact of lifetime alcohol consumption in 77 adults who drank over 40 g/day of ETOH. The median lifetime cumulative alcohol intake was 24 g-years. On multivariate analysis, increasing age was associated with severe liver disease, while alcohol consumption over 24 g-years was associated with the less severe disease with an OR of 0.26, 95% CI 0.07-0.97, $P = .04$. Patients who continued to consume alcohol or had been abstinent for ≤1 year had less severe disease. In this study, the authors concluded that some degree of regular alcohol intake over a lifetime compared to negligible intake appears to have a protective effect on liver histology severity among patients with NAFLD.[8] However, in 2009, Ekstedt et al investigated whether low alcohol intake in 71 NAFLD patients with histological re-evaluation and a mean follow-up of 13 years was associated with the progression of fibrosis.[9] At follow-up, 17 patients, or 24% of the studied patients fulfilled the criteria for significant fibrosis progression. The proportion of patients reporting heavy episodic drinking at least once a month was higher among those with a significant progression of fibrosis. Also, a trend towards higher weekly alcohol consumption was observed. Thus, moderate alcohol consumption in patients with biopsy-proven NAFLD might be associated with the progression of fibrosis. Heavy episodic drinking should be avoided in these patients to avoid the progression of fibrosis. Many studies recently showed that alcohol intake within the current definition's safe limits poses a significant risk for the progression of liver disease. In a study of 58,927 Korean subjects with NAFLD and low baseline fibrosis scores assessed by NAFLD fibrosis score and Fibrosis-4, either light (1.0-9.9 g/d) or moderate (10.0-29.9 g/d; 10.0-19.9 g/d for women) alcohol intake was independently associated with worsening of liver fibrosis over a median of 4.9 years of follow-up, compared to individuals without any alcohol consumption (0 g/d).[10] This study concluded that in patients with NAFLD, even moderate alcohol intake could be harmful. Prospective studies are necessary to confirm these data for the presence of steatohepatitis with or without fibrosis at baseline, if possible, in different cohorts.

Hart et al evaluated two prospective cohorts in Scotland to investigate the additive factor of alcohol consumption and BMI and the increased risk of liver disease. Patients were categorized according to alcohol consumption into zero drinks, 1 to 14 drinks/week and 15 or more drinks per week, and BMI as normal, overweight and obese. They found that raised BMI and alcohol consumption are related to liver disease, with evidence of a supra-additive interaction between the two, since the relative excess risk as a result of the interaction between BMI and alcohol consumption was observed.[11]

The relation between alcohol ingestion and ischaemic heart disease has also been evaluated in the study that assessed alcohol use and the Global Burden of Diseases.[4] Overall, women, particularly in high social demographic index locations, experienced some protective effects for ischaemic heart disease and diabetes after 60 years of age, although this risk was surpassed by the risk of cancer. Only high social demographic index and low high social demographic index locations had noticeable protective effects for ischaemic heart disease in men. However, this does not necessarily support alcohol-drinking habits since poorer alcohol-related outcomes were described as increasing diabetes in both genders.

The impact of alcohol intake under 14 drinks for women and 21 for men was evaluated in 570 NAFLD patients followed for 25 years

Key points

- NAFLD is a highly prevalent disease.
- The assessment of other causes of liver disease should be part of the initial evaluation.
- Cardiovascular disease is the primary cause of morbidity and mortality among these patients.
- There are no approved drugs for the treatment of NAFLD.
- Lifestyle changes, including physical activity and dietary re-education, are the leading measures to be followed by patients.

in the CARDIA study. Among those, 58% were drinkers. There were no significant differences in risk factors for either cardiovascular disease markers or subclinical cardiovascular disease when drinkers and non-drinkers were evaluated by a multivariate adjustment analysis, suggesting that alcohol use might not decrease cardiovascular risk disease in NAFLD patients.[12]

The established outcomes related to MAFLD non-NAFLD compared to MAFLD NAFLD will need to be investigated in the future. Thus far, most studies have associated alcohol use with the worst outcomes related to liver disease. Thus, additional public health strategies should be developed worldwide to decrease alcohol consumption, mainly in specific populations such as NAFLD patients.

3 | NAFLD AND INVESTIGATION OF COMORBIDITIES

The new acronym for NAFLD, MAFLD, includes an algorithm that considers its diagnosis in any individual with liver steatosis plus obesity or diabetes, or any individual with steatosis who has at least two risk factors that suggest metabolic deregulation.[5]

Even before this consensus, the coexistence of NAFLD with other chronic liver diseases was frequently observed. It is highly important to diagnose additional comorbidities in daily practice to better define adequate interventions that could improve patient outcomes.

Before the NAFLD epidemic, the most frequently identified liver diseases worldwide were chronic hepatitis B and C.[13] Patients with hepatitis C and a sustained virological response with steatosis present worse extrahepatic outcomes such as cardiovascular events and T2DM.[14]

Regarding chronic hepatitis B, Kim et al showed that among 587 patients treated with nucleos(t)ide analogues, 11.9% presented with MetS. The diagnosis of MetS had a significant prognostic impact for the cumulative occurrence rates of viral breakthrough, genotypic resistance, HCC, disease progression and overall adverse outcomes.[15] These data show that the occurrence of both viral hepatitis and NAFLD is not negligible and should be managed carefully to improve prognosis.

Autoantibody testing is performed in a variety of situations to clarify if there are signs of autoimmune diseases. Notably, autoantibodies are commonly performed to investigate the aetiology of enzyme increase in patients with abnormal liver enzymes. However, most antibodies are not sensitive/specific enough to make the diagnosis alone. Anti-smooth muscle antibody and anti-nuclear antibodies are present in many liver and non-liver conditions. Viral hepatitis, as well as alcohol intake, can also be associated with these antibodies. Markers of autoimmune liver diseases may also be observed in NAFLD patients with a prevalence of around 20% without more severe histological disease or worse follow-up outcome.[16] Nonetheless, the definition of the presence of autoantibodies and its association with the diagnosis of autoimmune liver diseases, mainly autoimmune hepatitis, may be challenging in patients with NAFLD. Additional diagnostic tests may need to be performed, and a liver biopsy may also be necessary to clarify the possible presence of autoimmune hepatitis associated with NAFLD rather than an epiphenomenon without prognostic implication.[17]

Similarly, a vigorous investigation must be performed when interviewing patients to exclude the long-term use of any herbal or non-prescribed supplements, over-the-counter medications or 'health foods', supplements or herbal products used as 'joint pain remedies'. Moreover, the use of certain drugs such as steroids, amiodarone or methotrexate, among others[18] could potentially induce drug-induced fatty liver disease or cause liver fat infiltration.

Elevated ferritin levels are observed in around 20%-30% of patients with NAFLD. In these cases, hyperferritinaemia is a dysmetabolic iron overload syndrome representing an acute phase reactant.[19] Transferrin saturation is a valuable screening test in patients with hyperferritinaemia, since a value of >45% suggests a diagnosis of hereditary hemochromatosis. In contrast, in the presence of a normal percent saturation of haemoglobin, ordering the hemochromatosis-associated genetic mutations or imaging for hepatic iron concentration would not be appropriate.[19]

Previous studies have shown that fatty liver disease is frequent in patients with coeliac disease, and in contrast, coeliac disease is also more common in patients with hepatic steatosis. Inflammation mediates the interplay in the pathogenesis of coeliac disease and hepatic steatosis, and they may be interconnected with a common background.[20] For the diagnosis of coeliac disease, serum tissue anti-transglutaminase-IgA (tTG-IgA) and endomysium-IgA (EMA-IgA) antibodies can be investigated. Both have similar sensitivities and have a high negative predictive value in patients with a low suspicion of coeliac disease. Additionally, both have high specificity for the diagnosis, even in low-risk patients. In the presence of suspected coeliac disease, the most appropriate initial tests are tTG-IgA and IgA levels.

Other rare diseases can resemble fatty liver such as Wilson's disease, glycogenoses, lysosomal acid lipase deficiency, and should be included in the differential diagnosis of liver steatosis in children and adolescents.

Thus, it is essential to identify other chronic liver diseases and manage them accurately in NAFLD patients. In Table 1, the primary investigation of common chronic liver diseases is presented.

4 | CARDIOVASCULAR DISEASE

The routine evaluation of cardiovascular disease and its risk factors is imperative in patients with NAFLD, since cardiovascular events are additional risk factors of morbidity and mortality. It should be remembered that there is no difference in mortality rates associated with cardiovascular disease in individuals with NAFLD and NASH.[21]

NAFLD is a predictor of atherosclerosis, and the evaluation and treatment of dyslipidaemia, when necessary, should be a therapeutic target in these patients.[22] It is important to emphasize that statins

TABLE 1 Baseline screening tests in managing patients with liver steatosis or MAFLD with potential comorbidities

Disease	Test	Interpretation
HCV infection	Anti-HCV	If (+), an HCV-RNA is necessary to define chronic HCV
HBV infection	HBsAg	If HBsAg (+), additional investigation is necessary
Autoimmune hepatitis (AIH)	ANA antibody, Anti-Smooth Muscle antibody, IgG	A liver biopsy may be necessary for a definite diagnosis
Primary Biliary Cholangitis (PBC)	AMA, IgM, Cholesterol	AMA (+) plus cholestasis is diagnostic of PBC
Primary Sclerosing Cholangitis (PSC)	MRCP, p-ANCA	Chronic cholestasis, repeated cholangitis
Hereditary Hemochromatosis	Transferrin saturation	If over 45% and ferritin levels are above 300 ng/mL in men and 200 ng/mL in women, further genetic tests are needed as well as hepatic iron evaluation by image (MRI)
Wilson's Disease	Ceruloplasmin	If <10 highly suggestive of Wilson's Disease
Alpha-1 antitrypsin deficiency	Alpha-1 antitrypsin	Low levels suggest the disease
Coeliac Disease	TTG-IgA – IgA IgA levels	A (+) tTG-IgA – IgA It is highly suggestive of Coeliac Disease

Abbreviations: ANA, anti-nuclear antibody; anti-HCV, Anti-hepatitis C virus antibody; HBsAg, Hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; IgA, Immunoglobulin A; IgG, immunoglobulin G, IgM, Immunoglobulin M; MRCP, Magnetic Resonance Cholangiopancreatography; MRI, Magnetic Resonance Imaging; p-ANCA, perinuclear anti-neutrophil cytoplasmic antibodies; tTG-IgA, transglutaminase antibodies IgA type.

can be used safely in these individuals, and not employing them can cause more damage.[23]

Arterial hypertension is also associated with NASH.[24] Thus, the severity of liver disease should be addressed in the initial evaluation of patients with arterial hypertension.

5 | LIFESTYLE CHANGES

Lifestyle modifications remain the most effective treatment for NAFLD/NASH and include a combination of dietary modifications and physical activity. It is well known that low levels of moderate-intensity physical activity and high amounts of sedentary time are associated with insulin resistance, MetS, T2DM[25] and NAFLD.[26] The relationship between diet and the development of NAFLD is complicated and is certainly related to dietary patterns and amount of food.

Increasing physical activity reduces intrahepatic triglyceride content and hepatocellular injury markers in patients with NAFLD, independent of weight loss.[27] A systematic review and meta-analysis from 17 studies on the impact of exercise training and associated weight loss on intrahepatic triglycerides (IHTGs) in individuals with NAFLD showed that exercise reduced IHTG levels, independently of significant weight change. However, the benefits achieved were substantially more significant when weight loss occurred.[27] The guidelines from the European Associations for the Study of the Liver (EASL), Diabetes (EASD) and Obesity (EASO) recommend 150-200 min/week of moderate-intensity aerobic physical activity for NAFLD patients, in three to five sessions.[18]

The four most common dietary patterns include the low-carbohydrate diet, the low-fat diet, the Dietary Approaches to Stop Hypertension diet (DASH) and the Mediterranean diet.[28] Newer diets are regularly proposed but the most important element is to prescribe a diet that can be followed by patients, taking into consideration local food, cost and habits.

6 | MEDICATIONS

The EASL guideline for the management of NAFLD proposes treating patients with significant fibrosis or with less severe disease but at high risk of disease progression (ie with diabetes, MetS, persistently increased ALT, high necroinflammation).[18] There are no drugs approved by regulatory agencies for NASH. Therefore, no specific therapy can be firmly recommended, and any drug treatment would be off label. In 2010, Sanyal et al published a study showing the beneficial effects of vitamin E and pioglitazone treatment in patients without diabetes, compared to placebo, for 2 years.[28] Vitamin E (800 IU/d) improved steatosis, inflammation and ballooning, and

induced NASH resolution in 36% of patients (21% in the placebo arm). Pioglitazone improved all histological features (except for fibrosis) and induced NASH resolution more often than placebo.[29] The histological benefit occurred together with improvements in ALT and partial correction of insulin resistance. Despite the potent antioxidant action, vitamin E should be used with caution since it has been associated with all causes of death and because of its possible side effects such as prostate cancer (men over 50 years) and an increased risk of intracranial bleeding. Pioglitazone may be associated with weight gain, congestive cardiac failure (rarely) and bone loss. Like vitamin E, pioglitazone's benefits must be balanced against the reported risks. Also, the optimal duration of therapy is unknown. Only liraglutide, a glucagon-like peptide-1 agonist used for weight loss, has been found to improve liver histology in NASH patients in a phase II study.[30]

7 | CONCLUSION

Non-alcoholic fatty liver disease is a condition that requires a multidisciplinary approach. In the initial evaluation, it is essential to identify other possible causes of liver diseases. Identifying comorbidities such as diabetes and cardiovascular diseases, and the immediate treatment of these conditions can significantly change the prognosis. Currently, there are no approved medications for NAFLD, and treatment should focus on lifestyle changes.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

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Management of NAFLD patients with advanced fibrosis

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Abstract

The prevalence of non alcoholic fatty liver disease (NAFLD) has increased to 25% in the general population and could double by 2030. Liver fibrosis is the main indicator of morbidity and mortality and recent estimations suggest a substantial number of individuals with undiagnosed advanced liver disease. Strategies to monitor advanced fibrosis are essential for early detection, referral, diagnosis and treatment in primary care and endocrine units, where NAFLD and consequently liver fibrosis are more prevalent. Blood-based non-invasive methods could be used to stratify patients according to the risk of the progression of fibrosis and combined with imaging techniques to improve stratification. Powerful new diagnostic tools such as MRE and PDFF are emerging and might prevent the need for liver biopsy in the near future. The current therapeutic landscape of NAFLD is rapidly evolving with an increasing number of molecules that treat key factors involved in its progression, but that still have a limited or no ability to effectively reverse fibrosis. Management of this disease will probably require a combination of sequential and personalized treatments as a result of its complex and dynamic pathophysiology. Lifestyle interventions are still the most effective therapeutic option and should be better integrated into patient management together with specific programs of bariatric endoscopy/surgery for morbidly obese patients.

KEYWORDS

advanced liver disease, liver fibrosis, NAFLD, NASH, screening

Key points

- Strategies to identify and treat patients with or at risk of advanced fibrosis as a result of NAFLD must be given priority.

Abbreviations: ADAPT, Age, presence of DiAbetes, PRO-C3 and plaTelet count; ALT, Alanine transaminase; APRI, AST-to-Platelet Ratio Index; AST, Aspartate transaminase; BMI, Body mass Index; CI, Confidence Interval; CPA, Collagen Proportionate Area; CVD, Cardiovascular disease; ECM, Extracellular Collagen Matrix; ELF, Enhanced Liver Fibrosis; FIB-4, Fibrosis Score-4; FXR, Farnesoid X receptor; HFS, Hepamet Fibrosis Score; HOMA, Homeostatic Model Assessment for Insulin Resistance; IQR, Interquartile range; MRE, Magnetic Resonance Elastography; NAFLD, Non-Alcoholic Fatty Liver Disease; NASH, Non-Alcoholic SteatoHepatitis; NASH-CRN, NASH Clinical Research Network; NFS, NAFLD Fibrosis Score; NITs, Non-invasive Tests; NPV, Negative Predictive Values; p-2D-3D SWE, (point-2D-3D) Shear Wave Elastography; PPAR, Peroxisome Proliferator Activated Receptor; PPV, Positive Predictive Values; PRO-C3, N-terminal type III Collagen Propeptide; TD2M, Type II Diabetes Mellitus; TE (VCTE), (Vibration controlled) Transient Elastography.

- Composite scores for the assessment of fibrosis are easy-to-use tools that help identifying patients with minimal or advanced fibrosis, and should be implemented in primary care health centres and endocrine units.
- Patient management should focus on treating comorbidities and risk factors that are more likely to worsen fibrosis and include active and well-designed standardized lifestyle interventions.

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is known to be the most prevalent chronic liver disease worldwide. The estimated pooled prevalence in the general population is 25% for NAFLD and ranges from 3% to 5% for non-alcoholic steatohepatitis (NASH) with wide geographical variations across the world. NAFLD has traditionally been described as a group of nosological entities characterized by a high accumulation of fat in the liver cells (steatosis) in the absence of any other cause of liver disease, alcohol consumption or steatogenic drug use. However, the last decade has provided ample evidence of a complex interplay between NAFLD and many other diseases, especially type 2 diabetes mellitus (T2DM) and obesity, with a prevalence of 55.5% which can reach up to 90% in extremely obese patients.[1] NAFLD alone is a risk factor for cardiovascular disease, the most common cause of death in these patients. NAFLD has also been associated with the development of numerous diseases including extrahepatic malignancies, chronic kidney disease, certain endocrinopathies including polycystic ovary syndrome and osteoporosis, brain aging and cognitive impairment.[2]

The spectrum of NAFLD ranges from simple steatosis, a relatively benign form of the disease, to NASH, which may or may not be associated with liver fibrosis. NASH and fibrosis seem to promote the development of diabetes mellitus, dyslipidaemia and arterial hypertension in patients without baseline metabolic disturbances.[3] Because of the strong association of this disease with general metabolic disorders as well as the coexistence of metabolic risk factors with some level of alcohol consumption in a substantial proportion of the population, alternative names have recently been proposed for this disease such as metabolic associated fatty liver disease (MAFLD) or dysmetabolism-associated fatty liver disease (DAFLD).[4,5] Whatever term best defines or classifies this disease,[6] it is clear that the global increase in obesity and dysmetabolic disorders together with an ageing population makes NAFLD a serious public health problem.

Chronic injury from NAFLD inhibits the regenerative capacity of the liver because of a state of overnutrition that generates an imbalance in the hepatic lipid metabolism that promotes cellular stress, apoptosis and liver injury. In these cases, fibrosis is a result of a complex crosstalk among different organs and also among most of the different cell types in the liver, in particular hepatic stellate cells (HSCs) and immune cells, which are the key drivers of fibrosis. Diet-induced accumulation of lipid overload and intrahepatic insulin resistance are considered to be key factors that trigger NASH through persistent accumulation of lipotoxic and glucotoxic damage, which mainly takes place in hepatocytes. Lipotoxicity and glucotoxicity eventually trigger apoptosis and liver injury along with a production of pro-inflammatory cytokines, chemokines and damage-associated molecular patterns (DAMPs) which upregulate the activation of Kupffer cells and monocyte-derived macrophages. This activation further promotes the transdifferentiation of hepatic stellate cells into myofibroblasts. In the long run, dendritic cells activate CD4 + T cells, which polarize Th1 and Th17 into pro-inflammatory lymphocytes worsening liver damage and inflammation.

Hepatic fibrosis is an adaptive mechanism whose main goal is to repair damaged tissue and is characterized by an accumulation of extracellular matrix (ECM). If the insult persists chronic liver injury may lead to cirrhosis, which is characterized by a distortion of the hepatic architecture generating abnormal blood flow and, in certain cases, portal hypertension, the major cause of clinical complications, including hydropic decompensation, bleeding events and hepatic encephalopathy. Liver fibrosis also progressively restricts normal liver regeneration increasing the risk of liver failure, and generates a favourable micro-environment for the development of liver cancer through mechanisms that have not been completely clarified.[7]

Although the prevalence of NAFLD is high, not all patients are at risk of developing severe complications. In 2017, one meta-analysis including 1,495 NAFLD patients evaluating the risk of all-cause mortality and liver-related mortality reported a linear increase in all-cause mortality as fibrosis progresses and a more sudden increase in liver-related mortality after stage 2.[8] A more recent meta-analysis including 4,428 biopsy-proven NAFLD patients reached a similar conclusion. It is important to note that this study did not find evidence of an additional risk of NASH compared to patients with simple steatosis or NASH and the same stage of fibrosis.[9] A nationwide longitudinal study evaluating 11,154 participants for a median follow-up of 14.5 years with 1795 registered deaths concluded that NAFLD per se was not associated with higher mortality [1.05; 95% confidence interval [CI]: 0.93-1.19]. On the other hand, high APRI (>1.5), NFS (>0.676) and FIB-4 (>2.67) values, three non-invasive scores to determine the risk of advanced fibrosis, were associated with mortality even after adjustment for other known predictors (NFS: hazard ratio, 1.69; 95% CI, 1.09-2.63; APRI: hazard ratio, 1.85; 95% CI, 1.02-3.37; FIB-4: hazard ratio, 1.66; 95% CI, 0.98-2.82).[10] Overall, these data suggest that although NASH plays a key role in driving and/or accelerating the progression of fibrosis in patients with NAFLD, liver fibrosis is probably the most important factor to be taken into account when evaluating patient prognosis.

