

EASL Clinical Practice Guidelines on hepatitis delta virus[☆]

European Association for the Study of the Liver^{*}

Summary

Hepatitis D virus (HDV) is a defective virus that requires the hepatitis B virus to complete its life cycle and cause liver damage in humans. HDV is responsible for rare acute and chronic liver diseases and is considered the most aggressive hepatitis virus. Acute infection can cause acute liver failure, while persistent infection typically causes a severe form of chronic hepatitis which is associated with rapid and frequent progression to cirrhosis and its end-stage complications, hepatic decompensation and hepatocellular carcinoma. Major diagnostic and therapeutic innovations prompted the EASL Governing Board to commission specific Clinical Practice Guidelines on the identification, virologic and clinical characterisation, prognostic assessment, and appropriate clinical and therapeutic management of HDV-infected individuals.

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Introduction

Hepatitis D or delta virus (HDV) is a defective virus, as its life cycle depends on the hepatitis B virus (HBV), from which HDV borrows all three HBV envelope proteins (HBV surface antigen [HBsAg]) to both enter and egress from the hepatocyte and sustain its productive infection.¹ HDV has a negative-sense single-stranded RNA genome of about 1,700 nucleotides. Recent proposals suggest classification into the *Deltavirus* genus of the *Kolmioviridae* family, part of the *Ribozyviria* realm.² Within the infected cell nucleus, HDV utilises the host RNA polymerase II to replicate via double rolling circle RNA synthesis. Newly synthesised, multimeric linear RNAs undergo autocatalytic cleavage and the resulting monomers are circularised via host cell-mediated ligation³ (Fig. 1). HDV replication is independent of HBV, with both *in vitro* and *in vivo* studies demonstrating that HDV may persist during liver regeneration by transmission of HDV RNA through cell division, even in the absence of HBV.⁴ Interestingly, several HDV-like viruses have recently been identified in different animal species (birds, fish, amphibians, snakes and invertebrates) without any association with a Hepadnavirus infection, suggesting that HDV has a long evolutionary history, and the HDV-HBV association may be specific to humans.^{5,6}

HDV encodes a single structural protein (hepatitis D or Delta antigen, HDAg), expressed in two isoforms that are identical except for an additional 19 residues located at the C terminus of the large form (L-HDAg), though they have distinct biological functions. While the small protein (S-HDAg) is required for viral replication, L-HDAg, which results from an editing event induced on the antigenomic RNA by the host's adenine deaminase,⁷ shuts down viral replication, promoting the

packaging of mature virions; this is facilitated by an isoprenoid prosthetic side chain covalently bound to its C-terminus by a host cell farnesyltransferase⁸ (Fig. 1). The two HDAg proteins bind to the HDV RNA genome to form a ribonucleoprotein which is then surrounded by an envelope containing all three HBsAg isoforms.⁹ Due to its structure, HDV binds to the same cell receptor as HBV, *i.e.* sodium taurocholate cotransporting polypeptide (NTCP), via interaction with the pre-S1 domain of the L-HBsAg isoform, thus mediating HDV entry into hepatocytes.¹⁰

The unique features of HDV, such as the tight and mandatory interplay with HBV on the one hand and the ability to persist in the absence of the helper virus on the other, explain why it is so difficult to clear HDV infection. Furthermore, HDV RNA acts as a ribozyme and self cleaves to replicate; it does not encode any protein with enzymatic activity and borrows the enzymes required for replication from the infected cell: this poses an additional challenge to the identification of HDV-specific targets for antivirals.

HDV can infect susceptible hosts via coinfection with HBV, or by superinfecting chronic HBV carriers. HBV/HDV coinfection, which may result in the clearance of both viruses, usually leads to acute hepatitis, with a wide clinical spectrum ranging from asymptomatic/mild hepatitis to acute liver failure. Severe cases of acute hepatitis are more frequent in HBV/HDV coinfection than in primary HBV mono-infection.¹¹ HDV superinfection of an HBsAg-positive individual – as a rule – leads to persistence of HDV resulting in chronic hepatitis D (CHD), which is associated with a worse clinical outcome than HBV mono-infection, with more rapid and more frequent progression to cirrhosis.¹² Studies conducted in Italy in the late '80s re-

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ported a rapid progression to cirrhosis (in less than 3 years) in about 15% of cases, particularly among people who inject drugs (PWID).^{13,14} However, CHD is not always rapidly progressive and a mild form of chronic hepatitis has been reported in a sizeable proportion of patients, mainly in more recent studies.^{15,16} Nevertheless, liver damage persisting over a long period of time may lead to cirrhosis, which is occasionally diagnosed in elderly patients with long-lasting CHD and mild biochemical activity.¹⁷

The natural history of both HDV infection and disease are inextricably linked with the patterns and dynamic changes of HBV and HDV epidemiology in different areas of the world. In addition, the genetic heterogeneity of both HBV and HDV may have an impact on the pathogenetic interplay between the two viruses, the complexity of which remains to be further

investigated. HDV is present worldwide and its epidemiology to some extent mirrors that of HBV, although accurate data on prevalence and health burden are patchy and incomplete, due to the lack of systematic population-based surveys. Poor awareness, the fact that hepatitis D is still considered a rare disease confined to high-risk settings and the lack, until recently, of accurate diagnostic assays have all been major obstacles to the full appraisal of HDV's global impact. Furthermore, the great geographical variation in HDV prevalence is driven by factors such as variability in transmission routes, hygienic and socio-economic conditions, timing and coverage of HBV vaccination, migration flows, as well as HDV heterogeneity (differences in virulence of HDV genotypes or genetic susceptibility of HBV-infected patients to HDV superinfection).^{18,19} While Northern Europe, North America, and

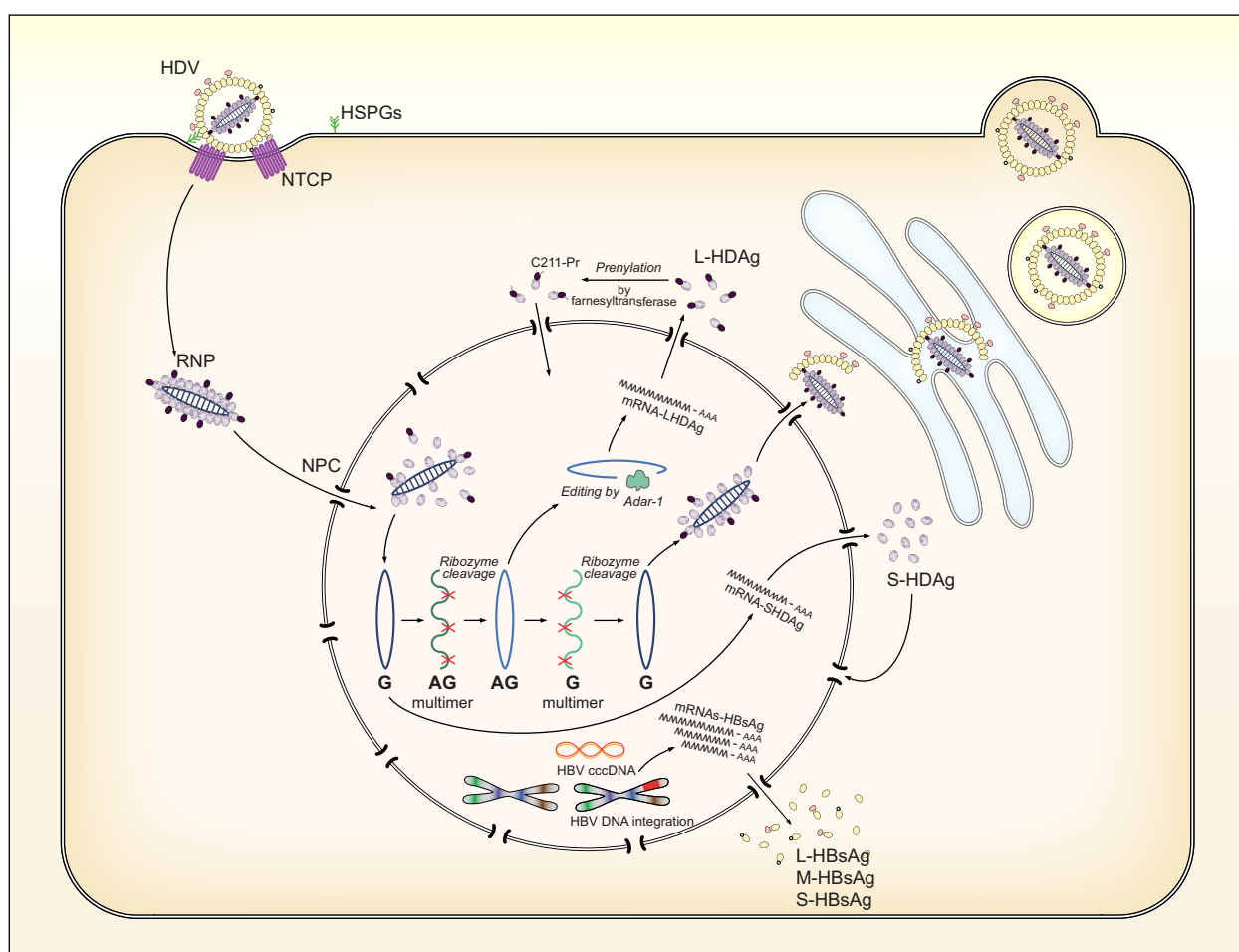


Fig. 1. HDV life cycle. Firstly HDV virions attach to HSPGs on the surface of hepatocytes and then specifically bind the NTCP receptor. After viral entry, the HDV RNP complex is transported to the nucleus via the NLS of HDAG. Within the nucleus of the infected cell, the HDV genome (G, negative strand) undergoes its first rolling circle amplification step, serving as a template for the synthesis of the antigenomic sense HDV (AG, positive strand) by the nuclear RNA polymerase II. The resulting AG multimers are cleaved by the intrinsic HDV ribozyme activity and ligated into circular monomers. During a second rolling circle step the RNA polymerase II uses AG as a template to synthesise HDV G multimers, which are further cleaved and ligated into circular G. A portion of AG is edited by ADAR1, yielding an extended HDAG ORF encoding for L-HDAG that is prenylated by farnesyl transferase activity. The HDV genome serves as a template for transcription of mRNAs which are translated into S-HDAG and L-HDAG. After their synthesis, HDV proteins are transported into the nucleus where they regulate viral replication or bind to circular G to form the RNP complexes which are exported to the cytosol. Finally, the RNP complexes are enveloped within a lipid coat embedded with HBsAg and complete HDV virions are secreted into the blood stream. ADAR1, adenosine deaminase RNA-specific; HDAG, HDV antigen; HDV, hepatitis D virus; HSPGs, heparan sulfate proteoglycans; L-HDAG, large HDAG; NLS, nuclear localisation signal; NTCP, sodium taurocholate cotransporting peptide; ORF, open reading frame; RNP, ribonucleoprotein; S-HDAG, small HDAG.

