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REVIEW ARTICLE

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Hepatitis B: Who to treat? A critical review of international guidelines

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Abstract

Chronic hepatitis B remains a global problem, affecting more than 250 million individuals worldwide. Around one-fifth of infected individuals develop advanced fibrosis or hepatocellular carcinoma (HCC). The World Health Organization (WHO) guidelines as well as the 2016 American Association for the Study of Liver Diseases (AASLD) guidelines are based on robust data and relied on multiple external systematic reviews to answer identified questions. In contrast, the latest guidelines from the European Association for the Study of the Liver (EASL), Asia Pacific Association for the Study of the Liver (APASL) and AASLD (2018 version) were developed by consensus of expert panels. Treatment is generally recommended for individuals at a high risk of disease progression, namely those with high alanine aminotransferase (ALT) levels, active viral replication and advanced fibrosis or cirrhosis. Although guidelines generally agree on treatment indications for special populations, current guidelines do not factor in clinically relevant factors such as age, gender and genotype into the treatment decision process. There is an unmet need for a better predictive model to select high-risk individuals, thus, more high-quality studies are needed.

KEYWORDS

evidence-based practice, GRADE approach, guideline, hepatitis B, chronic, patient selection

1 | INTRODUCTION

Chronic hepatitis B affects an estimated 257 million people worldwide and resulted in 887 000 deaths in 2015 alone.[1] The prevalence of hepatitis B s Antigen (HBsAg) is 3.61%.[2] Twelve to 20% of patients with chronic hepatitis B (CHB) develop cirrhosis. Of these patients, 20%-23% develop decompensated cirrhosis and 6%-15% develop hepatocellular carcinoma.[3] Once cirrhosis is established, the 5-year incidence of hepatocellular carcinoma (HCC) is 10%.[4] Hepatitis B is responsible for around 50% of HCC cases worldwide.[5]

Thus, there is a need to reduce the disease burden created by CHB. Possibly the most effective strategy was the development of the hepatitis B virus (HBV) vaccine. The use of HBV vaccines, introduced in the 1980s, has resulted in a reduction of chronically infected children under the age of five from 4.7% in the prevaccination era to 1.3%. Following the 1992 WHO recommendation for HBV vaccination at birth, HBV birth dose coverage increased to 84% globally in 2015.[1] However, the effectiveness of the HBV vaccination in preventing new cases of CHB does little for chronically infected patients.

Unlike chronic hepatitis C, where the endpoint of sustained virological response is a cure, which is clearly associated with improved clinical outcomes such as survival and HCC, the situation for CHB is less clear. A currently acceptable endpoint is a functional cure, also known as HBsAg loss 24 weeks off-therapy, which was decided at

Abbreviations: AASLD, American Association for the Study of the Liver; ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; CHB, chronic hepatitis B; EASL, European Association for the Study of the Liver; GRADE, Grading of Recommendations Assessment, Development, and Evaluation; HBeAg, hepatitis B e Antigen; HBsAg, hepatitis B S Antigen; HCC, hepatocellular carcinoma; LFTs, liver function tests; NA, nucleo(t)side analogue; PICO, population, intervention, comparison, outcomes; WHO, World Health Organization.

a consensus workshop by the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD). In a meta-analysis, HBsAg loss was associated with improved clinical outcomes such as HCC and survival.[2] However, this endpoint is difficult to achieve with currently existing antiviral therapies. Thus, current guideline goals for therapy are to improve survival and reduce the complications of liver disease such as cirrhosis and HCC. Since achieving the endpoint of HBsAg loss is unlikely in most CHB patients, the goals of therapy need to be based on obtaining sustained viral suppression either with long-term nucleo(t)side analogue (NA) therapy or short-term immunomodulators (pegylated interferon) which may result in 'immune control'.

The natural history of CHB includes different phases that change the risk of developing complications, and consequently the need for therapy. For instance the immune tolerant phase in which younger CHB patients have a high viral load and are hepatitis Be Antigen (HBeAg) positive with normal liver function tests (LFTs) is not generally recommended for therapy,[3] nor are inactive CHB carriers with low levels of virus who are HBeAg negative with normal LFTs and without significant fibrosis,[3] as these two phenotypes are at a lower risk of developing the complications of liver disease or death. Thus, the guidelines recommend treatment in those at risk of disease progression.

2 | GUIDELINE DEVELOPMENT AND RECOMMENDATIONS

The current standard for the development of clinical practice guidelines is the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) working group[6,7] which has been adopted by over 80 organizations worldwide including WHO and the Cochrane Collaboration. The fundamental basis of GRADE is the quality of evidence. GRADE provides a framework for a system that includes a transparent and rigorous methodology for rating the quality of evidence, an explicit balance of the benefits and harms of healthcare interventions, as well as explicit acknowledgement of the values and preferences that underlie the recommendations and whether the intervention represents a wise use of resources. Thus, much of the reliance on evidence is based on performing high-quality systematic reviews and meta-analyses. There are two important components to the guidelines. The first is the strength of the evidence. In the hierarchy of evidence, systematic reviews are the 'gold standard', then there is the strength of the recommendation which is either 'strong' or 'weak'. Weak evidence is not necessarily followed by a weak recommendation. For instance in HIV/HBV co-infection, antiretroviral therapy should be initiated in all those with evidence of severe chronic liver disease, regardless of CD4 count; and in all patients with a CD4 count ≤500 cells/mm^3, regardless of stage of liver disease (strong recommendation, low quality of evidence, WHO guidelines).[3] The framework of recommendations for CHB therapy is therefore based on two important hypotheses. First, there is a greater prognostic risk of increased liver-related complications and mortality in CHB phenotypes, depending, for example on HBeAg status, HBV DNA levels, the extent of liver fibrosis and ALT levels and secondly, that treatment of patients with these risk factors reduces the liver-related disease outcomes. While the evidence confirming the prognostic risk is good, randomized clinical trials showing the therapeutic benefits of antiviral therapy in improving liver-related mortality and outcomes are lacking.

There are four important clinical practice guidelines for CHB: AASLD,[8] EASL,[9] Asia Pacific Association for the Study of the Liver (APASL)[10] and WHO[3] guidelines. The WHO and the 2016 AASLD guidelines[11] were performed according to the GRADE standard, whereas the other two organizations did not perform a systematic evaluation of the evidence to draft the guidelines.

Key points

- Among the existing hepatitis B guidelines, the WHO and 2016 AASLD guidelines were developed based on the validated GRADE approach, which uses multiple systematic reviews to answer pre-identified questions from an expert panel.
- The 2018 AASLD guidance article, EASL and APASL guidelines were developed from consensus-based expert panels.
- Existing guidelines are fairly similar in their recommendations for treatment indications.
- Clinically relevant predictors of advanced disease including gender, age, hepatitis B genotype and family history should be incorporated into the treatment indications and represent a limitation to existing guidelines.

3 | METHODOLOGY OF GUIDELINE DEVELOPMENT

The 2016 version of the AASLD guidelines was developed in accordance with the standards of the Institute of Medicine for practice guidelines using the GRADE approach.[11] The guidelines development committee drafted a list of nine questions that physicians commonly encounter in clinical practice, and numerous systematic reviews were performed to answer these clinical questions. The 2018 AASLD updated guidance article was intended to complement the 2016 guideline and was developed by consensus of an expert panel. Unlike the 2016 AASLD guideline, the 2018 guidance article did not use formal systematic review or the GRADE approach. The 2018 guidance article provided additional recommendations that were not covered in the 2016 guidelines, including treatment of individuals with viral co-infections, acute hepatitis B, recipients of immunosuppressive therapy and transplant recipients. These additional recommendations in the 2018 updated guidance article were not supported by systematic review and did not use the GRADE framework. The grading of evidence and recommendations from

each guideline are summarized in Table 1, and a comparison of the guideline development process is summarized in Table 2.

The WHO guideline was developed using the GRADE framework and standards. There was an initial scoping and planning process to identify questions most relevant to patients with hepatitis B, especially those living in low- to moderate-income countries. These questions were structured in PICO (population, intervention, comparison, outcomes) format and outcomes were identified for each question. Systematic reviews were performed to address the identified PICO questions, and recommendations were presented using the GRADE method.

The 2015 APASL and 2017 EASL guidelines were developed by consensus of an expert panel, based on evidence from existing publications and expert opinions if evidence was not available.

4 | EXISTING GUIDELINES FOR CHB

The guidelines for indications to treat CHB can be divided into indications for treatment of CHB disease, and guidelines for treatment of CHB with co-morbidities or special populations (co-infection with HCV, HIV, HDV; extrahepatic manifestations, pregnancy, liver transplantation, immunosuppression or chemotherapy, acute HBV).

4.1 | Non-cirrhotic CHB

All the guidelines base their recommendations on three parameters: HBV DNA level must be above a designated threshold, ALT levels should be abnormal and there should be evidence of significant histological disease (Table 3). AASLD and APASL use a HBV DNA threshold of 20 000 IU/mL in HBeAg-*positive* non-cirrhotic CHB patients, whereas EASL uses a lower threshold of 2000 IU/mL. Similarly, AASLD and APASL use a threshold of ALT 2× ULN, whereas EASL accepts > ULN. In HBeAg-*negative* CHB patients, AASLD, APASL and EASL guidelines accept a lower threshold for HBV DNA of 2000 IU/mL. AASLD and APASL continue to use the ALT threshold of 2× ULN, whereas EASL accepts > ULN. The APASL recommendation is listed as B1, the AASLD guidance paper as moderate evidence, strong recommendation and EASL as evidence level 1, grade 1 recommendation. AASLD, APASL and EASL continue to recommend that patients with a histological examination showing moderate inflammation and fibrosis, or significant fibrosis should be treated (APASL C1). The WHO uses the same variables of HBV DNA, ALT and fibrosis assessment and state that non-cirrhotic patients over the age of 30 with persistently abnormal ALT and HBV DNA > 20 000 IU/mL are at risk of disease progression, based on large population-based cohort studies. These patients should be recommended for antiviral therapy (WHO strong recommendation, moderate quality of evidence). Unlike the AASLD guidelines, the WHO recommendations do not distinguish HBeAg status, they use the same threshold of DNA > 20 000 IU/mL for treatment in both cases. The ALT upper limits of normal of 30 U/L for men and 19 U/L for women have been endorsed by the WHO and the 2016 AASLD guidelines, although the AASLD updated the threshold to 35 U/L and 25 U/L, respectively, in the 2018 version.

EASL provides an additional recommendation (Evidence level III, grade 2 recommendation) to consider treatment in individuals older than 30, with persistently normal ALT and high HBV DNA levels, regardless of fibrosis stage.[9] However, this is based on a single study with low level evidence and the recommendation is not strong. AASLD does not recommend antiviral therapy for immune tolerant CHB, with moderate evidence and a strong recommendation. AASLD also recommends NAs for CHB patients >40 years old with normal ALT, a viral load >1 000 000 IU/mL and significant necroinflammation or fibrosis (low evidence and conditional recommendation). WHO does not make any recommendations for immune-tolerant CHB individuals older than 30.

EASL allows therapy in CHB patients with a family history of HCC, cirrhosis or extrahepatic manifestations (evidence level III, grade 2 recommendation).

4.2 | Special populations

4.2.1 | Cirrhosis, compensated and decompensated

In compensated cirrhosis, APASL (APASL C2) uses a HBV DNA threshold of >2000 IU/mL to start antiviral therapy, whereas AASLD (low evidence, conditional recommendation), EASL (EASL evidence level 1, grade 1 recommendation) and WHO (strong recommendation, moderate quality of evidence) accept any detectable level of HBV DNA (Table 3). For decompensated cirrhosis, all guidelines (AASLD moderate evidence, strong recommendation; EASL evidence level II, grade 1 recommendation, WHO strong recommendation, moderate quality of evidence) agree that antiviral therapy should be started in the presence of any detectable HBV DNA (APASL A1) (Table 3). HBeAg status and ALT levels should not be a consideration. The WHO recommendation for the treatment of cirrhosis is a strong recommendation, with a moderate quality of evidence.

4.2.2 | HCV, HDV and HIV co-infection

Patients with HCV co-infection are at risk of HBV reactivation and these patients should be regularly monitored. However, regular prophylaxis is not needed and indications to restart therapy are based on the same guidelines as those for HBV mono-infection, and are therefore determined by HBV DNA, ALT levels and the extent of liver inflammation/fibrosis, about which guidelines agree (APASL B1; EASL evidence level 1, grade 1 recommendation) (Table 4). In the presence of reactivation after HCV therapy, WHO also supports the use of NA. Recommendations for co-infected individuals are included in the AASLD 2018 guidance article but not in the 2016 guidelines.

The recommendations in patients with HDV co-infection include pegylated interferon therapy if HDV RNA is elevated with

TABLE 1 Grading of evidence and recommendation

Guideline	AASLD[a, 11]	APASL 2015[10]	EASL 2017[9]	WHO[3]
Quality of evidence	High/moderate quality: RCT Low/very low quality: Observational data	High quality (A): Meta-analysis or randomized trials without important limitations or double-upgraded observational studies. Further research is very unlikely to change our confidence in the estimate of effect Moderate quality (B): Downgraded randomized trials; upgraded observational studies. Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. Low quality (C): Double-downgraded randomized trials; observational studies. Very low quality (C): Triple-downgraded randomized trials; downgraded observational studies; case series/case reports. Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. Any estimate of effect is uncertain	I: Randomized, controlled trials II-1: Controlled trials without randomization II-2: Cohort or case-control analytical studies II-3: Multiple time series, dramatic uncontrolled experiments III: Opinions of respected authorities, descriptive epidemiology	The GRADE system classifies the quality of evidence as high, moderate, low and very low. RCTs are initially rated as high-quality evidence but may be downgraded for several reasons, including the risk of bias, inconsistency of results across studies, indirectness of evidence, imprecision and publication bias. Observational studies are initially rated as low-quality evidence but may be upgraded if the magnitude of the treatment effect is very large, if multiple studies show the same effect, if evidence indicates a dose-response relationship or if all plausible biases would underestimate the effect. The higher the quality of evidence, the more likely a strong recommendation can be made
Strength of recommendation	Strong recommendation: • Population: Most people in this situation would want the recommended course of action and only a small proportion would not. • Health care workers: Most people should receive the recommended course of action. • Policy makers: The recommendation can be adapted as a policy in most situations. Conditional recommendation: • Population: Most people in this situation would want the recommended course of action, but many would not. • Health care workers: Be prepared to help patients make a decision that is consistent with their values using decision aids and shared decision making. • Policy makers: There is a need for substantial debate and involvement of stakeholders	Strong recommendation (1): Factors influencing the strength of the recommendation included the quality of the evidence, presumed patient-important outcomes and cost. Weaker recommendation (2): Variability in preferences and values or greater uncertainty: more likely a weak recommendation is warranted. Recommendation is made with less certainty; higher cost or resource consumption	1: Strong recommendation. Factors influencing the strength of the recommendation included the quality of the evidence, presumed patient-important outcomes, and cost. 2: Weaker recommendation. Variability in preferences and values, or more uncertainty: more likely a weak recommendation is warranted Recommendation is made with less certainty: higher cost or resource consumption	A strong recommendation is one for which the Guidelines Development Group was confident that the desirable effects of adhering to the recommendation outweigh the undesirable effects. A conditional recommendation is one for which the Guidelines Development Group concluded that the desirable effects of adhering to the recommendation probably outweigh the undesirable effects but the Guidelines Development Group is not confident about these trade-offs. The implications of a conditional recommendation are that, although most people or settings would adopt the recommendation, many would not or would do so only under certain conditions. The reasons for making a conditional recommendation include the absence of high-quality evidence, imprecision in outcome estimates, uncertainty regarding how individuals value the outcomes, small benefits and benefits that may not be worth the costs (including the costs of implementing the recommendation)

[a]AASLD 2016 guideline. AASLD 2018 guideline was developed by consensus of an expert panel, without formal systematic review or use of the Grading of Recommendations Assessment, Development, and Evaluation system.

TABLE 2 Comparison of the guideline development processes and scope

Guideline	AASLD[a,11]	APASL 2015[10]	EASL 2017[9]	WHO[3]
Formulation of questions to be answered by guideline	Specific questions were specified a priori for evaluation by the guidelines committee, albeit not presented in a PICO (population, intervention, comparison, outcomes) format	Process not explicitly stated	Process not explicitly stated	An initial scoping and planning process to formulate questions across the continuum of hepatitis B care and treatment and determine patient-important outcomes. These questions were structured in PICO format and patient-important outcomes were identified for each research question. These outcomes were refined and ranked based on their importance for the patient population
Search for evidence	A separate group of AASLD content experts collaborated with an independent research group with expertise in conducting systematic reviews to synthesize the available evidence informing these key questions. By multiple face-to-face meetings, phone conferences and electronic communication, the systematic review group finalized evidence summaries following the GRADE approach	Manuscripts and abstracts of important meetings published through January 2015 have been evaluated. The clinical practice guidelines are based on evidence from existing publications or, if evidence was unavailable, on the experts' personal experience and opinion after deliberations	The CPGs have been based as far as possible on evidence from existing publications, and, if evidence was unavailable, on the experts' personal experience and opinion. Manuscripts and abstracts of important meetings published since the last CPG and prior to December 2016 have been evaluated	Systematic reviews and meta-analyses of the primary literature were commissioned externally to address the research questions and patient-important outcomes. Criteria for inclusion and exclusion of literature (eg study design, sample size, duration of follow-up) for the reviews were based on the evidence needed and available to answer the research questions

[a]AASLD 2016 guideline. AASLD 2018 guidance was developed by consensus of an expert panel, without formal systematic review or use of the Grading of Recommendations Assessment, Development, and Evaluation system.

abnormal ALT levels (EASL evidence level 1, grade 1 recommendation). This is associated with oral antiviral therapy for HBV if HBV DNA levels are elevated (EASL evidence level II-2, grade 1 recommendation). APASL recommends evaluating which virus is dominant and treating with pegylated interferon if indicated (A1) (Table 4). The WHO does not make any recommendations for HDV co-infection because data are too limited to provide firm guidelines.

In patients with HIV co-infection, all guidelines recommend starting HBV therapy regardless of the CD4 count (APASL B1, EASL evidence level II-2, grade 1 recommendation, WHO strong recommendation, low quality of evidence) with drugs that are active against HBV such as tenofovir or a TAF-based regimen that is active against both HBV and HIV (EASL evidence level II-1, grade 1 recommendation).

4.2.3 | Pregnancy

Other than the usual prophylaxis with vaccination and HBIg, mothers with a high viral load, and especially those who are HBeAg positive, are at an increased risk of mother-to-child transmission of HBV. Oral antiviral therapy in the third trimester reduces HBV DNA and decreases the risk of mother-to-child transmission, thus, it is recommended. However, the HBV DNA threshold to start antiviral therapy varies from 4 $\log_{10}$ IU/ml (AASLD low evidence and conditional recommendation; EASL evidence level 1, grade 1 recommendation) to >6 $\log_{10}$ IU/mL (APASL B2) (Table 4). The WHO did not make recommendations for antiviral therapy to reduce mother-to-child transmission because of a lack of evidence when the guidelines were drafted.

4.2.4 | Children

AASLD recommends antiviral therapy in children aged 2-18 years with abnormal ALT levels and HBV DNA > 10^6 IU/mL (AASLD moderate evidence, conditional recommendation) (Table 5). The WHO highlighted the low cure rates with NA and IFN in children as well as concerns over long-term safety and drug resistance, thus suggesting a conservative approach unless the individual has severe disease such as cirrhosis or histological evidence of severe necro-inflammatory disease. WHO notes that entecavir has been approved in children ≥2 years old and tenofovir in children 12 years or older. EASL states that children usually have mild liver disease and therefore recommends considering treatment with caution, although those who fulfil treatment criteria can receive antiviral therapy (EASL evidence level II-2, grade 2 recommendation).

4.2.5 | Liver transplant recipients

HBsAg-positive patients on the waiting list should be treated with NA (EASL evidence level II, grade 1 recommendation) (Table 5).

TABLE 3 Treatment indications for non-cirrhotic and cirrhotic individuals with chronic hepatitis B

Guideline	AASLD 2016 guideline[11]	APASL 2015[10]	EASL 2017[9]	WHO[3]
HbeAg-positive CHB	ALT > 2 ULN or evidence of significant histological disease plus elevated HBV DNA above 20 000 IU/mL (Quality of Evidence: Moderate; strength of Recommendation: Strong). Adults >40 y of age with normal ALT and elevated HBV DNA (>1 000 000 IU/mL) and liver biopsy showing significant necroinflammation or fibrosis (Quality and of Evidence: Very Low; strength of Recommendation: Conditional)	HBV DNA > 20 000 IU/mL and persistent ALT > 2 ULN or significant inflammation/fibrosis. (B1) ALT 1-2X ULN: Biopsy should be considered if non-invasive tests suggest evidence of significant fibrosis, age >35 y, ALT persistently elevated, or there is a family history of HCC or cirrhosis. Treat, if moderate to severe inflammation or significant fibrosis. (B1)	HBV DNA > 2000 IU/mL, ALT > ULN and/or at least moderate liver necroinflammation or fibrosis (Evidence level I, grade of recommendation 1). Adults >30 y with normal ALT and high HBV DNA levels may be treated regardless of the severity of liver histological lesions (Evidence level III, grade of recommendation 2)	Treatment is recommended for adults with CHB who do not have clinical evidence of cirrhosis (or based on APRI score ≤2 in adults), but are aged more than 30 y (in particular), and have persistently abnormal ALT levels and evidence of high-level HBV replication (HBV DNA > 20 000 IU), regardless of HBeAg status (Strong recommendation, moderate quality of evidence)
HbeAg-negative CHB	ALT > 2 ULN or evidence of significant histological disease plus elevated HBV DNA above 2000 IU/mL. ALT 1-2X ULN with significant fibrosis. Persistent ALT > ULN but <2 ULN with HBV DNA > 2000 IU/mL (Quality of Evidence: Moderate; strength of Recommendation: Strong)	HBV DNA > 2000 IU/mL and ALT > 2 ULN or significant inflammation/fibrosis. (B1) ALT 1-2X ULN: Biopsy should be considered if non-invasive tests suggest evidence of significant fibrosis, age >35 y, ALT persistently elevated, or there is a family history of HCC or cirrhosis. Treat, if moderate to severe inflammation or significant fibrosis. (B1)	HBV DNA > 2000 IU/mL, ALT > ULN and/or at least moderate liver necroinflammation or fibrosis (Evidence level I, grade of recommendation 1)	
Cirrhosis	Patients with viraemia (even <2000 IU/mL) should be treated with antiviral therapy (Quality and Certainty of Evidence: Very Low; strength of Recommendation: Conditional)	HBV DNA > 2000 mL for compensated cirrhosis (C2). HBsAg-positive patients with decompensated cirrhosis and detectable HBV DNA require immediate antiviral treatment with NA(s) (A1)	Patients with compensated or decompensated cirrhosis need treatment, with any detectable HBV DNA level and regardless of ALT levels (Evidence level I, grade of recommendation 1)	As a priority, all adults, adolescents and children with CHB and clinical evidence of compensated or decompensated cirrhosis should be treated, regardless of ALT levels, HBeAg status or HBV DNA levels. (Strong recommendation, moderate quality of evidence)

TABLE 4 Treatment indications for co-infected and pregnant individuals with chronic hepatitis B

Guideline	AASLD 2018 guidance[a, 8]	APASL 2015[10]	EASL 2017[9]	WHO[3]
HCV co-infection	HBV treatment is determined by HBV DNA and ALT levels as per the AASLD HBV guidelines for mono-infected patients	Antiviral treatment may be selected using the same criteria as for those patients with mono-infection (A1)	Patients fulfilling the standard criteria for HBV treatment should receive NA treatment (Evidence level II, grade of recommendation 1). HBsAg-positive patients undergoing DAA therapy should be considered for concomitant NA prophylaxis until week 12 post-DAA and monitored closely (Evidence level II-2, grade of recommendation 2)	HBV DNA monitoring is necessary as there is a potential risk of HBV reactivation during treatment or after clearance of HCV, which can be treated with NAs
HDV co-infection	If HBV-DNA levels are elevated, concurrent therapy with NA using preferred drugs (entecavir, TDF, or TAF)	In patients with co-infection of HBV and HDV, it is important to determine which virus is dominant and the patient should be treated accordingly with pegylated interferon alfa for 12-18 mo. Patients should be monitored for 6 mo post-treatment and beyond (A1)	In HDV-HBV co-infected patients with ongoing HBV DNA replication, NA therapy should be considered (Evidence level II-2, grade of recommendation 1)	-
HIV co-infection	Patients who are already receiving effective ARVT that does not include a drug with antiviral activity against HBV should have treatment changed to include TDF or TAF with emtricitabine or lamivudine	Tenofovir combined with emtricitabine or lamivudine plus a third agent active against HIV should be used (A1)	HIV-HBV co-infected patients should be treated with a TDF or TAF-based ART regimen (Evidence level I for TDF, II-1 for TAF, grade of recommendation 1)	In HBV/HIV-co-infected individuals, ART should be initiated in all those with evidence of severe chronic liver disease, regardless of CD4 count; and in all those with a CD4 count ≤500 cells/mm^3, regardless of stage of liver disease (Strong recommendation, low quality of evidence)
Pregnancy	HBV DNA level >200 000 IU/mL at 28-32 wk of gestation (Quality of Evidence: Low; strength of Recommendation: Conditional).	HBV DNA above 6-7 log10 IU/mL from week 28-32 of gestation. NAs can be administered after discussion with the patient, even in patients with lower DNA levels (B2)	HBV DNA levels 200 000 IU/mL or HBsAg levels [4 log10 IU/mL, antiviral prophylaxis with TDF should start at week 24-28 of gestation (Evidence level 1, grade of recommendation 1)	—

[a]AASLD 2018 guidance was developed by consensus of an expert panel, without formal systematic review or use of the Grading of Recommendations Assessment, Development, and Evaluation system.

TABLE 5 Treatment indications for special populations with Hepatitis B

Guideline	AASLD[8,11]	APASL 2015[10]	EASL 2017[9]	WHO[3]
Children	Antiviral therapy in chronic hepatitis B (CHB) HBeAg-positive children (ages 2-<18 y) with both elevated ALT and measurable HBV-DNA levels, with the goal of achieving sustained HBeAg seroconversion. (Quality and Certainty of Evidence: Moderate; strength of Recommendation: Conditional)	Patients with moderate to severe activity or significant fibrosis with any ALT level should be considered for treatment (A1). Treatment may be started in precirrhotic chronic HBV-infected patients if they have persistently elevated ALT levels [2 times upper limit of normal (ULN) (at least 1 mo between observations) and HBV DNA [20 000 IU/mL if they are HBeAg-positive and [2000 IU/mL if HBeAg-negative, even without a liver biopsy (B1)	In children or adolescents who meet treatment criteria, ETV, TDF, TAF and Peg IFNa can be used in this population (Evidence level II-2, grade of recommendation 2)	Children with CHB and clinical evidence of compensated or decompensated cirrhosis should be treated, regardless of ALT levels, HBeAg status or HBV DNA levels. (Strong recommendation, moderate quality of evidence) The FDA has approved tenofovir for use in adolescents and children above the age of 12 y for HBV treatment (and 3 y or older for HIV treatment). FDA has approved entecavir for children with CHB above 2 y of age
Liver transplant recipients	HBsAg-positive patients undergoing liver transplantation should receive prophylactic therapy with NAs ± HBIG[a]. Patients who receive HBsAg-negative but anti-HBc-positive grafts should receive long-term NAs[a]	Among low risk patients (ie with undetectable HBV DNA levels at the time of transplant), HBIG free regimens can be used. High potency NAs (entecavir or tenofovir) should be used for life (B1)	NA ± HBIG is recommended after liver transplantation (Evidence level II-1, grade of recommendation 1). HBsAg-negative patients receiving livers from donors with evidence of past HBV infection (anti-HBc positive) are at risk of HBV recurrence and should receive antiviral prophylaxis with a NA (Evidence level II-2, grade of recommendation 1)	—
Recipients of immunosuppressive/ cytotoxic therapy	HBsAg-positive patients should initiate anti-HBV prophylaxis before immunosuppressive or cytotoxic therapy[a]. HBsAg-negative, anti-HBc-positive patients receiving anti-CD20 antibody therapy or undergoing stem cell transplantation should be treated with NAs[a]	Prophylactic antiviral therapy should be given to HBsAg-positive cancer patients who receive cytotoxic or immunosuppressive therapy (A1). Physicians should be aware of the risk of HBV reactivation in HbsAg-negative, HBc-positive patients receiving rituximab (B1)	All HBsAg-positive patients should receive ETV or TDF or TAF as treatment or prophylaxis (Evidence level II-2, grade of recommendation 1). HBsAg-negative, anti-HBc-positive subjects should receive anti-HBV prophylaxis if they are at high risk of HBV reactivation (Evidence level II-2, grade of recommendation 1)	—
Acute Hepatitis B	Only indicated for those patients with acute liver failure or who have a protracted, severe course, as indicated by total bilirubin >3 mg/dL international normalized ratio >1.5, encephalopathy, or ascites[a]	Treatment is only indicated for patients with fulminant hepatitis B or for those with severe or protracted acute hepatitis B (C2)	Only patients with severe acute hepatitis B, characterized by coagulopathy or protracted course, should be treated with NA (Evidence level II-2, grade of recommendation 1)	Persons with fulminant or severe acute hepatitis may benefit from NA therapy with entecavir or tenofovir, to improve survival and reduce the risk of recurrent hepatitis B

(Continues)

TABLE 5 (Continued)

Guideline	AASLD[8,11]	APASL 2015[10]	EASL 2017[9]	WHO[3]
Extrahepatic manifestations	Indication for treatment independent of liver disease severity[a]	HBsAg-positive patients with extrahepatic manifestations and active HBV replication may respond to antiviral therapy (B1)	Patients with replicative HBV infection and extrahepatic manifestations should receive antiviral treatment with NA (Evidence level II-2, grade of recommendation 1)	HBsAg-positive persons with HBV-related extrahepatic manifestations and active HBV replication may respond to NA antiviral therapy
Family history of HCC	Consider treating in patients with a family history of HCC or cirrhosis, even if ALT < 2 ULN and HBV DNA below threshold[a]	Liver biopsy if patient does not reach the ALT or HBV DNA threshold for treatment. Treat if moderate to severe inflammation or significant fibrosis. (C1)	Patients with family history of HCC or cirrhosis can be treated even if typical treatment indications are not fulfilled (Evidence level III, grade of recommendation 2)	—

[a]AASLD 2018 guidance was developed by consensus of an expert panel, without formal systematic review or use of the Grading of Recommendations Assessment, Development, and Evaluation system.

HBsAg-positive recipients should receive NA + HBIg to prevent HBV reactivation in the setting of immune suppression (EASL evidence level II-1, grade of recommendation 1). However, APASL recommends the use of high potency NA alone in HBV DNA-negative individuals (APASL B1), whereas the use of HBIg is recommended for 1 year and NA continued in patients with positive HBV DNA at liver transplant. WHO and the AASLD 2016 guidelines do not provide recommendations for treatment of CHB in liver transplantation, whereas the 2018 AASLD guidance article recommends NA with or without HBIG for HbsAg-positive recipients, depending on patient and virological factors.

AASLD and EASL guidelines (EASL evidence level II-2, grade 1 recommendation) recommend the use of long-term NA to prevent HBV recurrence in HBsAg-negative patients receiving livers from anti-HBc-positive donors, but there are no guidelines from APASL.

4.2.6 | Immunosuppression/chemotherapy

AASLD 2018 guidance, EASL and APASL agree that HBsAg-positive patients receiving immunosuppressive therapy or cytotoxic therapy should receive prophylactic NA (APASL A1; EASL evidence level II-2, grade 1 recommendation) (Table 5).

AASLD specifies that anti-HBc-positive patients receiving anti-CD20 therapy or stem cell transplant should receive NA prophylaxis, whereas APASL (APASL B1) recommends NA prophylaxis in patients receiving anti-CD20 therapy. EASL only recommends antiviral prophylaxis if they are at high risk of HBV reactivation (EASL evidence level II-2, grade 1 recommendation). WHO and AASLD 2016 guidelines does not provide a specific recommendation for individuals undergoing immunosuppressive treatment.

4.2.7 | Acute hepatitis B

All guidelines agree that patients with acute liver failure should receive antiviral therapy, and AASLD 2018 guidance and APASL (APASL C2; EASL evidence level II-2, grade 1 recommendation) also include patients who have protracted severe acute hepatitis B (Table 5). WHO also support the use of antiviral therapy in fulminant or severe acute hepatitis.

4.2.8 | Extrahepatic manifestations

HBsAg-positive patients with extrahepatic manifestations and active viral replication may respond to antiviral therapy (APASL B1). The AASLD 2016 guideline highlights that the presence of extrahepatic manifestations provides an indication for treatment independent of liver disease severity, whereas WHO mentions that extrahepatic manifestations such as glomerulonephritis and vasculitis may be indications for treatment.

4.2.9 | Before or after locoregional or curative surgery for HCC

HBsAg-positive patients with HBV-related HCC should have antiviral prophylaxis before or after treatment for HCC if they have a detectable viral load (APSAL B2).

5 | MODELS OF INDICATIONS FOR TREATMENT

The use of risk variables such as viral load, ALT and liver fibrosis to determine the eligibility for treatment is incomplete, confusing and outdated. It is clear from prognostic studies such as REVEAL[12,13] and the HCC risk calculator[14] that high-risk variables include age, gender, ALT level, a family history of HCC, HBeAg status, genotype and HBV DNA levels. The limitation of this study is that it was performed in Asia thus these data must be confirmed in a Western population. Moreover, it mainly included patients over the age of 40 and therefore may not reflect immunotolerant phases. Finally, cirrhosis was excluded, thus this important variable was not represented. These variables were distilled into a risk calculator which predicted the 5- and 10-year risk of HCC, but a similar extrapolation could be made for cirrhosis. Risk calculators are extensively used in clinical diseases in particular the cardiac risk calculator which uses age, gender, ethnicity, cholesterol, systolic blood pressure, diabetes and smoking to calculate the risk of developing heart disease. Risk calculators provide information about the prognostic risk and randomized controlled trials are needed to determine how to translate this risk to reduce disease progression, outcomes and complications. However, the modifiable factors in the REVEAL risk calculator have not been tested in this context and cannot be recommended in guidelines since they are not supported by evidence. For instance a male >50 years who has ALT < 45 and HBV DNA of 20 000 IU/mL without liver fibrosis is not currently eligible for treatment but has a REVEAL risk score of 12- and a 10-year risk of developing HCC of 13.4%. Whether antiviral therapy reduces this outcome is unproven and would require a large study, including a few thousand participants with a high REVEAL risk score, monitored for at least 5 years to ensure that outcomes can be obtained. Support would be needed from both industry and government agencies and it is probably the most pressing unmet need today on this topic. This study would simplify indications for therapy and provide a more precise estimate of risk and risk improvement.

6 | CONCLUSIONS

Current indications for treatment of CHB are based on viral load, ALT levels, HBeAg status and the extent of liver fibrosis with certain differences among the different institutional organizations. However, the most well constructed guidelines were drafted by WHO based on a thorough examination of the evidence and systematic reviews of available evidence. Most existing guidelines are in agreement, with minor differences. However, the current indications are somewhat outdated and although the use of a risk calculator should be adopted, the studies to support this initiative are lacking.

CONFLICT OF INTEREST

Seng Gee Lim: Advisory Board: Gilead Sciences, Springbank, Roche, Abbvie, Abbott, Kaleido; Speakers Bureau: Gilead Sciences, Abbott, Roche; Research support: Abbott, Merck Sharpe and Dohme, Roche, Gilead Sciences. Daniel Huang has no conflicts of interest to declare.

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REVIEW ARTICLE

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Optimal management of chronic hepatitis B patients receiving nucleos(t)ide analogues

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Abstract

Management of chronic hepatitis B (CHB) is still considered a challenge in clinical practice. Patients must be carefully evaluated before starting therapy. This includes virology and laboratory assessments, an estimation of fibrosis by invasive and/or noninvasive methods, and an estimation of the risk of hepatocellular carcinoma (HCC). Nucleos(t)ide analogues (NAs) with a high barrier to resistance (tenofovir disoproxil fumarate [TDF], entecavir [ETV] and tenofovir alafenamide [TAF]) are the most frequently used treatments because of their good long-term efficacy and tolerability. None of these options has been shown to be more effective than the other, but certain factors should be considered when selecting the best therapy for specific populations. Most patients achieve a virological and biochemical response to these agents, with a low rate of emerging resistance during long-term treatment. However, the rate of hepatitis B surface antigen (HBsAg) loss is low and in most cases NAs therapy is lifelong. Safety concerns for long-term NA use have become a priority in the management of CHB, in particular, the risk of impaired kidney function and bone marrow density loss described with TDF regimens. The risk of HCC is not completely eliminated by NAs. Thus, patients at higher risk should be identified and provided with appropriate surveillance.

KEYWORDS

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

1 | INTRODUCTION

According to the World Health Organization (WHO), an estimated 257 million people are chronically infected with hepatitis B virus (HBV) worldwide, with a prevalence of 0.7% in non-endemic areas and up to 8.5% in highly endemic regions (West Africa and East Asia). Overall, only 10% of these individuals have access to care and treatment, which means that even with the ongoing interventions, an estimated 63 million new cases of HBV and 17 million HBV-related deaths will occur between 2015 and 2030.[1]

Strategies to control HBV infection are effective, affordable and available. Thus, elimination of hepatitis B, defined as a 90% reduction in the incidence of this infection and a 65% decrease in related deaths, is the goal advocated by the WHO for 2030.[1]

Following acute HBV infection, the disease becomes chronic in approximately 90% of newborns and 5%-10% of adults, depending on their different immunological capacities and host-virus interactions. Untreated chronic hepatitis B (CHB) is usually a progressive disease that leads to cirrhosis in up to 40% of cases. Hepatocellular carcinoma (HCC) usually develops in HBV patients with cirrhosis, although it can also occur in the absence of significant fibrosis because

Abbreviations: ALT, alanine-aminotransferase; APRI, aminotransferase-platelet ratio index; BMD, bone marrow density; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; DAA, direct active antivirals; eGFR, estimated glomerular filtrate rate; ESLD, end-stage liver disease; ETV, entecavir; FIB-4, fibrosis score based on four factors; HAART, highly active antiretroviral therapy; HBcrAg, hepatitis B core-related Antigen; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; LAM, lamivudine; mPAGE-B, modified PAGE-B score; NA, nucleos(t)ide analogues; qHBsAg, quantitative HBsAg; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; WHO, World Health Organization.

of the genomic integration of viral DNA and activation of molecular pathways.

The main goal of current HBV therapies is to reduce the development of fibrosis and HCC, which results in longer survival and better life quality for patients. Nucleos(t)ide analogues (NAs) with a high barrier to resistance such as tenofovir disoproxil-fumarate (TDF), entecavir (ETV) and tenofovir alafenamide (TAF) have good safety profiles and are effective for suppressing viral replication. However, a functional cure, defined as the loss of hepatitis B surface antigen (HBsAg) with undetectable serum HBV-DNA, occurs in only 5%-10% of patients receiving NAs. Thus, most patients require lifelong treatment.[2] A virological cure (elimination of viral DNA from liver cells) cannot be attained with NA therapy because a reservoir of covalently closed circular DNA (cccDNA) remains in the cell nucleus.

The purpose of this review is to define the optimal management of CHB patients receiving NAs. It first requires a complete initial evaluation, including the identification of those at high risk of developing HCC. The best analogue for each individual case is then chosen and the safety of the treatment and patient's response is appropriately monitored.

Key points

- The aim of CHB treatment is to prevent the development of cirrhosis, liver decompensation and HCC.
- TDF, ETV and TAF are the recommended NAs due to their high long-term efficacy and tolerability.
- HBsAg loss is rare with all NA regimens.
- Long-term TDF treatment has been related to kidney and bone side effects; TAF and ETV are preferred in patients with risk criteria.
- HCC risk is not completely suppressed with NA treatment. HCC surveillance is needed.

2 | INITIAL EVALUATION

The initial management of CHB starts with a complete clinical history and evaluation is summarised in Figure 1.[2] The HBV markers tested should include hepatitis B envelope antigen (HBeAg), anti-HBeAb and HBV-DNA. The phase of infection can be confirmed with these markers together with alanine-aminotransferase (ALT) levels, although more than one determination may be needed because of the dynamic nature of HBV infection. HBV genotyping is not needed in patients who are candidates for NA treatment. Ruling out hepatitis C and D viruses (HCV, HDV), and HIV coinfection is essential. Evidence of liver inflammation has been traditionally based on the persistent or intermittent elevation of ALT levels or the presence of histopathological changes in liver biopsy specimens (METAVIR ≥ F2, Ishak ≥ 3). Several HBV biomarkers such as quantitative HBsAg (qHBsAg), HBV-RNA and hepatitis B core-related antigen (HBcrAg) have potential implications in the natural history and diagnosis of the infection and in monitoring the response to therapy. The largest experience is with qHBsAg, a commercially available test that has been shown to be useful for classifying the various phases of infection.[3] HBsAg levels are higher in HBeAg-positive than HBeAg-negative patients and in HBV A and E genotypes.[3]

Liver biopsy is still considered the gold standard for assessing necroinflammation and fibrosis. Nevertheless, the invasive nature of this procedure limits its indications. Noninvasive methods can be a useful substitute for liver biopsy in individuals who do not fulfil the treatment criteria. Transient elastography is one of the most frequently used noninvasive procedures for this purpose. It has been shown to be accurate and easily reproducible, but is more useful for ruling out cirrhosis than detecting intermediate stages of fibrosis. In CHB patients, the cut-offs associated with significant fibrosis have been defined as between 9 and 13 kPa.[2] Higher liver stiffness values are related to a greater risk of HCC. Liver stiffness values should be interpreted with caution in patients with elevated ALT levels because they can be misleading high.

Serum biomarkers can also be used. The aminotransferase-platelet ratio index (APRI) and fibrosis score based on four factors (FIB-4) are the most widely used laboratory tests to estimate fibrosis as they include routine laboratory parameters and do not require additional cost. However, their utility has been less extensively evaluated in CHB than in chronic hepatitis C. A recent study in CHB patients evaluated FIB-4 and APRI in relation to liver biopsy findings. The authors reported a new cut-off of (<0.7) for the FIB-4 to exclude cirrhosis in patients >30 years old, with a sensitivity of 90.9% and a negative predictive value of 96.6%. Furthermore, FIB-4 was more accurate than APRI for diagnosing cirrhosis in CHB patients.[4]

3 | CHOICE OF TREATMENT WITH NUCLEOS(T)IDE ANALOGUES

Highly potent NAs (TDF, ETV and TAF) have replaced low-barrier NAs (lamivudine [LAM], adefovir disoproxil and telbivudine) and are currently the recommended first-line monotherapy regimens.[2] These agents strongly inhibit the HBV polymerase, suppressing viral replication. The most recently developed NA, TAF, is a TDF pro-drug that shares the same mechanism of action as TDF with improved bioavailability.[5]

Both ETV and TDF have been shown to be more effective than low- and intermediate-barrier NAs in phase III trials and real-life studies in CHB patients with or without compensated cirrhosis.[2] No significant differences in antiviral efficacy were detected between these two agents.[6] TDF and ETV therapy leads to high rates of HBV-DNA suppression (94%-99% viral suppression with up to 10 years' follow-up) in both HBeAg-positive and -negative patients.[7,8] However, HBsAg loss is infrequent, with annual rates of <1%.[7] HBeAg loss in HBeAg-positive individuals under long-term treatment with either TDF or ETV has been reported to be between 49% and 53%.[2,7] A biochemical response (ALT normalisation) was

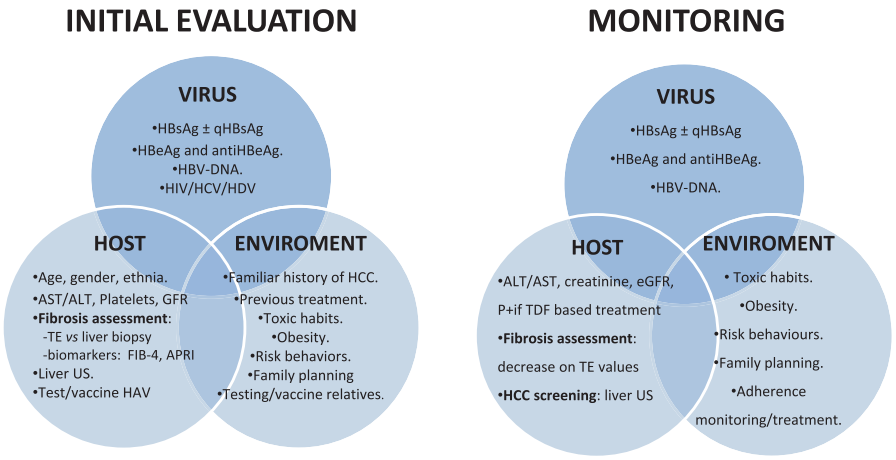

FIGURE 1 A, Initial evaluation required in CHB patients. B, Evaluation required in the monitoring of CHB patients under NA *Virological response* refers to HBV-DNA suppression (<10 IU/mL). HBV-DNA must be performed every 3 months after the beginning of therapy to exclude *primary non response*. *Serological response* includes HBsAg loss and HBeAg seroconversion. HBsAg must be performed yearly and HBeAg/Anti-HBeAg should be done every 6 months in HBeAg positive patients. *Biochemical response* is defined by ALT normalisation (<40 IU/mL). Liver enzymes and liver function tests should be performed every 3 months at the beginning of treatment and every 6 months thereafter. ALT, alanine aminotransferase; AST, aspartate aminotransferase; APRI, aminotransferase-platelet ratio index; eGFR, estimated glomerular filtrate rate; FIB-4, fibrosis score based on four factors; HAV, hepatitis A virus; (anti)HBeAg, (anti) hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; HBV-DNA, hepatitis B virus DNA; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; P+, serum phosphate; qHBsAg, quantitative hepatitis B surface antigen; TE, transient elastography; US, ultrasound

achieved in 77%-83% of CHB patients according to the HBeAg status after 10 years of TDF treatment.[7]

Long-term studies on TDF and ETV have reported the regression of fibrosis and cirrhosis and benefits in liver function.[9] Signs suggesting the regression of portal hypertension (small oesophageal varices) have also been observed after long-term viral suppression with NAs in HBeAg-negative patients.[10] The progression of liver disease decreases with NA regimens, preventing liver decompensation and lowering the risk of HCC in patients with cirrhosis, although the risk of HCC does not completely disappear.[11] A large nationwide observational study including 24 156 CHB patients receiving either ETV or TDF has suggested that individuals receiving TDF have a significantly lower risk of developing HCC possibly due to a potential carcinogenic effect of ETV in animals.[12] A closer analysis showed statistical errors that led to the misinterpretation of overall mortality and transplantation rates. After correction for these errors, the risk of HCC was significantly lower in TDF than in ETV regimens (HR 0.68; 95% CI, 0.59-0.77).[12] Later studies have not found any difference in the incidence of HCC, mortality, or liver-related complications in patients receiving these drugs.[13] Moreover, a recent meta-analysis including only retrospective studies showed a significantly lower incidence of HCC in patients receiving TDF regimens (rate ratio 0.66, 95% CI 0.49, 0.89)[14] with no differences in mortality or the incidence of transplantation.[14] Additional clearer data are needed to determine whether there is a difference in HCC risk between TDF and ETV. TAF has shown non-inferiority for viral suppression compared to TDF, with higher ALT normalisation rates at 96 weeks of treatment, although long-term treatment studies are still lacking.[15]

Table 1 summarises the recommended drugs in the different HBV populations.

3.1 | Treatment of specific patient populations

3.1.1 | Patients with decompensated cirrhosis

The goal of treatment in patients with end-stage liver disease (ESLD) is to improve the outcome of liver disease and survival and avoid the need for liver transplantation by the suppression of viral replication.[6] Because IFN is contraindicated, a potent high-barrier NA is the only acceptable therapeutic option. TDF and ETV have been shown to be effective in decreasing the MELD score and reducing transplantation, but ETV must be given at higher doses than usual. Although kidney impairment is a common concern because of the potential nephrotoxicity of TDF and ETV, and the compromised kidney status in patients with ESLD, there is insufficient evidence to recommend TAF in this group of patients. No significant differences in the incidence of kidney failure have been observed between patients receiving ETV or TDF.[16] Close follow-up in specialised units and assessment for liver transplantation is recommended in all of these patients.

3.1.2 | Patients with hepatocellular carcinoma

Reactivation of HBV replication has been documented after the use of various approaches to cure HCC in HBV patients and high

TABLE 1 Recommended antiviral drug and dosage in HBV-infected especial populations

	Tenofovir disoproxil fumarate (245 mg)	Tenofovir alafenamide (25 mg)	Entecavir (0.5 mg)
Decompensated cirrhosis	✓	✖ Not enough evidence.	✓! Increased usual dose (1 mg/d)
Kidney function impairment	Dose adjustment if eGFR <50 mL/min	✓ Not enough evidence for CrCl <15 mL/min and hemodyalisis	✓ Dose adjustment if eGFR <50 mL/min
HIV coinfection	✓ As part of HAART	✓ As part of HAART	✖ Not in monotherapy
Chronic hepatitis Delta	✓	✓	✓
Children	✓ Children aged >2 y	✓! Children aged >12 y	✓ (Children aged >2 y)
Women planning family and pregnancy	✓	✓	Not recommended

Abbreviations: CrCl, creatinine clearance; eGFR, estimated glomerular filtrate rate; HAART, highly active antiretroviral therapy.

HBV-DNA levels have been related to a poor outcome in this population.[2] Antiviral therapy with NAs leads to improvement in overall survival and reduces the recurrence of HCC but it has no effect on controlling extra-hepatic disease. Regression of portal hypertension has been associated with NA treatment and this could be a preoperative requirement for a curative option in some HBV-related HCC patients.[10] Some studies have also shown that NAs have a favourable impact on the postoperative prognosis of CHB-related HCC.[2]

3.1.3 | Patients with impaired kidney function

TDF, ETV and TAF are secreted by the kidney, and TDF and ETV require dose adjustments in relation to the estimated glomerular filtrate rate (eGFR). Conditions such as arterial hypertension, diabetes mellitus and nephrotoxic treatments must also be assessed and controlled. TDF and ETV are not contraindicated in patients with underlying kidney disease, but patients should be closely monitored because of a higher associated risk of adverse effects. TAF reduces TDF plasma levels and systemic exposure by up to 90% resulting in a better safety profile.[5,15]

3.1.4 | Women of childbearing age and family planning

Women who plan to conceive in the near future are only candidates for NA treatment when there is significant fibrosis or cirrhosis. Telbivudine and TDF are the only safe options during pregnancy and TDF is the preferred drug.[2] No relevant side effects have been reported during long-term treatment in the mother or the fetus during pregnancy in women with HIV and some studies have found that there are no side effects with TDF during breastfeeding. Treatment should be continued in women already receiving NA therapy if there is advanced fibrosis or cirrhosis and the patient is receiving TDF. A switch to TDF monotherapy is recommended if women are receiving ETV or another NA.[2]

3.1.5 | Patients with HIV and HBV coinfection

Liver-related deaths and overall mortality are reported to be significantly higher in patients with HIV-HBV coinfection than in those with either HBV or HIV monoinfection. The incidence of HCC is higher in coinfected patients and is associated with HBV-DNA levels and a lower $CD4^+$ cell count.[17] TDF and TAF have shown to have anti-HIV activity amongst high-barrier NAs. Thus, one of these agents must be included as a part of highly active antiretroviral therapy (HAART).[2] Lower HBV-DNA suppression rates have been described in HBV-HIV patients receiving TDF. Up to 10% of these patients have detectable HBV-DNA after long-term follow-up and proper adherence even when resistance has not been detected, but the clinical consequences of this have not been clearly established.[18] Hepatic flares and/or HBeAg seroconversion may occur in 20% to 25% of cases after initiating HAART as a part of the immunological reconstitution inflammatory syndrome.[19]

3.1.6 | Patients with HBV and HCV coinfection

HBV-HCV coinfection causes rapid progression of liver disease and is associated with a higher risk of HCC. Although this has not been clearly shown in in vitro models, coinfection leads to some HBV suppression, with low DNA and qHBsAg levels.[20] HBV-DNA increases in HBsAg-positive individuals and HBV reactivation has been identified in anti-HBc-positive patients in IFN-based HCV regimens and more recently, during and after treatment with direct-acting antivirals (DAAs). A systematic review including DAA regimens reported a reactivation rate (HBV-DNA ≥ 20 IU/

mL when previously undetectable) of 1.4% in anti-HBc-positive/HBsAg-negative individuals with no major clinical events. The overall estimated risk of reactivation in HBsAg-positive patients with no previous indication for NA therapy was 24%, with the elevation of ALT of two or more times the upper limit of normal in 9% of cases.[21] These data support the current recommendation of mandatory assessment of HBV status before starting DAAs and prophylactic NAs in HBsAg-positive patients up to 12 weeks after DAAs discontinuation. NA prophylaxis in anti-HBc-positive/HBsAg-negative patients is not recommended due to the low risk of HBV reactivation.

3.1.7 | Patients with chronic hepatitis delta (CHD)

The current treatment of choice for CHD is a peg-IFNα for 48 weeks, which achieves HDV-RNA suppression in 25%-30% of patients. Nevertheless, most HBV-HDV coinfected patients are not eligible for IFN-based therapies due to advanced liver disease or comorbidities. Treatment with high-barrier NAs is currently recommended to control HBV replication in patients with advanced liver disease or HBV-DNA ≥ 2000 IU/mL, even though long-term NA-suppressed CHD patients are at a higher risk of decompensation, HCC development, liver transplantation and death than individuals with CHB.[22]

3.1.8 | Children and adolescents

Children and adolescents with CHB should follow the standard criteria for starting therapy, with success rates that are similar to those seen in the general population for ETV and TDF.[2] Table 1 shows recommendations according to the patient's age. A recent real-world study including a small group of patients with HBeAg-positive chronic infection showed that the initiation of LAM in children under the age of 1 was much more effective than IFN therapy after the age of 1, achieving HBsAg loss in 83% and 36% (P = .0023) of patients, respectively, at 12 months of follow-up. Nevertheless, further studies are needed to support these findings.[23]

3.2 | Patients with treatment failure

Treatment failure in patients receiving high-barrier NAs is defined as a *partial virological response* (decrease of HBV-DNA > 1 $\log_{10}$ IU/mL, but detectable after at least 1 year of treatment) or a *virological breakthrough* (>1 $\log_{10}$ increase in DNA from nadir during treatment).[2] Most cases of failure are due to the lack of adherence. *Primary non-response* has not been described during treatment with high-barrier NAs and most cases of partial response are resolved by maintaining the selected NA, because it is often due to very high baseline HBV-DNA levels.

When non-adherence to therapy has been excluded, a partial response with a plateau in HBV-DNA levels or a virological breakthrough may be related to emerging resistance. TDF resistance is very unusual and has not been identified in long-term studies.[6-9] The resistance rate to ETV in naïve patients can reach 1.2% and rescue therapy with TDF is clinically effective. A recent study described a quadruple mutation in two patients with previous exposure to NA who developed a virological breakthrough during TDF treatment.[24] One patient died due to HCC and the other did not achieve virological suppression following a TDF/ETV combination and a new capsid inhibitor was started. Cross-resistance mechanisms between low- and high-barrier NAs have been described in patients previously exposed to these drugs. Cross-resistance to ETV is present in individuals with LAM resistance due to the rtM204V/I mutation. The addition or presence of rtT184, rtS202, or tM250 has also been related to lower efficacy of ETV.[2] TDF has been shown to be effective in LAM-resistant variants, without the development of resistance. There were no differences in a comparison between the TDF/ETV 300/1 mg combination and TDF monotherapy, which is considered to be a good option in these cases. Although consistent evidence on NA combinations is scarce, they could be considered in patients failing potent NA monotherapy. A recent report described that a combination of ETV-resistant mutation sites (rtL180M/T184L/M204V) and rtA200V conferred resistance to TDF monotherapy in vivo and in vitro. The patient achieved viral suppression with an ETV/TDF combination.[25]

4 | MONITORING THERAPY

Monitoring patients who are receiving NAs requires periodic biochemical and serological assessments to determine virological response and promptly detect emerging side effects. Examinations and recommended testing intervals are shown in Figure 1.

4.1 | Efficacy monitoring

A *biochemical response* is defined as the normalisation of ALT levels (<40 IU/mL) during treatment and is achieved in most patients. Factors such as steatosis and alcohol consumption are usually related to failure to normalise liver enzymes.[7] A *virological response*, defined as HBV-DNA levels <10 IU/mL, is achieved in most patients and is usually faster in HBeAg-negative cases because of initially lower HBV-DNA levels. A recent study showed the infectious potential of serum from patients with chronic suppression by NA treatment who infected naïve hepatocytes in mice despite undetectable viremia by conventional tests.[26] A *serological response* should be monitored in HBeAg-positive patients to detect seroconversion. Testing of baseline HBsAg level and its kinetics are useful during therapy to predict HBeAg and HBsAg loss. A significant reduction in qHBsAg (>1 $\log_{10}$ at 24 weeks) after the initiation of TDF in HBeAg-positive individuals has been proposed as a predictor of HBsAg clearance.[27] In HBeAg-negative CHB patients, qHBsAg usually decreases slowly, and a greater reduction seems to predict a virological response.

Noninvasive markers play an important role in the evaluation of the changes in fibrosis because serial liver biopsies are not feasible in clinical practice. A significant decline in liver stiffness in patients receiving NAs has been reported in several studies, probably due to the regression of fibrosis, decreased inflammation, or both, and it has been shown to be predictive of liver-related events. A recent study suggested that a decrease to ≤8 kPa in liver stiffness could be predict reduced recurrence of HBV-related HCC in patients receiving NAs.[28]

4.2 | Safety

In most patients with CHB NA treatment should be lifelong. Long-term studies have shown good tolerability and both TDF and ETV have been associated with a low incidence of kidney impairment.[7,16] There is a risk of nephrotoxicity and Fanconi syndrome with long-term TDF treatment, especially in HIV coinfected patients. Ten years of treatment with TDF in patients with CHB resulted in the reduction in kidney function in 5% of cases.[7] Furthermore, stimulation of phosphate loss due to proximal tubular injury could affect bone turnover and increase bone density loss. A decrease in bone marrow density (BMD), especially in the hip, has been detected after long-term TDF treatment, but the clinical relevance of these findings remains to be established.[7] Fanconi syndrome has been reported in isolated cases. Tubular injury and BMD loss (as well as Fanconi syndrome) seem to be reversible following TDF withdrawal and switching to ETV or TAF.[5,15] Long-term ETV has been associated with a low incidence of lactic acidosis (<0.3%).[6] EASL does not recommend TDF as first-line treatment because of its safety profile in individuals older than 60 with kidney impairment (CrCl <60 mL/min, albuminuria, serum phosphate <2.5 mmol/dL) or bone disease. TAF is recommended rather than ETV in patients previously exposed to NA.[2] A recent real-life study in 565 patients receiving long-term TDF treatment showed that 66% of cases were candidates to switch to ETV or TAF according to EASL criteria.[29]

These data suggest that creatinine levels and eGFR should be regularly monitored in all patients receiving NAs. Serum phosphate evaluations should be included in the follow-up of TDF-treated patients. Closer monitoring is required in individuals with risk factors for kidney disease, such as older age, diabetes mellitus, or nephrotoxic treatments, especially patients with ESLD receiving diuretic treatment.[6]

4.3 | Adherence

Incomplete adherence can reach 39% in NA-treated patients and is the main reason for treatment failure. Certain patient characteristics such as younger age, poor medication-taking routine and misconceptions about the disease, are the predictors of inadequate adherence. A large percentage of CHB patients in high-income countries are immigrants from endemic areas. Thus, language and cultural barriers must also be taken into account.

4.4 | Identifying patients with a risk of HCC

HCC remains higher than in the general population in this group even after long-term viral suppression.[11] Older age, male sex, poor quality living habits, a family history of HCC and African/Asian origin are risk factors for the development of HCC in HBV patients.[11] Although several scores (GAG-HCC, REACH-B, CU-HCC) have been proposed to identify individuals at a higher risk of developing HCC, they are not sufficiently accurate in non-Asian patients.[2]

The PAGE-B score (including age, sex, and platelet count as risk factors) was initially proposed for Caucasian CHB patients receiving NA treatment and was later validated in an Asian population. The accuracy of PAGE-B is similar to that of the GAG-HCC and CU-HCC, and significantly higher than the REACH-B score. The results of the modified PAGE-B score (mPAGE-B), which includes serum albumin levels, are better for the prediction of CHB after 5 years of treatment in Asian patients. Proposed cut-offs are ≥10 for PAGE-B and ≥13 for mPAGE-B.[30]

5 | COUNSELLING AND PREVENTION

Individuals living with CHB must be informed about the mechanisms of transmission and prevention. Testing and vaccination must be offered to sexual partners, first-grade relatives and housemates. Preservative use should be recommended even in patients with long-term suppression if the vaccination status of a sexual partner is unknown. Special efforts must be made for compliance and linkage to care in women of childbearing age.

Factors increasing the risk to the liver such as alcohol consumption and being overweight must be specifically addressed in CHB patients. Vaccination against hepatitis A should be ensured. In patients with other specific risk behaviours such as intravenous drug use, harm reduction strategies must be offered to avoid HCV, HDV and HIV infection.

CONFLICT OF INTEREST

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REVIEW ARTICLE

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Approach to the patient with chronic hepatitis B and decompensated cirrhosis

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Abstract

Patients with chronic hepatitis B virus (HBV) can develop progressive fibrosis, cirrhosis and hepatocellular carcinoma. Patients with chronic HBV and cirrhosis are at risk of developing hepatic decompensation and have high mortality without antiviral therapy and/or liver transplantation. Treatment of chronic HBV with antiviral therapy is indicated in all patients with cirrhosis whatever the HBe-antigen status and serum alanine aminotransferase (ALT), so that hepatic decompensation can be prevented. Initiating antiviral therapy in patients with decompensated cirrhosis can improve liver function, Child-Turcotte-Pugh (CTP) and model for end-stage liver disease (MELD) scores, as well as the need for liver transplantation and mortality. Patients with chronic HBV and cirrhosis who do not respond to antiviral therapy with normalization of ALT may have a co-existent liver disorder. One of the most common co-existent liver disorders present in patients with chronic HBV is non-alcoholic fatty liver disease (NAFLD). Patients with chronic HBV, NAFLD and cirrhosis may be at risk of developing decompensated cirrhosis and require a liver transplant. If patients with chronic HBV require liver transplantation, infection of the liver graft with HBV can be prevented with antiviral therapy.

KEYWORDS

chronic hepatitis B, cirrhosis, HBV DNA, non-alcoholic fatty liver disease

1 | INTRODUCTION

Chronic hepatitis B virus (HBV) is a global health concern. Approximately 250 million persons are chronically infected with HBV worldwide and are at risk of developing progressive fibrosis, cirrhosis, liver failure and/or hepatocellular carcinoma (HCC).[1] The prevalence of patients with chronic infection varies considerably around the world. The highest prevalence is seen in Asia and sub-Saharan Africa. The lowest rates are seen in North America, Western Europe and Australia. In these areas, most patients with chronic HBV are immigrants from parts of the world with a higher prevalence of HBV. Highly effective antiviral therapy that suppresses HBV replication has been available for nearly two decades. However, these agents rarely cure HBV and most persons with active disease who receive treatment require life-long therapy.[2] Mortality becasue of chronic HBV remains one of the top 15 causes of death worldwide including 40% of all deaths becasue of malignancy.[3] There are several reasons for this high mortality rate. In particular, most patients with chronic HBV live in areas of the world with lower income and reduced access to routine healthcare and treatment is life-long. Moreover, in developed countries with higher incomes, chronic HBV is often undiagnosed becasue of a lack of

Abbreviations: AASLD, American Society for the Study of Liver Diseases;; ALT, alanine aminotransferase;; APASL, Asian-Pacific Association for the Study of the Liver;; CTP, Child-Turcotte-Pugh;; DNA, deoxyribonucleic acid;; EASL, European Association for the Study of the Liver;; HBV, chronic hepatitis B virus;; HCC, hepatocellular carcinoma;; HCV, chronic hepatitis C virus;; MELD, model for end-stage liver disease;; NAFLD, non-alcoholic fatty liver disease.

universal screening as well as language, cultural and financial barriers that limit access to healthcare for many immigrants.

Chronic HBV infection is defined by the persistence of the HBV surface antigen and has several phases defined by HBV e-antigen status, serum levels of HBV deoxyribonucleic acid (DNA) and alanine aminotransferase (ALT) according to the guidelines developed by the American Society for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL) and the Asian-Pacific Association for the Study of the Liver (APASL).[4-6] These phases are summarized in Table 1. Patients with chronic active HBV are at risk of the progression of fibrosis and cirrhosis. This risk is most closely correlated to HBV DNA serum levels and it increases stepwise when the HBV DNA level increases >2000-20 000 international units per millilitre (IU/mL).[7] The annual risk of developing cirrhosis in patients with HBe-antigen-positive chronic active HBV infection is 2%-6%, while the annual risk in patients with HBe-antigen-negative infection is 8%-10%.[8,9] Thus, patients with HBe-antigen-negative chronic active HBV infection have a higher risk of the progression of fibrosis and the development of cirrhosis than HBe-antigen-positive patients with active disease, even though the level of HBV DNA tends to be 10-fold lower in HBe-antigen-negative patients than in HBe-antigen-positive ones. The reason for this is that many patients with HBe-antigen-positive chronic active HBV will spontaneously seroconvert to inactive HBV over time. This is associated with a marked decline in HBV DNA to <2000 IU/mL, a decline in serum ALT to the normal range and a marked reduction in hepatic inflammation. Patients who seroconvert to inactive HBV infection are at a very low risk of any further progression of fibrosis, and generally fibrosis will regress over time. In contrast, patients with HBe-antigen-negative chronic active HBV infection cannot seroconvert, generally remain within the active state and are at a life-long risk of the progression of fibrosis and cirrhosis.