1.1 | Screening advanced liver disease in the general population

Liver biopsy is still the reference method for the diagnosis of NAFLD. It determines the grade of steatosis, necroinflammation and fibrosis simultaneously and is still the only available technique to effectively diagnose NASH. The staging of fibrosis is usually based on the NASH-CRN score, which uses the Kleiner score to classify fibrosis, with moderate accuracy for intermediate stages because of a variability in inter- and intra-observer agreement of almost 25% for overlapping stages of fibrosis.[11] Several alternative methods have been developed to provide more objective quantification of fibrosis. Morphometry provides a finite-quantitative scale of the amounts of collagen, the Collagen Proportionate Area (CPA), which has already been used in certain clinical trials for Hepatitis C but it is time consuming and has a non-linear relationship with the stage of fibrosis.[12] Q-fibrosis, a technique that has been shown to improve the underestimation of staging in suboptimal biopsies (<15 mm) and under- and over-scoring by different pathologists ($P < .001$), has recently been modified and applied to NAFLD to improve the discrimination between F1 and F2 patients.[13] Liver biopsy is still the best method to evaluate the progression and regression of fibrosis but it is limited by cost, accuracy, a risk of adverse events and invasiveness so that it is unsuitable for large-scale screening.

Non-invasive techniques (NITs) provide a continuous measurement estimated by the integration of different sets of biological and/or physical properties in a dynamic algorithm. These algorithms usually integrate anthropometric parameters and the levels of certain components, which can be quantified in serum or blood samples. NITs can also be based on a subset of imaging techniques, which are usually performed to help estimating liver fat content and/or liver stiffness, an intrinsic physical property of the liver parenchyma. Serum biomarkers range from simple, inexpensive tests such as the AST-to- Platelet ratio Index (APRI), Fibrosis-4 (FIB-4), NAFLD fibrosis score (NFS) or Hepamet Fibrosis Score (HFS) to more sophisticated and patented tests such as the FibroTest®, Fibrometer®, ELF, Hepascore and PRO-C3. Several potential new NITs are currently being investigated and use various combinations of cytokines, chemokines, genetic polymorphisms, microRNAs and post-translational modified glycoproteins to assess fibrosis. Imaging techniques include vibration-controlled transient elastography (VCTE or Fibroscan) and magnetic resonance elastography (MRE), which use mechanical drivers to generate shear wave and measure its velocity using sonographic Doppler or MR techniques, and shear wave elastography (pSWE 2D-SWE, 3D-SWE), which uses high frequency ultrasound impulses for shear wave generation from one or multiple frequencies in real-time using ultrasound. These methods are usually accurate enough to exclude the presence of advanced liver disease, but not to effectively classify a significant number of patients that remain in the grey zone. None of them has proven so far a robust ability to dynamically monitor disease progression over time.

The ability of NITs to rule in or rule out liver fibrosis varies significantly depending on the cut-off value, which can be modified depending on the desired endpoint. Current available NITs have usually low to moderate positive predictive values and, therefore, a limited ability to confirm significant and advanced fibrosis, which often requires additional clinical information for a clear diagnosis. In contrast, the negative predictive value (NPV) of NITs is generally strong, allowing the clinician to confidently exclude advanced fibrosis or cirrhosis. The estimated prevalence of advanced fibrosis and cirrhosis in the population being studied, as well as certain comorbidities (diabetes, obesity, age), can influence the results of NITs for the diagnosis of advanced fibrosis. Differences in ethnicity can also influence certain NITs such as FIB-4 and NFS, whose results have been shown to be less reliable in South Asians than in Caucasians. All of these factors should be taken into consideration in the study design as well as the conclusions.[14] Table 1 summarizes the ability of several NITs to predict significant and advanced fibrosis according to four recent metanalyses.[15-18]

None of the existing NITs provides an analysis of fibrosis comparable to liver biopsy. However, NITs can be used to identify high-risk patients in the global population. Implementing targeted diagnostic screening programs in primary care and outpatient clinics could greatly reduce the number of patients with undiagnosed advanced liver fibrosis, which could represent 6-7% of the population.[19] Screening should be performed in patients with obesity, diabetes or individual components of the metabolic syndrome as well as in those with increased liver enzymes or steatosis. It is important to note that abnormal liver blood enzymes are not specific for the diagnosis or exclusion of fibrosis, so they must be incorporated into algorithms or associated with other tools to assess the extent of fibrosis.

Most of the algorithms and screening protocols proposed combine a two-stage evaluation. First, a non-invasive test with a single cut-off is performed in primary care or endocrinology units to exclude patients with a low risk of advanced fibrosis. FIB-4 or NFS are inexpensive, easy-to-perform tests with good NPV for the exclusion of advanced fibrosis using a single cut off (NFS<−1.455 and FIB4 < 1.3), and can be used as a first screening option for intermediate-to-high–risk patients. Both these tests may be influenced by age and should use a different cut-off for patients aged > 65 (NFS < 0.12 and FIB-4 < 2.0). FIB4 is easier to perform in primary care than NFS because the latter also requires albumin. Patients with available HOMA-IR scores can also be assessed for advanced fibrosis using a single cut off with the Hepamet Fibrosis Score (HFS < 0.12). HFS has been shown to be better than NFS and FIB-4 for the exclusion of advanced fibrosis, to significantly reduce the grey zone and seems to be less influenced by BMI and ALT levels. This test also improves classification of non-diabetic patients probably because the formula includes the HOMA index (https://www.hepamet-fibrosis-score.eu/) (Figure 1A).[20]

When advanced fibrosis cannot be excluded, patients should then undergo transient elastography. The cut-off for advanced fibrosis with TE is confirmed with 8 or 6.2 kPa (M and XL probes, respectively) for the exclusion of advanced fibrosis. The XL probe

TABLE 1 Summary of sensitivity, specificity and AUC values to identify significant (≥F2) and advanced fibrosis (≥F3). AUC: Area under the curve; SUM: Summary; Rest of acronyms and abbreviations are included in the abbreviations list

		SIGNIFICANT FIBROSIS (F ≥ 2)					ADVANCED FIBROSIS (F ≥ 3)				
Xiao et al 2017[15]	APRI	4 (864)	0.43-1.5	59.3 (33.3-71.1)	77.1 (66.2-90.6)	11 (2352) 0.70 [0.64-0.76] (0.54-0.87)	5 (729) 7 (1,351) 4 (1101) 4 (682)	0.452-0.50 0.54-0.98 1.00 1.50	72.9 (50.0-87.4) 68.6 (61.0-76.2) 43.2 (27.0-67.0) 32.9 (6.3-70.0)	67.7 (43.1-91.0) 72.7 (59.4-86.0) 86.1 (81.0-89.0) 90.5 (74.5-97.0)	29 (6746) 0.75 [0.72-0.77] (0.60-0.92)
	BARD	1 (242)	2	44.3	70.4	5 (1330) 0.64 [0.53-0.75] (0.50-0.73)	1 (242) 14 (3057) 5 (736)	1.5 2 3-4	83.0 75.2 (41.7-100) 59.4 (33.3-85.2)	59.0 61.6 (32.5-88.9) 75.1 (59.9-91.8)	30 (7791) 0.73 [0.71-0.75] (0.64-0.87)
	FIB-4	4 (819)	0.37-3.25	64.4 (54.4-77.8)	70.0 (60.0-87.5)	12 (2330) 0.75 [0.70-0.79] (0.59-0.84)	10 (2759) 8 (1533) 6 (1910) 6 (1890) 4 (543)	1.24-1.45 1.51-2.24 2.67 3.25 5.31-10.62	77.8 (63.0-90.0) 77.0 (70.6-89.5) 31.9 (12.0-63.2) 37.3 (5.0-56.0) 67.5 (50.0-100)	71.2 (55.5-88.0) 79.2 (67.1-93.6) 95.7 (88.3-98.7) 95.8 (89.0-100) 80.8 (54.0-100)	34 (8245) 0.80 [0.77-0.84] (0.37-0.95)
	NFS	2 (432)	-1.1	65.5 (60.9-70.1)	82.5 (68.7-96.3)	11 (2098) 0.72 [0.65-0.79] (0.55-0.88)	2 (106) 10 (3057) 5 (963) 14 (3896) 1 (235)	[−26.93]-[−2.16] -1.455 [−1.31]-[0.156] 0.67-0.676 0.735	80.5 (78.0-83.0) 72.9 (22.7-96.0) 78.2 (69.0-86.4) 43.1 (8.3-100) 68.4	69.5 (69.0-70.0) 73.8 (42.9-100) 71.7 (60.0-83.0) 88.4 (25.0-100) 88.3	38 (9245) 0.78 [0.75-0.81] (0.52-0.96)
	TE (M probe)	3 (559) 10 (1496) 8 (1110)	5.8 6.65-7 7.25-11	91.7 (90.0-94.0) 74.1 (58.8-100) 65.7 (52.5-84.0)	57.4 (42.0-80.0) 68.6 (44.7-84.3) 84.5 (70.0-93.2)	15 (2207) 0.83 [0.79-0.86] (0.67-1.00)	3 (393) 9 (1540) 5 (1157) 5 (773)	6.95-7.25 7.6-8 8.7-9 9.6-11.4	69.2 (66.7-70.4) 88.9 (65.0-100) 83.3 (76.0-88.4) 80.1 (69.0-100)	66.3 (64.5-67.7) 77.2 (65.9-90.2) 78.0 (62.9-88.0) 89.9 (83.8-96.9)	16 (2495) 0.87 [0.83-0.90] (0.75-0.98)
	TE (XL probe)	4 (654)	4.8-8.2	75.8 (57.0-92.0)	64.8 (37.0-90.0)	3 (318) 0.82 [0.75-0.89] (0.80-0.85)	3 (579)	5.7-9.3	75.3 (57.0-91.0)	74.0 (54.0-90.0)	3 (318) 0.86 [0.78-0.94] (0.84-0.90)
	SWE (pSWE-2d-SWE)	2 (233)	2.67-9.4	85.0 (84.0-86.0)	94.4 (88.9-100)	2 (233) 0.89 [0.46-1.00] (0.85-0.92)	3 (429)	3.02-10.6	89.9 (88.2-91.5)	91.8 (90.0-94.0)	3 (429) 0.91 [0.82-1.00] (0.88-0.95)
	MRE	3 (384)	3.4-3.62	73.2 (65.7-87.3)	90.7 (85.0-95.7)	3 (384) 0.88 [0.83-0.92] (0.86-0.89)	5 (628)	3.62-4.8	85.7 (74.5-92.2)	90.8 (86.9-93.3)	5 (628) 0.93 [0.90-0.97] (0.89-0.96)

(Continues)

TABLE 1 (Continued)

		SIGNIFICANT FIBROSIS (F ≥ 2)					ADVANCED FIBROSIS (F ≥ 3)				
Vali et al 2020[16]	ELF	5 (550)	7.70 9.80 10.51	97 (88-99) 57(40-73) 35 (22-50)	10 (03-26) 89(73-96) 97 (89-99)	5 (550) 0.81 (0.66-0.89) -	11 (4452)	7.70 9.80 10.51	93 (82-98) 65 (49-77) 51 (0.31-0.70)	34 (13-65) 86(77-92) 93 (85-96)	11 (4452) 0.83 (0.71-0.90) -
Jiang et al 2018[17]	TE M or XL probe	10 (?)	6.7-11.0	77 (70-84)	80 (74-84)	10 (?) 0.85 [0.82-0.88] -	11 (1753)	8.0-12.5	79 (69-87)	89 (84-92)	11 (1753) 0.92 [0.89-0.94] -
	SWE	6 (733)	1.16-1.32	70 (59-79)	84 (79-88)	6 (733) 0.86 [0.83-0.89] -	9 (982)	1.34-1.77	89 (73-96)	88 (82-92)	9 (982) 0.94 [0.91-0.95] -
Liang et al 2020[18]	MRE	12 (910)	3.4-3.62	87 (74-97)	86 (71-94)	12 (910) 0.93 [0.90-0.95] -	12 (910)	3.62-4.8	89 (81-94)	84 (63-94)	12 (910) 0.93 [0.90-0.95] -

(A)

	FORMULA/METHODS	ADVANTAGES	LIMITATIONS
FIB-4	age (yr) x AST [U/L]/(platelets [10^9/L] x (ALT [U/L])1/2	1.Cost effective 2. Easy to use and to implement in outpatient clinics and primary care 3. High negative predictive value for advanced fibrosis	1. Low positive predictive value. 2. High percentage in the grey zone. 3. Sensitive to obesity, age, AST and TD2M.
NFS	(-1.675 + 0.037 x age (yr) + 0.094 x BMI (kg/m2) + 1.13 x IFG/diabetes (yes = 1, no = 0) + 0.99 x AST/ALT ratio - 0.013 x platelet count (x10^9/L) - 0.66 x albumin [g/dl])		
HFS	1 / (1 + e[5.390–0.986 × age[45–64 years] − 1.719 × age [≥ 65 years] + 0.875 × male sex − 0.896 × AST[35–69 UI/L] − 2.126 × AST[≥ 70 UI/L] − 0.027 × albumin[4–4.49 g/dL] − 0.897 × albumin[< 4 g/dL] − 0.899 × HOMA [2–3.99 with no T2D] − 1.497 × HOMA [≥ 4 with noT2D] − 2.184 × T2D − 0.882 × platelets[155–219 × 1.000/μL] − 2.233 × platelets [< 155 × 1.000/μL])	1.Cost effective 2. Easy to use and to implement in outpatient clinics and primary care 3. High negative predictive value for advanced fibrosis 4.Less sensitive to obesity, AST and TD2M 5. Not sensitive to age	1. Low positive predictive value. 2. High percentage in the grey zone. 3. Assesment in non-diabetics requires HOMA-IR
TE	TE should be performed by an experienced operator (>100 examinations) following a standardized protocol with the patient, fasting for at least 2 hours, in the supine position, right arm in full abduction, on the midaxillary line with the probe-tip placed in the 9th to 11th intercostal space with a minimum of 10 shots with > 60% valid measurementes (IQR <0.3).	1. Higher diagnostic accuracy than most blood based NITs 2. Best validated imaging technique	1. Less available and/or more costly than NITs 2. Lack of parenchymal assessment 3. Sensitive to ascites, morbid obesity, cholestasis, inflammation from acute hepatitis, and heart failure 4. Operator and experience dependency
MRE	MRE generates mechanical waves generated in a drum device over the liver are imaged for about 15 seconds provides a color-coded liver stiffness map. Use should only be considered if the evaluation with TE is inconclusiv or for research purposes.	1. Highest diagnostic accuracy 2. Not influenced by BMI severe steatosis & hemochromatosis	1. Cost and availability 2. Limited experience & validation 3. Influenced by implanted metallic devices, claustrophobia and iron overload.

(B)

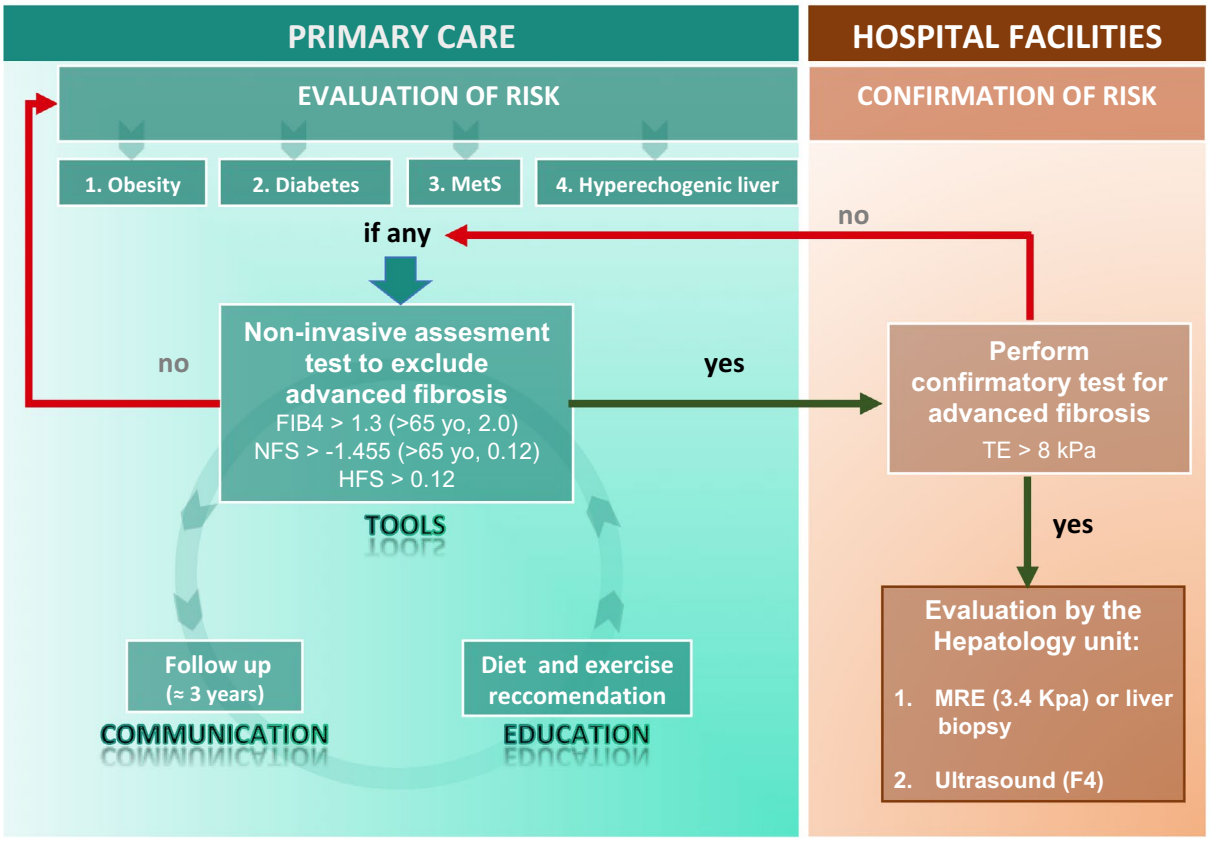

FIGURE 1 Referral care pathway proposed to improve the detection of advanced fibrosis in primary care or non-specialized units. (A) Methods, advantages and disadvantages of NITs proposed in the algorithm. (B) Referral care pathway including cut-off scores. Acronyms and abbreviations are included in the abbreviations list.

is highly recommended in obese patients. Advanced fibrosis can also be assessed using improved non-invasive blood panels such as PRO-C3/ADAPT and ELF (<7.7), or alternative imaging techniques such as MRE (3.4 kPa) ir 2D-SWE (8 kPa). Iron-overload can significantly influence MRE results and should be assessed with other sequences.[16,17] Patients above the recommended thresholds should be referred to a hepatologist for a possible liver biopsy to confirm the diagnosis, or ultrasound to confirm cirrhosis. Patients below the threshold should be followed in primary care using serum-based NITs if there are no other clinical symptoms suggesting advanced liver disease (2-3 years) (Figure 1B).

1.2 | Weight loss: a key cornerstone in NAFLD management

Interventions of diet and exercise as well as other strategies to induce weight loss have been shown to be useful for the treatment of both NASH and fibrosis, as well as to improve many of the comorbidities and risk factors associated with NAFLD. A single-arm trial with 293 patients showed that NASH and fibrosis regress in 90% and 45%, of patients who lost ≥10% weight at 1 year respectively.[21] A recent meta-analysis that included 22 studies (n = 2588) comparing a high percentage of weight loss, no weight loss or less weight loss found that after a median of 6 months of intervention weight loss was significantly associated with improvements in: 1) ALT (standardized mean difference: −9.81 U/L; 95% CI, −13.12 to −6.50); 2) steatosis (−1.48; 95% CI, −2.27 to −0.70); 3) NAS score (−0.92; 95% CI, −1.75 to −0.09); 4) liver stiffness (−1.11 kPa; 95% CI, −1.91 to −0.32), but did not find significant changes in: 1) histologic liver fibrosis (−0.13; 95% CI, −0.54 to 0.27); 2) inflammation (−0.01; 95% CI, −0.10 to 0.07) or ballooning (−0.11; 95% CI, −0.26 to 0.04).[22] This suggests that the percentage of weight loss plays an important role in the potential benefit of these interventions because the average weight loss observed (−3.61 kg; 95% CI, −5.11 to −2.12) was clearly below the 5%-10% decrease in body weight needed to resolve NASH and the regression of fibrosis in the previous study.