Table 1. Grades of recommendation.

Grade	Wording	Criteria
Strong	Shall, should, is recommended. Shall not, should not, is not recommended.	Evidence, consistency of studies, risk-benefit ratio, patient preferences, ethical obligations, feasibility
Weak or open	Can, may, is suggested. May not, is not suggested.	

Table 2. Level of evidence based on the Oxford Centre for Evidence-based Medicine (adapted from The Oxford 2011 Levels of Evidence).

Level	Criteria	Simple model for high, intermediate and low evidence
1	Systematic reviews (SR) (with homogeneity) of randomised-controlled trials (RCT)	Further research is unlikely to change our confidence in the estimate of benefit and risk
2	Randomised-controlled trials (RCT) or observational studies with dramatic effects; systematic reviews (SR) of lower quality studies (<i>i.e.</i> non-randomised, retrospective)	
3	Non-randomised controlled cohort/follow-up study/control arm of randomized trial (systematic review is generally better than an individual study)	Further research (if performed) is likely to have an impact on our confidence in the estimate of benefit and risk and may change the estimate
4	Case-series, case-control, or historically controlled studies (systematic review is generally better than an individual study)	
5	Expert opinion (mechanism-based reasoning)	Any estimate of effect is uncertain

Japan are traditionally considered as low endemicity areas, HDV infection is hyperendemic in certain geographic hotspots and populations called “endemic pockets” with the highest reported prevalence in HBsAg-positive individuals of Mongolia, Pakistan, Moldova, sub-Saharan Africa, Central Asia, Pacific Islands, the Amazon Basin, and Eastern Europe.^{12,20,21} Although the reliability of epidemiological data may be sub-optimal, some recent cohorts have shown HDV prevalence rates ranging between 10% and 70% in HBsAg-positive patients from certain low- and middle-income countries, particularly in the sub-Saharan African region, India, Mongolia and Western Brazil, where HBV is endemic,¹⁸ underlying the potential risk that migration flows could lead to the spread of HDV to low endemic areas.

So far, the management of CHD has been encompassed within the HBV guidelines and this stems from the recognition that CHD is an HBV-dependent rare disease. Accordingly, it has been designated as an orphan disease (ORPHA:402823), as it affects a relatively small fraction of HBsAg-positive individuals in absence of approved drugs with anti-HDV specific activity. More recently, our understanding of HDV pathogenesis has advanced significantly, leading to the identification of new therapeutic targets. For the first time since the discovery of HDV in the '70s, HDV-specific antiviral agents such as bulevirtide (BLV) and lonafarnib (LNF), have reached phase III clinical trials and consistent/substantial data on their efficacy will be available soon. Meanwhile, newly developed standardised methods enable better characterisation of both the clinical and virological phases throughout the natural history of HDV infection. Since the complexity of the clinical management of patients with CHD has increased significantly in recent years and in view of the newly available knowledge and therapeutic perspectives, the European Association for the Study of the Liver (EASL) has commissioned the first international Clinical

Practice Guidelines (CPGs) on the management of HDV-infected patients.

Methodology

The EASL Governing Board selected a panel of experts tasked with developing the present document, according to a standardised method adopted for other recently published international guidelines.²²

The present guidelines are intended for clinicians of all specialties who may deal with the management and care of patients with HDV infection (hepatologists, gastroenterologists, infectious diseases specialists). Based on practicalities, the authors decided to address treatment options currently available at the time of writing, although they were fully cognisant that future updates may be needed as new data from phase III randomised-controlled trials becomes available.

The CPG panel held multiple teleconferences and two face-to-face meetings. The process started with the identification of six main topics: i) screening; ii) diagnosis and stratification of HDV-infected individuals according to their virologic profile (HDV and HBV) and liver disease (grading and staging); iii) clinical aspects, natural history and cofactors influencing outcomes; iv) monitoring of HDV-infected individuals and selection of candidates for treatment; v) therapeutic approaches: antiviral treatment according to the viral target (HDV, HBV, or both) and liver transplantation; vi) treatment endpoints: virologic markers (HDV, HBV), biochemical tests (aminotransferases/liver function), liver imaging (stiffness; ultrasonography), histology, and clinical events. Two experts for each topic had the task of formulating the key questions, according to the PICO format (P - Patient, Problem or Population; I - Intervention; C - Comparison, Control or Comparator; O - Outcome). The panel agreed to adopt this format, although it did not appear to be optimal for a rare disease with a limited number of large

prospective studies and for which diagnostic tools are continuously evolving. Consequently, for many specific issues the data are scarce, with only low quality evidence available. PICO questions were submitted to the Delphi panel, composed of 29 experts, including patient representatives. Each question had to receive at least 75% agreement to be approved and, according to the Delphi panel suggestions, 13 PICO questions were defined to cover the six topics. After approval of the PICO questions, an extensive literature search was performed using PubMed, Embase, Google Scholar and Scopus. At the time of writing, a significant amount of data from ongoing studies (notably those from randomised-controlled trials of new drugs), have not yet been published *in extenso*, and therefore the experts agreed to include, as bibliographic references, the abstracts presented at international meetings. The quality of evidence was scored according to the Oxford Centre for Evidence-based Medicine (OCEBM) (adapted from The Oxford 2011 Levels of Evidence)²³ (Table 1). For each PICO question, one or more recommendations were written by the same experts who formulated the questions. The strength of the recommendations in these guidelines has been graded according to the OCEBM into two categories: strong or weak²⁴ (Table 2). In addition, the panel chose to formulate statements to address issues that were considered more controversial.

The recommendations were discussed and approved unanimously by the expert panel before they were sent to the Delphi panel for consensus agreement, defined as follows: less than 50% approval: re-write recommendation and resubmit to the Delphi panel; 50-75% approval: re-write/improve the recommendation, but no resubmission to the Delphi panel; 75-90% approval: consensus, no need to re-write the recommendation but the document will take into account the comments; ≥90% approval: assumed as strong consensus, no change needed but small corrections possible. The final version of the CPG with the corrections suggested by the Delphi panel was then sent to the EASL Governing Board for approval.

Screening

How and which HBsAg-positive individuals should be screened for HDV infection?

Recommendations

- Screening for anti-HDV antibodies should be performed with a validated assay at least once in all HBsAg-positive individuals (**LoE 3, strong recommendation, strong consensus**).
- Re-testing for anti-HDV antibodies should be performed in HBsAg-positive individuals whenever clinically indicated (e.g., in case of aminotransferase flares, or acute decompensation of chronic liver disease) (**LoE 3, strong recommendation, strong consensus**), and may be performed yearly in those remaining at risk of infection (**LoE 5, weak recommendation, strong consensus**).

Recently three large meta-analyses reported a prevalence of HDV infection between 0.11% and 0.98% in the general population, between 4.5% and 13.02% in all HBsAg-positive

carriers, and between 14.6% and 18.6% among those attending hepatology clinics. These figures correspond to an estimated burden of 12 to 72 million people living with serological evidence of HDV exposure worldwide.^{12,20,21} The wide variation in the estimated global prevalence of HDV infection (reflecting methodological hurdles and the geographical heterogeneity of HDV infection), the diagnostic limitations and the lack of highly effective treatments are three major factors that underpin the different approaches to HDV screening among countries and scientific societies. While the HBV CPGs from the EASL and Asian-Pacific Association for the Study of the Liver (APASL) recommend HDV testing in all HBsAg-positive patients,^{25,26} the American Association for the Study of Liver Diseases (AASLD) guidance on hepatitis B recommends only risk-based screening for HDV.²⁷

Recent studies showed that risk-based screening misses a sizeable number of HDV cases and that anti-HDV screening is performed in a minority of HBsAg-positive carriers even in countries where it is recommended for all HBsAg-positive individuals.²⁸⁻³¹ These findings underline the need to increase clinicians' awareness of the importance of testing for anti-HDV among HBsAg-positive carriers. Notably, the application of reflex testing for anti-HDV in all individuals who tested positive for HBsAg led to a 5-fold increase in diagnoses of HDV infections: most anti-HDV-positive individuals were young, 60% did not have common risk factors for infection, while 60% had advanced fibrosis.³¹ These data argue in favour of universal anti-HDV screening in HBsAg-positive individuals, as the early diagnosis of HDV infection is key to providing adequate personalised counselling and reducing the risk of transmission to anti-HDV-negative HBV carriers. Accordingly, given the dramatic infectivity of HDV among HBsAg-positive individuals, the identification of HDV-infected individuals would help to prevent its transmission by enabling the implementation of specific preventive actions among high-risk groups and within households and by promoting adherence to current anti-HBV vaccination programmes. Furthermore, personalised counselling of patients with CHD could help to prevent or reduce liver disease progression (e.g. by helping patients avoid or mitigate against the impact of disease cofactors), and to define monitoring and treatments according to the individual risk of disease progression. Despite potential biases (i.e., when studies were conducted) due to the evolving epidemiological pattern of HDV, as a consequence of HBV vaccination and migrant flows from highly endemic areas,^{19,32,33} anti-HDV prevalence is higher among selected high-risk populations, with a reported prevalence of 15% in patients coinfecting with human immunodeficiency virus (HIV), 30% in institutionalised persons, and up to 67% in PWID.^{25-27,34} In a meta-analysis,²⁰ the prevalence of anti-HDV was more than 3-fold higher in HBsAg-positive PWID than in individuals without risk factors (37.6% vs. 10.6%). Furthermore, a recent systematic review and meta-analysis showed that the prevalence of anti-HDV is higher in haemodialysis recipients (pooled odds ratio [OR] 3.4), men who have sex with men (pooled OR 16.0) and patients who are positive for anti-hepatitis C virus (HCV) antibodies (pooled OR 10.0).²¹ As a consequence, due to the risk of HDV superinfection, HBsAg-positive individuals with high-risk behaviour or living in countries or communities with high HDV prevalence should be tested repeatedly or whenever they present aminotransferase flares or liver disease decompensation, that cannot otherwise be explained.³⁵

The implementation of anti-HDV reflex testing in routine clinical practice is hotly debated; nevertheless, some hospitals and metropolitan areas already apply it.^{31,36} Before its general implementation, further studies are needed to estimate its cost-effectiveness, which may vary in different healthcare systems according to the prevalence of HBV and HDV. However, reflex testing may not only increase early diagnosis rates but also improve our current knowledge of HDV epidemiology.³⁷

Diagnosis and stratification

Which diagnostic test should be used to diagnose ongoing HDV infection?