Patients with chronic HBV infection are also at an increased risk of developing HCC. This risk is present in patients with or without cirrhosis. The risk of developing HCC in patients without cirrhosis is 1% annually and increases to 2%-5% per year[10] in patients who have developed cirrhosis. Moreover, the risk increases with the level of HBV DNA.[7]

Key points

- Patients with chronic hepatitis B virus (HBV) can progress to cirrhosis, liver failure and/or develop hepatocellular carcinoma.
- Treatment of patients with chronic hepatitis B virus and cirrhosis with oral antiviral drugs can reduce the risk of hepatic decompensation.
- Treatment of patients with chronic hepatitis B virus and hepatic decompensation with oral antiviral drugs can reduce the need for liver transplantation and improve survival.
- Treatment of patients with chronic HBV who require a liver transplant with oral antiviral drugs can prevent hepatitis B virus infection of the liver graft.

The presence of a co-existent liver disorder accelerates the progression of fibrosis to cirrhosis in patients with chronic HBV infection.[4-6] This includes co-infection with chronic hepatitis C virus (HCV) or hepatitis D virus, chronic alcohol use and non-alcoholic fatty liver disease (NAFLD). NAFLD is now recognized as the most common cause of liver disease worldwide and is estimated to be present in up to 25%-33% of the global population.[11] Virtually no countries are exempt from NAFLD with the highest prevalence now reported from countries in the Middle East. The presence of a co-existent liver disorder is one of the most common reasons for discordance between ALT, HBe-antigen status and HBV DNA when assessing a patient with chronic HBV. In most cases, a liver biopsy will be required to determine if elevated ALT levels are becasue of chronic HBV infection or the co-existent liver disorder and whether HBV is active, requiring treatment or whether it is inactive.

The following is a case of a patient with chronic HBV infection with cirrhosis who subsequently developed decompensated cirrhosis. Several issues related to the natural history and treatment of

TABLE 1 Phases of chronic HBV infection

	Immune tolerant	Active	Discordant	Inactive
ALT	Normal	Elevated	Normal or elevated	Normal
HBsAg	+	+	+	+
HBeAg	+	+ or −	+ or −	−
Anti-HBe	−	+ or −	+ or −	+
HBV DNA	>1 million	>20 000 if eAg + >2000 if eAg −	Variable	<2000
Histology	Minimal	Active	Variable	Minimal

Note: Most society guidelines define elevated ALT in patients with active HBV infection as twice the upper limit of normal.

Histology refers to the level of inflammation seen on liver biopsy.

Patients with discordance do not meet criteria for active or inactive disease based upon the ALT level, HBe-antigen status and the level of HBV DNA.

chronic HBV and management of patients with cirrhosis and decompensated cirrhosis are then discussed.

2 | CASE PRESENTATION

The patient is a 45-year-old male who immigrated to the United States from Afghanistan 10 years earlier. Elevated liver enzymes results were reported 5 years ago. The patient also had diabetes mellitus type 2 and hypertension, both well controlled. The Body Mass Index (BMI) was 30.5 kg/m^2. Laboratory studies showed elevated serum AST and ALT, 85 and 71 IU/L, respectively. All tests of synthetic hepatic and metabolic function were normal and the platelet count was 135 000/mm^3. The serologic assessment showed that the patient was HBsAg-positive, HBeAg-negative, anti-HBe-positive, with HBV DNA of 1200 IU/mL. HBV surface antigen titre was 1200 IU/mL. Serology for all other causes of chronic liver disease was negative. An ultrasound showed an echogenic liver consistent with hepatic steatosis. No liver nodules were identified. Sheer wave elastography showed a liver stiffness of 15 kilopascals (kPa) and Continuous Attenuation Patameter (CAP) score of 285. The remainder of the evaluation was unremarkable. All laboratory studies including an assessment of fibrosis are summarized in Table 2.

The patient was diagnosed with chronic hepatitis B and cirrhosis. Child-Turcotte-Pugh (CTP) score 5; MELD score 8. Oral antiviral therapy was initiated. HBV DNA became undetectable within 3 months. AST and ALT declined somewhat but remained elevated in the 60-70 IU/L range. An ultrasound was repeated every 6 months to screen for HCC. Over the next 5 years, there was a gradual decline in serum albumin and platelet count, and a gradual rise in serum creatinine. HBV DNA remained undetectable on antiviral therapy (Table 2). The patient developed hepatic encephalopathy and ascites and was found to have spontaneous bacterial peritonitis. He was treated with diuretics and antibiotics. The CTP score was now 10, the MELD score was 19 and the patient was evaluated for liver transplantation. A repeat ultrasound identified a 2 cm hypoechoic lesion in the right hepatic lobe. The lesion was enhanced on dynamic MR imaging, with washout with rim enhancement, Liver Imaging Reporting And Data System (LIRADS)-5. HCC was treated with embolic therapy. The patient was accepted as a candidate for liver transplantation, awarded a MELD upgrade for HCC and underwent liver transplantation. The liver explant showed cirrhosis, NAFLD and stained positive for HBsAg.

The patient was given intravenous Hepatitis B Immune Globulin (HBIG) during the anhepatic phase of transplant surgery and for 5 days thereafter. Oral antiviral therapy for HBV was started within 48 hours of the transplant. One year following the transplant, HBV DNA and HBsAg remained undetectable and liver transaminases are normal.

TABLE 2 Case presentation: summary of laboratory and clinical data

	Baseline	5 y
Body mass index (kg/m^2)	30.5	33.1
AST (IU/L)	85	91
ALT (IU/L)	71	75
ALP (IU/mL)	110	115
Total bilirubin (mg/dL)	1.0	2.4
Albumin (gm/dL)	3.6	2.4
INR	1.1	1.2
Sodium (mEq/L)	141	135
Creatinine (mg/dL)	0.9	1.9
WBC	8.2	4.6
Haemoglobin (g/dL)	13.1	11.9
Platelet count	135 000	94 000
HBsAg	Positive	Positive
HBeAg	Negative	Negative
Anti-HBe	Positive	Positive
HBV DNA (IU/mL)	1200	Undetectable
Sheer wave elastography	15 kPa, Continuous Attenuation Patameter 285	
CTP score	5	10
MELD score	7	19

3 | DISCUSSION

3.1 | Treatment of chronic HBV in patients with cirrhosis

AASLD and EASL guidelines recommend that patients with chronic HBV infection and stable cirrhosis be treated with antiviral therapy whatever the HBe-antigen status, HBV DNA levels and ALT.[4-6] APASL guidelines recommend treatment for patients with chronic HBV infection and stable cirrhosis when HBV DNA level is >2000 IU/mL regardless of HBe-antigen status or ALT.[6] However, if the HBsAg titre is >2000 IU/mL in a patient with HBV DNA <2000 IU/mL, as in our case, the risk of the progression of fibrosis and HCC has been shown to be increased.[12] Most hepatologists would initiate antiviral treatment in this case. The rationale for antiviral therapy in patients with cirrhosis is to prevent sero-reversion from inactive to active HBV infection, which could result in an HBV DNA flare and subsequent hepatic decompensation. The preferred therapy is either tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF) or entecavir.[4-6] All three agents are highly potent with an extremely low rate of viral resistance. The only exception is in patients who have previously developed antiviral resistance to lamivudine, and have an increased risk of antiviral resistance to entecavir. Higher doses of entecavir are recommended to reduce this risk.[13] The most common reason for patients not to become HBV DNA undetectable, or to develop recurrent viraemia after complete viral suppression, is that they are not compliant. Antiviral therapy should be continued life-long in patients with cirrhosis.[4-6] Peg interferon is contraindicated in patients with chronic HBV infection and cirrhosis because HBV DNA could flare with seroconversion and result in hepatic decompensation.[14]

Once antiviral therapy has been initiated in a patient with chronic HBV infection and cirrhosis, they should be monitored on a regular basis to ensure that HBV DNA has been suppressed to undetectable levels and serum ALT has declined to the normal range. Patients with cirrhosis must also be monitored for the development of HCC every 6 months with ultrasound.[15] Alpha-fetoprotein is also used to survey for HCC but it is not a substitute for ultrasound.

If ALT fails to decline to normal ranges after antiviral therapy has been initiated, another co-existent liver disease is probably present and additional tests should be performed to determine the aetiology of this liver disease. If the co-existent liver disease cannot be effectively treated, the patient remains at high risk of developing progressive liver injury, hepatic decompensation and will probably require liver transplantation. This was the case in our patient. Close monitoring of these patients is essential if hepatic decompensation is to be identified in timely fashion.

3.2 | Discordance between serum liver transaminases and HBV DNA

The biochemical, serologic and virologic features of active and inactive HBV infection are summarized in Table 1. In general, patients with chronic active HBV infection have elevated HBV DNA >2000-20 000 IU/mL based upon HBeAg status and elevated serum ALT.[4-6] In contrast, patients with inactive HBV infection are always HBe-antigen-negative, have HBV DNA <2000 IU/mL and have normal ALT. Discordance, as in our case, occurs when patients have elevated ALT but low levels of HBV DNA. A liver biopsy is recommended in patients with discordant values for HBV DNA and serum ALT to help differentiate active HBV infection from inactive HBV infection and/or to determine if there is another cause for elevated serum ALT.[4-6] In our case, a liver biopsy was not performed because the patient had evidence of cirrhosis and antiviral therapy was indicated regardless of the HBV DNA level and whether this was active or inactive HBV infection. Serology had excluded other treatable forms of chronic liver disease and features of the metabolic syndrome suggested NAFLD.

Discordance also occurs in patients with elevated HBV DNA and normal serum ALT. If the patient has very high HBV DNA and is HBe-antigen-positive, he is probably in the immune tolerant phase. However, if the HBV DNA is >2000 IU/mL when HBeAg is negative or >20 000 IU/mL when HBeAg is positive and the ALT is normal, a liver biopsy may also be necessary to determine if active hepatitis is present. Antiviral therapy should be initiated in these cases.[4-6]

3.3 | Treatment of HBV in decompensated cirrhosis

Patients with chronic HBV and decompensated cirrhosis have a CTP score >5, and/or ascites or hepatic encephalopathy. These patients are at a high risk of worsening liver failure and death and should always be treated with antiviral therapy whatever the HBV DNA level and referred for liver transplantation unless there is an obvious contraindication for transplant surgery.[4-6] Several studies have shown that treatment of chronic HBV infection with decompensated cirrhosis can cause stabilization and subsequent improvement in liver function, CTP and MELD scores.[16,17] Transplant-free survival can be as high as 80% and up to 33% of patients can be removed from the liver transplant waiting list.[18] However, patients who do not rapidly improve after antiviral therapy is started are at high risk of not recovering. Thus, liver transplantation assessment should not be delayed just because antiviral therapy is initiated. Peg interferon therapy is contraindicated in patients with decompensated cirrhosis. Although TAF has not been studied in these patients, there is no reason to suspect that this agent would not be just as effective as TDF.

Hepatic decompensation in patients with chronic HBV infection can occur because of active HBV infection or, in our case, because of a co-existent liver disease in association with inactive HBV infection. In either case, antiviral therapy should be initiated and the patient should be evaluated for liver transplantation unless s/he is not a candidate for liver replacement therapy. Our patient developed hepatic decompensation while on antiviral therapy and with undetectable HBV DNA clearly indicating that some other cause of progressive liver disease was present in addition to chronic HBV infection.

3.4 | NAFLD and chronic HBV infection

NAFLD is now recognized as the most common liver disease worldwide with a global prevalence of 25%-33%.[11] It is therefore highly likely that chronic HBV infection and NAFLD will co-exist in many patients. Several studies have suggested that the risk of NAFLD may be reduced in patients with chronic HBV infection, although this remains controversial.[19,20] Overall, the data suggest that if patients with chronic HBV infection also have features of metabolic syndrome, they are at a similar risk of developing NAFLD as patients without chronic HBV infection. It is also unclear whether the co-existence of NAFLD in patients with chronic HBV infection leads to more rapid progression of fibrosis. To date, the data are inconclusive.[21,22] This is probably because many of these studies do not have data on liver biopsies and because of the difficulty of differentiating non-alcoholic fatty liver, which rarely causes progressive liver injury, from non-alcoholic steatohepatitis.

Like HBV, NAFLD and insulin resistance are risk factors for the development of HCC. As a result, it would not be surprising if the co-existence of these two liver disorders was associated with a high risk of HCC. Indeed, several studies have shown that the risk of developing HCC in patients with NAFLD and chronic HBV infection may be three to seven-fold higher than in patients with chronic HBV infection alone.[23,24] The patient in our case developed HCC.

3.5 | Treatment of HBV after liver transplantation

Any patient with chronic HBV infection, either active, inactive or suppressed with antiviral therapy, who requires a liver transplant is at risk

of developing HBV infection on the liver graft. This can be prevented by administering HBIG at the time of the transplantation and initiating lifelong antiviral therapy.[25,26] Both TDF and entecavir can be used in liver transplant recipients. TAF has not yet been studied in liver transplant recipients. However, with identical efficacy and a lower risk of kidney injury and osteoporosis compared to TDF in the non-transplant setting, there is no reason to suspect this agent would be any less effective or have any higher risk of adverse events in the liver transplant recipient.

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CONFLICT OF INTEREST

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REVIEW ARTICLE

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Can we cure hepatitis B virus with novel direct-acting antivirals?

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Abstract

Current treatments against chronic hepatitis B (CHB) include pegylated interferon alpha (Peg-IFNα) and nucleos(t)ide analogs (NAs), the latter targeting the viral retrotranscriptase, thus inhibiting de novo viral production. Although these therapies control infection and improve the patient's quality of life, they do not cure HBV-infected hepatocytes. A complete HBV cure is currently not possible because of the presence of the stable DNA intermediate covalently closed circular DNA (cccDNA). Current efforts are focused on achieving a functional cure, defined by the loss of Hepatitis B surface antigen (HBsAg) and undetectable HBV DNA levels in serum, and on exploring novel targets and molecules that are in the pipeline for early clinical trials. The likelihood of achieving a long-lasting functional cure, with no rebound after therapy cessation, is higher using combination therapies targeting different steps in the hepatitis B virus (HBV) replication cycle. Novel treatments and their combinations are discussed for their potential to cure HBV infection, as well as exciting new technologies that could directly target cccDNA and cure without killing the infected cells.

KEYWORDS

cccDNA; HBV cure, chronic hepatitis B, direct acting antiviral

1 | INTRODUCTION

Although an effective hepatitis B virus (HBV) vaccine is available, more than 250 million individuals live with chronic hepatitis B (CHB) worldwide (WHO fact sheet) and are at risk of severe liver complications. Nearly 1 million deaths occur yearly throughout the world because of the development of cirrhosis and hepatocellular carcinoma (HCC), thus CHB continues to be a major global public health issue and novel therapeutic approaches are sorely needed. Approved antiviral treatments, nucleos(t)ide analogs (NAs) and interferon alpha (IFNα), can successfully keep the infection under control and improve the patients' quality of life. However, the ideal endpoints of a functional cure, which include loss of Hepatitis B surface antigen (HBsAg) and undetectable HBV DNA levels in serum, are rarely achieved with existing treatments. This is mainly because of the presence of the covalently closed circular DNA (cccDNA) minichromosome, which remains untouched in the nuclei of infected hepatocytes, and to the exhaustion of HBV-specific $CD8^+$ T cells and B cells. Because of the continuous presence of cccDNA, relapse of viraemia is frequent after treatment discontinuation. Thus, finding novel cccDNA inhibitors that target its formation, trigger its degradation and/or restore the HBV-specific antiviral immune responses

Abbreviations: CAMs, capsid allosteric modulators; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; CRISPR, clustered regularly interspaced short palindromic repeats; DAAs, direct acting antivirals; HBc, HBV core protein; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HDACs, histone deacetylases; HDV, hepatitis D virus; IFNα, interferon alpha; LNA-SSOs, locked nucleic acids SSOs; MyrB, myrcludex B; NAPs, nucleic acid polymers; NC, nucleocapsid; NAs, nucleos(t)ides analogues; NTCP, sodium taurocholate cotransporting polypeptide; Peg-IFNα, pegylated interferon α; pgRNA, pregenomic RNA; PHHs, primary human hepatocytes; rcDNA, relaxed circular DNA; RNAi, RNA interference; SSOs, single-stranded oligonucleotides.

by lowering viral antigen load to kill infected hepatocytes are the main goals for achieving a complete cure.

The HBV life cycle presents multiple potential targets for antiviral therapies. HBV infection is dependent upon the presence of its cellular receptor sodium taurocholate cotransporting polypeptide (NTCP)[1] and recent data suggest that it is enhanced by the presence of epidermal growth factor (EGFR).[2] Once HBV is internalized, it releases its nucleocapsid to the cytoplasm, which travels to the hepatocyte nucleus to release the relaxed circular DNA (rcDNA). cccDNA originates from the incoming rcDNA by mechanisms that are not fully understood and associates with histone and non-histone proteins to build a minichromosome that acts as the only transcriptional template for all viral RNA.[3] Pregenomic RNA (pgRNA) is packaged inside newly formed nucleocapsids and reverse transcribed by the viral polymerase. Nucleocapsids can then be released from cells by the secretory pathway or they can be recycled back to the nuclei to replenish the cccDNA pool (Figure 1).

cccDNA is an extrachromosomal plasmid-like molecule lacking centromeres. Its fate during cell division is unclear, although a recent study suggests that it is symmetrically distributed to daughter cells.[4] Several studies have also shown evidence of the presence of persistently infected primary human hepatocytes (PHHs) that can act as viral reservoirs.[5] Furthermore, the rapid resolution of acute HBV infection suggests that cytopathic mechanisms and compensatory cell proliferation could be synergistic, clearing HBV-infected cells while maintaining a functional liver. Thus, further knowledge of

Key points

- Current anti-HBV therapies can efficiently reduce HBV load and improve the patient's quality of life but cannot eliminate the infection or prevent the risk of rebound after treatment interruption.
- New combination therapies using direct and indirect acting antivirals, reducing viral antigen load and boosting the immune system are essential to eliminate HBV.
- The goal of novel direct acting antivirals (DAAs) under investigation is to reduce the viral reservoir cccDNA. However, larger and longer clinical trials are still needed to demonstrate the effectiveness of these compounds.

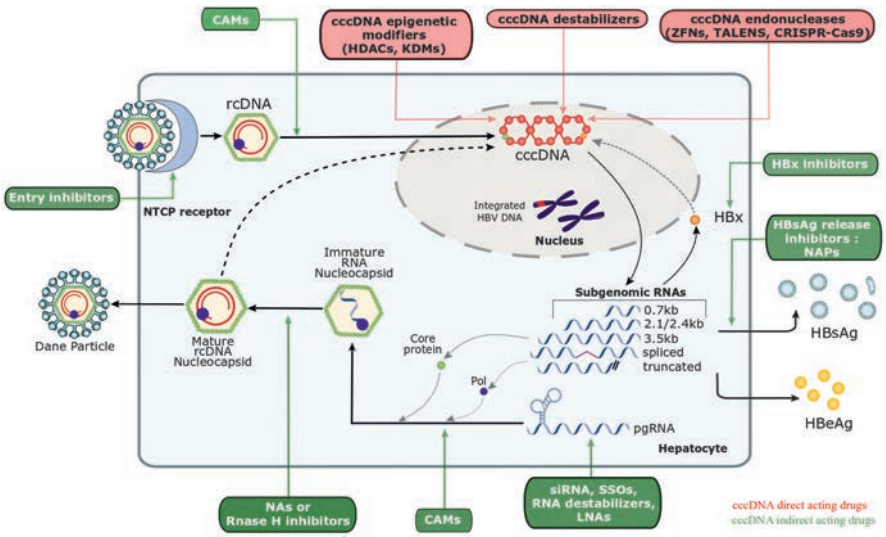

FIGURE 1 Novel therapeutics targets in the HBV Life Cycle (adapted from ref. [8]). cccDNA direct-acting drugs are shown in red. Three major therapeutic strategies are under pre-clinical consideration: cccDNA endonucleases (CRISPR-Cas9, ZFNs and TALENs), cccDNA destabilizers and cccDNA epigenetics modifiers. cccDNA indirect-acting drugs are shown in green. HBV entry into the hepatocyte can be inhibited by MyrB, which competes for binding to NTCP receptor. HBx protein, which control the cccDNA expression, or the interaction between HBx and cccDNA are potential targets for new antiviral drugs. Different RNA species are transcribed from cccDNA and these RNAs can be targeted for degradation by several drugs (siRNA, SSOs, RNA destabilizers and LNAs). NAPs can prevent virus budding and reduce HBsAg release. CAMs can reduce the release of infectious viral particles by destabilizing the HBV Core protein assembly, lead to the formation of aberrant capsids or morphologically normal capsids without genetic material. CAMs can present secondary effects inhibiting the recycling of rcDNA into the nucleus thus leading reduction in the cccDNA pool. NAs inhibit the retrotranscription by the viral polymerase and reduce the formation and synthesis of viral DNA from the pgRNA. RNAseH inhibitors causes the accumulation of long RNA:DNA heteroduplexes and blocks the plus-polarity DNA strand production. CAMs, capsid assembly modulators; cccDNA, covalently closed circular DNA; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats associated protein 9; DNA, deoxyribonucleic acid; HBV, hepatitis B virus; HDACs, histone deacetylases; KDMs, lysine demethylases; LNA, locked nucleic acid; NA, nucleoside analogue; NAPs, nucleic acid polymers; NTCP, sodium-taurocholate cotransporting polypeptide; pgRNA, pregenomic RNA; rcDNA, relaxed-circular DNA; RNA, ribonucleic acid; siRNA, small interfering RNA; SSOs, single-stranded oligonucleotides; TALENs, transcription activator-like effector nucleases; ZFNs, zinc finger nucleus

TABLE 1 Clinical trials with novels therapeutics in pipeline for chronic hepatitis B[8,15]

Drug name	Mechanisms	Therapeutic class	Delivery	Clinical trial	Comments
Entry inhibitors					
MyrcludexB	Competition with NTCP	Peptide	Subcutaneous injection	II	HBsAg loss in 26% of HBV/HDV co-infected patients after 48 wk of treatment with MyrB+peg-IFN and 24 wk treatment-free follow-up
CRV-431	Competition with NTCP and block of protein folding	Small molecule	Oral administration	I	Safe and well-tolerated at several doses (75-525 mg) in eight healthy volunteers
Transcriptional control					
Nitazoxanide	Inhibition of HBx–DDB1 protein interaction	Small molecule	Oral administration	I	After 4-20 wk of treatment, a pilot study showed an undetectable serum HBV DNA in eight of nine patients (89%). Phase I is ongoing (NCT03905655)
FXR agonist	Inhibition of FXR binding to cccDNA	Small molecule	Oral administration	I	Safe and well-tolerated in 46 healthy volunteers with several doses (30-800 mg/d) for 15 d. Phase II is ongoing
Post-transcriptional control inhibitors					
JNJ-3989/ARO HBV	RNA degradation	siRNA	Subcutaneous injection	II	Forty patients received weekly or monthly doses of JNJ-3989 with 14 d follow-up after third dose: 80% patients had HBsAg < 100 IU/mL after three injections. No serious adverse events reported
ARB-1467	RNA degradation	siRNA	Intravenous infusion	II	All 28 patients enrolled experienced decrease in HBsAg. Greater HBsAg reduction was more frequent with bi-weekly dosing than monthly dosing. Clinical development has been discontinued
ARC-520	RNA interference	siRNA	Intravenous infusion	I	Fifty-four healthy volunteers were enrolled in this study without moderate hypersensitivity reactions with oral antihistamine pre-treatment (ARC-520 found to induce a histamine release through mast cell degranulation)
GSK-3389404	RNA degradation	ASO	Subcutaneous injection	I	GSK 3389404 dosing has been tested up to 120 mg for 4 wk without serious adverse events.
RO-7062931/ RG-6004	RNA interference	LNA-SSOs	Subcutaneous injection	I	Phase I is ongoing (NCT03505190)
VIR-2218	RNA degradation	RNA interference	Subcutaneous injection	I	Phase I is ongoing (NCT03672188)
DCR HBVS	RNA degradation	siRNA	Subcutaneous injection	I	Phase I is ongoing (NCT03772249)
AB-729	RNA interference	siRNA	Subcutaneous injection	I	Phase Ia/b is ongoing and a phase II in combination with AB-506 (capsid assembly modulator) also
RG-7834	RNA interference	Unknown	Oral administration	I	Phase I is ongoing (NCT02604355)
Capsid assembly modulators					
JNJ-6379	Capsid assembly modulator	Small molecule	Oral administration	II	48 HBV-treatment-naïve patients received a dose of 25, 75, 150 and 250 mg. Median serum HBV DNA level decrease of 2.16-2.89 logIU/mL and median serum HBV RNA decrease of 1.43-2.30 logIU/mL compared to placebo after 4 wk. A phase 2a study is ongoing in treatment-naïve and virologically suppressed HBeAg-positive and negative patients alone and in combination with NAs (NCT03361956)

(Continues)

TABLE 1 (Continued)

Drug name	Mechanisms	Therapeutic class	Delivery	Clinical trial	Comments
RO-7049389/ RG7907	Core-protein binding	Small molecule	Oral administration	II	Twenty-one chronic-HBV infected patients received a dose of 200, 400 and 600 mg. Median serum HBV DNA level decrease of 2.7-3.2 logIU/mL and median serum HBV RNA level decrease of 1.3-2.3 logIU/mL after 28 d without serious adverse events
GLS-4	Core-protein binding	Small molecule	Oral administration	IIa	After 20 wk of treatment, serum HBV DNA levels declined of 1.48-6.09 logIU/mL with a twice or thrice daily administration
ABI-H0731	Core-protein Binding	Small molecule	Oral administration	IIa	After 12 wk of treatment with Entecavir (interim data), serum HBV DNA and RNA decrease of 4.5 and 2.6 logIU/mL. The total treatment duration is 24 wk (results no available)
ABI-H2158	Core-protein Binding	Small molecule	Oral administration	Ia	Safe and well-tolerated in 48 healthy volunteers
JNJ-440	Capsid assembly modulator	Small molecule	Oral administration	I	Single-dose up to 4000 mg and multiple once-daily doses to 2000 mg for 7 d were well-tolerated in 100 healthy volunteers
NVR 3-778	Capsid assembly modulator	Small molecule	Oral administration	I	NVR 3-778 was safe in 73 naïve-treatment HBV-infected patients. Median serum HBV DNA and RNA levels decrease of 1.43 and 1.42 logIU/mL after 4 wk of treatment. Clinical trials phase II is ongoing
AB-506	Core-protein binding	Small molecule	Oral administration	I	Treatment was well-tolerated in 33 healthy volunteers
EDP-514	Core-protein binding	Small molecule	Oral administration	Ia/Ib	Phase I is ongoing (NCT04008004)
QL-007	Core-protein binding	Small molecule	Oral administration	I	Phase I is ongoing (NCT03770624)
HBsAg release inhibitors					
REP-2139 & REP-2165	HBsAg binding	Nucleic Acid Polymer	Intravenous infusion	II	In combination with TDF and PegIFN, 60% of patients had HBsAg loss at the end of the study and at 24 wk treatment-free follow-up. ALT flares detected in all patients
Polymerase inhibitor					
Tenofovir exalidex	Polymerase inhibitor	Small molecule	Oral administration	Ia/b	Treatment was safe in 50 patients, with a serum HBV DNA decreased up to 3.9 logIU/mL
Besifovir dipivoxil maleate	Polymerase inhibitor	Small molecule	Oral administration	III	Besifovir had an antiviral efficacy comparable to Tenofovir after 48 wk

Abbreviations: ASOs, antisense oligonucleotides; cccDNA, covalently closed circular DNA; DDB1, damage DNA-binding protein 1; DNA, deoxyribonucleic acid; HBV, hepatitis B virus; HDV, hepatitis D virus; IFN, interferon; LNA, locked nucleic acids; NTC, national clinical trials; NTCP, sodium-taurocholate cotransporting polypeptide; RNA, ribonucleic acid; siRNA, small interfering RNA; SSOs, single-stranded oligonucleotides; TDF, tenofovir.

these potential reservoirs is fundamental to achieve complete viral elimination.

In this review we discuss the latest advances in novel direct acting antivirals (DAAs) targeting different steps in the HBV lifecycle, with the ultimate goal of cccDNA eradication to achieve a functional cure for HBV infection. Ongoing clinical trials evaluating these different targets to treat CHB and identify new molecules that can achieve a sustained antiviral effect will be discussed.

1.1 | Inhibition of HBV replication to control HBV infection

1.1.1 | Entry inhibitors

Recent studies suggest that *de novo* infection via the HBV cellular receptor NTCP is required to maintain the cccDNA pool.[1,5] Thus, blocking viral entry in naïve hepatocytes could lead to reduction of the

intrahepatic load of HBV genomes and could be an effective therapeutic target. Compounds that bind and block NTCP can compete with the virus for the use of the cellular receptor.[6] Mutational studies on a myristoylated preS peptide of 47 amino acids derived from HBsAg led to the discovery of the first HBV entry inhibitor not internalized by NTCP, myrcludex B (MyrB or bulevirtide). Ongoing clinical trials are testing the efficacy of combining MyrB with interferon alpha (IFNα) in patients co-infected with hepatitis D virus (HDV; Table 1). HDV exploits HBV envelope proteins to propagate, thus entry inhibitors targeting the NTCP receptor would inhibit internalization of both viruses. A phase II clinical trial (MYR203) showed that, in contrast to pegIFN monotherapy, the combination with MyrB leads to high rates off-treatment HDV RNA suppression and loss of HBsAg in a substantial proportion of patients. Whether these effects endure over time remains to be determined and thus MyrB is moving along the pipeline to phase III studies where extended monotherapy or in combination with IFNα will be assessed in HBV/HDV patients.

HBV entry inhibitors do not directly target cccDNA and because of the slow kinetics of cccDNA decay and hepatocyte turnover, MyrB monotherapy is not expected to result in complete depletion of cccDNA in infected cells. However, combination therapies with antiviral agents with a different mechanism of action could have a potent synergistic effect and ultimately lead to elimination of the cccDNA pool. Combinations of NAs and entry inhibitors could accelerate the elimination of infected cells. While NAs effectively block viral replication, residual viruses can be produced from chronically infected cells. HBV entry inhibitors could help neutralize residual viruses, protecting naïve hepatocytes from infection and accelerating the elimination of infected cells because of natural liver turnover.

Small molecules such as cyclosporine A and ezetimibe can influence NTCP bile transport functions and interfere with HBV infection. Whether bile salt transport function of NTCP is needed for HBV infection is still unclear. At present only hepatitis B immunoglobulins (HBIGs) are clinically approved as entry inhibitors. HBIGs do not target NTCP, they bind to HBV surface proteins preventing virus interaction with its cell receptors.[6]

1.1.2 | Nucleocapsid assembly modulators

The HBV core protein (HBc) is essential for genome packaging, reverse transcription and potentially for modulation of cccDNA. Therefore, targeting HBc would affect multiple aspects of the viral life cycle potentially leading to strong antiviral effects.[7] A slow nucleation rate and weak dimer-dimer association characterizes normal nucleocapsid (NC) assembly. Changing the strength or association rate could affect production of the normal NC. Several chemical classes of capsid allosteric modulators (CAMs) have been described to target the interdimer interphase of HBc, destabilizing its assembly rate.[8] Depending on their chemical structure CAMs lead to the formation of either aberrant or morphologically normal capsids lacking genetic material, both preventing the release of viral genetic material into newly infected cells and blocking the transport of incoming NC to the nucleus, thereby affecting cccDNA levels (Figure 1). In addition, some CAMs present secondary effects that prevent NC disassembly and inhibit the formation of cccDNA in de novo-infected cells or directly reduce HBe secretion[9-11] (Figure 1). Thus, CAMs are exciting new molecules in early clinical trials (Table 1). Phase I clinical trials on monotherapy showed effective reduction of circulating HBV DNA and RNA together with a good safety profile. However, serum antigens were not affected and rebound was observed after withdrawal. Longer treatment is necessary to evaluate the sustained antiviral effect of CAMs. The combination of NAs and CAMs could result in a synergistic interaction because of the different mechanisms of action of these two agents. This combination could lead to lower HBV viral loads. Whether further reducing viral loads would restore HBV-immune responses leading to viral clearance or whether a boost in the immune system is required is still unclear.

1.1.3 | Nucleos(t)ide analogues

Treatment with NAs leads to reduced levels of HBV DNA in patient serum and normalization of serum alanine transaminase, thus controlling the progression of liver disease and improving the patient's quality of life. The main target of NAs is the viral reverse transcriptase/polymerase, preventing the formation of new HBV DNA from pgRNA. However, cccDNA formation from incoming rcDNA does not seem to require the viral reverse transcriptase/polymerase activity, and thus NAs have only minimal effects on the existing pool of cccDNA or newly formed cccDNA molecules from de novo infection and viral relapse occurs after withdrawal of antiviral therapies.[12] A rapid decay of HBV DNA in serum is observed following the beginning of treatment but the decline of serum HBsAg and cccDNA is slow and incomplete in the liver, and cccDNA is detected in the liver of patients even after years of NAs treatment.[12] Since low levels of HBV replication persist, naïve hepatocytes can be constantly infected and cccDNA recycling can continue contributing to the replenishment of the intrahepatic pool of cccDNA during long-term NA therapy.[13] The development of more potent NAs that cause more profound suppression of viral production could lead to the prevention of intracellular cccDNA recycling or new rounds of infection with long-term treatment.

HBV RNAseH destroys HBV RNA after it has been retrotranscribed into DNA. In vitro studies have shown that targeting RNaseH function leads to truncation of minus-polarity DNA strands, accumulation of RNA/DNA heteroduplexes within viral capsids and failure to synthesize the plus-polarity DNA strand. RNAseH inhibitors target specific viral enzymatic activity that is different from NAs and thus could represent good candidates for combination therapies with NAs.[14]

1.2 | HBsAg serum level suppression

Immune exhaustion is a key feature to progression to chronic HBV infection. It is a major obstacle in HBV eradication and is believed

to be mainly because of the presence of HBsAg. RNA interference (RNAi) can directly target HBV transcripts and reduce the HBsAg load leading to reduced budding of viral and subviral particles, although the lowest HBsAg threshold needed to modify HBV-specific T-cell exhaustion profile is not known[15] (Figure 1). Several molecules are under evaluation. These agents differ in both HBV genome targeting and the delivery strategy used to introduce them into hepatocytes. ARC-520 was one of the first siRNA taken to clinical trials and was shown to successfully reduce HBsAg in HBeAg-positive patients. However, its effect was lost in HBeAg-negative patients or those receiving long-term NAs treatment highlighting the importance of the circulating HBsAg derived from integrated HBV.[16] Thus, while siRNA could reduce HBsAg, its design should target both cccDNA and integrated HBV DNA.[16] Preliminary results in a second generation of siRNAs (JNJ3989 and AB-729) suggest that HBsAg reduction was similar in HBeAg positive and negative patients[17,18] (Table 1).

New technologies are also being explored to reduce HBsAg. Single-stranded oligonucleotides (SSOs) are small nucleic acids complementary to their target RNA. Once bound, they promote RNA degradation via an RNAseH-dependent pathway. In vitro and in vivo studies of newer generation SSOs show a decrease in HBsAg and efficient inhibition of HBV gene expression and replication from cccDNA (Table 1). Non-conjugated SSOs naturally accumulate in different tissues leading to cell toxicity. Thus, locked nucleic acid SSOs (LNA-SSOs) have been engineered to be liver-specific and more stable, reducing viral replication and HBsAg both in vitro and in vivo.[19] Nucleic acid polymers (NAPs) are single SSOs that work independently of their sequence and thus, with an antisense effect. Their antiviral activity is dependent on their length and amphipaticity.[15] NAPs can block HBV during and after viral entry, providing a novel NAPs-specific post-entry activity in HBV. Small proof-of-concept clinical trials in HBeAg-negative patients have suggested that the add-on of NAPs in NAs+pegIFNa2a therapy blocks HBsAg release from infected cells leading to a functional cure in 39% of patients.[20] However, more research is needed to understand this post-entry mechanism and the ALT flares experienced by most patients. Pre-clinical models of RNA destabilizers such as AB-452 and RG7834 were shown to destabilize HBV RNA in a highly specific manner, leading to reduced HBsAg load.[21,22] In vitro data suggest that the mechanism of action of RNA destabilizers is different from siRNAs, with preferential reduction of subgenomic RNAs. These compounds could target and modify viral RNAs directly or indirectly promoting their degradation, but their target and exact mechanism of action requires further studies.

Because of the long half-life of cccDNA in infected hepatocytes, monotherapies whose goals are to suppress HBsAg cannot effectively affect the cccDNA pool within the span of a lifetime. For curative therapy, these treatments should be followed by a boost to induce immune restoration, perhaps in combination with DAAs targeting HBV replication.

1.3 | Direct targeting of cccDNA

1.3.1 | cccDNA targeting using genome editing strategies

Inhibiting cccDNA formation is a potentially solid strategy to directly target cccDNA. However, more information on the transformation of rcDNA to cccDNA is necessary and cccDNA present in chronically infected cells would not be affected by this approach. Thus, designer nucleases play a central role in potential CHB treatment to eliminate cccDNA. Different gene therapy strategies are under study to silence, edit or epigenetically modify HBV. The 'clustered regularly interspaced short palindromic repeats' (CRISPR)/Cas9 system is an attractive approach because of the simplicity of the gRNAs design to target specific DNA sequences, and the flexibility of using multiple gRNAs sequence targets. Several publications have presented proof-of-concept for the use of the CRISPR/Cas9 system to target the HBV genome (reviewed in ref. [23]). However, the results of in vitro studies are controversial on the fate of cccDNA after gene editing. Mutations on cccDNA could be over the repair threshold leading to its degradation. In contrast, the cell repair system could fix cccDNA leading to mutated cccDNA (Figure 1). The possibility that a Cas9-induced DNA double-stranded break (DSB) on cccDNA could lead to a linear HBV molecule and thus to an increased rate of integration and genomic instability must be investigated. The fate of cccDNA must be more clearly understood using more relevant models such as PHHs or liver-humanized mice models. Despite the obstacles of CRISPR/Cas9 for HBV therapy, it is still promising and the only potential target leading to direct mutagenesis of cccDNA. The gene editing field is constantly evolving by investigating novel nucleases with lower off-target effects and increasing their efficiency and safe delivery. Thus, understanding the biology of HBV after gene editing is an essential topic.

Recent studies have described a novel cccDNA destabilizer that not only achieved sustainable HBsAg reduction, potentially boosting the adaptive immune system but also rapidly and markedly reduced intrahepatic cccDNA levels in murine models.[24] This molecule is highly promising; however, further studies are needed to determine its mechanism of action using more relevant HBV infection models.

1.3.2 | Epigenetic modifiers to silence cccDNA

cccDNA transcription regulation is similar to human chromatin and thus follows the 'histone code' which states that specific combinations of epigenetic modifications in a specific genomic region can lead to either activation or repression of gene expression.[25] Thus, modulation of epigenetic modifiers such as histone deacetylases (HDACs) have been shown to suppress HBV transcription.[26] However, this could lead to pleiotropic effects affecting cell homeostasis. Thus, the targeting of specifically viral factors is under evaluation. Several

studies have shown a link between HBx expression and cccDNA epigenetic control.[27] The mechanism of HBx-mediated cccDNA regulation through interaction with structural partners is an interesting avenue for the use of small molecules to target this interaction. Another potential model explaining HBx stimulation of cccDNA transcription is associated with the rapid hypo-acetylation of histones in cells infected with an HBx mutant. This leads to repression of viral transcription and replication that does not affect the pool of cccDNA in the absence of HBx.[28] A better understanding of the epigenetic regulation of cccDNA is necessary to identify virus-specific factors that could be specifically targeted to permanently silence cccDNA.

1.4 | Ongoing clinical trials and perspectives

Current treatments for CHB reduce viral replication and improve quality of life and survival in treated patients. This goal can only be achieved if national healthcare systems make it possible for patients to comply to treatment protocols and attend follow-up consultations. However, this is not the case for most patients living with CHB, who do not have access to medical care, and have not even been diagnosed. Thus, making treatment accessible to at-risk populations to obtain a long-term functional cure in a limited period is the next challenge.

There are still several barriers to achieve a functional cure. These include, cccDNA, which is not affected by existing treatment, immune system exhaustion, which may be mediated by HBsAg and the presence of integrated HBV sequences, which can actively express viral proteins. Approved monotherapies such as NAs and IFNα do not effectively eradicate CHB infection and they cannot target HBV cccDNA or integrated DNA. CHB therapies can be classified into two functional categories: immune modulators to boost innate and/or adaptive immune mechanisms, and DAAs. High HBsAg serum load is associated with antigen-specific immune tolerance, thus new approaches to reduce viral antigens and/or viral release are being investigated. This approach hypothesizes that reducing HBsAg load would help overcome CHB-induced immune exhaustion. DAAs can be further classified into infection control (entry inhibitors), pre- or post-cccDNA targeting (CAMs, NAs, HBsAg transcriptional modulators) or direct cccDNA targeting. Therapies such as gene editing or epigenetic silencing that directly target cccDNA could eradicate viral replication and lead to a complete cure. Gene editing strategies targeting the HBV DNA would either lead to viral DNA to degradation, if mutations are beyond repair, or modify the viral genome leaving only defective molecules, thus no viral replication/production would be possible. Epigenetic silencing would not lead to HBV DNA eradication. Instead, the intact viral genome would still be present in the infected cells, but it would be transcriptionally silent. Thus, direct cccDNA targeting could potentially achieve a complete HBV cure. However, for the moment no direct cccDNA-targeting strategies have advanced to clinical trials.

Although there are several promising indirect cccDNA-targeting compounds, elimination of all forms of replicating HBV has not been achieved by monotherapy because none of them directly targets cccDNA. Thus, viral replication can reactivate at any stage. It is currently accepted that combination therapy should both disrupt the HBV replication cycle and restore the immune system. Both components of therapy should work together; however, it is still not determined which combination of drugs and protocol would be most effective for the treatment of HBV. Reducing viral antigens to allow T cells to rest, then directly increasing HBV-specific T cells and/or boosting the innate immune response, while at the same time targeting the viral replication cycle to reduce circulating HBV DNA could lead to the desired functional cure. Pre-clinical studies in animal models support this approach.[29,30] Furthermore, they suggest that it is important for reduction of circulating HBsAg to precede boosting the immune system to overcome HBV-immune tolerance and restore effective antiviral immune response. It is possible that the level of HBV-specific T-cell exhaustion might be different in mouse models and in patients with CHB who usually have very long-term infection. Moreover, a specific effect on the intrahepatic cccDNA pool has not yet been proven in these regimens and optimal timing and indications for combination therapies are the focus of several research groups.

There are several pre-clinical phase programs to reduce HBsAg levels in serum. They explore methods to enhance delivery to hepatocytes and evaluate the safety and efficacy of the chosen sequences (Table 1). A few siRNAs have already entered human studies with mixed results. One important result of these studies showed that a significant percentage of circulating HBsAg was derived from integrated sequences rather than cccDNA. Thus, novel siRNAs targeting different regions of the HBV genome are being evaluated in pre-clinical studies.

Several aspects of the definition of a functional cure remain unclear. The goal of a loss of HBsAg is based on the observation that patients who spontaneously lose HBsAg have better outcomes over time. However, it is possible that the improved outcome in this population is because of specific genetic determinants rather than the loss of the antigen itself. Whether integrated HBV will be a main barrier to achieve a functional cure is also a subject of debate and the notion that integrated sequences must be targeted to achieve a functional cure has been challenged. However, further studies on how integrated HBV sequences can affect genome instability and how their transcription products can affect the host are essential to clearly define a complete HBV cure.

In conclusion, although existing therapies for CHB are safe and beneficial, new strategies are still urgently needed to achieve a functional cure. Clinical trials on novel DAAs are in the early stages but are promising. Larger and longer trials are needed to understand if these molecules can consistently provide high functional cure rates. To replace existing therapies, new protocols should have extremely good safety profiles. In order to achieve this goal, combination therapies will probably be needed and their synergistic and unwanted effects should be carefully evaluated.

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REVIEW ARTICLE

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Will we need novel combinations to cure HBV infection?

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Abstract

Chronic hepatitis B is a numerically important cause of cirrhosis and hepatocellular carcinoma. Nucleoside analogue therapy may modify the risk. However, maintenance suppressive therapy is required, as a functional cure (generally defined as loss of HBsAg off treatment) is an uncommon outcome of antiviral treatment. Chronic hepatitis B is a numerically important cause of cirrhosis and hepatocellular carcinoma. Nucleoside analogue therapy may modify the risk. However, maintenance suppressive therapy is required, as a functional cure (generally defined as loss of HBsAg off treatment) is an uncommon outcome of antiviral treatment. Currently numerous investigational agents being developed to either interfere with specific steps in HBV replication or as host cellular targeting agents, that inhibit viral replication, and deplete or inactivate cccDNA, or as immune modulators. Synergistic mechanisms will be needed to incorporate a decrease in HBV transcription, impairment of transcription from HBV genomes, loss of cccDNA or altered epigenetic regulation of cccDNA transcription, and immune modulation or immunologically stimulated hepatocyte cell turnover. Nucleoside analogue suppressed patients are being included in many current trials. Trials are progressing to combination therapy as additive or synergistic effects are sought. These trials will provide important insights into the biology of HBV and perturbations of the immune response, required to effect HBsAg loss at different stages of the disease. The prospect of cures of hepatitis B would ensure that a wide range of patients could be deemed candidates for treatment with new compounds if these were highly effective, finite and safe. Withdrawal of therapy in short-term trials is challenging because short-term therapies may risk severe hepatitis flares, and hepatic decompensation. The limited clinical trial data to date suggest that combination therapy is inevitable.

KEYWORDS

antiviral therapy, Hepatitis B, nucleoside analogues, treatment of hepatitis B, viral eradication

1 | INTRODUCTION

Chronic hepatitis B is a numerically important cause of cirrhosis and hepatocellular carcinoma (HCC) worldwide. HBV can cause HCC in the absence of cirrhosis, but most cases worldwide occur in patients with cirrhosis (70%-90%). Indications for antiviral therapy using nucleoside analogues or pegylated interferon (PEG IFN) have been expanding to include earlier treatment to prevent progression to cirrhosis. The prospect of cures of hepatitis B would ensure that a wide range of patients could be deemed candidates for treatment with new compounds if these were highly effective, finite and safe.

Nucleoside analogue suppression of HBV replication modifies the risk of HCC amongst patients with chronic hepatitis B. Nucleoside analogue therapy is associated with a considerable reduction in risk but not elimination of HCC in patients with chronic hepatitis B. In many cases long-term treatment is required, although treatment may be discontinued in a proportion. Finite, circumscribed courses of treatment with nucleoside analogues and functional cures (generally defined as loss of HBsAg off treatment) are an uncommon conclusion of antiviral treatment. Current HBV therapies suppress viral replication but do not generally cure the disease, as they do not eliminate cccDNA, or integrated viral genomes. Thus, most of patients with chronic hepatitis B require maintenance suppressive therapy. A complete sterilizing cure with viral eradication and elimination of all HBV gene products from the host is improbable at present.

2 | HBsAg CLEARANCE

Spontaneous clearance of HBsAg occurs infrequently at rates of 1% per year in treatment naïve adults with chronic hepatitis B.[1,2] Typically, HBsAg loss has been observed in those with inactive disease and lower degrees of replication encountered in HBeAg-negative infection, and low quantitative HBsAg concentrations. However, HBsAg loss can occur after intense exacerbations of chronic disease in patients with high levels of HBV replication, (or during and after cessation of nucleoside analogue and PEG IFN therapy).[3] Lower concentrations of HBsAg (<1000 IU/mL or lower, and HBV DNA concentrations < 2000 IU/mL) favour HBsAg loss.[4]

HBsAg loss in patients treated with nucleoside analogues is similarly unusual (<1%), but can modify the aftermath of disease.[5-7] The rates are ostensibly higher in patients treated with PEG IFN or combinations of nucleoside analogues and PEG IFN.[8,9] Baseline and on-treatment HBsAg levels that go some way to predicting loss of HBsAg and determine interferon therapy failure have been identified.[10,11]

Incident cirrhosis after HBsAg seroclearance is rare, if it has not developed beforehand. The incidence of HCC is reduced but not prevented if HBsAg loss is achieved.[12-16] A large registry of treated patients in Hong Kong indicated that achieving HBsAg seroclearance reduced the risk of HCC to patients, compared to achieving complete viral suppression with prolonged nucleoside analogue treatment; the complications of cirrhosis were not however altered. Moreover, only 2.1% of patients lost HBsAg in this cohort during follow-up.[17] The potential advantage of HBsAg loss may be lost, however, if it first occurs in patients > 50 years old with pre-existing cirrhosis.[18,19]

Key points

- Several steps in the replication of HBV are potential druggable targets.
- Numerous investigational agents are being developed
- HBsAg loss comprises a recognizable endpoint for ongoing clinical trials of new HBV treatments
- The appropriate combinations and sequence of treatments are being investigated in current trials
- The target patient populations for antiviral and immunomodulatory trials and the appropriate sequence of treatment for patients at different stages may differ.

3 | FUNCTIONAL CURES: ACHIEVING THE GOAL OF HBsAg LOSS WITH IMPROVED TREATMENTS

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. This definition recognizes that HBV genomes are not cleared from the liver. Although a functional cure cannot be considered a true cure, because of persistent integrated viral genomes, HBsAg loss is a recognizable endpoint for ongoing clinical trials of new HBV treatments. HBsAg loss, and thus an improved clinical outcome could be obtained in a higher proportion of patients than is now possible with nucleoside analogues or PEG IFN. A reduction in the HBsAg antigen load could improve immunomodulatory strategies. Measurements of HBsAg are assays are standardized (if the sensitivity of detection and lower limits of quantification are defined). Once HBsAg loss is achieved, the rationale is that there will be no further need for therapy. Based on the observed effects of a reduction in HBV replication, it is hoped that a functional cure, achieved at a relatively young age, will prevent progression to cirrhosis and HCC. However, follow-up of patients with past chronic hepatitis B, especially those with cirrhosis, will be needed to confirm a long-term benefit.

4 | VIROLOGY OF HEPATITIS B AND PROSPECTS FOR A CURE

Hepatitis B virus (HBV) is a member of the hepadnaviridae virus family. The virion comprises an enveloped, 42 nm diameter DNA virus. The complete virion encloses a partially double stranded relaxed circular DNA (rcDNA) genome of 3200 base pairs. The

life cycle of HBV involves several steps: viral entry, uncoating, nuclear import, transcription, nucleocapsid assembly reverse transcription and viral secretion from host hepatocytes. HBV enters hepatocytes through its receptor, sodium taurocholate cotransporting polypeptide (NTCP). After uncoating the nucleocapsid, relaxed circular DNA (rcDNA) is released, and imported into the nucleus. Replication commences by repair and conversion of the rcDNA to covalently closed circular DNA, the circular DNA minichromosome in the nucleus. (cccDNA). The stable episomal cccDNA acts as the template for transcription of HBV mRNAs for subsequent translation to the viral proteins. cccDNA is thought to be synthesized from rcDNA, derived from incoming virions, and replenished from intracellular nucleocapsids which shuttle double-stranded DNA genomes to the nucleus. Four distinct viral transcripts, the pre-genomicRNA (pgRNA), preS1, preS2 and HBx RNAs are synthesized and subsequently translated into seven viral proteins. Core protein (HBcAg) and the HBV polymerase are transcribed from pgRNA, while secretory e antigen (HBeAg) is transcribed from precore RNA. Two types of virions are secreted from the hepatocyte: a population of complete DNA containing virions containing mature nucleocapsids with the partially double-stranded, rcDNA genome and a larger population containing an empty capsid with no DNA or containing RNA.

HBV DNA genomic fragments integrate into the genome of hepatocytes, but it is not thought that integration is mandatory for replication of HBV. In addition to infectious virions, HBV replication results in the excess production and release of subviral empty envelope particles, devoid of viral capsid or genome. The high HBsAg antigen load found in persistent infection, particularly in HBeAg -positive patients may cause profound antigen-specific immune dysfunction and exhaustion.

HBsAg can be transcribed from ccc/DNA but in HBeAg negative patients the probable predominant source of HBsAg is from RNAs transcribed from integrant HBV DNA.[20] This source of HBsAg is relatively inaccessible without cell loss. A better understanding of differences in the composition and source of subviral particles of HBsAg derived from covalently closed circular DNA vs integrated HBV DNA will assist therapeutic strategies.[21]

5 | STRATEGIES TO PROMOTE HBsAg LOSS

HBsAg loss may be promoted by cessation of nucleoside analogues but the results vary and most patients relapse. The strategy cannot be used in patients with severe liver disease because of the risk of decompensation if the disease is exacerbated. Nucleoside analogues can be discontinued after HBeAg seroconversion, in patients with undetectable HBV DNA and consolidation with 12 months or more of additional treatment. The guidelines in HBeAg-negative patients differ. EASL guidelines indicate that nucleoside analogue treatment can be stopped in certain patients (without cirrhosis) who have had undetectable HBV DNA in blood for >3 years. AASLD guidelines advise indefinite treatment unless HBsAg is undetectable. Relapse is common and reports of HBsAg loss vary considerably. Numerous studies have summarized the variability in response in heterogenous groups of patients. Rates of HBsAg loss also vary considerably in prospective studies. Combinations of PEG IFN and nucleoside analogues result in higher rates of HBsAg loss than nucleoside analogues therapy alone. IFN alfa decreases cccDNA transcription via epigenetic modification in experimental systems.[22]

Several steps in the replication of HBV are potential drug targets. Numerous agents are under development as immune modulators, to interfere with specific steps in HBV replication or as host targeting agents that inhibit viral replication by modifying host cellular function, or as immune modulators. Combination strategies will likely invoke deepening the inhibition of HBV replication, lowering viral antigen concentrations (particularly HBsAg) and enhancing the immune response. The investigational treatment landscape is discussed elsewhere in this issue. These potential treatments include targeted HBV entry inhibitors, inhibitors of cccDNA formation, inducers of cccDNA cleavage or transcription inhibition and epigenetic modifiers, core and capsid inhibitors, or perturbations of capsid morphogenesis, RNA interference therapies, HBsAg interaction and assembly or release inhibitors, and multiple immunomodulatory agents including toll like receptors agonists, immune checkpoint inhibitors, therapeutic vaccines, immunological engineering of T cells and cytokines including pathogen receptor agonists. HBX protein, a regulatory protein, transcribed from HBx RNA enhances cccDNA transcription and is an attractive viral target to silence cccDNA transcription.

6 | CURRENT STUDIES OF COMBINATION ANTIVIRAL THERAPY

While HBsAg loss remains an attractive goal, the target patient populations for antiviral and immunomodulatory trials and the appropriate sequence of treatment in patients at different stages of the disease are unknown. The unique virology of HBV poses difficulties for cure: HBV genomic fragments integrate into the genome of the hepatocyte. HBsAg can be transcribed from both cccDNA and integrated viral genomes. The latter source of HBsAg is relatively inaccessible without cell loss of DNA editing. HBsAg antigen load may causes profound antigen specific immune dysfunction and exhaustion. Nonetheless, several steps in the replication cycle are druggable targets and numerous investigational agents that interfere with specific steps in HBV replication or host cellular targeting are being tested in clinical trials. The evidence is still being gathered, however both HBeAg-positive and HBeAg-negative patients are being included in current trials. Empirical trial design and adaptive methodology are being applied to clarify appropriate combinations until we acquire insights into the mechanisms that determine response. Synergistic mechanisms will probably be needed to achieve a decrease in HBV transcription, impairment of transcription from HBV genomes, loss of cccDNA or altered epigenetic regulation of

cccDNA transcription, or hepatocyte cell turnover. Nucleoside analogue suppressed patients are being included in many current trials. Clinical trials are evaluating combination therapy.

Entry inhibitors of HBV have been identified, including neutralizing antibodies targeting the antigenic loop of the S domain, or epitopes expressed in the pre-S1 region; or attachment inhibitors that bind to the virus. Small molecules substrates of sodium taurocholate cotransporting peptide (NTCP); and irreversible NTCP inhibitors for example myrcludex B (bulevertide) block the entry of HBV and HDV. Elimination of both HDV and HBV poses a major challenge. Although viral entry is blocked by the entry inhibitor, HDV propagation through cell division is refractory. However, combination studies demonstrate synergism, blocking both spreading pathways.[23] Studies of myrcludex in combination with PEG-IFNalpha2a have shown promising reductions in HDV RNA concentrations: in the open label MYR203 trial, the combination of myrcludex B 2 or 5 mg and PEG-IFNalpha2a resulted in undetectable HDV RNA in 12/30 (40%) patients 24 weeks after 48 weeks of treatment. HBsAg concentrations reduced by 1 log in 40% of those treated with 2 mg myrcludex B, and in 13% treated with 5 mg in combination with PEG-IFNalpha2a. A long duration of treatment will be required to clear HDV RNA permanently but studies of 2 to 3-year duration are being planned. A marked effect on HDV RNA (−6.09 log) as also been observed with the combination of mycludex B 10 mg qd with 180 g once weekly PEG IFN alpha vs PEG IFN alpha alone (−1.29 log) after 48 weeks. 86% had undetectable HDV RNA at treatment week 48.[24-29] A similar difference in HDV RNA reduction was noted with the combination of myrcludex 10 mg and tenofovir, vs tenofovir (−2.84 vs −4.58 log respectively). The combination of lonafarnib, (an inhibitor of HDV assembly) ritonavir and PEG IFN lambda for 24 weeks in a recent phase 2a trial in 26 patients has shown a median HDV RNA decline of 3.4 log; 10/19 (53%) achieved HDV RNA undetectable or <LLOQ. Gastrointestinal symptoms were the most common adverse effect.[30] REP 2139, a nucleic acid polymer blocks the assembly of HDV and subviral particles preventing release of HDV and HBsAg: intracellular HBsAg is lowered. A 3 year follow-up of study REP 301-LTF, (NCT02876419) has been recently reported: 5/11 participants continue to show functional control of HBV and of HDV.[31]

Covalently closed circular DNA, and cccDNA transcription are targets of new therapies: several cytokines including IFNalpha and lymphotoxin beta receptor agonists lead to upregulation of APOBEC3A and B deaminases to degrade cccDNA. Oral HBV cccDNA destabilizers have been described[32]; HBsAg and HBV DNA reductions, and elimination of cccDNA like molecules in the HBV circle mouse model has been reported.[33-35] Endonucleases, zinc-finger nucleases, TAL effector nucleases, or CRISPR-associated nucleases to specifically target and cleave cccDNA remain experimental compounds.[36-38] Nitazoxanide, an antimicrobial approved for enteric protozoan infection inhibits the HBx-DDB1 protein interaction, to restore Smc5 protein levels and thus may counter the action of HBx, to reduce viral transcription.[39]

Single-stranded oligonucleotides are being investigated in combination. Small interfering (siRNA) to target HBV transcripts, and thereby engender their destruction by the RISC/Ago2 complex are being studied in combination with capsid assembly modulators in both naïve and nucleoside analogue treated patients. Encouragingly, ARC-520 given as a single dose in combination with entecavir resulted in decreases in HBV DNA in HBeAg-positive and HBeAg-negative patients and a decrease in HBsAg concentrations in HBeAg-positive patients. The initially observed lack of effect in HBeAg-negative patients, (because of truncated viral sequences derived from integrated HBV genomes) has been countered by re-designed siRNAs that target all HBV transcripts. GalNac delivery improves uptake.[40-43] GSK3389404, a second-generation liver targeted antisense oligonucleotide has been combined in a phase 2 study with nucleoside analogue treatment. 66 subjects were randomized to either placebo, GSK 404 30 mg weekly, 60 mg weekly or 120 mg biweekly or 120 mg weekly subcutaneous injections for 12 weeks to assess efficacy and safety. Dose-dependent HBsAg declines have been observed in both HBeAg-positive and negative subjects, with mean HBsAg declines ranging from 0.13 to 0.75 log IU/ML in the 120 mg per week treatment arm by day 85. Some subjects showed HBsAg log reductions ranging from 1.54 to 2.72. The Phase 2 study is ongoing.[44]

Sequential combinatorial therapy ideally should result in a rapid and precipitous decrease in HBsAg to precede immunomodulatory therapy. The effect of profound reductions in HBsAg upon restoration of immune reactivity, perhaps coupled with immunomodulatory therapy will be of interest, but restoration of immune reactivity has not yet been proven. Nonetheless, GalNac small interfering RNA knockdown of HBsAg in transgenic mice followed sequentially by an adjuvanted therapeutic vaccine has provided a sophisticated proof of concept.[45]

New capsid assembly modulators (CpAMs) (reviewed elsewhere in this edition) definitively deepen inhibition of HBV replication by disrupting early and late stages of HBV replication. Class I CpAMs induce capsid formation of disrupted morphology. The classification and terminology of CpAMS is currently being reviewed: broadly one class of CpAMs allow the assembly of morphologically normal capsids which are devoid of pgRNA, i.e. empty capsids. A second class results in the formation of abberrant capsids. CpAms inhibit encapsidation of pgRNA and may inhibit both cccDNA formation or replenishment via inhibition of the capsid uncoating step after viral entry and capsid shuttling.[46] These agents result in a decrease in HBV DNA and HBV RNA. Current trials are examining the efficacy of dual therapy with CpAMs plus a nucleoside analogue: The combination of ABI-HO731 (300 mg) plus entecavir for 24 weeks deepened HBV DNA and HBV RNA suppression in both naïve and nucleoside analogue suppressed HBeAg-positive patients compared to entecavir alone.[47] New phase 2 trials will combine siRNA targeting and CpAM administration. They may require prolonged administration to effect HBsAg clearance.

As discussed above, silencing HBV RNA transcripts together with other antiviral compounds is being actively investigated. Preliminary data indicate that triple therapy with RNAi designed to silence HBV transcripts both from episomal cccDNA and

integrated HBV DNA, may prove promising. JNJ73763989 (previously ARO-HBV) given subcutaneously to both HBeAg-positive and negative patients, and to nucleoside analogue experienced or naïve patients has been assessed in a recent study.[48] Patients received three subcutaneous doses of 25, 50, 100, 200, 300 or 400 mg JNJ73763989 every 4 weeks on days 1, 27 and 57; treated patients continued nucleoside analogue therapy beyond the end of the JNJ73763989 dosing. At day 113 the mean HBsAg $\log_{10}$ reduction from day one ranged from 1 to 1.75. In interim data the proportion of patients who achieved a greater than 1 $\log_{10}$ reduction in HBsAg from day one at nadir ranged from 4/8 of those given 25 mg to 8/8 receiving the higher doses. Thus 97% of patients (31/32) achieved a greater than 1 $\log_{10}$ reduction in HBsAg. Evaluation is ongoing. In an exploratory triple combination trial, RNAi JNJ73763989, was given by subcutaneous injection together with a CpAM (JNJ-56136379, at a dose of 250 mg orally daily) for 12 weeks to HBeAg positive and negative patients on entecavir or tenofovir. The combination resulted in a mean 1.7 log change in HBsAg from day 1.[49]

Nucleic acid polymers, which block assembly and release of subviral particles, followed by pegylated interferon or with tenofovir, led to reductions in HBsAg (and anti-HBs development). The exact mode of action is not clear but accelerated intracellular degradation of HBsAg is a putative possibility.[50-54] New classes of phosporamide nucleic acid polymers (STOPS) such as ALG-10,093 are a class of potent oligonucleotides that appear to reduce HBsAg secretion possibly by affecting protein trafficking perhaps resulting in putative degradation of HBsAg protein.[55]

Mechanisms to correct undetectable or weak HBV specific CD8 + T cell response and T cell exhaustion- probably as a result of chronic antigenic stimulation and loss of T cell effector function- are being sought. Immunotherapeutic strategies, (including interferon) either used alone, de novo or in combination with antiviral agents currently include TLR-7 and TLR-8 agonists, therapeutic vaccines, checkpoint modulators, a RIG-I agonist and anti-HBV antibodies. However serious adverse events have recently been reported in patients receiving inarigivir.[56] In a woodchuck model blocking PD-L1 together with HBV therapeutic vaccination reduced HBV DNA concentrations.[57,58] Unfortunately it has proven more difficult to validate the efficacy of immunomodulator therapies in human studies.[59-65] A recent combination of a TLR7 agonist RO7011785 in 3 cohorts of chronic hepatitis B patients, including virologically suppressed patients has been primarily designed to show the safety of the combination. The combination shows evidence of immune activation but the short 6 week dosing period to date does not allow an effect on HBsAg pharmacodynamics as yet. Longer duration studies are planned.[66] GS −9688, an oral selective small molecule agonist of TLR8 has been evaluated in a phase 2 study of 48 HBeAg-positive or negative patients suppressed on nucleoside analogue therapy. 3.0 mg, 1.5 mg or GS-9688 placebo were given once a week for 24 weeks in combination with nucleoside analogues. One HBeAg negative patient in the study (1.5 mg group) achieved the primary endpoint of ≥1 log10 IU/ml decline in HBsAg concentrations at week 24 and two other patients in the study (HBeAg-negative and positive) achieved HBsAg loss at week 24.[67]

7 | EXPERIMENTAL COMBINATIONS

The combination of a liver targeted HBV locked nucleic acid antisense oligonucleotide with RO7020531, a TLR7-agonist in the AAV HBV mouse model in mice treated for 8 weeks showed that the combination reduces HBsAg and HBV DNA concentrations compared to monotherapy. The combination also delayed rebound for several weeks after the end of treatment.[68] Treatment of HBV infected hepG 2-NTCP cells with HBV specific siRNA inhibited HBV replication and suppressed HBV antigen production. The reduced antigen production initially suppresses CD8 T cell recognition, but CD8 T cell recognition shows evidence of subsequent recovery after siRNA treatment.[69] A hypothesis has been proposed that a liver targeted PD-L1 locked nucleic acid antisense oligonucleotide can result in effective suppression of PDL 1 expression in the liver with the potential to overcome the immune tolerance observed in hepatitis B virus infection.[70]

Experimental combination therapy with a CaPM (Bay41-4109 alone or combination with IFN alpha activates an innate immune response in HBV infected HepG2-hNTCP cells, primary human hepatocytes and human liver chimeric TK-NOG mice.[71]

New putative serum biomarkers of infection include HBV core related antigen (HBcrAg), theoretically comprising hepatitis B core antigen, HBeAg and the 22 kDA precore protein encoded by the precore-core gene.[72] HBV RNA is detectable in serum. Therefore, HBcrAg or HBV RNA may be useful viral markers that are independent of HBV DNA for monitoring the antiviral effect of nucleoside analogues. These biomarkers can remain detectable in serum in patients with undetectable HBV DNA; HBcrAg and HBV RNA may correlate with cccDNA and provide an important clinical surrogate and marker of transcriptional activity of cccDNA; reductions in pgRNA result in a reduction in subsequent viral reverse transcription.