Regular physical exercise has several beneficial effects on overall health. While decreasing body mass and adiposity are not the primary outcomes, exercise can mediate several diseases that accompany obesity including T2DM and cardiovascular disease (CVD). Several studies have shown that weight loss can also result in a dose-dependent remission of T2DM. A weight loss of ~15 kg, as part of an intensive management program, can result in remission of T2DM in ~80% of patients with obesity and T2DM. An observational analysis of participants in the Look AHEAD (Action For Health in Diabetes) study (n = 5,145) examined the association between the extent of weight loss and changes in CVD risk factors at 1 year and found that weight changes were significantly correlated with changes in glycaemic control, blood pressure, HDL cholesterol and triglycerides. All of these results suggest that significant weight loss has a clear benefit in patients with NAFLD and most, if not all of the range of comorbidities and risk factors associated with it.[23,24]

Nevertheless, diet- and exercise-based interventions have several important limitations. The difficulty of long-term adherence and the maintenance of initial weight loss are probably one of the major drawbacks of this approach, and strategies to improve it are needed. A meta-analysis including 49 studies identified several energy intake-reducing behaviours and energy expenditure-increasing behaviours associated with long-term adherence and found consistent evidence that demographic factors were not predictive of weight-loss maintenance. On the other hand, behavioural and cognitive factors that promote a reduction in energy intake, an increase in energy expenditure and monitoring this balance were predictive factors. Specifically, self-monitoring factors were found to have a PPV for the maintenance of weight loss. Moreover, several cognitive-psychological factors also indirectly influence the maintenance of weight loss, ie high personal efficacy for exercise and weight management.[25]

Another major limitation is the lack of a general consensus for diet and exercise recommendations and of methods to assess whether patients are actively following intervention programs. Lifestyle protocols are usually at the discretion of the researcher and vary from study to study. There is also a risk of site-specific differences that confound study outcomes even when the same standardized lifestyle recommendations are applied to all participants. The Liver Forum Standard of Care Group recently reviewed this topic evaluating 46 clinical trials available on PubMed and clinicaltrials.gov, and showed that 52% of randomized and investigator-initiated controlled trials did not describe lifestyle modifications at all, 22% had undefined recommendations for diet and/or exercise and 26% had nutritional counselling and/or exercise recommendations. Interpretation of results is challenging without this basic information, especially when early-phase studies also fail to demonstrate a therapeutic response in treatment arms compared to placebo. This group has provided a series of recommendations for early- and late-stage studies that will most likely improve assessment of both diet- and exercise-based interventions.[26]

Surgery can be an option in patients in whom diet and exercise interventions are difficult. Bariatric surgery provides marked long-term weight loss and can prevent the development of the risk factors of CVD such as T2DM, hypertension and dyslipidemia.[27] A recent 5-year longitudinal study in patients who underwent bariatric surgery reported the resolution of NASH in 84% of patients (n = 64; 95% CI, 73.1-92.2) and the regression of fibrosis in 70.2% (95% CI, 56.6-81.6), which completely resolved in 56% (95% CI, 42.4-69.3) including 45.5% of patients with baseline bridging fibrosis.[36] It is interesting to note that in the presence of persistent NASH there was no decrease in fibrosis and less weight loss (reduction in BMI of 6.3 ± 4.1 kg/m^2 in persistent NASH vs 13.4 ± 7.4 kg/m^2 in NASH resolution; P = .017).[28] A recent meta-analysis including 32 cohort studies and 3093 biopsy specimens from bariatric patients showed a biopsy-confirmed resolution of steatosis in 66% of patients (95% CI, 56%–75%), inflammation in 50% (95% CI, 35%–64%), ballooning in 76% (95% CI, 64%–86%) and fibrosis in 40% (95% CI, 29%–51%). This intervention, however, also resulted in new or worsening features

of NAFLD, such as fibrosis, in 12% of patients (95% CI, 5%–20%).[29] Finally, this surgery with its associated risk factors cannot be indicated on a large scale to treat a disease as prevalent as NASH thus, dietary and exercise-based approaches remain the best strategy to manage this disease.

1.3 | Therapeutic landscape for NAFLD

Treatments for NASH and liver fibrosis differ in their mode of action but tend to result in one or more of these outcomes: 1. hepatocyte protection through active elimination of sources that trigger damage; 2. inhibition of signals that drive HSC activation; 3. immune modulation and 4. inhibition of fibrotic scar formation and propagation.

Most treatments in late clinical trials that have included a histological evaluation of tissue have been found to have limited or no efficacy in reversing NASH and fibrosis (Table S1). Emricasan, a pan caspase inhibitor, did not reach the primary objective of improvement in fibrosis without the worsening of NASH (emricasan 5 mg: 11.2%; emricasan 50 mg: 12.3%; placebo: 19.0%; $P = .972$ and .972, respectively) or the secondary objective of resolution of NASH without worsening of fibrosis (emricasan 5 mg: 3.7%; emricasan 50 mg: 6.6%; placebo: 10.5%; $P = .070$ and .335 respectively) [NCT02686762]. Selonsertib, an Ask1 inhibitor, did not improve the regression of fibrosis without worsening NASH in F3 patients (10% 18mg or 12% 6mg vs 13% placebo; $P = .49$ and $P = .93$, respectively) [NCT03053050], or compensated F4 (14% 18 mg or 13% 6 mg vs 13% placebo; $P = .56$ and $P = .93$, respectively) [NCT03053063]. Elafibranor, a PPAR-α and δ dual agonist, has been shown to resolve NASH without worsening fibrosis in a stage 2 trial, but has no effect on liver fibrosis. In addition, a recent press release from the Golden phase III trial reported that Elafibranor did not meet the primary endpoint of histological improvement of NASH (19.2% vs 14.7%; $P = .066$) or fibrosis (24.5% vs 22.4%; $P = .44$) in the interim analysis [NCT02704403]. Similarly, the GLP-1 inhibitor liraglutide has been shown to promote the resolution of NASH in a stage II trial (39% vs 9% placebo) but did not significantly improved fibrosis (26 vs 14%; $P = .46$) [NCT01237119]. The resolution of NASH has also been reported in a preliminary analysis in a stage II trial in which diabetic patients with NASH were treated with semaglutide (59% vs 17% in placebo) [NCT02970942], another GLP1 analogue. Both these agents require further evaluation in larger trials and evaluation for the resolution of fibrosis. Treatment with Aramchol, a liver-targeted SCD1 modulator, resulted in the resolution of NASH (19.2% vs 7.5%; $P = .0462$) as well as resolution of NASH without worsening fibrosis (16.7% vs 5.0%; $P = .0514$) and also a higher, but not significant, proportion of patients with a one-point improvement in fibrosis without the worsening of NASH in Aramchol 600mg vs placebo (29.5% vs 17.5%; $P = .2110$) [NCT02279524]. Cenicriviroc, an antagonist of C-C chemokine ligands 2 and 5 (CCL2 and CCL5) which promote liver fibrosis through activation of inflammatory signalling and immune cell infiltration, resulted in a significant reduction of one stage of fibrosis after 1 year (20% CVC vs 10% placebo; $P = .02$) but this difference was not significant after 2 years of treatment (15% CVC vs 17% placebo) [NCT02217475]. Post-hoc analysis comparing patients with advanced liver disease (F3) showed a greater but non-significant improvement in patients treated with CVC (15.8% vs 4.8% placebo $P = .18$).[30] Finally, Semaglutide has recently proven his ability to revert efficiently NASH (59% vs 17%; $P < .001$) but did not significantly improve fibrosis (43% vs 33%; $P = .48$) [NCT02970942]. The numerous reasons for the high rate of failure in these large trials were recently reviewed.[31,32]

There are currently more than 30 on-going trials (≥ stage2) of new therapies for NAFLD with a histological evaluation of fibrosis (Table S2). Thus far, obeticolic acid, an FXR agonist, is the only compound that has been found to modestly improve fibrosis in a phase III clinical trial interim analysis (resolution of fibrosis by at least 1 stage without worsening of NASH 23% 25 mg dose vs 12% in placebo). This improvement was not accompanied by a resolution of NASH, although several components of the histological NAFLD activity score did improve [NCT01473524]. Pioglitazone is a PPAR-γ analogue that been shown to promote the resolution of NASH in prediabetic and diabetic patients [NCT00994682] but has not been found to significantly improve fibrosis in randomized studies (Table S1). However, a recent meta-analysis including data from 5 trials suggest that this compound could also improve advanced fibrosis (OR, 3.15; 95% CI, 1.25-7.93; $P = .01$) and any stage of fibrosis (OR, 1.66; 95% CI, 1.12-2.47; $P = .01$), even in non-diabetics (OR, 2.95; 95% CI, 1.04-10.90; $P = .02$; for advanced fibrosis and OR, 1.76; 95% CI, 1.02-3.03; $P = .02$ for any stage fibrosis).[33]

1.4 | Concluding remarks

The management of NAFLD requires a multidisciplinary approach to increase detection and referral of patients with advanced fibrosis from primary care centres and non-specialist units, mainly endocrine to hepatology clinics. Patient management should focus on treating comorbidities and risk factors that are more likely to worsen fibrosis and include active and well-designed standardized lifestyle interventions. This disease also requires educational programs to improve awareness of the impact of this silent disease with long-term asymptomatic periods on quality of life and survival. Educational programs, tools and information to central laboratories and outpatient clinics as well as strategies to facilitate easy referral of patients between professionals are needed.

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We would like to apologise to all authors whose work has not been cited directly in this review owing to the restriction applied to the maximum number of references allowed.

CONFLICT OF INTEREST

DMM, RGD, JA and PD have no potential conflict of interest. MRG has served as a speaker for AbbVie, Bristol-Myers Squibb, GENFIT, Gilead Sciences, Intercept, MSD and Roche; an advisory

board member for GENFIT, Gilead Sciences, Intercept, Janssen-Cilag, Kaleido, NovoNordisk, Medimmune and Prosceinto; and has received research grants from Abbvie, Gilead Sciences and Intercept.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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SUPPLEMENT ARTICLE

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Non-alcoholic fatty liver disease in type 2 diabetes – A specific entity?

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Abstract

Individuals with obesity or type 2 diabetes (T2D) have an increased risk of developing non-alcoholic fatty liver disease (NAFLD). In insulin-resistant states, altered adipose tissue function may be the initial abnormality underlying NAFLD. Hepatic lipid oversupply interferes with insulin signalling and mitochondrial function. In obese individuals, adaptation of hepatic mitochondrial respiration fails with the progression of NAFLD and can activate pro-inflammatory pathways. T2D as well as type 1 diabetes are associated with altered hepatic mitochondrial function. Screening for NAFLD remains challenging especially in those with diabetes because liver enzymes are often in the normal range and the performance of NAFLD scores is limited. Patients with T2D and severe insulin-resistant diabetes (SIRD) have the highest prevalence of NAFLD at diagnosis and the greatest risk of progression. In this subgroup, the single-nucleotide-polymorphism (SNP) rs738409(G) of the patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene is associated with high liver fat content and adipose tissue insulin resistance. This frequent SNP is also known to be associated with lean NAFLD so that genetic testing for this and other SNPs could improve future screening strategies to identify high-risk individuals. Although lifestyle modifications are effective, this approach is limited owing to difficulties with compliance and several classes of drugs are being tested to treat NAFLD. Antihyperglycaemic drugs such as glucagon-like peptide 1 receptor agonists (GLP-1 RA), sodium-glucose cotransporter 2 inhibitors (SGLT2i) and pioglitazone are promising and halt the progression of NAFLD. In conclusion, although NAFLD in diabetes may not be a separate entity, there are specific features to its pathogenesis and clinical management.

KEYWORDS

adipose tissue insulin resistance, diabetes mellitus, NAFLD, PNPLA3, SIRD

Abbreviations: acyl-CoA, acyl-coenzyme A; ADIPOQ, adiponectin; AGE, advanced glycation end product; AGER-1, advanced glycation end-product receptor 1; ALT, alanine aminotransferase; APOB, apolipoprotein B; APOC3, apolipoprotein C3; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate transaminase; ATP, adenosine triphosphate; BMI, body mass index; ChREBP, carbohydrate response element binding protein; CKD, chronic kidney disease; CVD, cardiovascular disease; DAG, diacylglycerols; DNA, deoxyribonucleic acid; DNL, de novo lipogenesis; EASD, European Association for the Study of Diabetes; EASL, European Association for the Study of the Liver; EASO, European Association for the Study of Obesity; ELF, enhanced liver fibrosis; FFA, free fatty acid; FIB-4, fibrosis-4 score; FLI, fatty liver index; GCKR, glucokinase regulatory protein; GDS, German Diabetes Study; GFR, glomerular filtration rate; GIP, glucose-dependent insulinotropic polypeptide; GLP-1 RA, glucagon-like peptide 1 receptor agonists; HCC, hepatocellular carcinoma; IL6, interleukin 6; MARD, mild age-related diabetes; MBOAT7, membrane bound O-acyltransferase domain containing 7; MOD, mild obesity-related diabetes; MRS, magnetic resonance spectroscopy; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NFS, NAFLD fibrosis score; PKCε, protein kinase Cε; PNPLA3, patatin-like phospholipase domain-containing protein 3; Pro-C3, pro-peptide of type III collagen; RAGE, receptor for AGE; ROS, reactive oxygen species; SAID, severe autoimmune diabetes; SGLT2i, sodium-glucose cotransporter 2 inhibitors; SIDD, severe insulin-deficient diabetes; SIRD, severe insulin-resistant diabetes; SNP, single-nucleotide polymorphism; SREBF-2, sterol regulatory element-binding transcription factor; SREBP-2, sterol regulatory element-binding protein-2; T1D, type 1 diabetes; T2D, type 2 diabetes; TCF7L2, transcription factor 7-like 2; TG, triglyceride; TM6SF2, transmembrane 6 superfamily 2; TNF, tumour necrosis factor; US, ultrasound; VLDL, very low-density lipoprotein; WAT, white adipose tissue.

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is an increasingly frequent cause of chronic liver disease. This entity ranges from steatosis over non-alcoholic steatohepatitis (NASH) and liver fibrosis to cirrhosis and hepatocellular carcinoma (HCC).[1] NAFLD affects about 25% of individuals worldwide.[2] NASH is associated with advanced liver disease and progressive fibrosis, which has been identified as the main predictor of liver-related mortality in NAFLD. Moreover, the risk of liver failure and HCC is higher in NASH than in simple steatosis.[3] Genetic variants, such as Patatin-like phospholipase domain-containing protein 3 (PNPLA3), Transmembrane 6 superfamily 2 (TM6SF2), Glucokinase regulatory protein (GCKR) and Membrane bound O-acyltransferase domain containing 7 (MBOAT7), are associated with NAFLD and its progression[4] and may influence the course of NAFLD in people with diabetes. An increased risk of NAFLD and type 2 diabetes (T2D) was identified for a genetic polymorphism in the transcription factor 7-like 2 (TCF7L2), which was also associated with impaired beta cell function. It was also suggested for single-nucleotide polymorphisms (SNPs) of genes related to lipid metabolism such as sterol regulatory element-binding protein-2 (SREBP-2), sterol regulatory element-binding transcription factor (SREBF-2), adiponectin (ADIPOQ), apolipoproteins B and C3 (APOB and APOC3) and for the polymorphism rs780094 of the GCKR, a gene involved in hepatic glucose metabolism.[4] Of interest, the PNPLA3 variant rs738409 (G) was more frequently identified in patients with the SIRD cluster, which is characterized by high liver fat content.[5] Moreover, rs738409 (G) was associated with an increased risk of HCC.[1]

Obesity, insulin resistance and T2D are the main features of NAFLD.[6] NAFLD is diagnosed in about 70% of individuals with T2D, and 10%-20% of these are found to have NASH.[3] Compared to individuals without T2D, those with combined T2D and NAFLD have a higher risk of progressing to NASH, advanced fibrosis and HCC,[1] and T2D itself is an independent risk factor for HCC and cirrhosis.[1,4] NAFLD is correlated with more than two-fold increased risk of incident T2D.[4] In contrast to T2D, individuals with type 1 diabetes (T1D) usually do not develop steatosis within the first 5 years after diagnosis.[7] The risk of incident cardiovascular disease (CVD) is increased in people with NAFLD and T2D diabetes,[3] which is the major cause of morbidity and mortality in those with both conditions.[1] Moreover, patients with combined NAFLD and T2D present with an increased risk of developing chronic kidney disease (CKD)[3] and those with NAFLD and T1D have a 1.5- to 2-fold greater risk of CKD.[1]

Although peripheral and autonomic neuropathy are frequent complications of T2D, data on the relevance of NAFLD for the development and progression of these entities are limited. However, cardiovascular autonomic neuropathy was associated with hepatic steatosis in patients with T2D in the form of lower cardiovagal tone and baroreflex sensitivity and, thus, an increased risk of sudden death.[8]

Recently, cluster analyses have defined five diabetes subgroups: severe autoimmune diabetes (SAID), severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild age-related diabetes (MARD) and mild obesity-related diabetes (MOD).[9] These subgroups differ in diabetes-related comorbidities and complications.[9] Patients in the SAID (42%) and SIDD (29%) groups are already treated with insulin more often than those in the other subgroups (<4%) at baseline.[9] Although the highest prevalence of NAFLD was found in the SIRD subgroup (24.1%), this was based on two elevated alanine-aminotransferase (ALT) measurements and a BMI >28 kg/m^2 but no further imaging.[1,9] The SIRD subgroup also had the highest prevalence of diabetic nephropathy and over a period of 90 days 22.3% of the SIRD cohort presented with a glomerular filtration rate (GFR) <60 mL/min compared to <14% in the other subgroups respectively.[9] This cluster definition was validated in the German Diabetes Study (GDS) which determines insulin secretion and insulin sensitivity in people with T1D or T2D and a duration of disease <1 year using independent gold standard methods.[10] The GDS confirmed the results of the Swedish diabetes subgroups and also assessed diabetic neuropathy, showing a high prevalence of NAFLD (88%) and liver fibrosis (26%) in the SIRD cluster in a 5-year follow-up based on magnetic resonance spectroscopy (MRS), the fatty liver index (FLI), NAFLD fibrosis score and aspartate aminotransferase (AST)-to-Platelet Ratio Index (APRI).[10] After 5 years, diabetic neuropathy was significantly more frequent in the SIDD subgroup (50%) than in other subgroups (<18% respectively).[10] Diabetic nephropathy was present in 27% of the patients in the SIRD subgroup, compared to <5% in each of the other subgroups.[10]

Key points

- Adipose tissue insulin resistance and inflammation combined with altered hepatic mitochondrial capacity may be main drivers of obese NAFLD
- Severe insulin-resistant diabetes (SIRD) compared to other T2D subgroups presents with highest prevalence and risk of progression of NAFLD
- Single nucleotide polymorphism (SNP) rs738409(G) in the PNPLA3 gene associates with severe insulin-resistant diabetes (SIRD) and lean NAFLD
- Future screening for NAFLD in diabetes may comprise non-invasive imaging methods combined with biomarkers and risk factors as SNPs
- Antihyperglycaemic drugs GLP-1-RA, SGLT2i and pioglitazone improve metabolic alterations and seem promising to halt NAFLD progression in T2D

2 | IMPACT OF T2D ON PATHOGENESIS AND PROGRESSION OF NAFLD

Although the origin of NAFLD is still a subject of debate, accumulating evidence suggests that white adipose tissue (WAT) plays a central role in the initiation of both T2D and NAFLD.