Recommendation

- HDV RNA should be tested in all anti-HDV-positive individuals using a standardised and sensitive reverse-transcription PCR assay to diagnose active HDV infection (LoE 2, strong recommendation, strong consensus).

The presence of anti-HDV (IgG or total) antibodies identifies HBsAg-positive individuals who have been exposed to HDV; however, as anti-HDV antibodies persist after the clearance of HDV, testing for serum/plasma HDV RNA is needed to confirm an ongoing HDV infection.^{38,39} A recent systematic review and meta-analysis reported that the pooled proportion of detectable HDV RNA in 5,073 anti-HDV-positive individuals was 58.5% (95% CI 52.4–64.5). The rate of HDV RNA detection was higher in cohorts with higher prevalence of anti-HDV and in hepatology clinic populations than in the general population.²¹

In recent years, major efforts have been made to develop robust diagnostic assays by optimising and standardising HDV RNA detection protocols to meet the major challenges posed by the specific molecular features of the circular HDV genome, namely, strong self-base pairing and high sequence variability between different HDV genotypes.⁴⁰ Several in-house and commercial reverse-transcription PCR assays are currently available for the quantitative detection of HDV RNA using either dye- or probe-based methods, with amplification targets within well-conserved regions of HDAg or the ribozyme domains of HDV RNA. However, a high variability in diagnostic performance was shown by the first international external quality control assessment that used the World Health Organization international standard for HDV RNA genotype 1.^{41,42} Only 46.3% of the 28 laboratories participating in the quality control assessment properly quantified the serum panel, while 57.1% failed to detect up to 10 samples, and several others underestimated (>3 log IU/ml) HDV viral load for African genotypes (1 and 5-8).⁴² Every step in the real-time, reverse-transcription PCR of HDV RNA is critically sensitive, starting from pre-amplification procedures, in particular nucleic acid extraction. Up to 2 log₁₀ differences were reported in the lower limit of detection when comparing manual vs. automated extraction methods using the same amplification assay.⁴³ Thus, strict adherence to the procedures validated by the manufacturers is mandatory when using commercial assays, and any

modification of the original protocols must be further validated using the reference standard – the same applies for in-house protocols. Fully automated assays that enable more accurate and reliable quantitative HDV RNA detection of all HDV genotypes are eagerly awaited. The high genetic variability among the different HDV genotypes and some sub-genotypes has been proven to be responsible for underestimation of the viral load by several commercially available assays, mainly in the case of African sub-genotype 1 and African genotypes 5-8.⁴⁴ This critical issue must be considered in clinical practice when managing patients from these geographical areas. Therefore, the use of well-standardised real-time molecular assays for HDV RNA is recommended to assure an accurate diagnosis of ongoing HDV infection and to monitor antiviral treatment.⁴⁵ In both clinical trials and practice, HDV RNA should be quantified by a reference laboratory using well-standardised, validated assays and the results should be given in IU/ml to improve precision and comparability across laboratory test systems. Until there is harmonisation across the different assays, quantitative HDV RNA monitoring in sequential serum samples should be performed in the same laboratory and with the same assay to avoid inter-laboratory and inter-assay variability.

Standardised and validated real-time HDV RNA PCR assays are not currently available worldwide, highlighting an unmet need for the appropriate management of anti-HDV-positive individuals. Unluckily, there are no alternative serum markers of HDV replication: HDAg can be detected in the serum of patients with acute HDV infection only for the short time frame (about 2 weeks) preceding the appearance of a serological anti-HDV antibody response. Accordingly, serum HDAg is not detectable in the late phase of acute infection and in chronic infection because it is hidden within immune complexes formed with the homologous antibodies.³⁸ Thus, serum HDAg is not currently measured in clinical practice. Anti-HDV IgM is detectable within the first 2–3 weeks of acute HDV infection and persists when it progresses to chronicity; anti-HDV IgM levels are thus considered a surrogate marker of CHD. Anti-HDV IgM levels are associated with disease activity in chronic HDV infection.⁴⁶ In the past, when the availability of HDV RNA assays was scarce, anti-HDV IgM levels were used as a surrogate marker of viral replication.⁴⁴ However, anti-HDV IgM is not a direct marker of HDV replication and is not suitable for its monitoring.

In HDV-infected patients, persistence of HDV replication has been associated with the worst prognosis, with the converse applying to HDV RNA clearance.^{16,48–51} Preliminary reports suggest that viral load correlates with disease activity and progression; however, further studies with standardised assays are required to confirm these findings and define the prognostic role of quantitative HDV RNA monitoring in untreated patients.^{17,52–54} HDV RNA serum levels may fluctuate overtime, becoming temporarily undetectable; therefore, the definition of HDV infection status cannot be based on a single determination and requires repeated tests (at least two) 3 to 6 months apart.^{55,56} In addition, recent studies showed that the HDV viral load declines overtime in a significant proportion of patients, mainly those with cirrhosis, and may be associated with

reduced aminotransferase levels.⁵⁴ These findings suggest the need for serum HDV RNA re-testing not only to exclude temporary undetectability when characterising a newly diagnosed HDV infection,^{17,54} but also to confirm the possible clearance of serum HDV RNA in the case of persistent remission of disease activity.^{17,50,51,54}

Which HBV markers should be tested in patients with acute or chronic HDV infection?

Recommendations

- In patients with acute hepatitis, anti-HBc IgM should be used to distinguish individuals with HBV/HDV coinfection from HBsAg-positive individuals superinfected with HDV (**LoE 3; strong recommendation, consensus**).
- HBV e antigen (HBeAg)/anti-HBe status and HBV DNA levels should be tested because the presence of active HBV infection may worsen the outcome of hepatitis D (**LoE 3; strong recommendation, consensus**).

HDV infection can either be acquired simultaneously with HBV (HBV/HDV coinfection), resulting in both acute hepatitis B and acute hepatitis D, or as superinfection of a chronic HBsAg-positive individual (HDV superinfection).³⁵ Diagnosis of acute HBV/HDV coinfection is based on the simultaneous presence of markers of acute HBV infection (HBsAg, anti-HBc IgM and IgG) and acute HDV infection (anti-HDV IgM and IgG, and serum HDV RNA).⁵⁷ The most specific marker of HBV/HDV coinfection is the detection of anti-HDV IgM together with high levels of anti-HBc IgM. Acute hepatitis D acquired by coinfection is usually self-limited, progressing to chronicity in only 2% of cases.⁵⁸ On the contrary, HDV superinfection of an individual with chronic HBV infection often causes severe acute hepatitis that leads to chronic hepatitis D in up to 90% of cases. If the previous HBsAg status is unknown, it may be misdiagnosed as acute hepatitis B.⁵⁹ On the other hand, HDV superinfection may result in unexplained hepatitis exacerbation in an individual with previously known chronic hepatitis B (CHB). The absence of or low anti-HBc IgM levels may distinguish superinfection from coinfection,⁵⁹ the latter being characterised by high levels of anti-HBc IgM. In the setting of HDV superinfection, HBV replication can be suppressed.⁵⁷

CHD is diagnosed by the detection of high anti-HDV IgG levels, often associated with anti-HDV IgM, and of serum HDV RNA. Since the presence of active HBV infection has a critical impact on both the outcome of HDV infection and the disease course⁶⁰ in patients with CHD, an accurate characterisation of HBV infection is recommended and should be based on HBeAg/anti-HBe status and quantification of serum HBV DNA levels. Longitudinal studies have also shown fluctuations in HDV RNA and HBV DNA in the serum of patients with CHD, especially if they are HBeAg-positive.^{56,61} Thus, both HBeAg status and HBV DNA should be re-tested during follow-up, mainly in case of major changes in the liver disease profile, such as alanine aminotransferase (ALT) normalisation or hepatitis exacerbation, or in the case of HDV RNA clearance, as HBV DNA reappearance has been reported.⁶²

In seminal studies, HBsAg serum levels in untreated patients with CHD showed wider fluctuations than in untreated mono-infected patients with CHB. HBsAg levels declined significantly in the case of a spontaneous decline (>2 log) in or clearance of HDV RNA.^{54,56} Moreover, a significant, positive correlation between HDV RNA and HBsAg serum levels was observed.⁵³ However, at present, the associations of HBsAg serum levels and their fluctuation overtime with prognosis and clinical outcomes in patients with CHD remain to be defined. Conversely, in preliminary reports, quantitative monitoring of HBsAg serum levels proved useful in the identification of patients who responded to pegylated interferon- α (pegIFN α), as their reduction appeared to be a prerequisite for the achievement of definitive clearance of HDV RNA.^{63,64}

Data on the role of new HBV markers, such as hepatitis B core-related antigen (HBcrAg) and HBV RNA, in the management of CHD are scarce and preliminary, but suggest that these markers could better depict the interplay between HBV and HDV during both the natural course of the disease and treatment.^{53,65–67} Further studies are mandatory to assess the cost-benefit of their use in the clinical management of patients with CHD.

When should invasive (liver biopsy) and non-invasive tests (NITs) be used in the clinical management of patients with hepatitis D?

Statement

- Fully published data on the use of NITs in patients with CHD are currently limited and the correlation with liver histology is missing in a significant proportion of cases (**LoE 4, strong consensus**).

Recommendations

- Liver biopsy is recommended whenever it may contribute to the patient's management or for grading and staging liver disease when clinical signs or indirect evidence (by imaging techniques) of cirrhosis are absent (**LoE 3; strong recommendation, consensus**).
- NITs may be used to assess advanced liver disease, but specific cut-off values are not well established (**LoE 5, weak recommendation, strong consensus**).