8 | CONCLUSIONS

New compounds are currently limited to clinical trials and proof of mechanism and safety studies. Strategies with a combination of agents and additive or synergistic effects being sought. These agents will provide important insights into the biology of HBV and perturbations of the immune response, required to effect HBsAg loss at different stages of the disease. New combination therapies for HBV will require individualization but broad eligibility is sought. The safety of new therapies will be paramount given the safety of currently approved nucleos(t)ide analogues. Patients with cirrhosis are being excluded from early phase trials. Harm vs benefit in young adults will require careful consideration. Withdrawal of therapy in short-term trials is challenging because short-term therapies may have the risk of severe hepatitis flares, hepatic decompensation or

death. Therapies in development that rely on altering CD8 T cell recognition will require a deeper understanding of the effects of new inhibitory compounds and interactions between hepatocyte antigen expression, inflammatory cytokines and adaptive immune responses. Combination treatments appear to be an unavoidable strategy for improving functional cure. The cost of combination curative treatments may become problematic in low- and middle-income countries, given the low cost of generic nucleoside analogues and the minimal monitoring required.

CONFLICT OF INTEREST

None declared.

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REVIEW ARTICLE

Liver INTERNATIONAL WILEY

Hepatitis E, what's 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 in the world with an estimated 20 million cases every year and 70 000 deaths. Hepatitis E 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 the 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. The prevalence of positive HEV viraemia in blood donors in Europe ranges from 1/600 to 1/2500 in highly endemic European countries. HEV can cause neurological disorders and chronic infections in immunocompromised patients. The progression of acute hepatitis E is usually asymptomatic and resolves spontaneously. Diagnostic tools include anti-HEV IgM antibodies in serum and/or viral RNA detection in the blood or the stools by PCR. Ribavirin is used to treat 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 respectively encode the capsid protein and a protein involved in releasing new virions.[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] Among the 8 genotypes of *Orthohepevirus* A, only 1 (Asia and Africa) and 2 (Mexico and Africa) infect humans whereas 3 and 4 are endemic in pig, wild boar and rabbits and cause zoonotic infections. 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 essentially 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).[4]

2 | EPIDEMIOLOGY

In developing countries, HEV infection is characterized by large epidemics and a waterborne source of contamination. For example, the 1978 epidemic in the Kashmir valley in India was responsible for 52 000 symptomatic cases and 1700 deaths.[1] Sporadic cases occur in

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

between epidemics. Transmission is through direct ingestion of water contaminated by human faeces.[1] There is also a risk of maternal-fetal transmission of HEV, causing neonatal infections.[5] In these countries, 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.[6] There is an increased risk of maternal complications, mainly during the third quarter, with an increased risk of fulminant hepatitis and obstetric complications.

In developed countries HEV is a zoonosis and there is a risk of transmission by ingestion of contaminated meat (mainly pork, with HEV genotype 3 or 4).[7] Parenteral transmission by blood transfusion of blood products has been identified as a new potential mode of contamination.[8] In a recent study from England in a population of blood donors, 1 in 2848 had a positive HEV viraemia. Sixty-two contaminated blood products were transfused, leading to HEV infection in recipients in 42% of cases.[8] In France, the prevalence of positive HEV viraemia in blood donors is estimated to be 1/800.[9] A French study reported 23 cases of transfusion-transmitted infections between 2006 and 2016, including 14 with chronic hepatitis in immunosuppressed patients.[10]

In the 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.[2,7] Patients with chronic liver disease are at risk of decompensation and death, and maternal complications have not yet been described.

Keypoints

- Hepatitis E virus infection is usually a zoonotic infection in developed countries, transmitted by ingestion of contaminated food.
- It is a cause of severe decompensation in patients with cirrhosis and 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 show HEV viraemia in 1/600 to 1/2500 in highly endemic European countries and in 1/2300 to 1/14 500 in European countries with intermediate to low endemicity.[11] 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.[12] The symptoms are nonspecific and common to other viral hepatitis: asthenia, diarrhoea, 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.[7] HEV is a self-limiting infection that lasts for 4 to 6 weeks. There are severe forms in patients with cirrhosis and the elderly.[13] 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.

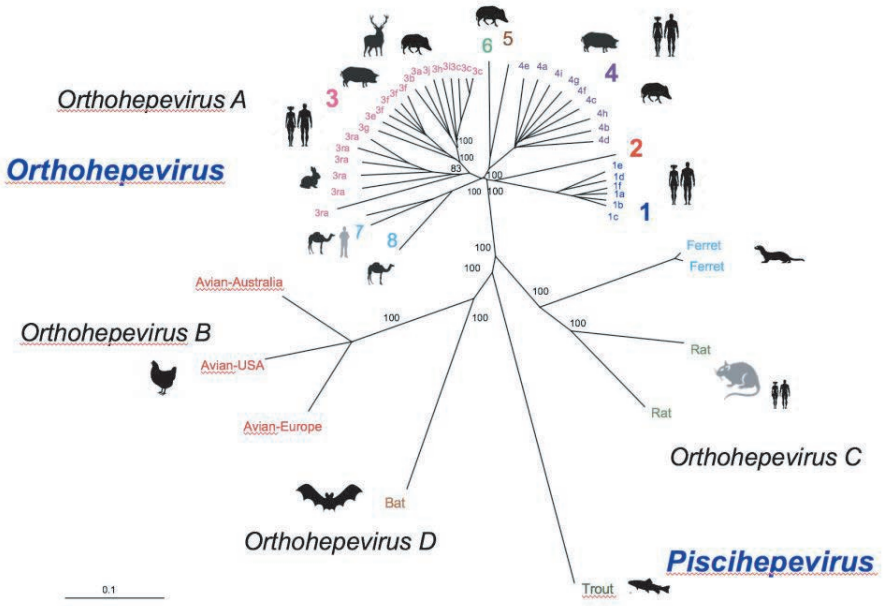

FIGURE 1 Hepeviridae phylogenetic tree

Acute hepatitis E can be mistaken for drug-induced hepatitis. Two studies describing patients who had an initial diagnosis of drug-induced hepatitis retrospectively tested for HEV found acute hepatitis E in 3% and 13% respectively.[14,15]

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.[16] 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.[12] Neurological injury include neuralgic amyotrophy (Parsonage Turner syndromes, PTS), Guillain Barré syndromes (GBS), meningoradiculitis and mononeuropathis multiplex.[17] 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%.[18] 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.[19,20] The prevalence of HEV infection in patients with GBS is 5%.[21]

The mechanisms by which HEV causes PTS or GBS are unknown, but it is probably an immune response induced by the virus. 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.

5 | CHRONIC HEPATITIS

HEV may cause chronic hepatitis in immunocompromised patients such as solid organ transplants,[22] patients undergoing chemotherapy for haematological malignancies,[23] and in some patients with HIV.

In these cases, acute hepatitis E is usually asymptomatic or pauci-symptomatic. Liver enzymes are usually moderately elevated and jaundice is rare. The incidence of infection with genotype 3 HEV after organ transplantation was 3.2/100 person-years in the southwest of France.[24] Sixty percent of these patients with acute hepatitis E will develop chronic hepatitis.[22] 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.[25]

Chronic hepatitis E has also been described in patients with haematological malignancies.[23] They are often asymptomatic. 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.[23]

Chronic hepatitis only occurs in patients infected with HIV and very low CD4 counts, always <250/mm^3.[26] 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, that is the same as in the general population.[27] The management of HIV treatment can be complicated during acute hepatitis E.

A recent French multicentre retrospective study was performed in patients with immunosuppressive therapy for inflammatory rheumatism.[28] Twenty-three cases of acute hepatitis E were reported including 18 patients treated with biotherapies (10 under anti-TNF).

6 | 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.[29] 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, appear early and last at least 16 weeks.[30] The sensitivity of tests in immunocompetent patients is excellent (>98%). Thus, the diagnosis in immunocompetent patients can be based on serology. In immunocompromised subjects, however, RNA detection is essential.

IgG appear shortly afterwards and last for years. The sensitivity of available commercial kits (Wantai) for the detection of IgG is excellent (>97% in immunocompetent patients) and specificity is very good (>99.5%) for IgM. 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.[31]

7 | TREATMENT AND PREVENTION

In most cases, the infection is self-limiting, and does not require treatment. Monitoring of liver function tests is recommended like in any viral acute hepatitis, 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.[32] 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. Risk factors for relapse are lymphopenia at the initiation of treatment, HEV RNA detected in serum after 1 month of treatment, the presence of HEV RNA in the stools at the end of treatment[32] and a decreased viral load <0.5 $\log_{10}$ copies/mL day 7 after treatment is initiated.

Chronic or persistent hepatitis in patients with HIV or receiving chemotherapy for haematological malignancies can be treated with ribavirin in the same manner.[23]

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

In developed countries where transmission is essentially due to 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). Its routine use began following a randomized placebo controlled phase III study between 2007 and 2009 in China.[33] 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.

8 | 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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REVIEW ARTICLE

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New epidemiology of hepatitis delta

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Abstract

Hepatitis D virus (HDV) is a defective pathogen that needs hepatitis B virus (HBV) for infection. Co-infection of HBsAg-positive individuals with HDV is commonly associated with a more rapid progression to cirrhosis, a higher incidence of hepatocellular carcinoma (HCC) and increased mortality. Initial studies have shown that about 5% of chronic HBV carriers worldwide (15-20 millions) were also infected with HDV. However, recent studies suggest that the prevalence of HDV is at least two- to three-fold higher than previous estimations. Improved diagnostic techniques have shown that HDV infection remains endemic in certain areas of the world. Injection drug users, individuals with high-risk sexual behaviour and patients co-infected with human immunodeficiency virus (HIV) represent the major reservoir of the disease in the Western world. Although the burden of HDV infection significantly decreased in Europe in the nineties, there has been no further decrease in the last decade, probably because of migration from HDV endemic countries. Until new and more effective therapies are available, public health measures should be reinforced by increasing prophylactic HBV vaccination programs, preventing transmission of the virus among parenteral drug users and implementing universal HDV screening of all HBV-infected individuals.

KEYWORDS
endemic, HBV, HDV, hepatitis D, prevalence

1 | INTRODUCTION

Hepatitis delta virus (HDV) is a defective pathogen that requires the helper functions of hepatitis B virus (HBV) for infection. It results in acute or chronic hepatitis, which may occur either by simultaneous co-infection with HDV and HBV or by HDV superinfection in a person who is already chronically infected with HBV.

HDV infection was initially identified in 1977 by Mario Rizzetto and colleagues who noted cases of severe hepatitis in individuals thought to be mono-infected with HBV.[1] Since the discovery of HDV 40 years ago, the unique virology of HDV and its relationship with HBV have been elucidated.

Assays for the antibody to HDV (anti-HDV) as the serological marker of HDV infection were developed soon after the discovery of the virus and became commercially available in 1984. The cloning and sequencing of the viral genome in 1986 and the subsequent virological research identified the features that make HDV unique among animal viruses.[2] Clinical studies in endemic areas in the 1980s showed that HDV was pathogenic, almost invariably causing liver disease that often led to cirrhosis and liver failure.

In this article, we present the current data on the burden and the global distribution of HDV infection, the recent changes in epidemiology and potential limitations in the accurate estimation of the prevalence of this disease.

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

2 | DISEASE PREVALENCE AND CHARACTERISTICS

The global disease burden of HDV infection is still a matter of debate. The 2017 WHO Global Hepatitis Report highlighted the uncertainty of the extent of the HDV burden. Previous studies in the 1980s showed that about 5% of chronic HBV carriers worldwide (15-20 million individuals) also had HDV infection. It was slightly more common in men than in women.[3] However, in a recent systematic review and meta-analysis, Chen et al suggested that more than 60 million individuals worldwide could be infected with HDV.[4] The authors analysed 182 studies from 61 countries and regions and showed that the overall prevalence of HDV was 0.98%. The prevalence of HDV in HBV carriers reached 10.6%, which is twice as high as previous estimations. Although several methodological issues have been raised about this otherwise well-conducted study, it clearly shows that the number of HDV-infected patients is probably much higher than previous estimations.

Chronic hepatitis D has commonly been described as a severe, progressive form of liver disease that is resistant to therapy. Long-term studies have reported that HDV infection in patients with chronic hepatitis B infection is associated with more rapid progression to cirrhosis compared to chronic hepatitis B alone, indicating that HDV infection tends to have a negative influence on the clinical outcome of chronic hepatitis B.[5] However, in epidemiological pockets such as the Greek island of Archangelos, HDV caused no or insignificant disease, suggesting that the clinical course of HDV infection may range from asymptomatic carriage of the virus to very severe disease.[6] More recently, a study performed in Italy reported a relatively low tendency to evolve into cirrhosis (4%) and a slow progression to major liver-related complications, such as HCC (4%), after a median follow-up of 25 years. Furthermore, in another study from Spain including 158 patients with chronic HDV infection who were followed for a median of 13 years, HBsAg clearance was observed in 8%, while 72% of the patients remained stable, 18% developed hepatic decompensation and 3% hepatocellular carcinoma.[7]

One factor that could influence the course of the disease is the HDV genotype.[8] Eight HDV genotypes have been identified to date. Genotype 1 has a wide distribution, genotypes 2 and 4 are found predominantly in Asia, genotype 3 is found in the Amazon basin and genotypes 5-8 are usually detected in Africa. In a study from Taiwan, patients infected with HDV genotype 1 had a lower remission rate and more adverse outcomes than those with genotype 2. HDV genotypes 2 and 4 seem to cause relatively mild disease, while genotype 3 has been associated with fulminant hepatitis in South America. Although the HBV genotype does not affect the interaction of HBsAg with HDV, the HDV genotype may influence the assembly with the HBsAg into virions.

Key points

- The global prevalence of hepatitis D virus (HDV) is probably higher than previously estimated.
- HDV infection remains endemic in many developing countries.
- Drug addicts, people with high-risk sexual behaviours and individuals co-infected with the human immunodeficiency virus are the major reservoir of HDV in the Western world.
- After an initial control of HDV infection in the nineties, HDV prevalence is increasing in Europe because of immigration from endemic areas.
- Public health measures and universal HDV screening of all persons infected with hepatitis B virus (HBV) remain critical for the elimination of HBV and HDV co-infection.

3 | HIGH PREVALENCE REGIONS

Certain high prevalence areas have been described and include 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.[9]

Initially, small studies were performed to establish local prevalence of HDV. There have been no systematic investigations and most studies were based on a few patients with diverse clinical features. Furthermore, in many countries there were no facilities or resources for local testing of HDV. Increased awareness and improved diagnostic testing have resulted in renewed evaluation of hepatitis D in the developing world.

The prevalence of HDV infection in HBsAg-positive populations in sub-Saharan Africa was recently reported in a systemic review and meta-analysis including 30 studies from 1995 to 2016.[10] In West Africa, the pooled seroprevalence of HDV was 7.3% in the general population and 9.6% in patients with liver disease. Specifically, the seroprevalence of HDV was high in Mauritania (20%), in Cameroon (14%), in pregnant women in Benin (11%) and in Ghana (8%), while it was low in Nigeria (5%), Senegal (3%), Burkina Faso (3%) and Gambia (2%). The seroprevalence of HDV in Central Africa was 26% in the general population and 38% in liver-disease populations. It is worth noting that 50% of patients with liver disease were HDV positive in the Central African Republic and that HDV was detected in 58% of HBsAg positive individuals and 17% of HBsAg pregnant women in Gabon. Interestingly, in East and South Africa, the seroprevalence of HDV was only 0.05% in the general population, although the data are insufficient to draw firm conclusions (Table 1). These findings suggest that there is a significant geographical variability, with highly endemic pockets in Central and West Africa. Overall, the seroprevalence of HDV in HBsAg-positive populations in several countries of West and Central Africa exceeds the estimated global seroprevalence, suggesting that they may contribute substantially to the burden of liver disease in sub-Saharan Africa.

Collective data from systematic reviews indicate that HDV is highly endemic in and around the East Mediterranean Region and

TABLE 1 Worldwide seroprevalence of antibodies against hepatitis D virus (HDV) among HBsAg-positive carriers in studies published after 1995[a]

Area/Country	No. of studies	Date of data collection	Subjects tested, n	HDV prevalence among HBsAg-positive carriers
Europe				
Italy	1	2008	1386	8.1%
UK (London)	1	2000-2006	962	8.5%
Spain	1	1998-2012	429	6.1%
Greece	1	1997-2010	2137	4.2%
France	1	1997-2011	4492	2%
Romania	1	2011	2761	23.1%
North and South America				
USA	1	1999-2013	2175	3.4%
Colombia	2	2011-2015	58	32.8%
Equador	1	1998	47	31.9%
Peru	1	1996	82	39%
Venezuela	1	2002-2004	54	11.1%
Brazil (Ama-zon basin)	6	1996-2006	291	36.8%
Argentina	1	2003-2009	109	0.9%
Asia including Middle East				
China	27	1997-2016	17 163	5.6%
Iran	13	2000-2010	4358	3.9%
Pakistan	3	2000-2009	21	28.8%
Turkey	12	1997-2003	4712	7.9% (West: 4.8%, South-East: 27.1%, Central: 12.1%)
Lebanon	1	2007	107	0.9%
Saudi Arabia	1	2004	60	3.3%
West & Central Africa				
Burkina Faso	2	2001 & 2015	217	3.2%
Benin	1	2011	44	11%
Gambia	2	2011	686	1.3%
Ghana	1	2015	107	8%
Mauritania	3	2008-2009	718	19%
Nigeria	1	2014	103	5%
Senegal	1	2003	175	3%
Cameroon	3	2007-2011	1903	13%
Gabon	3	2005-2015	307	43%
East & Southern Africa				
Mozambique	1	2007	146	0%
South Africa	2	2008	93	0%

[a]Data derived from references [4,10-12,15,16,24,26,27,30].

Middle East.[11] The pooled HDV prevalence in asymptomatic HBsAg carriers was 11% in Egypt, 26% in Sudan, 7% in Saudi Arabia, 5% in Iran and 18% in Pakistan. Overall, the prevalence of anti-HDV in these areas was 15% (Table 1).

In other parts of Asia, HDV remains a major medical problem in the South Eastern and Eastern parts of Turkey, where anti-HDV was detected in 28% and 45% of patients with chronic hepatitis B respectively.[12] HDV endemicity is consistent in Uzbekistan, where 80% of patients with HBsAg-positive cirrhosis were co-infected with HDV.[13] The median age of patients with HDV cirrhosis was 39 years old, confirming that HDV is the major cause of advanced liver disease and of juvenile cirrhosis in that country. In Mongolia, where the prevalence of hepatocellular carcinoma is the highest in the world, an exceedingly high rate of anti-HDV positivity (74%) was recently reported in the general HBsAg-positive population.[14]

There is a discrepancy between the high prevalence of endemic HDV in Taiwan and the very low prevalence in nearby Japan and Korea, even though these countries are all highly endemic for HBV. This suggests that HDV may have a different capacity to superinfect different populations of HBsAg carriers, possibly because of genetic resistance, host susceptibility to HDV or to viral genetics resulting in different relationships between HBV and HDV.[15]

A recently published review reported the estimated prevalence of anti-HDV in HBV carriers in South American countries, a geographical region that is traditionally endemic for HDV.[16] The pooled HDV prevalence was 22%, although it was less frequent in certain countries and populations. It was low in Argentina (2.6%) but high in Bolivia (22%), Colombia (27%), Peru (41%) and Venezuela (39%). Most of the studies were from Brazil, where the HDV prevalence is high in the Amazon basin (32%) but lower outside the Amazon basin (11%) (Table 1). However, those data should be interpreted with caution because of selection biases as a result of a lack of information from several countries far from the Amazon basin, as well as data from studies performed in the 1980s and 1990s, which resulted in an overestimation of the cases of HDV infection in the area.

4 | HIGH RISK POPULATIONS

The risk of HDV infection is high in injection drug users, patients with multiple sexual partners, men who have sex with men, individuals infected with human immunodeficiency virus (HIV) or hepatitis C virus (HCV) and people from endemic areas.[17] Soon after the discovery of HDV, injection drug users sharing needles were recognized as the major victims of this infection, with reported anti-HDV rates in HBsAg-positive patients in the 1980s ranging from 17% in Southern Europe to more than 90% in South Eastern Asia.

In the 1990s, the focus on HDV in drug users decreased in Europe because of the reduction in HBV in these communities. However, recent surveys in several European countries such as the United Kingdom, Germany, Spain and Switzerland have revealed that HDV is still endemic in communities of drug users.[18] The prevalence of HDV infection has also increased in injection drug users in the United States. In one injection drug user cohort in Baltimore, 50% of HBV infected individuals were found to be infected with HDV.[19] In a similar cohort of injection drug users assessed in San Francisco, 36% of HBsAg positive individuals were found to have HDV viral infection.[20] Therefore, even in developed countries, HBV infected injection drug users are at high risk of HDV infection.

The significant contribution of drug users to the burden of HDV infection has been clearly shown in a recently published review including data on the prevalence of HDV in the global population.[4] The authors found that the injection of illicit drugs is driving the HDV epidemic in HBsAg-positive individuals. The HDV seroprevalence in HBsAg-positive injection drug was three times higher than that in the population without any risk factors (37.6% vs 10.6%). The HDV seroprevalence was much higher in HIV-infected than HIV-noninfected injection drug users, which indicates that HIV co-infection might be an additional risk factor for HDV infection, perhaps because of high-risk behaviours. Moreover, a higher HDV seroprevalence was found in individuals with high-risk sexual behaviour (17%) either homosexual or heterosexual.

5 | CHANGING EPIDEMIOLOGY OF HDV INFECTION

After the discovery of HDV in Italy, prevalence rates >20% were reported in Southern Europe and HDV was considered endemic and an important cause of chronic hepatitis and cirrhosis in these areas. Elsewhere, in North America and Northern Europe, the infection was largely confined to intravenous drug addicts, in whom it was a major cause of severe and fulminant HBsAg hepatitis.[21]

At the end of the 1980s, the prevalence of HDV began to decrease in Europe. Better public health standards, HBV vaccination programs, disposable syringe practices and the effect of measures to control the spread of HIV resulted in a decrease in the number of HBsAg carriers and thus a decline in HBV-dependent HDV. In Italy, anti-HDV in HBsAg carriers with liver disease decreased from 25% in 1983 to 8% in 1997, with similar reductions in HDV seroprevalence reported in Turkey and Eastern European countries, based on better public health measures and HBV vaccination programs.[22]

By the end of the 1990s, the decrease was so significant in Southern Europe that it was hypothesized that HDV infection was one step away from eradication. However, in the last decade HDV has not declined further in Europe, probably because of migration from HDV endemic areas, which may counterbalance the residual cohort of long-term infections acquired in the epidemics of the 1970s and 1980s.

In a recent report from Italy, the overall prevalence of anti-HDV in chronic HBV patients was 10%, indicating that HDV infection has not continued to decline.[23] Similar figures have been reported in the United Kingdom (8.5%) and Germany (8%-14%) and are related to the increasing number of patients who have migrated from endemic areas.[24,25] Both in the UK and Germany, most HDV carriers are migrants from Eastern Europe, Africa, the Middle East and Turkey. In France, HDV infection is mainly seen in people from North Africa. The recent identification of HDV genotypes 5-7 in Europe could be a result of migration from Africa. In a prospective Greek study, the prevalence of anti-HDV in HBsAg-positive individuals was 4.2% and it was lower in native Greeks (2.8%) than in immigrants (7.5%) who contributed more than 50% of the HDV infection burden in Greece.[26] In Spain, as in other European countries, immigration has increased substantially in the last few years, with most immigrants originating from North Africa, Eastern Europe, and South America. A recent study from Northern Spain showed a decrease in the prevalence of HDV infection over the last 30 years, although it is still above 5% in chronic HBV patients. Moreover, in the last 5 years of the study, the downtrend stopped and a slight increase was observed. There was also a clear change in the epidemiology of HDV and migration from endemic regions is an important risk factor for HDV infection.[27]

Available data show that the current residual reservoir of HDV in Europe consists of two populations. One HDV population includes native patients who were infected in the 1970s and 1980s. A minority of this group has long-term indolent disease, while most of them have advanced liver disease. The second HDV population includes young immigrants with active chronic hepatitis D who have arrived from areas in which HDV infection is endemic. Although immigration will probably not affect local European populations who are protected by HBV vaccination, public health measures are needed to prevent the spread of HDV in immigrant communities. In contrast, HDV will probably not be controlled in hyperendemic areas where HBV is still unchecked and the presence of HDV infection has been reported in a substantial proportion of the HBsAg-positive population.

6 | CURRENT ISSUES IN THE ESTIMATION OF THE HDV PREVALENCE

The epidemiology of HDV infection is still not fully understood and the global prevalence of HDV remains unknown.[3] This is partially because of the use of less accurate serological tests in older studies. Although it is true that enzyme immunoassays, which are considered to be less reliable, were routinely used for the detection of anti-HDV, another underestimated but relevant factor that could artificially confuse epidemiological figures, is the lack of HDV testing. Because hepatitis D was considered to be a relatively uncommon disease in the late 1990s, it is highly probable that certain patients were not tested.

In a study performed in Northern California, Gish et al reported that the prevalence of HDV in HBsAg positive patients was 8%. However, in that study, only 42% of the 1191 HBV infected individuals were tested for HDV. It is important to note that 67% of the patients with HDV infection were diagnosed with cirrhosis compared to only 22% of the HBV mono-infected cohort.[28] Similarly, in a Midwestern US population of HBV-infected patients, Safaie et al found that 3.3% were positive for anti-HDV. However, only 12% of the 1007 HBsAg-positive patients were tested for anti-HDV.[29] Furthermore, in a retrospective Veterans Administration study, 7.8% of HBsAg-positive patients were tested for HDV and 3.6% of them were found to have HDV infection.[30]

These studies emphasize the need to achieve universal HDV screening in all HBV infected individuals. In addition, HBsAg carriers without prior HDV exposure should be counselled on their risk of HDV superinfection. Increasing coverage of prophylactic HBV vaccination remains critical for the elimination of both HBV and HDV.

7 | CONCLUSIONS

HDV infection represents a global health problem associated with increased liver related and overall mortality. Recent surveys suggest that the global HDV prevalence has been underestimated. Increased availability of reliable and sensitive diagnostic tests has shown that HDV infection is still endemic in many developing countries of the world. Illicit drug users, people with high-risk sexual behaviours and individuals with HIV co-infection are the high-risk groups in the Western world. Although a genuine decrease in HDV prevalence was achieved in the developed countries in the 1990s, no further decline has been noted since then and a slight increase was observed in the last decade. This increase in HDV prevalence is mainly linked to the migration of populations from endemic countries. Therefore, further action should be taken to control HBV infection through HBV vaccination campaigns and to implement universal HDV screening for HBV infected patients.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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REVIEW ARTICLE

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Future treatments for hepatitis delta virus infection

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Abstract

Around 15-20 million people develop chronic hepatitis delta virus worldwide. Hepatitis delta virus (HDV) is a defective RNA virus requiring the presence of the hepatitis B virus surface antigen (HBsAg) to complete its life cycle. HDV infects hepatocytes using the hepatitis B virus (HBV) receptor, the sodium taurocholate cotransporting polypeptide (NTCP). The HDV genome is a circular single-stranded RNA which encodes for a single hepatitis delta antigen (HDAg) that exists in two forms (S-HDAg and L-HDAg), and its replication is mediated by the host RNA polymerases. The HBsAg-coated HDV virions contain a ribonucleoprotein (RNP) formed by the RNA genome packaged with small and large HDAg. Farnesylation of the L-HDAg is the limiting step for anchoring this RNP to HBsAg, and thus for assembling, secreting and propagating virion particles. There is an important risk of morbidity and mortality caused by end-stage liver disease and hepatocellular carcinoma with HDV and current treatment is pegylated-interferon (PEG-IFN) for 48 weeks with no other options in patients who fail treatment. The ideal goal for HDV treatment is the clearance of HBsAg, but a reasonably achievable goal is a sustained HDV virological response (negative HDV RNA 6 months after stopping treatment). New drug development must take into account the interaction of HBV and HDV. In this review, we will present the new insights in the HDV life cycle that have led to the development of novel classes of drugs and discuss antiviral approaches in phase II and III of development: bulevirtide (entry inhibitor), lonafarnib, (prenylation inhibitor) and REP 2139 (HBsAg release inhibitor).

KEYWORDS

direct-acting antivirals, entry inhibitors, HBV DNA, secretion

Abbreviations: ADAR 1, adenosine deaminase acting on RNA 1; AE, adverse event; ALT, alanine aminotransferase; APOBEC3A/3B, Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A/3B; AST, aspartate aminotransferase; BLV, bulevirtide; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; CHD, chronic hepatitis D; ER, endoplasmic reticulum; ETV, entecavir; HAP, heteroarylpyrimidines; HBeAg + ve, hepatitis B e antigen-positive; HBeAg, hepatitis B e antigen; HBeAg - ve, hepatitis B e antigen-negative; HBsAg, hepatitis B surface antigen; HBV SVPs, hepatitis B virus subviral particles; 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 sulfate proteoglycans; IFN-α, interferon alpha; L-HDAg, large hepatitis delta antigen; LNF, lonafarnib; mRNA, messenger RNA; NA, nucleoside analogue; NI, nucleoside inhibitors; NNI, non-nucleoside inhibitors; NTCP, sodium taurocholate cotransporting polypeptide receptor; nucleocapsid, precore protein; ORF, open reading frames; PEG-IFN, pegylated-interferon; pgRNA, pregenomic RNA; PP, phenylpropanamides; QD, once daily; rcDNA, relaxed circular DNA; RTV, ritonavir; SBA, sulfamoylbenzamide; S-HDAg, small hepatitis delta antigen; siRNA, small interfering RNA; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TFV, tenofovir; TLR, toll-like receptor.

1 | INTRODUCTION

The hepatitis delta virus (HDV) was identified in 1977.[1] HDV infects up to 70 million people worldwide and 15-20 million of these develop chronic hepatitis delta (CHD), with different prevalences depending on the region. There is a significant risk of morbidity and mortality caused by end-stage liver disease and hepatocellular carcinoma (HCC). Because HDV requires hepatitis B surface antigen (HBsAg) to complete its life cycle, patients with hepatitis B virus (HBV) infection are at risk of having HDV co-infection and should be screened for HDV.[2] Early diagnosis and treatment should be considered. Although a prophylactic vaccine is available to protect against HBV and therefore HDV infection, vaccination campaigns are not well-implemented. Current treatment of HDV is pegylated-interferon (PEG-IFN) for 48 weeks with no other therapeutic options in patients who fail PEG-IFN. Nucleos(t)ide analogues (entecavir [ETV] and tenofovir [TDF]) have not been approved for HDV infection. Although significant advances have been made in the treatment of chronic viral hepatitis, targeting HDV is still a major challenge because of the unusual nature of this virus and the severity of the disease. Indeed, HDV does not encode its own polymerase but uses the host RNA polymerase II to replicate. Thus, unlike HBV, which possesses a virus-specific polymerase that can be targeted by specific inhibitors, the lack of an HDV-specific polymerase makes HDV a particularly challenging therapeutic target. Knowledge of the HDV viral cycle is important since each step of the cycle is a potential target for the development of new drugs. The ideal goal of HDV treatment is the clearance of HBsAg, which is equivalent to elimination, while an achievable goal is a sustained HDV virological response (negative HDV RNA 6 months after stopping treatment). Drugs under development include mainly entry inhibitors, prenylation inhibitors, and HBsAg release inhibitors. In this review, we will discuss the steps of the HDV cycle and direct antiviral approaches in phases II and III of development.

Key points

- Hepatitis delta virus (HDV) is a defective virus that requires the presence of the hepatitis B virus (HBV) for its own viral cycle infection.
- There is an important risk of morbidity and mortality caused by end-stage liver disease and hepatocellular carcinoma with HDV.
- HDV, like HBV, infects hepatocytes via a high specificity interaction with human sodium taurocholate cotransporting polypeptide receptor (NTCP) expressed on the basolateral membrane of hepatocytes.
- Current treatment of HDV is pegylated-interferon (PEG-IFN) for 48 weeks. There is no therapeutic option for patients who fail PEG-IFN.
- Drugs under development include mainly entry inhibitors, prenylation inhibitors and HBsAg release inhibitors.
- There is an urgent need to cure HDV infection, a cure to HBV will also lead to a cure to HDV.

2 | HDV VIROLOGY AND TARGETS FOR NEW DRUGS IN DEVELOPMENT

Identified in 1977, HDV is a small hepatotropic enveloped RNA virus, member of the *Deltavirus* genus.[1] HDV virus is a defective virus which highjacks the HBsAg of HBV for its own viral cycle infection.[2] The HDV virion is a glycolipidic spherical structure that measures approximately 36 nm in diameter on which HBsAg are exposed: small (S-HBsAg), medium (M-HBsAg) and large (L-HBsAg) HBsAg (Figure 1A).[3] The HDV lifecycle is illustrated in Figure 1B.

Like HBV, HDV infects hepatocytes by attachment on the cell surface with heparan sulfate proteoglycans (HSPGs) and via high specificity interaction with the human sodium taurocholate cotransporting polypeptide receptor (NTCP, SLC10A1) expressed on the basolateral membrane of hepatocytes (Figure 1B).[4,5] The key to HDV infectivity is the NTCP-binding domain (75 amino acids) present on the PreS1 domain of the HBV L-HBsAg.[5] Targeting the interaction between the virus and NTCP is one of the therapeutic strategies used to prevent HDV and HBV infection.[6,7] The peptidic inhibitor of NTCP, bulevirtide (BLV) (previously Myrcludex B), is based on this strategy and under evaluation in patients with HDV infection (see below).[6,7]

The HDV ribonucleoprotein complex (HDV RNP) is composed of a circular single-stranded RNA and multiple copies of the two forms of hepatitis delta antigen (HDAg), small (S-HDAg, 195 amino acids) and large HDAg (L-HDAg, 214 amino acids) (Figure 1B).[8] HDAg contains the nuclear localization signal (NLS) in its N-terminus, which allows RNP to be imported to the nucleus.[9]

The replication of HDV genomic RNA occurs in the nucleus following the double-rolling circle mechanism.[8,10] The HDV virus does not encode for its own RNA polymerase and uses the host's cellular machinery to replicate and translate its RNAs.[11] HDV RNAs are cleaved and self-processed by their own ribonuclease activity (ribozyme), and their subsequent ligation is assured by the host RNA ligases.[12,13]

The HDV genome is a 1.7 kilo-base RNA with negative polarity and contains a single open reading frame (ORF) for HDAg. During HDV viral replication, three different RNAs are synthesized: circular genomic RNA, circular complementary antigenomic RNA and linear polyadenylated antigenomic RNA (Figure 1B). Circular complementary antigenomic RNA is synthesized into the nucleolus from the HDV circular RNA genome.[8] Editing of HDV antigenomic RNA by cellular adenosine deaminase 1 (ADAR 1) allows L-HDAg transcription and translation while S-HDAg mRNAs are produced directly from the circular HDV genomic RNA transcript.[14,15] Indeed, the open reading frame of S-HDAg ends with the stop codon UAG at position 196. On the HDV antigenomic RNA, ADAR 1 deaminates the adenosine in the 196 position to generate an inosine (UAG → UIG) recognized as guanosine (UIG → UGG) generating

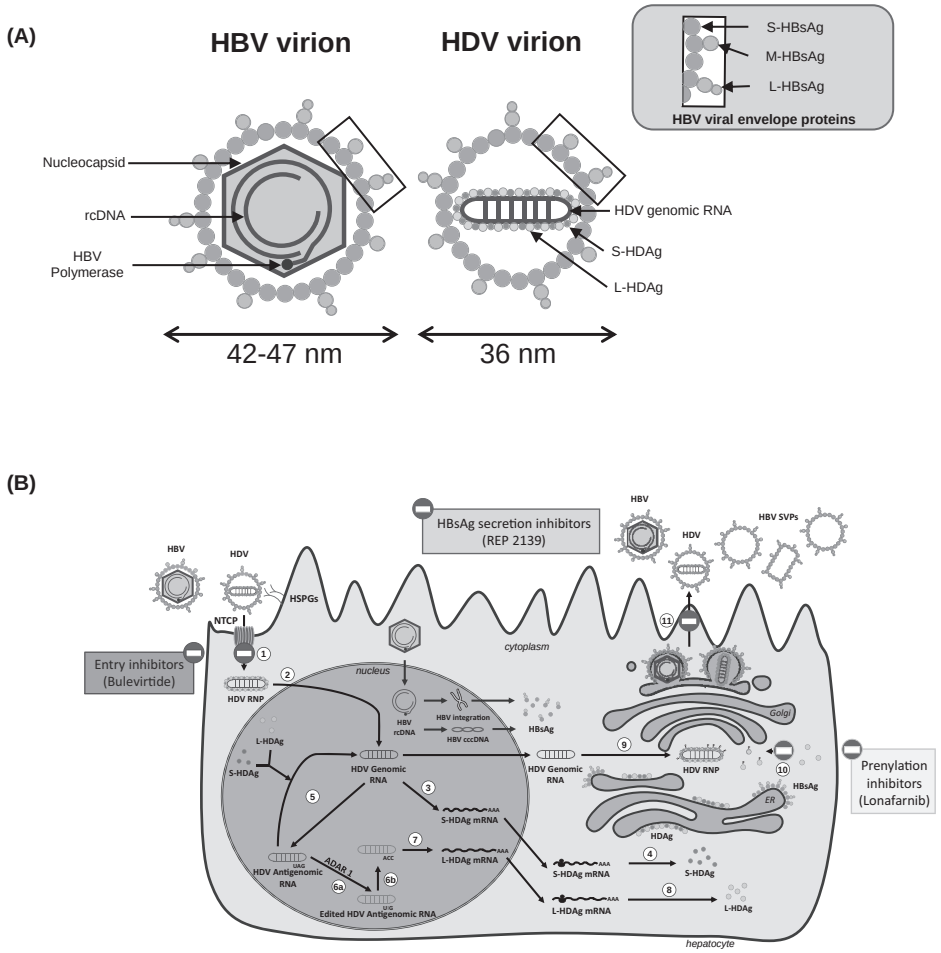

FIGURE 1 A, Comparison of HBV and HDV viral structure. HBV virion is a lipidic spherical structure measuring approximately 42 to 47 nm in diameter. HDV is a smaller virus than the HBV virion and measures approximately 36 nm in diameter. HDV and HBV are two hepatotropic viruses that share the same viral envelope composed of HBV HBsAg (S-HBsAg, M-HBsAg and L-HBsAg), which is important for viral entry. The HBV nucleocapsid is formed by hepatitis B core protein dimers and contains a partly double-stranded DNA genome in relaxed conformation. The HDV ribonucleocapsid is composed of HDAg (S-HDAg and L-HDAg) containing a single particle of circular single-stranded HDV RNA. The insert shows details of shared HBV and HDV envelope proteins. B, Lifecycle of hepatitis delta virus and targets for new drugs in development. (1) HDV virus infects hepatocytes after its attachment to HSPGs and binding to the NTCP receptor. (2) HDV RNA is transported to the nucleus. (3 and 4) S-HDAg is transcribed from HDV genomic RNA and translated into S-HDAg. (5) Replication of HDV genomic RNA with an intermediate form, the HDV antigenomic RNA readily used for new HDV RNP particles. (6a and 6b) HDV antigenomic RNA is edited by cellular ADAR 1. (7 and 8) Transcription and translation of L-HDAg from the edited HDV antigenomic RNA. (9) Assembly of the neosynthetized HDV RNP in the cytoplasm. (10) The farnesylation of L-HDAg allows its interaction with HBsAg. (11) Secretion of HDV virion out of the infected cell. In parallel, HBV capsid is translocated into the nucleus and HBV rcDNA repaired into HBV cccDNA. HBV rcDNA can also be integrated into the host genome and participate as well as HBV cccDNA at the HBsAg production. ADAR 1, adenosine deaminase acting on RNA 1; ER, endoplasmic reticulum; HBsAg, hepatitis B surface antigens; HBV cccDNA, hepatitis B virus covalently closed circular DNA; HBV SVPs, hepatitis B virus subviral particles; HBV: hepatitis B virus; HDV RNP, hepatitis delta virus ribonucleoprotein; HDV: hepatitis delta virus; HSPGs, heparan sulfate proteoglycans; L-HBsAg: large hepatitis B virus surface antigen; L-HDAg: large hepatitis delta antigen; M-HBsAg: medium hepatitis B virus surface antigen; NTCP, sodium taurocholate cotransporting polypeptide receptor; rcDNA: relaxed circular DNA; S-HBsAg: small hepatitis B virus surface antigen; S-HDAg: small hepatitis Delta antigen

a tryptophan codon UGG (Figure 1B). This change extends the open reading frame by 19 additional amino acids producing the L-HDAg. Although they partly share a common sequence, L-HDAg and S-HDAg have opposite effects on HDV replication: S-HDAg promotes viral replication while L-HDAg represses HDV viral replication.[16]

Furthermore, L-HDAg is crucial for HDV assembly and its farnesylation by host farnesyltransferase on cysteine at position 211, which anchors the RNP to HBsAg for the assembly of virion particles.[2,17] Thus, the ratio S-HDAg/L-HDAg is important for both the replication and assembly of viral particles, and interfering with virion assembly is one of the approaches used to target HDV replication. Certain drugs under development are designed to target the addition of the farnesyl lipid group on L-HDAg and thus prevent the interaction with HBsAg. The benefit of targeting the host farnesyltransferase by lonafarnib, which takes this approach, is discussed below.

After translation, S-HDAg and farnesylated L-HDAg interact with the neosynthetized HDV genomic RNA to form HDV RNP. HDV RNP particles then join the endoplasmic reticulum where they interact with the pool of excess HBsAg produced by HBV as non-infectious subviral particles. Finally, HDV virions are secreted using the Golgi secretion pathway (Figure 1B). Some recent in vitro data suggest that HDV RNP can be packaged by the glycoproteins of other viruses such as vesiculovirus, flavivirus and hepacivirus.[18] However, this HDV RNP package by other viruses has not yet been demonstrated in humans. HBsAg secretion inhibitors (discussed below) are under development to prevent the assembly of subviral particles using nucleic acid polymers such as REP 2139.

3 | HDV AND THE IMMUNE SYSTEM

HDV is considered to be a non-cytopathic virus and hepatic damage is immune mediated. However, HBV/HDV immune differences are not well understood. A recent study showed that, unlike HBV, HDV infection induces a strong IFN-β/λ response in innate immune-competent cell lines. Moreover, MDA5 was identified as the key sensor for recognition of HDV replicative intermediates and showed that HDV replication is not abolished by the endogenously induced IFN response or exogenous IFN treatment.[19]

In general, adaptive immune responses to HDV infections are weak.[20] In patients with chronic hepatitis D (CHD), helper T-cell responses are associated with a high frequency of secreting interleukin-10, which has immunomodulatory effects and inhibits interferon pathways.[21] Premature aging of immune cells and impaired T-cell functionality have been shown in patients with HDV infection.[22]Some HDV polymorphisms allow to escape detection by lymphocytes $CD8^+$ and to evade from the immune response.[23] These results provide insights into the mechanisms of adaptive immunity against HDV; however, more research is needed to fully clarify and understand the interaction of HDV with the immune system.

4 | HDV ENTRY INHIBITOR: BULEVIRTIDE

BLV is a candidate for the treatment of chronic hepatitis B (CHB) and CHD. BLV is a linear 47-amino acid peptide bearing an N-terminal myristoyl moiety and a C-terminal carboxamide. It is composed of naturally occurring L-amino acids and is derived from the N-terminal domain of the large HBsAg. BLV competitively binds to NTCP and inhibits attachment of HBV (and HDV) to NTCP.[24]

In an ongoing phase II trial (MYR202, NCT0354662) in patients with CHD, BLV monotherapy for 24 weeks induced a decrease in serum HDV RNA without affecting HBsAg.[6] In this study, 60 HBeAg-negative patients with CHD infection were randomly assigned to four groups. Patients received PEG-IFNα (180 μg once per week) alone, BLV (2 mg subcutaneous once per day) plus PEG-IFNα, BLV (5 mg once per day) plus PEG-IFN or BLV (2 mg once per day) alone for 48 weeks.

At the end of treatment (week 48), HDV RNA declined in all BLV groups. At week 48, HDV RNA was undetectable in 13% (2/15) of patients receiving PEG-IFN alone, 67% (10/15) of patients receiving 2 mg BLV plus PEG-IFN, 57% (8/14) of patients receiving 5 mg BLV plus PEG-IFN and 14% (2/14) of patients receiving BLV alone. At week 48, ALT normalization was obtained in 71% (10/14) of patients receiving BLV alone and in 29% (4/14) of patients receiving PEG-IFN alone. ALT normalization was achieved in 27% (4/15) of patients receiving 2 mg BLV plus PEG-IFN, and in 40% (6/15) of patients receiving 5 mg BLV plus PEG-IFN.

It is interesting to note that HBsAg declined by more than 1 $\log_{10}$ IU/mL in 47% (7/15) of patients receiving 2 mg BLV plus PEG-IFN and in 21% (3/14) of patients receiving 5 mg BLV plus PEG-IFN. No change in HBsAg was observed with monotherapy.

Eight paired biopsies were available from patients receiving BLV alone. At week 48, there was a reduction in necroinflammation in 75% (6/8) and in fibrosis in 50% (4/8). A median intrahepatic decrease in HDV RNA of 1.80 $\log_{10}$ IU/mL was observed. There was a strong reduction in HDAg-positive cells.

Recently, were reported 48 weeks data regarding BLV. Thirty HBe-negative CHD received for 48 weeks 10 mg BLV in either combination with PEG-IFNα or TDF, PEG-IFNα alone or TDF alone.[25] BLV was well tolerated. No Serious Adverse Event (SAE) was reported. At week 48, HDV RNA was undetectable in 86.7% in the BLV (10 mg) and PEG-IFNα arm, 40% in BLV (10 mg) and TDF arm, 13.3% in the PEG-IFNα monotherapy group and 13.3% in the TDF monotherapy group. No HBsAg decrease was observed at week 48. In conclusion, administration of 10 mg BLV in combination with PEG-IFNα is safe and well tolerated during 48 weeks. Strong antiviral responses against HDV confirmed previous results showing a strong synergism already at lower dosing of BLV. These results are promising and may require long-term administration.

Treatment was well tolerated with mild to moderate drug-related adverse events mainly caused by an increase in total bile acids. There was no pruritus. The main reported adverse events were related to PEG-IFN. No serious adverse events were reported.

Finally, BLV for 48 weeks alone and in combination with PEG-IFN-alpha was safe. Combination therapy showed a strong synergistic efficacy. Active (recruiting) trials (https://clinicaltrials.gov; access October 2019) are shown in Table 1.

BLV has been granted PRIME Eligibility by the EMA. In October 2018, it was granted Breakthrough Therapy Designation by FDA. In France, bulevirtide at the dose of 2 mg per day is available for patients with chronic hepatitis delta through an early access program (Autorisation temporaire d'utilisation [ATU]) since september 2019.

5 | L-HDAG PRENYLATION INHIBITOR: LONAFARNIB

Lonafarnib (LNF) is an oral inhibitor of farnesyl transferase, an enzyme involved in the modification of proteins through a process called prenylation. HDV uses this host cellular process inside hepatocytes to

TABLE 1 Active (recruiting) Phases II and III clinical trials for chronic hepatitis delta

Mode of action compound company	Official title	Number (participants)	Stage of development	Reference; Clinicaltrials.gov
Entry inhibitor; Bulevirtide; MYR GmbH	Open-label, Randomized Phase 3 Clinical Study to Assess Efficacy and Safety of Bulevirtide in Patients With Chronic Hepatitis Delta	150	3	NCT03852719
	A Multicenter, Open-label, Randomized Phase 2b Clinical Study to Assess Efficacy and Safety of Bulevirtide in Combination With Pegylated Interferon Alpha-2a in Patients With Chronic Hepatitis Delta	175	2b	NCT03852433
	A Multicenter, Open-label, Randomised, Comparative, Parallel-Arm, Phase II Study to Assess Efficacy and Safety of Myrcludex B in Combination With Peginterferon Alpha-2a vs Peginterferon Alpha-2a Alone in Patients With Chronic Viral Hepatitis B With Delta-agent	60	2b	NCT02888106
Prenylation Inhibitor; Lonafarnib; Eiger BioPharmaceuticals	A Phase 3, Matrix Design, Partially Double-Blind, Randomized Study of the Efficacy and Safety of 50 mg Lonafarnib/100 mg Ritonavir BID With and Without 180 mcg PEG IFN-alpha-2a for 48 Weeks Compared With PEG IFN-alpha-2a Monotherapy and Placebo Treatment in Patients Chronically Infected With Hepatitis Delta Virus Being Maintained on Anti-HBV Nucleos(t)Ide Therapy (D-LIVR)	400	3	NCT03719313
	Treatment of Chronic Delta Hepatitis With Lonafarnib, Ritonavir and Lambda Interferon	32	2A	NCT03600714

complete a key step in its life cycle. An important interaction between HDV and HBV proteins has been shown to be dependent upon the presence of the last four amino acids of the L-HDAg, making up a prenylation CXXX box motif, where C represents cysteine and X any other amino acid.[26] This amino acid sequence is required for the protein to be post-translationally modified by farnesyltransferase, an enzyme which covalently attaches a 15-carbon prenyl lipid-farnesyl-moiety to the cysteine of the CXXX box. Prenylation of the antigen-HDAg renders it more lipophilic, promotes its association with HBsAg and is essential for initiating the HDV particle formation process. Lonafarnib inhibits the prenylation step of HDV and blocks its replication. Since prenylation is a host process that is not under control of HDV, and LNF inhibits prenylation, a high barrier to resistance is expected.

The efficacy, safety and tolerability of LNF was assessed in a phase IIA proof-of-concept study in patients with CHD (NCT01495585).[27] This double-blind, randomized, placebo-controlled, dose ascending study evaluated two doses of LNF, 100 mg twice daily and 200 mg twice daily for 28 days. A dose-dependent decrease in HDV RNA levels of 0.7 $\log_{10}$ IU/mL with 100 mg BID and 1.6 $\log_{10}$ IU/mL with 200 mg BID was observed compared to a 0.08 $\log_{10}$ IU/mL decrease in the placebo arm after 28 days of treatment. The decline in HDV RNA viral levels was correlated to serum LNF drug levels. LNF was generally well tolerated with the most common adverse events being mild to moderate nausea and diarrhoea.

A subsequent phase II trial in patients with CHD, LOWR–1 (LOnafarnib With and without Ritonavir-1), was a parallel dose comparison study that randomized subjects to receive different doses of LNF with or without ritonavir (RTV) or PEG-IFN for 4 to 12 weeks (NCT02430181).[28] Since RTV inhibits CYP3A4 and LNF is extensively metabolized by CYP3A4, boosting LNF with RTV increases serum concentrations of the former, allowing the administration of lower doses of LNF. Data from 15 patients who received LNF alone or with ritonavir or in combination with PEG-IFN all led to decreased viral loads. High doses (200 mg twice daily or 300 mg twice daily) of LNF resulted in 1.6 and 2.0 $\log_{10}$ declines in viral loads after 4 weeks of treatment respectively. A lower dose of LNF (100 mg twice daily) with 100 mg daily RTV boosting or in combination with 180 mcg once weekly of PEG-IFN resulted in a 2.2 and a 1.8 $\log_{10}$ decline in viral load at week 4 respectively. At week 8, the mean viral load declines were 3.2 and 3.0 logs for subjects on LNF with RTV or LNF with PEG-IFN respectively. The most frequently observed adverse events were anorexia, nausea, diarrhoea, fatigue and weight loss, which appeared to be dose dependent. The results support further development of LNF with RTV boosting and exploration of the combination of LNF with PEG-IFN.

Recent data were reported regarding a study, which evaluated the safety and antiviral effects of combination therapy LNF boosted with RTV and PEG-IFN lambda in patients with CHD.[29] In this phase IIA open-label study, 26 adult patients with CHD were treated with oral LNF 50 mg and RTV 100 mg twice daily and subcutaneous PEG-IFN lambda 180 mcg weekly for 24 weeks and then monitored post-therapy for 24 weeks. TDF or ETV was started prior to therapy. At the end of therapy (19 of 26 subjects), the median HDV RNA decline was 3.4 log IU/mL with seven patients (37%) achieving undetectable HDV RNA. Adverse events were mostly mild to moderate and included gastrointestinal-related side effects, weight

loss, hyperbilirubinaemia and anaemia. Therapy was dose reduced in three patients and discontinued in four patients. The results are promising, and await longer follow-up.

LNF has been granted Orphan Drug Designation by the FDA and EMA, Fast Track Designation and Breakthrough Therapy Designation by the FDA and PRIME Eligibility Designation by the EMA.

6 | PLANNED PHASE III D-LIVR STUDY

D-LIVR (Delta Liver Improvement and Virological Response in HDV) is planned as an international, multicentre, phase III study in approximately 300 patients to evaluate an all-oral arm of LNF + RTV and a combination arm of LNF + RTV + PEG-IFN-α, with each arm compared to a placebo arm (background HBV nucleos(t)ide only), in HDV-infected patients. A PEG-IFN-α alone arm will be also available as a comparator. The LNF-containing arms will not be required to demonstrate superiority over PEG-IFN-α alone.

7 | HBSAG SECRETION INHIBITOR: REP 2139

Nucleic acid polymers, such as REP 2139 and REP 2165, block the assembly of subviral particles, preventing the release of HBsAg and allowing its clearance and restoration of functional control of infection when combined with various immunotherapies. The safety and efficacy of REP 2139 and PEG-IFN-alpha-2a were evaluated in a phase II trial in 12 patients with chronic HDV infection (NCT02233075).[30]

Nine patients had suppressed HBV DNA (<10 IU/mL) at the end of treatment, which was maintained in seven patients and newly established in an eighth patient at 1 year of follow-up. Eleven patients became HDV RNA-negative during treatment, with nine remaining HDV RNA-negative at the end of treatment. Seven of these patients were still HDV RNA-negative at 1 year of follow-up. Normalization of serum aminotransferases occurred in nine of 12 patients at 1 year of follow-up.

8 | CONCLUSION AND EXPERT OPINION

The success of direct-acting antivirals to cure hepatitis C virus infection has led to increased hope for a cure for HBV and HDV.[31] CHD is the most severe form of chronic viral hepatitis.

Improving knowledge of HDV virology and cycle is important for the development of new drugs. Ideally, the aim of treatment for HDV infection, like HBV infection, was to obtain a serological response with HBsAg loss and HBsAg seroconversion—that is, a functional cure—which is associated with an excellent prognosis.[32] HBsAg seroclearance is one of the most important endpoints of CHB and CHD, since it is associated with a reduced risk of HCC. Promising new treatment options in development include mainly entry inhibitors, prenylation inhibitors and HBsAg release inhibitors. Drugs developed for a HBV cure will also lead to a HDV cure. All pathways and combinations should be investigated to help achieve a functional cure defined by HBsAg loss.

BLV appears to be 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.

Viral response with LNF appears profound and early with antiviral efficacy in some cases, especially after 8 and 12 weeks of treatment. Therefore, it may be beneficial to use repeated courses of LNF-based regimens. Twelve weeks of treatment may also be considered in studies in the presence of 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-IFN. Thus, IFNs may be continued until more effective and well-tolerated immune modulators become available.

In addition to these new therapies, there is increasing research to identify new compounds to obtain a functional cure for CHB that could be useful in the treatment of CHD. Since HBV and HDV can be controlled by host immune responses, exploratory studies may include the investigation of innate and adaptive immune responses. Three areas of interest include capsid assembly modulators, immune system stimulators (toll-like receptor agonists and checkpoint inhibitors) and RNAi gene silencing. These studies in addition to those of NTCP receptor inhibitors, farnesyl transferase inhibitors, nucleic acid polymers in combination with interferon therapy will provide further insight in the management of this severe disease and hopefully a cure in the near future.

Current and future clinical trials must also consider HBV and HDV interactions because HDV suppression can lead to HBV reactivation. Therefore, a combination with nucleos(t)ide analogues might be maintained to control HBV replication in the treatment of CHD.

Future studies should not only investigate relative HDV RNA decline but also several secondary endpoints as surrogates for response including early virological responses during therapy, histological evaluation (histology activity and fibrosis), ALT normalization, HBs decline at the end or discontinuation of treatment.

It has been suggested that high HBsAg titres induce immune tolerance, which may represent a major obstacle to cure HDV and HBV. Decreasing HB levels by a different mode of action, such as with long-term nucleoside analogue treatment, or by targeting viral translation with siRNA inhibiting HBsAg release by nucleic acid polymers or by neutralizing HBsAg via specific antibodies, could potentially restore immunity. A combined strategy including reducing HBsAg levels and secretion with the above treatments and therapeutic targeting of B cells could induce anti-HBsAg antibodies and lead to a functional cure.

Finally, it should be mentioned that the treatments under evaluation are restricted to patients without cirrhosis or with compensated cirrhosis, and the rationale of including patients with decompensated cirrhosis should be considered.

CONFLICT OF INTERESTS

Tarik Asselah has acted as a speaker and investigator for Janssen, Gilead, Roche, and Merck. Nathalie Boyer has acted as a speaker and investigator for Janssen, Gilead, Roche and Merck. Corinne Castelnau has acted as a speaker and investigator for Janssen, Gilead, Roche and Merck. Patrick Marcellin has acted as a speaker and investigator for Janssen, Gilead, Roche and Merck. Dimitri Loureiro, Issam Tout and Abdel Mansouri declare no competing interests.

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REVIEW ARTICLE

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Hepatitis C elimination – Macro-elimination

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Abstract

In 2016 the WHO set a goal to obtain an 80% reduction in new chronic HCV cases, requiring a level of diagnosis of 90%, treatment coverage of 80% and resulting in a 65% reduction in HCV-related deaths by 2030. This goal is easier to reach in specific populations such as people who inject drugs (PWID), men who have sex with men (MSM) or blood-transfusion recipients before screening for HCV became mandatory and in high-income regions. It is much more difficult to achieve macro-elimination throughout the population especially in low-income areas with underdeveloped infrastructures, a high prevalence of HCV and limited economic resources. To achieve the WHO goals by 2030, awareness of HCV must increase and the cascade of care must be improved and implemented. Diagnostic procedures and treatment should be affordable and universally available. At the end of 2017 fewer than 15 countries were on track to reach these goals by 2030.

KEYWORDS

HCV, HCV care cascade, HCV screening, macro-elimination

1 | INTRODUCTION

With the development of effective direct acting antiviral agents (DAAs) for the treatment of hepatitis C virus (HCV) infection, the elimination of HCV as a major public health threat is possible.

In 2016 the WHO presented a strategy to eliminate HCV by 2030, defined as an 80% reduction in new chronic HCV cases, diagnosis of 90% of cases, treatment coverage of 80% and a 65% reduction in HCV-related deaths.[1]

Only a minority of the countries in the world is on track to reach this goal by 2030. Indeed, by the end of 2017 fewer than 15 countries were expected to be on track, according to WHO.[2,3]

Many important steps must be taken to achieve macro-elimination. These include increasing awareness of HCV in the public and in the health profession, identifying and diagnosing all existing cases, treating and curing all viraemic cases and reducing the risk of reinfection by effective measures as well as implementing surveillance for possible reinfections. This demands effective linkage to care with methods that are adapted to the different parts of the world. These challenges are easier to overcome in industrialized Western countries such as Iceland with small populations and a small number of infected individuals [4] while they are more difficult in developing regions, eg parts of Eastern Europe, Asia, South America and Africa, where there are more individuals with undiagnosed HCV.[5]

One limitation to having a clear view of the progress being made in the worldwide elimination of HCV is suboptimal and lack of standardized reporting in many countries and regions, especially in low-income areas. Recently, Safreed-Harmon et al published an article describing how to implement standard reporting to monitor progress towards the elimination of HCV.[6] If standard and accurate reporting became mandatory and feasible it would be easier to monitor the progress made towards reaching WHO goals worldwide.

Moreover, a recent review by Calcvaruso et al evaluated whether global elimination of HCV is a realistic goal.[7] They concluded that the differences in the prevalence of HCV throughout the world, as well as in populations, ages, risk factors and disparities in the costs of drugs and the availability of funds will make reaching this goal difficult.

Abbreviations: WHO, world health organization; HCV, hepatitis C virus; PWID, people who inject drugs; MSM, men who have sex with men; DAA, direct acting antivirals; HBV, hepatitis B virus; POC, point-of-care; NSP, needle syringe programs; OAT, opioid agonist treatment.

2 | HCV EPIDEMIOLOGY

Hepatitis C virus is a major health issue, with an estimated 69 million chronically infected patients worldwide.[8] The HCV epidemic affects all regions, but there are major differences among and within countries. The WHO Eastern Mediterranean Region and the European Region have the highest reported prevalence of HCV.[3]

Global access to affordable hepatitis testing is limited. According to WHO, a minority of people with viral hepatitis have been diagnosed, corresponding to 20% of the total number of persons infected with HCV (14 million). Only a small fraction of diagnosed patients has been treated. In 2015, only 7.4% of patients diagnosed with HCV infection, corresponding to 1.1 million persons, had started treatment. The cumulative number of persons treated for HCV reached 5.5 million in 2015, but only about half a million of these were treated with the newer DAAs. Moreover, in 2015 there were more newly detected HCV cases than patients who had started treatment.[3]

Unsafe healthcare procedures (iatrogenic infections) and injection drug use are the leading causes of new HCV infections, accounting for most of the 1.75 million new infections in 2015. The 2015 WHO estimates conclude that viral hepatitis resulted in 1.34 million deaths, similar to the number of deaths from tuberculosis, but higher than the number of deaths from HIV. Left untreated, HBV and HCV infection can lead to cirrhosis (720 000 deaths annually) and hepatocellular carcinoma (470 000 deaths annually). Furthermore, mortality from viral hepatitis has increased by 22% since 2000. Unless people with HBV and HCV infection are diagnosed and treated, the number of deaths caused by viral hepatitis will continue to increase.[3,9]

Key points

- WHO elimination goals are set to be reached by 2030
- These include HCV diagnosis of 90%, treatment coverage 80% and 65% reduction in deaths caused by HCV
- In 2017 only 12 countries were on track to reach these goals (Australia, Egypt, France, Georgia, Iceland, Italy, Japan, Mongolia, the Netherlands, Spain, Switzerland and UK)
- Barriers for elimination still remain (awareness of infection, economic restraints for testing and treatment, linkage to care and availability of DAAs)
- Reducing global hepatitis burden is dependent upon the success of prevention, implementation of outreach screening and treatment and progress made in key high-burden countries.

3 | SCREENING STRATEGIES AND DIAGNOSIS (RAPID DIAGNOSTIC TESTS, POINT-OF-CARE TESTING)

Screening to achieve micro-elimination in subgroups of infected individuals is usually focused on high-risk groups such as people who inject drugs (PWID), baby-boomers, men who have sex with men (MSM) and blood recipients transfused before general blood donor screening began (1992 in some regions).[9] In many low-income regions, screening is not widely available because of economic restraints and logistical problems. In a recent French study, population-based screening was found to be cost-effective even in fairly low prevalence countries such as France.[10] In low-income regions, screening needs to be simple and easy to perform, decentralized and available in the form of point-of-care (POC) testing.

3.1 | Point-of-care testing

In recent years more easily accessible tests have been developed to simplify screening and diagnosis. These tests do not require elaborate laboratories to diagnose HCV, facilitating testing outside the hospital and healthcare centres. However, specific training of healthcare personnel is required. Moreover, point-of-care testing is also possible using saliva or capillary blood, without venipuncture. Examples of point-of–care tests are rapid tests from saliva or blood (on-site anti-HCV and/or HCV RNA test) or dried blood-spot (anti-HCV and HCV RNA test, which require further laboratory processing).[11-14]

4 | MATHEMATICAL MODELLING

Several mathematical models have been developed to determine how to reach WHO goals. Although forecasts with mathematical modelling are dependent upon the quality of data input and may be uncertain, they still provide an indication of the level of intervention and input that may be required.

Modelling studies often try to examine the effects of scaled-up HCV treatment for the general population and sub-populations. Besides prevalence, incidence and data on HCV treatment, other preventive measurements that could affect prevalence such as combined interventions, may also be entered into the model. For example, in PWID a combined intervention includes extensive coverage of needle syringe programs (NSP) and increased access to opioid agonist treatment (OAT). This is the most effective combined intervention for the prevention of HCV in this population, and can achieve micro-elimination in this subset.[15-18]

Multiple and expanded micro-elimination efforts in different high-risk groups could result in macro-elimination on a country/regional basis. A study modelling the potential prevention benefit of a hepatitis C treatment-for-all strategy on global, regional and country levels was recently published.[19] This study concluded that significant prevention benefits were obtained from the WHO treat-all strategy, although there was more benefit per treatment when PWID were targeted.[19]

Several country-specific models on HCV elimination have been published. An individual-based micro-simulation Markov model was developed in a recent elimination model that simulated disease progression of HCV-infected patients in China from 2004 to 2050.[20] Four different scenarios with different assumptions about treatment and the natural history of disease were constructed including a natural history scenario, a pre-DAAs scenario, a DAA treatment scenario for all patients with advanced fibrosis/cirrhosis and a scenario for all patients whatever the stage of fibrosis. In this model, DAA treatment had a significant impact on the burden of HCV in China, but only if it was rapidly implemented and included all HCV-infected patients. In the scenario in which all patients were treated, the prevalence of chronic HCV was expected to peak at 10.8 million around 2020, and then decrease to 7.9 million in 2050.[20] The authors also concluded that if the future burden of HCV-related diseases is to be prevented, China must rapidly increase the number of treated patients [20] and expand HCV screening to identify more cases requiring rapid treatment. If these measures are not implemented the HCV burden in China will continue to remain high in the future.[20] In Europe, with an advanced healthcare infrastructure, substantial expansion of screening programs is also necessary to reach WHO targets.[21]

In middle- and especially in low-income countries, the cascade of care models for other diseases such as HIV can be used and implemented for HCV [9] For example, in Rwanda, as in many other regions, the government must further decentralize care and integrate HCV management into routine clinical services to provide better access to diagnosis and treatment for patients. Making rapid diagnostic tests available in public healthcare facilities would help increase diagnoses. Furthermore, increased public and private funding is essential to support care and treatment services.[22] In a territory-wide population-based study from Hong Kong the authors concluded that the reduction in the diagnostic, treatment and mortality rates was low, especially since treatment with peg-IFN was still used in some cases making make it difficult to reach the WHO hepatitis elimination target.[23] The authors concluded that generalized use of new DAAs was also urgently needed in this region.