2.1 | WAT dysfunction and lipid flux

Lipolysis is a central feature of WAT dysfunction and is a result of WAT insulin resistance and inflammation.[11] It is interesting to note that insulin resistance can exist without associated inflammation, as seen in models of lipodystrophy as well as after a high-fat challenge in which WAT insulin resistance precedes WAT macrophage infiltration.[11] However, WAT inflammation with excessive lipolysis can trigger fatty liver disease.[3] Thus, abnormal adipocyte function rather than fat mass per se causes spill over of free fatty acids (FFA) into the circulation.[11] Visceral adipose tissue shows higher rates of lipolysis and lower lipogenesis than subcutaneous WAT and an increase in visceral adipose tissue volume increases the level of lipid influx into the liver and, thus, metabolic dysregulation.[11] Hyperglycaemia further promotes WAT insulin resistance, which induces elevated WAT reactive oxygen species (ROS) levels and oxidative damage as well as inflammatory processes involving macrophage-induced release of cytokines such as tumour necrosis factor (TNF) or interleukin 6 (IL6).[11] The pancreas compensates for increased insulin resistance by enhanced insulin secretion, which in turn promotes hepatic de novo lipogenesis, hyperlipidaemia and WAT dysfunction and, thus, a greater risk of developing NAFLD.[11]

High levels of circulating FFA trigger hepatic FFA uptake.[11] In the hepatocyte, FFA may either be re-esterified and stored as triglycerides (TG) in lipid droplets,[11] channelled to mitochondria for beta oxidation or secreted as very low-density lipoproteins (VLDL) and thus re-enter the circulation for uptake by peripheral tissues.[4] Chronic carbohydrate-rich overnutrition further stimulates hepatic de novo lipogenesis (DNL) through transcription factors such as sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate response element binding protein (ChREBP) and, thus, promotes additional generation of FFAs and VLDL.[4] While ectopic lipid accumulation and its consequences have been termed "lipotoxicity", chronic hyperglycaemia causing glucose-induced insulin resistance, cellular damage and metabolic deterioration is called "glucotoxicity".[4] Lipotoxicity and glucotoxicity are closely related and enhance each other's actions.[4] Thus, hyperglycaemia in T2D promotes DNL and ectopic fat accumulation by stimulation of transcription factors ChREBP and liver X receptor, which in turn promotes transcription of genes involved in lipogenesis and also by increasing citric acid cycle activity, delivering acyl-coenzyme A (acyl-CoA) as a substrate for DNL.[4]

Intrahepatic fatty acid metabolism also generates lipid intermediates such as diacylglycerols (DAG) and sphingolipids (eg ceramides).[4,11] Increased DAG levels activate protein kinase Cε (PKCε) membrane translocation, which in turn phosphorylates insulin receptor Thr1160 residue, inhibiting insulin signalling and triggering hepatic insulin resistance.[11] Recent published findings suggest that in contrast to former results in mice, ceramide synthase does not play a major role in human obesity-induced insulin resistance.[12] However, circulating and hepatic sphingolipids may play a role in the progression of simple steatosis to NASH as specific sphingolipid species are correlated with hepatic oxidative stress and inflammation in severely obese humans.[13] Since certain dihydroceramide species, especially 18:0, occur up to 9 years before the diagnosis of diabetes, these species may be predictive markers for diabetes before its onset.[12]

2.2 | Hepatic energy metabolism

High levels of FFA can contribute to increased hepatic oxidative stress and mitochondrial alterations and damage.[4] Mitochondrial respiration rates in obese individuals with or without hepatic steatosis are 4-5 times higher than those in lean healthy individuals because of of adaptation to increased substrate availability.[14] However, this adaptation seems to be lost in individuals with NASH who present with severe hepatic insulin resistance as well as a 33% decrease in maximal hepatic mitochondrial capacity[14] and elevated markers of mitochondrial oxidative damage such as increased proton leak, production of ROS and lipid peroxidation, mitochondrial deoxyribonucleic acid (DNA) damage and reduced mitochondrial biogenesis.[14]

There is further evidence of defective hepatic energy metabolism in individuals with T2D as measured by ^{31}P magnetic resonance spectroscopy. Measurement of hepatic adenosine triphosphate (ATP) levels showed 26% lower ATP concentrations and 42% lower flux through ATP synthesis in patients with T2D compared to non-diabetic age- and body mass index (BMI)-matched healthy individuals.[15,16] It should be noted that similar alterations may already be present in patients with newly diagnosed T1D and may occur independently of increased liver fat content.[6] Thus, diabetes probably affects mitochondrial capacity per se, which is further supported by a recent comparison of the mitochondrial function of obese NASH patients with and without T2D.[6] In the hyperglycaemic state, advanced glycation end products (AGEs) are generated that may contribute to the pathogenesis and progression of NAFLD through the induction of receptor for AGEs (RAGE) and downregulation of the AGE clearance receptor AGE receptor 1 (AGER-1).[17] Long-term hepatocyte exposure to AGEs promotes an AGER1/RAGE imbalance with subsequent redox, inflammatory and fibrogenic activity.[17]

2.3 | Lean vs obese NAFLD

Obese individuals are not the only individuals at risk of developing NAFLD. An estimated 40% of the global NAFLD population are non-obese (BMI <30 kg/m^2) and about 19% are lean (BMI <25 kg/m^2).[18] Individuals with lean and non-obese NAFLD have metabolic alterations including visceral adiposity and peripheral insulin resistance that not only drive NAFLD but also increase the cardiovascular risk.[1,4] Moreover, a recent report showed an incidence of diabetes in the non-obese NAFLD population of 12.6 per 1000 person-years and the global estimate for adults in 2014 was 8.5%, confirming that individuals with non-obese NAFLD are at a substantial risk of diabetes,[18] although with a lower prevalence than overweight/obese people with NAFLD.[1]

Increased serum triglyceride levels in lean patients were shown to be correlated with the development and severity of NAFLD, comparable to that in obese/overweight people with NAFLD.[19] Genetic profiling of lean NAFLD suggests that there is a high prevalence of the SNPs rs58542926 (T) in TM6SF2 as well as of the rs738409 (G) polymorphism of PNPLA3. Both SNPs are associated with advanced liver disease, while rs58542926 (T) in TM6SF2 seems to protect against cardiovascular events.[4] Subclinical inflammatory processes, which are assessed by increased circulating IL-6, TNFα and leptin and reduced serum adiponectin levels, are thought to upregulate hepatic DNL and the hepatic pro-inflammatory state. However, unlike obese individuals with NAFLD, lean individuals presented with no relevant alterations of IL-6, TNFα or leptin levels compared to healthy humans. Data on circulating adiponectin concentrations in lean NAFLD were inconsistent.[19] Moreover, no alterations in hepatic mitochondrial oxidation rates or pyruvate cycling were found between lean individuals with and without NAFLD as measured by ^{13}C- magnetic resonance spectroscopy. Thus, factors other than inflammation or loss of mitochondrial capacity may be the major triggers in the pathogenesis of lean NAFLD.[20]

3 | SCREENING AND DIAGNOSIS

The gold standard for the diagnosis of NAFLD is still a histological classification by liver biopsy based on the staging of steatosis, inflammation and fibrosis.[1] However, liver biopsy is limited not only by its invasiveness and related complications, poor acceptability and high cost but also as a result of basic issues such as small tissue sample volumes, which may not be representative of all the alterations from NAFLD in the liver[2] and spontaneous changes in NAFLD stage over time. As a result, because data on reliable NAFLD screening tools are lacking and research is ongoing, current guidelines differ on recommendations for screening in individuals with T2D (Table 1).[1,21-23]

The joint European Association for the Study of the Liver (EASL)-European Association for the Study of Diabetes (EASD)-European Association for the Study of Obesity (EASO) guidelines on the management of NAFLD recommend routine screening for NAFLD in high-risk individuals, defined as patients with T2D, by an assessment of liver enzymes and steatosis either by ultrasound (US) or steatosis biomarkers (Fatty Liver Index, SteatoTest, NAFLD Fat Score).[1] It should be noted that this non-invasive screening strategy has its limitations. The risk of an increased severity of NAFLD, advanced hepatic fibrosis and the development of HCC are associated with T2D independently of serum aminotransferases levels.[1] Obese/overweight individuals with NASH were more frequently found to have increased ALT levels than individuals with lean NASH, suggesting that the evaluation of liver enzymes for the diagnosis of NAFLD is even more unreliable in lean than in obese/overweight NAFLD.[24] Thus, a correct diagnosis of the severity of NAFLD rather than its overall occurrence in T2D (70%, as mentioned above[3]) should be the target of current and future screening. Reliable data assessing non-invasive screening tools for NAFLD in lean or obese/overweight people, especially in those with T2D, are needed.

TABLE 1 Recommendations for screening and diagnosis of NAFLD in T2D

	EASL-EASD-EASO[1]	AASLD[21]	ADA[22]	NICE[23]
Perform screening	+	–[b]	+[a]	+
Consider T2D subgroups	–	–	–	–
Liver enzymes	+[c]	n	+	–
Fatty liver index	+	n	n	n
NAFLD liver fat score	+	n	n	n
NAFLD fibrosis score	+	+	(+)[d]	n
Fibrosis-4 index	+	+	(+)[d]	–
Enhanced liver fibrosis test	+	n	(+)[d]	+
Imaging	+(US)	+(TE,MRE)	+(US, TE)	+(US)

Note: +, recommended, –, not recommended, n, not included in recommendation.

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ADA, American Diabetes Association; EASL-EASD-EASO, European Association for the Study of the Liver-European Association for the Study of Diabetes-European Association for the Study of Obesity; MRE, magnetic resonance elastography; NAFLD, non-alcoholic fatty liver disease; NICE, National Institute for Health and Care Excellence; T2D, type 2 diabetes; TE, transient elastography; US, ultrasound.

[a]If liver enzymes are elevated or fatty liver in ultrasound.

[b]Only if high suspicion for NAFLD and NASH (eg symptoms or incidentally discovered hepatic steatosis).

[c]Any increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT).

[d]No specification of fibrosis biomarkers.

In the presence of steatosis but in the absence of abnormal liver enzymes, serum fibrosis markers should also be determined to estimate disease severity and to determine the need for referral to a specialist.[1] Recommended fibrosis markers include NAFLD fibrosis score (NFS), fibrosis-4 score (FIB-4) and the enhanced liver fibrosis test (ELF). In general, the performance of steatosis and fibrosis scores is rather modest in groups of T2D patients, although a combination of indices can improve their predictive value.[2] Ciardullo et al assessed the intermediate or high risk of hepatic fibrosis in patients with T2D according to EASL-EASO-EASD guidelines, as these patients should be referred to a specialist.[25] The number of participants requiring referral to a specialist was reduced using a sequential testing strategy which first identified patients with steatosis via FLI (>60), and then stratified patients for a low, intermediate or high risk of advanced fibrosis with the FIB-4 score.[25] The combination of FLI and FIB-4 score resulted in 43.7% people with steatosis and 28.3% of the total population who required referral to a specialist. However, this proportion could be further reduced to 20.7% and 13.4%, respectively, by applying age-adjusted FIB-4 cut-offs.[25] Sequential testing strategies to reduce the number of patients requiring liver biopsy to test for NASH were also assessed by Bril et al, showing that the use of plasma AST, then FIB-4, pro-peptide of type III collagen (Pro-C3), the APRI or NAFLD fibrosis scores could exclude certain patients.[26] First, these authors successfully excluded 44% of patients, then an additional 19% recommended for liver biopsy were excluded using Pro-C3, compared to about 8% using FIB-4, APRI or the NAFLD fibrosis score.[26] Whether sequential testing strategies are cost-effective and reliable to reduce the number of patients recommended for liver biopsy requires further investigation in larger cohorts.

Accurate determination of hepatic steatosis and liver stiffness by magnetic resonance or US-based (transient) elastography are increasingly available and may be the future first-line screening tools for NAFLD. The use of imaging together with biomarkers may further improve predictive values. Recently, liver stiffness measurements, controlled attenuation parameter and AST were combined to create a NASH screening tool with a better performance,[27] but this approach must still be validated in large external cohorts.

4 | CURRENT TREATMENT STRATEGIES FOR NAFLD IN T2D

Weight loss significantly improves NAFLD and an average weight loss of ≥10% not only completely reverses steatosis but also, to a certain extent, NASH and fibrosis.[1] Nevertheless, it is difficult for most people to achieve and maintain the required amount of weight loss.[3] Bariatric surgery is an option for long-term weight loss and reversal of NAFLD[1] and T2D, but may be accompanied by peri- and/or post-operative complications.[1]

There is no established pharmacological treatment for NAFLD. However, several drugs are being tested in different clinical trials. These studies focus on NAFLD treatment in mixed populations with and without T2D. Unfortunately, most novel drugs have not been successful thus far.

Antihyperglycaemic drugs used to treat T2D have been tested in people with NAFLD. Metformin was not effective in improving the histological components of NAFLD in patients with and without T2D, but may help reduce the risk of HCC,[2] although data on HCC from interventional studies are still pending. Off-label use of pioglitazone or vitamin E, either separately or in combination, may provide a treatment option for NAFLD, since both substances were found to improve the reversal of NASH with no worsening of fibrosis.[1] However, the safety profile of both pioglitazone and vitamin E is a subject of debate, thus limiting their potential use in routine clinical practice.[1] Newer pharmaceutical options such as glucagon-like peptide 1 receptor agonists (GLP-1 RA) and sodium-glucose cotransporter 2 inhibitors (SGLT2i) have recently been evaluated.

GLP-1 RA reduce cardiovascular risk and are therefore recommended as first-line therapy for people with T2D and established CVD or a high cardiovascular risk.[28] GLP-1 RA also improve the risk of chronic kidney disease, have been shown to be effective in lowering hepatic gluconeogenesis (and thus hepatic insulin resistance and glucose control) and induce weight loss (eg about 10% by semaglutide treatment).[4] The GLP-1 RA liraglutide has resulted in the reversal of NASH in 39% of patients compared to 9% in the placebo group in a population of NASH patients with and without T2D.[3] The results of a recent 72-week phase 2 trial showed that NASH was resolved but there was no improvement of fibrosis in participants receiving 0.1 mg, 0.2 mg or 0.4 mg subcutaneous semaglutide injections once a day (NASH resolution: 40%, 36% and 59%, respectively) compared to placebo (17%).[29] Combined GLP1-RA and glucose-dependent insulinotropic polypeptide (GIP) agonists might be another option for the treatment of NASH in T2D. Tirzepatide is expected to improve clinical outcomes more than GLP-1 RAs.[2] Histological data on the improvement in NASH are not yet available, however, a reduction in the biomarkers of liver damage and increased circulating adiponectin levels have been described in patients with T2D.[2]

SGLT2i inhibit glucose re-absorption in the proximal tubule of the kidney, lowering blood glucose concentrations and inducing moderate weight loss as a result of a calorie deficit.[2] SGLT2i reduce the cardiovascular risk and have also been reported to slow or even halt the progression of chronic kidney disease.[28] Recently, SGLT2i (alternatively GLP-1 RA) were recommended as first-line treatment for patients with T2D and CVD or a high cardiovascular risk.[28]

In a recent study, treatment with empagliflozin resulted in a reduction of 22% in liver fat content in patients with T2D compared to placebo after 24 weeks of treatment.[30] Moreover, serum uric acid levels decreased while adiponectin levels increased with empagliflozin compared to placebo, which may contribute to SGLT2i-mediated reduction in net lipid storage.[30] Although it improves hepatic insulin sensitivity in people with NAFLD, the relative reduction in liver fat content was associated with a weight loss ≥30% in participants treated with canagliflozin compared to placebo ($P < .01$), leading to the conclusion that canagliflozin could improve steatosis

through weight loss.[4] Moreover, a small-scale pilot study reported histological improvement in hepatic steatosis, ballooning and fibrosis and further reduction in NASH after 6 months of empagliflozin treatment in patients with T2D.[2] Mice models show that SGLT2i can inhibit the progression of both NASH and HCC,[2] however, more prospective studies are required and whether these results will be similar in humans remains unclear.

5 | SUMMARY/OUTLOOK

Obese NAFLD is a global epidemic and closely related to insulin resistance and T2D.[11] NAFLD in T1D is less frequent and seems to be related to chronic hyperglycaemia because of an absolute insulin deficiency.[7] The pathogenesis of lean NAFLD is mainly related to altered body fat distribution with greater visceral fat mass, reduced muscle mass and peripheral insulin sensitivity as well as a high frequency of specific NAFLD-related SNPs.[4,19] The SIRD subgroup of T2D as well as lean NAFLD were both recently found to be associated with the polymorphism rs738409 (G) in the PNPLA3 gene.[5,9] This finding may be useful in the future for targeted screening of groups at high risk of advanced liver disease. The antihyperglycaemic agents GLP-1 RA and SGLT2i may be effective therapeutic tools in patients with T2D because they improve the metabolic status including liver fat content.[3,4]

In conclusion, individuals with lean NAFLD present with differences in hepatic energy metabolism, frequency of NAFLD-related SNPs and risk of comorbidities such as diabetes compared to those with obese NAFLD, while hyperglycaemia may worsen liver disease independently from steatosis. It should be noted that the hepatic energy metabolism in diabetic patients is decreased compared to non-diabetic individuals. These findings suggest that NAFLD must be evaluated in specifically defined subgroups and as clear-cut entities to optimize the future management of NAFLD and take the next steps in precision medicine.

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CONFLICT OF INTEREST

BS and SK declare no conflicts of interest. MR has served on scientific advisory boards or received speaker's honoraria for Boehringer-Ingelheim Pharma, Eli Lilly, Fishawack Group, Novo Nordisk, Servier Laboratories, Target NASH. He is also a consultant for Terra Firma and has been involved with clinical trial research for Boehringer Ingelheim, Danone Nutricia Research and Sanofi-Aventis.

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SUPPLEMENT ARTICLE

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New drugs for NASH

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Abstract

Non-alcoholic steatohepatitis (NASH) is a result of inflammation and hepatocyte injury in the presence of hepatic steatosis which can progress to cirrhosis. NASH is the most rapidly growing aetiology for liver failure and indication for liver transplantation in the United States. Non-alcoholic fatty liver disease (NAFLD) is associated with obesity, type 2 diabetes, dyslipidaemia and metabolic syndrome. Because of the absence of approved pharmacotherapy, weight loss and lifestyle modifications remain the safest and most effective first-line treatment. However, this may not be effective in patients with advanced fibrosis or cirrhosis and long-term adherence is difficult to achieve. Therefore, effective drugs are urgently needed for the treatment of NASH. Drug development targeting pathological pathways in NASH have exploded in the past decade, with numerous new drugs under investigation. This review summarizes the results of pivotal finalized phase 2 studies and provides an outline of key active studies with trial data of drugs under development.

KEYWORDS

clinical trials, drug therapy, fatty liver, non-alcoholic steatohepatitis, pharmacotherapy

Key points

- Despite the high prevalence and potential consequences of NASH, there are currently no approved treatments for this disease.
- Given the scale of the problem and unmet needs, there are numerous agents in the development pipeline.
- The paradigm for the ideal drug is targeting both steatohepatitis and fibrosis and improvement of cardiometabolic risk factors.
- The main new drugs under investigation mainly focus on the pathogenesis of NASH to target inflammation and fibrogenesis.
- Because of the complexity of NASH, it will probably be necessary to combine different classes of drugs to increase their effectiveness.

Abbreviations: ALT, alanine aminotransferase; APRI, AST-to-Platelet Ratio Index; Aramchol, Arachidyl-amido cholanoic acid; ASK1, apoptosis signal-regulating kinase 1; AST, aspartate aminotransferase; CCR2, chemokine receptor type 2; CCR5, chemokine receptor type 5; FGF19, fibroblast growth factor 19; FXR, farnesoid X nuclear receptor; Gal-3, galectin-3; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide; HCC, hepatocellular carcinoma; HFF, hepatic fat fraction; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HVPG, hepatic venous pressure gradient; MPC, mitochondrial pyruvate carrier; MRI-PDFF, MRI-proton density fat fraction; NAFLD, Non-alcoholic fatty liver disease; NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis; OCA, Obeticholic acid; PPAR, peroxisome proliferator activated receptor; SCD1, Stearoyl-CoA desaturase 1; T2D, type 2 diabetes; THR β, thyroid hormone receptor β; TXR, Tropifexor.

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease in Western countries. It is currently the third leading cause of cirrhosis in the United States and is the fastest growing cause of hepatocellular carcinoma (HCC) in liver transplant candidates.[1,2] Non-alcoholic steatohepatitis (NASH) leads to progressive fibrotic remodelling of the liver culminating in HCC and cirrhosis.[3] The burden of end-stage liver disease caused by NAFLD is expected to increase two- to three-fold by 2030.[4] Therefore, there has been a significant increase in the number of studies investigating new drugs for more effective management of NAFLD. However, for the moment, no drugs have been approved for treating NASH and treating this disease remains a major unmet clinical need. Many phase 2 and 3 trials are ongoing and a new chapter is expected in NASH treatment in the near future (Table 1).[5] The specific agents under study are best evaluated according to their mechanism of action and the status of front-line therapeutics for NASH is reviewed below.