Histology remains the gold standard for the most accurate characterisation of liver disease, also enabling the categorical grading and staging of necro-inflammation and fibrosis, respectively.^{68,69} In addition, HDaG immunohistochemistry and HDV RNA detection contribute to estimating the burden of HDV infection, but unfortunately these additional assays are not available in most pathology laboratories.⁴⁷

In patients with CHD, liver biopsy can be performed when the definition of the disease grade and stage may help to modify the clinical/therapeutic management of individual patients, for instance when imaging and blood tests are conflicting, or when, in patients with multiple cofactors of liver disease, it is necessary to investigate the relative impact of HDV on the overall liver disease burden. Furthermore, liver biopsy can be useful to rule out or confirm the presence of an

autoimmune component in liver damage when anti-liver-kidney microsomal (LKM)-3 autoantibodies or other signs of autoimmunity are present.⁷⁰ Liver biopsy should be performed in clinical trials to study the correlation between serum markers of virological and biochemical response, grading and staging of liver disease and intrahepatic HDV expression and to rule out possible toxicity associated with the investigational drug.⁷¹ Conversely, liver biopsy as an invasive tool to diagnose cirrhosis is not required when imaging techniques (ultrasound, CT, MRI) identify specific features of cirrhosis such as a nodular liver with signs of portal hypertension (increased spleen longitudinal diameter, oesophageal varices, mild ascites). Liver biopsy is unsuitable for monitoring liver disease progression during the follow-up of patients with CHD, while the longitudinal assessment of NITs may provide useful information.^{68,69}

In chronic viral hepatitis, the categorical staging of fibrosis on histology or the presence of indirect signs of cirrhosis on imaging have been used as the gold standard comparator when assessing the diagnostic performance of non-invasive tests such as liver stiffness measurement (transient elastography [TE], shear wave elastography [SWE]) and fibrosis scores (APRI, FIB4, AAR, API, GUCI and Lok indexes, CDS and HUI scores).^{68,69} An advantage of NITs is that they enable the dynamic tracking of the overall disease burden before and after the development of compensated advanced chronic liver disease.^{68,69} In the setting of CHD, the disadvantage is that NITs have not been consistently validated in large multicentre studies.^{72–78} In addition, combined scores that use indirect markers of liver inflammation (*i.e.*, ALT) or techniques (TE and SWE) that are influenced not only by fibrosis, but also by inflammation and congestion, may overestimate fibrosis because of the significant impact of hepatic inflammation, which characterises a substantial proportion of CHD cases.^{68,69,79} It has been reported that 53% of 230 patients (only 29% with histologically proven cirrhosis) were misclassified by these scores as having cirrhosis.⁸⁰ A recent paper, where 108 patients with CHD were studied (50 of whom had pegIFN α -induced undetectable HDV RNA and low/normal ALT values), reported that the areas under the receiver operating characteristic curves (AUROCs) for NITs differed according to the detectability of viraemia and disease activity; additionally, the cut-offs for significant fibrosis based on either fibrosis scores or TE were higher in viraemic patients.⁸¹ Consistent with previous reports, the diagnostic performance of fibrosis scores was lower than that of TE. Nevertheless, GUCI and Lok indexes and APRI showed AUROCs of 0.90 for the identification of cirrhosis.⁸¹ In spite of the wide use of the well-standardised measurement of liver stiffness by TE in chronic viral hepatitis, its use in clinical practice to stage CHD is supported by only three full papers (two from the same group and with major overlaps in patient populations): overall 235 patients with CHD were studied.^{74,75,81} The AUROCs to identify cirrhosis ranged between 0.95 and 0.86, whereas the thresholds of 10, 12.5 and 12 kPa predicted cirrhosis with sensitivity/specificity of 95/75%, 77.8/82.5% and 75/81.5%, respectively.^{74,75,81} A cut-off of 14 kPa was proposed, with 78% sensitivity, 86% specificity, 93% negative predictive value (NPV) and 64% positive predictive value (PPV).⁷⁴ However, in a recent report including 330 patients, who had all undergone liver biopsy, the diagnostic performance for identification of cirrhosis was poor for both fibrosis scores (sensitivity [13% for AAR, 27% for APRI, 29%

for FIB-4, 31% for Fibro Test]; NPV [73%, 76%, 77% and 77%]) and TE (sensitivity 47%; NPV 77%) when using the cut-offs proposed in the literature (12.5 kPa for TE).⁸² Thus, the proposed thresholds require validation in large and well-characterised populations, to adequately weigh the impact of biochemical activity-inflammation and advanced fibrosis-cirrhosis in their definition. Overall, the available data suggest that TE performs better at ruling out than ruling in cirrhosis also in the setting of CHD.^{68,69}

To overcome such a critical problem, a specific score for HDV (delta-4 fibrosis score, or D4FS) was proposed, where TE is combined with the classic blood biomarkers of liver disease (gamma-glutamyltransferase [GGT], platelet count, ALT): an AUROC of 0.94 was obtained for the identification of cirrhosis in the validation cohort.⁷⁶ Other scores not including liver stiffness were proposed in recent years, such as the delta fibrosis score, which used GGT, age, albumin, and serum cholinesterase, and an even simpler score based on spleen size, platelet count and albumin levels, which showed an AUROC of 0.93 in predicting cirrhosis, though it was only evaluated in a highly selected group of patients aged between 18 and 25 years.^{73,77} Further studies in larger cohorts of patients with CHD are needed to develop and validate algorithms to stage CHD and monitor the efficacy of treatments.

However, as the influence of necro-inflammation on stiffness values declines with the increase of fibrosis, it is reasonable to use the TE thresholds proposed by Baveno VII, in order to identify patients with cACLD (compensated advanced chronic liver disease) and clinically significant portal hypertension (CSPH).⁸³

Clinical aspects, natural history and cofactors

Which factors should be considered to identify patients with CHD who are at higher risk of liver disease progression?

Recommendation

- Factors that should be considered to identify patients with CHD at higher risk of liver disease progression include elevated aminotransferases and GGT levels, advanced stage of liver disease, persistence of HDV viraemia, high serum HBV DNA levels and viral coinfections. Cofactors of chronic liver injury, such as alcohol abuse, obesity and diabetes, should also be considered (**LoE 4, strong recommendation, strong consensus**).

There are no large prospective studies aiming to evaluate the predictors of long-term outcomes of CHD, but since the '80s a number of longitudinal cohort studies have reported that the different profiles of HDV and HBV infection, the activity, stage and cofactors of liver disease, HIV coinfection and the patient demographics correlated with disease outcome (development of cirrhosis, liver decompensation, hepatocellular carcinoma [HCC]) and mortality (Fig. 2). These studies, which were conducted across different times and geographical areas, showed a heterogeneous prevalence of cirrhosis at the time of enrolment (ranging from 25 to 70%) and variable patterns of progression to liver-related

events,^{13,16} resulting from recruitment biases that are at least partly due to the dynamic epidemiology of HDV infection. Accordingly, an Italian study run in three tertiary referral centres reported a prevalence of cirrhosis of 29% and 75% in patients enrolled from 1977 to 1986 and from 1985 to 1996, respectively.⁸⁴ In the initial studies, the majority of patients progressed rapidly (within 5–10 years) to advanced liver disease^{11,13,85} and, in a subset, cirrhosis and decompensation developed even more rapidly (in less than 1 year).¹⁴ This very aggressive course was hypothesised to be correlated with the possible emergence of more pathogenic HDV strains through the rapid circulation of HDV in the drug-abusing community,^{14,16} but the underlying HBeAg-positive HBV infection with florid HBV replication (>200,000 IU/ml) that favours the rapid and massive intrahepatic spread of HDV was shown to be a major driving force.^{14,60} In addition, concomitant HIV infection could contribute to worse outcomes, as recently confirmed by the Swiss HIV cohort study.⁸⁶ Significant disease severity with rapid progression and poor survival was also confirmed by a study from Romania where the median overall survival of 166 patients with compensated cirrhosis was less than 5 years and the mean time for liver decompensation was less than 2 years; 12% of patients developed HCC; MELD (model for end-stage liver disease) >15 and gastrointestinal bleeding were independent factors associated with death.⁸⁷ More recent studies showed that CHD can have a less aggressive course, at least in a proportion of cases. One study from Italy reported that 42% (82 of 195) of patients with CHD developed cirrhosis after a mean follow-up of 116 months and the 20-year survival probability in the overall cohort of 299 patients was 86%; persistent HDV replication was the only independent predictor of increased mortality, whereas female sex, alcohol abuse and viral replication were associated with clinical decompensation.⁸⁸ Whether viraemia levels have a prognostic role remains to be clarified, even if available data show that higher viral load is associated with higher aminotransferase levels⁵³ and worse clinical

outcomes.⁵² Conversely, persistent viraemia has been shown to be consistently associated with worse outcomes^{15,16,51,88}: HDV RNA viraemia was associated with a 3.8-fold and 2.6-fold higher risk of liver-related events and HCC in a recent Swedish study where 337 anti-HDV-positive patients (233 of whom were viraemic) were enrolled at secondary care centres and followed up for a mean period of 6.5 years. The prevalence of cirrhosis at baseline among viraemic patients was 29.6% (compared to 8.8% in non-viraemic patients), and the probability of being free of cirrhosis among the 164 viraemic patients was 82%, 64% and 51% at 5, 10 and 15 years, respectively.¹⁶ Interestingly, 82% of the patients originated from outside Europe (44.8% from Asia), with origin found to be an independent predictor for liver-related events on multivariate analysis.¹⁶ The lack of virologic characterisation did not allow for an analysis of the impact of HDV and HBV genotypes on CHD progression. Indeed, data from Taiwan suggested that HDV genotype 1 infection is associated with a more severe outcome than HDV genotype 2, the same held true for HBV genotype C vs. HBV genotype B.⁸⁹ Among HDV genotypes, genotype 3, which is usually detected in the Amazon Basin, has been reported to be more frequently associated with advanced liver disease,⁹⁰ while genotype 5 seems to be associated with a slowly progressive liver disease and better response to IFN α .⁹¹ The latter observation has, at least in part, been challenged by a recent study on 1,112 anti-HDV-positive patients, where European genotype 1 and African genotype 5 HDV infections were shown to be associated with a higher risk of developing cirrhosis. However, overall sub-Saharan African patients were at a lower risk of cirrhosis development than European patients, but patients with genotype 5 HDV displayed a higher cirrhosis risk than African patients infected with other HDV genotypes.¹⁵ These findings suggest that HDV genotype and place of birth could be independent factors influencing the outcome of CHD.^{15,92} However, additional studies are needed to better dissect the role of HDV and HBV genotype, independently of ethnicity (host and/or

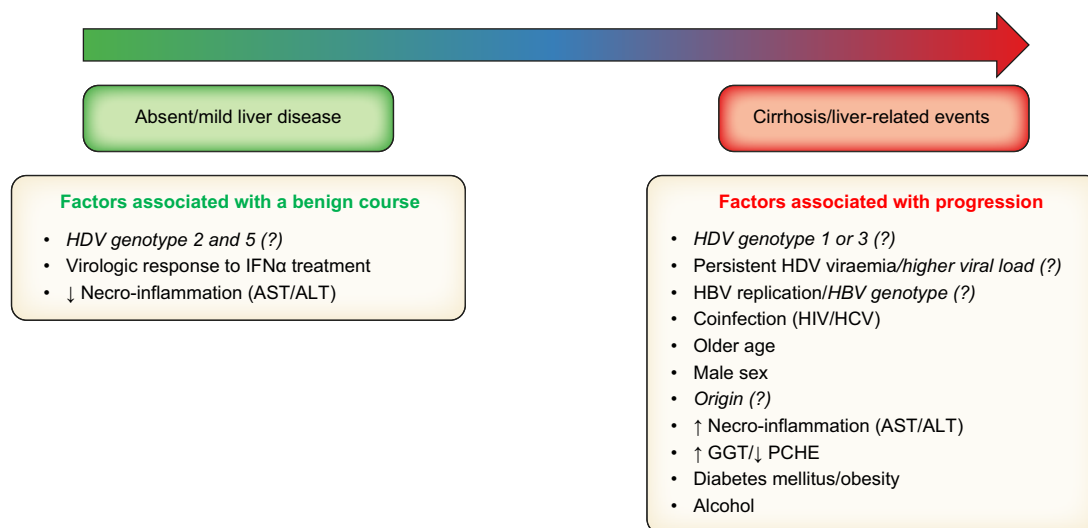


Fig. 2. Factors influencing the outcome of HDV infection and disease. HDV, HBV and host associated factors together with liver disease activity and comorbidities were shown to influence the outcome of HDV infection and disease. Note: Factors still under investigation are in *italics*. ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus; GGT, gamma-glutamyltransferase; HBV, hepatitis B virus; HDV, hepatitis D virus; PCHE, pseudocholinesterase.

environmental factors), and to define their relevance in the management of the individual patient in clinical practice (Fig. 2).