5 | THE HCV CARE CASCADE

Even with universal access to HCV treatment and sustained viral response (SVR) rates > 95%, patients still need to be linked to care to achieve macro-elimination of HCV. Key elements for this are presented in Table 1.

As several studies have shown, there are many factors that can negatively affect the 'HCV care cascade' or the 'retention cascade', which is defined as retention along every step of the care pathway from diagnosis to achieving an SVR.[3,9] Over time, from anti-HCV screening to confirmation with a HCV antigen or a HCV RNA test, then referral to a specialist with a follow-up visit for the assessment of fibrosis and finally starting treatment – a large proportion of patients is lost to follow-up, even in high-income regions such as the US that have full access to care. Results can substantially be improved if all of this is implemented.[24]

There is a specific challenge with the HCV care cascade in high-income countries because of concomitant comorbidities in patients such as drug use, psychiatric disorders or a lack of social stability. The treatment retention cascade can be improved if patients are treated geographically closer to where they originally access services, creating a 'one-stop-shop'.[25,26] Thus, in these cases, HCV treatment should be offered in dependency disorder clinics, OAT (opioid agonist treatment), clinics, prisons and at NSP (needle syringe programs). In the developing world treatment should also be available in remote healthcare centres by local personnel. This will increase linkage to care and make the process more decentralized and patient-centred, which has been successfully achieved in Australia.[12,27] The prevalence of HCV has declined throughout the population after just a few years in Australia based on decentralized care for patients with HCV including treatment for all PWID. Thus, the prevalence of viraemia may be reduced through intensive treatment in PWID.[28]

As stated above, the governments in low-income regions such as Rwanda must further decentralize care and integrate hepatitis C management into routine clinical services.[22]

6 | HARM REDUCTION MEASURES

In high-risk groups such as PWID, NSP and OAT are examples of harm reduction interventions. A recent global review of access to these services reported that only 79 countries in the world have implemented these two interventions for PWID and only four countries (Australia, Austria, the Netherlands and Norway) were considered to have high levels of NSP and OAT.[9] In the WHO strategy for HCV elimination, a goal of > 300 needle/syringes distributed per PWID per year is proposed for effective prevention of HIV and hepatitis transmission.[29]

HCV may be transmitted through both sharing of needle/syringes and other drug paraphernalia.[30-32] NSP is recommended to reduce the spread of HIV and hepatitis in PWID.[16,33-35] Certain reviews have concluded that there is evidence that NSP is reducing injection risk behaviour and transmission of HIV but the data are insufficient to confirm its effectiveness in preventing the transmission of HCV.[36-38] On the other hand, there are studies showing that NSP prevents HCV transmission, in particular when it is combined with OAT.[16,35] In a recent Cochrane report, Platt et al concluded that OAT was associated with a 50% reduction in the HCV transmission rate, while needle exchange programs (NEP) alone was less effective with a reduction of 21%. When stratified by region, a 56% risk reduction was found in settings with high NSP coverage in Europe and combined OAT/NEP was associated with a 74% reduction in the HCV transmission rate.[9]

In many regions safe handling of blood, sharp instruments, needles and syringes in the healthcare sector needs to be reinforced to prevent nosocomial infections.[9] In some regions blood supply and donors are not tested for HCV.[3,9]

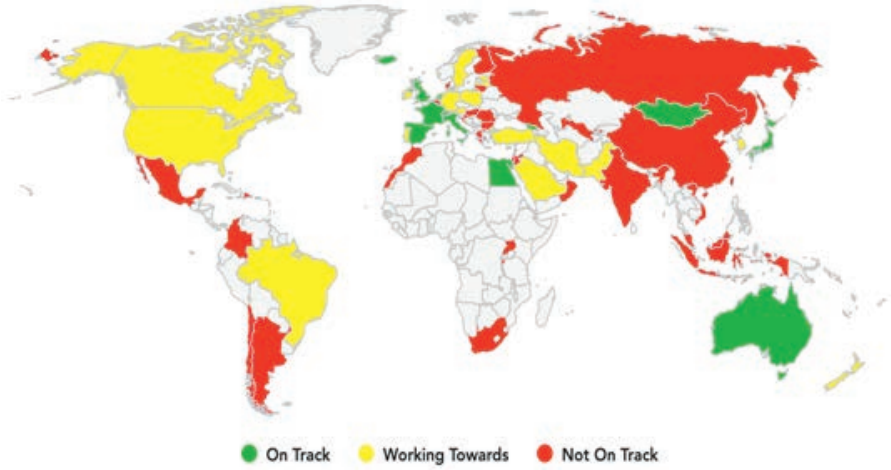

FIGURE 1 Countries on track to eliminate HCV by 2030.
Source: Polaris Observatory 2019, (http://cdafound.org/polaris/ September 30, 2019)

7 | DIFFERENT COUNTRIES'/REGIONS' ACHIEVEMENTS SO FAR

Overall only 12 countries have been shown to be on track to achieve the WHO goals by 2030 [39]: Australia, Egypt, France, Georgia, Iceland, Italy, Japan, Mongolia, the Netherlands, Spain, Switzerland and UK Figure 1.[2,3]

Iceland may be the first to reach this goal, probably by 2020.[4] This is not surprising because Iceland is a well-defined geographic area with devoted staff, excellent infrastructure, access to decentralized treatment for all and a relatively low number of infected persons. In Europe, however, there are wide variations in what has already been achieved and what remains to be done to reach the WHO target.[21]

As mentioned above Australia, has been a global benchmark for best practices and has had a very good start. However, they have recently experienced flattening treatment rates, and they need to diagnose more persons and link them to care.[21] Nevertheless, there has been a decline in overall HCV prevalence throughout the population through intensive treatment and cure in PWID.[28]

Egypt, a low-income country, has developed the largest national program for HCV treatment and has now successfully treated several million people. A prerequisite for their success has been the negotiation of low DAA prices and the ability to produce DAA locally.[40]

Many smaller countries such as Georgia, Mongolia and Iceland have received help from international drug companies to provide access to DAA drugs free-of-charge and logistical help from international societies in collaboration with the national teams to reach the WHO target before 2030.[9]

8 | NATIONAL PLANS

Although WHO recommends that all countries develop a national plan for the elimination of hepatitis many have not yet done so. A comprehensive package of prevention, screening and treatment interventions could prevent 15.1 million new infections and 1.5 million deaths from cirrhosis and liver cancer, corresponding to a 81% reduction in incidence and a 61% reduction in mortality compared to the 2015 baseline. This reaches the WHO HCV incidence reduction target of 80% but is just short of the mortality reduction target of 65%, which could be reached by 2032. Reducing the global hepatitis burden is only possible with successful prevention interventions, implementation of outreach screening and treatment and progress in key high-burden countries including China, India and Pakistan.[8]

TABLE 1 Key elements in macro-elimination of chronic HCV as a public health threat and linkage to care for chronic HCV infections

Awareness of HCV and prevention of new infections
Access to testing and diagnosis
Linkage to care
Access to medicine (DAA)
Development of national plans

Adopted and modified from: BCG. Road to elimination: barriers and best practices in hepatitis C management. Available at: http://image-src.bcg.com/image/BCG-Road-to-Elimination_tcm-166034.pdf (assessed Sep 2018)

9 | SUMMARY AND CONCLUSION

Despite the availability of highly effective therapeutic regimens based on direct acting antivirals, many barriers to the elimination of HCV still remain. These are related to awareness of the infection, the economic feasibility of testing, treatment and linkage to care, the availability of the therapeutic drug regimens and reinfection rates. Unless a prophylactic vaccine becomes available, HCV elimination by 2030, as proposed by WHO, appears difficult to accomplish in certain regions/countries.[41]

CONFLICT OF INTEREST

MK has received honoraria for lectures from AbbVie and Gilead, and MSD and received research grants from Gilead.

OW has consultancies with AbbVie, BMS, Gilea and MSD/Merck and has worked on speaker bureaus for AbbVie, BMS, Gilead and MSD/Merck.

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REVIEW ARTICLE

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Micro-elimination of hepatitis C virus

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Abstract

Background & Aims: HCV affects about 71 million people worldwide with 1.75 million new infections a year, mainly associated with healthcare, blood transfusion before screening of donors and drug use. Hepatitis C is a systemic disease with hepatic and extrahepatic manifestations resulting in increased morbidity and mortality in HCV-infected patients compared to cured or uninfected individuals.

Results: The goal of eliminating hepatitis C by 2030 is based on the following three main actions: strengthening and increasing outreach screening; increasing access to treatment; and improving prevention. Although the tools and the targets of HCV elimination have now been well established, micro-elimination, a cure in high-risk populations, is possible, but has not been achieved. These populations are mainly migrants, prisoners, drug users, HIV co-infected patients and psychiatric patients. New tools must be developed to achieve micro-elimination, in particular, rapid diagnostic orientation tests for better screening, delocalization of healthcare services to improve access to care, and training physicians to raise awareness of the disease, increase understanding of its pathogenesis and provide information on the availability of safe and effective treatment to cure chronic infection and reverse hepatic and extrahepatic manifestations.

Conclusion: Thus, while the goal of complete elimination of hepatitis C virus was feasible in Western countries, it was more difficult in high-prevalence countries where improvement in the detection of chronic infection (with rapid serological and virological diagnostic tests), outsourcing of diagnostic and therapeutic care and access to direct oral antivirals are urgently needed.

KEYWORDS

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

1 | INTRODUCTION

Between 1990 and 2013, viral hepatitis A, B (HBV), C (HCV), D and E increased from the tenth to the seventh leading cause of death worldwide, exceeding HIV, tuberculosis or malaria. They are the first cause of mortality of infectious origin.[1] Combined HBV and HCV chronic infections account for 96% of all viral hepatitis-related mortalities. Most mortality is because of chronic HBV and HCV-related hepatocellular carcinoma and cirrhosis. The disease burden of these two viruses varies depending on geographical regions with a greater proportion of HCV infection in Europe, the Middle East, the Americas and North Africa and of HBV infection in sub-Saharan Africa and in Asia.

In 2016 this led to the approval of the Global Health Sector Strategy to eliminate hepatitis infection by 2030. The World Health Organization (WHO) introduced global targets for the management

Abbreviations: AFEF, Association Française pour l'Etude du Foie; ANRS, Agence Nationale de Recherche sur le SIDA et les Hépatites; DAA, direct-acting antivirals; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MSM, men who have sex with men; PWID, people who inject drugs; RDOT, rapid diagnostic orientation tests; WHO, World Health Organization.

of HCV, including a 90% reduction in new cases of chronic hepatitis C, 65% reduction in hepatitis C-related deaths and the treatment of 80% of eligible individuals with chronic hepatitis C infections.[2] Interventions and targets are directed towards improvement in blood safety and injections, risk reduction policies, and hepatitis diagnosis and treatment. Micro-elimination policies, namely elimination of HCV infection in targeted populations, are a part of this elimination program.

2 | HCV ELIMINATION: REALISTIC IN EUROPE, PROBLEMATIC ELSEWHERE

HCV affects about 71 million people worldwide with 1.75 million new infections a year, mainly associated with healthcare and drug use.[3] The global prevalence of viraemic HCV estimated by the Polaris Observatory was 1% (95% uncertainty interval 0.8-1.1) in 2015, corresponding to 71.1 million (62.5-79.4) viraemic infections (the models were built for 100 countries (59 approved by country experts, 41 estimated using published data alone, while data were insufficient for the remaining countries to create a model). Genotypes 1 and 3 were the most common cause of infections (44% and 25% respectively).[4]

Hepatitis C is a systemic disease responsible for liver disease with cirrhosis and hepatocellular carcinoma (which is the leading cause of death) as well as cryoglobulinaemic vasculitis with a risk of purpura, polyarthritis, polyneuritis or membranous-proliferative glomerulonephritis or even clonal expansion leading to non-Hodgkin's lymphoma B.[5-7] There is also an increased risk of diabetes, cardio-, cerebro- or reno-vascular diseases, neurocognitive disorders and extrahepatic cancers (other than non-Hodgkin's lymphoma) because of chronic inflammation.[8] Finally, these hepatic and extrahepatic manifestations result in increased morbidity and mortality in HCV-infected patients compared to those cured or uninfected.[9] Thus, the treatment of all HCV-infected patients is a cost-effective approach to reduce hepatic and extrahepatic morbidity and mortality.[10] A recent prospective study by the AFEF-ANRS C0-22 HEPATHER cohort, including more than 14 000 HCV-infected subjects, has shown that the virological cure of treated patients reduces hepatic and extra-viral mortality as well as the risk of occurrence of hepatocellular carcinoma compared to untreated patients.[11]

The course of HCV infection and the safety and efficacy of treatment have significantly changed since the introduction of interferon-free regimens. A combination of 2 or 3 second-generation direct-acting antiviral agents (DAA) provides a sustained viral response, and thus a cure to hepatitis C infection, in more than 95% of cases. Antiviral treatment is generally well tolerated and the duration (8-12 weeks) depends on the choice of therapy, the stage of fibrosis, prior treatment and the presence of resistance-associated variants.[12,13]

A dozen countries have an elimination policy plan. While the challenge of elimination can easily be met in Iceland (about 350 000 inhabitants), it will be more difficult in Egypt (about 80 million inhabitants, 15% infected with HCV with expected treatment of 100 000 patients annually compared to 150 000 new cases per year) or in Georgia, which is the first country to have adopted an elimination policy with 6.5% of the population infected with HCV, mostly because of drug use. While elimination seems to be a realistic goal in Europe based on current policies for screening, treatment access and national plans (50%-60% of countries have a national plan for hepatitis C, but only 37%-55% have the funding),[4] this is less true in the USA, because of limited access to care and reimbursement of treatment as well as an epidemic of hepatitis C from drug use. Elimination is currently impossible in the countries of the South and particularly in sub-Saharan Africa.[4]

Key points

- The tools to eliminate HCV are well established: screening, access to care, treatment and post-cure follow-up
- The targets for HCV micro-elimination are well established: high-risk populations (migrants, PWID, MSM) and target groups (haemophilia, HIV infection)
- Diagnostic burn-out in which only already diagnosed people are being treated, and not enough people are being diagnosed every year with new or re-infections, limits complete HCV elimination
- HCV elimination is feasible in Western countries but uncertain in high prevalence and developing countries
- Increased screening, outsourcing of diagnostic and therapeutic care and access to direct oral antivirals are mandatory

The goal of eliminating hepatitis C by 2030 was based on the following three main actions: (a) improving access to hepatitis C treatment[2,3] by increasing treatment in city/hospital networks; (b) increasing outreach screening with rapid diagnostic orientation tests (RDOTs) that combine HIV, HCV and HBV; and (c) reinforcing prevention through innovative actions that target priority populations outside the healthcare system.

A recently published modelling study has estimated that to achieve WHO targets, unlimited treatment should increase from 150 000 patients in 2015 to 187 000 patients in 2025. Diagnosis needs to increase from 88 800 new cases annually in 2015 to 180 000 in 2025. The development of screening programs is, therefore, crucial to increase treatment and achieve the goals of the WHO.[14]

3 | IMPROVING SCREENING OF HCV INFECTION

Improved screening is critical to achieve the WHO elimination objective. Nearly two-thirds of the population with HCV are unaware of their infection and will need to be diagnosed before treatment

can be considered.[14] Of an estimated 150 000 French individuals infected with HCV, 70 000 are not aware of their status with a risk of progression of liver disease and contamination in at-risk populations. This emphasizes the importance of screening programs to identify undiagnosed patients and create protocols of linkage to care. To meet WHO targets, 90% of the individuals infected with HCV should be diagnosed by 2030, requiring national screening programs that expand beyond screening high-risk populations. Very few European countries have expanded screening to identify patients with a fibrosis score of F2 or higher after 2017. Without expanded screening it will be difficult to identify new, undiagnosed cases. The decision to treat should no longer be based on fibrosis, all viraemic patients must be treated, even those with minimal liver disease.[13] Patient follow-up and linkage to care must also be organized because previously diagnosed patients may be lost to follow-up in the presence of treatment restrictions (prioritization).[14] This requires new technologies such as RDOT or 'Point of Care' tests, which allow a serological diagnosis, and especially detection of positive viraemia within 20-30 minutes, for a 'test and treat' policy.[15] All these measures can help meet the challenge by improving 'diagnostic burn-out', with five times more viral C infections than diagnosed in 2016 and five times fewer cures than new infections.[16]

4 | IMPROVING ACCESS TO CARE BY DELOCALIZATION

While 25% of infected individuals are highly motivated and have already been diagnosed and treated, 25% of the infected population is difficult to identify because of precarious lifestyles outside the healthcare system. The diagnosis is less difficult in the remaining 50%. Thus, policies must improve the screening of at-risk populations, mainly migrants, prisoners, drug users, or men who have sex with men (MSM). For example, although 15% of the prison population is infected with HCV worldwide, with approximately 4%-7% in France, only 36%-70% of prisoners have been screened. Thus, this population could benefit from rapid diagnosis and treatment procedures, which take 8-12 weeks to reduce both individual medical risks and contamination in prisons. Harm reduction services, such as prison-based needle and syringe programs and treatment for opioid use disorders, are crucial for reducing the incidence of HCV in prisons and improving overall health outcomes.[16,17] Historically, many prisoners were not screened for HCV infection because there was limited knowledge about new treatment options and concerns that this population would not have access to treatment.[16] Other limitations in this population include difficult access to specialists who can prescribe DAA and payment for treatment. Finally, prisoners sentenced to less than 6 months may not be managed, despite the shorter treatment durations (2-3 months). HCV treatment for prisoners is still limited in many countries in Europe, with highly variable access to care in Germany, Italy, France and England. Policy and effective implementation are key to successful management of HCV in prisoners.[17]

Difficult access to care is also an issue for migrants and populations with remote healthcare systems. Multidisciplinary projects can improve the cascade of care from diagnosis to treatment in these cases. A policy of not diagnosing vulnerable populations who are considered to be difficult to treat has not been validated in randomized trials and real-life data, which show that all these at-risk populations have a cure rate of between 90% and 98% similar to the 'general' population.[18]

Socio-behavioural features may influence the access to care. In the French ANRS cohort of HIV-HCV co-infected patients (Hepavih), patients with unstable housing conditions were prescribed DAA less frequently than other populations. Patients receiving DAA treatment were less likely to report unstable housing (0.46 [0.24; 0.88]), cannabis use (regular or daily use: 0.50 [0.28; 0.91], non-regular use: 0.41 [0.22; 0.77]) and a history of drug injection (0.19 [0.12; 0.31]) on multivariate analysis.[19] Thus, improving access to DAAs remains a major clinical and public health strategy, in particular for individuals with high-risk behaviours. Prioritizing access to care in these at-risk populations is crucial since a DAA-associated cure reduces the incidence and prevalence of HCV infection in the community resulting in an individual but also a collective benefit to limit the epidemic.[20]

In high-income countries, sharing of needles and equipment among people who inject drugs (PWID) is the major route of transmission and harm and risk reduction policies (mobile risk reduction service, injection room and needle/syringe exchange) resulted in a reduction in the prevalence of HCV infection and in re-infection before access to DAA treatment.

Unlike HBV infection, the risk of sexual transmission of HCV has always been considered low.[21] This low risk was recently confirmed in 500 anti-HCV-positive, HIV-negative persons and their long-term HCV-negative heterosexual partners. The incidence rate of sexual HCV transmission was 0.07% per year or one infection per 190 000 sexual contacts.[22] However, in the mid-2000s, sexual transmission of HCV was identified in men who have sex with men (MSM),[20,23] At first this was considered to be a confounding factor because of frequent exposure of this population to injectable drugs. However, further evidence from Europe, the USA and Australia showing HCV in MSM who denied drug use relaunched the debate on the risk of sexual transmission of HCV.[24] Because new HCV infections were typically found in HIV-positive MSM, it was initially suggested that HIV status could be an important factor for sexually acquired HCV.[25] However, recent studies suggest that sexual transmission of HCV also occurs in HIV-negative MSM, indicating that this is not the only factor affecting susceptibility.[26] The frequency of sexual exposure to HCV and the use of drugs during sexual context, especially injected or snorted ('chemsex') drugs is also a major risk factor. The high rates of HCV reinfection in MSM who clear HCV (spontaneously or following successful treatment) show the importance of education on risky sexual or other behaviours in the transmission of HCV. Thus, routine HCV testing and behaviour counselling should be part of the follow-up in these patients. Finally, prompt HCV treatment may also play a

role in decreasing the prevalence and incidence of HCV, especially when they are combined with additional interventions as part of comprehensive sexual health services.[20]

Finally, screening activities should be relocated. Mobile teams have already been created to improve diagnostic efforts and provide immediate treatment to high-risk populations. This has been reported in local initiatives, where quick blood tests accompanied by a mobile Fibroscan (non-invasive evaluation of fibrosis) can provide an accurate evaluation of the stage of fibrosis and curative treatment.

5 | 'MICRO-ELIMINATION': CURE OF HIGH-RISK POPULATIONS

Although the tools and the targets of HCV elimination are now well established, micro-elimination, or a cure HCV in high-risk populations (migrant communities from high-prevalence regions, HIV-infected, PWID, MSM) and target groups (patients with advanced liver disease, haemophiliacs, prisoners, generational cohorts with high prevalence),[27] is a feasible goal that has not yet been achieved.

In populations with chronic kidney disease or HIV infection and high viraemia all patients have not yet been cured, even though the safety and efficacy as well as the reduced risk of infection or re-infection has been clearly proven when individuals are treated[20] and despite the high level of education of specialized physicians. Although HCV/HIV co-infected patients have been considered a priority in countries with prioritization of DAA since 2014, around one-third of have not been treated in France, even though the efficacy and safety of DAAs are similar to that in HCV mono-infected patients, and doctors are aware of the higher risk of liver deterioration with HIV co-infection.[28] Most HCV-infected kidney recipients have been treated with excellent results.[29] However, the treatment of dialysis patients is unsatisfactory, although this specific population has well-known over-morbidity and mortality because of more extrahepatic manifestations (diabetes, vascular disease) than hepatic complications.[30] The situation is even worse in oncology, rheumatology or haematology where less than one-third of patients receive a serological test and even fewer anti-HCV positive patients receive HCV RNA testing (personal data). This shows the urgent need for more extensive education on the diagnosis and treatment of HCV infection.

Another unmet need is the psychiatric population. These patients are 5-10 times more likely to be infected with HCV than the general population.[31] Although the risk factors for contamination are identified in two-thirds of cases, they are not identified in one-third, suggesting possible institutional contamination.[32] This high-risk population is greatly undertreated.

Obstacles to access to treatment are not only related to patients, but also to physicians, who must be made aware of the hepatic and extrahepatic consequences of this systemic disease, as well as the safety and high efficacy of treatment, and thus the possibility of eliminating HCV.

6 | CONCLUSION

We are in a unique position since chronic HCV infection is the only chronic viral infection that can be cured. With the development of effective policies in recent years (prioritization of treatment to the most severe patients, then to at-risk populations and finally to the entire population) and of non-invasive procedures for the evaluation of liver fibrosis (Fibroscan, Fibrotest, Fibrometer and other non-invasive biochemical fibrosis tests), the elimination of HCV is a realistic theoretical objective. It will require improved detection of chronic infection (rapid serological and virological tests), outsourcing diagnostic and therapeutic care, and access to direct oral antivirals. Even if a potential elimination of HCV infection is possible, the co-morbidities related to alcohol and the metabolic syndrome, which contribute to the aggravation of liver diseases, must not be forgotten. They require a model of multidisciplinary management in patients infected with HCV.

CONFLICT OF INTERESTS

Dr S Pol has received consulting and lecturing fees from Bristol-Myers Squibb, Janssen, Gilead, Roche, MSD, MYR-Pharma, Shionogi, Biotest and Abbvie, and grants from Bristol-Myers Squibb, Gilead, Roche and MSD. Dr L. Parlati and C. Hollande have nothing to disclose.

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REVIEW ARTICLE

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Natural history of NASH and HCC

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Abstract

Widespread unhealthy dietary habits associated with a sedentary lifestyle have made NAFLD the most frequent chronic liver disease worldwide, with a global prevalence of ~25%. Although NAFLD is mainly considered to be a benign disease, it can progress to severe liver fibrosis and hepatocellular carcinoma (HCC), with the latter found in non-cirrhotic livers in about 40% of cases. Factors favouring the progression of liver disease in NAFLD are only partially understood. Male sex, older age and Caucasian ethnicity have frequently been identified as factors accelerating the progression of fibrosis in NAFLD, although data are not consistent. Host genetic variants appear to be very important, especially in the gene coding for the patatin-like phospholipase domain-containing 3 (PNPLA3), and they may also play a role in the development of HCC, independent of activity and the extent of liver damage. However, the most important factors affecting disease progression are found in the metabolic syndrome, that is, obesity, type 2 diabetes and arterial hypertension. This mini-review will discuss the contribution of these factors to NAFLD-associated morbidity, emphasizing the importance of preventive measures such as physical activity and weight control in view of the current pandemic of the metabolic syndrome.

KEYWORDS

cirrhosis, diabetes, hepatocellular carcinoma, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, obesity

1 | INTRODUCTION

In a recent review[1] pooling data from 86 studies including 8.5 million subjects from 22 countries, the global estimated prevalence of non-alcoholic fatty liver disease (NAFLD) is 25.24%, with a peak prevalence in the Middle East and Latin America, and the disease increases with age. Thus, NAFLD is the most prevalent liver disease worldwide. Moreover 51.34% of patients are obese, and 22.51% have diabetes.[1] This short review will focus on the risk factors of the progression of NAFLD to advanced fibrosis and HCC.

2 | THE PROGRESSION OF LIVER FIBROSIS IN NON-ALCOHOLIC FATTY LIVER DISEASE

NAFLD is a slowly progressive disease, which does not lead to end-stage liver disease in most cases. It is difficult to estimate the rate of progression of NAFLD to severe fibrosis because of the high risk of selection bias when liver biopsies are performed in these patients, as well as the different criteria defining NAFLD in different cohorts. NAFLD may progress to non-alcoholic steatohepatitis (NASH),[2,3] which poses a significant risk of progression to hepatic

Abbreviations: BMI, body mass index; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL, high density lipoprotein; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing 3.

and non-hepatic complications and mortality. The average progression of liver fibrosis in NASH patients is 0.09% (95% CI: 0.06-0.12),[1] with an incidence of severe fibrosis of approximately 68 per 1000 person-years.

In a more recent systematic review and meta-analysis reporting fibrosis stage-specific mortality from five NAFLD cohort studies,[4] patients were at increasing risk of both liver-related and all-cause mortality as the stages of fibrosis increased. The estimated all-cause mortality rate ratio was 1.58 (in patients with stage 1 fibrosis) increasing to 2.52, 3.48 and 6.40 for stages 2, 3 and 4 fibrosis respectively. The increase was more dramatic in the liver-related mortality ratio, which sky-rocketed from 1.41 in patients with stage 1 fibrosis to 9.57, 16.69 and 42.30 in patients with stages 2, 3 and 4 fibrosis respectively.[4] Thus, fibrosis stage determines liver-related mortality, and its impact increases exponentially with progression to more severe lesions. It is clear that with the current epidemic of the metabolic syndrome, and the ageing of the affected population the global clinical and financial burden of NAFLD is bound to be staggering in the decades to come.

2.1 | Risk factors of progression

The risk factors for the progression of NAFLD to liver fibrosis are not fully understood. Although age has been associated with increasingly severe liver fibrosis in NASH patients, this is probably related to accumulating metabolic alterations occurring in the elderly with a long duration of liver disease. In a cross-sectional study in 432 patients, 26.8% with NASH and 17.4% with moderate to severe liver fibrosis, the independent predictors of moderate to severe fibrosis were male sex, Caucasian ethnicity, type 2 diabetes and increased liver enzymes, but not age.[5] The increased risk of fibrosis affects not only men, but also postmenopausal women and those who have undergone premature menopause.[6] Time from menopause is also directly associated with an increased likelihood of more severe liver fibrosis, after several adjustments (OR for each 5-year unit = 1.2, 95% CI 1.1-1.3, P = .002).[6] Ethnicity is also associated with NASH and fibrosis. However, although Hispanic ethnicity may predispose to NASH,[7] it is unclear whether this is also associated with increased progression of liver disease,[8] which has been suggested in Caucasians.[5] The differences among ethnic groups may be partly explained by genetic polymorphisms, most importantly an isoleucine to methionine protein variant at position 148 of the patatin-like phospholipase domain-containing 3 (PNPLA3) factor, a protein expressed in the liver and involved in lipid metabolism.[9] *PNPLA3* variants are associated with the progression of liver fibrosis and cirrhosis independent from liver inflammation and NASH.[10] Another rarer polymorphism that occurs in *TM6SF2* has also been shown to be associated with advanced liver fibrosis and cirrhosis independent from age, BMI, diabetes and *PNPLA3* genotype.[11]

Higher rates of fibrosis progression are observed in patients who are obese, or with type 2 diabetes.[2,3,12,13] In an important, well-designed prospective study in 52 patients with histologically proven NAFLD, who systematically underwent a biopsy after 36 months,[2] fibrosis progressed in 14 (27%), was stable in 25 (48%) and regressed in 13 (25%). It is important to note that these authors identified a decrease in BMI and waist circumference as independent predictors of stable liver disease activity and fibrosis.[2] Clinical and laboratory factors associated with the progression of fibrosis were also critically assessed in a meta-analysis of seven studies with paired biopsies.[14] Interestingly, this analysis found that the presence of arterial hypertension and low aspartate to alanine aminotransferase ratio at baseline liver biopsy were associated with the progression of fibrosis, but not age, ethnicity or diabetes. Higher grades of steatosis also seemed also to be more likely to lead to disease progression, but this was only based on two studies, where, oddly, the baseline severity of necroinflammation was not predictive of fibrosis progression. The authors correctly emphasized the heterogeneity among studies, and the difficulty of firmly establishing the independent factors of fibrosis progression in NAFLD patients, where several confounders, especially related to lifestyle, may play a role.

Keypoints

- Non-alcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease worldwide with an estimated global prevalence of ~25%.
- Although mainly benign, NAFLD may progress to non-alcoholic steatohepatitis, cirrhosis and hepatocellular carcinoma (HCC).
- Factors leading to the progression of NAFLD are only partially understood, a limitation that is particularly serious when considering that 40%-50% of HCC cases occur in non-cirrhotic livers, raising the question of whom and how to screen for this complication.

2.2 | Weight reduction has a beneficial role

Reduction in body weight has been identified as a major predictor of stability or improvement in liver lesions, including the stage of fibrosis.[15-17] This has also been reported in patients undergoing bariatric surgery to manage obesity,[18] although in these cases the mechanisms leading to improved liver lesions may also involve changes in intestinal hormone secretion.

A large prospective study analysed the effects of lifestyle changes to reduce body weight in 293 patients with histologically proven NASH, followed for 1 year.[17] Paired liver biopsies were collected at the beginning and end of the observation period in 261 patients. Improvement or even the resolution of NASH was more frequent in those who lost weight. In particular, NASH resolved in 90% of the patients who lost ≥10% of their weight and fibrosis regressed in 45%. However, a subsequent multicentre cross-sectional study of 1058 biopsy-proven NAFLD patients, emphasized the

importance of distinguishing obesity from metabolic status.[19] A metabolically healthy status was defined in that study by the absence of diabetes, low HDL, hypertriglyceridemia and arterial hypertension. The number of altered metabolic factors determined the risk of NASH and significant fibrosis. Interestingly, the latter was more frequently observed in the presence of adverse metabolic conditions in both obese and non-obese patients. Patients who were not obese but metabolically unhealthy more often had significant liver fibrosis than healthy obese patients (31.7% vs 11.4%, $P < .0001$). The authors mentioned that metabolically healthy obese patients are not entirely healthy and emphasized that the greatest impact on NASH and liver fibrosis is determined by a metabolically unhealthy status, which should be the real focus of patient counselling.

3 | PROGRESSION TO HCC

NAFLD may progress to hepatocellular carcinoma (HCC). According to a large, recent meta-analysis,[1] the incidence of HCC among persons with simple NAFLD is very low, that is, 0.44 per 1000 person-years (range, 0.29-0.66), which is much less than that commonly reported for chronic hepatitis B or C. In patients with NASH, on the other hand, the annual HCC incidence rate increases by more than 10-fold, that is, 5.29 per 1000 person years (95% CI: 0.75-37.56), which is remarkable but still less than that reported for other chronic liver disorders. These incidence rates should be considered in relation to two major epidemiological observations which have a significant public health impact. Firstly, the global prevalence of NAFLD is higher than that of any other chronic liver disorder such as chronic viral hepatitis B or C.[1] Secondly, the disease burden of HCC in NAFLD/NASH patients is increasing. In an analysis of the Scientific Registry of Transplant Recipients, including patients on the liver transplantation waiting list in the US between 2002 and 2016,[20] the proportion of patients with NASH and HCC increased 7.7-fold (from 2.1% to 16.2%, $P < .0001$). The prevalence of HCC with NASH on the same list increased 11.8-fold during the same period, showing it to be the fastest growth of all causes of liver disease listed for liver transplantation in the US, followed by chronic hepatitis B (6.0-fold), alcohol-related liver disease (3.4-fold) and chronic hepatitis C (2.3-fold). Global estimates confirm this tendency. A recent modelling study[21] assessing the future burden associated with NAFLD in China, France, Germany, Italy, Japan, Spain, United Kingdom and the US suggested that even if obesity and type 2 diabetes level off in the next few years, the prevalence of NASH could continue to increase with the long-term sequelae, including HCC, doubling until 2030, due to the ageing (and increase) in the world population.

3.1 | Risk factors of HCC

The risk factors of HCC in patients with NAFLD/NASH are only partly known. Although advanced liver fibrosis frequently precedes HCC, like in other chronic liver disorders, a significant proportion of HCCs occur in non-cirrhotic livers. Although early studies included patients who underwent surgical resection, liver transplantation or were recruited in tertiary referral centres,[22-26] creating a potential referral bias, recent studies with large databases have confirmed this observation. One important retrospective cohort study performed in patients with HCC diagnosed from 2005 to 2010 in the US Veterans Health Administration[27] investigated the risk factors for the development of HCC in the absence of cirrhosis. Medical records of 1500 patients were reviewed and 194 of them had no evidence of cirrhosis. A greater proportion of patients with HCC without cirrhosis had metabolic syndrome, NAFLD or had no risk factors of chronic liver disease compared to those with cirrhosis. Patients with NAFLD and HCC had a more than 5-fold higher risk of having HCC without cirrhosis, than those with hepatitis C virus (HCV)-associated HCC, for example. Similarly, patients with HCC and the metabolic syndrome had an unadjusted OR of 5.0 (95% CI 3.1-7.8) to have HCC without cirrhosis. Overall, 34.6% of patients with NAFLD and HCC had no evidence of cirrhosis, compared to 8.9% in patients with HCV, 7.7% in those with hepatitis B virus (HBV), and 11.1% in those with alcohol-related liver disease.[27] The lack of cirrhosis in a significant proportion of NAFLD patients has been extensively confirmed and may occur in about 30%-50% of patients.[28-31] The evidence was particularly convincing in a multicentre observational prospective study performed in Italy,[31] which not only confirmed that ~50% of patients with NASH and HCC were non-cirrhotic, but that less than half of them had been identified during routine screening programmes, raising the troublesome issue of how to identify curative HCC. It is important to note that HCC in NAFLD patients were larger at diagnosis and more frequently presented with an infiltrative pattern. Despite this, after careful patient propensity score matching, the survival rates of NAFLD HCC were similar to those observed in patients with HCV infection. Nevertheless, the authors emphasize the importance of identifying patients with NAFLD without cirrhosis who could benefit from surveillance, and respond to curative therapies.

Several additional studies have assessed the risk factors associated with the development of HCC in NAFLD/NASH which may be relevant to design targeted screening strategies. The features associated with the metabolic syndrome besides age and male sex, such as diabetes, arterial hypertension and increased BMI, are strongly and frequently among the independent risk factors. One study assessed whether obesity is an independent factor of HCC in patients with advanced cirrhosis undergoing liver transplantation.[32] In the 19 271 patients included in the study with an incidence of HCC of 3.4% (n = 659), obesity was an independent factor for HCC in patients with alcohol-related cirrhosis (OR 3.2; 95% CI, 1.5-6.6; $P = .002$) and cryptogenic cirrhosis (OR, 11.1; 95% CI, 1.5-87.4; $P = .02$), but not in those with HBV, HCV or other liver disorders, suggesting that closer surveillance is needed in the former.

Diabetes has also been repeatedly reported to increase the risk of HCC in patients with NASH. In a landmark study from the Mayo Clinic,[33] 253/354 (71%) patients with NASH and cirrhosis admitted between 2006 and 2015 had diabetes. After a median follow-up of 47 months, 30 patients developed HCC. Diabetes was independently

associated with an increased risk of HCC (HR = 4.2, 95% CI = 1.2-14.2, *P* = .02), together with age (each decade increasing by an HR of 1.8) and decreased serum albumin (HR = 2.1, 95% CI = 1.5-2.9, *P* < .01), while BMI and arterial hypertension were not. These results were validated in an analysis of liver transplantation registrants with NASH, where diabetes was still found to be an independent predictor of HCC (HR = 1.3, 95% CI = 1.0-1.7, *P* = .03).

A study performed in Taiwan in 23 820 persons followed for 14 years analysed the association between obesity, diabetes and HCC, depending on the presence or absence of HBV or HCV infection,[34] based on a link with the national tumour registry. Obesity was independently associated with an increased risk of HCC (RR 4.13; 95% CI, 1.38-12.4) in anti-HCV positive patients, but not in those with HBV. Importantly, persons without HCV or HBV had a two-fold increased risk of HCC after controlling for other metabolic factors (RR, 2.36; 95% CI, 0.91-6.17). Diabetes was associated with HCC irrespective of the presence or absence of HBV and HCV.

3.2 | Physical activity has a beneficial effect

Because the metabolic syndrome has a confirmed, negative impact on the risk of HCC, one could hypothesize that the level of physical activity should have a beneficial effect. Indeed, this association was first reported in a mouse model characterized by the spontaneous development of NASH and HCC.[35] Mice fed standard chow were randomized to an exercise (motorized treadmill) or sedentary routine for 32 weeks. At the end of the observation period, the exercising mice had fewer, smaller liver tumours, although no effect was identified on steatosis or NASH. Exercise resulted into increased phosphorylation of AMPK and its substrate raptor, leading to decreased mTOR activity. These experimental observations have been elegantly confirmed by recent data in humans. A landmark multinational cohort study (the European Prospective Investigation into Cancer and Nutrition Cohort, the EPIC Study) assessed the impact of vigorous physical activity on different types of liver cancer in more than 470 000 persons followed for a median of 14.9 months.[36] The multivariate-adjusted HR for HCC was 0.55 (95% CI 0.38-0.80) in active compared to inactive participants. Waist circumference and BMI explained respectively ~40% and 30% of the association. Vigorous physical activity for at least 2 h/wk was also found to be beneficial to the risk of HCC (HR 0.50, 95% CI 0.33-0.76) compared to no vigorous activity, after taking into consideration potential confounders. Interestingly, the presence and level of physical activity was not correlated to the risk of other liver cancers, such as intrahepatic bile duct cancers or non-gallbladder extrahepatic bile duct cancers.

Similarly, treatment of diabetes can reduce the risk of HCC. Indeed, this has been shown in several studies, especially in Asia. A nationwide study performed in Taiwan analysed 47 820 diabetic patients.[37] Independent factors associated with the risk of HCC were HCV, HBV, insulin use, cirrhosis and metformin use. In particular, each additional year of metformin use reduced the risk of HCC by about 7% in diabetic patients. Another study in 19 349 newly diagnosed diabetic patients and 77 396 control persons without diabetes recruited from the same Taiwan National Health Insurance Research Database showed a clear dose-dependent reduction in the risk of HCC in diabetics taking metformin. The risk reduction was greater than that in patients taking thiazolidinediones (51% vs 44% reduction).[37] Interestingly, statins also seem to protect from HCC. A nested case-control study from Korea in patients with newly diagnosed diabetes reported an adjusted OR of 0.36 (95% CI 0.22-0.60) in statin users vs non-users. Risk reduction was accentuated with an increase of cumulative defined daily doses.[38]

3.3 | The role of genetics

Finally, genetics seems to play a role in the risk of HCC in NAFLD patients. The association of *PNPLA3* variants and severe liver lesions has been confirmed in several studies. Heterogeneity at *rs738409* has also been associated with the risk of HCC in patients with NAFLD.[39] Variant frequencies were significantly different between 100 NAFLD-HCC cases (CC = 28, CG = 43, GG = 29) and 275 NAFLD-controls (CC = 125, CG = 117, GG = 33). After adjustment for age, gender, diabetes, BMI and cirrhosis, each copy of the *rs738409* G variant led to an additive risk for HCC with an OR of 2.26. The risk of HCC among GG homozygotes was five-fold vs wild type CC. These important results suggest that genetic variants could help stratify at risk patients in whom strict surveillance for HCC could be beneficial, independent of the presence of cirrhosis.[40] More data and prospective studies are clearly needed to further confirm this hypothesis.

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CONFLICT OF INTEREST

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REVIEW ARTICLE

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Non-invasive tests for liver fibrosis in NAFLD: Creating pathways between primary healthcare and liver clinics

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Abstract

Despite affecting around one-fourth of the general population worldwide, non-alcoholic fatty liver disease (NAFLD) remains a largely under-recognized disease in primary healthcare, with not more than 10% of patients diagnosed with NAFLD referred to specialists. The main challenge in clinical practice is the identification of those with advanced liver fibrosis or cirrhosis, as they are at the greatest risk of developing complications. Liver biopsy appears to be an unrealistic and unsuitable option because of the large number of high-risk patients and the well-known limitations of this technique. This has favoured the development of non-invasive tests, which have been an area of intensive research in the past decade. Transient elastography, FIB-4 and the NAFLD fibrosis score are the most extensively used and best validated tests, with summary AUROC values for detecting advanced fibrosis in NAFLD patients of 0.88, 0.84 and 0.84 respectively. Although much work remains to be done to establish cost-effective strategies for the screening for advanced fibrosis, the sequential use of non-invasive tests (serum biomarkers, then measurement of liver stiffness using transient elastography) appears to be the most promising strategy. The next step is to establish effective pathways in primary healthcare and/or diabetes clinics where most NAFLD patients are seen, to identify those who need to be referred to liver clinics for further assessment.

KEYWORDS

fibrosis, liver stiffness, NAFLD, non-invasive, serum biomarkers, transient elastography

1 | INTRODUCTION

Although it affects around one-fourth of the general population worldwide,[1] non-alcoholic fatty liver disease (NAFLD) remains a largely under-recognized disease in primary healthcare, with not more than 10% of patients diagnosed with NAFLD referred to specialists.[2] There may be several explanations for this. The lack of symptoms and of a good diagnostic marker, as well as the lack of awareness of many general practitioners plays an important role. However, most NAFLD patients will not progress, and only a minority, in particular those with non-alcoholic steatoheapatitis (NASH) and advanced liver fibrosis (histological stage Kleiner or SAF F3-4) are at the greatest risk of developing the complications of chronic liver disease. Indeed, advanced fibrosis, but not NASH, has been shown to be the major driver of long-term outcome and mortality.[3,4] For instance, in a recent meta-analysis[3] including 1495 NAFLD patients with 17 452 patient-years of follow-up, risk for all-cause mortality (mainly cardiovascular) and for liver-related mortality were increased in case of cirrhosis, with a mortality rate ratio (MRR) of 6.40 (95% CI 4.11-9.95) and 42.30 (95% CI 3.51-510.34) respectively. Therefore,

Abbreviations: 2D-SWE, two-dimensional shear wave elastography; MRE, Magnetic resonance elastography; NAFLD, non-alcoholic fatty liver disease ; NASH, non-alcoholic steatohepatitis ; NFS, NAFLD fibrosis score; pSWE/ARFI, point shear wave elastography/acoustic radiation force imaging; TE, transient elastography.

fibrosis has become the main focus in referral centres and screening NAFLD patients for advanced fibrosis is the current challenge in clinical practice.

Liver biopsy, the gold standard for the staging of liver fibrosis, appears unrealistic and unsuitable in this group because of the large number of high-risk patients and the well-known limitations of this technique. This has favoured the development of alternative non-invasive strategies, which have been an area of intensive research over the past decade. Although none of the non-invasive tests adequately discriminate NASH from simple steatosis in patients with NAFLD,[5] they are now extensively used in liver clinics to detect advanced liver fibrosis and are recommended by international guidelines.[6-9]

This review will discuss the performance, advantages and limitations of non-invasive tests for detecting advanced fibrosis in NAFLD patients and how they can be used to establish effective pathways between primary healthcare and/or diabetes clinics to identify patients who should be referred to specialists.

Key Points

- The challenge in clinical practice is to identify NAFLD patients with advanced liver fibrosis or cirrhosis, as they are at the highest risk of developing complications.
- The best way to meet this challenge is the sequential use of non-invasive tests to select patients who should be considered for liver biopsy.
- Transient elastography, FIB-4 and NAFLD fibrosis score are the most widely used and best validated tests.
- Availability, cost, applicability and the context of use are critical issues when using non-invasive tests.
- The next step is to establish effective pathways from primary healthcare and/or diabetes clinics where most NAFLD patients are seen to identify those who need to be referred to liver clinics for further assessment.

2 | AVAILABLE NON-INVASIVE TESTS AND IMPORTANT CONSIDERATIONS WHEN USING THEM

Non-invasive tests rely on two different but complementary approaches: either measuring the levels of serum biomarkers, or measuring liver stiffness using ultrasound- or magnetic resonance-based elastography techniques. Currently available non-invasive tests are summarized in Figure 1. Serum biomarkers range from simple and inexpensive (non-patented) tests such as AST/ALT ratio, AST to platelet ratio Index (APRI), Fibrosis-4 (FIB-4) and NAFLD fibrosis score (NFS) to more sophisticated patented tests such as the FibroTest® (Biopredictive, Paris, France), Fibrometer®, (Echosens, France), ELF™ score (Siemens Healthcare, Germany) and Hepascore (PathWest, University of Western Australia, Australia). Elastography includes ultrasound-based techniques, such as transient elastography (TE) (FibroScan, Echosens, France), point shear wave elastography (pSWE) using acoustic radiation force imaging (ARFI) and two-dimensional shear wave elastography (2D-SWE) and magnetic resonance elastography (MRE).

There are several critical issues that should be considered when using non-invasive tests: availability, cost, and 'context of use'.[5] For instance, non-patented serum biomarkers such as FIB-4 and NFS, which are based on simple, inexpensive and widely available parameters, seem particularly suited for first-line use in primary healthcare settings. In contrast, MRE, which is only available in a few expert tertiary referral centers, is time-consuming and expensive, and thus better suited for research or clinical trials (Figure 1). In addition, when evaluating the performance of non-invasive tests, applicability, which is defined as the sum of the reliability (the percentage of interpretable tests) plus the failure rate (absence of test results), should be taken into account. For instance the FibroTest® has been shown to have an excellent applicability (99%) [10] whereas the applicability of TE is lower in obese patients when the regular probe is used (M) (around

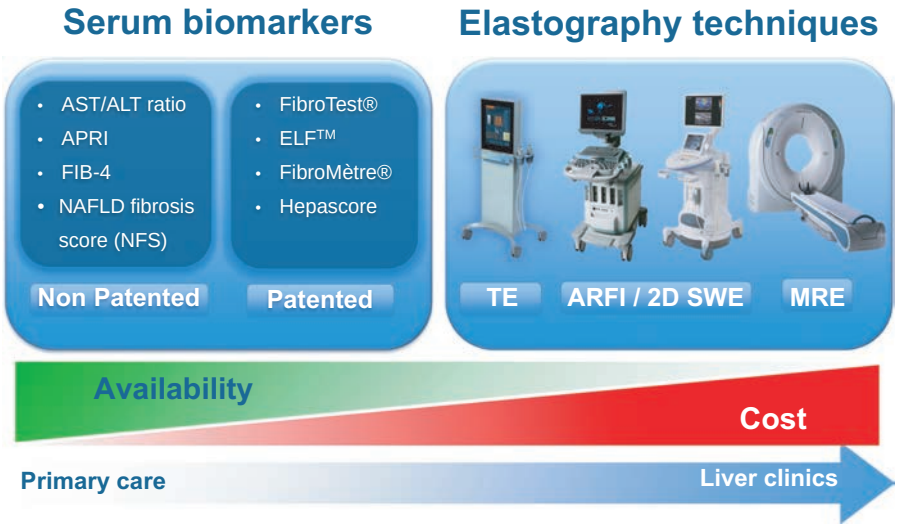

FIGURE 1 Currently available non-invasive tests. Importance of taking into account availability, cost and context of use. TE, transient elastography; pSWE/ARFI, point shear wave elastography/acoustic radiation force imaging; 2D-SWE, two-dimensional shear wave elastography; MRE, magnetic resonance elastography

80%).[11] However, recent studies in NAFLD patients have shown that TE applicability increased to 97% when the XL probe was used.[12,13]

3 | PERFORMANCE OF NON-INVASIVE TESTS FOR DETECTING ADVANCED FIBROSIS IN NAFLD

3.1 | Serum markers

A recent meta-analysis compared the performances of APRI, FIB-4 and NFS for diagnosing advanced fibrosis (based on 64 studies in 13 046 NAFLD patients) with summary AUROCS of 0.77, 0.84 and 0.84 respectively.[14] FIB-4 and NFS were the most accurate, with high-negative predictive values (>90%) for excluding advanced fibrosis. No meta-analyses independent from the developers are available for patented tests. When compared head-to-head in the same population, patented serum biomarkers tend to outperform non-patented serum biomarkers. For instance, in a French study in 452 patients with biopsy-proven NAFLD from the developer of Fibrometer®, comparing Fibrometer® to FibroTest® and Hepascore as well as FIB-4 and NFS, Fibrometer® outperformed all the other tests for diagnosing advanced fibrosis.[15] In another study from the same group [16] that compared Fibrometer® and ELF™ to FIB-4 and NFS in 417 NAFLD patients, Fibrometer® and ELF™ performed equally but outperformed FIB-4 and NFS. However, in a recent independent head-to-head comparison in a large cohort of 3202 patients with biopsy-proven NAFLD (71% with advanced fibrosis) from two phase 3 trials,[17] the performance of ELF™ and FIB-4 was similar for the diagnosis of advanced fibrosis (AUROCs 0.80 vs. 0.78) while the performance of NFS was lower (AUROC 0.74). Thus, despite a slight improvement in diagnostic accuracy compared to non-patented serum biomarkers, the limited availability of patented serum biomarkers and their cost could limit their widespread application, particularly in primary healthcare settings.

3.2 | Elastography

TE is the technique with the largest amount of evidence (around 4000 patients).[5] In the most recent meta-analysis based on 19 studies and 2495 NAFLD patients from different ethnic backgrounds, TE had excellent accuracy for diagnosing advanced fibrosis with a summary AUROCS of 0.88.[14] However, most of these studies used the M probe. Two recent multicentre prospective studies using the XL probe in large cohorts of NAFLD patients (around 400) in the UK [12] and in the USA,[18] confirmed these results with AUROCs of 0.80-0.83 for advanced fibrosis and 0.89-0.93 for cirrhosis. Interestingly, steatosis and the type of probe did not affect liver stiffness measurements. Similarly, in a large cohort of 496 NAFLD patients who underwent TE with both the M and XL probes, Wong et al[19] showed that a high BMI (>30 kg/m^2), but not severe steatosis, increased liver stiffness values. The authors concluded that the same cut-offs can be used without further adjustment for steatosis when the M and XL probes are used according to the appropriate BMI. Overall, these results suggest that TE is the best technique to confidently exclude advanced fibrosis and cirrhosis with a high-negative predictive value (around 90%) in NAFLD patients. For example, TE had a 94% to 100% negative predictive value at a cut-off < 8 kPa. In contrast, the positive predictive value did not exceed 64% at a cut-off > 10 kPa. Finally, TE is available in most liver clinics worldwide and is thus the technique of choice for the first-line screening of advanced fibrosis.

Data on other ultrasound-based techniques remain limited in NAFLD patients. One meta-analysis of pSWE/ARFI based on nine studies and 982 NAFLD patients reported summary AUROCs of 0.94 and 0.95 for advanced fibrosis and cirrhosis respectively.[20] A meta-analysis from the company, evaluating 2D-SWE in 156 NAFLD patients,[21] reported summary AUROCs of 0.93 and 0.92 for advanced fibrosis and cirrhosis respectively. Because of the limited amount of data, pSWE/ARFI and 2D-SWE are not included in current guidelines on the management of NAFLD.[6,7] In a recent meta-analysis based on five studies and 628 NAFLD patients, the pooled AUROC for advanced fibrosis with MRE was 0.96.[14] However, despite its excellent accuracy, MRE remains a research tool because of its limited availability and cost.

In summary, TE, FIB-4 and NAFLD fibrosis scores are the most widely used and best validated non-invasive tests in NAFLD patients.

4 | REFERRAL PATHWAYS FROM PRIMARY HEALTHCARE/DIABETES CLINICS TO LIVER CLINICS

Although most of the existing data on the use of non-invasive tests come from liver clinics, most NAFLD patients, with different prevalences and severities, are seen either in primary care or diabetes clinics.

4.1 | Primary healthcare

Because of the high prevalence and low severity of NAFLD (less than 5% of patients with advanced fibrosis) in primary healthcare, general practitioners must be actively involved in the screening and management of this group, with care pathways in place to identify patients at risk of liver disease for referral to liver clinics.[22] Because of their simplicity, wide availability and high-negative predictive values, FIB-4 and NFS seem to be well suited for use in primary care to identify NAFLD patients without advanced fibrosis who do not require further assessment. Indeed, a recent study performed in a low-risk cohort from the Hong Kong general population (n = 922) has shown that a negative result with FIB-4 or NFS was associated with a negative predictive value of 98%, indicating that people without advanced fibrosis can be confidently excluded, with a minimal chance of a missed diagnosis.[23] It should be kept in mind that FIB-4

and NFS do not adequately rule-in advanced fibrosis (with a high rate of false-positive results), thus further assessment with a more specific test is required in case of positive results. Several tests have been proposed for these cases, including ELF™,[24,25] Fibrometer®[26] and TE.[23,25-27] For instance, in a large, prospective study of a UK primary care referral pathway in 1,452 patients with NAFLD, Srivastava et al[24] recently showed that a two-step pathway (FIB-4 followed by ELF™ performed in those with an indeterminate (score 1.3-3.25) FIB-4) reduced unnecessary referrals to liver specialists by 81%, and also markedly increased (five-fold) the accurate referral of cases with advanced fibrosis. Importantly, because of the dual FIB-4 or NFS cut-offs, a significant proportion of patients (around 30%) fall in the intermediate risk category and cannot be correctly classified (indeterminate results). This may lead to unnecessary referral of these patients to liver clinics for further assessment. Indeed, performing a second test (ELF™) in case of an indeterminate FIB-4 rescued 84% of patients who were subsequently found to have advanced fibrosis or cirrhosis, and would otherwise have been falsely reassured by FIB-4 alone.[24] However, the cost and limited availability of patented serum biomarkers such as ELF™ might limit their widespread application in primary healthcare.

In practice, FIB-4, based on age, transaminases and platelet count, is easier to calculate than NFS (which requires albumin) and may thus be more suitable for use by general practitioners. Finally, the simplest and most pragmatic approach, which is more likely to be used by general practitioners, would be the use of a single cut-off strategy (refer all patients with FIB-4 > 1.3 for TE examination in the liver clinic), but with a risk of over-referral (Figure 2A).

4.2 | Diabetes clinics

The expected prevalence of advanced fibrosis in diabetes clinics is much higher (around 20%) than in primary care and close to that seen in liver clinics. In addition, it has been suggested that serum biomarkers of fibrosis, which were developed and validated in non-diabetic cohorts, underperform when applied to diabetic patients.[28] Also, the use of FIB-4 or NFS may lead to a higher proportion of patients in the intermediate category.[25] Thus, the direct use of a more specific test seems more suited to this setting (Figure 2B). This screening strategy has been tested by systematically performing liver stiffness measurements using TE in consecutive type 2 diabetes patients seen at diabetes clinics in Hong Kong (n = 1918)[29] and in France (n = 705).[30] As a result, 13 to 18% of patients had elevated liver stiffness and 50% of those who underwent a liver biopsy (94 and 47 respectively) had advanced fibrosis. This strategy seems promising but requires further validation.

In conclusion, screening programs using sequential algorithms (FIB-4 or NFS followed by TE) to identify NAFLD patients with advanced fibrosis in primary healthcare or TE in diabetes clinics, should be explored and adequate education of general practitioners and diabetologists on the follow-up and treatment of patients considered to be at low risk, is needed.

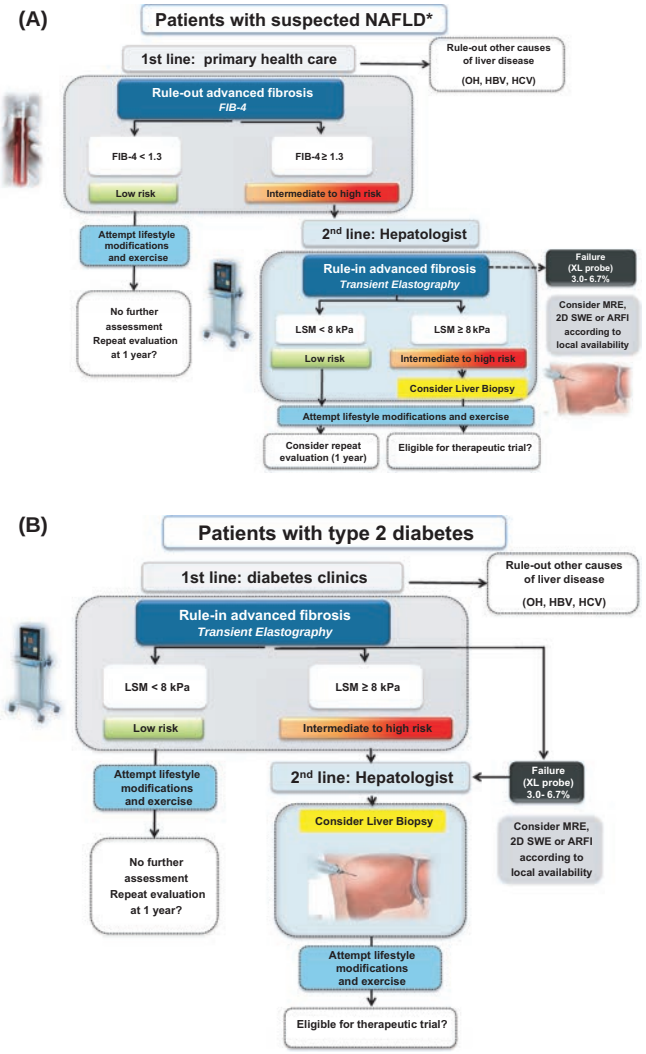

FIGURE 2 A suggested algorithm for the use of non-invasive tests for risk stratification of patients with suspected NAFLD in clinical practice in primary healthcare. *Suspicion of NAFLD is based on the presence of steatosis on ultrasound or abnormal liver tests (transaminases/γ-glutamyl transferase) in patients with risk factors (obesity, type 2 diabetes or metabolic syndrome). The proposed algorithm is based on expert opinion. The choice of non-invasive tools should be sequential, guided by local availability and the context of use. B, A suggested algorithm for the use of non-invasive tests for risk stratification of patients with type 2 diabetes in diabetes clinic. Adapted from ref 5

CONFLICT OF INTEREST

Speaker bureau of Abbvie, Echosens, Gilead, and Novo Nordisk. Consultant for Allergan, Gilead, Intercept, MSD, Pfizer and Servier.

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REVIEW ARTICLE

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A diabetologist's perspective of non-alcoholic steatohepatitis (NASH): Knowledge gaps and future directions

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Abstract

There is a close link between steatohepatitis (NASH) and Type 2 diabetes (T2DM). Recently, the American Diabetes Association (ADA) recommended screening for NASH and advanced fibrosis in patients with diabetes and hepatic steatosis or elevated plasma alanine aminotransferase (ALT). This is because as many as ~30% to 40% may have NASH and ~10% to 15% advanced fibrosis. The role of hyperglycemia and the natural history of NASH in diabetes remain poorly understood, as well as which diagnostic algorithm or interventions are most cost-effective. There is significant clinical inertia and most patients today are still not receiving adequate lifestyle intervention or pharmacological treatment with diabetes agents known to be effective against NASH. Lifestyle intervention improves steatohepatitis in proportion to the magnitude of weight loss, but this trend is not as consistent for regression of fibrosis. This limited success supports the need for concomitant pharmacological therapy. Pioglitazone has been shown to consistently induce resolution of NASH in both patients with or without diabetes in a total of 498 participants in five randomized controlled trials (RCTs), but with modest effects on liver fibrosis. Proof-of-concept studies suggest a potential role for GLP-1RAs and SGLT2 inhibitors. Combination therapy is on the horizon. Treating diabetes and NASH with a combination of pioglitazone, GLP-1RAs or SGLT2i, could be a cost-effective strategy to treat both diseases while reducing their high cardiovascular risk. Future combination therapies will likely combine existing diabetes agents with novel NASH-spechfic drugs under development. This review highlights current knowledge gaps and proposes future directions for the treatment of NASH in diabetes.

The worldwide increase in obesity has not only quadrupled the number of individuals with diabetes mellitus in the past three decades[1] but created an epidemic of steatohepatitis (NASH) of unsuspected proportions just a decade ago. The worldwide prevalence of non-alcoholic fatty liver disease (NAFLD) is at least 55% in patients with Type 2 diabetes (T2DM).[2] However, it is probably higher because more than 90% of the studies used liver ultrasound for the diagnosis, which is less sensitive for intrahepatic triglyceride content than magnetic resonance-based imaging techniques.[3] Recently, Younossi et al[4] reported the significant burden of NASH in patients with T2DM in the United States. The overall prevalence of NAFLD was found to be >70%, or more than 18 million patients with T2DM and NAFLD. Of these, the investigators estimated that one in four had NASH. In patients with simple steatosis (non-alcoholic fatty liver or NAFL) most of the cost was

Abbreviations: ADA, American Diabetes Association; CVD, Cardiovascular disease; CKD, Chronic kidney disease; DPP-IV, Dipeptidyl peptidase-4 inhibitor; FFA, Free fatty acids; GLP-1RAs, Glucagon like peptide-1 receptor agonists; IHTG, Intrahepatic triglycerides; 1H-MRS, Magnetic resonance and spectroscopy; MRI-PDFF, Magnetic resonance imaging–estimated proton density fat fraction; NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; RCT, Randomized placebo-controlled trial; SGLT2i, Sodium-glucose co-transporter-2 inhibitors; T2DM, Type 2 diabetes; VCTE, Vibration controlled elastography.

from diabetes care, but it increased to 25% of all diabetes care costs in patients with NASH. The total liver-related cost of NASH compared to NAFL was $2,275 vs $95 per person-year respectively. Over the next 20 years, NASH in T2DM is projected to account for 29% of all liver transplants and 812,000 liver-related deaths. Of note, the model did not include the potentially higher rates of diabetes-related micro- and macrovascular complications associated with NASH.[5] These findings should alarm diabetologists and prompt them to become proactive by viewing NASH as a common and serious complication of T2DM that should be systematically screened.[6]

1 | ROLE OF THE DIABETOLOGIST IN THE MANAGEMENT OF NASH

Current guidelines[7,8] remain vague about who should be screened for NASH-fibrosis. They discourage systematic screening because of the knowledge gaps about the cost-effectiveness of this approach, while at the same time they suggest "consider screening" in high risk patients such as those with obesity/metabolic syndrome or T2DM. This mixed message about screening and intervention is understandable as the impact of early intervention on the natural history of steatohepatitis is incomplete. For instance, controlled trials of lifestyle interventions have all been shorter than 1-year and few pharmacological interventions for NASH have gone beyond 2-3 years.[6-8] However, screening strategies for many diseases were originally not as evidence-based as desired and vague guidelines risk confusing clinicians and resulting in inaction in primary care.

A step forward in the field of diabetes has been the 2019 Standard of Care guidelines by the American Diabetes Association that recommended that "patients with T2DM and elevated liver enzymes aminotransferase (ALT) or fatty liver on ultrasound should be evaluated for the presence of NASH and liver fibrosis" (Recommendation 4.14, page S40).[9] This has helped raise awareness among primary care physicians and diabetologists to more actively participate in the management of NASH. One would expect that intensive lifestyle or pharmacological interventions (at least with pioglitazone as proven in five randomized controlled trials [RCTs]) see (Table 2) would reduce the risk of cirrhosis in this population. Furthermore, worse micro- and macrovascular complications may occur in patients with NAFL or NASH.[6,10] Diabetologists are becoming increasingly aware that NASH should be screened for in the same way as diabetic retinopathy or nephropathy.

2 | THE LINK BETWEEN DIABETES AND NASH: KNOWLEDGE GAPS AND FUTURE DIRECTIONS

The links between diabetes and NAFLD are complex and bidirectional, with one worsening the other and vice-versa.[10] The mechanisms remain poorly understood but include among others, genetic factors, insulin resistance, dysfunctional adipose tissue (lipotoxicity), chronic hyperglycemia ("glucose toxicity" or glucotoxicity), altered gut microbiome and many others. Recent in-depth reviews[10-14] have examined the many genetic and environmental factors at play. NASH may accelerate the progression of prediabetes to diabetes and make it more difficult to control. On the other hand, diabetes itself appears to accelerate steatohepatitis and the progression of fibrosis to cirrhosis,[10] although there are few controlled prospective studies to date. In a recent small head-to-head analysis[15] more significant fibrosis progression was found in 18 months in patients with diabetes than in those without.