2 | METABOLIC PATHWAYS

The goal of anti-NASH drugs targeting metabolic pathways is to reduce the accumulation of hepatic fat, including obeticholic acid (OCA), which acts on the farnesoid X receptor (FXR) involved in the metabolism of bile acids; peroxisome proliferator-activated receptor (PPAR) agonists such as pioglitazone, elafibranor and saroglitazar; analogues of fibroblast growth factor (FGF), such as FGF-21 and FGF-19, and inhibitors of de novo fat synthesis, such as aramchol, acetyl coenzyme A carboxylase (ACC; eg GS-0976) and incretin, such as liraglutide.[6]

2.1 | FXR agonists

2.1.1 | Obeticholic acid (OCA)

OCA is a classic agonist for FXR and is the most extensively studied FXR agonist for NASH. Although it is derived from the primary human bile acid, chenodeoxycholic acid, it stimulates FXR activity approximately 100-fold more than the latter, and is highly selective with minimal activity to G-protein-coupled bile acid receptor.[7] The FXR nuclear receptor is expressed in the liver and intestines and plays an important role in the synthesis and enterohepatic circulation of bile acids.[8] Its activation reduces bile acid synthesis by inhibiting the conversion of cholesterol into bile acids. Activation of FXR in the ileum also inhibits the uptake of bile acids by downregulating the sodium-dependent bile acid transporter. Its main function is to regulate cholesterol lipoprotein and bile acid metabolism to modulate immuno-inflammatory and fibrogenic responses.[8] The main trials evaluating OCA are the FLINT trial, a phase 2B study[9] and the ongoing REGENERATE phase 3 study.[10] The FLINT trial was a multicentre, double-blind, placebo-controlled, randomized

TABLE 1 List of clinical trials for the anti-NASH drugs

Drug	Mechanism of action	Phase in clinical trial	Trial identification
Obeticholic acid	FXR agonist	III	NCT02548351
Tropifexor	FXR agonist	IIb	NCT02855164
Elafibranor	PPAR-α/δ agonist	III	NCT02704403
Saroglitazar	PPAR-α/γ agonists	II	NCT03061721
Aramchol	SCD1 inhibitor	III	NCT04104321
Semaglutide	GLP-1 analogue	IIb	NCT02970942
Tirzepatide	GLP-1-GIP co-agonist	III	NCT03861039
Cotadutide	GLP-1-glucagon agonist	II	NCT04515849
NGM282	FGF19 analogue	II	NCT03912532 NCT04210245
MSDC-0602K	MPC inhibitor	IIb	NCT02784444
Resmetirom	THR-ß agonist	III	NCT03900429
Cenicriviroc	CCR2/CCR5 inhibitor	III	NCT03028740
Selonsertib	ASK1 inhibitor	III	NCT03053050 NCT03053063
Emricasan	Caspase Inhibitor	II	NCT03205345 NCT02960204 NCT02686762
Simtuzumab	Lysyl oxidase-like 2 inhibitor	II	NCT01672866 NCT01672879
GR-MD-02	galectin-3 inhibitor	II	NCT02462967 NCT02421094 NCT04365868

clinical trial in the USA in biopsy-proven NASH patients without cirrhosis. This study assessed treatment with 25 mg daily of oral OCA compared to placebo for 72 weeks. A total of 141 patients were randomly assigned to receive OCA and 142 to receive placebo in this trial. Forty-five per cent of patients achieved a significant improvement in their NAFLD activity score (NAS) and fibrosis. However, the resolution of NASH after treatment with OCA was not significantly different than with placebo. Twenty-three per cent of patients in the OCA group developed pruritus compared to only 6% in the placebo group. The conclusion of the FLINT trial was that OCA improved the histological features of NASH, but further studies were needed to determine the long-term benefits and safety of this agent.[9]

The global REGENERATE study compares the effects of OCA to placebo for histological improvement and liver-related clinical outcomes in patients with NASH and stage 2 or 3 liver fibrosis. This study has three arms and patients are randomized 1:1:1 as follows: OCA 10 mg, OCA 25 mg daily and placebo. A liver biopsy is obtained at screening, at 18 and 48 months and at the end of study. The primary endpoints are the proportion of OCA-treated patients vs placebo who achieve improvement of at least one stage in liver fibrosis with no worsening of NASH or the proportion of OCA-treated patients compared to placebo with a resolution of NASH and no worsening of fibrosis. The secondary endpoints are all-cause mortality and liver-related clinical outcomes. The estimated completion date of this study is October 2022. Nine-hundred and thirty-one patients with stage F2-F3 fibrosis were included in the primary analysis (311 in the placebo group, 312 in the OCA 10 mg group and 308 in the OCA 25 mg group). The endpoint for the improvement in fibrosis was achieved by 71 (23%) patients in the OCA 25-mg group ($P = .0002$), 55 (18%) in the OCA 10-mg group ($P = .045$) and 37 (12%) in the placebo group. Again, the main adverse event was pruritus. The evaluation of clinical outcomes is ongoing.[10] Accelerated approval was not given by the FDA in the USA based on the existing profile on the effects of OCA, which was considered to be insufficient. The trial to determine the influence of OCA on "difficult outcomes" such as liver-related events and also the generally accepted surrogate of "progression to cirrhosis" is ongoing.

2.1.2 | Tropifexor (LJN-452)

Tropifexor (TXR) is a highly potent, non-bile acid FXR agonist that has shown to have potent in vivo activity in rodent PD models by measuring the induction of FXR target genes in various tissues, and has been shown to be effective in preclinical models of NASH.[11] Recruitment for a phase 2 adaptive design study (FLIGHTFXR) in patients with NASH has recently been completed (results are awaited) (NCT02855164). In addition, a recent randomized, double-blind, multicentre, phase 2B study is evaluating the safety and efficacy of a combination of TXR and cenicriviroc (TANDEM) in patients with biopsy-proven NASH and liver fibrosis (stages F2/F3). This study includes a 48-week treatment period and 4 weeks of follow-up.[12] Data from the histological outcomes with this agent are expected in the last quarter of 2020.

2.2 | Peroxisome proliferation-associated receptor (PPAR) agonists

These are a heterogeneous class of compounds that engage the alpha, gamma and beta/delta subtypes of PPAR receptors. The individual compounds are either pure agonists of one subtype or bind more than one subtype. The pharmacological and clinical profile of each agent depends on the relative affinity and downstream signalling that is generated by binding of the drug to the individual agents. Pioglitazone is a classic PPAR-γ agonist and has been studied in a large number of phase 2B studies. This agent "defats" the liver and resolves steatohepatitis[13] but has modest effects on fibrosis. It also causes weight gain, fluid retention and increases the risk of osteopenia and fractures in older women.[14] Other agents are currently being actively evaluated.

2.2.1 | Elafibranor

Elafibranor (GFT-505; Genfit, Lille, France) is a PPAR-α/PPAR-δ dual agonist that regulates lipid and insulin metabolism. The GOLDEN study[15] was a phase 2B multicentre, double-blind, randomized controlled trial comparing elafibranor 80 and 120 mg daily to placebo for 52 weeks in patients with non-cirrhotic NASH. Although there was no reversal of NASH, in a post hoc analysis based on a modified definition, NASH was resolved without the worsening of fibrosis in a higher proportion of patients in the 120-mg elafibranor group than in the placebo group (19% vs 12%; odds ratio = 2.31; $P = .045$). Further causal analysis of 234 patients with NAS ≥ 4 showed that 120 mg of elafibranor resulted in a higher rate of NASH resolution than placebo based on the protocol definition (20% vs 11%; odds ratio = 3.16; $P = .018$) and the modified definitions (19% vs 9%; odds ratio = 3.52; $P = .013$). Patients in whom NASH resolved after receiving elafibranor 120 mg were found to have lower stages of liver fibrosis than those without resolution (mean reduction of 0.65 ± 0.61 in responders for the primary outcome vs an increase of 0.10 ± 0.98 in non-responders; $P < .001$). Elafibranor was well tolerated and improved patient cardiometabolic risk profile, although it caused mild but reversible increases in serum creatinine. A phase III trial to evaluate the efficacy and safety of elafibranor vs placebo in patients with NASH (RESOLVE-IT) (registration no. NCT02704403) was then begun in 2016. The study included 2000 NASH patients with NAS ≥ 4, with ≥1 of each component of the score and F1-F3 fibrosis. The primary outcome was histological improvement, defined as the resolution of NASH without worsening of fibrosis at 72 weeks with a composite outcome that would evaluate all-cause mortality, cirrhosis and "liver-related clinical outcomes" at 4 years. However, the

interim analysis did not show any histological benefit and studies for NASH have been discontinued in this agent.

2.2.2 | Saroglitazar

Saroglitazar is a PPAR-α/PPAR-γ dual agonist that has been shown to improve lipid and glycaemic parameters. A phase 2, prospective, multicentre, double-blind, randomized trial (EVIDENCES II; registration no. NCT03061721) in patients with NAFLD/NASH was performed to determine the efficacy and safety of saroglitazar magnesium compared to placebo. The study randomized 106 adult subjects with a body mass index ≥25 kg/m^2 and alanine aminotransferase (ALT) ≥50 U/L in a 1:1:1:1 ratio to receive 1, 2 or 4 mg of saroglitazar or placebo. The primary endpoint was the percentage of change in ALT levels from baseline to week 16 in the saroglitazar and placebo groups. All saroglitazar groups achieved the primary endpoint, saroglitazar 1 mg (−27.3%), 2 mg (−33.1%) and 4 mg (−44.3%) vs placebo (4.1%) ($P < .001$ for all). At week 16, saroglitazar 4 mg resulted in significant reduction in Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), triglycerides, total cholesterol and AST-to-Platelet Ratio Index (APRI) compared to the placebo ($P < .05$ for all). The secondary endpoints included the proportion of patients with a ≥50% reduction in ALT levels and a change in liver fat content (measured by MRI-proton density fat fraction (MRI-PDFF)) from baseline to week 16 in the saroglitazar vs placebo groups. A ≥50% reduction in mean ALT from baseline to week 16 occurred in 51.8% of patients with saroglitazar 4 mg compared to 3.5% in the placebo group ($P < .0001$). A >30% reduction in liver fat content was found in 40.7% of patients who received saroglitazar 4 mg vs 8% in the placebo group ($P = .006$). There was no significant change in the percentage of body weight between saroglitazar 4 mg and placebo (1.88% vs 0.28%, $P = .9$) and, overall, saroglitazar was well tolerated. Another phase 2B trial has been completed in North America and the results are expected in the last quarter of 2020.

2.2.3 | Arachidyl amido cholanoic acid (Aramchol)

Aramchol is a cholic-arachidic acid conjugate that targets Stearoyl-CoA desaturase 1 (SCD1), inhibiting de novo fat synthesis.[16] A 52-week phase 2B trial (NCT02279524) was performed to evaluate the effect of Aramchol (400 and 600 mg) on hepatic triglyceride content using MRI spectroscopy in biopsy-proven NASH patients without cirrhosis. The ARRIVE trial is a recent double-blind, randomized, placebo-controlled trial that tested the efficacy of 12 weeks of treatment with Aramchol vs placebo in HIV-associated NAFLD.[17] Fifty patients with HIV-associated NAFLD, defined by MRI-PDFF ≥ 5%, were randomized to receive either Aramchol 600 mg daily (n = 25) or placebo (n = 25) for 12 weeks. This study concluded that Aramchol did not reduce hepatic fat or change body fat or muscle composition based on an MRI assessment in patients with HIV-associated NAFLD.[17] A phase 2B trial (ARREST) of Aramchol in NASH showed a decrease in steatosis and inflammation and ballooning along with a modest but statistically non-significant improvement in fibrosis. This molecule is currently being evaluated in a Phase 3 global trial for NASH (NCT04104321).

2.2.4 | Incretins

Liraglutide is a glucagon-like peptide (GLP)-1 analogue that was evaluated in a phase 2 trial on NASH (LEAN trial; registration no. NCT01237119). The study showed that although liraglutide improves NASH and reduces patient's body weight, liver fibrosis was worsened in patients treated with this agent compared to the placebo group. Topline results from a rigorous well-conducted phase 2B trial of semaglutide for NASH have recently been released (registration no. NCT02970942). These results showed that NASH was resolved in 59% of patients with semaglutide compared to approximately 20% in the placebo arm. However, this was not associated with significant improvement in fibrosis. The full results of this important trial are awaited. Based on the potential utility of GLP-1, a combination of GLP-1 agents with glucose-dependent insulinotropic polypeptide (GIP) and/or glucagon is under active investigation. Tirzepatide is a GLP-1-GIP co-agonist. GIP improves glucose disposal and also reduces nausea associated with GLP-1 activity. It has been associated with significant weight loss in obese individuals.[18] Similarly, cotadutide is a GLP-1-glucagon agonist. Glucagon activates lipid oxidation and also improves mitochondrial turnover and function in NAFLD models. Both tirzepatide and cotadutide are undergoing phase 2B trials. Oral formulations of semaglutide have also been shown to reduce weight but less significantly than injectable formulations.[19] There are no existing data on the use of oral semaglutide for the treatment of NASH.

2.2.5 | Fibroblast growth factor (FGF) analogues

FGF19 is a hormone that regulates bile acid synthesis, glucose homoeostasis and energy homoeostasis. NGM282 is an engineered non-tumorigenic analogue of FGF19. A randomized, double-blind, placebo-controlled, phase 2 study was performed to evaluate the efficacy and safety of NGM282 in patients with biopsy-proven NASH.[20] Patients were assigned (1:1:1) to receive either 3 or 6 mg of subcutaneous NGM282 or placebo. The primary endpoint was an absolute change in liver fat content from baseline to week 12. Responders were patients who achieved a reduction in absolute liver fat content of at least 5% measured by MRI-PDFF. At 12 weeks, 20 (74%) patients in the 3-mg group and 22 (79%) in the 6-mg group achieved the primary endpoint vs two (7%) in the placebo group. Histological data have confirmed improvement in steatosis, steatohepatitis and also fibrosis with this molecule.[21] Side effects such as injection site reactions (34%), diarrhoea (33%), abdominal pain (18%) and nausea (17%) were diagnosed in 76/82 (93%) patients and were more frequent in the NGM282 groups. No life-threatening events or patient deaths occurred during this

study. The study concluded that NGM282 is associated with a reduction in liver fat content with an acceptable safety profile in patients with NASH.[20] This agent is now moving into Phase 3 trials and is also being evaluated in patients who have already progressed to cirrhosis.

2.2.6 | MSDC-0602K

MSDC-0602K is a second-generation insulin sensitizer that has been shown in initial studies to increase lipid oxidation and reduce de novo lipid synthesis and gluconeogenesis in the liver, both in vivo and in vitro, without the side-effects of first-generation insulin sensitizers.[22] It targets the mitochondrial target of thiazolidinediones (mTOT). A phase 2B 52-week double-blind study evaluated the efficacy and safety of MSDC-0602K in patients with biopsy-confirmed NASH (n = 392). Half of the patients had controlled type 2 diabetes (T2D). The primary endpoint was hepatic histological improvement of ≥2 points in NAS with a ≥1-point reduction in either ballooning or lobular inflammation, and no increase in fibrosis at 12 months. The secondary endpoints included improvement in NAS without worsening of fibrosis, resolution of NASH and a reduction in fibrosis. The exploratory endpoints included changes in insulin sensitivity, liver injury and liver fibrosis markers. All patients were randomized to receive a single daily dose of placebo or 62.5, 125 or 250 mg of MSDC-0602K. Although analysis of MSDC-0602K study data did not show any statistically significant effects on primary or secondary liver histology endpoints, effects on the non-invasive measures of liver cell injury and glucose metabolism were identified. The incidence of hypoglycaemia and PPARγ-agonist-associated events such as oedema and fractures were similar in the placebo and MSDC-0602K groups. However, the results of this trial were confounded by different interpretations of histological outcomes when the biopsies were reviewed by multiple pathologists, raising concerns about the reliability of subjective histological assessments of NASH.[23] There is currently no consensus on the best approach to evaluate liver histology in phase 3 trials of NASH, which remains an unresolved topic in this field. It has also stimulated research to improve the precision of histological assessments with machine-learned approaches. For the moment, these are emerging technologies which have not been approved by regulatory agencies to assess the benefit of therapies in NASH.

2.2.7 | Resmetirom (MGL-3196)

Thyroid hormones play a central role in controlling lipid metabolism through the activation of the β receptor, influencing the levels of serum cholesterol and triglycerides as well as on the accumulation of fat in the liver. The thyroid hormone receptor β (THR β) is highly expressed in hepatocytes and is responsible for regulating the metabolic pathways in the liver that are frequently impaired in NAFLD and NASH. Resmetirom (MGL-3196, Madrigal Pharmaceuticals Inc) is an oral THR β selective agonist that was developed to target dyslipidaemia but has also been shown to reduce hepatic steatosis in fat-fed rats, improving insulin sensitivity, promoting liver regeneration and reducing apoptosis.[24] A phase II double-blinded, randomized, placebo-controlled study was performed including patients with biopsy-proven NASH and ≥10% liver steatosis.[25] The primary outcome was the percentage of change from baseline in hepatic fat fraction (HFF) assessed by MRI-PDFF at 12 weeks for Resmetirom vs placebo. A total of 125 patients were included from October 2016 to July 2017 from 25 medical centres in the USA. A statistically significant improvement was found at 12 weeks in the relative decrease in liver fat in patients treated with Resmetirom compared to placebo. Resmetirom also significantly improved steatohepatitis but had somewhat modest effects on fibrosis.[25] Statistically significant reductions were also observed in ALT and AST levels, atherogenic lipids, lipo-protein(a), markers of inflammation and fibrosis as well as improvement in NASH on liver biopsies in Resmetirom-treated patients compared to placebo. Resmetirom was well tolerated, although it was associated with an increase in gastrointestinal adverse events, which were self-limited and did not result in study withdrawal. A multinational phase 3 placebo-controlled study of Resmetirom in patients with NASH and fibrosis is now recruiting (NCT03900429). Other thyroxine beta-receptor agonists are also currently being actively evaluated (Viking therapeutics) and have shown to have beneficial effects in phase 2B trials (NCT4173065).[26] This molecule is also entering phase 3 trials.

3 | OXIDATIVE STRESS AND INFLAMMATION

3.1 | Cenicriviroc

Cenicriviroc is an oral, dual antagonist of chemokine receptor type 2 and type 5, causing inhibition of overactive inflammatory signalling and disruption of signalling that activates stellate cells, targeting both inflammation and fibrogenesis.[27] Based on the phase 2B CENTAUR trial (NCT02217475), cenicriviroc did not reach the primary endpoint of a 2-point NAS decline, although there was a significant improvement in the grade of fibrosis.[28] A phase 3 AURORA trial (NCT03028740) is ongoing to evaluate the efficacy and safety of cenicriviroc on NASH-related fibrosis. The primary endpoints are improvement of at least one stage of fibrosis without worsening of NASH after 12 months of treatment as well as all-cause mortality and liver-related clinical outcomes at 5 years. The initial histological results from the trial are expected in 2021.

3.2 | Selonsertib (SEL, GS-4997)

Selonsertib is an apoptosis signal-regulating kinase 1 (ASK1) inhibitor. An open-label phase 2 trial evaluating NASH patients with

moderate and severe liver fibrosis reported a regression in fibrosis and other parameters of liver injury.[29] STELLAR 3 (http://www.natap.org/2019/HCV/050819_01.htm) and STELLAR 4 (http://www.natap.org/2019/HCV/022719_01.htm) are phase 3 trials that were begun to evaluate NASH patients with stage 3 fibrosis and cirrhosis respectively. Because STELLAR 4 did not reach the primary endpoint (at least 1-point reduction in fibrosis without worsening of NASH at 48 weeks) it was discontinued, and the STELLAR program was cancelled.

3.3 | Emricasan

Emricasan is a pan-caspase inhibitor that blocks liver cell-related apoptosis and inflammatory responses. Two phase 2b trials, ENCORE-PH (NCT02960204) and ENCORE-LF (NCT03205345), have investigated the effect of emricasan on improving event-free survival in patients with NASH-related cirrhosis or decompensated NASH-related cirrhosis respectively. ENCORE-NF (NCT02686762) enrolled patients with biopsy-proven NASH and F1-F3. Top-line results from ENCORE-LF and ENCORE-NF clinical trials did not meet its primary endpoints.[30] Further development of this molecule for NASH has been discontinued.

4 | ANTI-FIBROTICS (LYSYL-OXIDASE INHIBITOR AND GALECTIN)

The anti-lysyl oxidase-like 2 drug GS-6624 (simtuzumab) directly blocks the formation of collagen bands, resulting in an anti-fibrotic effect. Two phase 2B trials (NCT01672866 and NCT01672879) evaluated the efficacy of simtuzumab on collagen deposition in liver tissues in a NASH group with progressive fibrosis, and mapped its effects on hepatic venous pressure gradient (HVPG) in NASH-related cirrhosis. However, both clinical trials were terminated prematurely. Animal studies showed that the galectin-3 (Gal-3) inhibitor GR-MD-02 can reduce hepatic fibrosis.[31] A phase 2B clinical trial (NCT02462967) was performed to evaluate the safety and efficacy of GR-MD-02 for the treatment of NASH-related compensated cirrhosis and associated portal hypertension. However, although there was evidence of a reduced HVPG in a subset of patients, this trial did not reach the primary endpoint.[32] A second phase 2B trial is being performed to confirm these findings.

5 | THE INTESTINE

There are anti-NASH drugs that target the intestine and treat NAFLD by regulating enterohepatic circulation, which include anti-obesity preparations (eg orlistat), regulators of the intestinal flora (eg IMM-124e) and transplantation of fecal microbiota (eg solithromycin). However, none of these drugs has entered phases 2B or 3 clinical trials.

6 | COMBINATION THERAPY

A phase 2B ATLAS trial (NCT03449446) was begun by Gilead Sciences in 2018 to evaluate a combination of selonsertib, the ACC inhibitor GS-0976 and the FXR agonist GS-9674 in NASH patients with bridging fibrosis or compensated cirrhosis, as well as the incidence of adverse events and abnormal levels of hepatic biochemical indicators. Combination therapy may have greater anti-fibrotic and anti-steatotic effects than monotherapy.