The prevalence of anti-HCV antibodies among anti-HDV-positive individuals ranges from 6% to 70% according to the population studied and risk factors for parenteral transmission of viral infection (PWID, migrants, etc). Triple hepatitis viral infections are associated with viral interference and HDV is usually the dominant virus, with HCV RNA being detectable in 10–40% of triple-infected patients.^{15,93,94} However, fluctuations in HCV, HBV and HDV viraemia overtime have been described.⁵⁵ Overall, the available studies suggest that patients with triple infection have a more severe form of liver disease, mainly in the case of superinfection with HCV in a HBsAg/anti-HDV-positive patient or with HBV/HDV in an HCV-positive patient.^{93,95}

Among liver enzymes, aminotransferases are a surrogate marker of disease activity, as shown by their correlation with higher necro-inflammation at histology,⁸⁴ but their levels may decline during the course of the disease, mainly in the more advanced phases of cirrhosis.^{17,84} Therefore, their role in the management of patients with CHD varies in the different phases of the disease and low aminotransferase levels may be observed in patients with cirrhosis. Conversely, higher GGT levels have been associated with cirrhosis.⁹⁶ Interestingly, in the long-term follow-up of the HIDIT-1 study, high GGT independently predicted clinical outcomes on multivariate analysis.⁵⁰ GGT levels are usually elevated in patients with fatty liver, obesity and diabetes; these conditions have been associated with an aggressive course of chronic viral hepatitis, and with an increased rate of clinical decompensation events and HCC in patients with cirrhosis.^{16,97,98} (Fig. 2). Therefore, patients with CHD should be guided towards effective lifestyle modifications to obtain the best possible correction of dysmetabolic cofactors and limit liver disease progression.

Among terminal liver-related events, liver decompensation is thought to occur more frequently than HCC and to be responsible for more deaths in patients with CHD.^{87,88,99} This is despite evidence that the risk of HCC is also increased in CHD compared to HBV monoinfection, with development at younger ages.^{100–102}

How and when should HCC surveillance be performed in patients with CHD?

Recommendation

- HCC surveillance should be performed with abdominal ultrasound every 6 months in patients with CHD with advanced fibrosis or cirrhosis, regardless of anti-HDV therapy (**LoE 3, strong recommendation, strong consensus**).

HCC surveillance should be performed by ultrasound every 6 months in patients with CHD and advanced fibrosis (*i.e.* bridging fibrosis, METAVIR F3 or Ishak stage 4 or 5) or cirrhosis (METAVIR F4, Ishak stage 6), regardless of anti-HDV therapy. As discussed before, no specific thresholds for NITs have been

conclusively defined to identify advanced fibrosis or cirrhosis in CHD, thus the decision to start HCC surveillance should rely on imaging, clinical or bio-humoral signs of advanced liver disease.

Several studies have reported that patients with CHD have a higher risk of developing HCC compared to those with HBV infection alone.^{102–105} This notion is supported by a systematic review of 93 studies (N = 98,289) reporting a greater risk of HCC in HBV/HDV-coinfected than HBV-monoinfected patients (OR 1.28; 95% CI 1.05–1.57) that was even stronger in prospective cohort studies (OR 2.77; 95% CI 1.79–4.28; $I^2 = 0\%$). The exclusion of studies published before 2010, of those at a high risk of bias and/or those including patients with HCV/HDV/HDV coinfection resulted in a more evident difference in HCC risk between HBV/HDV-coinfected and HBV-monoinfected patients.¹⁰² Another meta-analysis found that patients with CHD had a 2-fold higher risk of developing liver cancer than patients infected with HBV alone.⁹⁵

While cirrhosis is a major risk factor for hepatocarcinogenesis in both chronic hepatitis B and C, this relationship has not been conclusively demonstrated in CHD. A subgroup analysis of five studies including 709 patients with CHD and histologically proven cirrhosis¹⁰² just failed to demonstrate an increased risk of HCC in patients with cirrhosis (pooled OR 2.17; 95% CI 0.96–4.9; $p = 0.06$; $I^2 = 21.46\%$). Similarly, in another meta-analysis, data from the pooled original studies did not confirm that the presence of advanced fibrosis/cirrhosis was associated with a significantly higher risk of HCC.⁹⁵ To further complicate matters, APRI, FIB-4, Fibrotest and Fibroscan do not seem to represent accurate predictors of cirrhosis in patients with CHD, although a combination of serum markers (APRI <2 or FIB-4 <3.27) and liver stiffness assessment (Fibroscan <12.5 kPa) had been proposed to rule out cirrhosis in a preliminary study.⁸⁰

The EASL CPGs on HCC management suggest that non-cirrhotic patients with stage 3 fibrosis, regardless of the aetiology of liver disease, as well as non-cirrhotic patients with chronic hepatitis B and a PAGE-B score >10 can be considered for surveillance based on individual risk assessment (evidence low, recommendation weak). The first recommendation stems from the notion that patients with chronic hepatitis C and bridging fibrosis are at risk of developing HCC, possibly related to rapid worsening of liver disease stage and difficulties defining the transition from advanced fibrosis to cirrhosis, particularly using non-invasive tests.¹⁰⁶ Regarding the PAGE-B score, there is a single report in patients with CHD, confirming that both intermediate (10–17) or elevated (≥ 18) scores were associated with an increased risk of HCC (hazard ratio 4.63 [95% CI 2.10–10.22] and 18.43 [95% CI 8.16–41.63], respectively, $p < 0.001$) in this setting too.¹⁵

Based on the above premise, while awaiting more consistent information about HCC incidence in non-cirrhotic patients with CHD, it seems reasonable to recommend HCC surveillance in cases with bridging fibrosis, especially in the presence of other HCC risk factors, such as alcohol, tobacco use, obesity, family history of HCC, potential exposure to aflatoxins

(e.g. in patients from sub-Saharan Africa), and HIV or HCV coinfection.

The mechanisms leading to the development of HCC in chronic HDV infection remain to be elucidated. The oncogenic effect of HBV infection, even in the absence of cirrhosis, is well recognised,¹⁰⁷ but recent data have shown that the molecular signature of HDV-associated HCC differs from that of HBV-associated HCC.¹⁰⁸ HDV activates a specific DNA methylation process, HDV-induced signalling pathways, epigenetic dysregulation and an altered expression of upregulated genes involved in cell-cycle/DNA replication.^{100,108–110} Thus, the question of whether HDV is an oncogenic virus remains unanswered and further studies are needed to investigate the direct oncogenic potential of HDV infection, as well as to identify new diagnostic markers that can help predict the development of HCC or enable its early diagnosis in patients with CHD.

Evidence regarding the impact of HDV viraemia on the risk of HCC is not conclusive. One study showed a non-significantly higher number of HCC cases in patients with HDV viraemia (8/95, 8.4% vs. 2/35, 5.7%; $p > 0.9999$),¹⁰⁴ whereas others reported that HDV viraemia contributed significantly to the development of HCC, with a higher cumulative HCC incidence (22.2%) in HDV RNA-positive vs. HDV RNA-negative patients (7.3%, $p = 0.01$).¹¹¹ In another study, the risk of HCC was 2.6-fold higher in viraemic vs. non-viraemic patients although the difference was not statistically significant.¹⁶ The evidence that HDV RNA may be spontaneously cleared in patients with long lasting infection and cirrhosis could at least in part account for these findings.¹⁷

Therefore, both HDV RNA-positive and -negative patients with CHD and advanced fibrosis/cirrhosis should be maintained on HCC surveillance. Several reports have described differences in the severity of liver disease according to HDV genotype; HDV-genotype 1 is associated with a significantly higher incidence of cirrhosis and mortality.¹⁵ Coinfection with HIV or HCV is associated with a higher risk of HCC; a 6- to 9-fold increase in HCC risk has been reported in HIV/HBV/HDV-triple-infected patients compared to HIV/HBV-coinfected patients.^{86,105}

The goal of surveillance is to detect HCC at early stages when curative therapies or liver transplantation can be considered. Six-monthly ultrasound surveillance is strongly recommended in the EASL guidelines.¹⁰⁶ The accuracy of serum alpha-fetoprotein (AFP) results is suboptimal for HCC surveillance. Nonetheless, the addition of AFP determination to ultrasound enhances detection rates of early- or any-stage HCC in patients with cirrhosis, although increased false-positive rates were observed in a systematic meta-analysis.¹¹² In both EASL and the AASLD HCC guidelines, AFP has been considered optional.¹¹³ When ultrasound evaluation of the liver is technically challenging (e.g., patients with severe obesity or hepatic steatosis), other imaging techniques such as CT or contrast-enhanced MRI can be considered.^{106,113} Finally, since the goal of HCC surveillance is to improve patient survival, it is cost-effective only in individuals who are eligible for cancer treatment or liver transplantation.

Patient monitoring and selection for treatment

How should untreated patients with CHD be monitored?

Recommendations

- Patients with CHD should receive regular work-up for liver disease at least every 6–12 months (**LoE 3, strong recommendation, strong consensus**).
- Virological parameters measured as part of the clinical work-up should ideally include quantitative assays for HBsAg, HBV DNA and HDV RNA (**LoE 5, strong recommendation, consensus**).