As summarized in Table 1, an area that deserves future attention is understanding the factors linked to glucotoxicity that may drive steatohepatitis. Most of our knowledge of the role of glucotoxicity are from in vitro or in vivo experiments. While there are obvious difficulties in conducting studies in humans, such as obtaining liver tissue or paired biopsies following an intervention, certain key studies are feasible in the clinical setting. These include long-term, prospective studies on the impact of chronic hyperglycemia to compare disease progression in obese individuals with or without T2DM. This would require a rigorous design where the severity of liver disease is established by histology (biopsy) at baseline and a liver biopsy repeated

Key points

- The risk of non-alcoholic steatohepatitis (NASH) with advanced fibrosis in Type 2 diabetes (T2DM) is being increasingly recognized among diabetologists. However, there are major gaps in knowledge: Why does the prognosis seem to be worse for NASH with diabetes? What is the optimal diagnostic algorithm? How can physicians best take advantage of available diabetes agents to treat both hyperglycaemia and NASH?
- There is significant clinical inertia about behavioural modification and pharmacological treatment. An intensive and ideally structured lifestyle intervention is essential for the successful treatment of NASH in diabetes.
- Pioglitazone is the only diabetes agent incorporated into treatment guidelines for NASH. Glucagon like peptide-1 receptor agonists (GLP-1RAs) and Sodium-glucose co-transporter-2 (SGLT2) inhibitors are being actively investigated.
- Given that pioglitazone, GLP-1RAs and SGLT2 inhibitors reduce cardiovascular disease, the major cause of death in patients with NASH, future studies should explore their role alone or in combination to improve both T2DM and steatohepatitis while reducing the cardiometabolic risk.
- Future work should focus on understanding why diabetes is associated with disease progression, on improving screening strategies in the primary care setting, and on examining the role of combination therapy between existing antidiabetic and novel agents in development.

TABLE 1 Potential mechanisms by which chronic hyperglycemia may play a role in NASH

- Hepatic inflammation and oxidative stress with formation of hydrogen peroxide/hydroxyl radicals leading to hepatocyte lipid peroxidation.
- Accelerate the production of advanced glycosylation end-products (AGEs) and development of inflammation in Kupffer and hepatic stellate cells (both have receptors for AGE).
- Alteration of the hepatocyte microenvironment by glucotoxicity in the same way as for micro- and macrovascular diabetic complications.
- Upregulation by hyperglycemia of genes involving key lipogenic the transcription of genes encoding key lipogenic and glycolytic pathways (i.e. transcription factor carbohydrate responsive element-binding protein or ChREBP; stimulation of liver-pyruvate kinase [L-PK] and many other).
- Activation by high-fructose diets of de novo lipogenesis with upregulation of inflammatory pathways (i.e. c-jun N-terminal kinase [JUN]-signaling pathway).

Abbreviation: NASH, Non-alcoholic steatohepatitis.

over time. A standardized assessment of the micro- and macrovascular complications of diabetes would be needed at inclusion and during regular follow-up to help determine their relationship to the severity of NASH over time. Although some of this information is being gathered from the placebo arms of clinical trials on new NASH drugs, it is often not reported, or detailed information on diabetic complications is lacking.[14] A study of this type would also provide long-term information on the role of glycemic control, weight gain or weight loss over time, or that of other relevant factors.

It is still not known if optimal control of hyperglycemia can reverse steatohepatitis or fibrosis, or at least slow its progression. A RCT on this topic would have to avoid the confounder of weight loss induced by some agents (e.g. with Glucagon like peptide-1 receptor agonists [GLP-1RA] or Sodium-glucose co-transporter-2 inhibitors [SGLT2i]), or of modifying insulin resistance per se (with pioglitazone). Optimal control might be achieved with a combination of metformin, sulfonylureas, dipeptidyl peptidase-4 (DPP-IV) inhibitors and insulin, which are all believed to have minimal or no effect on liver histology in NASH, while avoiding the use of GLP-1RA, SGLT2 inhibitors or pioglitazone. Patients could be kept on a stable dose of these agents at study entry in combination with the other agents to facilitate recruitment and study retention. Treatment would compare the impact of achieving normoglycemia vs a higher HbA_{1c} target, as seen in glycemic control trials.[16,17] The study would not need to be as long as glycemic control trials[16,17] because the primary outcome would be the effect of treatment on the reversal of steatohepatitis without worsening of fibrosis, which would be expected 12-18 months after intervention.

3 | THE CHALLENGE OF DIAGNOSING NASH IN PATIENTS WITH T2DM

While there is increasing awareness about NASH in patients with diabetes, the true challenge is to develop a cost-effective approach for the early diagnosis of moderate-to-advanced liver fibrosis (≥F2). It must be practical, pertinent for busy primary care clinicians and feasible as a standard of care, such as the screening approaches for retinopathy, neuropathy or nephropathy. The diabetologist or primary care physician must learn to distinguish between patients with simple steatosis (NAFL) who do not need to be referred to a specialist, from those with NASH and moderate-to-severe fibrosis requiring further evaluation, and eventually a liver biopsy.

Several diagnostic approaches have been suggested,[7,8,18] in particular an algorithm with a focus on primary care providers and diabetologists has been proposed by Bril and Cusi[6], and more recently by Castera et al[19]. As previously discussed,[6-8,18-20] the sensitivity of non-invasive tests (NITs) is limited for the diagnosis of steatohepatitis or the early stages of fibrosis. Overall, the specificity and negative predictive value of NITs is good for advanced fibrosis while the sensitivity and positive predictive value are less strong. For instance, in a recent study by Siddiqi et al[20] in 1,904 patients enrolled in the NIDDK NASH Clinical Research Network recruited from 2004 through 2018, FIB-4 and the NAFLD fibrosis score (NFS) outperformed other non-invasive models for detecting advanced fibrosis, although their area under the curve (AUC) was only 0.80 for FIB-4 and 0.78 for NFS. Similar results were reported by Anstee et al,[21] using baseline data from the STELLAR Trials in 3,202 patients with evaluable biopsies. The performance of individual NITs with single thresholds derived from the literature to discriminate advanced fibrosis (F3-F4) from less severe disease (F0-F2) had an AUC of 0.74 for NFS, 0.78 for FIB-4, and 0.80 for both Enhanced Liver Fibrosis (ELF) and for imaging by vibration controlled elastography (VCTE; FibroScan). The investigators found that a threshold with a high degree of sensitivity had an unacceptably low degree of specificity, and vice versa. A combination of NITs improved the predictive value somewhat, but the sensitivity remained low with an AUC ranging between 0.69 and 0.77. Further studies are needed to know how these data can translate into the "real world" given the high prevalence (71%) of patients with F3-F4 fibrosis in this study, which is very different from the population to be screened in primary care or by diabetologists. This makes the interpretation of the positive and negative predictive values difficult (although it would not influence sensitivity and specificity which are not affected by prevalence). In our experience, the performance of plasma biomarkers and diagnostic panels is worse in primary care or diabetes clinics than in cohorts of patients with advanced liver fibrosis (from liver clinics). Similar results were reported for VCTE in another recent study,[22] with AUCs of 0.77 in patients with F2 and 0.80 in those with F3. As expected, results were higher in F4; AUC = 0.89. It should be noted that 50% of these patients had diabetes, thus a future analysis from this cohort comparing VCTE results to those without diabetes will be of interest.

Most patients in primary care clinics will not have any fibrosis (F0) or will be "in the middle" of the disease spectrum (F1 or F2). Thus, the performance of plasma biomarkers and diagnostic panels is not expected to be as good. Future studies must develop algorithms in this setting. This is also our own personal experience in patients recruited in diabetes clinics.[23-26] A recent study compared several plasma biomarkers and diagnostic panels in 213 patients with T2DM not

recruited from hepatology clinics.[26] None of the diagnostic tests for NASH were better than plasma ALT (AUC: 0.78) (e.g. NashTest 2, HAIR, BARD or OWLiver). Similarly, none significantly outperformed plasma AST (AUC: 0.85) for advanced fibrosis (≥F3) (e.g. APRI; FIB-4; Fibrotest or NFS). Numerically, but without reaching statistical significance, the AUC of plasma fragments of propeptide of type III procollagen or PRO-C3 was greater than plasma AST (AUC 0.90 [0.85–0.95] vs. 0.85 [0.80–0.91]) and also complemented an initial AST ≥ 26 IU/L in a sequential model. However, the sequential model also led to acceptable results with non-commercial diagnostic tests such as FIB-4 or APRI.

Future studies in larger cohorts of patients with T2DM are ongoing and will probably help optimize screening strategies. Examples of large academic-industry consortiums for the development of biomarkers are NIMBLE (Non-Invasive BioMarkers of Metabolic Liver Disease; www.fnih.org) supported by the Foundation for the National Institutes of Health Biomarkers Consortium, and the LITMUS project (Testing Marker Utility in Steatohepatitis; https://litmus-project.eu) which is a combined effort by the European Innovative Medicines Initiative Joint Undertaking (IMI 2 JU) with companies from the European Federation of Pharmaceutical Industries and Associations (EFPIA). This work, and that of many other investigators in the field, will help validate current and future NITs and enter a new era of precision medicine to target therapy to patients at the highest risk of developing cirrhosis.

4 | THE FUTURE OF TREATING NASH IN PATIENTS WITH T2DM

4.1 | What have we learned so far?

Treatment options for NASH in patients with T2DM are expanding **(**Table 2**).** Vitamin E is effective in patients with biopsy-proven NASH without diabetes[27] and had a somewhat similar effect for the resolution of NASH in a recent small proof-of-concept study in patients with T2DM.[28] However, the main goal of the study was to examine the role of adding vitamin E to pioglitazone which did not result in an improvement in histological parameters compared to those with thiazolidinedione alone.[27,29-31] A large multicenter study is needed to determine the efficacy and safety of vitamin E in patients with diabetes.

Because diabetes and NASH often overlap, there is a strong rationale for treating both with antidiabetic agents. While metformin is not effective,[7,8] current treatment guidelines have incorporated pioglitazone as a therapeutic option in patients with or without T2DM.[6,7]

Table 2 summarizes the five pioglitazone RCTs that have treated a total of 498 patients for up to 3 years. More than 50% of patients (placebo-subtracted difference of ~30 to 40%) showed a consistent response with either resolution of NASH or improvement in individual histological parameters (such as ballooning or inflammation).[27-31] However, improvement of fibrosis was only reported in some of the studies.[29,31] We refer readers to recent in-depth reviews on the overall safety and efficacy of pioglitazone in NASH.[6,12-14,32] Unfortunately, confusion between rosiglitazone and pioglitazone (e.g. pioglitazone reduces CVD but not rosiglitazone) and misinformation about safety (e.g. weight gain; summarized in Table 2) have limited prescriptions and resulted in clinical inertia. Care must be taken when prescribing this agent, such as in patients with diastolic dysfunction (i.e. heart failure with preserved ejection fraction), congestive heart failure, severe obesity, especially if lower limb oedema is present, as well as of concomitant use with amlodipine or high-dose insulin, which may also promote lower extremity oedema or use in patients with a history of osteoporosis or bladder cancer. Most patients tolerate this thiazolidinedione well. Weight gain is dose-dependent (2%-5%) and greater in the absence of a lifestyle program. Most patients respond well to an intermediate dose of 30 mg/day with no need for the 45 mg/day maximal dose (personal experience). Future studies must explore the role of lower-dose pioglitazone (15 mg/day) and of combination therapy with GLP-1RAs or SGLT2 inhibitors in this population.

4.2 | What is the future role of GLP-1RAs for NASH?

As summarized in Table 2, most RCTs of GLP-1RAs have reported a reduction in ALT or intrahepatic triglyceride content.[32,33] Liver histology was also improved in a landmark proof-of-concept study.[34] The mechanisms remain unclear and were significantly related to weight loss, however, this may not explain it all.[14,32] In contrast, the results with DPP-IV inhibitors were largely negative for the treatment of NASH in placebo-controlled trials[32,33] **(**Table 2). Weekly semaglutide is a more potent GLP-1RA and has been found to be more effective in a head-to-head analysis than liraglutide in lowering plasma glucose and body weight.[35] Results with the daily injectable semaglutide formulation for patients with NASH will soon become available (Clinical.Trials.gov NCT02970942). Semaglutide is also being explored in combination therapy in dual and triple therapy with novel agents such as cilofexor (a farnesoid X receptor agonist) and firsocostat (an acetyl-CoA carboxylase inhibitor) (NCT03987074). Finally, a novel development is oral semaglutide. In recent studies, collectively known as the PIONEER trials, oral semaglutide led to better glycemic control and more weight loss when compared to liraglutide, empagliflozin or sitagliptin for the treatment of T2DM.[36] However, it should be noted that there was significantly less weight loss with oral semaglutide than with injectable formulations. In a recent systematic review of 11 RCTs including 9,890 patients, average weight loss was ~3.0 kg with oral semaglutide.[36] Prescriptions of GLP-1RAs for the management of T2DM will probably increase because of their cardiovascular and renal protection. This will greatly impact the future management of NASH, either alone or in combination therapy.

4.3 | Is there a future role for SGLT2i for the treatment of NASH?

Like GLP-1RAs, clinicians are increasingly prescribing SGLT2 inhibitors for the management of T2DM because of the benefit of these agents on cardiovascular and chronic kidney disease.[37] Early in vivo[38,39] and

TABLE 2 Randomized placebo-controlled trials examining the effect of diabetes medications for the treatment of NAFLD

Author	Medication	n	Duration (weeks)	Body weight	Intrahepatic TG[b]	Histology[c]
Pioglitazone						
Belfort et al[29]	PIO	55	24	↑2.7%	↓54%	Improved
Aithal et al[30]	PIO	74	48	↑2.9%	–	Improved
Sanyal et al[27]	PIO	163	104	↑4.8%	–	Improved
Cusi et al[31]	PIO	101	72[d]	↑2.5%	↓39%	Improved
Bril el al[28]	PIO + vit E	105	72	↑5.2%	–	Improved
GLP-1 RAs						
Smits et al[52]	Liraglulide	18	12	unchanged	unchanged	–
Armstrong et al[34]	Liraglutide	52	48	↓4.3%	–	Improved[e]
Vanderheiden et al[53]	Liraglutide	71	24	↓2.2%	↓33%	–
Frossing et al[54]	Liraglutide	72	26	↓5.6%	↓32%	–
DPP-IV inhibitors						–
Smits et al[52]	Sitagliplin	18	12	unchanged	unchanged	–
Cui et al[55]	Sitagliplin	50	24	unchanged	unchanged	–
Joy et al[56]	Sitagliplin	12	24	unchanged	unchanged	–
Macauley et al[57]	Vildagliptin	44	24	↓2.1%	↓27%	–
SGLT2 inhibitors						
Bolinder et al[43]	Dapaglifiozin	67	24	↓2.2%	unchanged	–
Eriksson et al[44]	Dapagliflozin	84	12	↓2.2%	↓10%[a]	–
Cusi et al[45]	Canaglifiozin	56	24	↓3.4%	↓18%[a]	–
Latva-Rasku et al[46]	Dapagliflozin	32	8	↓2.1%	↓3%	–
Kahl et al[47]	Empagliflozin	84	24	↓2.4%	↓22%	–

Arrows indicate statistically significant changes vs placebo.

AbbreviationS: DPP-IV inhibitors, dipeptidyl peptidase-4 inhibitors; GLP-1RA, glucagon-like peplide-1 receptor agonist; NAFLD, Non-alcoholic fatty liver disease ; PIO, pioglitazone; SGLT2 inhibitors, sodium-glucose co-transporter-2 inhibitors; vit E, vitamin E.

[a]Not significant compared to placebo.

[b]Placebo-corrected change in intrahepatic triglycerides with treatment measured by MR-based techniques.

[c]improvement by a NAS score and/or resolution of NASH and/or improvement in fibrosis.

[d]72-week RCT followed by 72 weeks open extension with 3-year final liver biopsy.

[e]Improvement on histology (NAS score) greater with liraglulide on paired liver biopsies.

proof-of-concept open-label clinical[40-42] studies suggested that this class could play a potential role in the treatment of NASH. However, their impact on liver histology remains to be established. Table 2 summarizes several recent RCTs in NAFLD.[43-47] Taken together, this class has been remarkably consistent in causing a 2% to 3% decrease in total body weight (1.5-3.0 kg) and in lowering plasma aminotransferases with a placebo-corrected reduction in intrahepatic triglyceride content of 10%-30%. However, decreases in intrahepatic triglyceride content must be interpreted with caution because changes in liver fat on imaging may or may not reliably correlate with necroinflammation or fibrosis on histology.[48] Most but not all trials[45] have failed to report an improvement in insulin sensitivity. In one study, more patients lost ≥5% of body weight and had a ≥30% reduction in liver fat on canagliflozin than with placebo (38% vs 7%, $P = .009$).[45]

At present there are only small uncontrolled studies reporting anecdotal benefits to liver histology with SGLT2 inhibitors[49-51] with improvement that is somewhat beyond what would be expected from the modest 2%-3% weight loss. Whether this is an indication of weight-independent mechanisms of action remains to be established. Future studies will show whether SGLT2 inhibitors, either as monotherapy or (more likely) in combination therapy, have a role to play in the management of patients with NASH.

5 | LOOKING AHEAD: HOW TO INTEGRATE NASH IN THE ROUTINE CARE OF PATIENTS WITH T2DM?

In conclusion, better awareness among diabetologists will help incorporate NASH into the routine management of patients with diabetes. Patients with elevated ALT or hepatic steatosis may be at a highest risk and should be screened as suggested by the most recent ADA Standard of Care guidelines.[9] Because of the risk of hepatic and cardiometabolic complications associated with NASH, it is important to

improve our understanding of the natural history of the disease in diabetes, to identify those at greater risk of rapid progression to cirrhosis, as well as to clarify whether NASH influences the complications of diabetes. Other questions remain including: What is the optimal diagnostic algorithm? How to combine NITs and imaging in the primary care and diabetology settings? Should we incorporate into guidelines the best diagnostic algorithms in development in order to overcome current clinical inertia and learn more about the true magnitude of the NAFLD epidemic?

We are also still at the dawn of establishing the best treatment for NASH. Weight loss and exercise will always be the cornerstone of management and leaders in the field must design long-term multicenter studies to establish the best and most sustainable strategy. Pharmacological approaches should take advantage of a "dual treatment strategy" that would be cost-effective for the simultaneous treatment of diabetes and NASH. Pioglitazone (low-cost, greatest RCT evidence in NASH, cardiovascular benefit) and GLP-1RAs (increasing evidence for the treatment of NASH, cardiovascular benefit, renal protection) will likely play an increasing role. The place of SGLT2 inhibitors (reduction of ALT and steatosis, cardiovascular benefit, renal protection) must be clarified, most likely as important agents to add-on to current or future therapies. Even if hypothetically the above 3 classes of agents (pioglitazone, GLP-1RAs or SGLT2 inhibitors) would have no effect in NASH, they should still play a bigger role in the management of these patients to reduce their high cardiovascular risk, which is the main cause of mortality in NASH.[37] Finally, many novel pharmacological agents are in advanced stages of development and will soon expand the treatment options.[14] Indeed, I envision that today's formidable clinical challenge to diabetologists with NASH, the "new complication of diabetes", will become routine care tomorrow and its devastating complications something of the past

CONFLICT OF INTEREST

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REVIEW ARTICLE

Liver INTERNATIONAL WILEY

Current management of non-alcoholic steatohepatitis

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Abstract

Non-alcoholic steatohepatitis (NASH) is the most common cause of liver disease in Western populations, and its prevalence is increasing rapidly. It is part of a multisystem disease affecting other organs such as the kidneys, heart and blood vessels, and is closely associated with the components of the metabolic syndrome. Physicians managing patients with NASH should not only focus on the management of NASH, but also on associated comorbidities in individual patients. The approaches to treatment of NASH include either limiting energy surplus alone, or in combination with targeting of downstream pathways of inflammation and fibrosis. In this mini-review, we discuss the currently available treatment options for NASH, as well as those in late-stage clinical trials. We discuss the challenges of managing these patients with a limited number of approved therapies, as well as managing advanced-stage patients with NASH and cirrhosis. We also discuss the specific management of comorbidities in NASH patients, in particular diabetes, hypertension, dyslipidaemia and cardiovascular diseases. Finally, we present the screening protocols for both hepatocellular carcinoma and extrahepatic malignancies in these patients.

KEYWORDS

management, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, treatment

1 | INTRODUCTION

Despite the rising prevalence of non-alcoholic steatohepatitis (NASH) and the increasing burden of disease, the efficacy of current drug therapies for NASH are limited, and there are no existing food and drug administration (FDA)-approved drugs. However, besides the liver, NASH is part of a multisystem disease that affects other organs such as the kidneys, the heart, the arteries and can precede the onset of both diabetes and the metabolic syndrome. Narrowly focusing on the liver may limit overall improvement in outcomes, especially considering that the top two causes of mortality in non-alcoholic fatty liver disease (NAFLD) patients are cardiovascular and malignancy-related.[1] This review describes our current approach to the management of NASH patients, including screening for and managing comorbidities.

NASH occurs when the liver is overwhelmed by chronic energy surplus. This energy surplus leads to lipotoxicity, cell death, inflammation and fibrosis. The two main approaches to the treatment of NASH include either targeting the root cause of energy surplus and excess adiposity, or specific anti-inflammatory and antifibrotic therapies.

2 | WEIGHT LOSS WITH LIFESTYLE MEASURES

By limiting energy surplus, weight loss has been shown to be a cornerstone in the management of NASH patients, with ≥7% weight

Abbreviations: ACE-i, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CKD, chronic kidney disease; FDA, food and drug administration; GLP-1, glucagon-like peptide-1; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; SGLT2, sodium glucose cotransporter 2.

loss improving histology. Not only can weight loss improve liver histology, but it also improves the cardiometabolic profile of these patients and their glucose homeostasis.

Dietary weight loss can be achieved by caloric restriction, leading to improvements in liver fat. Various diets have been proposed to aid in weight loss, including Atkins, Biggest Loser, Jenny Craig, Nutrisystem and Volumetrics. Newer diets such as the Ketogenic Diet and Intermittent Fasting have also shown to have potential benefits for weight loss. Despite the various strategies and nutrient compositions of these diets, the key factor to their effectiveness is a reduction in energy intake, with a proposed reduction of 500 kcal/d below energy requirements to help achieve weight loss.[2] More important than the composition of nutrients in these diets is the recommendation of a diet plan that an individual patient can adhere to. Independent from weight loss, adherence to the Mediterranean diet, coffee consumption and increasing the proportion of omega-3 polyunsaturated fatty acids in the diet have been shown to be beneficial to NASH patients.[3] Despite these recommendations, acceptance of dietary interventions is challenging because of the close association between dietary habits, culture and ethnicity. In addition, many patients with NASH cirrhosis have a caregiver with NASH, probably because of shared lifestyle-related risk factors.[4] Ideally, dietary interventions should be applied to the entire household. This can help with adherence to the diet as well as potentially benefit caregivers at risk of the metabolic syndrome and its associations. Patients and their families should receive advice from trained nutrition professionals on dietary intervention strategies to achieve weight loss.

Exercise has also been shown to help with weight loss and NASH. No obvious benefit has been demonstrated between aerobic and resistance exercises, or between high-intensity and low-intensity exercise. Resistance exercises may be more feasible in NASH patients who cannot tolerate aerobic exercise, whereas high-intensity exercises may increase adherence to exercise programmes with the reduced time commitment. Guidelines recommend >30 min/d of exercise, for at least 5 days in a week.[2] Given the frequency of multiple comorbidities in these patients, safety must be emphasized when recommending physical activity, with supervised exercise programmes initially in those with significant comorbidities. A combination of diet and exercise appears to be synergistic, resulting in greater weight reduction and liver fat reduction.[5]

The key to implementing these lifestyle changes is initiation and adherence to these changes. Only a minority of patients can achieve this and both patients and providers are often disappointed during clinical visits. Even when patients do achieve weight loss most return to their baseline weight within 3-5 years[6] for multifactorial reasons, including socioeconomic and psychological. Unfortunately, many physicians do not have the experience or time to unravel these complex issues during a regular clinic visit. We recommend a holistic lifestyle intervention plan incorporating diet, exercise and physical activity modifications, provided by appropriately trained professionals.

Key Points

- Despite the high prevalence and potential consequences of non-alcoholic steatohepatitis (NASH), there are currently no food and drug administration approved treatments for this disease.
- NASH should be managed as a multisystem disease in conjunction with the metabolic syndrome and its features.
- Weight loss by lifestyle measures, or aided by medical interventions help limit energy surplus.
- Specific medications can potentially be used to target inflammation and fibrosis.
- Management of other features of the metabolic syndrome must be taken into account in relation to the individual patient and his or her comorbidities.

3 | MEDICAL INTERVENTIONS FOR WEIGHT LOSS

Because very few patients manage to achieve and sustain weight loss with lifestyle measures alone, other options have to be considered to limit the chronic energy surplus in the liver. A number of pharmacological agents have been approved by the US FDA for long-term weight loss (Table 1). These medications function by impairing the absorption of calories, supressing the appetite or acting as a stimulant. Medications are indicated in patients who cannot achieve >5% loss of total body weight with lifestyle interventions, who cannot sustain weight loss, patients with a body mass index (BMI) ≥27 kg/m^2 and at least one metabolic comorbidity, or those with a BMI >30 kg/m^2 whatever the comorbidities.[2] Most of these medications have not clearly been shown to be beneficial to liver histology in NASH patients. Only Liraglutide, a glucagon-like peptide-1 (GLP-1) agonist, has been found to improve liver histology in NASH patients in a phase II study.[7]

Weight loss medications combined with lifestyle measures can help patients achieve and sustain 5%-10% total body weight loss, thus, it has become increasingly clear that to sustain weight loss, long-term administration of these medications may be necessary. Thus patients are exposed to a longer period of side effects from these medications. Lifestyle measures should be the first option, and should be continued when pharmacological agents are being taken. Patients should be closely monitored, and medications should be discontinued if patients do not achieve ≥5% weight loss. In addition to these medications, sodium-glucose cotransporter-2 (SGLT2) inhibitors have also been shown to be effective for weight loss, and may thus be beneficial in NASH. However, further studies are needed before they can be recommended for use.

Bariatric surgery has also been shown to have clear benefits for weight loss and was associated with improved cardiometabolic profiles in these patients. Although several studies have confirmed

TABLE 1 US Food and Drug Administration approved weight loss medications approved for long-term use

Medication	Mechanism of action	Reported benefits to the liver	Notable side effects
Orlistat	Pancreatic lipase inhibitor—impairs fat absorption	Possible improvements in histology	Fat malabsorption, diarrhoea, liver failure
Lorcaserin	Serotonin 2C agonist—appetite suppressant	Reductions in liver enzymes	Constipation, migraine, neurological side effects, serotonin syndrome
Phentermine-topiramate	Sympathomimetic—induces satiety		Constipation, tachycardia, insomnia, neurological side effects
Naltrexone-bupropion	Dopamine agonist and opioid receptor antagonist	Reductions in liver enzymes	Tachycardia, hypertension, headache, insomnia, seizure, suicidal thoughts
Liraglutide	GLP-1 agonist	Phase II trial demonstrating improvements in histology	Gastrointestinal side effects

Note: Medications currently approved by the US FDA for long-term use for weight loss.
Abbreviation: GLP-1, glucagon-like peptide-1.

improvements in liver histology after bariatric surgery, the largest prospective study to date demonstrated a possible worsening of fibrosis, with more severe fibrosis in almost 20% of patients 1-year after surgery.[8] The potential benefits must be weighed against the risks of bariatric surgery, especially in patients with more advanced NASH, advanced fibrosis and cirrhosis.

Certain novel, non-invasive mechanical devices have been developed to help weight loss by limiting energy surplus, with fewer side effects than pharmacological or surgical options. Gelesis 100, a hydrogel-based matrix was recently FDA approved for weight loss.[9] This compound works by occupying space in the stomach thus reducing the appetite. It is not associated with the side effects of other drugs such as nausea and vomiting with GLP-1 agonists. Other minimally invasive mechanical devices such as intragastric balloons and duodenal mucosal resurfacing may help with weight loss by limiting energy surplus. However, other than Gelesis 100, the use of these therapies for NASH are in the early stages, and further studies are needed to determine their efficacy and safety for the treatment of overweight.

4 | TARGETING DOWNSTREAM PATHWAYS IN NASH

Although they are not FDA approved, both Vitamin E and pioglitazone (a peroxisome proliferator-activated receptor-γ agonist) benefit patients with NASH. They have both been shown to improve disease activity scores, and fibrosis in certain cases.[10] Vitamin E reduces oxidative stress in the liver because of its powerful antioxidant properties. Despite the potential benefits, vitamin E has also been associated with increased all-cause mortality, increased intracranial bleeding and prostate cancer. However, NASH patients who might have benefited from histological improvement from vitamin E were not included in these studies. In addition, the higher mortality rates may have been because of the higher proportion of men, the failure to take into consideration smoking, as well as other drugs and supplements.[11] These risks must be balanced with the potential benefit in NASH patients, who have no options for treatment other than lifestyle measures. Certain subgroups of NASH patients have been shown to respond better to vitamin E, and individualized therapy may be a possible strategy.[12] Pioglitazone has been shown to benefit NASH patients without diabetes, prediabetes or overt diabetes.[10,13] This agent improves insulin sensitivity and enhances adipocyte fat storage. Pioglitazone causes weight gain, which discourages patients who are already struggling to adhere to weight loss interventions. In addition, it may cause congestive cardiac failure and bone loss. As with vitamin E, the benefits of the drug must be balanced against the reported risks.

Other than these two medications, a number of new drugs targeting downstream NASH pathways are in the late-stage clinical trials. These drugs treat the disease by inhibiting excess lipid delivery to the liver, de novo lipogenesis, apoptosis, inflammation,or fibrogenesis. A number of these drugs act as farnesoid X receptor agonists, inhibiting de novo lipogenesis, improving insulin sensitivity, and regulating the link between de novo lipogenesis and bile acid metabolism. Fibroblast growth factor analogues can also provide a similar effect. Another class of drugs increases β oxidation of fatty acids in the mitochondria and modulates the uptake of fatty acids in the liver via thyroid hormone receptors. The drugs currently in phase IIb and phase III trials and their mechanisms of action have been listed in Table 2.

The benefit of these drugs has not yet been confirmed in NASH, but prospects are good for an FDA approved drug for this indication in the near future. The FDA has created an alternative pathway for

TABLE 2 List of drugs currently being evaluated in phase 2b and phase 3 clinical trials

Drug(s)	Mechanism of action	Phase in clinical trial	Trial identification
Obeticholic acid	FXR agonist	III	NCT02548351
Cenicriviroc	CCR2/CCR5 inhibitor	III	NCT03028740
Elafibranor	PPAR-α/δ agonist	III	NCT02704403
Belapectin	Galectin-3 inhibitor	III	4th quarter 2019
Resmetirom	THR-ß agonist	III	NCT03900429
Aramchol	SCD1 inhibitor	III	3rd quarter 2019
Tropifexor	FXR agonist	IIb	NCT02855164
Pegbelfermin	FGF21 analogue	IIb	NCT03486899
NGM282	FGF19 analogue	IIb	NCT03912532
Seladelpar	PPAR-δ agonist	IIb	NCT03551522
Lanifibranor	PPAR-α agonist	IIb	NCT03008070
MSDC-0602K	MPC inhibitor	IIb	NCT02784444

Note: List of medications currently in late-stage clinical trials (phase 2b and phase 3) for NASH.
Abbreviations: CCR, C-C chemokine receptor; FGF, fibroblast growth factor; FXR, farnesoid X receptor; MPC, mitochondrial pyruvate carrier; PPAR, peroxisome proliferator-activated receptor; SCD-1, stearoyl-CoA desaturase 1; THR-ß, thyroid hormone receptor ß.

approving drugs, known as the Accelerated Approval Pathway (subpart H for drugs). This enables drug companies to apply for approval with trials using surrogate endpoints, shortening the time needed for FDA approval. In addition, drugs that have failed earlier clinical trials are being evaluated in combination with other drugs. All these efforts will probably result in an FDA approved drug in the near future.

We recommend carefully explaining the risks, benefits and limitations of lifestyle measures compared to currently available pharmacotherapy and allowing patients to make a choice based on an informed decision. The use of vitamin E or pioglitazone for NASH is considered 'off-label', and this must be clearly explained to patients. Participation in clinical trials can be offered to eligible patients.

5 | MANAGING PATIENTS WITH CIRRHOSIS AND NASH

The progression of NASH from significant fibrosis to cirrhosis is associated with a exponentially poorer prognosis. Once the patients develop cirrhosis, the only good treatment option is liver transplantation. Patients may require a liver transplant once they develop decompensated cirrhosis, hepatocellular carcinoma (HCC) or certain other complications of cirrhosis. NASH patients with cirrhosis may also develop sarcopenia and frailty, which can worsen their waiting list mortality.[14] After liver transplantation, these patients must take diabetogenic medications such as steroids, tacrolimus and mammalian target of rapamycin inhibitors to prevent allograft rejection because of their increased metabolic risk. Cardiovascular mortality has been shown to be a significant cause of post-liver transplant mortality, which may be increased in these patients.[15] A new strategy including sleeve gastrectomy at the time of liver transplantation was recently reported. Patients with a BMI ≥35 kg/m^2 who underwent a sleeve gastrectomy during transplantation maintained greater weight loss and had fewer components of the metabolic syndrome.[16] Although this study did not specifically evaluate NASH patients, the predominant liver disease in this study was NASH, and almost 50% of the patients had NASH cirrhosis. Further studies are needed before this can be recommended in routine practice.

6 | MANAGING DIABETES IN PATIENTS WITH NASH

NASH can precede diabetes and potentially increase the risk of incident type 2 diabetes. A prospective study reported that patients with NAFLD can have an up to two-fold increased risk of subsequent incident diabetes.[17] Patients with NASH who are not previously diagnosed with diabetes should be screened for type 2 diabetes. If not present, screening should be repeated at regular intervals in these patients. Fasting plasma glucose levels can be measured during routine follow-up to monitor for the development of diabetes or prediabetes in these patients.

Once a patient has diabetes, the management of type 2 diabetes in NASH patients must be evaluated in relation to his/her comorbidities. Metformin should be the first line treatment in diabetes.[18] If the haemoglobin A1c is still not sufficiently controlled, we recommend GLP-1 agonists, SGLT2 inhibitors, or thiazolidinediones. GLP-1 agonists have been shown to improve cardiovascular and all-cause mortality in high-risk patients with type 2 diabetes. They have been shown to reduce heart failure and the progression of chronic kidney disease (CKD) in certain patients, and are recommended in these patients by the American Diabetes Association clinical practice recommendations.[18] In addition, these agonists are associated with significant weight loss, and a phase 2 study reported improvement in

liver histology in patients with NASH.[7] SGLT2 inhibitors can also improve weight loss, cardiovascular outcomes and reduce the progression of CKD.[18] Finally, there is emerging evidence that these agents are beneficial to patients with NASH. Pioglitazone has been shown to improve steatohepatitis, disease activity scores and possibly fibrosis in non-diabetic patients. A recent trial has also demonstrated histological benefits in NASH patients with type 2 diabetes.[13] However, these results must be balanced against the weight gain, fluid retention and possible worsening of heart failure associated with this drug.

NASH patients with cirrhosis and diabetes who are eligible for liver transplantation must often reach glycaemic and weight loss targets to be listed on national transplant waiting lists. Insulin is commonly prescribed to achieve the glycaemic targets in these patients. This presents a management dilemma, as the associated weight gain with insulin may prevent patients from achieving weight loss targets. In addition, these patients are sarcopenic, and thus severely insulin resistant and metabolically inflexible. They often require much higher doses of insulin to achieve the glycaemic targets, further increasing weight gain and adiposity. We recommend the use of GLP-1 agonists or SGLT-2 inhibitors even in these patients, as they have been shown to help with weight loss, to improve survival and to stabilize CKD in this group.[18] Ideally, insulin should be used in cirrhotic NASH patients when glycaemic control is difficult with the above-mentioned medication combinations.

7 | MANAGING HYPERTENSION IN PATIENTS WITH NASH

NASH is associated with hypertension and the severity of NASH tends to increase in patients with hypertension.[19] The association with NASH does not appear to be as strong as that with diabetes. The benefits of weight loss, especially via lifestyle measures are also true for hypertension. Given the association of NASH with diabetes and CKD, many patients with NASH may need to receive an angiotensin-converting enzyme inhibitor (ACE-i)/angiotensin receptor blocker (ARB). There is a theoretical rationale for the use of ACE-i and ARB in NASH patients, as the latter can inhibit the activation of hepatic stellate cells and target pathways involved in the pathophysiology of portal hypertension. However, data on the comparative benefit of antihypertensive agents in NASH are limited. Thus we recommend management based on current guidelines. Additionally, given the relative ease and low cost of checking blood pressure, we recommend that NASH patients get regular blood pressure checks during clinical visits, with specific thresholds for intervention according to current guidelines. NASH patients may need to discontinue ACE-I or ARBs once they develop decompensated cirrhosis.

8 | ATHEROGENIC DYSLIPIDAEMIA IN NASH PATIENTS

Patients with NASH have poorer atherogenic profiles than non-NASH patients, with higher plasma concentrations of very low-density lipoprotein, low-density lipoprotein and small dense low-density lipoprotein. Patients with NASH should undergo screening for dyslipidaemia at regular intervals. Studies evaluating statin use in NASH patients have demonstrated improvements in biochemistry. A Greek study reported a histological improvement in NASH in patients receiving rosuvastatin.[20] However, these findings were not consistent with others. Thus, with the still unproven efficacy for NASH, the main aim of treatment of dyslipidaemia is the prevention of cardiovascular events, in particular atherosclerotic cardiovascular disease (ASCVD). Based on current evidence and guidelines, patients with increased LDL levels should have treatment based on their risk profiles. Statins can be safely used in patients with NASH, and should be the first-line treatment of LDL to prevent ASCVD. If the response to statins are insufficient, ezetimibe or a proprotein convertase subtilisin/kexin type 9 inhibitor can be added.[21] Fibrates can be considered in patients with concomitant hypertriglyceridaemia.

Many patients with NASH and dyslipidaemia are not prescribed statins, despite studies showing their safety in this population.[22] This is probably because of a fear of liver injury in this group. Withholding statins in these patients with an increased risk of cardiovascular disease can result in worse outcomes. Educating providers, especially non-hepatologists, is a key to allow NASH patients with elevated liver enzymes to continue to receive statins if needed to reduce ASCVD.

9 | CARDIOVASCULAR DISEASE IN NASH PATIENTS

NAFLD is associated with cardiovascular disease and worsening disease severity resulting in a higher rate of incident fatal and non-fatal cardiovascular events. NAFLD is a predictor of atherosclerosis, which has been shown to be independent of traditional cardiovascular risk factors.[23] In addition, NAFLD is associated with diastolic dysfunction, impaired ventricular relaxation and increased myocardial thickness, causing reduced exercise tolerance. Exercise tolerance lowers as liver fibrosis worsens.[24]

The main intervention in these patients should be aggressive risk factor modification, including smoking cessation and control of associated comorbidities in addition to the diet and lifestyle measures discussed above. Physicians managing patients with NASH should regularly evaluate the patient's cardiovascular risk profile with tools such as the ASCVD risk calculator. There is no firm evidence to recommend aggressive routine screening such as coronary artery calcium scoring in these patients.

10 | HCC IN NASH PATIENTS

Although it was traditionally believed to only occur after the onset of cirrhosis, studies have now demonstrated that between 13% and 20% of patients with NAFLD-related HCC do not have cirrhosis.[25] Because of the rapidly increasing prevalence of this disease, NAFLD-related HCC is the second most common indication for HCC

transplants in the USA, and may become the first indication. Patients with NAFLD-related HCC also tend to be diagnosed at a more advanced tumour stages compared to other causes of HCC.

While there is still insufficient evidence to recommend screening in non-cirrhotic NASH patients, those with NASH cirrhosis should undergo regular screening for HCC. A retrospective study showed that patients with NASH cirrhosis were inadequately screened, and those who did undergo screening had smaller tumours at diagnosis.[26] Physicians should identify patients who qualify for screening, especially those with early, well-compensated cirrhosis and minimal biochemical abnormalities.

11 | EXTRAHEPATIC MALIGNANCY SCREENING IN NASH PATIENTS

The second most common cause of mortality in patients with NASH is extrahepatic malignancies.[1] Increased rates of colon and breast cancer have been reported in male and female patients respectively. It remains unclear if this is because of NASH, or to the shared comorbidities in NASH, obesity and the metabolic syndrome. Current recommendations for cancer screening should be followed in this population. In particular, biennial screening mammograms for women aged 50-74, and colon cancer screening in patients age 50-75 are important.[27]

There are certain practical challenges when performing colorectal screening in NASH patients. A study from Hong Kong reported an increased incidence of adenomas and advanced neoplasms in NASH patients, with a higher rate of proximal colonic lesions[28], whereas proximal/right colon lesions are the most commonly missed lesions during colonoscopy. In addition to the increased risk of sedation and endoscopy in obese patients, diabetic patients tend to have more difficulty achieving adequate bowel preparation. This may further increase the rates of missed lesions in NASH patients, especially those with diabetes.

12 | VACCINATIONS IN NASH PATIENTS

The Centers for Disease Control and Prevention recommends vaccinations for patients with liver disease. Influenza, tetanus, diphtheria and pertussis, pneumococcal, hepatitis B, hepatitis A, varicella, and measles, mumps and rubella are recommended in patients with chronic liver disease including NASH.[29] Physicians managing these patients should follow a vaccination schedule. However, rates of vaccination, even for hepatitis A and B have been shown to be suboptimal in patients with chronic liver diseases managed by hepatologists.[30]

13 | CONCLUSION

Despite the limited number of pharmacological agents available for the treatment of NASH, the key to management is also effective treatment of comorbidities. The physicians caring for these patients should look beyond the liver to achieve good outcomes in this group, and preferably work with trained ancillary healthcare providers to provide care. In addition, because of this rapidly changing field, physicians should keep abreast of the current literature and emerging pharmacological agents when managing this group.

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CONFLICTS OF INTEREST

Arun Sanyal: Dr Sanyal is President of Sanyal Biotechnology and has stock options in Genfit, Akarna, Tiziana, Indalo, Durect and Galmed. He has served as a consultant to Astra Zeneca, Nitto Denko, Enyo, Ardelyx, Conatus, Nimbus, Amarin, Salix, Tobira, Takeda, Jannsen, Gilead, Terns, Birdrock, Merck, Valeant, Boehringer-Ingelheim, Lilly, Hemoshear, Zafgen, Novartis, Novo Nordisk, Pfizer, Exhalenz and Genfit. He has been an unpaid consultant to Intercept, Echosens, Immuron, Galectin, Fractyl, Syntlogic, Affimune, Chemomab, Zydus, Nordic Bioscience, Albireo, Prosciento, Surrozen and Bristol Myers Squibb. His institution has received grant support from Gilead, Salix, Tobira, Bristol Myers, Shire, Intercept, Merck, Astra Zeneca, Malinckrodt, Cumberland and Novartis. He receives royalties from Elsevier and UptoDate. Mark Muthiah: None.

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REVIEW ARTICLE

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New drugs for non-alcoholic steatohepatitis

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in Western countries. At present the safest and most effective first-line therapy for the management of non-alcoholic steatohepatitis (NASH) is lifestyle modification with diet and exercise. However, long-term adherence to lifestyle modification is rare in the target population, leading to progression of liver disease and its complications such as cirrhosis and hepatocellular carcinoma. Thus, new drugs that focus mainly on the pathogenesis of NASH to target inflammation and fibrogenesis are under investigation. This mini-review summarizes the results of pivotal finalized phase 2 studies, and provide an outline of ongoing phase 2 and phase 3 studies.

1 | INTRODUCTION

Despite the high prevalence and importance of non-alcoholic fatty liver disease (NAFLD), the incorrect idea that the progression of this disease is usually benign has considerably limited the development of drugs for non-alcoholic steatohepatits (NASH). With the awareness that NAFLD can progress to advanced stages of liver injury and is associated with possible complications such as hepatocellular carcinoma and hepatic failure, there has been a significant increase in the number of studies investigating new drugs for more effective management of liver disease. Although there are about 196 agents being evaluated for the treatment of NASH, none of these drugs has been approved to treat this disease so far. However, many phase 2 and 3 trials are ongoing and a new chapter is expected in NASH treatment in the near future (Table 1).

1.1 | Obeticholic acid

Obeticholic acid (OCA) is derived from the primary human bile acid, chenodeoxycholic acid, which stimulates the farnesoid X nuclear receptor (FXR) in humans.[1] OCA stimulates FXR activity approximately 100-fold more intensely than chenodeoxycholic acid, and is highly

Abbreviations: NAFLD, Non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OCA, Obeticholic acid; FXR, farnesoid X nuclear receptor; NAS, non-alcoholic fatty liver disease activity score; CVC, Cenicriviroc; CCR2, chemokine receptor type 2; CCR5, chemokine receptor type 5; TZD, thiazolidinedione; MPC, mitochondrial pyruvate carrier; PPAR, peroxisome proliferator activated receptor; T2D, type 2 diabetes; FGF19, fibroblast growth factor 19; MRI-PDFF, MRI-proton density fat fraction; ALT, alanine aminotransferase; APRI, AST to Platelet Ratio Index; THR β, thyroid hormone receptor β; HFF, hepatic fat fraction; AST, aspartate aminotransferase; TXR, Tropifexor; Aramchol, Arachidyl-amido cholanoic acid; SCD1, Stearoyl-CoA desaturase 1, MUFAs, monounsaturated fatty acids; ASK1, apoptosis signal-regulating kinase 1.

selective with minimal activity to G protein-coupled bile acid receptor, another bile acid receptor.[1] The FXR nuclear receptor is expressed in the liver, intestines, adrenal glands and kidneys and plays an important role in the synthesis and enterohepatic circulation of bile acids.[2] 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.[2] Other important function of farnesoid X receptor activation is to reduce bile acid synthesis by inhibiting the conversion of cholesterol to bile acids.

The main trials evaluating OCA are the FLINT trial, a phase 2b study[3] and the ongoing REGENERATE phase 3 study.[4] The FLINT trial was a multi-centre, double-blind, placebo-controlled, randomized clinical trial in the USA in biopsy-proven NASH patients without cirrhosis with a non-alcoholic fatty liver disease activity score (NAS) ≥4, with at least 1 point in each component of the score. This study assessed treatment with 25 mg daily of oral OCA compared to placebo for 72 weeks. The primary outcome was improvement in centrally scored liver histology defined as a decrease of at least 2 points in NAS without worsening of fibrosis from baseline to the end of treatment. A total of 141 patients were randomly assigned to receive OCA and 142 to receive placebo in this trial. Forty-five per cent of the 110 patients in the OCA group who were scheduled to undergo biopsies at baseline and at 72 weeks had improved liver histology compared to 21% of the 109 patients in the placebo group (relative risk 1.9, 95% CI 1.3-2.8; $P = .0002$). Twenty-three per cent of the 141 patients in the OCA group developed pruritus compared to only 6% of 142 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.

The main objectives of REGENERATE, an ongoing phase 3 global study are to compare 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. A liver biopsy is obtained at screening, at 18 and 48 months, and at the end of study. The estimated completion date of this study is October 2022. This study has three arms and patients are randomized 1:1:1 as follows: OCA 10 mg, OCA 25 mg daily and placebo. The primary endpoints are the proportion of OCA-treated patients vs placebo who achieve an 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 liver fibrosis. All-cause mortality and liver-related clinical outcomes will also be evaluated as secondary endpoints. The results of the interim 18-month analysis[5] showed that 1968 patients with stage F1-F3 fibrosis were enrolled and received at least one dose of treatment. 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 37 (12%) patients in the placebo group, 55 (18%) in the OCA 10-mg group ($P = .045$), and 71 (23%) in the OCA 25-mg group ($P = .0002$). NASH resolution was not achieved in any patient. As reported previously, the main adverse event was pruritus with 28% in the 10-mg group and 51% in the 25-mg group compared to 19% in the placebo group. The evaluation of clinical outcomes is ongoing.

Key Points

- Despite the high prevalence and potential consequences of NASH, there are currently no approved treatments for this disease
- There are many new drugs in the pipeline
- Specific medications can potentially be used to target inflammation and fibrosis
- The main new drugs under investigation are agonist or inhibitors of specific receptors
- The new drugs will likely be used in combination to increase their effectiveness

TABLE 1 List of drugs currently being evaluated in phases 2 and 3 clinical trials

Drug(s)	Mechanism of action	Phase in clinical trial	Trial identification
Obeticholic acid	FXR agonist	III	NCT02548351
Elafibranor	PPAR-α/δ agonist	III	NCT02704403
Cenicriviroc	CCR2/CCR5 inhibitor	III	NCT03028740
MSDC-0602K	MPC inhibitor	IIb	NCT02784444
NGM282	FGF19 analogue	IIb	NCT03912532
Saroglitazar	PPAR-α/γ agonists	II	NCT03061721
Resmetirom	THR-ß agonist	III	NCT03900429
Tropifexor	FXR agonist	IIb	NCT02855164
Aramchol	SCD1 inhibitor	III	3rd quarter 2019
Selonsertib	ASK1 inhibitor	III	NCT03053050

Abbreviations: ASK1, apoptosis signal-regulating kinase 1; CCR, C-C chemokine receptor; FGF, fibroblast growth factor; FXR, farnesoid X receptor; MPC, mitochondrial pyruvate carrier; PPAR, peroxisome proliferator-activated receptor; SCD-1, Stearoyl-CoA desaturase 1; THR-ß, thyroid hormone receptor ß.

1.2 | Elafibranor

Elafibranor is a peroxisome proliferator-activated receptor alpha-delta α/δ agonist. It regulates lipid and insulin metabolism, two key components in the pathophysiology of NAFLD and NASH. The GOLDEN study[6] was a phase 2b multi-centre (Europe and USA), double-blind, randomized controlled trial comparing elafibranor 80 mg and 120 mg daily to placebo for 52 weeks, including 276 patients with biopsy-proven, noncirrhotic NASH with NAS ≥3 and ≥1 point for each component in the score. Reversal of NASH, defined as the absence of at least 1 of either steatosis, ballooning or inflammation without progression to bridging fibrosis or cirrhosis, was not achieved. However, NASH resolved without fibrosis worsening in a higher proportion of patients in the 120-mg elafibranor group vs the placebo group (19% vs 12%; odds ratio = 2.31; P = .045), based on a post hoc analysis for the modified definition. In post hoc analyses of patients with non-alcoholic fatty liver disease activity score ≥4 (n = 234), elafibranor 120 mg resolved NASH in larger proportions of patients 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 with NASH resolution after receiving elafibranor 120 mg had reduced liver fibrosis stages compared with those without NASH resolution (mean reduction of 0.65 ± 0.61 in responders for the primary outcome vs an increase of 0.10 ± 0.98 in nonresponders; P < .001). Liver enzymes, lipids, glucose profiles, and markers of systemic inflammation were significantly reduced in the elafibranor 120-mg group vs the placebo group. Elafibranor was well tolerated and did not cause weight gain or cardiac events, but did produce a mild, reversible increase in serum creatinine.[6]

RESOLVE-IT (https://clinicaltrials.gov/ct2/show/NCT0270443) is an ongoing phase 3 study that will include 2,000 NASH patients with NAS ≥4, with ≥1 of each component of the score and F1-F3 fibrosis. The primary outcome is histological improvement, defined as the resolution of NASH without worsening of fibrosis at 72 weeks with a composite outcome that will evaluate all-cause mortality, cirrhosis and "liver-related clinical outcomes" at 4 years. Results are due in December 2021.

1.3 | Cenicriviroc

Cenicriviroc (CVC) is an oral, dual antagonist of chemokine receptor type 2 (CCR2) and type 5 (CCR5), located on Kupffer cells and hepatic stellate cells.[7] This mechanism of action drives the molecular engines that drive NASH via blockade of overactive inflammatory signalling and disruption of signalling that activates stellate cells, targeting both inflammation and fibrogenesis. CVC is administered in 150 mg daily tablets, since it has a long plasma life of 30-40 hours. CENTAUR[8] was a phase 2b, 24-month study that included 189 patients randomized 2:1:1 in three arms as follows: arm A with continuous administration of 150-mg CVC for 24 months, arm B with placebo for 12 months followed by an additional 12 months of CVC and arm C with placebo for 24 months. Included patients underwent a protocol liver biopsy at baseline, 12 months and at the end of 24 months. Eligible patients had biopsy-proven NASH with an NAS ≥4 and stage 1-3 fibrosis. The primary outcome of a ≥2 point decrease in NAFLD activity score with no worsening of fibrosis at 1 year was not achieved. However, a key secondary outcome was the improvement in liver fibrosis without worsening of NASH, which was achieved in 20% from the treatment group as well as lower levels of interleukin-6, C-reactive protein and fibrogen in this group.[8]

Currently, AURORA (https://clinicaltrials.gov/ct2/show/NCT03028740), a randomized, double-blind, placebo-controlled, multi-centre phase 3 study is ongoing to evaluate the efficacy and safety of CVC for the treatment of moderate to severe liver fibrosis in adults with NASH. The overall aim is to include 2,000 patients with NASH and F2-F3 fibrosis in a 2:1 CVR to placebo ratio in each of the arms. The first part of this study was designed to determine the superiority of CVC for the improvement of at least one stage of fibrosis without worsening of NASH after 12 months of CVC. The aim of the second part of this study is to evaluate the composite endpoint of histopathological progression to cirrhosis, liver-related clinical outcomes, and all-cause mortality over 5 years. A liver biopsy will be performed at baseline, at 12 months and at the end of this study, at month 60.

1.4 | MSDC-0602K

The first-generation insulin sensitizer pioglitazone, a thiazolidinedione (TZD), improved NASH but had many side effects which have limited its use.[9]

MSDC-0602K is a second-generation insulin sensitizer. It is an inhibitor of the mitochondrial pyruvate carrier (MPC) with minimal peroxisome proliferator activated receptor γ (PPAR) binding. Initial studies showed that MSDC-0602 could 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.[9]

The phase 2b 52-week double-blind study evaluating MSDC-0602K included 392 biopsy-confirmed NASH (NAS >4, ≥1 in each component) patients with histological evidence of F1-F3 fibrosis (at least 50% F2/F3). 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 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 mg, 125 mg or 250 mg of the compound. Although analysis of MSDC-0602K study data did not show any statistically significant effects on primary or secondary liver histology endpoints, the effects on the non-invasive measures of liver cell injury and glucose metabolism were identified. The incidence of hypoglycemia and PPARγ-agonist-associated

events such as oedema and fractures were similar in the placebo and MSDC-0602K groups. The authors concluded that further studies were needed to clarify the results obtained.[10]

1.5 | NGM282

NGM282 is an engineered analogue of FGF19, a hormone that regulates bile acid synthesis, glucose homoeostasis and energy homoeostasis. Previous studies have shown that mice expressing FGF19 have an increased metabolic rate, decreased adiposity and increased insulin sensitivity with no increase in the hormones most often associated with an increase in metabolic rate. However, the therapeutic potential of fibroblast growth factor 19 (FGF19) has been limited by its hepatocarcinogenicity.[11]

NGM282, a nontumorigenic variant of FGF19 was evaluated in a randomized, double-blind, placebo-controlled, phase 2 study, that included patients with biopsy-proven non-alcoholic steatohepatitis. Patients were assigned (1:1:1) to receive either 3 mg 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 at least a 5% reduction in absolute liver fat content measured by MRI-proton density fat fraction (MRI-PDFF).[11]

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. 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 authors concluded that NGM282 is associated with a reduction in liver fat content with an acceptable safety profile in patients with non-alcoholic steatohepatitis.[11]

1.6 | Saroglitazar

Saroglitazar, a dual-peroxisome proliferator-activated receptor agonist, has been shown to improve lipid and glycemic parameters through PPAR-α and γ agonist actions respectively (predominant PPAR-α and moderate PPAR-γ actions).

Recently, a phase-2, prospective, multi-centre, double-blind, randomized trial was performed to determine the efficacy and safety of saroglitazar magnesium compared to placebo in patients with NAFLD/NASH. A total of 106 adult subjects who had alanine aminotransferase (ALT) ≥50 U/L and body mass index ≥25 kg/m^2 were randomized in a 1:1:1:1 ratio to receive 1 mg, 2 mg or 4 mg of saroglitazar and placebo. The primary endpoint was the percentage of change in ALT levels from baseline to week 16 in the saroglitazar vs placebo groups. The secondary endpoints included the proportion of patients with ≥50% reduction in ALT levels and change in liver fat content (measured by MRI-PDFF) from baseline to week 16 in the saroglitazar vs placebo groups. The primary endpoint was achieved in all three groups with saroglitazar. A significant reduction in mean ALT from baseline to week 16 was observed with saroglitazar 1 mg (−27.3%), 2 mg (−33.1%) and 4 mg (−44.3%) vs placebo (4.1%) ($P < .001$ for all). A significantly higher proportion of patients had ≥50% reduction in mean ALT from baseline to week 16 with saroglitazar 4 mg compared to placebo (51.8% vs 3.5%; $P < .0001$). At week 16, saroglitazar 4 mg resulted in a significantly higher reduction in HOMA-IR, triglycerides, total cholesterol and AST to Platelet Ratio Index (APRI) than placebo ($P < .05$ for all). A significantly higher percentage of patients had >30% reduction in liver fat content with saroglitazar 4 mg than with placebo (40.7% vs 8%, $P = .006$). 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.[12]

1.7 | Resmetirom (MGL-3196)

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.[13] Resmetirom (MGL-3196, Madrigal Pharmaceuticals Inc), a highly selective THR β agonist, has been developed to target dyslipidemia but has also been shown to reduce hepatic steatosis in fat-fed rats, improving insulin sensitivity, promoting liver regeneration and reducing apoptosis.[14]

This double-blind, randomized, placebo-controlled phase 2 study included patients with biopsy proven NASH and ≥10% liver steatosis. 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. Eligible liver biopsies included stage 1-3 fibrosis with an NAFLD activity score of at least 4, including a score of at least 1 in each component according to the NASH clinical research network scoring system.[15]

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. Statistically significant reductions were also observed in ALT and AST levels in Resmetirom-treated patients. Statistically significant effects in the reduction of atherogenic lipids, lipo-protein(a), markers of inflammation and fibrosis were also found compared to placebo as well as improvements in NASH on liver biopsy. The evaluation of more advanced NASH was limited by the relatively low baseline NAS and the few patients with advanced stages of fibrosis. Resmetirom was well tolerated even if it was associated with an increase in gastrointestinal adverse events. These adverse events were self-limited and did not result in study withdrawal.[15]

A phase 3 in patients with NASH and fibrosis is now recruiting (https://clinicaltrials.gov/ct2/show/NCT03900429).

1.8 | Tropifexor (LJN-452)

Tropifexor (TXR) is a highly potent, nonbile acid FXR agonist that induces target genes at very low doses in vitro and in vivo, and

has been shown to be effective in pre-clinical models of NASH.[16] The tolerability and safety of TXR was shown to be favourable in a phase 1 study in healthy volunteers (unpublished results). A phase 2 adaptive design study (FLIGHTFXR) in patients with NASH is ongoing (https://clinicaltrials.gov/ct2/show/NCT02855164). In addition, a recent randomized, double-blind, multi-centre, phase 2b study is evaluating the safety and efficacy of a combination of TXR and cenicriviroc 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.[17]

1.9 | Arachidyl amido cholanoic acid (Aramchol)

Arachidyl amido cholanoic acid, a cholic-arachidic acid conjugate, targets Stearoyl-CoA desaturase 1 (SCD1), inhibiting the synthesis of monounsaturated fatty acids (MUFAs), the major fatty acid of triglycerides, cholesteryl esters and membrane phospholipids.[18] Aramchol (400 and 600 mg) was tested in biopsy-proven NASH patients without cirrhosis in a 52-week phase 2b trial (2018) to evaluate their effect on hepatic triglyceride content using MRI spectroscopy (https://clinicaltrials.gov/ct2/show/NCT02279524). A recent double-blind, randomized, placebo-controlled trial tested the efficacy of 12 weeks of treatment with aramchol vs placebo in HIV-associated NAFLD.[19] 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 and muscle composition based on an MRI assessment in patients with HIV-associated NAFLD.[19]

1.10 | Selonsertib (SEL, GS-4997)

Selonsertib is an inhibitor of apoptosis signal-regulating kinase 1 (ASK1) that causes apoptosis and fibrosis. An open-label phase 2 trial evaluating NASH patients with moderate and severe liver fibrosis identified a regression in fibrosis and other parameters of liver injury.[20] Therefore, phase 3 trials evaluating NASH patients with stage 3 fibrosis (http://www.natap.org/2019/HCV/050819_01.htm) – STELLAR 3 – or cirrhosis (http://www.natap.org/2019/HCV/022719_01.htm) – STELLAR 4 – were initiated. Because STELLAR 4 did not reach the primary endpoint (at least one-point reduction in fibrosis score, without worsening of NASH at 48 weeks) it was discontinued and the STELLAR program was cancelled.

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CONFLICTS OF INTEREST

Ana Carolina Cardoso: None. Claudio de Figueiredo Mendes: None. Cristiane A Villela-Nogueira is the principal investigator of the AURORA study at Federal University of Rio de Janeiro site in Brazil.

Arun Sanyal: Dr Sanyal is the President of Sanyal Biotechnology and has stock options in Genfit, Akarna, Tiziana, Indalo, Durect and Galmed. He has served as a consultant to Astra Zeneca, Nitto Denko, Enyo, Ardelyx, Conatus, Nimbus, Amarin, Salix, Tobira, Takeda, Jannsen, Gilead, Terns, Birdrock, Merck, Valeant, Boehringer-Ingelheim, Lilly, Hemoshear, Zafgen, Novartis, Novo Nordisk, Pfizer, Exhalenz and Genfit. He has been an unpaid consultant to Intercept, Echosens, Immuron, Galectin, Fractyl, Syntlogic, Affimune, Chemomab, Zydus, Nordic Bioscience, Albireo, Prosciento, Surrozen and Bristol Myers Squibb. His institution has received grant support from Gilead, Salix, Tobira, Bristol Myers, Shire, Intercept, Merck, Astra Zeneca, Malinckrodt, Cumberland and Novartis. He receives royalties from Elsevier and UptoDate.

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REVIEW ARTICLE

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The role of nutrition in non-alcoholic fatty liver disease: Pathophysiology and management

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Abstract

A healthy diet together with physical activity could induce weight loss and control the progression of non-alcoholic fatty liver disease (NAFLD). However, the composition of diet has not been clearly established. Macronutrients such as saturated fatty acids (SFA), trans-fats, simple sugars and animal proteins have a harmful effect on the liver. On the other hand, monounsaturated fats (MUFAs), polyunsaturated (PUFAs) omega-3-fats, plant-based proteins and dietary fibres are considered to be beneficial to the liver. The impact of specific micronutrients is less well-known. Nutrients are part of the food we eat. Food makes up our meals, which compose our dietary patterns. Non-alcoholic fatty liver disease patients usually follow Western diets which are rich in soda, frozen junk food, juice, red meat, lard, processed meats, whole fat dairy foods, fatty snack foods, take-away foods, cakes and biscuits and poor in cereals, whole grains, fruit, vegetables, extra virgin olive oil (EVOO) and fish. On the other hand, the Mediterranean diet (MD) is beneficial for NAFLD even when it is iso-caloric or there are no changes in body weight. A new approach, called 'nutritional geometry' considers the importance of integrating nutrition, animals and the environment. The goal of this approach is to combine nutrients and foods in a model to understand how food components interact to regulate the properties of diets affecting health and disease. The use of algorithms developed by artificial intelligence (AI) to create a personalized diet for patients can provide customized nutritional counselling to prevent and treat NAFLD.

KEYWORDS

artificial intelligence, dietary guidelines, macronutrients, micronutrients, nutritional geometry, personalized nutrition

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a major health problem because of its high prevalence. Non-alcoholic fatty liver disease is associated with obesity, insulin resistance, type 2 diabetes mellitus (DM2), hyperlipidemia, hypertension and metabolic syndrome.[1] Non-alcoholic fatty liver disease covers a wide pathological spectrum ranging from steatosis to steatohepatitis (NASH) progressing to different degrees of liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC). At present there is no clear consensus on the pharmacological treatment of NAFLD, however, it is clear that therapeutic approaches should focus on lifestyle modification. Diet and exercise interventions remain the

Abbreviations: AI, artificial intelligence; DASH, Dietary Approach to Stop Hypertension; DHA, docosahexanoic acid; DM2, type 2 diabetes mellitus; EPA, eicosapentanoic acid; EVOO, extra virgin olive oil; MD, Mediterranean diet; MUFA, monounsaturated fatty acids; NAFLD, non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

first line of therapy and studies have shown that a healthy diet and weight loss in the early stages of NAFLD could be sufficient to control disease progression.[2] However, despite clear evidence that dietary interventions are effective, the extent and the composition of the diet has not been clearly established. Moreover, patients often fail to follow dietary interventions. Thus, simple, multidisciplinary, nutritional guidelines are needed that target the disease mechanisms. In addition, recent findings increasingly support an approach involving personalized nutrition and the role of artificial intelligence (AI) in this. Thus, the purpose of this review was to analyse the role of nutrients in the pathophysiology of NAFLD, focusing on the design of tailored diets.

Key points

- Dietary modifications have been shown to be effective in controlling non-alcoholic fatty liver disease (NAFLD).
- Modifications in the composition of specific macro- or micro- nutrients in the diet are not a central point.
- The Western diet is associated with a greater risk of disease progression in NAFLD while the Mediterranean diet with an improvement in NAFLD.
- Nutritional geometry can be an excellent tool to study the relationships between the various aspects of diet and NAFLD pathophysiology.
- The use of algorithms developed by artificial intelligence for personalized nutritional counselling would be useful to prevent and treat NAFLD.