CONFLICTS OF INTEREST

Dr Albhaisi has no conflicts of interest. Dr Sanyal is President of Sanyal Biotechnology and has stock options in Genfit, Akarna, Tiziana, Indalo, Durect, Exhalenz and Hemoshear. He has served as a consultant to Astra Zeneca, Conatus, Coherus, Bristol Myers Squibb, Blade, Tobira, Takeda, Siemens, Merck, Genentech, Tern, Gilead, Lilly, Poxel, Artham, Boehringer Ingelheim, Novo Nordisk, NGM Bio, Birdrock, Novartis, Pfizer and Genfit. He has been an unpaid consultant to Intercept, Echosens, Perspectum, Immuron, Galectin, Fractyl, Affimune, Chemomab and Nordic Bioscience. His institution has received grant support from Gilead, Salix, Tobira, Intercept, Bristol Myers, Shire, Merck, Astra Zeneca, Malinckrodt, Cumberland and Novartis. He receives royalties from Elsevier and UptoDate.

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SUPPLEMENT ARTICLE

Liver INTERNATIONAL WILEY

End-stage liver disease: Management of hepatorenal syndrome

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Abstract

Hepatorenal syndrome (HRS) is a serious complication of cirrhosis with high morbidity and mortality rates. Recently, the definition of HRS type 1 has been updated and is now called HRS-AKI. This new definition reduces the risk of delaying HRS treatment and eliminates the need to establish a minimum creatinine cut-off for the diagnosis of HRS-AKI. From a pathophysiological point of view, newly identified mechanisms involved in the development of HRS are related to the inflammatory response, conditioning the development of extrahepatic organ dysfunction in patients with cirrhosis. One of the main challenges for the diagnosis of HRS is the validation of new biomarkers to obtain an early and differential diagnosis of kidney injury (eg HRS vs. ATN). Treatment of HRS is based on the use of vasoconstrictive agents in combination with albumin and terlipressin is the most widely used vasoconstrictor drug, with a high response rate. The effects of a continuous infusion of terlipressin at a dose of 2-12 mg/day was similar to bolus administration, but with lower rates of adverse events. Finally, MELD/MELD-Na which includes creatinine as one of its main determinants gives AKI-HRS patients priority on the waiting list (WL) for liver transplant (LT). However, the MELD and MELD-Na scores are reduced in responding patients, resulting a longer waiting time in these patients than in non-responders. Thus, the initial MELD/MELD-Na score (pre-treatment value) should be used to prioritize patients on the WL for LT in these cases.

KEYWORDS

AKI, cirrhosis, HRS, liver Transplant

1 | INTRODUCTION

Hepatorenal syndrome (HRS) is a serious complication of cirrhosis that is associated with high morbidity and mortality rates. Its development is associated with functional circulatory changes in the kidneys which are a maladaptive response of physiological compensatory mechanisms leading to a significant decrease in the estimated glomerular filtration rate (eGFR).[1] Moreover, this circulatory condition is reversible if renal blood flow is reestablished, either by liver transplantation or by the use of vasoconstrictor therapy.[2] The terminology, definition, and classification of HRS have changed considerably in the last 10 years, mainly due to changes in the diagnosis and staging of acute kidney injury (AKI) and improved characterization of the natural history of

Abbreviations: ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; ATN, acute tubular necrosis; CCM, Cirrhotic cardiomyopathy; CKD, chronic kidney disease; CSPH, clinically significant portal hypertension; CysC, Cystatin C; eGFR, estimated glomerular filtration rate; FENa, fractional excretion of sodium; HRS, hepatorenal syndrome; HRS-AKD, HRS Acute kidney disease; HRS-CKD, HRS Chronic kidney disease; ICA, International Club of Ascitis; LT, liver transplant; N-GAL, neutrophil gelatinase-associated lipocalin; RRT, renal replacement therapy; SBP, spontaneous bacterial peritonitis; SLKT, simultaneous liver-kidney transplant; TLR4, Toll-like receptor 4; WL, waiting list.

acute kidney disease in patients with cirrhosis.[3,4] Thus, one of the main challenges of clinical practice is to differentiate HRS from acute tubular necrosis (ATN), which is important because the use of vasoconstrictors is not indicated in the latter patients. Also, one of the main topics of debate is whether HRS and ATN should be considered a continuum instead of different entities.[5,6] Emerging biomarkers can help differentiate these two conditions and even provide prognostic information on the recovery of kidney function after liver transplantation (LT), as well as help decide on the need for simultaneous liver-kidney transplant (SLKT).[7] The present review describes the recent advances that have shaped the current definitions, diagnosis and management of HRS.

Key points

- Hepatorenal syndrome (HRS) is a deterioration of renal function caused mainly by the presence of systemic circulatory dysfunction. However, it has recently been discovered that systemic inflammation and the presence of cirrhotic cardiomyopathy also play a role in its pathogenesis. The development of HRS is associated with poor survival.
- The diagnosis of HRS is based on the new criteria of the International Club of Ascites-Acute Kidney Injury (ICA-AKI) and Hepatorenal Syndrome-Acute Kidney Injury (HRS-AKI), which are essential to exclude the presence of intrinsic kidney disease (hematuria, proteinuria or abnormal renal ultrasound).
- Currently, two types of hepatorenal syndrome are recognized depending on the time of presentation and the progression of kidney injury. The first, HRS-AKI, represents the acute deterioration of renal function, while the second represents exacerbated chronic kidney dysfunction, HRS-CKD.
- The treatment of HRS includes the early use of terlipressin with albumin. However, liver transplantation continues to be the treatment with the greatest benefit to survival, and therefore, timely referral for transplant evaluation is crucial in preventing permanent kidney damage and if necessary to determine the need for a simultaneous liver and kidney transplant.
- The use of new renal biomarkers in clinical practice can improve both the diagnosis and prognosis of this population. In particular, NGAL is a promising biomarker in cirrhosis with a significant impact in clinical practice for the differentiation between ATN and HRS, showing that improved prognostic accuracy has significant implications in organ allocation.

2 | DEFINITIONS

Acute deterioration of renal function, determined by an increase in serum creatinine, is a prevalent condition (19%-26%) in hospitalized patients with cirrhosis.[8] Although it is widely used, serum creatinine is known to have serious limitations in patients with decompensated cirrhosis. Creatinine synthesis is reduced in patients with cirrhosis, either because of reduced muscle mass or reduced protein intake. Moreover, there is a gender bias.[9] Therefore, creatinine is a sub-optimal biomarker for risk stratification in this population. New, alternative, more precise biomarkers such as cystatin C (CysC), are promising in patients with cirrhosis both because of the possibility of early diagnosis and their ability to establish a prognosis.[10] Nevertheless and despite its limitations, serum creatinine continues to be the most affordable and available biomarker for eGFR and thus, the definition of acute renal failure has evolved in the last two decades due to the variability of this serological biomarker. Recent modifications in the diagnostic criteria for AKI by the International Club of Ascites (ICA), based on an absolute increase in serum creatinine of at least 0.3 mg/dl or 50% from baseline, have been shown to be more effective for the early detection of patients at a higher risk of a longer hospital stay, multiple organ failure, admission to intensive care units, in-hospital mortality, and mortality at 90 days.[11-13] (Table 1).

Recently, the ICA also updated the definition of type 1 HRS, which is now called HRS-AKI. One of the main changes of this new definition is that the two-week interval required to double the serum creatinine in the previous definition has been modified because it creates a risk of delaying the beginning of treatment for hepatorenal syndrome. It has also been shown that the higher the serum creatinine at the start of vasoconstrictor treatment, the lower the probability of reversing HRS.[14] Thus, this new definition has eliminated the need for establishing a minimum creatinine cut-off for the diagnosis of HRS-AKI.[3] In contrast, the new ICA definition states that functional kidney injury which does not meet HRS-AKI criteria is now called HRS-NAKI (that is, not AKI) and is defined by eGFR instead of serum creatinine. The presence of NAKI is divided into HRS Acute kidney disease (HRS-AKD) if the eGFR is less than 60 ml/min/1.73 m2 for less than three months and HRS Chronic kidney disease (HRS-CKD) if it is less than this for more than three months (Table 1).

3 | PATHOGENESIS OF HEPATORENAL SYNDROME

3.1 | Circulatory dysfunction

The main driver in the development of the complications of cirrhosis is clinically significant portal hypertension (CSPH). The consequent splanchnic arteriolar vasodilation is a key factor in the pathophysiology of HRS-AKI.[2] In the early stages of cirrhosis, the increase in intraportal hypertension is modest along with

TABLE 1 Definition of AKI according to international club of ascites

ICA AKI in Cirrhosis	Increase in sCr>= 0.3 mg/Dl (26.5 umol/L) within 48 hours OR sCr percentage increase >= 50% x baseline, which is known or presumed to have occurred within the prior 7 days
ICA Determining Baseline sCr in Cirrhosis	SCr value obtained in the previous 3 months should be used, when available if multiple sCr values within previous 3 months, value closest to admission sCr should be used. If no previous sCr available, admission sCr serves as baseline value
ICA AKI Staging in Cirrhosis	Stage 1: Increase in sCr >= 0.3 mg/Dl (26.5 umol/L) within 48 hours OR increase in sCr 1.5-2 x baseline
	Stage 2: Increase in sCr 2-3 x baseline
	Stage 3: Increase in sCr > 3 x baseline OR sCr > 4 mg/dl (353.6 umol/L) with an acute rise > 0.5 mg/dl (44 umol/L) OR initiation of RRT
OLD NAME **HRS type 1**	**NEW NAME** **HRS-AKI**
- Doubling of serum creatinine to a concentration >= 2.5 mg/dL within 2 weeks	- Increase In serum creatinine of >= 0.3 mg/Dl within 48 hours OR - Increase in serum creatinine >= 1.5 times from baseline (creatinine value within previous 3 months, when available, may be used as baseline, and value closest to presentation should be use)
- No response to diuretic withdrawal and 2-day fluid challenge with 1 g/kg/day of albumin 20%-25%	- No response to diuretic withdrawal and 2-day fluid challenge with 1 g/kg/day of albumin 20%-25%
- Cirrhosis with ascities	- Cirrhosis with ascities
- Absence of shock	- Absence of shock
- No current or recent use of nephrotoxic drugs (NSAIDs, contrast dye, etc)	- No current or recent use of nephrotoxic drugs (NSAIDs, contrast dye, etc)
No signs of structural kidney injury - Absence of proteinuria (> 500 mg/day) - Absence of hematuria (> 50 RBCs per high power field) - Normal findings on renal ultrasonography	No signs of structural kidney injury - Absence of proteinuria (> 500 mg/day) - Absence of hematuria (> 50 RBCs per high power field) - Normal findings on renal ultrasonography
HRS type 2 - Gradual increase in serum creatinine, not meeting criteria above	HRS-NAKI *HRS-AKD* - Estimated glomerular filtration rate < 60 mL/min/1.73 m^2 for < 3 months in absence of other potential causes of kidney disease. - Percentage increase in serum creatinine < 50% using last available value of outpatient serum creatinine within 3 months baseline value *HRS-CKD* - Estimated glomerular filtration rate < 60 mL/min/1.73 m^2 for >= 3 months in absence of other potential causes of kidney disease

a decrease in systemic resistance caused by vasodilation. This vasodilation, which is the main cause of HRS, is triggered by the overproduction of vasodilator substances (nitric oxide, carbon monoxide and endocannabinoids) and their reduced degradation due to increased portal hypertension and the leaking of these substances into the general circulation. Increased cardiac output, heart rate and the activation of powerful vasoconstrictor systems and the renin-angiotensin-aldosterone system are triggered as compensatory physiological measures. In the same way, the development of liver complications shows that these initially adaptive measures are no longer efficient, causing deterioration of renal blood flow.[15] These consequences are associated with the retention of sodium and free water with the accumulation of ascites and oedema.[16] Later, renal vasoconstriction becomes even more pronounced, eGFR decreases, and SHR may develop. Finally, if extreme renal vasoconstriction is not corrected in time, it may lead to the development of acute tubular necrosis, although this evolution is still controversial.[5,6] (Figure 1).

3.2 | Systemic inflammation

The presence of a systemic inflammatory response syndrome was identified in almost half the patients with HRS-AKI, independently from the presence of infection.[17] Systemic inflammation occurs as a result of increased intestinal permeability which leads to pathological bacterial translocation from the intestine to the systemic circulation, changes in the quantity and quality of microbiome and immune dysfunction associated with cirrhosis.[18]

Bacterial translocation induces a broad spectrum of genes that encode molecules responsible for triggering an inflammatory response through specific receptors called pattern

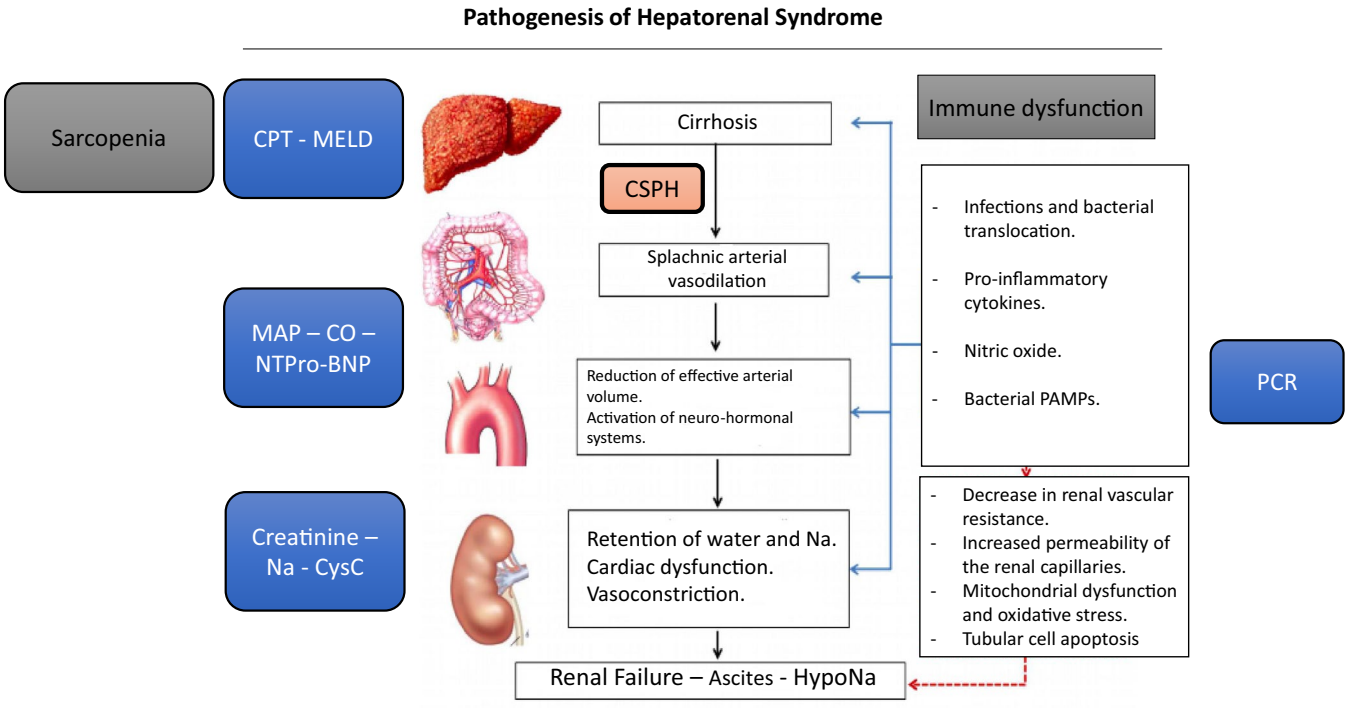

FIGURE 1 Pathophysiology of hepatorenal syndrome

recognition receptors.[19] The Toll-like receptor 4 (TLR4) is the main pattern recognition receptor that has been studied. Tubular TLR4 overexpression has been described in patients with cirrhosis and renal dysfunction.[20] A subset of patients diagnosed with hepatorenal syndrome showed TLR4 overexpression in tubular cells and evidence of tubular cell damage, suggesting an overlap in the pattern of kidney damage and not a pure form of HRS-AKI.[20] The inflammatory components can spread to the systemic circulation and peripheral organs, conditioning the development of dysfunction of extrahepatic organs, including the kidney. Immune dysfunction and changes in systemic inflammation can contribute to systemic circulatory changes associated with the development of HRS. Clear evidence of this situation is represented by high levels of pro-inflammatory cytokines (TNF-a and IL-6).[21] (Figure 1).

3.3 | Cirrhotic cardiomyopathy

Cirrhotic cardiomyopathy (CCM) is a silent condition that is difficult to identify in a stable setting but can become symptomatic during a decompensating event and is clearly involved in the pathophysiology of HRS, mainly because it greatly alters renal perfusion. Recent studies have shown that the presence of CCM, either based on echocardiographic parameters or biomarkers such as NT-proBNP (Diaz JM et AASLD 2020 - Poster number 1846), is directly related to the development of HRS through its dynamics and decreased cardiac output.[22] (Figure 1).

4 | CLINICAL APPLICATION OF KIDNEY BIOMARKERS

Despite the numerous limitations of creatinine as a renal biomarker, it continues to be the most widely accepted parameter worldwide. However, the development of new, more clinically useful, renal biomarkers is promising.[9] One of the main uses of urinary biomarkers is to clarify the aetiology of renal failure, more specifically by differentiating ATN from HRS-AKI. The most extensively investigated biomarker thus far is neutrophil gelatinase-associated lipocalin (N-GAL), which has been shown to be robust in differentiating ATN from HRS-AKI and thus to be useful when deciding on vasoconstrictor therapy.[7] The best diagnostic performance of N-GAL for differentiating ATN has been found at a cut-off of 220 ug/g with approximately 86% of the diagnoses of ATN with values above this threshold, while 88% of those with HRS-AKI and 93% of prerenal-AKI had values below this cut-off.[23,24] (Figure 2A) However, despite its discriminative capacity, this urinary biomarker is not easily accessible in daily practice worldwide, thus, more readily available, simpler tools are needed.

The use of the fractional excretion of sodium (FENa) continues to be useful in differentiating between functional and structural damage. In case of functional damage, the tubules are usually intact, allowing greater Na reabsorption due to renal hypoperfusion. However, circulatory disorders, especially in patients with advanced cirrhosis, could cause chronic renal hypoperfusion and therefore affect the estimated values of FENa < 1%. Despite this, different studies in HRS-AKI have shown that FENa values < 0.2% adequately

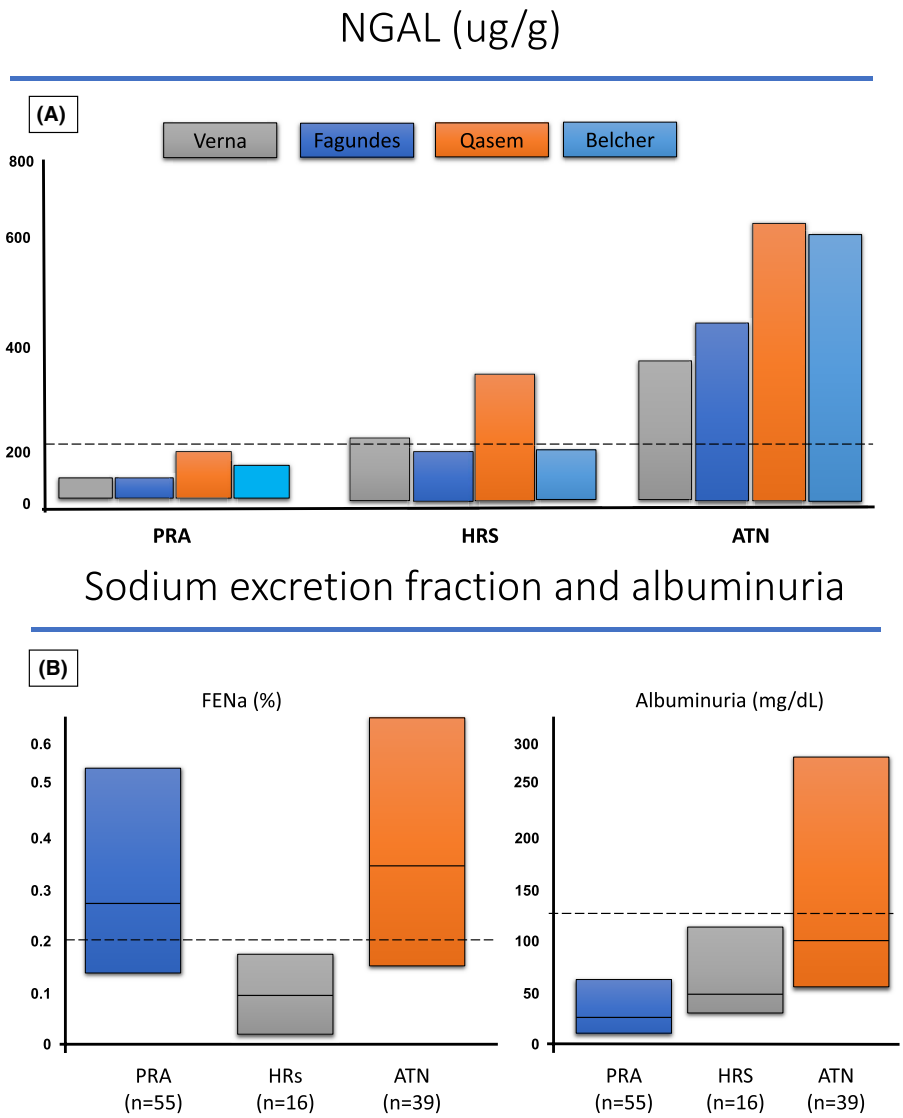

FIGURE 2 Urinary biomarkers in the differential diagnosis of hepatorenal syndrome vs. acute tubular necrosis

differentiate HRS-AKI from ATN.[23,25] (Figure 2B) Moreover, recent studies have shown similar results in the diagnosis of ATN based on high levels of albuminuria.[26]

Finally, the use of serum CysC has become more relevant to identify patients at risk of developing renal events independently of muscle mass or sex, as well as for its predictive value for the development of acute on chronic liver failure (ACLF) and mortality on the waiting list (WL) for LT.[10] Figure 3.