In patients with CHD the baseline diagnostic-assessment process is essential to correctly define the phase of HBV/HDV infection and to evaluate activity, stage and cofactors of liver disease, including signs of autoimmunity such as increased Ig levels and presence of autoantibodies (LKM-3). Notably, the available data indicate that, despite the high heterogeneity of patient cohorts and the evidence that milder disease forms may be observed in a sizeable number of cases,¹⁶ at least 25% of patients have cirrhosis at first evaluation²¹ and cirrhosis may be present at a young age.⁹³ The lack of standardisation of NITs in the setting of CHD implies that the information obtained from clinical history, blood tests (of disease activity, liver function and platelet count), ultrasound (size of the liver, capsular contour, echo pattern of the parenchyma, spleen size) has to be combined to accurately characterise liver disease at the single patient level.⁶⁸

A regular work-up of HBV/HDV infection and liver disease ensures the identification of changes in disease profile requiring antiviral treatment, such as the transition from mild to severe disease activity (significant and persistent aminotransferase elevations), eventually associated with worsening of liver stiffness, or with blood tests or ultrasound evidence of disease progression. Conversely, in patients with more advanced liver disease who cannot be treated (either because of pegIFN α contraindication or without access to new drugs) monitoring is required to identify signs of disease progression (worsening of portal hypertension, hepatic decompensation or HCC development) to warrant specific treatments of these complications and a timely referral to liver transplant centres.^{87,88,99} The follow-up intervals (every 3 months vs. 6 months vs. yearly) have to be personalised depending on individual risk factors, stage of liver disease and the distinct clinical setting.^{87,88,99,114}

Persistence of HDV viraemia is associated with poor outcomes,^{16,51} but recent studies report that HDV RNA may become spontaneously undetectable in a significant proportion of cases (up to 28%) and this may be associated with a reduction in aminotransferases.^{17,54} Whether spontaneous HDV RNA clearance has a positive impact on long-term clinical outcomes requires further investigation, as no difference was observed in clinical outcome between those with or without HDV RNA clearance in the preliminary reports, though most patients had advanced liver disease.^{17,54} Thus, HDV RNA should be monitored at least yearly in untreated patients and

repeated tests (at 3 or 6 months) are recommended in case of its clearance in order to differentiate HDV clearance from spontaneous fluctuations in viral replication.^{51,55,56} Even if, at present, data supporting a prognostic role of HDV RNA serum levels in untreated patients are scarce,^{52,53} the monitoring of HDV viraemia should be performed using commercially available standardised assays to generate reliable and quantitative results.^{42,43}

In patients with advanced fibrosis or cirrhosis, clinical, biochemical and imaging follow-up should be maintained irrespective of HDV RNA clearance because progression of liver disease may still occur.^{17,54} HBV DNA and HBsAg serum levels should be monitored yearly or when major fluctuations of HDV RNA or ALT flares are observed, because relapses of HBV replication have occasionally been reported in the case of HDV clearance,⁶² while decline/loss of serum HBsAg has also been observed in the case of ≥ 2 log reductions or clearance of HDV RNA.^{17,54}

Which patients with CHD should be considered for antiviral treatment?

Recommendations

- All patients with CHD should be considered for antiviral treatment (**LoE 3, strong recommendation, consensus**).
- Patients with decompensated cirrhosis should be evaluated for liver transplantation (**LoE 3, strong recommendation, strong consensus**).
- Patients with HCC may be considered for antiviral treatment on an individualised basis (**LoE 5, weak recommendation, strong consensus**).

Despite recent reports suggesting that CHD may have a less aggressive course than initially described in the '80s, CHD is still a progressive liver disease for which remission is rare.^{16,49,50} Furthermore, cirrhosis can be diagnosed in a proportion of patients without a previous history of significant liver damage, suggesting that advanced liver disease may develop subtly in patients with mild but long-lasting liver necro-inflammation.¹⁷ Accordingly, in older cohorts, it was described that about 10% of patients had a mild CHD with an uneventful course during a relatively short follow-up.⁸⁴ The driving factor of disease progression is the persistence of viral replication,^{16,88} whereas treatment-induced suppression of viral replication results in clinical benefit.^{49,50,115,116}

Therefore, all patients with CHD are potential candidates for antiviral therapy; nevertheless, the decision on whether to start treatment should be made at an individual patient level after careful evaluation.

Whether the presence of cirrhosis influences the response (end of treatment and 24 weeks after treatment) to IFN α (standard or pegylated) has not been ascertained. Cirrhosis did not show any impact on response in patients treated in the HIDIT-1 and HIDIT-2 trials^{117,118}; whereas, in a retrospective study of 99 patients with CHD treated with IFN α , a higher platelet count was an independent predictor of off-treatment viral response, suggesting that patients without advanced liver disease are more likely to achieve a virological response.¹¹⁵ Nevertheless, only about 29% (24-34%) of

patients respond to IFN α , and relapse occurred in about 50% of them during long-term follow-up.^{119,120} Furthermore, IFN α treatment is contraindicated in patients with major extrahepatic comorbidities or advanced liver disease and is associated with side effects that may significantly impact quality of life during treatment or lead to treatment discontinuation.²⁵ As a consequence, IFN α use in patients with mild liver disease (F0-F1) should be carefully weighed, taking into account the new therapeutic approaches that are under development.¹²¹ Preliminary data on BLV suggest that the on-treatment response at week 48 is not influenced by the presence of cirrhosis at baseline.¹²² Therefore, all patients with active liver disease, advanced liver fibrosis or compensated cirrhosis should be considered for treatment, as a successful treatment may result in improved long-term clinical outcomes, as indicated by IFN α -based antiviral treatments^{49,50,115,116,119} (Fig. 3).

Currently, there are no licensed treatments for patients with CHD-related decompensated cirrhosis. Therefore, such patients must be evaluated for liver transplantation, which is associated with an excellent outcome in the setting of CHD.¹²³ If liver transplantation is not possible, a best-supportive-care strategy is recommended.

In patients with CHD and HCC, the optimal treatment for HCC (including liver transplantation) should be prioritised, whereas antiviral treatment may be considered on a case-by-case basis, depending on the overall prognosis and potential benefit.

Therapeutic approaches

Which patients with CHD can be treated with PegIFN α ?

Statement

- IFN α has been used since the '90s for the treatment of CHD. Mono- and multicentre studies have been conducted with IFN α , with only two randomised phase II studies published. Nevertheless, long-term data on clinical benefit and safety are available (**LoE 2, strong consensus**).

Recommendations

- All patients with CHD and compensated liver disease, irrespective of whether they have cirrhosis or not, should be considered for treatment with PegIFN α (**LoE 2, strong recommendation, consensus**).
- PegIFN α for 48 weeks should be the preferred treatment schedule (**LoE 3, strong recommendation, consensus**).
- Personalised treatment durations may be considered based on HDV RNA and HBsAg kinetics and treatment tolerability (**LoE 3, weak recommendation, strong consensus**).

Until recently, standard IFN α and mostly its pegylated form (pegIFN α) was the only treatment option for CHD.²⁵ IFNs are molecules with broad antiviral efficacy against many viruses, including HBV and HCV and the synergism between the antiviral and immunomodulatory activities of IFN α are believed to play a major role in the control of CHB.²⁵ In CHD, effective IFN α

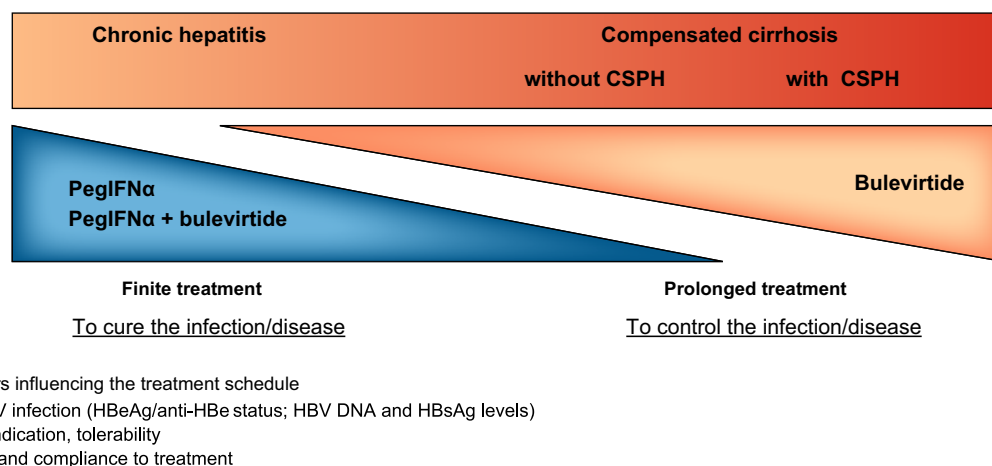


Fig. 3. Management of antiviral treatment in patients with CHD. Finite or prolonged treatments are the two approaches used in CHD aimed to cure or control the infection and disease. A major factor influencing the choice of treatment is the stage of liver disease. HBV, hepatitis B virus; CHD, chronic hepatitis D; CSPH, clinically significant portal hypertension; HBeAg, HBV e antigen; HBsAg, HBV surface antigen; IFNα, interferon-α; pegIFNα, pegylated-interferon-α. (This figure appears in color on the web.)

treatment is associated with a decline of both HBV and HDV markers, suggesting that a combined action on the two viral infections is essential to achieve full control of HDV infection.^{63,66,115} The specific mechanism of action of IFNα on HDV is not entirely delineated. *In vitro* studies suggest that IFNα marginally inhibits HDV replication in stably infected cells.^{125,126} A recent study suggests that its action may be viral strain-dependent because HDV viraemia was effectively inhibited by pegIFNα in human liver chimeric mice infected with HDV-1p and HDV-3, but not by HDV-1 (actually the first HDV that was cloned after serial passages in chimpanzees and woodchucks).¹²⁷ In addition, in recent years, *in vitro* studies unveiled new functions of IFNs.^{126,128} Notably, they showed that both IFNα and IFNλ significantly reduce HDV infection when given at an early stage of the infection, suggesting an inhibitory effect on viral entry.¹²⁶ Furthermore, both IFNs suppress cell division-mediated HDV spread, possibly by increasing the elimination of HDV replicative intermediates during mitosis.¹²⁸ This specific mode-of-action has fostered the investigation of IFNs in combination with other drugs that interfere with the biological life cycle of the virus (BLV or LNF). At present, the type of immune modulatory activity played by IFNs on innate and adaptive immune responses in CHD has not been fully elucidated.^{129–131}