2 | INFLUENCE OF DIETARY MACRONUTRIENTS ON NON-ALCOHOLIC FATTY LIVER DISEASE

Several studies have confirmed the role of specific macronutrients in the onset and progression of NAFLD. However, it is very difficult to separate the role of each separate macronutrient, in relation to the amount of energy provided, their proportion in the diet and the food they contain. The macronutrient composition of a diet is associated with NAFLD/NASH, independent of energy intake. Macronutrients such as saturated fatty acids (SFA), trans fats, simple sugars (sucrose and fructose) and animal proteins damage the liver. These modulate the accumulation of triglycerides and antioxidant activity in the liver, which affects insulin sensitivity and postprandial triglyceride metabolism.[3] In contrast, monounsaturated fatty acids (MUFA), PUFA ω3 fats, plant-based proteins and dietary fibres appear to be beneficial to the liver.[2]

2.1 | The role of fats

We can distinguish three types of fats at a nutritional level, saturated, monounsaturated and polyunsaturated. Despite a general consensus that the intake of saturated fats should be reduced, the issue of dietary fatty acid composition remains controversial. The SFA diet was associated with a marked increase in liver fat, probably because of an increase in de novo liver lipogenesis and an increase in lipolysis of adipose tissue. In contrast, unsaturated fat intake was associated with a decrease in lipolysis, preventing the accumulation of fat in the liver.[4] The SFA diet has also been linked to impaired glutathione metabolism and an increase in oxidative stress, which leads to the progression of NAFLD.[5] However, at present, it is not clear whether different sources of SFA (for example, dairy vs meat) can have different effects on liver fat content. On the other hand, it is also important to consider that the effects of saturated fats seem to depend on a patient's genetic background.[6] The specific effects of trans fats on the human liver have not been adequately evaluated because most studies have been performed in mice models.

Studies on MUFA have reported different, sometimes contradictory conclusions. This may be because of both differences in methodology and the origin of MUFAs. A negative relationship between MUFA consumption and the progression of NAFLD has been reported, mainly in cross-sectional studies in which the origin of MUFA was not considered. On the contrary, studies in which extra virgin olive oil (EVOO) was the source of MUFA suggest that its intake could improve fatty liver. In addition, a randomized controlled study in DM2 patients[6] showed that a MUFA-enriched isocaloric diet, induced a significant reduction in liver fat compared to a diet high in carbohydrates and fibre. In addition, the consumption of 20 g/day for 12 weeks in hypocaloric diets attenuated the degree of fatty liver in patients with NAFLD. However, it is difficult to isolate the effects of MUFA from other components (polyphenols), present in EVOO and the importance of the hypocaloric diet.[7]

Polyunsaturateds, including mainly ω3 and ω6 fats have also been evaluated in the progression of NAFLD in particular the essential PUFAS, α-linolenic acid (ALA; ω3) and linoleic acid (LA; ω6). LA is metabolized to arachidonic acid (AA; 20:4 n-6), and ALA is metabolized to eicosapentanoic acid (EPA; 20:5 n-3) and docosahexanoic acid (DHA; 22:6 n-3). The metabolic products of AA are proinflammatory, prothrombotic and proaggregatory. On the other hand, EPA and DHA modulate the liver's lipid composition, increasing anti-inflammatory mediators and decreasing insulin resistance.[8] In fact, low EPA and DHA liver values could tilt the balance towards liver fatty acid lipogenesis, instead of fatty acid beta-oxidation Figure 1.

Therefore, the ratio ω6/ω3 fats plays an important role in increasing the prevalence of chronic metabolic diseases (mostly a ω6/ω3 imbalance). Nevertheless, a double-blind randomized trial showed that a long-term hypercaloric diet rich in ω6 PUFA intake prevents liver fat accumulation in overweight individuals.[4]

Several clinical trials have addressed the potential benefits of omega-3 PUFAs on NAFLD/NASH. A systematic review and meta-analysis of controlled intervention studies on the effects of ω3 PUFAs in NAFLD patients[9] indicates that supplementation with ω3 decreases liver fat content and the steatosis score. However, the effects of ω3 supplementation on improving severe liver injury markers,

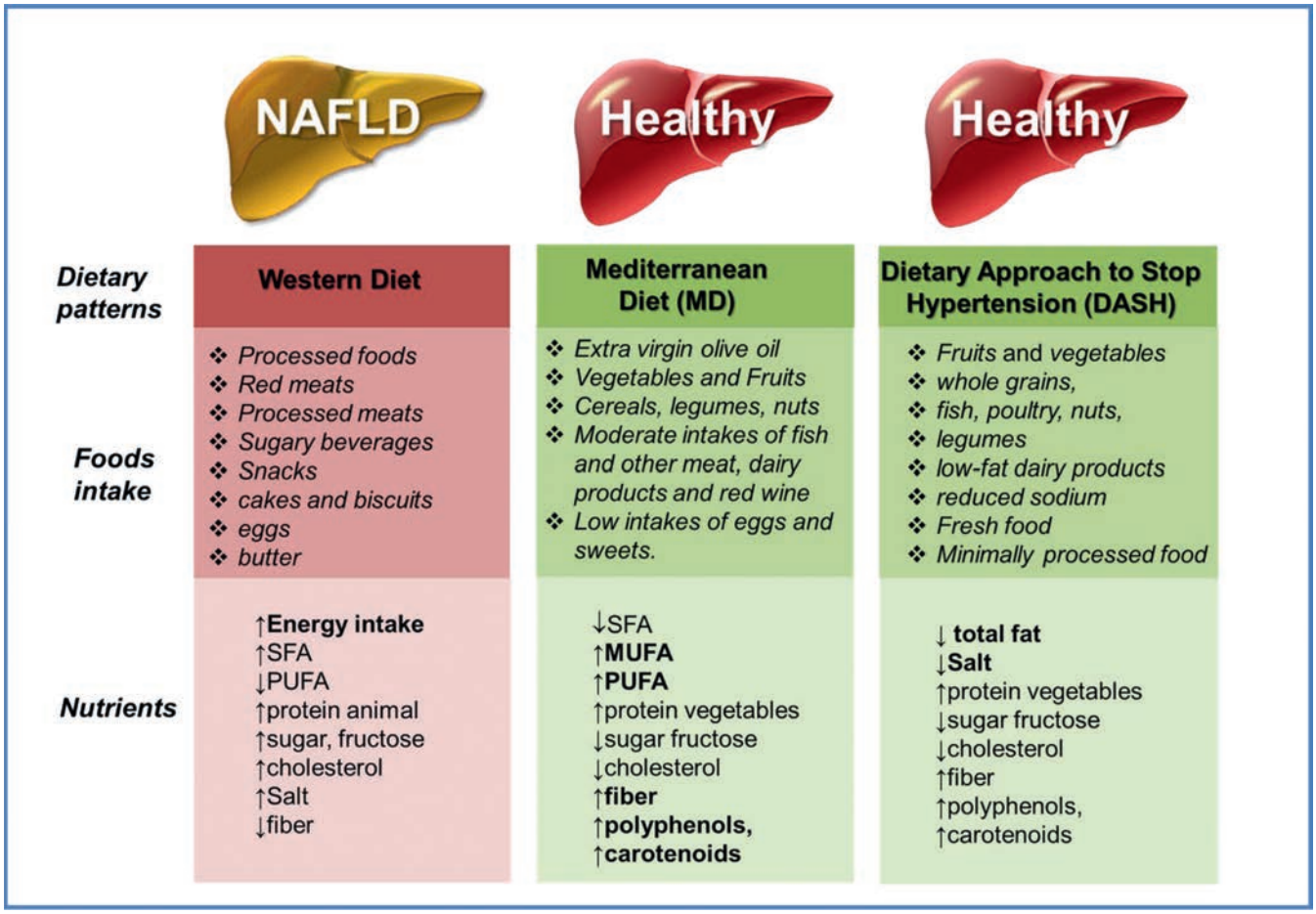

FIGURE 1 Non-alcoholic fatty liver disease (NAFLD) dietary patterns/food/nutrients chart. The Western diet is associated with NAFLD. This type of diet contains excessive amounts of refined and processed foods, red meat, processed meat, sugary drinks, snacks, cakes, biscuits, eggs and butter. It involves an excess of calorie consumption, saturated fats, animal protein, sugar, cholesterol and salt. The Mediterranean diet has beneficial effects on NAFLD. This diet is based on the high intake of extra virgin olive oil, vegetables, fruits, cereals, nuts and legumes; moderate intakes of fish and other meats, dairy products and red wine and low intakes of eggs and sweets. So, it provides a large amount of monounsaturated fatty acids, polyunsaturated fatty acids, vegetable proteins, fibre and antioxidants; and low amounts of sugar, cholesterol and saturated fats. Dietary approach to stop hypertension has beneficial effects on NAFLD. This diet is rich in fruits, vegetables, whole grains, fish, poultry, nuts, legumes and low-fat dairy products; it has low levels of sodium, added sugars and fat. Finally, this diet emphasizes on the consumption fresh food. This diet provides low intakes of total fat, salt, sugar and cholesterol; and high intakes of vegetable protein, fibre, and antioxidants.

such as inflammation and fibrosis are not well-established. It is important to consider that the controversial results on ω3 could be because of differences in methodology, the duration of the nutritional intervention, levels of intake, their sources, the EPA/DHA relationship, the chemical composition of ω3 and the patient's genetic background.[10]

The contribution of dietetic cholesterol in NAFLD is not clear. Certain nutritional studies suggest that high-cholesterol diets are involved in the development of NAFLD.[3] However, the same studies show that patients had high fat intake Figure 1.

2.2 | Role of carbohydrates

In the past twenty years, there has been substantial evidence to confirm the adverse metabolic effects of over consumption of simple carbohydrates. However, studies have cast doubts on the real role of monosaccharides and disaccharides when they are naturally contained in foods in NAFLD. On the contrary, numerous epidemiological studies have presented convincing evidence that there is an association between added sugars (sucrose, fructose and high fructose corn syrup) and NAFLD.[11] Overall, the dietary source of monosaccharides and disaccharides is essential to determine their effect on NAFLD.

Numerous studies have found a positive association between the risk of NAFLD and high-fructose products (cakes, soft drinks and sugary snacks).[2] The liver is the primary site of fructose metabolism, with nearly 60% oxidation of fructose ingestion. Furthermore, fructose metabolization in the liver is much higher than that of glucose. The hepatic metabolism of fructose stimulates de novo lipogenesis in the liver, increasing liver fat.[12] The most recent meta-analysis of controlled clinical trials concluded that the isocaloric exchange of carbohydrates for glucose does not induce NAFLD. However, when

fructose is the source of a hypercaloric diet, patients with NAFLD have increased liver fat and plasma alanine aminotransferases.[13] In addition, Abdelmark et al[14] showed that in adult patients with NAFLD, an increase in fructose consumption increased fibrosis and swelling Figure 1.

The role of non-digestible carbohydrates (fibre) in NAFLD has not been extensively studied. A decrease in fibre consumption is thought to be related to NAFLD. The proposed rationale is that low fibre intake, along with other dietary patterns induces dysbiosis, which modifies the microbiota inducing endotoxemia, systemic inflammation, insulin resistance and liver inflammation and damage. An alteration of gut microbiota has been observed in NAFLD patients. Prebiotic intake has also been shown to improve liver phenotype in NAFLD patients (Figure 1).[15]

2.3 | Role of proteins

The role of protein intake in the development of NAFLD is unclear. Existing studies do not provide evidence for or against. This may be because of the methodology used in the different studies, the origin of the protein source used (vegetable or animal), as well as the foods containing it (Figure 1).

3 | CONTRIBUTION OF MICRONUTRIENTS TO NON-ALCOHOLIC FATTY LIVER DISEASE

Micronutrients are important for the development of NAFLD. To date, the micronutrients involved in NAFLD are zinc, copper, iron, selenium, magnesium, vitamins A, C, D and E and carotenoids.[16] The proposed mechanisms of action are their antioxidant, antifibrotic, immunomodulatory and lipoprotective effects.

Non-alcoholic fatty liver disease patients have been shown to have decreased levels of serum zinc, copper, vitamins A, C, D, E and carotenoids. Moreover, an iron and selenium excess have been reported to play a role in the severity of NAFLD.[16]

Lipid soluble vitamins have been linked to NAFLD, mainly low serum levels of vitamin A.[17] Because vitamin A may be beneficial, there are some concerns about supplementation. Vitamin A has many other effects. Treatment with vitamin E showed a decrease in transaminase levels and liver lobular inflammation, improved liver fibrosis and reduced steatosis.[18] Vitamin E supplementation is a common practice in NAFLD patients. Vitamin E has antioxidant effects and NAFLD patients present with increased oxidative stress. Nevertheless, vitamin E supplementation could have different side effects, including an increase in the risk of certain types of cancer or of hemorrhagic stroke, which are the key factors reducing its use in clinical practice.

A mix of micronutrients could be proposed to help in the treatment of NAFLD. However, the interactions between different vitamins and between vitamins and macro/micronutrients must be taken into consideration. Moreover, identifying the contribution of specific micronutrients is difficult because human diets are complex and vary and may not correspond to experimental dietary models. Thus, it is difficult to recommend diets with specific micronutrients.

4 | FROM MAJOR FOODS GROUPS TO DIETARY PATTERNS: EVIDENCE FROM NON-ALCOHOLIC FATTY LIVER DISEASE

4.1 | Relationship between food group intake and non-alcoholic fatty liver disease

Nutrients are contained in the foods that people eat, thus a more physiological approach is an analysis of the intake of food groups and their relationship with NAFLD. There is a general consensus that the intake of a variety of foods is important to prevent the development of NAFLD.[19]

The foods that are considered to be beneficial for the prevention and progression of NALFD are whole grain cereals, fruits and vegetables, fatty fish (mainly high in ω3) and EVOO. On the other hand, foods that are considered to adversely effect NALFD include red meat and processed meats, soda, processed foods, cakes and biscuits.[14] Patients with NAFLD have been shown to consume fewer cereals, grains, fruits and vegetables than healthy subjects. NAFLD patients have a higher intake of cooking oils, candy, pastry, desserts, salty food, spicy food, sauce, dressings and soft drinks.[20]

A recent study showed that patients with NAFLD had a higher intake of red and processed meats. The effect was independent of saturated fat and cholesterol intake.[21] Moreover, cooking meat at high temperatures for a long period could be an important factor.

Extra virgin olive oil is a 'protective' food and exerts its healthy effects through MUFAs (especially oleic acid) and phenolic compounds. It has been suggested that EVOO should be included in the diets of NAFLD patients since it reduces insulin resistance and blood triglycerides, thus inducing downregulation of lipogenic genes.[22] In a randomized, double-blind clinical trial, the consumption of 20 g/d of olive oil attenuated the fatty liver grade in NAFLD patients.[7] Finally, a randomized trial in prediabetic patients with an isocaloric diet rich in EVOO, reported a decrease in liver fat and an improvement in both hepatic and total insulin sensitivity.[23]

Because people consume different amounts of various food groups and because of the limited number of large clinical trials, in some cases the impact of different foods are not clear, for example dairy products, coffee and rice. The results of studies on the consumption of dairy products were inconclusive in relation to NAFLD[20] while those on coffee were contradictory (Figure 1).

4.2 | Healthy dietary patterns help reduce the risk factors of non-alcoholic fatty liver disease

Another approach is to analyse the role of diet in NAFLD. In this case, data are based on habitual food consumption, which is therefore

more realistic. Western dietary patterns are often associated with the development of NAFLD independent of physical activity.[2] This diet is generally hypercaloric with inadequate intake of fruits, vegetables, whole grains, legumes, fish and low-fat dairy products and excessive refined and processed foods, alcohol, salt, red meats, sugary beverages, snacks, eggs and butter. In addition to the role of the different foods found in the diet, the excess amount of calories are a risk factor for NAFLD.[1]

In the last decade, several studies have analysed the beneficial effects of certain dietary patterns on NAFLD, in particular the Mediterranean Diet (MD) and the dietary approach to stop hypertension (DASH).

A good general definition of MD is the high intake of EVOO, vegetables including leafy greens, fruits, cereals, nuts and pulses/legumes; a moderate consumption of fish and other meats, dairy products and red wine and a low intake of eggs and sweets. Adherence to this diet is measured by a score. At present, several studies (observational studies and short-term trials) have demonstrated that this type of diet is beneficial for NAFLD by improving liver status, in particular hepatic insulin sensitivity and lipid profile.[24] Moreover, this diet may improve NAFLD without changing body weight, which is an important obstacle in lifestyle changes. To better confirm its beneficial effects, long-term trials with more patients with histological outcomes are required.

The DASH diet, which was designed in the 1990s to regulate blood pressure, has also been found to have beneficial effects on NAFLD.[25] The DASH diet is rich in fruits, vegetables, whole grains, fish, poultry, nuts, legumes and low-fat dairy products. Moreover, there is reduced sodium, added sugars, as well as saturated and total fats. DASH emphasizes the consumption of minimally processed and fresh foods.

Both of these diets are probably beneficial because of their macro- and micronutrient components, but other aspects should also be considered. For example, the diet-induced modifications in gut microbiota could be an important factor. Inadequate changes in gut microbiota increase gut permeability and the translocation of bacteria and their products to blood. This induces endotoxemia, which has been found to contribute to liver inflammation in NASH patients.[26] A recent study established a relationship between gut microbiota and NAFLD.[27] Thus, additional studies are needed to investigate the mediation of NAFLD and microbiota through diet (Figure 1).

5 | NUTRITIONAL GEOMETRY: A KEY CONCEPT FOR PERSONALIZED NUTRITION IN NON-ALCOHOLIC FATTY LIVER DISEASE

When studying the role of nutrition/foods/diets on metabolic diseases, the involvement of specific nutritional parameters of these diseases may be unclear. Moreover, the relationship between biology and environment is complex. Finally, foods are composed of a mixture of nutrients which are combined into meals to make up a person's diets and dietary patterns. Diets are known to be more than the sum of their components. As a result, the single-nutrient model paradigm (the relationship between a particular disease and a specific nutrient) is not a useful approach in metabolic diseases, and the large number and huge variety of nutrients, foods, diets and dietary patterns that constitute human nutrition must be taken into consideration. The level of focus must be changed to give priority to foods, diets and dietary patterns. A methodology that takes into account the interactions among nutrients in foods and diets and metabolic diseases should be developed. This methodology should define and quantify the effects of different diets on different health outcomes.

In response to this, the notion of 'nutritional ecology' has been developed[28] based on the idea that there is a dynamic interface between the organism and its environment from a nutritional point of view. The goal of this concept is to combine nutrients and foods into a model to understand the way food components interact to regulate the properties of diets affecting health. This is called 'nutritional geometry'.

This approach models the relationship of different levels of a nutritional combinatorial hierarchy (nutrients, foods, meals and diets) using the right-angle mixture triangle geometric model.[28] The model can use micronutrients, a combination of macro- and micronutrients, bioactive compounds or other food components. Usually, the model represents three-dimensional macronutrients (fats, carbohydrates and proteins) of foods and how to combine them into a meta-mixture: for example, meals and diets.[28] The levels of the hierarchy of meta-mixtures represented in the model can change depending on the question being addressed. Instead of macronutrients, we can choose foods, dishes, daily meals or dietary patterns. An important conclusion of studies using nutritional geometry is that the balance of macronutrients affects food, energy intake and various physiological functions in varied manners.[28] (Figure 2)

Some questions addressed by nutritional geometry are as follows: (a) how does dietary macronutrient balance relate to energy intake? (b) how does the range of energy intake relate to energy balance? (c) what is the relationship between dietary macronutrient ratios and total energy intake? (d) what is the relationship between dietary macronutrients ratios and socioeconomic status? and (e) how does dietary balance influence protein intake? Another unexplored issue that can be analysed by nutritional geometry is the quality of the macronutrients.

Thus, nutritional geometry accommodates multiple diet components with different animal models and particular health issues. There is only one study to date using nutritional geometry to evaluate the relationship between diet and NAFLD.[29] The authors found that the development of NAFLD increases during old age once carbohydrate intake is >25 kj/d. When protein intake >10 kj/d, the probability of avoiding NAFLD increases. Finally, the highest probability of suffering from severe NAFLD occurs with low-protein, high fat diets. Moreover, the authors concluded that the ratio and quality of macronutrients could be as important as the diet's energy content.

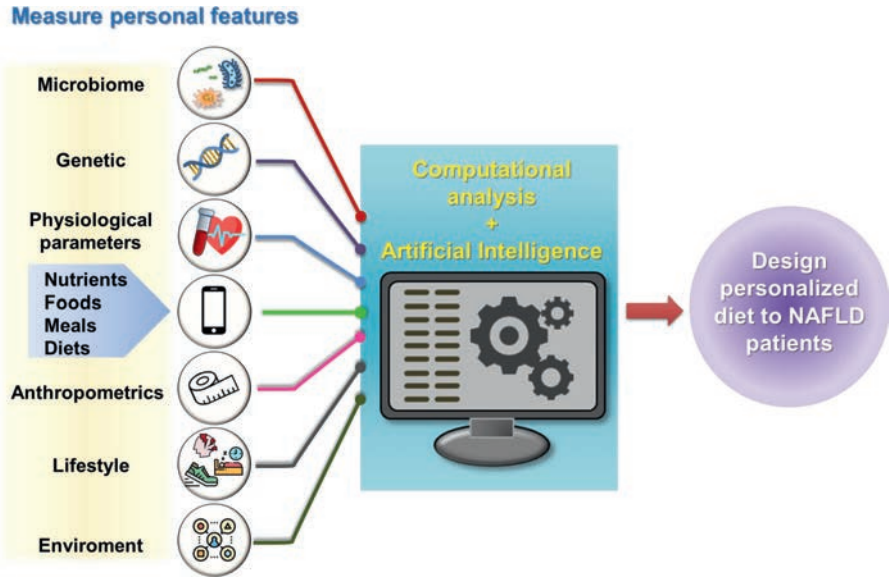

FIGURE 2 Personalized nutrition. With the advances in technology, big data analysis and artificial intelligence, we can design diets and dietary patterns in a personalized way according to the specific situation of non-alcoholic fatty liver disease patients
Source: Adapted from Zeevi et al.[30]

6 | LOOKING TO THE FUTURE: THE ROLE OF PERSONALIZED NUTRITION

Dietary guidelines are based on the assumption that diets affect everyone equally, without taking into consideration the heterogeneity and individuality of the metabolism, microbiota and lifestyle. Each person is unique and reacts differently to the same foods or dietary patterns. Zeevi et al[30] measured the postprandial glucose responses to 46.898 foods in 800 individuals and found a great variability in responses to the same foods. These data suggest that the use of general dietary recommendations may be limited. These authors developed a machine-learning algorithm based on these data that integrates several traits (blood parameters, dietary patterns, anthropometric values, physical activity and gut microbiota). This algorithm precisely predicted individual post-prandial glycemic values to real-life meals. This type of study will make it possible to develop more personalized diets to predict the potential role of nutrients/food/diets on the pathophysiology and progression of NAFLD and to design personalized nutritional interventions for more effective control of the disease (Figure 2).

7 | CONCLUSIONS

The relationship between nutrients/food/meals, dietary patterns and NAFLD has been extensively studied in the last decade. Research into the role of nutrition for the management of NAFLD patients is a major challenge. This is particularly important because lifestyle modifications including diet, exercise and weight loss have been found to be effective in controlling NAFLD. The long-term effects of calorie-restricted diets result in an improvement in several features of NAFLD. The specific macronutrient composition of the diet seems to be less important, although further studies are needed to clarify this issue. However, hypocaloric diets, either high fat/low carbohydrate or low fat/high carbohydrate intake are known to be equally effective in reducing liver lipids. The Western style diet is associated with a greater risk of NAFLD while the Mediterranean diet results in significant improvement in steatosis, even in the absence of weight loss. One important difficulty in the research of nutrition and NAFLD is the slow progression of the disease. Furthermore, prospective long-term trials with liver biopsies are required to monitor histopathological endpoints. Nutritional geometry could be an excellent tool in these cases to study the relationships between the various aspects of diet, nutrients and liver health. Models can be used to understand the multiple dimensions and relationships between nutritional issues and NAFLD. Another important contribution will be algorithms developed by AI to create a personalized diet for patients. Except for certain broad nutritional guidelines, the idea that there is no single optimal diet is gaining ground. In upcoming years, patients will probably wear devices to register data on what they eat. This information will be processed by deep learning and integrated by AI, with multiple data (physical activity, level of stress, sleep, microbiome, physiological constants, medications and genome) to provide customized dietary recommendations and personalized nutritional counselling, to prevent and treat NAFLD.

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CONFLICT OF INTEREST

The authors (GB and MRG) declare no conflict of interest.

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REVIEW ARTICLE

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Optimizing curative management of hepatocellular carcinoma

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Abstract

The goal of curative management of hepatocellular carcinoma is to provide the best chance of remission. However, recurrence rates for both local and distant relapse are high. Patient subgroups at higher risk of these events can be identified based on histological patterns that are closely linked to specific molecular subtypes. Patient outcome has improved with more effective therapeutic strategies thanks to technological advances in surgical techniques and percutaneous ablation. The main goal of controlling the cause of liver disease is to decrease distant/late recurrence and prevent deterioration of hepatic function. Ongoing trials testing the combination of neoadjuvant and/or adjuvant regimens with these procedures as well as routine tumour molecular analysis may modify therapeutic algorithms for hepatocellular carcinoma in the future.

KEYWORDS

ablation, adjuvant, biopsy sample, hepatocellular carcinoma, resection

1 | INTRODUCTION

Increasing the rate of HCC patients eligible for curative procedures is key to improving the poor prognosis in these patients. This goal can be achieved by promoting surveillance of at-risk populations with chronic liver disease, in particular those with cirrhosis. Patients with very early-stage (BCLC 0) and early-stage (BCLC A) HCC have preserved liver function and solitary lesions or up to three nodules that are <3 cm in diameter.[1] These patients can benefit from resection, transplantation or percutaneous ablation (PA), with a median survival of between 50% and 70% at five years.[2] However, the term « curative» resection or ablation of HCC in patients with cirrhosis is misleading, since tumour relapse is frequent following these procedures.[3] It is therefore highly important to identify predictors of recurrence and promote continued improvement of procedures and therapeutic strategies to decrease this risk.

Early and late recurrence must be considered independently because they are associated with different risk factors. Early recurrence, arbitrarily defined as occurring within two years following a curative procedure, is related to the development of intrahepatic metastases and is linked to tumour burden including large size, an incomplete tumour capsule and vascular invasion. Late recurrence is more likeky to be due to a de novo carcinogenic process with risk factors related to the extent of patient's liver disease (presence of cirrhosis, persistence of the cause of hepatic insult) and high alpha-foetoprotein (AFP) levels.[4,5] Except for these easy-to-assess characteristics, information obtained by analysis of both tumour and non-tumour biopsy specimens refines the estimates of the risk of recurrence. Figure 1 summarizes

Abbreviations: HCC, hepatocellular carcinoma; Io, immunotherapy; IRE, irreversible electroporation; OS, overall survival; PA, percutaneous ablation; RFA, radiofrequency ablation; RFS, recurrence-free survival; TKI, tyrosine kinase inhibitor.

these different prognostic factors and the potential actions that can be taken to optimize curative HCC management (except transplantation) in routine practice, which are developed in the present review. This includes advances in surgical or ablation procedures, control of the cause of liver disease and neoadjuvant/adjuvant approaches currently tested in clinical trials.

Key points

- The goal of optimizing curative hepatocellular carcinoma management is to decrease the recurrence rates
- Histological analysis of a biopsy specimen can further clarify the prognosis
- Surgical and ablation procedures provide tumour remission in increasing numbers of patients
- Controlling the cause of liver disease also plays a key role in these patients
- Neoadjuvant and/or adjuvant approaches and molecular analyses will provide new therapeutic strategies

2 | REFINEMENT OF PROGNOSIS ACCORDING TO PATHOLOGICAL AND MOLECULAR INFORMATION OBTAINED BY TUMOUR BIOPSY

There are three goals to obtaining a tumour biopsy for the therapeutic strategy of HCC: making a diagnosis, assessing the prognosis and predicting the response to therapy.

As recently described by Calderaro et al,[6] distinct morphological phenotypes of HCC have been found to be associated with the different genetic defects and biological pathways that drive tumour progression. Next generation sequencing (NGS) using whole-exome, whole genome and RNA sequencing have highlighted the key mutations in the driver genes involved in liver carcinogenesis: somatic alterations in the *TERT* promoter (40%-60%), *TP53* (15%-40%), *CTNNB1* (20%-30%), *AXIN1* (5%-15%), *ARID1A* (5%-18%), *ARID2* (4%-15%), *RB1* (3%) and *CDKN2A* (2%-12%).[7] Moreover, integrative analysis of transcriptomic data together with genetic alterations has helped identify major molecular subgroups of HCC, G1 to G6. G1 to G3 subgroups are the 'proliferative' subclasses associated with chromosomal instability and *TP53* mutations, whereas the G4 to G6 subgroups are 'non-proliferative' subclasses associated with chromosomal stability. G1 to G2 subgroups are associated with Hepatitis B Virus (HBV) infection and *AXIN1* mutations with the G1 subgroup are enriched in stem cell features with a high serum AFP level. G3 subgroups are enriched in *FGF19* amplifications and *TSC1/2* mutations together with dysregulation of cell cycle genes at the transcriptomic level. The G4 subgroup is associated with a transcriptomic profile close to that of the mature hepatocyte. Finally, G5 to G6 subgroups are strongly associated with the somatic mutations of *CTNNB1*, coding for β-catenin.

Since NGS is not routinely performed, different morphological phenotypes have been identified linked to the different molecular subclasses at the histological level, and can be classified as follows.[6]

A *CTNNB1* mutated HCC, observed in 20%-30% of resected HCC, is characterized by the activation of the WNT/ß-catenin pathway, with strong glutamine synthase immune-histochemical expression and corresponding to the G5 to G6 subgroup. These HCC are morphologically well-differentiated with microtrabecular and/or pseudoglandular architectural patterns, intratumour cholestasis and a lack of immune infiltration. This subclass has not been found to influence prognosis. However, molecular profiling of tumour biopsies from advanced HCC has suggested an altered response to immune checkpoint inhibitors.[8] This could be important for future adjuvant/neoadjuvant strategies (see below).

The second phenotype is the macrotrabecular-massive (MTM) HCC, which is closely associated with the G3 molecular subgroup. This subclass is defined by an architectural pattern composed of large trabeculae (>6 cell-thick) of tumour cells, whatever their cytological aspect (hepatocytic, clear cells, undifferentiated) surrounded by endothelial cells, with empty spaces between these tumour clusters or macrotrabeculae.[9] It is important to note that the prognostic value of this phenotype has been validated in resected liver or biopsy samples of patients undergoing surgery or

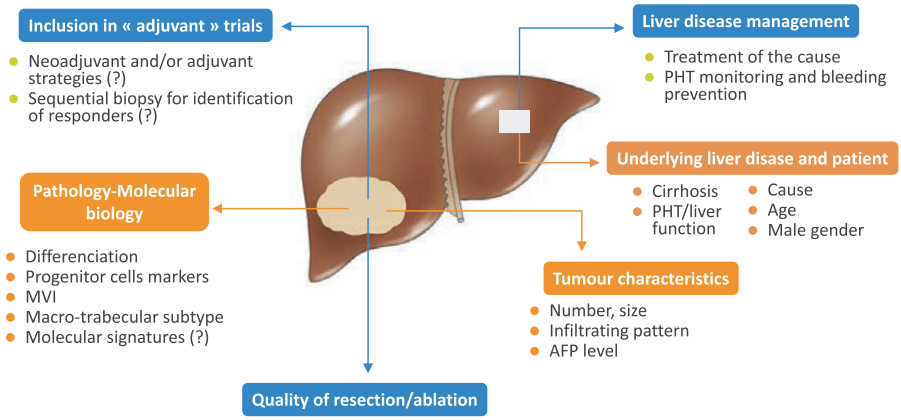

FIGURE 1 Risk factors for recurrence following HCC curative management (orange) and potential optimization strategies (blue)

ablation showing a poorer prognosis linked to angiogenesis activation and vascular micro-invasion. Thus, detection of any MTM-HCC subtype component in a biopsy sample (10%-20% of patients) eligible for curative treatment may require more intensive therapeutic strategies.

A third subtype is the steato-hepatitic subtype observed in about 10% of HCC, and belonging to the G4 molecular subclass. It is characterized by steatosis, cell ballooning, pericellular fibrosis and inflammatory infiltrate. These tumours are often well-differentiated and develop more frequently in patients with metabolic syndrome. Although steato-hepatitic HCC is rarely associated with microvascular invasion and satellite nodules, no clear-cut relationship with prognosis has been established so far in biopsy samples.

The progenitor subtype, another HCC subtype, can be defined in biopsy samples by the immune-histochemical expression of markers of a biliary lineage such as CK19 and or EpCAM in more than 5% of cells. This subtype may represent 5% of HCC and is associated with *TP53* and *RPS6KA3* mutations as well as with G1 molecular subclasses. These HCCs express stemness-related markers and have been regularly linked to more aggressive behaviour with increased recurrence and shorter survival.[10]

The immune microenvironment also influences prognosis independent from HCC histological subclass, since tumour infiltrating T lymphocytes were associated with a favourable outcome and a lower risk of relapse after surgery.[11] Finally, screening for microvascular invasion has been performed in biopsy specimens,[12] and two immuno-histochemical markers – PIVKA-II and H4K16 – were identified as a major prognosis factor.

3 | IMPROVEMENT OF CURATIVE PROCEDURES THROUGH TECHNICAL INNOVATION AND ACCURATE PATIENT SELECTION

3.1 | Advances in surgical treatments

The feasibility of liver resection depends on the quality of the parenchyma and the extent of resection, while oncological suitability depends on the biological features of the tumour.

Major hepatectomy (resection of 3 or more Couinaud segments) is only feasible in the absence of portal hypertension and with a MELD score <9.[13] Even when these criteria are met, preliminary portal vein embolization is recommended to assess the liver regeneration in patients with cirrhosis in whom right hepatectomy is planned. As growth of the future liver remnant is slower in patients with cirrhosis, chemoembolization is often performed first to prevent tumour progression while awaiting the benefit of portal embolization. Recently, simultaneous occlusion of both portal inflow and hepatic venous outflow of the planned resection target showed greater and more rapid growth of the future liver remnant than portal embolization alone, although the concomitant increase in liver function may be somewhat delayed compared to the increase in volume.[14]

Until the most recent EASL guidelines, portal hypertension was considered to be a contraindication to hepatectomy, but these were revised based on several papers showing that resection can be safely performed in the presence of indirect signs of portal hypertension such as platelet count <100 000/μL and/or splenomegaly, as long as liver function remains normal (MELD score <9).[1] It may be possible to further broaden the criteria by incorporating other factors such as an assessment of liver/spleen stiffness. In the absence of clinically detectable ascites, the risk of liver decompensation after minor hepatectomy has been shown to be very low if liver stiffness as measured by elastometry is <12 kPa.[15] Measurements between 12 and 20 kPa make up a 'grey zone', and measurement of splenic stiffness and/or volume may increase the accuracy of the noninvasive assessment of resectability.

Several technical improvements and innovations have helped improve the prognosis of patients with HCC. For instance, laparoscopic resection is now extensively used for HCC despite the absence of randomized controlled trials comparing this approach to open surgery. A recent meta-analysis analysed 51 retrospective studies with or without propensity score matching to compare laparoscopic and open resection for HCC in 6812 patients.[16] Laparoscopy was associated with decreased operative blood loss, lower 30-day morbidity and mortality, and a shorter hospital stay, with a similar rate of R0 resection. There was a trend towards lower HCC recurrence and increased long-term survival with laparoscopy. Other innovative technologies, including intra-operative near-infrared (NIR) imaging fluorescence-guided surgery, are highly promising in the field of HCC.[17] Indocyanine Green (ICG), a vital dye that is extensively used to assess liver function, binds to plasma proteins and emits light with a peak wavelength of around 830 nm when illuminated with NIR light. ICG is excreted in bile, and tends to concentrate in HCC enabling intra-operative detection by the NIR camera. While the ultimate role of this technology must be determined, interest is particularly high among laparoscopic surgeons where there is a need for alternative means of tumour detection because of the absence of tactile feedback.

3.2 | Innovation in percutaneous ablations

Innovation in percutaneous ablative techniques including radiofrequency ablation (RFA), microwave ablation (MWA), cryotherapy and irreversible electroporation (IRE) is one of most effective strategies to improve the outcome of patients presenting with the broadened early stages of HCC.[5] Underlying cirrhosis and the presence of comorbidities still limits curative resection in selected patients despite recent developments in hepatobiliary surgery. Furthermore, the worldwide shortage of cadaveric donors makes liver transplantation within 6 months after diagnosis of HCC unrealistic in many more selected patients. Indeed, in most patients with early-stage HCC, ablation is the only opportunity to obtain long tumour progression-free survival that is similar to that reported after resection. Moreover, when feasible, ablation should be considered to be the first line treatment in transplantable patients when bridging treatment is

required. Similarly, if straight resection cannot be attempted without preparing portal embolization, ablation may be a suitable alternative in selected cases. In both cases ablation has the advantage of decreasing the risk of shifting the treatment from a curative to a palliative strategy, which has been associated with deterioration of outcome.[1]

Improving the visibility of the target on imaging is the key to improving the efficacy of tumour ablation.[18] Although real-time and multiplanar ultrasound (US) is the reference guidance method for percutaneous ablative techniques, inconspicuous US targets remain a major cause of technical contraindications to ablation. In patients with unresectable tumours, this usually results in endo-arterial approaches, mainly trans arterial chemoembolization (TACE), which has been associated with a less complete response and shorter survival. Fusion image technologies including the co-registration of real-time US with pretherapeutic CT or MRI 3D-data sets allow US guided ablation of tumors that are normally poorly visible. If necessary, an intravenous contrast bubble injection during the procedure improves US target conspicuity and allows intra-operative delineation of the ablation zones. In practice, percutaneous ablation is not denied in patients because of tumour visibility on US in expert centres that routinely use these tools. Many advanced technologies based on multimodal fused imaging such as cone-beam CT or angiography-CT suite are also now available for more difficult ablative procedures, especially those requiring multiplicator insertions in critical locations. Thus, at present, technical contraindications to percutaneous ablations for liver tumours because of imaging limitations have been almost completely overcome.

Superficial HCC locations have long been considered a contraindication because it was assumed there was a higher risk of bleeding, tumour seeding and collateral damage to neighbouring critical structures such as the diaphragm, digestive tract and gallbladder. The experience acquired worldwide in the last two decades using common intratumourous irradiating ablative methods (monopolar RFA, MWA) has shown that puncturing the target through the non tumourous liver parenchyma and systematic tract ablation is safe for subcapsular tumours in relation to the risk of haemorrhage and tumour seeding. Artificial ascites involving filling the peritoneal cavity with isotonic serum to separate the target from critical adjacent structures are effective in preventing thermal damage. Furthermore, artificial ascites can help improve the visibility of some subdiaphragmatic tumours on ultrasound. However, recent improvements in laparoscopic liver surgery should be considered before choosing ablation of subcapsular HCC because while resection is now more often safe, some reports have suggested that ablation using standard irradiating intratumoural methods such as monopolar RFA may be less effective than in intraparenchymal locations.[5]

Advances in ablative technologies have obviously contributed to optimization of curative management of patients with HCC. Monopolar RFA has been the most frequently used technique for ablation of HCC by far. Two decades ago, monopolar RFA was adopted worldwide rather than ethanol injections and any other chemical methods for the treatment of unresectable tumours presenting with HCC within the Milan criteria. This is because a mean four times fewer procedures was needed to obtain a complete response, resulting in better local recurrence-free and overall survivals. However, up to 25% of local recurrences have been reported after monopolar RFA and systematic pathological examinations of the explanted liver in transplanted patients after first line RFA showed up to 40% of remnant viable tumours at the ablation site. Thus, the efficacy of ablative methods must be improved to increase their 'curative' value. Nodules >3 cm and the 'heat sink effect' of large neighbouring vessels (>3 in diameter) are the two main predictive factors for incomplete ablation after monopolar RFA. Developments in MWA technologies that heat the tissue faster and at higher temperatures should extend these limits. In clinical practice, despite a trend to induce larger ablation volumes in shorter times, the results of MWA and monopolar RFA are similar for local control of HCC.[5] Cryotherapy has the advantage of clear delineation of the ice ball when CT is used for monitoring. However, only a few centres use this technique for HCC ablation and its limitations seem similar to those reported with monopolar RFA and MWA.[5] Finally, to extend the existing limits of ablative techniques, the type of energy and the number of applicators could be less than the diffusion of energy around and between the applicators.[19] Thus, we developed a classification of ablative technologies according to two modes of energy diffusion. The most common mode including monopolar RFA, MWA and cryotherapy is centrifugal from each applicator, while the other including only multibipolar RFA and IRE is centrifugal between each combination of applicators. The main consequence for clinical application is better prediction of the boundaries of the ablation zone by the centripetal mode compared to the centrifugal mode,which implies a limited diffusion of energy outside of the applicators. Thus, indications for ablation can be extended to larger tumours (up to 8 cm) with centripetal energy devices, even infiltrative tumours with limited portal invasion (Vp1-3).[20] For standard indications (tumour <5 cm), the no-touch approach can be implemented with centripetal methods, especially multibipolar RFA, which involves inserting applicators outside the tumour (extratumourous method). The local recurrence rate in HCC up to 5 cm (including for those <3 cm) is markedly decreased with no-touch multibipolar RFA compared to monopolar RFA.[19] In addition, no-touch ablation allows safe and effective ablation of a wide spectrum of subcapsular tumours. Irreversible electroportation (IRE), which is also a centripetal ablative method, is the only nonthermal technique. IRE is currently a unique option for curative treatment of central HCC abutting the main bile ducts. In addition, because IRE spares the collagenic skeleton and the microvessels of surrounding non tumourous tissue, this technique appears more suitable than other thermal methods in fragile patients with poor liver function and severe comorbidities.[21]

4 | DECREASING RATES OF RECURRENCE

4.1 | Control of underlying liver disease

This step is pivotal because the goal is to preserve liver function and potentially decrease the rates of distant/late recurrences.

4.1.1 | HCV infection

It is not clear whether the benefit of viral clearance is related to a potential impact on the oncological process or a reduction in end-stage liver disease (ESLD)-related risk of death independent from HCC management. Most of the available data for the impact of HCV eradication on the risk of HCC recurrence have been obtained by meta-analyses or meta-regressions performed in the interferon (IFN) era. However, although there was speculation on the potential benefits of IFN on HCC recurrence, based on its antiviral, anti-inflammatory and anti-angiogenic effects, randomized controlled trials did not demonstrate the efficacy of IFN-based adjuvant therapy in patients undergoing curative HCC procedures.[22] Moreover, the controversy on the potential harmful impact of direct antiviral agents (DAAs) on the risk of HCC recurrence has probably not only limited prescriptions in these otherwise priority candidates for antiviral treatment, but also prevented any definite conclusions from being drawn based on rigorous prospective data.[23] However the findings of prospective studies and the latest reports in large American populations[24] have challenged these hypotheses and recently provided further support of the safety of DAAs in patients who have achieved effective HCC remission. Finally, it is tempting to speculate that longer follow-up may be required to identify any differences in long-term HCC recurrence (more than 2-3 years). This is probably the time needed for the decreased inflammatory and/or fibrotic processes induced by viral suppression to affect liver carcinogenesis.

Overall, achieving a sustained virological response (SVR) in patients with HCV-related cirrhosis is not clearly associated with a modified risk of short- or medium-term tumour recurrence following a curative procedure. Nevertheless, HCV eradication favours optimal HCC management by preventing deterioration of liver function, leading to improved overall survival, whatever the antiviral regimen. While awaiting confirmation from larger prospective studies with longer follow-up, patients with HCV-related cirrhosis, complicated or not by HCC who are eligible for curative procedures, must be prioritized for access to antiviral treatment.

4.1.2 | HBV infection

Maintained HBV virosuppression in patients with HBV-related cirrhosis has tertiary prophylactic properties in addition to increased overall survival (OS) and decreased HCC occurrence, and is independently associated with a reduced risk of late recurrence after local curative-intent treatment of HCC. At least three meta-analyses including more than 23 000 HBV-related HCC patients, mainly from Asia, showed that nucleoside analogue (NA) therapy significantly reduced the risk of recurrence after surgical resection and improved both disease-free and OS compared to no treatment. In patients with recurrent HCC, the use of HBV antiviral therapy was associated with both preserved liver function at recurrence and an increased proportion of patients eligible for curative HCC treatment.[25]

4.1.3 | Other causes of chronic liver disease

Scientific evidence of the benefits of controlling non-virological factors involved in underlying chronic liver disease are scarce, but meaningful.[26] Abstinence from alcohol consumption and intensive care of diabetes mellitus has been related to an improved prognosis in HCC patients. Conversely, inadequate blood glucose maintenance in diabetic patients is a significant risk factor for recurrent HCC and poor survival after curative RFA therapy.

4.2 | Inclusion of patients eligible for curative procedures in ongoing neoadjuvant/adjuvant trials

The high rates of intrahepatic local HCC recurrence strongly influence patient prognosis, which could be improved by implementation of neoadjuvant and/or adjuvant strategies. However, many adjuvant therapies have failed to improve recurrence-free (RFS) or overall survival (OS), including sorafenib.[27] However, the results of these trials are limited by selection biases and tyrosine kinase inhibitors (TKI) side-effects.

Several studies have shown that surgery and particularly ablation procedures may significantly alter the immune microenvironment. Therefore, it could be hypothesized that adding immunotherapy (Io) before and/or after these curative procedures could lead to improved RFS by inhibiting immune related pro-tumour effects.[28] In particular, the rationale for combining RFA and immunotherapy is based on boosting the immune response that is triggered by the necrosis induced by percutaneous treatment. Based on positive results in studies evaluating immune checkpoint inhibitors in advanced HCC,[29] which reported encouraging results and a fair safety profile, a number of studies are testing the combination of immune checkpoint blockade in addition to curative procedures. Table 1 summarizes ongoing Phase II or III trials testing neoadjuvant and/or adjuvant strategies before/following effective curative procedures (resection or PA). Most studies are designed to include patients with a high risk of relapse (multiple tumours or single >3 cm), with RFS as the main endpoint. All trials are being performed in academic centres worldwide and will recruit several hundred patients thus providing a potential opportunity for patients being managed in routine practice.

5 | PERSPECTIVES

Curative management of HCC patients will continue to improve as progress is made in prognosis, systemic therapy and innovation

TABLE 1 Ongoing trials testing various drugs or immunotherapies in neoadjuvant and/or adjuvant settings of HCC eligible for curative procedures

Drug(s)	Setting	Intervention	Phase	Planned recruitment	NCT identifier and study acronym
Nivolumab	Adjuvant	Resection, ablation	III	530	NCT03383458 (CheckMate 9DX)
Atezolizumab plus bevacizumab	Adjuvant	Resection, ablation	III	662	NCT04102098 (IMbrave050)
Durvalumab and/or bevacizumab	Adjuvant	Resection, ablation	III	888	NCT03847428 (EMERALD-2)
Pembrolizumab	Adjuvant	Resection, ablation	III	950	NCT03867084 (KEYNOTE-937)
Lenvatinib	Adjuvant (MVI)	Resection	III	377	NCT04053972
Nivolumab	Neoadjuvant and adjuvant	Ablation (IRE)	II	50	NCT03630640 (NIVOLEP)
Pembrolizumab	Neoadjuvant and adjuvant	Resection, ablation	II	50	NCT03337841
Cemiplimab	Neoadjuvant and adjuvant	Resection	II	94 (various cancer types)	NCT03916627
Gefitinib	Adjuvant	Resection	II	40	NCT00282100

in surgical or ablative procedures. Molecular biology will allow personalized intervention strategies, as several non-tumour and tumour molecular prognostic signatures have been validated in both resected liver and biopsy specimens of small HCC,[30] although they must still be prospectively validated for use in clinical practice. Ultimately, combining precision medicine and new drugs with liver resection or percutaneous ablation in neoadjuvant and/or adjuvant approaches might improve patient outcomes in the future.

DISCLOSURES

Dr Nahon has received honoraria/grants from Abbvie, AstraZeneca, Bayer, Bristol-Myers Squibb, Gilead, Ipsen and Roche.

CONFLICT OF INTEREST

The authors do not have any disclosures to report.

AUTHOR CONTRIBUTIONS

All authors contributed to the drafting and critical revision of the manuscript.

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REVIEW ARTICLE

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Liver transplantation as therapy for hepatocellular carcinoma

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Abstract

Liver transplantation can provide curative therapy in selected patients with hepatocellular carcinoma. Well-established criteria include tumours that are within the Milan criteria and without evidence of vascular or extrahepatic involvement. Modest expansion of the original Milan criteria has been shown to achieve similar recurrence-free survival rates. Overall, HCC recurrence occurs in about 10%-15% of LT recipients, most within the first 2 years. Predictors of post-transplant recurrence include high alpha-foetoprotein, macrovascular invasion, as well as tumour size and number. Once HCC recurs after transplantation, prognosis is poor, though better if detected early. There is no established role for systemic prophylactic post-transplant chemotherapy.

1 | INTRODUCTION

Liver transplantation (LT) was established as an effective therapy for small, hepatocellular carcinoma (HCC) in a landmark study by Mazzaferro and colleagues in 1996. Candidates had a single lesion no larger than 5 cm, or up to three lesions, no larger than 3 cm, without macrovascular invasion or spreading outside of the liver and survival was similar to that in patients transplanted without HCC.[1] Although the "Milan criteria" are extensively applied in transplant centres, many have expanded the criteria to provide more patients with treatment options while maintaining acceptable recurrence-free transplant survival. HCC is the indication for approximately 30% of the LT performed in the US and Europe.

2 | SELECTION OF BEST CANDIDATES FOR LIVER TRANSPLANTATION

The goal of allocation strategies is to minimize wait-list deaths while achieving acceptable post-LT graft and patient survival. With the well-recognized shortage of donated livers worldwide, prioritizing patients with HCC, many with compensated cirrhosis, vs those with decompensated cirrhosis, without creating a disparity in access is challenging. Policies continue to evolve. The Model of End Stage Liver Disease (MELD) score prioritizes patients on the LT list. Patients requiring LT for HCC may be underserved on the transplant list by their natural MELD score, as they often do not have underlying decompensated liver disease. Patients with stage T2 lesions, defined as falling within the Milan criteria (Table 1) based upon lesion size and or growth characteristics (on cross-sectional, contrast imaging performed on equipment that meets minimal technical specifications), are eligible for MELD exception points in the US. The scans must be interpreted by a radiologist at a liver transplant centre. If the lesion is atypical, a biopsy must be positive for HCC. Once a patient is listed for LT with a MELD exception, there is a 6-month waiting period for implementation of the exception, partly to decrease the risk of being transplanted too early with an HCC that exhibits aggressive tumour biology resulting in a poorer post-transplant prognosis. The MELD exception points are capped at 34, thus improving access for patients with decompensated cirrhosis with a MELD >34. These changes have increased the waiting times for patients with HCC.

The maximum tumour size and number considered suitable for LT is now extended beyond the Milan criteria. The University of California at San Francisco (UCSF) criteria, published in 2006, (Table 1) have now been adopted across the US. The criteria state that an initial tumour burden of a single lesion ≤6.5 cm or up to 3 lesions, each ≤4.5 cm and with an aggregate diameter ≤8 cm are acceptable for LT. However, to be eligible for the MELD exception, the HCC must be downstaged

Abbreviations: AFP, alpha-foetoprotein; CNI, calcineurin inhibitor; DBD, donation after brain death; DCD, donation after cardiac death; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; LDLT, living donor liver transplantation; LRT, locoregional therapy; LT, liver transplantation; MELD, model of end-stage liver disease; mTOR, mammalian target of rapamycin; MVI, microvascular invasion.

using locoregional therapy (LRT) to within the Milan criteria. Patients transplanted meeting the UCSF criteria and downstaged to the Milan criteria have outcomes similar to those initially within the Milan criteria.[2] Another model for predicting the outcomes of LT is Metroticket 2.0, which predicts 5-year survival and the risk of HCC-related death after LT based upon the sum of viable tumour size in cm plus the number of lesions, and the latest AFP, as well as a model using explant pathology[3] (Table 1). Of note, the model predicts a worse outcome for patients with hepatitis C virus (HCV) infection, but the study was performed in patients transplanted before direct-acting antivirals (DAAs).

Alpha-foetoprotein (AFP) has repeatedly been shown to identify patients with HCC with a high risk of recurrence after LT.[4,5] Patients with HCC within the Milan Criteria but an AFP >1000 ng/mL have a 5-year recurrence-free survival of only 20%.[5] Thus, these patients must demonstrate an AFP response to LRT to be considered for LT. In a recent analysis of UNOS data evaluating patients undergoing downstaging of HCC, an AFP ≥100 ng/mL was an independent predictor of post-LT HCC recurrence.[4]

Since patients may receive multiple treatments for HCC over an extended period of time, dynamic models could improve prediction of post-LT HCC recurrence. The Hazard Associated with Liver Transplantation for Hepatocellular Carcinoma (HALTHCC) score was developed to predict overall post-LT survival[6] and was recently validated in an international cohort.[7] It uses pre-LT AFP, MELD-sodium, and tumour burden as a continuous risk metric. There was a strong correlation between pre-LT risk and explant pathology predicting recurrence, but further prospective studies are needed to test this score.

Key points

- Hepatocellular carcinoma (HCC) is the indication for ~30% of the liver transplants performed in the US and Europe
- Patients with T2 HCC (within Milan criteria) or those downstaged by locoregional therapy to within the Milan criteria are acceptable candidates for liver transplantation.
- Treatment of concurrent chronic viral hepatitis in patients with HCC improves waiting list survival. Direct-acting antivirals for HCV do not increase the risk of recurrent HCC after locoregional therapy.
- Models including the RETREAT and MORAL scores can be used to predict post-liver transplant recurrence of HCC to tailor surveillance and management.
- A multidisciplinary approach to treatment should be considered to optimize outcomes in patients with recurrent HCC after liver transplantation, including surgical resection, locoregional therapy, adjustment of immunosuppression and systemic chemotherapy.

3 | MANAGEMENT OF PATIENTS WITH HCC ON THE WAITING LIST

Most patients on the waiting list with HCC will undergo LRT as a bridge to LT. Modalities include trans-arterial chemoembolization, radio-embolization, radiofrequency ablation, microwave ablation, and stereotactic body radiation therapy. LRT prevents wait-list drop-off because of tumour progression. In a propensity matched analysis from the UK, one session of LRT was associated with a 49% lower risk of delisting for progression of HCC and/or post-LT recurrence.[8] This advantage was lost if more than 3 LRTs were needed, which might suggest that poorer tumour biology and/or longer waiting times are risks of a worse outcome. The goal of these bridging therapies is to achieve a complete response, defined by the absence of viable tumour on cross-sectional imaging and AFP <100 ng/mL. Patients with a complete response had the best outcomes in a study evaluating the Modified Response Evaluation Criteria in Solid Tumours (mRECIST) classification immediately following the first LRT.[9]

3.1 | Downstaging to within the Milan criteria

To be eligible for LT and the MELD exception, patients with a tumour burden outside of the Milan criteria must undergo LRT to downstage the tumour burden to within the Milan criteria. Moreover, downstaging may allow time to determine whether the biology of a patient's HCC is favourable for LT. A multicenter study evaluated 187 consecutive patients with HCC and initial tumours outside the Milan criteria (single lesion ≤8 cm, 2-3 lesions each ≤5 cm or 4-5 each ≤3 cm with an aggregate ≤8 cm), who were enrolled in a downstaging protocol. Successful downstaging to within the Milan criteria followed by LT was achieved in 58% of patients.[10] Tumour progression occurred in 32% of patients and 5% had liver-related deaths without LT. On multivariate analysis, a pretreatment AFP >1000 ng/mL (HR 3.3) and Childs Pugh Class B or C (HR 1.6) were associated with treatment failure, suggesting that these patients were unlikely to benefit from downstaging.[10] In a US analysis of 3819 patients with HCC from 2012-2015 in the UNOS database, the 3-year post-LT HCC recurrence was 6.9% in those within the Milan criteria, 12.8% in those downstaged through UNOS downstaging criteria and 16.7% in those downstaged from beyond the UNOS downstaging criteria.[4] Independent predictors of post-LT deaths in the downstaged groups were an AFP ≥100 at the time of LT (HR 2.4) and being in short (<3 months) or medium wait-time (3-9 months) vs long wait-time (>9 months) regions (HR 3.1),[4] suggesting that wait-time can help define "low-risk" tumour biology.

3.2 | HCC-treatment in patients with viral hepatitis

Concurrent treatment of the underlying liver disease is important, as this can reduce the risk of further liver decompensation and increase the patient's ability to receive and tolerate LRT. All patients

TABLE 1 Criteria for transplanting patients with hepatocellular carcinoma

Criteria	Tumour burden limits	Indicators of poor prognosis	Outcomes
Milan	1 lesion ≤5 cm or up to 3 lesions, each ≤3 cm (1)	AFP >1000 ng/mL predicts recurrence and poor survival[5]	4-y overall/recurrence-free survival 85%/92% Recurrence probability at 3 y 6.9% (3)
UCSF	1 lesion ≤6.5 cm Up to 3 lesions, each ≤4.5 cm, aggregate diameter ≤8 cm (2)	Survival of 90% and 75.2%, at 1 and 5 y, respectively, but 50% 1-year survival if these criteria were exceeded (2).	5-y survival 80% (5)
Metroticket 2.0	[a]	Based upon maximal viable tumour size, plus number of tumours and AFP (3)	
UNOS-Downstaging	1 lesion <8 cm 2-3 lesions <5 cm and the aggregate diameter <8 cm or 4-5 each ≤3 cm with an aggregate ≤8 cm (5)	Pre-treatment AFP >1000 ng/mL and/or Childs Pugh Class B or C predict downstaging failure (8) AFP ≥100 ng/mL at LT predicts recurrence; Increased risk of post-LT death in short and medium wait time regions (5)	3-y post-OLT survival 79.1% 3-y recurrence probability 12.8% (5)

[a]Provides a risk assessment post-LT based upon the sum of viable tumour size in cm plus the number of lesions and the AFP predicts outcomes. http://www.hcc-olt-metroticket.org/#calculator_pre

with chronic hepatitis B virus (HBV) infection should be on antiviral therapy, preferably entecavir or tenofovir. Treatment with DAAs can be considered in patients with HCV, although experts suggest waiting until a complete response to LRT has been achieved. Although the rates of a sustained virological response are modestly reduced in patients with HCC compared to those without (~80%), this should not prevent treatment.[11] HCV-infected patients with HCC on the waiting list have better waiting-list survival and lower risk of delisting if HCV treatment is given than if it is withheld.[12]

4 | TRANSPLANT-SPECIFIC CONSIDERATIONS

4.1 | Donor selection in patients with HCC

Timely access to LT can affect LT outcomes in patients with HCC. One study has suggested that to minimize the risk of post-LT recurrence in patients with HCC the optimal waiting time was at least 6 months and no more than 18 months.[13] The use of extended criteria donors is an attractive way to increase access to donors in areas with longer LT waiting-times. Because donor quality can influence graft outcomes, investigators have evaluated whether extended criteria donors and living donors have a different risk of recurrent post-LT HCC.

Donation after cardiac death (DCD) recipients have higher rates of biliary complications and poorer overall survival than donation after brain death (DBD) recipients. However, in the largest single-centre experience examining the impact on HCC recurrence (340 DBD and 57 DCD), there was no difference in recurrence-free survival or recurrence rate of HCC after LT.[14] Transplanting livers from donors who are positive for hepatitis B core antibody, indicating possible prior exposure to HBV, can be safely and successfully done with appropriate post-LT viral prophylaxis. With the introduction and widespread availability of DAA therapy for HCV, livers from HCV-viremic donors are now being used in HCV-uninfected recipients. However, the data are insufficient to determine if these livers will influence post-LT HCC recurrence.

Living donor liver transplantation (LDLT) provides another opportunity to expand donor options in patients with HCC and is particularly valuable in regions where waiting times are long and patients are at risk of delisting because of tumour progression. A multicenter study from France compared the outcomes of patients with cirrhosis and HCC who underwent LDLT (n = 79) and DBD (n = 782). There were no differences in the groups for pre-LT treatment of HCC or tumour burden in the explant exceeding the Milan criteria, but the AFP was higher in the LDLT group. There were no differences in overall intent-to-treat survival at 5 years (73.2% vs 66.7%, respectively) or post-LT HCC recurrence (10.9% vs 11.2% respectively). It is important to note that the delisting rate in the DBD group was 20.7%, mostly because of tumour progression compared to none in the LDLT group.[15] In considering the risk of HCC recurrence, the waiting-time before LDLT is relevant to the risk of HCC recurrence, because short waiting-times may not provide sufficient time for evaluation of tumour biology and response to LRT, which could improve stratification for the risk of post-LT recurrence.

4.2 | Immunosuppression and prophylactic systemic chemotherapy

Patients at high risk of recurrent HCC may benefit from adjustment of their post-LT immunosuppression. Immunosuppression has been shown to be involved in accelerated growth of recurrent post-LT HCC. The use of higher levels of calcineurin inhibitors (CNI) earlier in the post-LT phase has been associated with an increased risk of HCC recurrence.[16] The mammalian target of rapamycin (mTor) inhibitors, sirolimus and everolimus, has been associated with antineoplastic effects on HCC,[17] and their use has been associated with lower HCC recurrence rates compared to CNI.[18] In a prospective, randomized, open-label, international trial of 525 LT recipients with HCC, including sirolimus or not as part of the immunosuppression regimen, sirolumus was associated with statistically better recurrence-free survival at year 3 (HR 0.7; CI 95% 0.48-1.00) but not at year 5 (HR 0.84; 95% CI, 0.62-1.15).[19] Thus, early adjustment of immunosuppression limiting the use of CNI and using m-TOR inhibitors could help reduce HCC recurrence rates. The outcomes of preemptive sorafenib, a multitargeted, orally active tyrosine kinase inhibitor, in LT recipients at high risk of HCC recurrence have been mixed,[20,21] and systemic chemotherapy is not routinely recommended to prevent recurrent HCC, even in high-risk patients.

5 | HCC RECURRENCE AFTER LIVER TRANSPLANTATION

HCC recurrence occurs despite careful selection of LT recipients and is because of tumour cell dissemination from circulating cancer cells and micrometastases before or during total hepatectomy.[22] HCC recurrence usually occurs early, a median of 12.3 months after LT. Late recurrence more than 2 years after LT is rare.[23] Recurrent HCC developed in 29 of a series of 132 LT patients for HCC in Italy (15.9%). Fifteen (71%) of these occurred within the first 18 months, and only 2 (7%) more than 2 years later.[23] The most common sites of HCC recurrence in a study of 84 cases of recurrent HCC were the lung (44.0%), bone (29.8%), liver (26.2%) and peritoneum (26.2%).[24]

Overall, HCC recurs in about 10%-15% of LT recipients.[20,25] Several factors have been associated with post-LT recurrence. Microvascular invasion (MVI) on the explant has been associated with increased HCC recurrence and decreased survival.[21] There is an approximately 3.8- to 4.9-fold increase in HCC recurrence with MVI.[24,26] Pre-LT assessment of MVI and tumour grade has been limited by the poor accuracy of preoperative biopsy, which often does not correlate with the grade or presence of microvascular invasion on the final pathology report. Recent efforts have been made to non-invasively predict explant microvascular invasion through radiogenomics. Radiogenomics maps the imaging features of the tumour to corresponding gene expression profiles to determine the genetic characteristics of the tumour. Certain features of HCC on CT have been discovered through radiogenomics, including the presence of internal arteries in the tumour and hypodense halos around the tumour. Radiological features are promising for the prediction of microvascular invasion in the resected liver.[27] Further prospective studies are needed to determine the validity of this growing field to predict outcomes.

Elevated AFP levels are increasingly recognized as a sign of worse tumour biology and increased HCC recurrence after LT[5] The number of tumours, higher tumour grade, shorter waiting time before LT, and poor response to LRT have all been found to be predictive of higher HCC recurrence rates after LT.[28-31]

Several models have been developed to predict HCC recurrence after LT. The Risk Estimation of Tumour Recurrence After Transplant (RETREAT) score was developed through a multicenter study to risk-stratify HCC recurrence after LT. The study showed that in multivariate analysis, elevated AFP, the presence of MVI on the explant, and the largest viable tumour diameter plus the number of viable tumours on the explant was predictive of HCC recurrence. The RETREAT score was developed with these factors and assigning 0-3 points for increasing AFP levels, 2 points for the presence of MVI, and 0-3 points for the increasing size of the largest viable tumour diameter plus the number of viable tumours for a total score of 8 (Table 2). A RETREAT score of 0 is predictive of a 1- and 5-year recurrence risk of only 1.0% (95% CI, 0.0%-2.1%) while a score of 5 or higher predicted 1-year and 5-year recurrence rates of 39.3% (95% CI, 25.5%-50.5%) and 75.2% (95% CI, 56.7%-85.8%) respectively. The score performed well in the validation cohort of the study with a c-statistic of 0.82 (95% CI, 0.77-0.86), and using the net reclassification index, the score outperformed the Milan criteria at predicting HCC recurrence 1 year (0.40, $P = .01$) and 5 years after LT (0.31, $P < .01$).[24]

The Model of Recurrence After Liver Transplantation (MORAL) developed by investigators at Columbia University incorporates the pre-LT neutrophil-lymphocyte ratio (NLR) to its prediction model. A pre-LT NLR ≥5 had been shown to predict poor outcomes in HCC.[31] The pre-MORAL score includes a combination of pre-LT NLR >5, AFP >200 ng/mL, and largest tumour size >3 cm (pre-LT values). The post-MORAL score (post-LT findings) includes the presence of grade 4 tumour, vascular invasion, largest tumour size >3 cm and >3 tumours on the explant. The resulting combined, combo-MORAL score was found to have a c-statistic of 0.91 (95% CI, 0.87-0.95) with a better performance than the Milan criteria (0.63; 95%, 0.54-0.71) and UCSF criteria (0.57; 95% 0.47-0.66).[26] Further multicenter studies are needed to confirm the validity of these findings.

Accurate models to predict HCC recurrence can help tailor surveillance after LT. High risk patients can undergo more frequent surveillance imaging while low risk patients may be able to reduce or even avoid unnecessary surveillance imaging after LT. While there are no standard guidelines for post-LT HCC surveillance, the authors of the RETREAT study propose more frequent HCC surveillance for RETREAT scores of ≥5, consisting of multiphasic, cross-sectional imaging of the abdomen as well as CT of the chest and AFP every 3-4 months for 2 years, then every 6 months from years 2-5. The authors recommend surveillance every 6 months for 2 years for a score of 1-3 and every 6 months for 5 years for a score of 4. They do not recommend surveillance in patients with a RETREAT score of 0 because of the low risk of HCC recurrence in this group (1.0%; CI 95%, 0.0%-2.1%) at 5 years (Table 3).[24]

TABLE 2 RETREAT Score[a]

Risk factor	RETREAT points
1. AFP at LT, ng/mL	
0-20	0
21-99	1
100-999	2
≥1000	3
2. Microvascular invasion	2
3. Largest viable tumour diameter (cm) plus number of viable tumours	
0	0
1.1-4.9	1
5.0-9.9	2
≥10	3

[a]The RETREAT score ranges from 0-8 and is calculated from the sum of the 3 risk factor points.