5 | PREVENTION OF HEPATORENAL SYNDROME

5.1 | Prevention of circulatory dysfunction

Numerous predictors have been described for the development of HRS: hyponatremia, high plasma renin activity,[27] the degree of ascites,[28] and elevated CysC values.[29] However, the main factors associated with HRS-AKI are the acute hemodynamic changes associated with infections and large volume paracentesis without albumin administration, while the development of AKI without a clear triggering factor is very rare (1.8%).[28]

The prevalence of HRS-AKI in the presence of spontaneous bacterial peritonitis (SBP) or other bacterial infections is 30% and is a sign of a poorer short-term prognosis.[30-33]

Post-paracentesis circulatory dysfunction occurs after large-volume paracentesis (≥5 L) and is associated with hypotension, hyponatremia, and an increased risk of HRS-AKI. Albumin administration after large-volume paracentesis significantly reduces this risk and improves overall survival in these patients.[1] This protective effect appears to be unique to albumin, compared to other volume expanders, suggesting that albumin has an additional benefit other than as a plasma expander.[34]

Moreover, the development of HRS-AKI can be prevented by the administration of intravenous albumin in addition to the early initiation of effective antibiotic treatment in the presence of SBP (8.3%

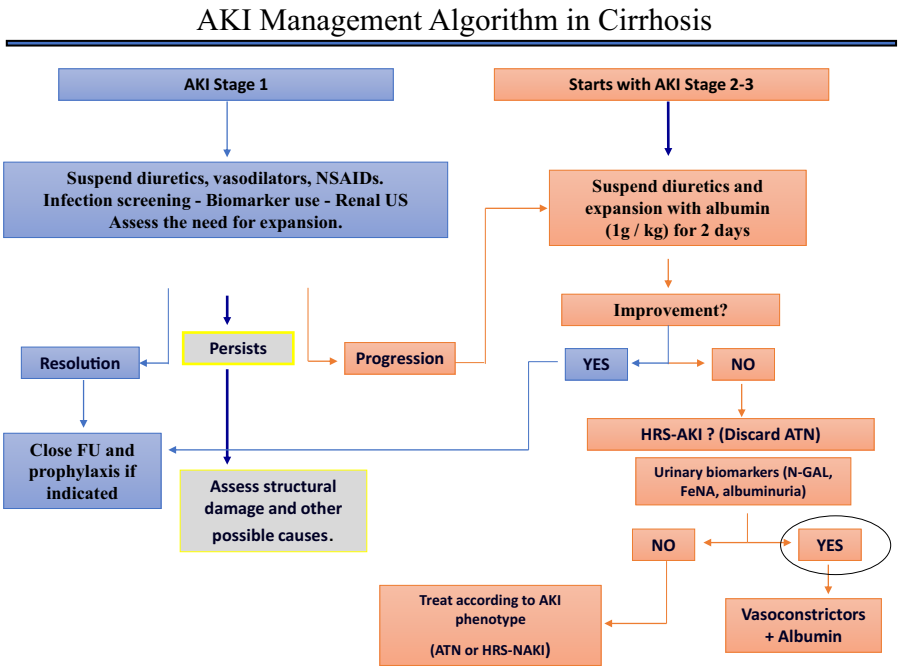

FIGURE 3 Algorithm for management of acute kidney injury in patients with cirrhosis

vs 30.6% with antibiotics alone; $P = .01$), leading to a reduction in overall mortality (16% vs 35.4%; OR: 0.34).[30,35] In contrast, although the administration of albumin to patients with non-SBP infections can improve circulatory function and delay the development of renal dysfunction,[36] it has not been shown to prevent the development of HRS-AKI or improve survival.[37]

The evidence on the prolonged use of albumin as a preventive strategy in decompensated cirrhosis is controversial. This hypothesis has been evaluated in a recent RCT in which weekly albumin administration was added to standard treatment for 18 months and was shown to improve overall survival (77% vs 66%; $P = .028$) as well as to reduce the incidence of HRS-AKI (OR:0.39).[38] In contrast, a similar trial evaluating the long-term use of albumin and midodrine in 196 patients with decompensated cirrhosis on the WL for LT did not show a one-year survival benefit, or any prevention of the complications of cirrhosis.[39] In conclusion, although there is biological plausibility for the use of albumin, future trials such as PRECIOSA12 or ATTIRE trial, are expected to shed light on long-term albumin in this population.

5.2 | Antibiotic prophylaxis

Prophylactic antibiotics to prevent SBP and after gastrointestinal bleeding, have been shown to decrease the incidence of HRS-AKI. The risk of SBP is identified by lower concentrations of protein in ascites fluid (<1.5 mg/dl) associated with liver and/or kidney dysfunction (bilirubin > 3 mg/dl, serum sodium < 130 mEq/L, Child-Pugh score > 10, and/or serum creatinine > 1.2 mg/dl) In these cases antibiotic prophylaxis prevents both the development of SBP as well as significantly reducing the risk of HRS-AKI and overall mortality.[40]

6 | MANAGEMENT AND TREATMENT OF HEPATORENAL SYNDROME

At present, vasoconstrictor agents in combination with albumin are the first-line treatment for HRS-AKI.[41-45] Terlipressin, a vasopressin analogue, is the most commonly prescribed drug. The efficacy of terlipressin plus albumin in the treatment of hepatorenal syndrome has been evaluated in a large number of patients, with a response rate ranging from 25% to 75%. Terlipressin can first be administered intravenously at 0.5-1 mg every 4-6 hours, then gradually increased to a maximum dose of 2 mg every 4 hours. Treatment should be maintained until a complete response is obtained or for a maximum of 14 days. The side effects of terlipressin are related to vasoconstriction, with a risk of myocardial infarction and intestinal or peripheral ischemia.

Continuous infusion of terlipressin at a dose of 2-12 mg/d has been shown to have effects similar to a bolus administration but with lower rates of adverse events in one study.[46] Baseline serum creatinine and the degree of ACLF (the higher the degree, the greater the inflammation) are inversely associated with the response to terlipressin.[43,47] Other vasoconstrictive agents have been proposed in combination with albumin. Although norepinephrine at a dose of 0.5-3 mg/h, IV is an alternative treatment that has been shown to be effective in small studies[48-50] a recent controlled trial suggests that this agent is not as effective as terlipressin in reversing HRS-AKI, renal replacement therapy (RRT)

requirements, or overall survival in ACLF.[45] The combination of midodrine plus octreotide, used in countries where terlipressin is not yet available, has been shown to be less effective than terlipressin in a single-centre study.[41]

7 | IMPLICATIONS OF HRS-AKI TREATMENT IN LIVER TRANSPLANTATION

Although the response to vasoconstrictor therapy plus albumin has clearly been found to be beneficial in restoring renal function, LT is the therapy with the greatest benefit to survival.[51] On one hand, the fact that MELD/MELD-Na includes creatinine as one of its main determinants, means that patients with HRS-AKI are prioritized on the WL for LT. However, responding patients present with a reduction in the MELD and MELD-Na scores, and thus have to wait for a graft about twice as long as those who do not respond, and have a lower possibility of LT in the short term.[52] This issue has been addressed by experts in the field who suggest using the baseline MELD/MELD-Na score (pre-treatment value) for giving priority on the LT WL to responders to terlipressin and albumin. This strategy is reasonable, especially because for any given MELD score value, patients with HRS have shorter expected survival than candidates for LT with chronic liver disease.[12]

Patients responding to terlipressin and albumin present with less severe AKI episodes after LT and less need for RRT than those who do not respond to vasoconstrictor therapy, which lowers post-LT survival rates.[52] The most widely accepted hypothesis on the impact of the lack of response to vasoconstrictor therapy in pre-LT and the consequences after LT, is based on the presence and/or the progression to ATN, where tubular injury markers are frequently higher or which increase over time as HRS-AKI evolves. However, the lack of robust data supporting the hypothesis of a progression from AKI-HRS to AKI-ATN shows the need for additional well-designed studies, possibly with new biomarkers of tubular injury.[5,6]

Finally, although the response to treatment with terlipressin plus albumin reduces the risk of CKD one year after LT in patients with HRS-AKI, strategies are needed to improve prioritization for responders on the WL for LT to prevent long-term kidney damage and thus its impact on post-LT survival.

8 | THE DIFFICULT DECISION BETWEEN LIVER OR SIMULTANEOUS LIVER–KIDNEY TRANSPLANTATION

Predicting the outcome of kidney function after LT is a challenge because it is difficult to accurately evaluate the relative contribution of kidney disease itself, perioperative events, and post-LT immunosuppression on kidney dysfunction after LT.

The presence of AKI before LT has been shown to be associated with a higher risk of long term chronic kidney disease (CKD) after LT as well as an increased risk of mortality.[53]

The treatment of choice for patients with HRS-AKI is liver transplantation, and in case of pure HRS without any other renal disease, kidney function should be fully restored post-LT. However, there are several issues that should be taken into account when deciding on transplantation. First, kidney recovery after LT in patients with HRS-AKI is less probable in the presence of associated ATN. Moreover, this complication is associated with decreased survival. In addition, other intrinsic CKD could also play a role. Thus, the decision to perform SLKT rather than LT alone is based not only on the increased risk of post-LT mortality, but also on the risk that the kidney might not recover.

The decision is clearly not easy. The duration of AKI and dialysis and any evidence of CKD are factors that can help. In the most difficult cases, a (usually transjugular) kidney biopsy should be performed to reach the best decision.

CONFLICT OF INTEREST

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SUPPLEMENT ARTICLE

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Acute-on-chronic liver failure: Where do we stand?

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Abstract

Acute-on-chronic liver failure (ACLF) is defined by the rapid development of organ(s) failure(s) associated with high rates of early (28-day) mortality in patients with cirrhosis. ACLF has been categorized into three grades of increasing severity according to the nature and number of organ failures. In patients with grade 3 ACLF, 28-day mortality is >70%. While the definition of ACLF has been endorsed by European scientific societies, North American and Asian Pacific associations have proposed alternative definitions. A prognostic score called the CLIF-C ACLF score provides a more precise assessment of the prognosis of patients with ACLF. Although bacterial infections and variceal bleeding are common precipitating factors, no precipitating factor can be identified in almost 60% of patients with ACLF. There is increasing evidence that cirrhosis is a condition characterized by a systemic inflammatory state and occult infections or translocation of bacteria or bacterial products from the lumen of the GUT to the systemic circulation which could play a role in the development of ACLF. Simple and readily available variables to predict the occurrence of ACLF in patients with cirrhosis have been identified and high-risk patients need careful management. Whether prolonged administration of statins, rifaximin or albumin can prevent ACLF requires further study. Patients with organ(s) failure(s) may needed to be admitted to the ICU and there should be no hesitation in admitting patients with cirrhosis to the ICU. No benefit to survival was observed with albumin dialysis and rescue transplantation is the best option in the most severe patients. One-year post-transplant survival rates exceeding 70%-75% have been reported, including in patients with grade 3 ACLF but these patients were highly selected. Criteria have been proposed to define futile transplantation (too ill to be transplanted), but these criteria need to be refined to include age, comorbidities and frailty in addition to markers of disease severity.

KEYWORDS

Acute-on-chronic liver failure, cirrhosis, inflammation, liver transplantation, multiple organ failure

Abbreviations: ACLF, Acute-on-chronic liver failure; AKI, Acute kidney injury; APASL, Asian Pacific Association for the Study of the Liver; ATN, acute tubular necrosis; CLIF, Chronic liver failure Consortium; DAMPs, damage-associated molecular patterns; EASL, European Association for the Study of the Liver; HCC, hepatocellular carcinoma; HRS, hepatorenal syndrome; ICU, Intensive Care Unit; INR, International normalized ratio; MELD, the Model for End-Stage Liver Disease; NASCELD, North American Consortium for the Study of End-Stage Liver Disease; PAMPs, pathogen-associated molecular patterns; WBC, white blood cell count.

Key points

- Acute-on-chronic liver failure is characterized by the development of organ failures associated with high early mortality in patients with cirrhosis
- Systemic inflammatory response plays a central role in the development of ACLF
- To date, extracorporeal liver support proved ineffective in the management of ACLF
- Salvage liver transplantation is the best option in the sickest patients but some patients may be too sick to be transplanted

1 | NATURAL HISTORY OF CIRRHOSIS: THE PLACE OF ACUTE-ON-CHRONIC LIVER FAILURE

The natural history of cirrhosis is characterized by a long asymptomatic phase, called compensated cirrhosis.[1,2] Compensated cirrhosis can last for years before the development of complications which is then defined as decompensated cirrhosis. These complications include variceal bleeding, ascites, encephalopathy and bacterial infections, among others. Portal hypertension plays a central role in the transition from compensated to decompensated cirrhosis. While portal hypertension is non-clinically significant (hepatic venous pressure gradient ≤10 mm Hg) in the early phase of compensated cirrhosis, portal pressure increases with disease progression and patients with clinically significant portal hypertension (hepatic venous pressure gradient >10 mm Hg) are more likely to have large oesophageal varices (grade II-IV).[3] These patients are also more likely to present with the complications defining decompensated cirrhosis. Based on a systematic review of the literature, the clinical course of cirrhosis has been summarized into four stages of increasing severity: stages 1 (no varices and no ascites) and 2 (varices and no ascites) corresponding to compensated cirrhosis; and stages 3 (ascites ± varices) and 4 (bleeding ± ascites) corresponding to decompensated cirrhosis.[1] One-year mortality rate ranges from 1% in stage 1 patients to 57% in stage 4 patients. However, the course of cirrhosis varies greatly depending upon age, gender, the cause of the underlying liver disease and the progression of this disease. In parallel, patients with cirrhosis may develop hepatocellular carcinoma (HCC) at a rate of 3%-5% per year, which obviously influences survival. HCC will not be discussed in this review.

Beyond this classic representation of the course of cirrhosis and its complications, which are used to define the different stages of disease, it is well known that certain patients with either compensated or decompensated cirrhosis may suddenly begin to deteriorate with extra-hepatic organ failure(s) and high short-term mortality. Organ/system failures associated with abrupt deterioration include renal failure, circulatory failure, respiratory failure, neurological failure and coagulation failure. With the implementation of the Model for End-Stage Liver Disease (MELD) score to prioritize patients at high risk of early mortality in transplantation[4,5] and the advent of new therapeutic options to manage critically ill patients with cirrhosis,[6,7] there has been renewed interest in better defining the subgroup of patients with rapid deterioration and organ failure(s) and in having a better understanding of the underlying mechanisms of this event. This has resulted in new terminology for this syndrome which has been called acute-on-chronic liver failure (ACLF).

2 | THE COMPLEX DEFINITION OF ACUTE-ON-CHRONIC LIVER FAILURE

2.1 | European definition

To define ACLF as a distinct syndrome, a prospective study was performed in Europe to determine the characteristics of patients with cirrhosis and organ(s) failure with a ≥15% mortality rate at 28 days, with or without prior decompensation.[8,9] This study included 1343 consecutive patients with cirrhosis enrolled in 2011. Liver failure was defined by serum bilirubin (Table 1). Extra-hepatic organ failures included kidney failure defined by serum creatinine, cerebral failure defined by the grade of encephalopathy, coagulation failure defined by international normalized ratio (INR), circulatory failure defined by blood pressure and/or the need for vasopressors and respiratory failure defined by PaO_2/FiO_2 or SpO_2/FiO_2.[8] These definitions were adapted from the SOFA score, which has been extensively used in general intensive care in the last two decades.[10] Patients received 0 to 4 points for each variable of the so-called CLIF (for Chronic Liver Failure Consortium)-SOFA score, according to different thresholds of increasing severity (Table 2). The CLIF-SOFA score ranges from 0 to 24 points, covering a wide spectrum of disease severity. Three grades have been proposed to more easily categorize patients with ACLF.[8] ACLF grade 1 is defined as (i) kidney failure alone, (ii) failure of the liver, coagulation, circulation or respiration alone with serum

TABLE 1 Definition of organ failure according to the CLIF-SOFA score

Organ/system	Failure
Liver	Serum bilirubin ≥ 12mg/dL (204 μmol/L)
Kidney	Serum creatinine ≥ 2 mg/dL (176 μmol/L)
Brain	Grade III-IV encephalopathy[a]
Coagulation	INR ≥ 2.5 or platelet count ≤ 20 x 10^9/L
Circulation	Need for vasopressors[b]
Lung	PaO_2/FiO_2 < 200 or SpO_2/FiO_2 < 214

[a]According to the West-Haven classification.

[b]Including dopamine, terlipressin, epinephrine or norepinephrine.

creatinine ranging from 1.5 to 1.9 mg/dL or mild to moderate encephalopathy or (iii) patients with cerebral failure alone with serum creatinine ranging from 1.5 to 1.9 mg/dL. ACLF grade 2 is defined as failure of two organs. ACLF grade 3 is defined as failure of three or more organs. These grades were defined not only on the basis of the type and number of failed organ(s) but also to identify subgroups of patients with homogeneous rates of 28- and 90-day mortality. In the seminal study that defined ACLF, grades 1, 2 and 3 represented 11%, 8% and 3.5% of patients at enrolment respectively.[8]

2.2 | Limitations of the European definition

Since 2013, the European criteria based on the CLIF-SOFA score have been extensively validated and widely accepted to define ACLF. This definition has been endorsed by the European Association for the Study of the Liver (EASL) and is based on objective and readily available variables as well as clearly defined cut-off values, thus facilitating comparisons between different populations and different studies. However, the European EASL-CLIF definition of ACLF has certain limitations. Liver failure is defined by increased serum bilirubin which is basically inaccurate. Bilirubin may be increased from a number of conditions other than impaired liver function, including a systemic inflammatory response, sepsis and bile duct obstruction. Coagulation failure is defined by increased INR which is questionable in the presence of chronic liver disease because the decrease in coagulation factors is mainly related to impaired liver function. Cerebral failure is essentially defined by different grades of encephalopathy according to the West-Haven classification[11] and it is well known that encephalopathy is a functional disorder that is usually reversible. On one hand, incorporation of encephalopathy improves the accuracy of prognostic scores.[12,13] On the other hand, encephalopathy only represents a single cause of impaired central nervous system function. Kidney failure is defined by serum creatinine >2mg/dL (204 μmol/L), a value which can be considered too high (Table 1). Indeed, it is well known that due to reduced muscle mass and impaired creatinine metabolism, serum creatinine overestimates the glomerular filtration rate in advanced cirrhosis.[14,15] Patients with serum creatinine within the normal range may have a markedly decreased glomerular filtration rate.[16] For instance, according to the most recent EASL recommendations and the International Club of Ascites, acute kidney injury is defined by an increase in serum creatinine ≥0.3 mg/dL (26.5μmol/L) within 48 hours or a percentage increase in serum creatinine ≥50% from baseline within 7 days. [17] Circulatory failure is defined by a need for vasopressors in patients with a mean arterial pressure <70 mm Hg who are unresponsive to fluid administration, which corresponds to the recommendations in general intensive care units (ICU). However, hypotension is common in patients with advanced cirrhosis, and a target of 60-65mHg is generally accepted in patients who receive vasopressors.[6] While all the variables incorporated in the MELD and MELD-Na scores are weighed by a coefficient that reflects each variable's impact on the risk of mortality, the weight of organ failures is equal in the CLIF-SOFA.[18] However, it is reasonable to assume that respiratory failure in cirrhosis is associated with a much higher mortality risk than encephalopathy, which is reversible.