Studies of IFNα for HDV infection include two randomised-controlled trials and many uncontrolled trials with prospective and retrospective designs, in which, despite a consistent definition of response (HDV RNA undetectability 24 weeks after the end of treatment), the sensitivity of the PCR assays for HDV RNA detection significantly changed overtime from >1,000 IU/ml to 6 IU/ml. Thus, the results of the different studies are not fully comparable. After re-testing 372 sera samples from 120 pegIFNα-treated patients with a more sensitive reverse-transcription PCR assay, 31% of the samples previously classified as negative with in-house PCR were HDV RNA-positive, indicating the potential for misclassification by assays with suboptimal sensitivity.¹³² Despite these limitations, pegIFNα was shown to be more effective than standard IFNα, with response rates of about 25% vs. 17%¹³³; accordingly, a recent meta-analysis of 13 studies reported a virologic response at 24 weeks post-treatment in 29% (17–47%) of patients receiving pegIFN.¹¹⁹ However, more than 50% of patients with virologic

response at 24 weeks post-treatment developed virological relapse later, up to 10 years after the end of IFNα treatment.^{50,120} Nevertheless, despite HDV RNA recurrence, long-term follow-up of the HIDIT-I trial showed that patients with undetectable HDV RNA at 6 months after the end of treatment, or at any time in the post treatment follow-up, had better outcomes (with less liver-related events) than non-responders.⁵⁰ The prolongation of (peg) IFNα treatment to 2 years in most studies does not appear to result in an increased rate of virologic response,^{117,134–136} but liver histology improved in most patients who were treated for 96 weeks in the HIDIT-II trial.¹¹⁷ Furthermore, anecdotal cases and cohort studies suggest that some patients with CHD may benefit from prolonged or repeated treatments, with higher rates of HDV RNA undetectability, which are associated with higher rates of HBsAg loss in long-term follow-up.^{115,137} Currently, prolongation or re-treatment with IFNα may be considered in patients with good compliance to treatment, with slow virologic response (in a subset of patients, the decline in viral load becomes more pronounced after the first 24 weeks of treatment) or with a progressive HBsAg decline.^{63,64,66,115,124,137} The combination of pegIFNα with HBV-specific nucleos(t)ide analogues (NAs),^{117,124,138–141} or ribavirin^{134,142,143} does not appear to improve the virologic response, as discussed in the later section on NAs.

Regarding the baseline predictors of response to IFNα, infection sustained by HDV genotype 5, low HDV RNA and HBsAg serum levels were shown to be associated with higher rates of virologic response.^{63,91,115,117} The data on the effect of liver disease stage are in some way conflicting, even if most of the studies suggest that IFNα is equally effective in patients with advanced or non-advanced liver disease.^{49,117,118} However, among patients with cirrhosis, the chances of response could be lower in those with clinically significant portal hypertension (*i.e.* with low baseline platelets).^{115,134} The identification of on-treatment predictors of response or futility rules has been addressed by several studies, but the high variability of HDV RNA kinetics during treatment has made it challenging to determine how to use the timing/extent of viral load decline to guide therapy at the individual patient level.¹⁴⁴ Serum HDV RNA levels at week 24 of treatment appear to be the strongest predictor of response, as undetectable HDV RNA at this time point

had a PPV of 100% for the identification of virological response 24 weeks after the end of treatment. The diagnostic performance of HDV RNA response at week 24 (1 log HDV RNA decline or less) in the identification of non-response (<1 log HDV RNA decline at end of therapy) was modest, with 67% sensitivity, 85% specificity, 67% PPV and 91% NPV.⁶⁴ Thus, even if most of the patients without significant HDV RNA declines at week 24 have a low probability of response 24 weeks after the end of treatment, some patients still respond after the first 6 months of therapy. An increasing number of studies suggest that a combination of HDV (HDV RNA) and HBV (HBsAg and HBcrAg) markers could be used to develop algorithms to tailor pegIFN α treatment at the single patient level.^{63,66} HBsAg serum levels <1,000 IU/ml at week 24 of treatment were shown to differentiate responders (both HBsAg and HDV RNA undetectable at the end of follow-up) and partial responders (HBsAg detectable and HDV RNA undetectable at end of follow-up) from non-responders (59% vs. 10%, $p < 0.001$), and a 1.61 log HDV RNA decline at week 24 was the best cut-off to differentiate responders or partial responders from non-responders (AUROC 0.791, sensitivity 86%, specificity 68%).⁶³

Retrospective cohort studies have shown that IFN α treatment favourably affects the natural history of CHD when compared to no treatment or treatment with NAs, while significant histological improvements with clearance of necro-inflammation and reduced fibrosis were shown on follow-up liver biopsies.^{49,50,116,120,142,145} In addition, long-term clinical complications and death from liver disease develop less frequently in responders compared to non-responders, particularly in patients without cirrhosis at baseline.^{50,115,120}

Overall (peg)IFN α has been used in patients with CHD, including compensated cirrhosis, but it is contraindicated in patients with decompensated cirrhosis.²⁵ In patients with compensated cirrhosis, special attention should be given to diagnose CSPH and oesophageal varices: in this subset of patients, the cost/benefit of pegIFN α treatment should be carefully evaluated because of a higher risk of major adverse events during treatment (Fig. 3). IFN α treatment is usually well tolerated in patients with CHD, even when treatment exceeds the canonical 48 weeks^{115,117} and the rate of treatment discontinuation is not higher than for other forms of viral hepatitis.^{117,124} Besides the common side effects of IFNs, specific attention should be paid to identifying autoimmune hepatitis in a timely manner in the setting of CHD, as this can be triggered by the treatment.^{124,146,147} Accordingly, markers of autoimmunity, notably anti-LKM-3 antibodies, which are frequently detected in patients with CHD (up to 13% in Italian cohorts), can be associated with autoimmune hepatitis in a minority of patients.^{70,148}

Which patients with CHD can be treated with BLV?

Statement

- Despite the lack of data on long-term efficacy and safety, or on the optimal duration of BLV treatment, preliminary results from phase II studies (with BLV given as monotherapy or in combination with pegIFN α), on-treatment data from a phase III trial of BLV monotherapy and real-life studies suggest consideration of BLV as a treatment option for CHD whenever available (**LoE 3, consensus**).

Recommendations

- All patients with CHD and compensated liver disease should be considered for treatment with BLV (**LoE 3, strong recommendation, consensus**).
- The optimal dose and duration of treatment have not yet been defined (**LoE 5, consensus**). Until further data become available, long-term treatment with BLV, 2 mg once daily, may be considered (**LoE 5, weak recommendation, consensus**).
- The combination of pegIFN α and BLV may be considered in patients without pegIFN α intolerance or contraindications (**LoE 5, weak recommendation, consensus**).

BLV (or bulevirtide, formerly myrcludex B), a synthetic myristoylated lipopeptide consisting of 47 amino acids of the preS1 domain of the HBV large surface protein, blocks the attachment of HBsAg to the cell entry receptor NTCP.¹⁰ *In vitro* and animal model studies showed that BLV interferes with the cellular entry of both HDV and HBV. Accordingly, in non-infected hepatocytes, BLV blocks the formation of both covalently closed circular DNA and HDV replicative intermediates and its continuous administration decreases the fraction of infected cells by blocking the HBV-mediated spread of HDV.^{149–151} However, during BLV treatment, intrahepatic HDV spreading due to the HBV/NTCP-independent, cell division-mediated mechanism persists, antagonising the eradication of HDV infection.⁴

Different doses (2, 5 and 10 mg) of BLV have been investigated either in monotherapy or in combination with pegIFN α .^{122,152–155} In the first human study in CHD, daily subcutaneous injections of 2 mg BLV led to a significant decline in HDV RNA (1.67 log cp/ml) after 24 weeks and its combination with pegIFN α led to a higher mean on-therapy HDV RNA decline (2.59 log cp/ml) and undetectability rates (7/7 vs. 2/7 with BLV monotherapy).¹⁵² The primary endpoint of this pilot study was a 0.5 log decrease in quantitative HBsAg levels at any time, which was not reached in any patient. In a subsequent phase II study (MYR202), the significant viral load reduction induced by BLV monotherapy was confirmed, as 24 weeks of 2, 5 and 10 mg BLV resulted in median HDV RNA declines of 2.140, 2.021 and 2.702 log IU/ml; ≥ 2 log HDV RNA reductions in 53%, 53% and 81% of patients; and undetectable HDV RNA in 4%, 6% and 3% of patients, respectively.¹⁵³ Paired liver biopsies were available for 22 patients and showed a significant decline of intrahepatic HDV RNA and a reduction of HDV-infected cells. A combined response (viral load decline ≥ 2 log and normal ALT) was observed in 21%, 28% and 37% of patients treated with 2, 5 and 10 mg BLV and treatment was associated with a reduction of liver stiffness values (-2.85, -2.58 and -3.38 kPa in the 2, 5 and 10 mg BLV groups, respectively), suggesting a positive impact on intrahepatic necro-inflammation.¹⁵³ However, 49 of the 55 (89%) patients with virologic response experienced a relapse of viral replication after treatment discontinuation, which was associated with aminotransferase flares in 22% of cases.¹⁵³ Furthermore, the study showed the absence of a dose-dependent efficacy, supporting

use of the lower BLV dose (2 mg) that does not saturate the bile acid transporter. HBsAg serum levels did not significantly decline during the 6 months of BLV monotherapy.¹⁵³ The overall findings of phase II study were further strengthened by an interim analysis at week-48 of the ongoing phase III study (MYR301), where BLV 10 mg did not provide an efficacy advantage over BLV 2 mg.¹²² Accordingly, BLV 2 or 10 mg were associated with HDV RNA declines of ≥ 2 log at week 48 in 71% and 76% of patients, respectively, while response rates from week 24 to 48 went from 66% to 76% in the 10 mg group and from 55% to 71% in the 2 mg group.¹²² After 1 year of treatment, undetectable HDV RNA and combined (virologic and biochemical) response were achieved in 12% and 45%, respectively, of the patients treated with 2 mg BLV and in 20% and 48%, respectively, of those treated with 10 mg BLV. Since the optimal duration of BLV therapy associated with the achievement of a durable virological response is unknown, extending BLV treatment beyond 1 year currently appears to be the most appropriate strategy to further increase or to maintain the virologic response (Fig. 3). Accordingly, in July 2020, BLV 2 mg received conditional marketing authorisation by the EMA for the treatment of CHD, with the recommendation to maintain the treatment until clinical benefit is observed.¹⁵⁶