Adapted from Mehta N, et al Validation of a risk estimation of tumour recurrence after transplant (RETREAT) score for hepatocellular carcinoma recurrence after liver transplant. JAMA Oncol. 2017;3:493-500.

TABLE 3 Proposed HCC surveillance schedule after LT based on RETREAT score

RETREAT score	Surveillance schedule[a]
0	No surveillance recommended
1-3	Every 6 mo for 2 y
4	Every 6 mo for 5 y
≥5	Every 3-4 mo for 2 y, followed by every 6 mo from years 2 through 5

[a]Recommended surveillance consists of cross-sectional, multiphase imaging (CT or MRI) of abdomen along with chest CT and AFP.

Adapted from Mehta N, et al Validation of a risk estimation of tumour recurrence after transplant (RETREAT) score for hepatocellular carcinoma recurrence after liver transplant. JAMA Oncol. 2017;3:493-500.

5.1 | Treatment of recurrent HCC after liver transplantation

Overall survival if recurrent HCC is detected after LT is poor. Multifocal recurrence and early recurrence after LT (≤6 months) are associated with worsened survival.[32,33] At present there is no consensus algorithm for the treatment of recurrent HCC. An earlier diagnosis of recurrence could help improve outcomes as several case series have shown improved survival in patients who underwent resection for localized recurrence compared to patients with multifocal recurrence.[23,32,33] Early use of sorafenib and other mTOR inhibitors may also be options as well as resection or LRT, though more data are needed.[34] In a metaanalysis of 1021 LT recipients with HCC recurrence, the median survival after diagnosis of recurrence was 13 months. A variety of treatments were used including surgical resection, systemic therapy with sorafenib, LRT, and the best supportive care. Surgical resection of localized recurrent HCC (42 months) and sorafenib use (18 months) were associated with higher survival rates, although, sorafenib use was associated with increased adverse effects.[35] The best supportive care was associated with the lowest survival rate (3.3 months).[35] If feasible, resection or LRT should be attempted in combination with the use of sorafenib and mTOR inhibitors as any form of therapy appears to have better overall outcomes than supportive care.

The use of checkpoint inhibitor immunotherapy has increased for the treatment of HCC with promising results as second-line therapy for HCC if sorafenib fails.[36] At the time of this writing, 2 checkpoint inhibitors, nivolumab and pembrolizumab, have been approved for the treatment of HCC by the US Food and Drug Administration. Both are anti-PD1 monoclonal antibodies that release the "brakes" of the immune system to control HCC. Although pre-LT treatment data have been promising for HCC, no current data exists to show a benefit for post-LT HCC recurrence. In fact, the use of checkpoint immunotherapy must be carefully considered because of potential uncontrolled immune reactivation. Several case reports of severe acute T-cell-mediated rejection and antibody-mediated rejection have been described in the post-LT setting with graft loss and death.[37] Further efficacy and safety studies are needed before routine use of these medications.

6 | CONCLUSION

Liver transplantation is effective therapy in patients with HCC within the Milan criteria or in those who can be downstaged to within the Milan criteria. Expansion of eligibility criteria is being explored to identify the upper limits of tumour burden that can be effectively managed with LT. However, the limitations of access to donor organs prevent large numbers of patients from having LT as definitive treatment. Thus, expanding donor options, including living donor LT, is important. Outcomes for LT with recurrent HCC are poor, but are improved by early detection, highlighting the importance of surveillance in "at-risk" recipients. To improve the outcomes of patients with post LT HCC recurrence, a multidisciplinary approach to treatment should be considered including surgical resection, locoregional therapy, adjustment of immunosuppression and systemic chemotherapy.

CONFLICT OF INTEREST

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REVIEW ARTICLE

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What's new in portal hypertension?

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Abstract

Portal hypertension is defined as increased pressure in the portal venous system. The most common cause of portal hypertension is cirrhosis. In this setting, there is an increase in intrahepatic resistance leading to an increase in portal pressure. By increasing portal blood flow, splanchnic vasodilation further aggravates portal hypertension. New pathogenic pathways are being established which might result in new therapeutic strategies. The presence of varices at endoscopy and/or other abdominal portosystemic collaterals confirms the diagnosis of portal hypertension. The role of non-invasive and imaging tests in the diagnosis and prognosis of portal hypertension has been clarified. Non-selective beta-blockers decrease both the risk of variceal haemorrhage and hepatic decompensation. Terlipressin, somatostatin or octreotide, in combination with early endoscopic therapy, are recommended for the treatment of acute variceal haemorrhage. Early Transjugular intrahepatic portosystemic shunt (TIPS) is effective as salvage therapy in acute variceal bleeding in selected patients and prevents rebleeding more effectively than endoscopic and medical therapy resulting in an increased survival.

KEYWORDS

carvedilol, cirrhosis, gastro-esophageal varices, HVPG, liver stiffness, portal hypertension, propranolol, TIPS, variceal haemorrhage

1 | INTRODUCTION

Portal hypertension (PH) is defined as increased blood pressure in the portal venous system. It is most often observed as a complication of chronic liver disease (CLD). Cirrhosis is the most frequent cause of PH and its complications, in particular gastro-oesophageal varices (GEVs), variceal haemorrhage (VH), ascites, spontaneous bacterial peritonitis and other infections, as well as hepatorenal syndrome and portosystemic encephalopathy.[1] These complications represent the first cause of death and the main indication for liver transplantation in these patients.

Since the main cause of PH is cirrhosis, the present chapter will focus on new aspects of cirrhosis-related PH.

Cirrhosis is a heterogeneous disease currently classified into two main stages: compensated and decompensated cirrhosis.[2]

A new entity called compensated advanced chronic liver disease (cACLD) has been proposed to show that severe fibrosis and cirrhosis are a continuum in asymptomatic patients, and that determining the difference between the two stages is often clinically impossible.[3] Portal hypertension may occur before an established histological diagnosis of cirrhosis.

Hepatic venous pressure gradient (HVPG) measurement is the gold-standard method to assess the presence of PH.

Invasive methods to confirm cACLD include liver biopsy to identify severe fibrosis or established cirrhosis, upper GI endoscopy showing gastro-oesophageal varices and/or HVPG measurement

Abbreviations: cACLD, compensated advanced chronic liver disease; CLD, chronic liver disease; CSPH, clinically significant portal hypertension; EVL, endoscopic variceal ligation; GEVs, gastro-oesophageal varices; GI, gastrointestinal; GOV1, gastrooesophageal varices type 1; GOV2, gastro-oesophageal varices type 2; HE, hepatic encephalopathy; HRVs, high-risk varices; HSCs, hepatic stellate cells; HVPG, hepatic venous pressure gradient; IGV, isolated gastric varices; LSM, liver stiffness measurement; NSBB, non-selective beta-blockers; PH, portal hypertension; PVT, portal vein thrombosis; RCT, randomized controlled trial; TE, transient elastography; TIPS, Transjugular Intrahepatic Portosystemic Shunt; VH, variceal haemorrhage.

with values >5 mm Hg indicating sinusoidal PH or >10 mm Hg indicating clinically significant PH (CSPH) Figure 1.

1.1 | What's new in the pathogenesis of portal hypertension?

The increased intrahepatic resistance in cirrhosis is a result of a combination of structural changes in the hepatic sinusoids (fibrosis, regenerative nodules) and vasoconstriction in the intrahepatic circulation because of decreased production of vasodilators from sinusoidal cells.

A new approach to the pathogenesis of PH which may affect the therapeutic management of this complication, is that the traditional concept of cirrhosis as a pro-haemorrhagic condition has changed and cirrhosis is now considered to be both a pro-haemorrhagic and prothrombotic disease.[4] The prothrombotic state may result in the worsening of hepatic fibrosis and PH.[5] This hypothesis is supported by the fact that anticoagulation leads to a reduction in hepatic fibrosis and PH.

Another factor that has been shown to contribute to the worsening of PH is the translocation of bacterial or bacterial products from the intestinal lumen into the system circulation.[6]

Gut-derived bacterial products stimulate hepatic stellate cells (HSCs) and Kupffer cells, leading to activation of HSCs, as well as fibrogenesis secondary to the activation of Kupffer cells.

Bacterial-induced inflammation also contributes to a deterioration of the systemic hyperdynamic circulation.[7]

1.2 | What's new in the diagnosis of portal hypertension?

Non-invasive tests are useful for staging liver fibrosis in patients with chronic liver disease.[8] The introduction of transient elastography (TE) in clinical practice allows the early identification of patients with CLD at risk of developing CSPH. Liver stiffness by TE can suggest cACLD in asymptomatic subjects with known causes of CLD. A good correlation has been reported between HVPG and liver stiffness measurements (LSM) in patients with advanced liver fibrosis or cirrhosis.[8] The best correlation between HVPG and LSM occurs with HVPG values between 5 and 10 to 12 mm Hg. This is the range for mild PH, which is driven by increased intrahepatic resistance. The correlation persists but decreases markedly with levels >12 mm Hg when increased hepatic blood flow is an additional factor contributing to PH (CSPH).

LSM can discriminate between patients with and without CSPH. LSM values >13.6 kPa or LSM >21 kPa have been found to have a

Key points

1. Transient elastography may be useful for the early identification of patients at risk of developing clinically significant portal hypertension. When uncertainty remains, measurement of sinusoidal pressure by determination of hepatic venous pressure gradient is essential.
2. Etiological treatment of the underlying liver disease can reduce portal hypertension in part.
3. Dose reduction or discontinuation of non-selective beta-blockers can be considered in patients with refractory ascites who develop low blood pressure and impaired renal function.
4. Carvedilol, statins and anticoagulants are promising for the management of portal hypertension.
5. Early Transjugular Intrahepatic Portosystemic Shunt placement after variceal haemorrhage in high-risk patients with cirrhosis leads to improved survival in selected patients.

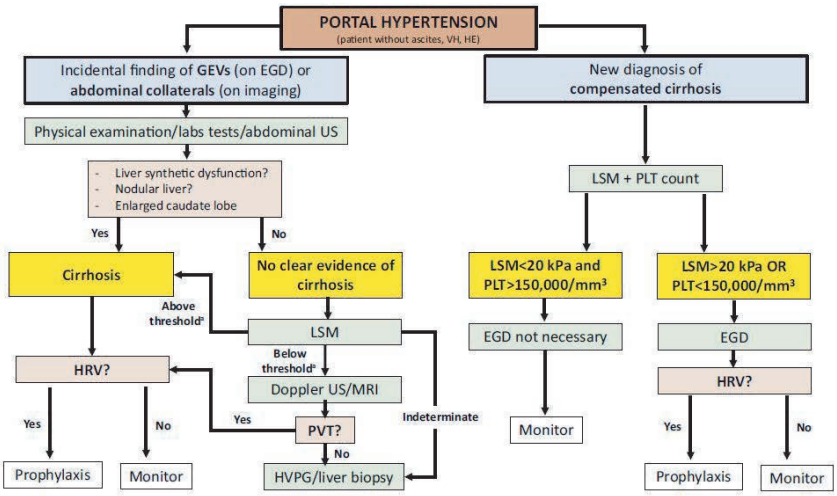

FIGURE 1 Diagnostic algorithm in portal hypertension (with permission of L Turco and G Garcia-Tsao6). Liver stiffness measurement thresholds vary depending on cause of chronic liver disease. HE, hepatic encephalopathy; PLT, platelets (count); PVT, portal vein thrombosis

90% sensitivity and a 90% specificity for the diagnosis of CSPH.[9] The Baveno VI Consensus Conference recommended that an LSM of 21 kPa or more be used to indicate CSPH in these subjects based on the results of these studies.[3]

Determining the presence of oesophageal varices and the severity of PH is essential for the management and prognosis of patients with cirrhosis. However, endoscopy is not risk-free and the HVPG is usually only performed in specialized centres. Therefore, numerous studies have evaluated non-invasive tests to determine the presence of varices or PH.

Patients with a liver stiffness <20 kPa and with a platelet count >150.000 have a very low risk of having oesophageal varices that require therapy. These patients do not need endoscopic screening.[3]

Twenty-one percent of endoscopies could be avoided with these criteria and fewer than 5% of patients with varices would be missed. A recent study in patients with HCV-related cirrhosis who achieved an SVR and in treated patients with hepatitis B-virus-related cirrhosis showed that Baveno VI criteria can also be applied to these subjects. Results showed that 25% of gastrointestinal endoscopies could have been avoided and only 1% of subjects with oesophageal varices would have been missed.[10]

When uncertainty remains, measurement of sinusoidal pressure by hepatic vein catheterization and determination of the HVPG is essential.

1.3 | What's new in the treatment of portal hypertension?

Treatment of PH differs depending on the stages of cirrhosis, because the prognosis, mechanisms of the disease and the therapeutic targets are different.

Treatment of PH includes the prevention of first bleeding, the treatment of acute variceal bleeding and the prevention of recurrent bleeding.

Etiological treatment of the underlying liver disease can reduce PH and prevent complications in patients with confirmed cirrhosis.

Viral elimination in chronic hepatitis C has been shown to prevent progression to more advanced stages of fibrosis/cirrhosis. Furthermore, in patients with compensated cirrhosis, viral elimination has been shown to prevent or delay the development of varices, decompensation and hepatocellular carcinoma.

Disease regression is also an important outcome that depends on the stage of liver disease when antiviral therapy is started.

In patients with decompensated cirrhosis, regression to a compensated stage may result in delisting of certain patients from the transplant list.[11] However, the structural changes in the liver architecture have reached a point of no return in these patients, and it is probably impossible for the liver to regress to a non-cirrhotic stage.[6] Regression to a non-cirrhotic stage is only possible in patients with compensated cirrhosis.

The regression of fibrosis has been reported in a group of patients with post-transplant recurrent HCV with at least stage F1 fibrosis (METAVIR) before therapy.[12] The regression of fibrosis occurred in patients with lower pretreatment HVPG than in those without regression. Patients with cirrhosis (in particular those with CSPH) are less likely to regress.

Forty years ago, Lebrec et al showed that propranolol significantly reduces the risk of rebleeding from EV.[13] Since then, non-selective beta-blockers (NSBB) have become one of the most effective preventive therapies against VH in patients with cirrhosis, both for primary and secondary prophylaxis (combined with EVL).

There is no indication for beta-blockers to prevent the formation of varices[3] in patients without or with small varices.

Patients with small varices and red wale marks or Child-Pugh C have an increased risk of bleeding and should be treated with NSBB. Patients with small varices without signs of increased risk may be treated with NSBB to prevent bleeding.[10]

Traditional NSBB (propranolol, nadolol) and carvedilol are valid first line treatments.

Carvedilol may be an alternative to NSBB for the prevention of VH (especially for primary prophylaxis) and is the first choice in patients who do not respond to propranolol.[14]

Measurement of the HVPG for a response to therapy provides additional relevant information since a decrease in the HVPG of at least 10% from baseline or to <12 mm Hg after chronic treatment with NSBB is associated with a significant reduction in the risk of variceal bleeding.[15] However, beta-blockers should be prescribed when indicated, independent of the possibility of measuring HVPG.

Patients with medium-large varices should be treated either with NSBB or endoscopic band ligation for the prevention of the first episode of variceal bleeding.[3] The choice of treatment should be based on local resources and expertise, patient characteristics, contraindications and adverse events. However, NSBBs are preferable because they also have other potential beneficial effects, in particular decreasing the incidence of hepatic decompensation and death. This last effect of beta- blockers deserves additional information.[16]

A recently published RCT (PREDESCI study) performed in 201 patients with compensated cirrhosis and CSPH treated with either NSBB (propranolol or carvedilol depending on the HVPG response) or placebo, has shown that HVPG responders had a significant decrease in the incidence of a first clinical decompensation compared to the placebo group.[17] The decrease in portal pressure was less marked in patients with subclinical PH than in those with CSPH after acute administration of beta-blockers, suggesting that NSBB are more suitable for the prevention of decompensation in patients with CSPH.

This new information has important clinical implications, and suggests that patients with compensated cirrhosis should be screened for CSPH, and that therapy with NSBB should be started at detection of CSPH, which can be easily diagnosed using non-invasive

tools. This new indication for NSBB could strongly influence patient outcomes.

The safety of NSBB in subgroups with end-stage disease (refractory ascites and/or spontaneous bacterial peritonitis) has been questioned.[18] In a meta-analysis of 3 RCTs and 8 observational studies, NSBB use was not associated with increased all-cause mortality in patients with ascites or in those with refractory ascites.[19] Close monitoring is necessary in patients with refractory ascites, and dose reductions or discontinuation can be considered in those who develop low blood pressure and impaired renal function. If NSBB are stopped, endoscopic band ligation should be performed.[3]

Cyanoacrylate injections appear to be more effective than beta-blockers in preventing a first episode of bleeding in patients with large type 2 gastro-oesophageal varices or isolated type 1 gastric varices. Further studies are needed to evaluate the risk/benefit of using cyanoacrylate in this setting.[3]

The use of statins to reduce portal pressure is a promising new strategy. Statins have antioxidative, antiproliferative and anti-inflammatory effects, and can improve endothelial dysfunction.[20] These agents may also decrease the structural component of increased intrahepatic resistance by their antifibrotic properties. In a large RCT in patients with cirrhosis and PH, the addition of simvastatin to standard therapy (EVL + NSBB) alone did not decrease rebleeding at 2 years but was associated with a decrease in mortality (9% vs 22%).[21] The benefit to survival was only observed in patients with Child-Pugh A or B cirrhosis and not in Child-Pugh C patients. Statins are generally well-tolerated and significant side effects are not frequent, although the risk increases with higher doses. Further studies are needed since strong clinical evidence of a beneficial effect of statins on the complications of PH or survival is still lacking.

Patients with cirrhosis have an increased risk of venous thrombotic events, mainly portal vein thrombosis,[22] which occurs in approximately 20% of patients and is associated with poor outcomes. In a meta-analysis of 8 studies, patients with cirrhosis and portal vein thrombosis who receive anticoagulant therapy have increased recanalization and decreased progression of thrombosis, compared to those who do not receive anticoagulants, with no increase in bleeding and a lower incidence of VH.[23]

Therefore, in the absence of contraindications anticoagulants are often the first-line therapy for patients with cirrhosis and portal vein thrombosis.[3]

Studies evaluating anticoagulants in the prevention of portal vein thrombosis in cirrhosis are lacking.

In one RCT evaluating a 12-month course of enoxaparin (4000 IU/d, subcutaneously) vs no treatment in preventing portal vein thrombosis in patients with cirrhosis, enoxaparin significantly delayed the occurrence of hepatic decompensation and improved survival.[24]

There are ongoing studies evaluating the effects of anticoagulation in patients with cirrhosis and without portal vein thrombosis. The primary outcomes of these studies are survival and as well as the occurrence of liver-related complications.

When an acute bleeding episode occurs in patients with cirrhosis and PH, Child-Pugh class C, the updated MELD score and failure to achieve primary haemostasis are the variables most consistently found to predict six-week mortality.[3] Patients with acute variceal bleeding should be considered for ICU. The goal of resuscitation is to preserve tissue perfusion. Volume restitution should be initiated to restore haemodynamic stability. Packed red blood cell transfusion should be performed with a target haemoglobin level of between 7 and 8 g/dL. Antibiotic prophylaxis is mandatory and should be started at admission. Intravenous ceftriaxone (1 g/24 h) should be considered in patients with advanced cirrhosis in settings with a high prevalence of quinolone-resistant bacterial infections and in patients on quinolone prophylaxis. Either lactulose or rifaximin can prevent hepatic encephalopathy. However, further studies are needed to evaluate the risk/benefit and to identify high-risk patients before a formal recommendation can be made. Vasoactive drugs should be started as soon as variceal bleeding is suspected, ideally before endoscopy. Vasoactive drugs (terlipressin, somatostatin, octreotide) should be used in combination with endoscopic therapy and continued for up to five days.

To date, terlipressin is the only drug that has been shown to decrease mortality in acute VH.[25]

Endoscopy should be performed within 12 hour of presentation in patients with cirrhosis presenting with GI bleeding. The airway should be protected if endoscopy is performed in patients with altered consciousness. Ligation is the recommended form of endoscopic therapy for acute oesophageal variceal bleeding. Endoscopic therapy with a tissue adhesive (eg N-butylcyanoacrylate) is recommended for acute bleeding from isolated gastric varices (IGV) and type 2 gastro-oesophageal varices (GOV2) that extend beyond the cardia. EVL or tissue adhesive can be used in bleeding from type 1 gastro-oesophageal varices (GOV1).[3]

Early TIPS (Transjugular Intrahepatic Portosystemic Shunts) placement with PTFE-covered stents within 72 hour (ideally 24 hour) improves survival in high-risk patients with acute variceal bleeding.[26,27] It should be considered in patients bleeding from EV, GOV1 and GOV2 at high risk of treatment failure (eg Child-Pugh class C < 14 points or Child-Pugh class B with active bleeding) after initial pharmacological and endoscopic therapy. The criteria for high-risk patients are being redefined.

Results from a prospective study of early TIPS in a large cohort of high-risk patients were recently reported by Hernandez-Gea et al.[28] Early TIPS reduced the 1-year probability of treatment failure and rebleeding compared to drug and endoscopic therapy (92% vs 74%; $P < .01$) with no increase in hepatic encephalopathy.

One-year mortality was also lower in Child-Pugh C patients who received early TIPS. Mortality rates did not differ in Child-Pugh B patients with active bleeding at endoscopy.

Despite these promising findings, adherence to early TIPS is low. In a recent study by Thabut and colleagues,[29] only 6.8% of patients with variceal bleeding eligible for early TIPS actually underwent the procedure because of lack of availability of TIPS or because the physician did not believe in the beneficial effects of early TIPS.

Given the high incidence of severe adverse events, balloon tamponade should only be used in refractory variceal bleeding as a temporary 'bridge' (for a maximum of 24 hour), until definitive treatment can be instituted.[3]

Persistent bleeding despite combined pharmacological and endoscopic therapy is best managed by PTFE-covered TIPS. Rebleeding during the first five days may be managed by a second attempt at endoscopic therapy. If rebleeding is severe, PTFE-covered TIPS is probably the best option.

Recurrent VH can be prevented with a combination of NSBB (propranolol or nadolol) + endoscopic band ligation.[3] Ligation should not be used as monotherapy unless there are intolerance/contraindications to NSBB. NSBB should be used as monotherapy in patients with cirrhosis who cannot be treated with band ligation. Covered TIPS placement is the treatment of choice in patients that fail first line therapy (NSBB + band ligation).

In patients with portal hypertensive gastropathy, NSBB are the first line therapy to prevent recurrent bleeding.[3] TIPS may be considered in patients in whom NSBB and/or endoscopic therapies fail.

Frailty and sarcopenia have recently been described as independent predictors of mortality in patients with cirrhosis and PH. Sarcopenia is defined as the total or functional loss of muscle mass and is usually associated with high rates of complications, such as susceptibility to infections, hepatic encephalopathy, ascites and even increased mortality in waiting list patients.[30] The gold standard for the diagnosis of sarcopenia is the direct quantification of skeletal muscle mass through cross-sectional imaging in L3 by CT scan. Since the routine and repeated use of CT imaging is limited in clinical practice because of cost and exposure to radiation, new direct tools (handgrip strength or muscle thickness by ultrasound analysis) and indirect scores[31] have been proposed to establish the diagnosis and follow-up. Portal hypertension helps perpetuate sarcopenia because of impaired gut motility, decreased nutrient absorption and the development of protein losing enteropathy. Nutritional interventions have been shown to improve survival and quality of life in patients, especially when they occur early on. Avoidance of fasting is mandatory to prevent accelerated starvation. Similarly, physical activity and regulated exercise is an anabolic stimulus that helps improve muscle mass and functionality, aerobic capacity and even decrease HVPG.

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REVIEW ARTICLE

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Optimal management of ascites

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Abstract

Ascites is the most common complication of cirrhosis, which develops in 5%-10% of patients per year. Its management is based on symptomatic measures including restriction of sodium intake, diuretics and paracentesis. Underlying liver disease must always be treated and may improve ascites. In some patients, ascites is not controlled by medical therapies and has a major impact on quality of life and survival. TIPS placement and liver transplantation must therefore be discussed. More recently, repeated albumin infusions and Alfapump® have emerged as new therapies in ascites. In this review, the current data on these different options are analysed and an algorithm to help the physician make clinical decisions is suggested.

KEYWORDS

ascites, cirrhosis, portal hypertension

1 | INTRODUCTION

Ascites is the most common complication of cirrhosis, with 5%-10% of patients with cirrhosis developing this complication per year. Ascites has a major impact on quality of life and is associated with a poor outcome. Management involves two different approaches. The first approach is symptomatic, based on restriction of sodium intake, diuretics, albumin infusion and paracentesis. These symptomatic options should always be associated with treatment of the underlying cause of liver disease to improve liver function. Most patients recover with medical therapy.

When medical therapy fails, transjugular intrahepatic portosystemic shunts (TIPS) are the first-line treatment to be discussed in these patients as TIPS have been shown to improve ascites as well as survival compared to repeated paracentesis. TIPS are contraindicated in patients with the most severe presentation, with a high MELD or a high Child-Pugh score, or with hepatic encephalopathy (HE), and liver transplantation is the only curative option. An age of more than 65 or 70 years old is another important issue, as it may be a contra-indication for both TIPS placement and liver transplantation.

In this review, we will first focus on the pathophysiology of ascites in cirrhosis, and then discuss the different therapeutic options. Finally, we will suggest an algorithm to help the physician in different clinical situations. The management of hepatorenal syndrome, a severe complication that has the same pathophysiology than ascites, will not be discussed in this review.

2 | PATHOPHYSIOLOGY OF ASCITES IN CIRRHOSIS

Ascites is defined as an accumulation of fluid in the peritoneal cavity and is because of cirrhosis in about 80% of cases. It can be graded according to its severity: grade 1 (mild ascites) if only detectable by ultrasound, grade 2 (moderate ascites) with moderate symmetrical distension of abdomen and grade 3 (large ascites) with marked abdominal distension.[1] Ascites affects 5%-10% of patients with compensated cirrhosis per year and is considered to be the most common complication of cirrhosis. Moreover, its prognosis is poor (2-year mortality of 40%). It appears later than

Abbreviations: ACLF, acute-on-chronic liver failure; AP, Alphapump®; HE, hepatic encephalopathy; LT, liver transplantation; LVP, large volume paracentesis; MDRO, multidrug-resistant organisms; MELD, model for end-stage liver disease; PTFE-covered, polytetrafluoroethylene-covered; RA, recurrent ascites; RCT, randomized controlled trial; SBP, spontaneous bacterial peritonitis; SMT, standard medical treatment; TIPS, transjugular intrahepatic portosystemic shunt; XDRO, extended drug-resistant organisms.

variceal bleeding in the natural history of cirrhosis, with a more severe outcome.[2]

Ascites is known to be multifactorial and seems to result from the combination of portal hypertension and liver insufficiency. Several hypotheses have been suggested to explain its pathophysiology, in particular that ascites reflects a reorganization of haemodynamics in cirrhosis. Indeed, the reorganization of the hepatic structure in cirrhosis is responsible for an increase in hydrostatic pressure in the sinusoid capillaries, which leads to an increase in local synthesis of vasodilator substances, such as nitric oxide. Thus, there is a decrease in splanchnic arterial resistance.[3] Compensatory mechanisms then occur, especially an increase in cardiac output and activation of metabolic pathways to increase effective volaemia (sympathetic nervous system and renin-angiotensin-aldosterone pathway). Synthesis of anti-natriuretic substances is then increased and results in sodium and water retention in the proximal tubule, loop of Henle and distal tubule.[3] This can result in dilutional hyponatraemia, which may worsen the prognosis and makes treatment of ascites more difficult. In a final stage, the severe systemic vasodilation and subsequent renal vasoconstriction are responsible for acute kidney injury by decreasing renal blood flow, defining the hepatorenal syndrome. Moreover, hypoalbuminaemia owing to hepatic insufficiency is responsible for a decrease in oncotic pressure, which facilitates the fluid leakage from the intravascular sector to interstitial space.[3] Because of the reorganization of the hepatic structure in cirrhosis, the capillaries are no longer fenestrated and protein concentration is then poor in this fluid.

Finally, some studies suggest that bacterial translocation, which is frequent in cirrhosis and responsible for local and systemic inflammation, plays a role. This mechanism may increase permeability of capillaries and facilitate fluid leakage to the peritoneal cavity.[3]

3 | OPTIMAL MANAGEMENT OF ASCITES

We will focus on the treatment of ascites in patients: (a) without refractory ascites, (b) with refractory ascites and (c) with spontaneous bacterial peritonitis (SBP). In patients with complicated ascites, that is with either refractory ascites or SBP, liver transplantation (LT) must be discussed.

3.1 | Patients without refractory ascites

3.1.1 | Classical treatments

The treatment of ascites is based on symptomatic therapies, including sodium restriction and diuretics, as patients with ascites have a positive sodium balance. Dietary sodium should be moderately restricted (80-120 mmol/day) to prevent a reduced calorie intake, which could impair nutritional status. The aim of diuretic therapy is weight loss of <0.5 kg/day (or 1 kg/day in the presence of peripheral oedema). Patients should receive an anti-mineralocorticoid drug alone, starting at 100 mg/day, with a stepwise increase to a maximum of 400 mg/day. Furosemide should be added in non-responders or in patients who develop hyperkaliaemia, at a dose of 40 mg/day to a maximum dose of 160 mg/day. Other general measures and treatments have also been evaluated: (a) it has not been shown that prolonged maintenance of the supine position improves the resolution of ascites; (b) there is evidence that the treatment of underlying liver disease can improve ascites, such as abstinence from alcohol or viral suppression; (c) the use of several drugs is contraindicated to avoid renal impairment, such as non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors or aminoglycosides (except in patients with severe bacterial infections); (d) other treatments such as midodrine, terlipressine or clonidine are not recommended.

3.1.2 | New therapeutics in patients without refractory ascites: albumin and TIPS

Hypoalbuminaemia and the synthesis of dysfunctional albumin are increasingly recognized as key factors in the pathophysiology of the complications of cirrhosis including ascites. Patients with moderate ascites were considered to be the most appropriate candidates to evaluate the efficacy of repeated albumin infusions to improve survival, prevent further complications of cirrhosis including encephalopathy, sepsis, as well as to reduce ascites. In the ANSWER study,[4] patient with ascites who were receiving diuretics and were not considered refractory received either albumin (40 g twice a week for 2 weeks and then 40 g weekly) or standard medical treatment (SMT). Patients in the albumin group showed a 38% decrease in the mortality hazard ratio, fewer episodes of HE and sepsis, and a later need for paracentesis. Finally, during the 18 months of follow-up, fewer patients developed refractory ascites. Interestingly, a post hoc analysis (ILC 2019 presented data) of the ANSWER study showed that the albumin level after 1 month of treatment was strongly predictive of survival. In particular, 18-month survival reached 90% when the albumin level was >40 g/L. This suggests that the amount of albumin infused is highly important and may need to be adapted on a case by case basis. In another RCT, patients awaiting liver transplantation received either midodrine 15-30 mg/day and albumin 40 g/day or placebo. There was no difference between the groups, for survival on the waiting list for the complications of cirrhosis or control of ascites.[5] However, rapid access to LT (median treatment 80 days in both groups) may have prevented this trial from more significant results.

TIPS placement induces decompression of the portal circulation by shunting an intrahepatic portal branch into a hepatic vein. Its indications in the treatment of refractory ascites are better defined and will be discussed later in this manuscript. However, the benefit of TIPS insertion in less severe patients, such as in those with recurrent ascites (RA) remains uncertain. RA was first

defined in a 1996 consensus as ascites that recurs at least three times within 12 months despite sodium restriction and diuretic treatment.[6] Recently, EASL guidelines defined early RA as ascites that recurs earlier than 1 month after initial control.[1] None or very few of these patients were included in initial RCTs comparing TIPS with bare metal stents to standard medical treatment (SMT). Recently, a study by Bureau et al compared the prognosis of patients with RA receiving either TIPS with PTFE-covered stents or SMT.[7] However, these patients were more severe than the previous definition of RA. To be included, they had to have required at least two LVPs at least 3 weeks apart. It is important to note that 30% of patients had a history of variceal bleeding, and 20% had a history of renal failure, showing the severity of their circulatory dysfunction. There was a significant increase in 1-year survival without transplantation (93% vs 52% $P = .003$) in the TIPS group, which was the primary endpoint of the study. It is interesting to note that hepatic encephalopathy (HE) did not occur more frequently in the TIPS group. These results, obtained in patients with RA, moderate hepatic insufficiency and an absence of previous overt HE, illustrate the importance of defining which patients are the best candidates for TIPS and the more severely ill patients, who should be listed for transplantation.

3.2 | Patients with refractory ascites

3.2.1 | Definition of refractory ascites

According to the International Ascites Club, refractory ascites is defined as "ascites that cannot be mobilized or the early recurrence of which cannot be satisfactorily prevented by medical therapy."[6] This definition includes diuretic-resistant ascites, (ascites that cannot be mobilized or the early recurrence of which cannot be prevented because of a lack of response to sodium restriction and diuretic treatment), and diuretic-intractable ascites, (ascites that cannot be mobilized or the early recurrence of which cannot be prevented because of the development of diuretic-induced complications that preclude the use of an effective diuretic dosage). From a practical point of view, it is very difficult to reach the maximal doses of diuretics and 90% of patients have intractable ascites. HE, renal failure, hyponatraemia, hypo- or hyper-kaliaemia and muscle cramps are the main reasons for the withdrawal of diuretics.[1]

3.2.2 | Large volume paracentesis

Large volume paracentesis (LVP) is the first-line treatment of refractory ascites.[6] Plasma volume expansion is needed to prevent post-paracentesis dysfunction. In a meta-analysis of randomized controlled trials, albumin infusion has been shown to be more effective than other plasma expanders in the prevention of post-paracentesis dysfunction.[8] Albumin infusion should therefore be performed in patients undergoing LVP >5L (8 g/L of ascites removed).[1]

3.2.3 | Albumin

Long-term administration of albumin has also been shown to reduce mortality in patients with refractory ascites. The single center, non-randomized study by Di Pascoli et al, evaluated the prognosis of patients with refractory ascites treated with 40 g albumin twice a week vs SMT.[9] Two-year mortality, which was the primary endpoint, was significantly lower in the albumin group (41.6% vs 65.5%, $P = .032$). This study has many limitations including TIPS as an alternative therapy in these patients. However, long-term administration of albumin, which was shown to improve survival in more severe patients, such as those with refractory ascites, could be an interesting option in selected patients, especially liver transplantation candidates.

3.2.4 | Transjugular intrahepatic portosystemic shunt

Transjugular intrahepatic portosystemic shunt (TIPS) placement induces decompression of the portal circulation by shunting an intrahepatic portal branch into a hepatic vein. In an evaluation of refractory ascites, six prospective randomized controlled trials (RCT) compared non-covered TIPS and LVP for recurrence of ascites, hepatic encephalopathy and survival (Tables 1 and 2).[10-15] The results were analysed in several meta-analyses. In the meta-analysis of individual data by Salerno et al ascites recurrence and transplant-free survival were better in the TIPS group, compared to LVP.[16] However, the average number of HE episodes was higher in the TIPS group. It seems important to note that these studies were published before the use of PTFE-covered stents. More recent results obtained in recurrent ascites suggest that the earlier results would probably have been better using covered stents.[7] To date, no prospective controlled trial has been published using covered stents in refractory ascites. In the study published by Bureau et al, patients were included in case of recurrent ascites, defined by two LVPs at least 3 weeks apart, and excluding those who had undergone >6 LVP in the last 3 months. These criteria were quite different from both the historical definition of recurrent ascites and those of refractory ascites, as previously discussed.

TIPS insertion is contraindicated in patients with heart failure, advanced liver failure, defined by a Child-Pugh score >13 or a MELD score >19, and significant HE. Thus, patients must be carefully selected for TIPS placement. Although exclusion criteria in RCT were heterogeneous, there were certain similarities, such as >70 or 75 years old, HE on the day of TIPS placement, Child-Pugh >11, HCC outside of the Milan criteria and heart failure.

Three main complications negatively influence prognosis after TIPS placement: (a) liver failure and death; (b) refractory HE and (c) heart failure. One study presented a simple predictive model of survival combining platelet count and total bilirubin level[17] and showed that the actuarial 1-year survival rate in patients with both a platelet count >75 × 10^9/L and a total bilirubin level <50 μmol/L

TABLE 1 Main studies comparing LVP and other therapeutics in patients with refractory or recurrent ascites

			Enrolled patients (n)		Improvement of ascites (%)		Development of hepatic encephalopathy (%)		Survival (%)	
			TIPS	LVP	TIPS	LVP	TIPS	LVP	TIPS	LVP
Bare TIPS	Refractory ascites	Randomized controlled studies								
		Lebrec et al[10] 1996	13	12	38	0	15	6	29	60
		Gines et al[11] 2002	35	35	51	17	60	34	26	30
		Sanyal et al[12] 2003	52	57	58	16	38	21	35	33
		Narahara et al[13] 2011	30	30	87	30	20	5	20	5
	Refractory + recurrent ascites	Rössle et al[14] 2000	29	31	84	43	23	13	58	32
		Salerno et al[15] 2004	33	33	79	42	61	39	59	29
		Meta-analysis for refractory ascites								
		Salerno et al[16] 2007	149	156	58	11	58	38	56	50
Covered TIPS	Recurrent ascites	Randomized controlled study								
		Bureau et al[7] 2017	29	33	89	29	34	33	93	52

Abbreviations: LVP, large volume paracentesis; TIPS, tranjugular intrahepatic portosystemic shunt.

TABLE 2 Outcome of Alfapump® for patients with cirrhosis and refractory ascites

			Number of LVP per patient per month (median)		Development of adverse effects (n)				Child-Pugh score (mean)			MELD score (mean)				Improvement of Quality of life (HRQoL score)	
	Enrolled patients (n)	Length of follow-up (months)	At baseline	At the end of follow-up	Infections	Catheter issues	Pump malfunction	Alfapump® explant	At baseline	At 6 mo	At the end of follow-up	At baseline	At 6 mo	At the end of follow-up	Death, n (%)	Abdominal symptoms	Activity scores
Observational studies																	
Bellot et al[20] 2013	40	6	3.4	0.2	24 (60%)	10 (25%)	2 (5%)	3 (7.5%)	8.5	8.6	8.6	12.6	11.7	11.7	9 (22%)		
Stirnimann et al[21] 2017	56	24	2.2	0.2		28 (50%)	11 (20%)	27 (48%)	8.8	9.8	7.5	13.42	17.04	9.11	23 (41%)		
Randomized controlled study																	
Bureau et al[22] 2017	27	6	1.6	0.3	25 (93%)	5 (9%)	4 (7%)	3 (5.4%)	8.2			12.2			15 (56%)	+1.25	+0.80

Abbreviation: LVP, large volume paracentesis.

[3 mg/dL] was 73.1% compared to 31.2%, in patients with a platelets count <75 × 10^9/L or a total bilirubin level >50 μmol/L. In another study several risk factors for the worsening of HE were described: older age, poor liver function, a previous episode of HE, sarcopenia and minimal hepatic encephalopathy. Nevertheless, there is no predictive model for the selection of patients according to their risk of developing HE. We recently recommended excluding TIPS as a non-urgent option in patients with a history of at least two episodes of HE, or with HE on the day of TIPS placement.[18] Moreover, our group suggested that TIPS placement be discussed on a case-by-case basis in patients older than 70. Finally a very recent prospective study has shown that cardiac decompensation occurs in about 20% of patients following TIPS placement.[19] The authors reported that a combination of a BNP <40 pg/mL and a NT-proBNP <125 pg/mL before TIPS and the exclusion of diastolic dysfunction on echocardiography excluded the risk of cardiac decompensation.

3.2.5 | Alfapump®

Alfapump® (AP) is a fully implantable, programmable and rechargeable pump system that automatically diverts ascitic fluid from the peritoneal cavity to the urinary bladder, allowing fluid removal by micturition (Tables 1 and 2).[20,21] In a recent multicenter RCT in patients with refractory ascites, AP significantly reduced the number of LVP and improved the quality of life as well as nutritional parameters.[22] Quality of life was also shown to improve by AP in another study.[23] AP is contraindicated in patients with chronic renal failure, because it can cause acute, but reversible, renal failure. Moreover, some patients, especially with HE, may experience technical difficulties. Thus, it seems reasonable not to recommend AP as an alternative therapy in patients with HE unless there is a relative to take care of the device.

3.2.6 | Liver transplantation

LT should be discussed in all patients with refractory ascites because of the poor survival in this group. Nevertheless, despite the poor prognosis of this complication, some patients will present with a low MELD score that can delay LT. Liver transplantation could be prioritized based on a MELD score exception in these patients. However, prioritization can only be considered in patients with a strict contraindication to TIPS placement.[24] Thus, TIPS should be the first option in these patients.

3.2.7 | Summary of available therapeutics, indications

As previously mentioned, LVP should be performed in patients with refractory ascites (Figure 1). If LVP is the first-line treatment, a second line therapy has to been considered as soon as the diagnosis is made to improve the prognosis. A careful clinical, biological and morphological examination must be performed. This includes obtaining a clinical history, including age, and a systematic search for a previous episode of HE or heart decompensation. The physical examination should screen for confusion, flapping, sarcopenia and left or right signs of heart failure. The biological evaluation should include routine blood exams, hepatic function, renal and cardiac function with BNP and NT-proBNP. Finally, the morphological evaluation should include an abdominal ultrasound exam, CT scan and echocardiography. TIPS seems to be the best therapeutic option in patients under the age of 65, with no previous episodes of HE, a Child-Pugh score <13, a MELD score <19, total bilirubin levels <50 μmol/L, a platelet count >75 × 10^9/L, normal BNP/NT-proBNP values and normal echocardiography. TIPS should be contraindicated in patients over 70 years old, with history of more than two episodes of HE. AP can be considered in the latter unless they present with normal renal function (Cl Creat ≥50 mL/min). A case-by-case discussion is needed in patients considered to be at high risk, according to liver function, cardiac function, and the risk of HE after TIPS. As there is a theoretical risk of developing either liver failure or refractory HE after TIPS, we believe that liver transplantation should be discussed in all patients.

3.3 | Patients with spontaneous bacterial peritonitis

SBP is the most frequent site of bacterial infection in patients with cirrhosis. SBP is still associated with high mortality and may trigger worsening of liver function and other complications of cirrhosis such as HE, renal failure and bleeding. The increasing prevalence of multidrug-resistant organisms (MDRO) is a concern in the treatment of SBP. This mainly includes extended spectrum beta lactamases producing Enterobacteriaceae and beta lactams-resistant Gram-positive bacteria. The emergence of extended drug-resistant organisms (XDRO), in hospitalized patients but also in the community in some parts of the world, emphasizes the need for data on the use of new antibiotics in patients with cirrhosis. European data support a high prevalence of MDRO infections in decompensated or acute on chronic liver failure (ACLF) patients. About 29% of the strains isolated in the 264 culture-positive infections among the 1146 patients with decompensated cirrhosis or ACLF followed in the CANONIC cohort (2011) were MDRO.[25] There are large discrepancies among centres and countries, with a higher prevalence in Western European countries in these almost 10-year-old data. The only factors significantly associated with the occurrence of MDRO were nosocomial infections, hospitalization within the previous 3 months and intensive care unit admission. It is important to note that long-term exposure to norfloxacin was not identified as a risk factor. More recent data (2017-2018) in 883 European patients with decompensated cirrhosis showed that 39.7% of culture-positive infections among the 284 patients who developed infection were MDRO, which is a nearly 10% increase compared to 2011 data. It is interesting to note that there was a shift towards a higher prevalence in Eastern and Southern European countries. Worldwide, the study by Piano et al reported 1302 infections in hospitalized patients with cirrhosis.[26] MDRO were isolated in 34% of cases. Risk factors were nosocomial or healthcare-associated infections, antibiotic exposure within

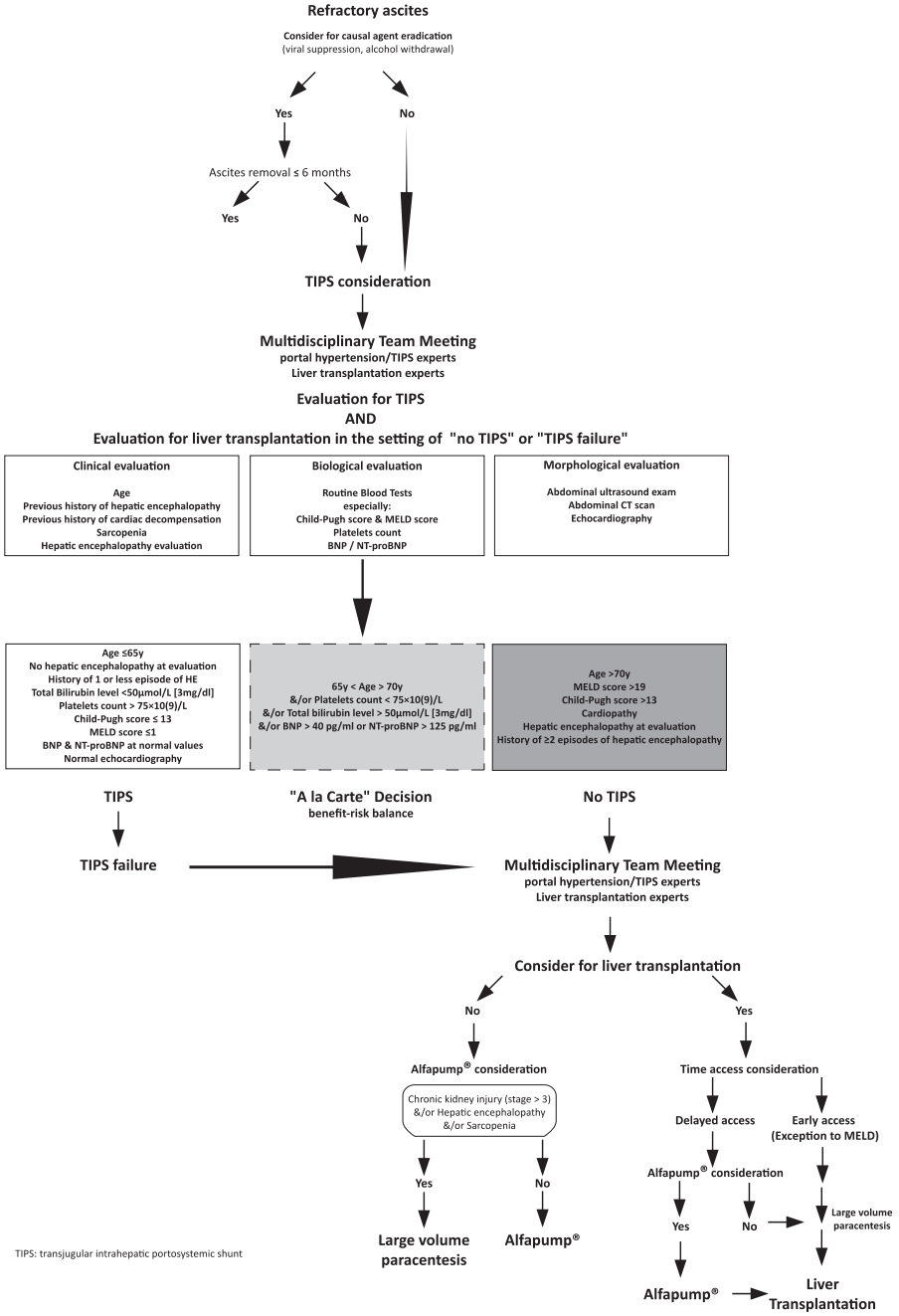

FIGURE 1 Algorithm for the management of refractory ascites in patients with cirrhosis

the previous 3 months but also geographical origin, in particular India where the rate of MDRO and XDRO was the highest. The sites most concerned by MDR infections were pneumonias and urinary tract infections. Prevalence was lower in SBP (27%), like in the CANONIC cohort (13.9% for SBP vs 29.3% all sites included). An Italian RCT has compared initial antibiotic therapy with meropenem plus daptomycin vs ceftazidim to treat nosocomial SBP. There was a significantly higher response to treatment for the decrease in neutrophils count in ascites in the meropenem plus daptomycin group, but 90-day transplant-free survival was similar in both groups. On multivariate analysis, ineffective first-line treatment was a significant predictor of mortality, as described in previous studies. Thus, recommendations about antibiotic therapy for SBP are very difficult and highly important for clinical outcome. They must depend on the local bacterial ecology and individual risk factors such as previous antibiotic therapy, healthcare associated, or nosocomial infections. The EASL guidelines for community acquired SBP recommend third generation cephalosporins or piperacillin plus tazobactam.[1] Meropenem is recommended for nosocomial SBP, in association with linezolid or daptomycin when the prevalence of drug-resistant Gram-positive bacteria is high. Administration of 20% albumin is also recommended during SBP at the dose of 1.5 g/kg at day 1 and 1 g/kg at day 3. Indeed, in the study by Sort et al, this treatment resulted in a significant decrease in hospital mortality and occurrence of renal failure compared to antibiotic therapy with cefotaxime

alone (10% vs 29%, respectively, P = .01) and (10% vs 33%, respectively, P = .02).[27] Severe patients (serum creatinine ≥88 μM or total bilirubin ≥68 μM) seemed to benefit most from treatment. Whether it should be administrated to all patients with cirrhosis is therefore a subject of debate.

Prophylaxis of SBP is another clinically important issue. Norfloxacin is the only drug recommended and concern is growing about its safety and efficacy because of the increasing prevalence of MDRO. Frequent neurological and osteo-articular side effects have led drug regulation agencies to issue warnings about this drug and advise limiting its use to when no alternative is available. In primary prophylaxis, norfloxacin is recommended when ascites fluid protein level is below 15 g/L in association with severe cirrhosis (Child-Pugh score ≥9 and total bilirubin level ≥3 mg/dL (51 μmol/L), with either impaired renal function or hyponatraemia).[1] A French RCT compared norfloxacin to placebo in Child Pugh C patients without previous SBP.[28] Six-month mortality was only significantly lower in patients with low ascitic fluid protein levels (<15 g/L), confirming that primary prophylaxis should be restricted to the most severe patients. However, these are not recent data (2010-2014) and cannot take into account the change in susceptibility of Gram-negative bacteria to fluoroquinolones, which may affect the effectiveness of prophylaxis. Norfloxacin use in secondary prophylaxis is an even greater issue because of the high prevalence of recurrent SBP after a first episode. It was shown to be effective in a single RCT published in 1990, significantly decreasing the rate of recurrent SBP from 68% in the placebo group to 20% in the norfloxacin group. There are no similar more recent results. However, a recent German observational study in patients receiving primary or secondary prophylaxis with norfloxacin suggests a significantly greater risk of SBP in patients with quinolone-resistant Gram-negative bacteria. These results in a population with a 50% rate of baseline MDRO highlight the importance of resistance to fluoroquinolones and suggest that routine screening patients for MDRO could be advisable. Finding an alternative to oral fluoroquinolones is also important. Preliminary results and a recent meta-analysis support the effectiveness of rifaximin in primary or secondary prophylaxis of SBP.[29] However, the results of a RCT including a larger number of patients, comparing rifaximin to oral fluoroquinolones are still awaited.

4 | CONCLUSION

Prognosis is poor in patients with complicated ascites, including refractory ascites or SBP. In these situations, TIPS placement and liver transplantation must both be discussed, because TIPS may be either contraindicated or with an uncertain outcome in patients at high risk of developing further liver failure, HE or cardiac decompensation. The recent study by Bureau et al conducted patients with recurrent ascites suggests that TIPS placement could be indicated at an earlier stage, before the development of refractory ascites. We believe that there should be a multidisciplinary discussion to improve selection of patients for the best therapeutic option.

CONFLICT OF INTEREST

MR: speaker for Gore, Gilead, Abbvie; MM: none; PS: none; CB: speaker for Gilead, Abbvie; DT: consultancy for Gore, Alfasigma, Gilead, MSD, AbbVie, Medday.

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REVIEW ARTICLE

Liver INTERNATIONAL WILEY

EASL clinical practice guidelines for the management of occupational liver diseases

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Abstract

The Clinical Practice Guidelines (CPG) on Occupational Liver Diseases (OLD) of the European Association for the Study of the Liver (EASL) have been developed to increase awareness, recognition and improve management of patients with OLD. Indeed, although workplace exposure has been associated with virtually the entire spectrum of acute and chronic liver diseases, data on the epidemiology of OLD are scarce. These diseases may be a result of high-level accidental exposure or prolonged lower level exposure to a variety of chemicals including solvents, pesticides, metals and other agents. While acute liver diseases related to OLD are uncommon and easily recognized, chronic liver diseases are relatively more frequent but often overlooked because of their asymptomatic course and an insidious onset which is often accompanied by comorbidities. Because of the absence of data in observational studies and meta-analyses or systematic reviews, the evidence and recommendations in these guidelines have been graded according to the Oxford Centre for Evidence-based Medicine, which assesses evidence according to diagnostic, prevalence, aetiological, prognostic or preventive categories. They can still generate grades of recommendation even when the evidence is inconclusive.

KEYWORDS

drug-induced liver injury, hepatocellular carcinoma, liver angiosarcoma, occupational liver disease, toxicant-associated steato hepatitis

1 | INTRODUCTION

In 2016 the European Association for the Study of the Liver decided to publish recommendations on the management of liver diseases diagnosed in persons who are exposed to chemicals in the work place.[1] One major aim was to fill the gap in the standardization of the diagnosis of occupational liver diseases (OLD) and to position the role of liver experts in a field of medicine where a multidisciplinary approach is often required. Indeed, one major barrier to the understanding and management of OLD is the lack of specific tests for toxicity. Histopathological examination of the liver, which is of major importance in the management of many liver diseases, may not be helpful in all cases of OLD. The assessment of internal doses of chemicals is usually not decisive. As a result, the diagnosis of OLD is challenging and based on exclusion criteria, thus explaining the need for a multidisciplinary approach in most cases. EASL recommendations do not cover liver diseases acquired in the healthcare setting such as viral hepatitis or halotane toxicity. The panel of experts who participated in these recommendations included a hepatologist (M. Colombo, Milan Italy), and experts in toxicology (C. Stove, Ghent, Belgium), pharmacology (M. Lucena, Malaga, Spain), pathology (V. Paradis, Clichy, France), occupational medicine (M.Lotti, Padua, Italy) and epidemiology (C. LaVecchia, Milan, Italy).

2 | HOW TO DIAGNOSE OLD?

The type and severity of liver injuries are based in part on criteria for drug-induced liver injury (DILI) Figure 1.

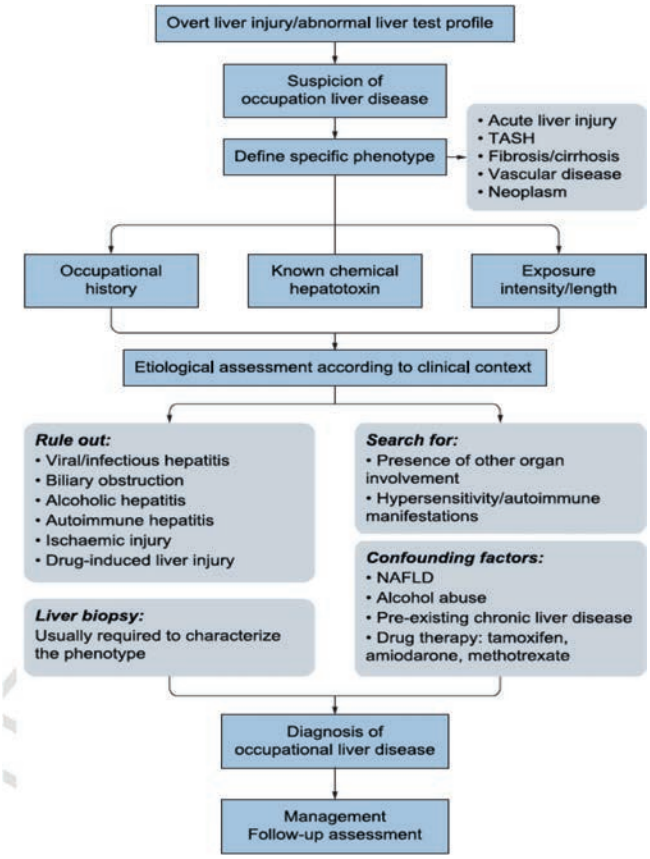

FIGURE 1 Diagnostic algorithm of occupational liver disease TASH: toxicant-associated steato-hepatitis, NAFLD non-alcoholic fatty liver disease. [1] Schematic approach to the assessment and diagnosis of occupational liver disease. OLD, occupational liver diseases; TASH, toxicant-associated steatohepatitis

3 | EPIDEMIOLOGY OF NON-NEOPLASTIC LIVER DISEASE

Data collection is biased by difficulties in adjusting for covariates and confounding factors. The higher prevalence rates of self-reported unspecified 'liver diseases' observed in blue-collar or unskilled workers, often associated with tobacco and alcohol use, makes it difficult to determine causation.[2] This was the case in European workers with high level exposure to vinyl chloride monomer (VCM), resulting in 203 deaths from cirrhosis.[3.] The association of shift work with non-alcoholic fatty liver disease (NAFLD), was disproven by a cross section of the National Health and Nutrition Examination Survey (NHANES) where the OR of developing NAFLD was 1.11 with a 95% CI of 0.87-1.43.[4]

4 | EPIDEMIOLOGY OF LIVER MALIGNANCIES

Liver angiosarcoma (AS) was first associated with the manufacture of VCM in 1974,[5] and a causative link was firmly established between high occupational exposure to VCM (among autoclave workers) and this deadly cancer. The onset of the tumour has a long incubation phase as suggested by the analysis of nearly 10,000 cases, where deaths from liver AS are thought to occur after an average of 40 years of follow-up. In 1987 the International Agency for Research on Cancer (IARC) concluded that VCM exposure also causes hepatocellular carcinoma (HCC), a statement that was confirmed in 2012 following the re-analysis of VCM-exposed European and US workers [6,7..] These studies included 60 deaths, mainly from HCC, but also from liver cancer of unspecified or undefined histology, thus raising the possibility of a misclassification with AS (reviewed by [3]). However, a more recent re-analysis of the US cohort found that the increased risk of HCC was restricted to workers with very high estimated cumulative exposures, that is over 1000 ppm-years—approximately 25 times the working lifetime at the exposure limit of 1.0 ppm of the liver. Since occupational exposure to VCM was controlled in the mid-1970s, few additional cases of VCM-related AS of the liver are anticipated in the future and thus of other liver cancers, either.

Recommendation: Ultrasound surveillance for the development of liver neoplasms should be discussed in workers exposed to high levels of VCM in the past, that is until the mid-1970s, as defined by their job title (reactor cleaners). Grade D.

Evidence: Extrapolation from level 2 studies (historical cohort studies).

Key points

- Collecting the occupational history is crucial. Patients must be assessed by a multidisciplinary team.
- Acute liver disease is rare compared to chronic liver injury. There are many sensitizing cofactors.
- Liver angiosarcoma is clearly associated with high exposure to vinyl chloride (in the past only), HCC is possible, cirrhosis is unlikely.
- Hepatitis and cholestasis are the dominant occupational lesions, often obscured by comorbidities such as alcohol and metabolic steatohepatitis.
- The unmet needs: availability of specific tests of toxicity.

5 | CONTROVERSIAL ASSOCIATIONS WITH LIVER CANCERS

5.1 | Trichloroethylene (TCE)

Trichloroethylene (TCE), which has been widely used for decades to degrease metal parts and for dry cleaning, can induce a variety of cancers in selected strains of rats and mice, including liver/kidney tumours, and lymphomas.[8] The evidence from epidemiological studies is not conclusive.

5.2 | Tetrachloroethylene (or perchloroethylene)

A systematic review of 18 studies did not show any consistent association between exposure and liver cancer, even in studies with a large number of observed events/exposed cases or a strong exposure-assessment approach.[9]

5.3 | Polychlorinated biphenyl (PCB) exposure

Epidemiological evidence does not support an association between occupational exposure to PCBs and liver cancer risk. Nevertheless, PCBs are currently classified as Group 1 'Carcinogenic to humans' by the IARC [10] largely based on evidence of a risk of melanoma in humans.

5.4 | Pesticides

Biomarker-based studies performed in Chinese populations have suggested that certain organochlorine serum levels, mainly of dichlorodiphenyl trichloroethane (DDT), may be associated with an increased risk of liver cancer.[11] In a systematic review of 15 studies on pesticide exposure and liver cancer (mainly HCC), most studies, particularly those relying on self-reported exposure and occupation, job-exposure matrices and rural residence, found no association.[12]

Recommendation: Workers with potential exposure to hepatotoxic chemicals should receive a document listing the chemicals used in the factory. This document may be made available to the workers without request. Grade D.

Evidence: Level 5 (Expert opinion).

6 | WHAT MAKES WORKERS SUSCEPTIBLE TO DEVELOP LIVER INJURY?

Liver injury in the workplace is often the result of an interaction between environmental risk factors and genes.[13] While the hepatotoxic potential of some chemicals, such as phosphorus or pyrrolizidine alkaloids, depend more on the extent of exposure than on host susceptibility, the opposite is true for halothane and paracetamol. Thus, risk assessment should also take into account host-environmental interactions that are likely to modulate the severity of liver injury, a fact that adds complexity to the diagnosis of OLD. Like in DILI, a higher percentage of adipose tissue in women favours lipophilic chemicals that enhance the risk of liver injury.[14,15] The concurrent administration of drugs and exposure to chemicals may have unpredictable consequences through the induction or inhibition of microsomal activity, an interaction that may take place either directly or via the generation of reactive metabolites. A mixed exposure to chlorinated organic solvents, including dichloromethane, 1,2-dichloropropane and trichloroethylene, has been incriminated in severe acute hepatitis.[16]

Recommendation: Caregivers, as well as workers exposed to liver enzyme inducers and/or using enzyme inducing drugs should be informed of the possibility of interactions with anticonvulsant drugs. Grade C.

Evidence: Level 4 (case series).

Along these lines, special attention should be given to alcohol consumption because it can increase the hepatotoxic effects of other compounds that are taken simultaneously through its inducing effect on the cytochrome P450 system (CYP), in particular the isoform CYP2E1. It is well established that excess alcohol intake potentiates the effects of occupational exposure to CCl_4 and other chemicals that are activated by the same cytochrome P450 enzymes.[17]

Recommendation: Caregivers and workers should be informed by the attending physician that alcohol can be toxic to the liver and potentiates liver toxicities from occupational exposure. Grade C.

Evidence: Extrapolation from 2c studies (outcome research and mechanistic studies).

Pre-existing liver diseases may also play a role in modulating the severity of OLD, as it may synergize with professional exposure to toxins, resulting in increased susceptibility to these agents. This may be the case in patients with NAFLD and obesity where CYP2E1 is upregulated. This favours the metabolism of toxins such as VCM into reactive metabolites, which could ultimately increase the susceptibility to the development of toxicant-associated steatohepatitis (TASH). Pre-existing NAFLD increases the risk of acetaminophen overdose-induced acute liver injury.[18]

Recommendation: Occupational workers with classic risk factors for fatty liver may be advised by the attending physician to undergo baseline screening for NAFLD/NASH, alcoholic fatty liver disease (AFLD) and alcoholic steatohepatitis (ASH) and close follow-up. Grade D.

Evidence: Level 5 (Expert opinion).

Regular alcohol intake above predefined thresholds (>20 g/day (women), >30g/day [men]) should be investigated in patients exposed to occupational chemicals, because it increases the risk of AFLD and ASH.[19]

Recommendation: Screening for the presence of NAFLD/NASH, AFLD/ASH and/or residual fibrosis is suggested in workers with a hepatitis C cure or controlled chronic hepatitis B virus infection. Screening for clinical data suggesting NAFLD/NASH, AFLD/ASH is suggested to better define their risk profile when exposed to chemicals. Grade D.

Evidence: Level 5 (Expert opinion).

Screening for liver cancer is not recommended in trichloroethylene and other chlorinated solvent-exposed workers, workers exposed to polychlorinated biphenyl or workers exposed to pesticides.

Recommendation: Workers with potential exposure to hepatotoxic chemicals should receive a document listing the chemicals used in the factory. This document may be made available to the workers without request. Grade D.

Evidence: Level 5 (Expert opinion).

7 | DEFINITION OF LIVER INJURY

Serum markers reflecting hepatocellular necrosis and inflammation are used to detect liver injury in workers exposed to potential hepatotoxins.[20] Although these biomarkers are not specific for any form of toxic liver disease, lack mechanistic insight and may not have prognostic value, the similar clinical and histopathological features of OLD and drugs make it appropriate to adopt the consensus criteria for DILI to define acute liver injury in workers exposed to toxicants.[1]

1. ALT level ≥5 x Upper Limit of Normality (ULN),
2. Alkaline phosphatase (ALP) level ≥2 x ULN (particularly if concomitantly elevated gamma-glutamyl transpeptidase (GGT) in the absence of bone disease) or
3. ALT level ≥3 x ULN and simultaneous total bilirubin (TB) level >2 x ULN.

There is a tendency for the pattern of liver injury to shift to a cholestatic/mixed biochemical signature.[15] Liver biopsy can increase the diagnostic accuracy. Unlike DILI, however, biochemical and histological correlations are lacking for OLD data. Insidiously atypical phenotypes of toxic liver injury include steatosis, toxicant-associated steatohepatitis (TASH), fibrosis, cirrhosis, vascular liver disorders and liver cancer.[22] Thus, the sensitivity of DILI criteria for acute liver injury may be low for detecting chronic liver damage from occupational exposure.

Definition:

Acute liver injury in occupational workers should be classified as hepatocellular, cholestatic and mixed, according to the liver biochemistry from the first laboratory assessment.

Evidence: Level 5 (expert opinion).

In chemically induced acute liver failure often accompanied by the simultaneous involvement of other organs, a delay of 26 weeks between the onset of jaundice and the development of encephalopathy is unlikely.[23] Rather, fulminant liver failure, with symptoms appearing 24-48 hours after chemical exposure, such as that observed with carbon tetrachloride poisoning, may be the standard for acute exposure to occupational toxicants.