Three grades of increasing severity have been defined to facilitate categorization of patients with ACLF.[8] While grades 2 and 3 are logical (two and three or more organ failures respectively), grade 1 ACLF includes different combinations of organ failure with different threshold values, thus representing a heterogeneous group of patients.

2.3 | Acute-on-chronic liver failure in North America and Asia

Alternative definitions of ACLF have been proposed by North American and Asian Scientific Societies. According to the North

TABLE 2 The CLIF-SOFA score

Organ/System	0	1	2	3	4
Liver (bilirubin, mg/dL)	<1.2	≥ 1.2 to < 2	≥ 2 to < 6	≥ 6 to < 12	≥ 12
Kidney (creatinine, mg/dL)	<1.2	≥ 1.2 to < 2	≥ 2 to < 3.5	≥ 3.5 to < 5 or RRT	≥ 5 or RRT
Cerebral (encephalopathy grade)	No encephalopathy	I	II	III	IV
Coagulation (INR and platelets)	<1.1	≥ 1.1 to < 1.25	≥ 1.25 to < 1.5	≥ 1.5 to < 2.5	≥ 2.5 or platelets ≤ 20 x 10^9/L
Circulation (mean arterial pressure, mm Hg)	≥ 70	< 70	Dopa ≤ 5 or Dobu or Terli	Dopa > 5 or Epi ≤ 0.1 or NEpi ≤ 0.1	Dopa > 15 or Epi > 0.1 or NEpi > 0.1
Lungs					
PaO_2/FiO_2 or	>400	>300 to ≥ 400	>200 to ≥ 300	>100 to ≥ 200	≤100
SpO_2/FiO_2	>512	>357 to ≥ 512	>214 to ≥ 357	>89 to ≥ 214	≤89

Note: RRT denotes renal replacement therapy; Dopa denotes dopamine; Dobu denotes dobutamine; Terli denotes terlipressin; Epi denotes epinephrine; NEpi denotes norepinephrine; PaO_2 denotes partial arterial oxygen pressure; FiO_2 denotes fraction of inspired oxygen; SpO_2 denotes pulse oximetric saturation.

American Consortium for the Study of End-Stage Liver Disease (NASCELD), ACLF is defined by two or more organ failures of the four described. Brain failure is defined as grade 3 or 4 encephalopathy according to the West-Haven classification.[11,19] Renal failure is defined by the need for renal replacement therapy. Respiratory failure is defined by the need for bilevel positive airway pressure or mechanical ventilation. Circulatory failure is defined by the need for vasopressors, mean arterial pressure <60 mm Hg or a reduction of >40 mm Hg in systolic blood pressure from baseline despite adequate fluid resuscitation.[20] The NASCELD-ACLF definition has been validated in the prediction of 30-day mortality in hospitalized patients with cirrhosis, and the higher the number of organ failures, the higher the mortality rate. Mortality rates were higher in patients with than without infection, whatever the number of organ failures.[20]

According to the Asian Pacific Association for the Study of the Liver (APASL), ACLF is defined as an acute hepatic insult manifesting as jaundice (serum bilirubin ≥5mg/dL) and coagulopathy (INR ≥ 1.5) complicated within 4 weeks by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease/cirrhosis, and which is associated with high 28-day mortality.[21] EASL and APASL definitions are very different, which complicates comparisons between European and Asian populations. Of note, the APASL criteria were defined in populations that were mainly patients with hepatitis B virus (HBV) infection while 60% of patients in EASL-CLIF criteria had cirrhosis related to alcohol abuse.[8]

3 | MECHANISMS INVOLVED IN ACUTE-ON-CHRONIC LIVER FAILURE

ACLF is usually multifactorial but systemic inflammation, which plays a central role, is correlated with the severity of the syndrome. Several studies have shown that patients with ACLF have an intense inflammatory response and oxidative stress, a profile that is not observed in decompensated cirrhosis.[22-24] Bacterial infection is the most common precipitating factor of ACLF[23,24] and it was observed in 39% of the European cohort.[8] Alcoholic hepatitis and variceal bleeding may also trigger ACLF. The pattern of systemic inflammation differs according to the precipitating event.[23] Furthermore, it is important to note that 23.3% of patients who develop ACLF did not have previous episodes of decompensation and no precipitating event could be identified in 58.9%. In these patients, occult infections and/or bacterial translocation or bacterial products in the systemic circulation related to portal hypertension may trigger the inflammatory response.[25]

Although the intrinsic mechanisms of ACLF have been clarified, none of them is specific. In patients with sepsis as the precipitating event, the systemic inflammatory response is triggered by recognition of pathogen-associated molecular pattern (PAMPs) by pattern recognition receptors.[26,27] The systemic inflammatory response is exacerbated, resulting in organ damage, cell death and the release of damage-associated molecular patterns (DAMPs) which perpetuate the inflammatory response. In patients without evidence of sepsis, systemic inflammation may be related to the release of DAMPs from injured hepatocytes, but occult infection or bacterial translocation of PAMPs may also play a role.[9] Severe systemic inflammation and oxidative stress result in several changes, including increased NO production by splanchnic vessels, decreased effective arterial blood, which is a characteristic feature of the hyperkinetic state in cirrhosis, immune-mediated tissue damage and mitochondrial dysfunction, resulting in decreased production of ATP. These changes may induce organ failures that define ACLF.[9,28]

Systemic inflammation requires a significant amount of energy. In a recent study, blood metabolome was explored in patients with ACLF and patients with decompensated cirrhosis without ACLF.[29] Thirty-eight metabolites were distinctive of ACLF and represented a fingerprint which increased across ACLF grades. The higher the fingerprint intensity, the higher the release of inflammatory markers (tumour necrosis factor-α, soluble CD206, soluble CD163).[29] The metabolomic profile of ACLF was characterized by increased glycolysis, decreased mitochondrial production of ATP and extra mitochondrial amino acid metabolism producing metabolotoxins. All these changes may play a role in the development of extra-hepatic organ failures.

4 | PROGNOSIS OF ACLF AND PROGNOSTIC SCORES

By definition, the early mortality rate is high in ACLF and mortality increases in parallel with the number of organ failures. In the seminal study that defined ACLF, 28-day mortality rate was 23.3%, 31.3% and 74.5% in ACLF grades 1, 2 and 3 respectively.[8] Ninety-day mortality was 40.8%, 55.2% and 78.4% in ACLF grades 1, 2 and 3 respectively. Independent of the organs/systems criteria that define ACLF, the mortality rate was higher in patients with a high white cell count and/or high C-reactive protein level, highlighting the deleterious role of systemic inflammation.[8] However, the respective role of both the specific type/combinations of organ failures and the severity of each of these individual organ failures on mortality have not been clearly assessed. In addition, only 24% of patients who had ACLF either at enrolment or within 28 days after enrolment were transplanted.[8] Bacterial infections were associated with a worse prognosis in patients with ACLF.[24] In patients with ACLF at admission, organ failures improved in 49.2%, fluctuated without significant improvement in 30.4% and worsened in the remaining 20.4%.[30] There is significant disagreement on the incidence of ACLF depending on the definition. In an independent series of patients with compensated cirrhosis, the incidence of EASL-CLIF ACLF was 20.1/1,000 persons/year compared to 5.7/1,000 person/years for APASL ACLF. In parallel, 28-day mortality was 37.6% for EASL-CLIF ACLF compared to 41.9% for APASL ACLF.[31] The prognostic value of the EASL-CLIF ACLF scoring system has been validated in independent populations.[32]

A simplified scoring system called the CLIF-organ failure score system and a prognostic score have been developed to predict

mortality in patients with ACLF according to the EASL-CLIF criteria (Table 3).[33] Based on this simplified scoring system called 'CLIF-OFs', a continuous prognostic score was derived including two variables with independent prognostic value in addition to those entered in the scoring system: age and white blood cell count (WBC). The so-called 'CLIF-C ACLFs' which ranges from 0 to 100 points read as follows: CLIF-C ACLFs = 10 x ((0.33 x CLIF-OFs) + (0.04 x age) + (0.63 x ln (WBC count)) − 2).[33] There was a linear correlation between the CLIF-C ACLF score and 28-day mortality in the derivation as well as in the validation cohort. However, the predictive value of this score was relatively modest with a c-statistic of only 0.76 for 28-day mortality. [33] The CLIF-C ACLF score was better than the MELD and MELD-Na scores to predict early mortality in this population. The course of ACLF also influences mortality. Independent of the initial ACLF grade, 28-day transplant-free mortality is low to moderate (6%-18%) in patients with a non-severe early course (final no ACLF or ACLF-1) and high to very high (42%-92%) in those with a severe early course (final ACLF-2 or −3). [30]

5 | MANAGEMENT OF ACUTE-ON-CHRONIC LIVER FAILURE

5.1 | General management

Patients with brain, kidney, circulation and/or lung failure should be referred to an intensive care unit. The first step is to identify and to treat the precipitating event. The prevalence of infection is high and patients with suspected bacterial infection should be rapidly administered antibiotics. The choice of the antibiotics depends upon the local practices but large spectrum antibiotics are recommended with de-escalation if a susceptible agent is identified. Fungal infections are uncommon.

Supportive management is needed in patients with organ failures. Acute kidney injury (AKI), which is common, should be treated with volume expansion including albumin.[6] If AKI does not improve with volume expansion and if the patient meets the other criteria for hepatorenal syndrome (HRS)-AKI,[17]intravenous terlipressin is the treatment of choice. Continuous infusion of terlipressin is as effective as intravenous boluses but with fewer adverse events.[34] The response to terlipressin is observed in about 50% of patients. However, the higher the number of organ failures, the lower the probability of response. For instance, the rate of response in grade 3 ACLF is of about 30%.[35] Theoretically, terlipressin is not justified in patients with acute tubular necrosis (ATN). However, it may be difficult to clearly differentiate ATN from HRS-AKI at an early stage and these two entities may represent a continuum.

Circulatory failure justifies volume expansion followed by vasopressors if adequate blood pressure is not restored. Norepinephrine is the most commonly used vasopressor and a target mean blood pressure of 65 mm Hg is generally accepted. Terlipressin is an alternative which was found to be better than norepinephrine in one controlled study including patients with cirrhosis and septic shock.[36] However, this has not been validated in other populations. One study suggests that non-selective beta blockers are protective in ACLF with a significantly lower mortality rate even though the difference is numerically modest.[37] However, once patients develop circulatory failure, beta blockers should be discontinued.

Even in the absence of hypoxaemia, endotracheal intubation and mechanical ventilation may be needed for airway protection in patients with grade 3-4 encephalopathy. Patients should be sedated with short-acting agents such as propofol. Benzodiazepines should be avoided because the metabolism of these agents is usually reduced in cirrhosis, resulting in prolonged sedation. A controlled study has shown that albumin dialysis with MARS® improves severe encephalopathy better than standard therapy outside the context of ACLF.[38] No evidence supports the use of MARS in patients with ACLF and it is still unclear if MARS® is better at improving encephalopathy than conventional haemofiltration in critically ill patients with cirrhosis.

There is still reluctance to admit critically ill patients with cirrhosis to the ICU due to an especially poor prognosis in those with multiple organ failures. However, several surveys performed in the last decade have shown that acceptable survival rates can be achieved in

TABLE 3 The simplified CLIF-organ failure score system (CLIF-OFs)

Organ/system	Subscore = 1	Subscore = 2	Subscore = 3
Liver	Bilirubin < 6mg/dL	Bilirubin ≥ 6 mg/dL	Bilirubin ≥ 12 mg/dL
Kidney	Creatinine < 2 mg/dL	Creatinine ≥ 2 mg/dL and < 3.5 mg/dL	Creatinine ≥ 3.5 mg/dL or RRT[a]
Brain (encephalopathy according to West-Haven score)	Grade 0	Grade 1-2	Grade 3-4 or MV for encephalopathy[b]
Coagulation	INR < 2	INR ≥ 2 and < 2.5	INR ≥ 2.5
Circulatory	MAP[c] ≥ 70 mm Hg	MAP[c] < 70 mm Hg	Use of vasopressors
Respiratory			
PaO_2/FiO_2	>300	≤ 300 and > 200	≤ 200
SPO_2/FIO_2	>357	≤ 357 and > 214	≤ 214

[a]RRT, renal replacement therapy.

[b]MV, mechanical ventilation.

[c]MAP, mean arterial pressure.

patients with cirrhosis admitted to the ICU and this reluctance is no longer justified.[39-44] Practically, a trial of unrestricted intensive care could be proposed to critically ill patients with cirrhosis which would be limited in those with three or more non-haematological failures, 3 days after admission.[39]

5.2 | Extracorporeal liver support

Conventional haemofiltration/haemodialysis devices are designed to remove water, electrolytes and small hydrophilic molecules that accumulate due to markedly decreased or absent glomerular filtration. These devices are highly effective in preventing fluid overload, restoring acid-base balance and removing potentially toxic hydrophilic compounds. However, haemofiltration/haemodialysis is not effective in removing non-hydrophilic compounds that accumulate due to impaired liver function and which may contribute to ACLF. Since these compounds have a high affinity for proteins, systems combining haemofiltration and blood or plasma exchange against a dialysate enriched in albumin have been developed to remove non-hydrophilic molecules such as bilirubin. Importantly, these extracorporeal liver assist devices do not include human or xenogenic liver cells. The most commonly used liver assist devices based on exchanges with albumin are MARS® and Prometheus®.[45-47] Two controlled trials have been conducted with these devices in patients with ACLF, and none of them were shown to improve survival.[48,49]

5.3 | Liver Transplantation

Since the implementation of the MELD or MELD-Na score-based allocation policy in most Western countries, priority is given to the most severely ill patients to minimize waiting list mortality.[4] Patients with ACLF are typically a group at high risk of early mortality who will have significantly improved survival with transplantation. However, salvage transplantation in patients with cirrhosis and organ failure is still a challenge. Because of the scarcity of organs, post-transplant survival should be similar to that of patients with decompensated cirrhosis or HCC, even at the cost of higher post-transplant morbidity and a longer hospital stay.

Several series of liver transplantation in critically ill patients with cirrhosis and/or patients with ACLF from North America and Europe have been published in the last decade.[30,50-57] In the US series from the UNOS/OPTN registry, defining ACLF was difficult due to missing data or missing items. In the absence of details on PaO_2 or SpO_2 at registration or transplantation, mechanical ventilation was considered to be a surrogate marker of respiratory failure which is questionable since patients with normal gas exchange can be put on mechanical ventilation due to encephalopathy.[56,57] Nonetheless, these series showed that good results could be obtained in patients with organ failures and/or ACLF grade 3 with 1- to 5-year survival rates exceeding 70% (Table 4). However, substantial differences could be observed between series due to different definitions and selection criteria. Indeed, important selection criteria must also be taken into consideration. Age, frailty, comorbidities, sepsis and the course of the disease should be taken into account in the decision to transplant ,and all the authors insisted that the patients were highly selected. A recent survey was performed with a multidisciplinary panel of 35 experts from North America and Europe to determine a consensus on which critically ill patients with cirrhosis are too severely ill to be transplanted.[58] The survey was performed according to the Delphi method and the panel recommended delaying transplantation in cases of frailty, persistent fever or less than 72 hours of appropriate antibiotics in patients with sepsis. Respiratory, circulatory and metabolic failure were considered to be the most important organ/system failures to be considered. Finally, it was suggested that respiratory failure with PaO_2/FiO_2<150 mm Hg, norepinephrine dose > 1 μg/kg/min and/or serum lactate level > 9mmol/L could be contraindications for transplantation.[58]

Prioritization for transplantation is also an issue in patients with ACLF. The MELD score is arbitrarily capped at 40 points.[4] However, it has been shown that patients with a calculated MELD score > 40 are at a higher risk of waiting list mortality than those with a MELD score of 40.[59] No difference in post-transplant survival was observed between patients with a MELD score >40 and those with a MELD score of 40, suggesting that an uncapped MELD score would be better than the existing MELD score in the most severely ill patients.[59] However, even an uncapped MELD score is not appropriate in patients with ACLF, as it only takes into account the liver (bilirubin) coagulation (INR) and the kidney (creatinine). A specific score taking into account lungs, circulation and brain is still needed.

6 | PERSPECTIVES

As discussed in previous paragraphs, ACLF is a severe condition with high mortality rates. A first step is to identify patients at a high risk of developing ACLF and to prevent precipitating factors. A multicentre study called PREDICT was recently performed in Europe to explore predictive factors of the development of ACLF 3 months after enrolment. More than 1,000 patients with acute decompensation of cirrhosis were included and three groups were identified: (i) pre-ACLF patients who developed ACLF and had 3-month and 1-year mortality rates of 53.7% and 67.4% respectively, (ii) unstable decompensated patients with cirrhosis who required ≥ 1 readmission but did not develop ACLF and had a 3-month and 1-year mortality rates of 21% and 35.6% respectively and (iii) stable compensated patients with cirrhosis who were not readmitted, did not develop ACLF and had 1-year mortality rate of 9%. [60] In this series of patients with acute decompensation of cirrhosis, the predictive factors of rapid progression to ACLF were age, presence of ascites, white blood cell count, serum albumin, serum bilirubin and serum creatinine at study enrolment. A score called 'CLIF-C ACLF-D' including these variables with their relative weight was created and its prognostic accuracy was better than the CLIF-C AD, MELD, MELD-Na and Child-Pugh scores.[60] This score was highly specific

TABLE 4 Liver transplantation in critically ill cirrhotic patients and/or patients with acute-on-chronic liver failure

Author	Year	Country/region	Population	Post-transplant outcome
Alexopoulos S.[50]	2013	United States	38 patients with MELD score > 40 MV, 13%; RRT, 84%; vasopressors, 34%	One-year survival: 77%
Gustot T. [30]	2015	Europe	25 patients with ACLF MV, 40%; RRT 28%	One-year survival: 75%
Knaak J. [53]	2015	North America	42 ICU patients with MV	One-month survival: 94%
Levesque E. [54]	2017	France	140 with ACLF MV, 21%; RRT, 11%; vasopressors, 17%	ACLF grade 1, one-month survival: 85% ACLF grade 2, one-month survival: 83% ACLF grade 3, one-month survival: 60%
Nekrasov V. [55]	2017	United States	207 patients with MELD score ≥ 40 MV 26%, RRT 57%	One-year survival: 80%
Artru F. [51]	2017	France	73 patients with ACLF grade 3	One-year survival: 80%
Thuluvath PJ. [57]	2018	United States	11,204 patients with organ failures	Five-year survival Two organ failures: 74% Three organ failures: 73% Four organ failures: 67% Five or six organ failures: 67%
Sundaram V. [56]	2019	United States	5,355 patients with ACLF 3	One-year survival: 81.8%
Artzner T. [52]	2020	France	152 patients with ACLF 3	One-year survival: 67.1%

Abbreviations: ACLF, acute-on-chronic liver failure; MV, mechanical ventilation; RRT, renal replacement therapy.

(95%) but had a low sensitivity (38%). Although this score has not been validated in other populations, it may be a useful tool to identify patients with decompensated cirrhosis who are likely to progress to ACLF at an early stage.

Once patients at high risk of developing ACLF have been identified, several preventive measures should be applied. The prevention of variceal bleeding with beta blockers, elastic band ligation or TIPS remains an important step. However, beta blockers can be harmful in patients with end-stage cirrhosis, especially those with refractory ascites as well as in patients with impaired cardiac function.[61,62] In particular, beta blockers may trigger hepatorenal syndrome by decreasing cardiac output.[63] One recent controlled trial has shown that long-term administration of norfloxacin in patients with ascites and low protein concentrations improves survival.[64] Another controlled study has shown that the long-term administration of albumin in patients with uncomplicated cirrhosis also improves survival.[65] Other options including long-term administration of a combination of simvastatin and rifaximin are being explored in multicentre controlled trials.[66] Whether these approaches will be effective in preventing ACLF in the population of high-risk patients will need to be evaluated but the evidence is promising.

Albumin dialysis failed to improve survival in patients with established ACLF.[48,49] Another approach, plasma exchange, has been tested in ACLF, mostly in Asian countries where the criteria for ACLF are different from European criteria.[67] Although results are encouraging, more evidence is needed. A large multicentre European trial is ongoing.

Systemic inflammation is central in the development in ACLF. The pathways involved in systemic inflammation during ACLF have been clarified but are not fully elucidated.[9] In the near future, possible targets to block the inflammatory cascade that results in organ failures may be identified and agents that interact with these targets could be explored.

Finally, as shown in Table 4, good results have been reported with salvage transplantation in patients with life-threatening ACLF, even in those with ACLF grade 3. However, these patients were carefully selected in centres with significant experience in the management of acutely ill patients. One unresolved issue is the futility of treatment in patients who are too ill to be transplanted. Although consensus recommendations have been published,[58] objective markers to define futility still need to be determined.

CONFLICT OF INTEREST

The authors do not have any disclosures to report.

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