In OLD, extrahepatic organ failure may be a result of either the direct effect of the chemical or multiorgan failure because of severe liver damage. In selected cases, however, such as in workers exposed to VCM, methylene dianiline and dimethylformamide (DMF), hepatotoxicity is the main clinical feature (reviewed by [1]).

Recommendation: The severity of acute chemical liver injury can be evaluated using the adapted severity index scale designed for DILI. Grade D.

Evidence: Level 5 (expert opinion).

Recommendation: The diagnosis of OLD should be based on the judgement of an expert occupational physician. The assessment of OLD can be further improved on a case-by-case basis by consultation with a multidisciplinary team including hepatologists, pathologists, toxicologists and epidemiologists. Grade D.

TABLE 1 Pathological patterns and morphological features of liver disease associated with workplace-related toxicants

Pathological patterns	Morphological features	Toxicants
Acute damage		
Hepatocellular	Hepatocellular necrosis + lobular inflammation	CCl4, chloroform, toluene, TNT, PCBs, chloronaphtalene, DMF, hydrazine, 2-nitropropane, phosphorus, DMA, halothane, TCE, tetrachloroethane, 1, 4-dichlorobenzene
	Microvesicular steatosis	DMF
Cholestatic / Mixed	Cholestasis, cholangitis Combined features	Methylenedianiline Nitrobenzene, paraquat, methylenedianiline
TAFLD	Steatosis (macro/microvesicular) Steato-hepatitis (steatosis + lobular inflammation + hepatocellular ballooning)	Chloroalkenes (PCE, TCE), VCM, chloroform, CCl4, volatile organic compounds (benzene, toluene, styrene, xylene), dioxins, chlordecone, DMF, hydrazine, arsenic, mercury, POPs, pesticides, and some nitro-organic compounds
Vascular	Sinusoidal obstruction syndrome Peliosis	VCM, dioxin, pyrrolizidine alkaloids, arsenic, copper sulfate VCM
Chronic damage		
Fibrosis	Periportal fibrosis Extensive fibrosis/cirrhosis	VCM, PCBs, chloronaphthalene, Tetrachloroethane VCM
Vascular tumors	Hepatoportal sclerosis	VCM, sprays containing copper sulfate and lime
Epithelial hepatocellular carcinoma		Arsenic, dimethylnitrosamine
Cholangiocarcinoma		1,2-Dichloropropane, dichloromethane
Vascular Angiosarcoma		VCM, arsenic
Epithelioid hemangioendothelioma		VCM

Abbreviations: DMF, dimethylformamide; PCBs, polychlorinated biphenyls; POPs, persistent organic pollutants; VCM, vinyl chloride monomer

Evidence: Level 5 (expert opinion).

8 | OBTAINING THE OCCUPATIONAL HISTORY

Bearing in mind that occupational exposure usually occurs from inhalation and through the skin, the diagnostic process requires a multidisciplinary approach that includes occupational health physicians and industrial hygienists, as well as toxicologists and epidemiologists in certain circumstances. The latter are needed because of discrepancy between the large number of chemicals that have been shown to cause liver toxicity experimentally but with little or no evidence in humans.[24] There are no long-term biomarkers to identify historical exposure to a potentially hazardous chemical. Exposure data could be used to consult workplace exposure limit databases, such as those from the national list of occupational limit values (OEL) from EU member states, including Germany (DFG-MAK Commission), The Netherlands (DECOS) and France (ANSES), and also from other sources such as the Scientific Committee on Occupational Exposure Limits (SCOEL),[25] the Occupational Safety and Health Administration (OSHA), the National Institute of Safety and Health (NIOSH) or the American Conference of Governmental Industrial Hygienists (ACGIH). Data from urine and blood biomonitoring are better than those obtained from workplace monitoring systems because they provide personalized assessment of exposure, which can help when exposure is relatively recent or ongoing. Segmental hair analysis may be used or alternatively, DNA adducts can be monitored in blood (135,136). Many of the toxicants listed in Table 1 will be converted to reactive intermediates in the liver, which form covalent adducts with macromolecules such as DNA and proteins. Haemoglobin adducts are detectable up to more than 100 days following exposure, because their disappearance is linked to the lifespan of red blood cells, which is about 4 months. It should be noted that there is not necessarily a link between the level of exposure and the extent of damage, as this is compound-dependent.

9 | NON-INVASIVE STAGING OF LIVER INJURY

Liver damage is effectively identified and staged for multiple causes by non-invasive markers such as transient elastography, Fib-4 and albumin to platelet ratio index (APRI).[26] These tests can help in cases of occupational exposure by 1) identifying subclinical hepatic injury without symptoms and/or abnormal serum liver blood tests, 2) staging the severity of overt chronic liver disease and 3) evaluating resolution of acute liver injury or suspected chronic disease after 12 months of persistently altered results (such as in DILI). While workers exposed to toxins may develop a variety of histopathological liver lesions, TASH mimics the histopathological changes observed in NASH, making it extremely difficult to characterize the former. When the individual with liver disease is no longer exposed, this does not necessarily provide evidence of a relationship with the work environment.

Recommendation: Staging of OLD can require a dynamic evaluation including repeated liver tests and liver stiffness measurements by transient elastography or serum predictors of fibrosis such as Fib-4 and APRI after the patient is removed from occupational exposure to suspected toxins. Grade D.

Evidence: Level 5 (Expert Opinion).

Although liver biopsy is the most reliable technique for the diagnosis and staging of any type of liver disease, it is limited by cost, sampling error and procedure-related morbidity and mortality. In patients with more than one risk factor, liver biopsy remains the most robust diagnostic procedure to define the cause of underlying liver abnormalities.

Recommendations:

1. Liver biopsy may be performed in patients with persistently abnormal non-invasive liver tests, depending on the clinical context and the extent of the liver abnormalities. Grade D.
   Evidence: Level 5 (Expert Opinion).
2. When performing a liver biopsy to diagnose a liver mass, sampling of the non-tumoral liver is suggested. Grade D
   Evidence: Level 5 (Expert Opinion).

10 | PATIENT MANAGEMENT

Management of patients with OLD is mainly based on the nature and severity of disease. Patients with acute injury should be removed from further exposure while the degree of liver impairment is being determined. In severe and ongoing cases, consideration should be given to the appropriate setting in the hospital and the need for liver transplantation.[27]

Recommendation: The relevant health authorities and/or compensation agencies can be informed of a documented or suspected case of OLD. Grade D.

Evidence: Level 5 (Expert Opinion).

11 | PREVENTION

Areas of high risk still exist, particularly in developing countries. The two broad approaches to prevent workers from being affected by liver toxicants include primary prevention (elimination or control of exposure through interventions in the working environment) and secondary prevention (identification of excessive exposure and early clinical effects in individuals.) The severity of OLD will determine the decision to temporarily or permanently remove affected workers from the workplace, which will also depend on the working environment and social factors. While most countries have their own exposure limits, these are broadly similar to the American ACGIH-derived Threshold Limit Values [28] which are revised on a regular basis, and the most influential worldwide values. Environmental exposure can

be controlled individually by biological monitoring as a part of medical surveillance [29] and based on the analysis of substances or metabolites in blood, urine or breath. Biological limit values have also been suggested for certain substances. Traditional liver function tests may be normal even in the presence of liver damage and specific tests for toxicity are not available. Raising worker awareness about the risks of exposure by providing information, instruction and training is one of the most important and effective aspects of prevention.

12 | CONCLUSIONS

The diagnosis and management of OLD require a multidisciplinary approach and overcoming the many barriers because of the absence of specific markers of occupational exposure. More attention must be paid to improving safety in the workplace by collecting cohort data from occupational registries including clinical, biochemical and follow-up information, to obtain incidence figures of hepatotoxicity and trends in re(emerging) OLD. Another unmet need is the development and quantification of sensitive and specific biomarkers of liver damage caused by toxicants. This could help fine-tune differential diagnoses, and reduce the need for histological examination of the liver while providing clues to the prognosis. More effective risk stratification algorithms could be developed if progress is made in the field of biomarkers, while providing mechanistic insights that could help the development of safe and effective treatments.

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REVIEW ARTICLE

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Recent developments in the field of vascular liver diseases

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Abstract

Knowledge in the field of vascular liver disease is continuously expanding. The present update will discuss recent data on i) the Abernethy malformation in adults; ii) portal vein thrombosis in cirrhosis; iii) advancing expertise in recanalization of the portal vein and iv) experience in using direct oral anticoagulants in the field of vascular liver disease.

KEYWORDS

anticoagulants, cirrhosis, congenital extrahepatic portsystemic shunt, direct oral anticoagulants, non-specific beta blockers, portal flow velocity, portal hypertension, portal vein thrombosis

1 | INTRODUCTION

Knowledge in the field of vascular liver diseases is continuously growing. A previous update at the 2018 PHC meeting focused on portosinusoidal vascular disease and portal vein thrombosis complicating cirrhosis. The present update will discuss recent data on i) the Abernethy malformation in adults; ii) portal vein thrombosis in cirrhosis; iii) advancing expertise in the recanalization of the portal vein and iv) results with direct oral anticoagulants in the field of vascular liver disease.

2 | ABERNETHY MALFORMATION IN ADULTHOOD

Congenital extrahepatic portsystemic shunt is a rare disorder in which blood flowing out of the spleen and intestine is diverted to the systemic circulation and bypasses the liver through abnormal communications. Although this entity was formerly called 'congenital absence of the portal vein', this is misleading and should no longer be used because the apparently absent extra- and intrahepatic portal venous system can still be identified in many patients after occlusion of portosystemic shunts.

In 2012, a review analysed 265 children with this disorder between 1979 and 2012.[1] Recently, a large collaborative study from the vascular liver disease interest group (VALDIG) focused on predominantly adult patients (median age 21 years old) identified in the last 3 decades in 23 participating liver centres. Sixty-six patients were finally included.[2]

The cumulative 30-year incidence of hepatic encephalopathy was 28%, which is probably overestimated by an unknown percentage. Indeed, on one hand encephalopathy is a major sign leading to a diagnosis of Abernethy malformation and a source of overestimation, whereas on the other hand, non-specific or subclinical features of encephalopathy are probably overlooked in patients with a recognized malformation.[2] Risk factors could not be identified among demographic characteristics, clinical and laboratory features (including serum ammonia levels), brain MRI findings or associated conditions.

As in many conditions, Abernethy malformation results in altered liver perfusion with portal blood and a high incidence of liver nodules as detected on vascular enhanced imaging.[3] In the VALDIG survey, benign hepatic nodules were identified in 24 of the 66 patients, hepatic adenomas in 10, and hepatocellular carcinoma in 8. These figures are similar to the findings in patients with Budd-Chiari syndrome, but appear to be lower than those in patients with vascular portosinusoidal disease or portal cavernoma.[2] An imbalance between increased arterial and decreased portal blood perfusion probably explains these changes, although the exact mechanisms remain unknown. Except in the presence of fully characteristic images of focal nodular hyperplasia, liver biopsy is required for complete characterization of liver nodules.

Abbreviations: MELD, model for end-stage liver disease; TIPS, tranjugular intrahepatic portosystemic shunt; VALDIG, vascular liver disease interest group.

Although adenomas mostly affect women, hepatocellular carcinoma mainly affects men. Increased AFP levels are certainly suggestive of hepatocellular carcinoma but not highly specific, and not sensitive enough to be used as a screening tool.[2]

In the VALDIG survey, pulmonary arterial hypertension and hepato-pulmonary syndrome were found in eight and two patients with dyspnoea, and two and none of the 19 asymptomatic patients that were screened for these complications respectively. Pulmonary arterial hypertension therefore appears to be more common in this population than in patients with cirrhosis.[2] Still, a bias might be introduced because dyspnoea is one of the presenting features of otherwise asymptomatic Abernethy malformation.

No clear recommendations can be made on management because of limited data on treatment and outcome.[4] Shunt closure was performed by surgery or interventional radiology in 15 (23%) of the VALDIG survey patients.[2] A one- or two-step procedure was performed depending on the apparent impact of an occlusion test. Overall tolerance was good. No new complications related to congenital extrahepatic portosystemic shunts developed after a fairly limited follow-up (median 2.5 years). In 19 patients with hepatic encephalopathy, improvement was reported after medical therapy in four patients, shunt closure in four patients and liver transplantation in two patients. A decrease in the size of benign nodules and an increase in liver volume were reported after shunt closure in a handful of analysable patients. Shunt closure was found to be a good option for pulmonary arterial hypertension in a small number of reported cases. Liver transplantation (in 6 patients) was only performed in patients with hepatocellular carcinoma or hepatic adenoma with or without hepatic encephalopathy.

Based on these observations, the algorithm proposed for the management of patients with Abernethy malformation suggests considering shunt closure for patients with encephalopathy but also for prophylaxis. This approach seems reasonable considering the minimal morbidity reported in experienced centres. However, because most patients probably have no manifestations until they are older, prognostic features for the development of complications remain a major unmet need.

3 | PORTAL VEIN THROMBOSIS IN PATIENTS WITH CIRRHOSIS

Portal vein thrombosis is common in patients with cirrhosis. Indicators for an increased risk of portal vein thrombosis have been studied in several cross-sectional studies. Features of severe portal hypertension and advanced liver disease have consistently been identified (see a recent review by Stine et al [5] and Chen et al [6]) and the role of these factors has been confirmed in longitudinal studies, as recently reviewed in Nery et al.[7] Recent surveys have focused on portal haemodynamic factors. Moreover, the entity of transient portal vein thrombosis has been singled out.

3.1 | Portal vein blood flow velocity

Because the well-known Virchow's triad of factors for venous thrombosis includes slowing of blood flow, portal vein blood flow velocity has been repeatedly evaluated as a potential risk factor. Results have been inconsistent. Two prospective longitudinal studies [8,9] and one retrospective case control study [10] did find a significant and independent association between baseline portal vein blood flow velocity and later development of portal vein thrombus. Another prospective study found an association on univariate analysis. However, multivariate analysis was not performed and the AUC using portal flow velocity for predicting portal vein thrombosis was small (0.709).[11] Nevertheless, another retrospective survey of longitudinal cases [12] and a prospective longitudinal study [7] did not confirm these results. Furthermore, when the development of portal vein thrombosis was analysed according to the changes in portal blood flow velocity preceding the event, no independent relationship could be shown.[13]

Interpretation of these inconsistencies is difficult for several reasons. First, there is a well-known inter- and intra-observer variability in the assessment of portal flow velocity using Doppler ultrasound.[14] There is also a well-known strong association between portal flow velocity and the severity of portal hypertension or severity of cirrhosis.[15-17] The latter association makes it difficult to clearly confirm an independent relationship between portal flow velocity and portal vein thrombosis after adjustment for portal hypertension and the severity of cirrhosis. Finally, the overall characteristics of patients differ in studies, particularly for the severity of liver disease at baseline. Therefore, it is probable that decreased portal flow velocity is related to the severity of portal hypertension/cirrhosis and plays a major role in portal vein thrombosis, besides the hypercoagulable state and other known or still unknown factors. The impact of portal vein blood flow velocity could be more marked in patients with advanced cirrhosis (where the incidence of portal vein thrombosis is highest and the slowing of portal blood flow is significant) than at earlier stages (where the incidence of portal vein thrombosis is lower and portal vein blood flow velocity is well preserved).

3.2 | Beta-adrenergic blocking agents

Non-specific beta-adrenergic blockers have long been shown to decrease portal tributary blood flow in patients and animals with portal hypertension, which is probably one of the major reasons these agents decrease both the portosystemic pressure gradient and the risk of gastrointestinal bleeding. As a result, they decrease portal vein blood flow velocity and could contribute to an increased risk of portal vein thrombosis in patients with cirrhosis. This issue has recently been addressed in several longitudinal studies. Univariate analyses have shown an association between portal vein thrombosis and the use of non-specific beta-adrenergic blockers.[7,13,18] However, this association was not found in a recent cross-sectional

study.[19] In many centres, non-specific beta-adrenergic blockers are so widely prescribed in patients with varices at risk of bleeding that they can be considered a surrogate for this feature. Therefore, it is not surprising that multivariate analyses adjusting for the presence of oesophageal varices at baseline have not all identified an independent role for non-specific beta-adrenergic blockers.[7,13,20] Moreover, in these studies the way oesophageal varices were taken into account in patients treated endoscopically (either with a 'history of' or at 'baseline endoscopy') probably significantly influenced the results of multivariate analyses. In particular, after endoscopic therapy, an absence of varices at inception actually means that there were varices at high risk of bleeding. Furthermore, the size of recent prospective studies has been small.[20] We explored whether the haemodynamic changes induced by non-specific beta blockers could be related to the development of portal vein thrombosis [7] and found that their effect on portal vein thrombosis was not linked to changes in portal vein blood flow velocity or to changes in heart rate. Other haemodynamic mechanisms related to beta-adrenergic blockade could be involved, for example increased reduction in portal venous territory blood flow with stress associated with the release of adrenergic catecholamines.[21] Clearly, further long-term studies are needed to clarify these points.

3.3 | Transient portal vein thrombosis

Large long-term studies have clearly shown that most portal vein thrombi documented in patients with cirrhosis only partially occlude the lumen of the portal vein. Moreover, when studied longitudinally, a high proportion regress in size or resolve, but then recur later in many patients.[22] Therefore, the causes or risk factors, and the prognostic value and consequences of partial occlusion could differ from those of complete and permanent occlusion, which is typical in non-cirrhotic portal vein thrombosis and occasionally seen in patients with cirrhosis.

A precise definition of transient portal vein thrombosis, which is a condition where portal vein thrombus resolves, is needed. In particular, the diagnostic imaging method, the schedule for imaging follow-up to identify clearance or persistence, and possible regression without complete resolution must be clarified. Computed tomography and magnetic resonance imaging after vascular contrast enhancement are the preferred options for a precise delineation of the portal vein thrombus. These methods also provide precise quantification of the luminal occupancy by the thrombus, which is needed in clinical studies,.[23,24] Based on a randomized prospective study comparing 3-month and 6-month intervals for a repeated Doppler ultrasound examination, a 3-month interval appears to be optimal for the characterization of transience.[13]

Currently available epidemiological data indicate that approximately 30% of portal vein thrombi are transient.[22] However, a systematic meta-analysis emphasized the significant heterogeneity of the different studies analysed.[10] The heterogeneity was probably based on several factors, including different time frames for evaluation, different selection criteria for the study population (Child-Pugh class, hepatocellular carcinoma, waiting list for liver transplantation or control groups in of retrospective studies on anticoagulation therapy) as well as an evaluation of abstracts vs full papers.[7]

Limited data suggest that although spontaneously transient portal vein thrombosis is associated with severe liver disease it has no independent impact on outcome and prognosis after adjustment for the severity of portal hypertension and cirrhosis.[7,12,13,23,25] Apparently, this does not agree with an increasing body of data on the impact of recanalization during anticoagulation therapy. Recanalization during anticoagulant therapy is related to improved event-free survival after adjustment for the severity of liver disease and portal hypertension,[26-30] improved liver function tests [31,32] as well as decreased mortality, particularly in patients with the most severe liver disease.[29] It is frustrating to note that no predictors for the resolution of portal vein thrombus have been identified.[12,22,23,33] Results on analyses to identify predictors for recanalization on anticoagulation therapy have been varied. The delay in initiating anticoagulation is the only consistent factor.[30-32,34-36]

Two low-powered studies evaluated recurrence after spontaneous resolution, which was found to occur in 9 out of 20 patients [12] and 19 out of 89 patients[13] respectively.

In conclusion, although the entity of transient portal vein thrombosis could account for a large proportion of cirrhotic portal vein thromboses, it can only be identified by repeated imaging. A consensus definition must be drafted that specifies the time frame for re-evaluation and imaging procedure to be used. Data on the impact of this entity on prognosis and outcome are still insufficient. Longitudinal studies are needed. However, this entity should be taken into consideration when deciding on anticoagulation therapy by asking the questions: (a) Is transient portal vein thrombosis really related to the risk of extrahepatic thrombus extension? And (b), is transient portal vein thrombosis a surrogate for intra/extrahepatic activation of coagulation which could per se, be the therapeutic target?

4 | RECANALIZATION OF THE THROMBOSED PORTAL VEIN

4.1 | In the absence of cirrhosis

4.1.1 | Recent portal vein thrombosis

In the last two decades, early anticoagulation has become the mainstay for achieving recanalization in patients with recent portal vein thrombosis in the absence of cirrhosis.[37,38] According to observational retrospective and prospective studies, this option is associated with complete portal vein recanalization in almost 40% of patients, and partial recanalization in an additional 14%, whereas spontaneous recanalization can only be expected in up to 17%.[39-41] This is associated with a very low risk of intestinal infarction, almost complete prevention of extensive intestinal resection, and an absence

of short-term mortality related to portal vein thrombosis or anticoagulation therapy.[40,42] However, non-recanalization after 6 months corresponds to permanent occlusion and results in the development of a portal cavernoma and permanent portal hypertension. The independent risk factors identified for an absence of recanalization include splenic vein thrombosis and detectable peritoneal fluid at presentation. The severity of initial features does not appear to influence recanalization or protection from intestinal ischaemia.[42] While one retrospective study in 38 patients has suggested that a delayed initiation of anticoagulation therapy (7 to 30 days after the first symptoms compared to within 7 days) affects recanalization ($P < .05$),[43] this was not found in a prospective multicentre study in 95 patients.[42]

A suboptimal rate of portal vein patency and/or a perceived higher risk of intestinal infarction in patients with more severe or protracted symptoms have prompted several groups to attempt recanalization using systemic and/or local thrombolysis. A systematic review of reported experiences did not support routine use of the latter approach because of a similar rate of complete recanalization (40%) and a high rate (up to 60%) of severe procedure-related and sometimes fatal, bleeding.[40] In recent years, retrospective studies have focused on a gradual approach in patients with persistent symptoms (mostly pain) or with imaging results of extension of the clot burden several days after initiation of anticoagulation therapy. Benmassaoud et al reported low-dose systemic thrombolytic agent administration as the second step followed by catheter-directed thrombolysis.[44] Three different approaches to the portal vein have been reported: transhepatic,[45-47] transjugular [44,45,48,49] or per operative.[50,51] Generally, mechanical thrombectomy was attempted first, followed by catheter-directed thrombolysis. When the transjugular route was used it was not systematically followed by TIPS placement [45,49,52] or TIPS was inserted first [44,48] or not inserted.[46] Overall, complete recanalization was achieved in about 50% of patients, and partial recanalization in 25%. Procedure-related complications occurred in 40 to 75% of patients and severe complications in 10 to 40%. One patient died because of a transhepatic procedure.[45] The transhepatic and per-operative approaches appear to be associated with a higher risk of complications than the transjugular approach.[16,45] It is difficult to interpret these results because the criteria for failed anticoagulation, which triggers a more aggressive approach, were not clearly defined. It should be remembered that the short- and medium-term outcome of acute portal vein thrombosis is excellent with anticoagulation alone and that chronic portal hypertension without liver disease is a manageable condition with an apparently acceptable quality of life in most patients. Mortality from bleeding because of portal hypertension in the absence of liver disease is extremely low, even on anticoagulation therapy.

In conclusion, randomized controlled trials are needed to compare the outcomes of anticoagulation and catheter-directed thrombolysis in similarly selected patients with recent portal vein thrombosis in the absence of cirrhosis. The favourable benefit risk balance of systemic low-dose thrombolysis as a first step must be confirmed. The transjugular approach appears to be the best option. The criteria for inserting TIPS following successful catheter-directed thrombectomy/thrombolysis must be clarified. Safety issues must be carefully evaluated.

4.1.2 | Portal cavernoma

It remains unclear whether the portal vein can be recanalized after the development of cavernoma. The definitions in various studies differ and it is uncertain whether so-called chronic portal vein thrombosis really corresponds to a well-developed cavernoma. Recent reports of selected cases suggest that recanalization may be achieved through percutaneous manoeuvres. Several approaches have been used. In particular, successful recanalization has been achieved by the transjugular transvenous route alone, usually followed by a TIPS insertion.[48,53-57] Successful recanalization has also been obtained by the transhepatic route alone,[58] or a combination of a transhepatic or transplenic approach with secondary TIPS.[59-61] Although the technical advances are impressive, the heterogeneity of the reported procedures, uncertainty about the case definitions, short follow-up, the small reported series and the risk of a publication bias prevent drawing any conclusions on the clinical elements of these cases. In any case, clinicians should be extremely cautious before making any decisions to perform invasive procedures in patients with portal cavernoma because of the efficacy of pharmacological and endoscopic treatment for portal hypertension, the absence of significant parenchymal liver disease and the excellent long-term prognosis except for the underlying diseases. At present patients with cavernoma with the poorest outcomes usually have involvement of the superior mesenteric and splenic veins,[62] which renders portal vein recanalization irrelevant.

4.2 | In patients with cirrhosis

The most recent systematic review including meta-analyses of recanalization and the progression of PVT in patients with cirrhosis comparing the effects of anticoagulation therapy to no therapy included eight studies with 353 patients.[63] This study showed that portal vein recanalization was more frequent with anticoagulation than without (complete or partial 71% vs 42%, complete 53% vs 33%, respectively, $P < .0001$), and that progression was less common (9% vs 33%, respectively, $P < .0001$). Furthermore, the overall bleeding rate was similar, but the rate of variceal bleeding was significantly lower ($P = .04$) in patients receiving anticoagulants. Since this paper was published, a dozen, mainly retrospective studies have been published, largely confirming these findings in a total of 653 patients receiving anticoagulation, and including 312 patients who received anticoagulants compared to 251 patients who did not.[28-32,35,64-67]

These recent studies show the high risk of recurrence following discontinuation of anticoagulation (43% in 219 patients at risk). Even more interestingly, these studies provide data on additional points that are important for the interpretation of mainly morphological

data. Not surprisingly multivariate analyses in these retrospective observational studies showed significant differences between patients who received anticoagulants and those who did not. In particular, a lower proportion of past variceal bleeding or cavernoma, lower blood urea levels,[30] a smaller proportion of alcoholics, severe liver disease or cavernoma [35] were found as well as an increased proportion of women or non-specific beta-blockers[31] and a higher proportion of involvement of the superior mesenteric vein or multiple venous sites.[29] These factors were not taken into account in the 2017 meta-analysis.[63]

Although many studies were small, most performed multivariate analyses to identify factors associated with recanalization. However, no solid conclusions can be drawn from the extremely heterogeneous findings except for the negative influence of delaying the initiation of anticoagulation therapy by more than 6 months in two studies [32,34] as previously suggested.[26] In particular, results on the influence of the severity of liver disease (assessed by the MELD or Child Pugh scores) differed and showed a higher rate in most severe patients [29,30] or in compensated patients.[32,34] Extension and the degree of lumen occupancy were rarely identified as prognostic indicators for recanalization.[34]

Finally, an increasing number of studies have evaluated the most important issue, which is the impact of recanalization on survival and the need for liver transplantation. None of these studies except one [28] found any influence,[29,30,32,35,64,67] although univariate analyses often suggested that recanalization was favourable. On the other hand, several studies have suggested that anticoagulation therapy has an independently positive effect, regardless of recanalization or the severity of liver disease.[30,31,35] Thus a dissociation similar to that already noted in a randomized controlled trial [68] and a prospective observational study [13] was found between portal vein obstruction, disease severity and the influence of anticoagulation.

5 | DIRECT ORAL ANTICOAGULANTS AND SPLANCHNIC VEIN THROMBOSIS

The limitations of heparins and vitamin K antagonists in general and in patients with portal hypertension in particular are well documented.[69,70] The use of direct oral anticoagulants has been evaluated in splanchnic vein thromboses. Although the use of direct oral anticoagulants for Budd-Chiari syndrome and portal vein thrombosis is rapidly increasing,[71-74] studies remain limited if not anecdotal. Indeed, the small numbers of patients in studies evaluating Budd-Chiari syndrome, recent portal vein thrombosis and portal cavernoma in the absence of cirrhosis,[31,52] and portal vein thrombosis in patients with cirrhosis,[65] prevents drawing any conclusions and making any recommendations. Experience is increasing in patients with cirrhosis without splanchnic vein thrombosis but with other indications (mostly atrial fibrillation).[73,75] Although all these retrospective analyses suggest that the use of direct oral anticoagulants is feasible, much data are still missing, in particular on the optimal dose as well as on monitoring and pharmacological reversal. Furthermore, head-to-head comparisons of these various agents are needed because of the markedly different pharmacokinetic profiles, as well as potential liver toxicity.[76] One randomized but not blinded trial comparing warfarin with rivaroxaban for the treatment of portal vein thrombosis in patients with cirrhosis identified a clear advantage for rivaroxaban [77] and one unblinded randomized controlled trial comparing rivaroxaban to placebo is ongoing in patients with non-cirrhotic portal vein thrombosis (NCT02555111). Randomized double-blind trials are also ongoing for the prophylactic treatment of portal vein thrombosis in patients with cirrhosis comparing rivaroxaban to placebo. Until these data become available, it is safe to conclude that the use of direct oral anticoagulants in patients with portal hypertension is feasible but based on limited results. This should remain an individual option, and decided in relation to other published results with enoxaparin, but not with vitamin K antagonists.

CONFLICT OF INTEREST

I declare that I have no conflict of interest with respect to this paper or with any other matter.

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REVIEW ARTICLE

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The future of autoimmune liver diseases – Understanding pathogenesis and improving morbidity and mortality

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Abstract

Autoimmune liver diseases (AILD), namely autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), are rare diseases. These days, patients with PBC almost never require liver transplantation. When treated early with ursodeoxycholic acid patients have a normal life expectancy if the disease is diagnosed at an early stage and the patients respond to treatment. Patients with AIH often go into remission with first-line therapy including corticosteroids alone or in combination with azathioprine. Nevertheless, about one quarter of patients already developed cirrhosis at diagnosis. Those who do not respond to first line standard of care (SOC) have significant liver-related morbidity and mortality. No approved second- or third-line treatments are available and the drugs are selected based on limited case series and personal experience. Larger trials are needed to develop efficient therapies for difficult-to-treat AIH patients. No treatment has been found to alter the natural course of disease in patients with PSC except for liver transplantation. Identifying PSC patients at risk of developing cholangiocarcinoma (CCA) is another unmet need. Current research in all AILD including AIH, PBC and PSC, focuses on improving our understanding of the underlying disease process and identifying new therapeutic targets to decrease morbidity and mortality.

KEYWORDS

autoimmune hepatitis, cholangiocarcinoma, primary biliary cholangitis, primary sclerosing cholangitis, regulatory T cells

1 | INTRODUCTION

The term autoimmune liver diseases (AILD) includes three different entities: primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH). The aetiologies are unknown and the pathogenesis of these diseases is poorly understood. A better understanding of the aetiopathogenesis of these entities should improve the diagnosis and treatment of AILD patients and prevent liver-related mortality and transplantation.

Abbreviations: AIH, autoimmune hepatitis; AILD, autoimmune liver diseases; ALP, alkaline phosphatase; CCA, cholangiocarcinoma; FGF19, fibroblast growth factor 19; FMT, faecal microbiota transplantation; FXR, farnesoid-X-receptor; IBD, inflammatory bowel disease; IL-2, interleukin-2; OCA, obeticholic acid; PBC, primary biliary cholangitis; PPAR, peroxisome proliferator-activated receptor; PSC, primary sclerosing cholangitis; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

2 | PRIMARY BILIARY CHOLANGITIS

Primary biliary cholangitis, a non-suppurative destructive cholangitis, is the archetype of an autoimmune disease. Women are affected more often (approximately 9:1) than men and well-defined antibodies targeting the E2 subunit of the pyruvate dehydrogenase complex are a diagnostic hallmark. PBC is associated with a variety of other autoimmune disorders, for example celiac disease, autoimmune thyroid disorders and systemic lupus erythematosus, to name a few. Originally PBC was called primary biliary cirrhosis because patients were often diagnosed in advanced stages of the disease. At present, due to improved techniques, PBC patients are diagnosed in earlier stages with less advanced fibrosis. In the 1980s, PBC was a leading indication for liver transplantation but, nowadays, liver transplantation for PBC is a rare event. Therefore, the name was changed to PBC to mirror the current prognosis of PBC. PBC patients, who are diagnosed at an early stage of the disease and who respond to ursodeoxycholic acid (UDCA) therapy have a life expectancy similar to age- and sex-matched controls. These patients no longer develop end-stage liver disease and the need for liver transplantation is rare. Disease progression is stopped if alkaline phosphatase (ALP), a surrogate marker for inflammation of the bile ducts, normalises or drops by more than 40% within one year after the initiation of UDCA therapy (13-15 mg/kg body weight daily) (Barcelona criteria).[1,2] There are multiple definitions for UDCA treatment failure. UDCA treatment failure occurs in 25%-50% of these patients depending on the criteria.[3] Non-response one year after treatment initiation places these patients at a higher risk of disease progression, including the development of hepatocellular carcinoma and end-stage liver disease.[4] The POISE (Phase 3) trial evaluated obeticholic acid (OCA), a farnesoid-X-receptor (FXR) agonist for the treatment of patients with PBC who did not achieve a reduction in ALP with UDCA to less than 1.67 × upper limit of normal (ULN) or in bilirubin to below 2 × ULN. The primary endpoint was ALP <1.67 × ULN with at least 15% ALP-reduction and normalization of bilirubin at 12 months. This endpoint was reached both in the 5-10 mg OCA group (with or without UDCA) and the 10 mg OCA (with or without UDCA) group in 46% and 47% respectively. Patients in the placebo group (with or without UDCA) reached the primary endpoint in 10% of cases. As expected, the major side effect of OCA treatment was dose-dependent pruritus, which led to discontinuation of treatment in up to 10% of patients. In addition, an increase in LDL-cholesterol was observed and the long-term cardiovascular risk in PBC patients treated with OCA must still be determined.[5] In 2018, the BEZURSO (phase 3) trial was published, evaluating bezafibrate in combination with UDCA in non-responders. The primary endpoint was complete biochemical remission after 24 months.[6] Roughly one third of all patients reached this primary endpoint and two thirds achieved normalized ALP values with bezafibrate. The main side effects in the bezafibrate group were a 5% increase in creatinine values due to a pharmacological effect, not caused by reduced kidney function, and myalgia in 20% of patients (10% of patients had myalgia in the placebo group).[6] Although bezafibrate has not yet been approved for the treatment of PBC, it is an appealing off-label treatment option in non-responders to UDCA treatment, with a favourable side-effect profile and low treatment costs. A recent small trial from Leuven, Belgium, evaluated the additive effects of bezafibrate in patients who did not respond adequately to dual therapy with OCA and UDCA. Triple therapy (bezafibrate, OCA, UDCA) lowered cholestatic parameters and improved pruritus in most patients.[7] There are ongoing trials to clarify the mechanism of action of bezafibrate, which is an agonist for the peroxisome proliferator-activated receptor (PPAR). While this agent works as an agonist on different PPARs, selective PPAR-δ- (Seladelpar; CYMABAY) or PPAR-α/δ-agonists (Elafibranor; GENFIT) are also under evaluation in phase III trials for PBC to improve the efficacy and reduce side effects in patients who do not respond to UDCA therapy. Seladelpar demonstrated anti-cholestatic properties and improved pruritus in both patients with and without cirrhosis (Child A)[8] and elafibranor has also been shown to have anti-cholestatic effects associated with a reduction in pruritus.[9] Another trial in PBC is evaluating the use of the fibroblast growth factor 19 (FGF19) agonist NGM282 (NGMBio). FGF19 is mainly located in the ileum and serves to regulate bile acid metabolism. Production of FGF19 is induced by bile acid-dependent activation of the FXR receptor. FGF19 translocates via the portal system to the liver and suppresses expression of the rate-limiting enzyme for de novo bile acid synthesis in the liver (CYP7A1). In a small 28-day trial, NGM282 improved ALP by at least 15% from baseline.[10]

In summary, PBC, as a role model for autoimmune diseases in general and liver diseases in particular, is no longer a major indication for liver transplantation. PBC patients who are diagnosed early and respond to UDCA have a normal life expectancy. New PBC treatments, in particular OCA, have recently been approved in non-responders to UDCA. New agents are under investigation in PBC patients to further improve efficacy and tolerance.

Key points

- Disease modifying treatments for primary sclerosing cholangitis (PSC) are desperately needed. There is no existing treatment that alters the natural course of the disease.
- New biomarkers for the risk-stratification of both PSC and autoimmune hepatitis (AIH) are needed.
- New biomarkers are needed to predict the non-response to ursodeoxycholic acid (UDCA) in primary biliary cholangitis (PBC)
- Salvage therapies are an unmet need in AIH.
- Selective peroxisome proliferator-activated receptor (PPAR) and fibroblast growth factor 19 (FGF19) agonists are under clinical development for the treatment of PBC patients that do not respond to UDCA.

3 | PRIMARY SCLEROSING CHOLANGITIS

Primary sclerosing cholangitis is a rare liver disease that significantly affects quality of life, morbidity and mortality. The aetiology of PSC is unknown and its pathogenesis is poorly understood. The only existing life-saving treatment option is liver transplantation. Nevertheless, PSC reoccurs in 20%-37% of patients after liver transplantation.[11] No drugs have been shown to be effective in preventing death, liver transplantation or cholangiocarcinoma (CCA). Although UDCA improves ALP, it does not improve survival. The role of ALP as a surrogate marker for treatment response is a subject of debate. The prognosis is poor in patients with PSC; up to 40% require liver transplantation and up to 31% develop CCA. Inflammatory bowel disease (IBD) is present in most cases.[12,13] The diagnosis of PSC is mainly based on imaging, in particular endoscopic retrograde cholangio-pancreatography and magnetic resonance cholangio-pancreatography. Strictures and prestenotic dilatations of the large and medium-sized intra- and extrahepatic bile ducts are characteristic features. Sclerosing cholangitis from other causes must be excluded, for example, ischemic-type biliary lesions after liver transplantation, secondary sclerosing cholangitis usually after long-term intensive care, or IgG4-related cholangitis. Liver histopathology typically shows concentric 'onion-skin-like' fibrosis around the bile ducts, which is often accompanied by elevated cholestatic laboratory test results. It has been hypothesized that the pathogenesis of PSC is multifactorial, but it is poorly understood. Various therapeutic agents are under investigation for PSC with multiple molecular targets (e.g. FXR, PPAR, adhesion molecules and the microbiota) in different organs (e.g. liver and gut). Improving the understanding of the aetiopathogenesis of this disease can help identify future therapies. Recent published studies support a potential role for bacterial translocation from the gut to the liver due to intestinal barrier dysfunction. Nakamoto et al[14] showed that *klebsiella pneumoniae*, *proteus mirabilis and enterococcus gallinarum* were prevalent in patients with PSC and responsible for bacterial translocation as well as for hepatobiliary inflammation in gnotobiotic mouse models. The progression of hepatobiliary disease was promoted via a TH17 response.[14] Therefore antibiotic therapy might be beneficial in the natural course of PSC.

It is also urgent to improve the risk stratification of unfavourable outcomes in patients with PSC. Recent studies have shown that elevated Immunoglobulin G (IgG) at diagnosis is an independent predictor of transplant-free survival.[15] The Amsterdam-Oxford model has been proposed to stratify the risk of liver transplantation or death in PSC patients using seven available variables (albumin, platelet count, age, PSC subtype, aspartate-aminotransferase, ALP and bilirubin).[16] New biomarkers are needed to diagnose PSC, predict disease outcome and serve as monitoring parameters. Bile proteomic profiles might help identify PSC patients at risk of developing CCA, thus facilitating early diagnosis of CCA and improving the prognosis by reinforcing screening for malignancy.[17] Because UDCA has not been found to improve the outcome of liver disease in patients with PSC, new therapies are urgently needed. Recent evidence has supported a link between the liver and the gut in the pathogenesis of PSC. The potential impact of IBD therapies on PSC were evaluated based on the strong association between PSC and IBD. A retrospective analysis by Tse et al found initial improvement in ALP in patients with PSC and concomitant IBD who were treated with the anti-tumour-necrosis factor alpha (TNF) antibody, adalimumab.[18] However, these findings were not confirmed when PSC patients were treated with another anti-TNF antibody, infliximab. No improvement in liver biochemistry was seen in patients with IBD and concomitant PSC receiving vedolizumab, an $\alpha 4\beta 7$ integrin inhibitor.[18] A recent trial investigated the feasibility and safety of faecal microbiota transplantation (FMT) in patients with PSC. Ten patients with PSC and concomitant IBD and an ALP >1.5 × ULN were included and received a single FMT via colonoscopy. A more than 50% decline in ALP levels was observed in 30% of patients and no adverse events occurred.[19] Thus, further evaluation of FMT is needed in patients with PSC and IBD. The non-steroidal FXR-agonist, cilofexor has been shown to improve cholestasis and liver injury in a small trial with 52 patients who were randomized to receive a placebo and two groups with different doses of cilofexor. Treatment with the maximum dose of cilofexor over a period of 12 weeks led to a 21% decrease in ALP and to significant improvement in aminotransferase levels.[20] Nor-UDCA, a side-chain modified derivate of UDCA, had dose-dependent beneficial effects on ALP values in a phase II trial.[21] A phase III study with NorUDCA for PSC is ongoing. Rupp et al showed that endoscopic bile duct dilatation was beneficial in patients with PSC in a large retrospective study.[22] PSC patients with a dominant bile duct stricture received either endoscopy with or without bile duct dilation at defined intervals or in case of clinical symptoms, an approach that does not follow current recommendations from European and American guidelines. The outcome in patients who received scheduled dilation therapy was significantly better for transplant-free survival and time to transplantation, with a reasonable safety profile and no increase in intervention-associated adverse events. This study is worth noting because it chose hard endpoints such as transplant-free survival while previous studies have only used ALP as a surrogate marker. Nevertheless, hard endpoints such as death or the need for liver transplantation are difficult to assess in clinical trials of such a rare disease. New biomarkers are needed that are more robust than biochemical markers such as ALP. Finally any new drug for PSC should be evaluated for hard endpoints such as death or transplant-free survival. Furthermore, risk stratification of PSC subgroups must be improved to effectively analyse potential improvements in mortality or the need for liver transplantation with any new agents. Because patients with PSC with or without associated IBD are a heterogeneous group with a variety of disease phenotypes, a more individualized approach may be needed for multimodal treatment options in the future.

4 | AUTOIMMUNE HEPATITIS

Autoimmune hepatitis is characterized by elevated aminotransferases, hypergammaglobulinaemia, the presence of characteristic

auto-antibodies (e.g. anti-nuclear antibodies [ANA] and smooth muscle antibodies [SMA] in AIH type 1, liver-kidney-microsome antibodies [LKM] in AIH type 2) and histopathological features such as plasma cell enriched infiltrates, emperipolesis and interface hepatitis. Neither histopathology, auto-antibodies or hypergammaglobulinaemia alone can diagnose AIH. All of these features and the exclusion of other liver diseases are necessary to make the diagnosis of this disease, which can be confirmed by a response to corticosteroids. New diagnostic markers are needed to diagnose AIH as well as to monitor treatment response. Existing auto-antibodies either lack sensitivity or specificity or both. Although the sensitivity and specificity of ANA and SMA are acceptable, they are not specific for AIH. Anti-soluble-liver-antigen antibodies and LKM-antibodies are highly specific but their sensitivity is limited.[23] Although anti-asialoglycoprotein receptor antibodies have the best overall performance, they are not widely available.[24] We recently showed that anti-huntingtin-interacting protein 1-related protein (anti-HIP1R protein) antibodies, measured using enzyme-linked immunosorbent assay, were elevated in AIH patients with a higher specificity than and equal sensitivity to ANA and SMA.[25] Normalization of IgG and aminotransferases as well as a lack of inflammatory activity in liver histology are the main treatment goals, called complete remission. A subgroup of patients fails to achieve remission with corticosteroids and/or azathioprine, which are the only approved agents for the treatment of AIH. Large randomized, controlled trials that evaluate the efficacy and safety of other immunosuppressive drugs except for budesonide, are lacking.[26] Second- and third-line therapies are used off-label in daily clinical practice for treatment failures or intolerance to standard of care (SOC) with corticosteroids alone or combined with azathioprine. Mycophenolate mofetil is the second treatment of choice in patients who do not tolerate azathioprine. Several drugs are used in non-responders to first line treatment with SOC, including calcineurin inhibitors such as cyclosporine A and tacrolimus. However, none of these drugs have been approved for this indication. Although Weiler-Norman et al showed that anti-TNF-antibodies induced remission in a series of difficult-to-treat patients,[27] infectious complications developed in up to 70% of these patients. However, several case reports indicate that TNF-antibodies may even cause AIH.[28] Rituximab, a B-cell depleting agent, has been shown to induce remission in hard-to-treat AIH patients with lower complication rates.[29] Another B-cell depleting monoclonal antibody targeting the B-cell activation factor receptor is under investigation in a phase II/III trial in AIH patients who do not respond to SOC.[30] Furthermore, there is a reduced balance of regulatory and effector T cells in patients with AIH who do not achieve remission. Interleukin-2 (IL-2) is a key cytokine for T-cell tolerance and IL-2 therapy has shown to restore immune tolerance by restoring impaired regulatory T-cell function. A case report of low dose IL-2 therapy with 1 million units five times a month for six months showed an increase in regulatory T cells and induced remission in one of two treated AIH patients.[31] Data on the transfer of regulatory T cells in humans for this indication will soon be available. Both IL-2 therapy and the transfer of regulatory T cells are promising strategies, which require further investigation in larger trials. Because AIH is a rare disease and most patients respond to first line therapy, large prospective multicentre studies are difficult. Nevertheless, a multicentre international trial is urgently needed to perform robust studies for salvage therapies in patients with AIH and an incomplete response or intolerance to SOC. The European Reference Network (ERN) for rare liver diseases could facilitate the implementation of these studies in the future.

5 | SUMMARY

Overall, PBC is probably the best understood and well managed autoimmune liver disease. Two approved treatments and one off-label treatment are used in clinical practice. PBC patients have a normal life expectancy if they are diagnosed early, and if they respond to treatment. The goal of current trials for PBC is to maximize the response rate in non-responders to UDCA while maintaining a good safety profile. Research in AIH is focused on a better understanding of the pathogenesis of the disease to improve diagnosis and treatment. Available diagnostic tests lack specificity and sensitivity. Thus, the diagnosis of AIH often remains a clinical challenge because specific tests are not available and the diagnosis is based on several parameters and the exclusion of other liver diseases. New biomarkers are under investigation to meet this need. Treatment is mainly focused on patients who fail to achieve complete remission with the SOC (corticosteroids and/or azathioprine). Several second- and third-line agents, several of them approved for other autoimmune diseases, are available. New agents either targeting B lymphocytes or enhancing regulatory T lymphocytes are under investigation for the treatment of AIH patients who do not respond to first line SOC. PSC remains the 'black box' of hepatology. The aetiology is not understood and there are no treatment options except for liver transplantation. PSC is a significant risk factor for the development of CCA. Because the suggested aetiology is multifactorial, future management of PSC may be based on multiple approaches combining risk-stratification, effective screening strategies and drugs targeting different molecules and pathways optimizing the outcome of these patients. AILD are rare diseases. Multicentre prospective cohorts of patients with AILD are necessary for robust future trials to evaluate biomarkers, risk-scores and treatment options. The European Reference Network on hepatological diseases (ERN RARE-LIVER) might be the appropriate group to help implement these trials.

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CONFLICT OF INTEREST

Bastian Engel declares no conflict of interest related to this work. Richard Taubert is inventor of the patent application for the use of anti-HIP1R for the diagnosis of AIH. Elmar Jaeckel is inventor of the patent application for the use of anti-HIP1R for the diagnosis of AIH. Michael P. Manns is or was principal investigator for Falk Pharma, Gilead, Intercept and Novartis. He received grants from Falk Pharma, Gilead and Intercept. Michael Manns is or was a consultant for Falk Pharma, Gilead, Intercept, Roche and Novartis and he lectured for Falk Pharma and Gilead. Travel

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REVIEW ARTICLE

Liver INTERNATIONAL WILEY

Imaging of liver tumours: What's new?

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Abstract

Liver tumours are very common and malignant tumours represent a major cause of cancer-related death. Imaging plays an important role at many different stages of the care pathway. This review discusses new aspects and new roles for imaging and for MRI, in particular. MRI is already the best tool for the characterization and staging of benign and malignant liver tumours and it could also become a useful screening tool, especially for hepatocellular carcinoma. Liver imaging will be increasingly quantitative in the future, integrating new approaches such as those of artificial intelligence.

1 | INTRODUCTION

In this review article, we will focus on innovations in liver imaging. Liver imaging is still based on three pillars: ultrasound (US) and contrast-enhanced US, computed tomography (CT), and magnetic resonance imaging (MRI). Each imaging technique has improved in the last few years with better spatial and contrast resolution, shorter acquisition times, and decreases in radiation doses for CT. However, there has not been a revolution in liver imaging, instead, most changes have been based on new indications and not breakthrough technical improvements. The main innovations include: (a) the role of imaging at different stages of disease: screening, diagnosis, staging, and monitoring tumour response; (b) the shift to a more quantitative approach; and (c) an integrated approach including other sources such as pathology for 'integrated diagnostics'. This article will discuss the different steps of this process, except for post-therapeutic imaging.

2 | WHAT'S NEW FOR SCREENING MALIGNANT LIVER TUMOURS?

2.1 | Hepatocellular carcinoma

Approximately 90% of hepatocellular carcinomas (HCC) are associated with a known underlying cause, usually chronic viral hepatitis (B and C), alcohol intake, aflatoxin exposure or non-alcoholic fatty liver disease (NAFLD) associated with metabolic syndrome. According to EASL guidelines, HCC surveillance is recommended in patients with cirrhosis, in HBV patients without cirrhosis with an intermediate or high risk of HCC and Fibrosis 3 patients without cirrhosis, regardless of aetiology.[1] US is the method of choice and is often indicated to monitor other conditions outside of HCC surveillance, such as the development of portal hypertension, including the onset of ascites or portal vein thrombosis. US should be performed by experienced personnel every 6 months. However, US is very challenging in patients with chronic liver disease, especially those with a coarse liver texture. A recent meta-analysis based on 32 studies (including 13 367 patients) characterized the sensitivity of US with or without AFP measurement for the detection of HCC in patients with cirrhosis.[2] Although ultrasound detected any stage of HCC with a sensitivity of 84% (95% CI, 76%-92%), the sensitivity for early-stage HCC was only 47% (95% CI, 33%-61%).

The question is whether CT or MR imaging could play a role in the surveillance of HCC. One trial comparing CT and ultrasound did not show any significant improvement in early HCC detection with CT. Indeed, there is a risk of radiation with CT due to repeated exposure and potential contrast-induced nephrotoxicity.[3]

MRI-based surveillance is usually reported to have a high specificity and sensitivity, including for the detection of early HCC. One trial comparing US and MR imaging showed that MRI was significantly more sensitive than US for early HCC detection. However, MRI is associated with well-known limitations such as high cost and availability.[4] To compensate for these drawbacks, several studies have evaluated the diagnostic performance of abbreviated MRI

Abbreviations: US, ultrasound; HCC, hepatocellular carcinoma; HCA, hepatocellular adenoma; LLCER, lesion to liver contrast enhancement ratio; ADC, apparent diffusion coefficient.

protocols. Several sequences are more sensitive than others for tumour detection in the liver, such as T2-weighted, diffusion-weighted, and hepatobiliary phase sequences after administration of hepatobiliary MR contrast agents.

Marks et al retrospectively showed that a simulated abbreviated MR protocol including T2-weighted and gadoxetic acid-enhanced hepatobiliary phase sequences has a high negative predictive value. The inclusion of a diffusion-weighted sequence did not significantly change the diagnostic performance of the abbreviated protocol.[5]

In another retrospective study in patients at risk of HCC, a simulated abbreviated MRI protocol including a T1-weighted, a hepatobiliary phase and a diffusion-weighted sequence was shown to have a sensitivity and negative predictive value of 80.6% and 80% respectively. This was considered to be acceptable, although it was lower than the contrast-enhanced protocol (90.3% and 94.9%).[6]

Lee et al chose another approach comparing dynamic contrast-enhanced MR sequences (using extracellular gadolinium contrast agent) to a conventional liver MRI protocol in patients at risk of HCC. They did not find any difference for liver reporting imaging and data system (LI-RADS) categorization between the abbreviated and the full MRI protocols in 93%-96%. The estimated time to run this abbreviated MRI was approximately 7-10 minutes.[7]

In summary, this is a very interesting topic and abbreviated MRI will probably play a greater role as a surveillance tool in patients at risk of HCC. Whether it will be used in all patients as a first-line surveillance tool or in patients in whom US is difficult such as those with metabolic syndrome or very high risk patients, will be discussed in the future.

2.2 | Liver metastases

Liver metastases are the most common cause of malignant liver lesions worldwide. Because they are often the main prognostic factor in these patients, early detection can strongly influence management. Patients with extrahepatic cancer who are at risk of lung and liver metastases are usually followed by serial chest and abdominopelvic CT. Until recently, the added value of MRI was not clear. Han et al investigated the impact of liver MRI in the staging assessment of newly diagnosed colorectal cancer patients and showed that the diagnostic yield of MRI for liver metastases in patients with tiny (too small to be characterized) liver lesions on CT or negative CT was very low.[8] Indeed, this clinical context is different from follow-up screening of liver metastases.

Canellas et al recently reported the results of a preliminary study of an abbreviated MRI protocol in patients with colorectal liver metastases including T2-weighted, diffusion-weighted and hepatobiliary phase sequences and showed a sensitivity and area under ROC curve >90%, which was not different from full MRI.[9] The acquisition time was less than 10 minutes. Thus, MRI plays a crucial role in the staging of liver metastases, while its role in screening for liver metastases has not been sufficiently assessed but seems promising.

Key Points

- The diagnostic performance of ultrasound in detecting early-stage hepatocellular carcinoma during surveillance programmes is low. MRI could become a first-line tool in these cases.
- Besides HNF1A-inactivated and inflammatory hepatocellular adenomas, which are well recognized on MRI, new imaging features could help recognize other subtypes.
- THE 2018 EASL guidelines for diagnosing hepatocellular carcinoma propose a step by step algorithm for contrast-enhanced imaging.
- MRI is the modality of choice for staging malignant liver tumours.
- Liver imaging is moving from morphological imaging to quantitative data.

3 | WHAT'S NEW FOR DIAGNOSING HEPATOCELLULAR ADENOMAS?

More than a decade ago, hepatocellular adenomas (HCA) were classified into molecular subtypes characterized by mutations inactivating HNF1A, activating β-catenin or inflammatory pathway.[10] The two most common subtypes, HNF1A-inactivated HCAs (HHCA) and inflammatory HCAs (IHCA) have been shown to have characteristic MR features enabling their diagnosis with high sensitivity and specificity.[11,12]

Zucman-Rossi et al recently defined a new subgroup of HCA characterized by sonic hedgehog activation (shHCA), which corresponds to 4% of previously unclassified HCAs. shHCAs are associated with obesity and symptomatic bleeding (71% of bleeding in shHCA vs 14% in other subgroups).[13] Although there are very few reports of imaging findings of shHCA, this subtype should be suspected when tumours exhibit haemorrhagic foci or large cystic spaces in a steatotic liver.

The two main predisposing factors for malignant transformation are HCA in men and beta-catenin exon 3 (bex3) HCA. Recognition of bex3 HCA could be clinically important, especially in women. The first description of imaging findings of bex3 HCA was non-specific with lesion heterogeneity on all MR sequences.[11] A first study showed that some HCAs retained gadoxetic acid in hepatobiliary phase MRI using this agent: six of 21 IHCAs, five of six bex3 HCA, but none of the 10 HHCAs and none of the six unclassified HCAs.[14]

Because retention of gadoxetic acid was not shown to differentiate IHCA from bex3 HCA, other researchers quantitatively evaluated lesion uptake by measuring the lesion-to-liver contrast enhancement ratio (LLCER) comparing the precontrast and hepatobiliary phases. Results showed that all IHCAs had a negative LLCER,

indicating that HCAs did not take up gadoxetic acid, while six of seven bex3HCAs had a positive LLCER, indicating that HCAs had retained gadoxetic acid.[15] This suggests that positive contrast uptake on hepatobiliary phase MRI might be a good indicator of the presence of bex3 activation.

In summary, studies are evaluating the MRI findings in shHCA, which often show bleeding on pathology. Quantitative MRI seems to be an interesting approach to differentiate bex3 HCA from other HCAs, which retain gadoxetic acid on hepatobiliary phase sequences. This may require quantitative assessment but could be important in clinical practice.

4 | WHAT'S NEW FOR DIAGNOSING HEPATOCELLULAR CARCINOMAS?

There have been several recently updated HCC guidelines. We will focus on the North American (AASLD) and the European (EASL) recommendations, which have included several changes:

- traditionally, the diagnostic algorithm of HCC in patients with chronic liver diseases begins with US. Indeed, US is the primary imaging modality in patients with chronic liver diseases and the recommended surveillance tool. Nevertheless, CT or MRI often identifies new nodules in patients with chronic liver diseases, especially in those who have already been treated for HCC. The new EASL algorithm considers any imaging modality (US, CT or MRI) as the entry point for nodule detection.

- while Asian guidelines (Japanese, Korean and Asia-Pacific) have recommended the use of hepatospecific MR contrast agents (and gadoxetic acid in particular) for the diagnosis of HCC, Western recommendations (AASLD and EASL) have always focused on extracellular MR contrast agents. The 2018 EASL guidelines have integrated all MR contrast agents including extracellular, gadobenate dimeglumine (with late hepatobiliary phase) and gadoxetic acid (with early hepatobiliary phase). When hepatobiliary MRI agents are used, EASL guidelines recommend that the transitional phase and the hepatobiliary phase not be taken into consideration for a non-invasive diagnosis. Thus, the question of which MRI contrast agents should be used is still a subject of debate in the literature, although recent publications have shown that extracellular MR contrast agents have a higher diagnostic performance than gadoxetic acid.[16,17] The advantages of extracellular contrast agents include better visualization of hyperenhancement on hepatic arterial phase sequences (with fewer respiratory artefacts), the use of delayed phase sequences, which improves visualization of tumour washout, and higher specificity.

4.1 | The role of contrast-enhanced US (CEUS)

The role of CEUS was questioned in the 2012 EASL guidelines because of the potential risk of misdiagnosis of cholangiocarcinoma, which appeared to occur at a rate of 2-5% of all new nodules in cirrhosis.[1] Studies have shown that the onset of washout takes place less than 60 seconds after contrast administration in most cholangiocarcinomas, while this is rare in HCC, and that the intensity of portal phase washout is more marked in cholangiocarcinoma than in HCC. This has led to a more precise definition of the typical features of HCC on CEUS, which is therefore arterial hyperenhancement followed by mild and late (>60 seconds) washout. According to the most recent EASL guidelines, CEUS is not a first-line imaging modality for diagnosing HCC but can be performed after a negative or inconclusive CT or MRI. Interestingly, the specificity of CEUS for diagnosing HCC is quite high and the sensitivity for diagnosing 20-30 mm HCC after a negative CT or MRI ranges from 54% to 62%.[18]

- The LI-RADS classification is constantly evolving. The 2018 version was endorsed by the AALSD. Major criteria are based on lesion size (10-19 mm or larger), the presence of non-rim hyperenhancement on hepatic arterial phase and the presence of additional findings such as washout, enhancing capsule and threshold growth. A lesion is classified as LI-RADS 5 (definitive HCC) if it is 20 mm or larger, shows hepatic arterial phase non-rim hyperenhancement and has any of the three additional findings of washout, enhancing capsule or threshold growth. A 10-19 mm lesion is classified as LI-RADS 5 (definitive HCC) if it shows hepatic arterial phase non-rim hyperenhancement with washout or threshold growth.

5 | WHAT'S NEW FOR STAGING MALIGNANT LIVER TUMOURS?

Here, we will focus on intrahepatic staging and we will not consider extrahepatic staging.

MRI is the modality of choice for intrahepatic staging, whatever the malignant tumour (HCC, cholangiocarcinoma or liver metastases).

5.1 | Hepatocellular carcinoma

HCC treatment is based on tumour characteristics (defined by the number and size of nodules, the presence of vascular invasion and extrahepatic spread), liver function (defined by Child-Pugh class, bilirubin, albumin, clinically relevant portal hypertension and ascites) and general tumour-related health status. Contrast-enhanced CT or MRI is mandatory to assess tumour characteristics and choose the best therapeutic option. While both of these tools accurately diagnose vascular invasion, visualization of multicentric and small satellite tumours is better with MRI. Again, the best sequences are T2-weighted, diffusion-weighted and hepatobiliary phase using MR contrast agents. Choi et al compared the diagnostic performances of CT and gadoxetic acid-enhanced MRI for assessing BCLC stage according to the final diagnosis. MRI outperformed CT and BCLC stage was changed correctly after gadoxetic acid-enhanced dynamic MRI in 14% of the cases.[19]

In another study, the Barcelona liver clinic cancer (BCLC) stage was changed following gadoxetic acid-enhanced MRI in 28% of patients and the treatment decision in 19%.[20] Gadoxetic acid-enhanced MRI detects not only additional HCC when the tumour is considered single but also identifies nodules that are not hyperenhanced on hepatic arterial phase but hypointense on hepatobiliary phase sequences. The presence of these nodules before treatment significantly influences recurrence-free survival after both hepatic resection and tumour ablation and can change treatment options.[21]

Gadoxetic acid-enhanced MRI may also be used to select potential candidates for liver transplantation. Kim et al have reported that satellite nodules and peritumoural hypointensity on hepatobiliary phase sequences on preoperative hepatobiliary MRI were associated with a higher tumour recurrence rate in patients transplanted either within or outside the Milan criteria.[22]

5.2 | Intrahepatic cholangiocarcinoma

The most important prognostic factors for intrahepatic cholangiocarcinoma are the presence of vascular invasion, tumour multiplicity, local extension, periductal infiltration and lymph node metastases. MRI should be used rather than CT because it is better at detecting additional tumours, especially small ones.

5.3 | Liver metastases

The treatment that provides the best overall survival in patients with colorectal liver metastases is surgical resection, often associated with perioperative chemotherapy. The goal of surgery is to obtain complete resection, and therefore, intrahepatic staging is highly important. MRI is known to be more sensitive than CT and fluorodeoxyglucose (FDG)-positron emitting tomography (PET) for detecting colorectal liver metastases on a per-lesion basis. The question is how to perform MRI and which contrast agents should be used. Thus, we performed a meta-analysis comparing the diagnostic performance of the two best MRI sequences: diffusion-weighted and gadoxetic-enhanced sequences.[23] Thirty-nine articles were included (1,989 patients, 3,854 metastases). Sensitivity estimates for diffusion-weighted, gadoxetic acid-enhanced MRI and the combined sequence for detecting liver metastases on a per-lesion basis was 87.1%, 90.6% and 95.5% respectively. A combination of the two sequences was significantly more sensitive than gadoxetic acid-enhanced MR imaging. Similar results were observed in liver metastases <1 cm.

Is liver MRI indicated to detect liver metastases in patients with potentially resectable pancreatic ductal carcinoma and normal liver CT? This question was evaluated in two recent studies because 13%-23% of patients who undergo a surgical procedure are found to have unresectable disease because of vascular involvement, peritoneal carcinomatosis or undiagnosed liver metastases. Liver metastases were seen in both studies on MRI in 10%-23% of the patients and were later confirmed, avoiding unnecessary laparotomy.[24,25]

Because surgical resection of pancreatic adenocarcinoma is still associated with a significant risk of morbi/mortality and there may be a certain number of unidentified liver metastases on CT, preoperative liver MRI should be performed in these patients.

6 | WHAT'S NEW FOR QUANTITATIVE IMAGING OF LIVER TUMOURS?

Most of the diagnostic imaging tools for detecting and characterizing liver tumours are still based on morphology: shape, contour, relative echogenicity/attenuation/signal and tumour enhancement. Although many quantitative variables are under investigation, few are used in daily practice, except for diffusion-weighted imaging, which exploits the random motion of water molecules in the tissues. This impedance of water molecule diffusion can be quantitatively assessed using the apparent diffusion coefficient (ADC) value. Malignant liver tumours have a much lower mean ADC than benign tumours. However, ADC values depend on several factors, for example, the type of sequence and b values, and therefore reproducibility is low. In practice, diffusion-weighted sequences should be systematically performed in liver MRI because they are quite sensitive for tumour detection, although the characterization and assessment of tumour response based on ADC values is still a topic of research.

There has been also great enthusiasm for liver perfusion obtained in CT perfusion studies or dynamic contrast-enhanced MRI. These techniques measure tissue perfusion, blood flow, and vascularity by analyzing the signal enhancement curve of tissue. Quantitative analysis requires computation-based curve-fitting algorithms, which are obtained with mathematical models. Although this approach could be used to determine tumour perfusion, an acquisition protocol that is not available in clinical practice is required to obtain the data.

6.1 | Radiomics

Instead of looking at one quantitative parameter, it is now possible to rapidly extract numerous quantitative features from CT or MRI images. Radiomics is defined as the conversion of digital medical images into mineable high-dimensional data. The idea is that medical images contain information (not visible to the naked eye) that reflects underlying pathophysiology and that these relationships can be determined via quantitative image analyses.[26] Quantitative image features based on intensity, shape, size or volume and texture offer information on tumour phenotype. Radiomics is well suited for oncology and is being evaluated for several indications including detection, diagnosis, assessment of prognosis and prediction of response to treatment. Results have been encouraging in several studies evaluating the use of radiomics in HCC, particularly for the pretreatment prediction of biological tumour characteristics, the risk of recurrence and survival.[27]

6.2 | From radiomics to artificial intelligence

Artificial intelligence machine-learning algorithms are boosting the power of radiomics for prognostic prediction and factors associated with treatment strategies such as survival time, recurrence, adverse events and subtypes. Therefore, radiomic approaches combined with artificial intelligence could have practical applications in precision medicine.[28]

6.3 | Integrated diagnostics

Although both radiology and pathology are essential for liver tumour characterization, the two medical specialties have worked in silos for many years. However, things are changing because: (a) pathology is not necessarily the ultimate method of reference and (b) radiology can explore microscopic changes and therefore provide additional information. We should now consider integrated diagnostics, which is defined as the seamless collaboration among the diagnostic specialists in particular pathologists and radiologists. The goal of integrated diagnostics is to reduce the time and expense of diagnostic processes and provide clinicians with practical, implementable results. There are many examples in liver tumours, in particular hepatocellular adenoma with a strong genotype-phenotype relationship.

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