The immune modulatory and antiviral activities of IFN α on both HBV and HDV, and its notable ability to inhibit cell division-mediated HDV spread, are the rationale for combining it with BLV, with the aim of achieving a durable virologic response.^{25,128} In the MYR203 study, a 48-week combination therapy resulted in 24-week off-treatment HDV RNA undetectability (<10 IU/ml) rates of 53%, 27% and 7% in patients treated with 2, 5 or 10 mg of BLV, respectively.¹⁵⁴ A ≥ 1 log HBsAg serum decrease was observed in 40%, 13% and 13% of patients depending on the BLV dose (2, 5 and 10 mg BLV, respectively). HBsAg clearance occurred in 4/15 (27%) and 1/15 (7%) patients treated with BLV 2 mg and 10 mg, respectively.¹⁵⁴ Persistently undetectable serum HDV RNA at 24 weeks after treatment was only reported in patients with a decline in serum HBsAg, confirming the importance of combined control of HBV/HDV to maintain response.¹⁵⁴ In the ongoing MYR204 study, patients were randomised to receive BLV 2 or 10 mg in combination with pegIFN α for 48 weeks followed by an additional 48 weeks of BLV, or 10 mg BLV monotherapy for 96 weeks. The 24-week on-treatment results confirmed that the HDV RNA decline was higher in patients receiving BLV 2 and 10 mg plus pegIFN α (88% and 92%) compared to BLV monotherapy (72%) and only patients receiving the combination therapy achieved a ≥ 1 log serum HBsAg decline (12% and 8% of the patients treated with pegIFN α plus 2 and 10 mg BLV, respectively).¹⁵⁵ The preliminary data on *de novo* combination therapy are promising, but the number of patients enrolled in the phase II study was quite small and some results (*i.e.* the striking difference in the virologic response and HBsAg clearance between the 2 and 5/10 mg doses) remain to be explained. Even if combination therapy appears to be an attractive finite therapy for CHD, additional studies are needed to identify the optimal treatment schedule before it may be proposed as first-line therapy in this setting. In current clinical practice, BLV plus pegIFN α should be

offered to compliant patients and possibly within clinical protocols (Fig. 3).

Several reports on real-life data from over 500 patients treated in France, Germany, Austria and Italy have been presented at international meetings,^{157,158} but a detailed description of treatment outcomes has only been published in full manuscripts for approximately one-third of these patients.^{67,159–164} With the limitation of heterogeneous treatment schedules and follow-ups, the overall data confirm the combined response rates and safety reported in clinical trials.¹⁶⁵ Notably, preliminary data from the French Early Access Cohort suggested an increasing virologic response (≥ 2 log HDV RNA decline) from month 12 (33%) to month 24 (68%).¹⁵⁷ A case report series from Italy and Austria showed that BLV treatment for 3 years in patients with advanced cirrhosis was associated with a significant improvement in liver function tests, disappearance of oesophageal varices and the resolution of autoimmune hepatitis associated with HDV infection.⁶⁷ The German real-world experience over a mean observation period of 38 ± 17.6 weeks, confirmed high rates of virologic response (≥ 2 log HDV RNA decline in 74% of patients and undetectable viraemia in 22%), whereas a <1 log HDV RNA decline was reported in 9% of patients and viral breakthrough (≥ 1 log increase) was reported in 12% of virologic responders. In the latter cohort, five patients had hepatic decompensation at baseline (Child-Pugh B in four and C in one): the treatment was well tolerated without major side effects; all patients achieved a virologic response, and ALT declined and platelets increased in all but one; a patient with refractory ascites experienced a temporary improvement of ascites.¹⁵⁹ Finally, the serum and intrahepatic clearance of HDV RNA (despite HBsAg persistence) was recently shown to be maintained at 1 year after discontinuation of 72-week BLV treatment in a patient with cirrhosis and oesophageal varices.¹⁶⁶ The case report suggests that the clearance of HDV infection may be favoured by prolonged BLV treatment and that patients with more advanced disease and lower intrahepatic burden of HDV infection¹⁷ could benefit more from BLV treatment.

BLV treatment was well tolerated without drug-related serious adverse events or treatment discontinuations.^{152–154} A minority of patients complained of mild symptoms like fatigue, nausea, headache, dizziness or showed a reduction of platelets or white blood cells; adverse reactions at the injection site were mild, transient and only occasionally required specific treatment.^{167,168} Since BLV inhibits the bile acid transporter function of NTCP,^{169,170} as expected a transient increase of total bile acids was reported in all studies; it was dose related (median values up to 20 and 80 $\mu\text{mol/L}$ in BLV 2 and 10 mg groups) and symptomless (including pruritus).^{122,153–155} Notably, the genetic deficiency of NTCP leads to extreme elevations of plasma bile acids without clinical signs of hepatic dysfunction.¹⁷¹ The implications of BLV binding within the pore of NTCP on bile acid transports and the physiological variability of NTCP expression need to be further investigated to better understand the virologic response to BLV.^{172–174}

Blocking hepatocellular uptake of bile acids may also have metabolic effects, as using 5 mg of BLV led to a decline in serum LDL cholesterol and an increase in HDL cholesterol in

volunteers with LDL cholesterol levels of >130 mg/dl.¹⁷⁵ When possible, coadministration of NTCP substrates should be avoided during BLV treatment. This includes distinct statins like fluvastatin, atorvastatin, pravastatin or rosuvastatin and thyroid hormones. *In vitro*, BLV can partially inhibit OATP1B1 and OATP1B3 at higher doses, which are usually not reached with the approved subcutaneous dose of 2 mg.¹⁷⁶ Moreover, in healthy volunteers, coadministration of tenofovir and BLV led to a decreased clearance of the CYP3A substrate midazolam.¹⁷⁷ Thus, potential drug-drug interactions should be considered when treating patients with BLV.

Overall, the data on BLV are compelling, as 40% to 65% of patients treated with BLV monotherapy for 48 weeks achieve a ≥ 2 log HDV RNA decline and normal ALT, a surrogate endpoint indicating a likely improvement of intrahepatic necro-inflammation, as also suggested by the reduction of liver stiffness.^{67,122,153,161} Nevertheless, several questions remain to be answered about the correlation between HDV RNA and ALT kinetics: why do some patients experience an ALT reduction despite a poor virological response? Conversely, why do other patients, despite a ≥ 2 log decline of viral load, not show a biochemical response? Is there a viraemia threshold that correlates with the resolution of HDV-induced liver damage and could baseline viraemia influence the extent of HDV RNA decline required to improve the liver damage? Furthermore, does prolonged BLV treatment promote a further increase in the response rate or conversely do a proportion of patients lose their response? Similarly, what about safety over time? Will the BLV and pegIFN α combination qualify as an effective finite treatment option for a sizeable number of patients? Finally, the correlation between the achievement and maintenance of surrogate endpoints and long-term clinical benefits remains to be demonstrated.

Meticulous virological, biochemical and clinical monitoring of patients on BLV has to be maintained to identify potential recurrence of viral replication or safety issues. Nevertheless, given the high relapse rate after 6 months of treatment, prolonged treatment is recommended until further data are available (Fig. 3).

When should NAs be used in patients with CHD?

Recommendations

- NAs should be given in patients with decompensated cirrhosis irrespective of the presence of detectable HBV DNA (LoE 5, strong recommendation, strong consensus).
- NAs should be given in patients with compensated cirrhosis and detectable HBV DNA (LoE 5, strong recommendation, strong consensus).
- NAs should be given in patients without cirrhosis if HBV DNA levels are higher than 2,000 IU/ml (LoE 5, strong recommendation, strong consensus).

Over the last two decades several studies investigated NAs either as monotherapy or in combination with IFN α in CHD, reporting poor efficacy with respect to the control of HDV infection. Famciclovir, lamivudine, clevudine, entecavir, adefovir and tenofovir have been given for 6 to 12 months and found to be ineffective.^{117,124,178–181} Similarly, the combination with pegIFN α was not associated with higher rates of virologic response, although the combination with adefovir was associated with a significant HBsAg decline¹²⁴ – this finding was not confirmed when tenofovir was used.¹¹⁷ The only exception were some studies in HIV/HDV-coinfected patients, in whom long-term treatment with tenofovir was associated with HDV RNA declines and improvements in liver stiffness.^{182,183} It has been hypothesised that the effect on HDV RNA could be mediated by immune reconstitution favouring better control of HDV infection rather than a direct antiviral effect on HBV/HDV.¹⁸⁴ However, the results of these small, retrospective cohorts were not confirmed in other independent cohorts.^{185–187} Accordingly, at present, NAs are not recommended as part of the antiviral therapy aimed at controlling CHD.

Conversely, NAs are indicated when control of HBV replication is appropriate, mainly in two scenarios: active HBV replication (HBV DNA >2,000 IU/ml) or when prevention of reactivation of HBV is clinically mandated.

The presence of significant HBV replication (HBV DNA >2,000 IU/ml) may contribute directly to liver damage; in addition, it has been shown to have a negative impact on the outcomes of patients with CHD; thus, its inhibition by NAs is recommended.^{25,60} Furthermore, several studies have shown that major fluctuations of HDV and HBV replication may occur over time^{17,54–56} and that the progressive decline of HDV viral load and eventually its clearance in the more advanced stages of liver disease may be associated with a recurrence of HBV replication.^{55,62} Accordingly, in patients with cirrhosis, NA treatment should be started in all decompensated patients, independently of the presence of HBV viraemia, and in all patients with compensated cirrhosis if they have detectable serum HBV DNA.²⁵

Finally, the availability of a new drug, BLV, blocking the entry into the hepatocytes of both HBV and HDV and therefore interfering with the life cycle of both viruses^{122,188} exposes patients to the risk of HBV reactivation in case of treatment discontinuation. Thus, treatment with NAs should be considered at the time of BLV discontinuation and initiated in case of relapse of HBV replication.

Which is the best prophylactic strategy for prevention of post-transplant hepatitis D recurrence?

Recommendation

- Patients who have undergone liver transplantation for CHD should receive hepatitis B immunoglobulin (HBIG) combined with a high genetic barrier NA after transplantation (LoE 3, strong recommendation, strong consensus).

Statement

- During the early post-transplant period the optimal HBIG dose has not been defined and varies among centres. Most experienced centres give HBIG at 10,000 IU intravenously in the anhepatic phase, followed by 600–1,000 IU intramuscularly/intravenously daily for 7 days, then weekly for 3 weeks, and then monthly until month 3–6 (**LoE 3, n.a.**).

Recommendation

- After the early post-transplant period (6 months), HBIG should be administered at the dose that maintains anti-HBs serum levels >100 mIU/ml (**LoE 3, strong recommendation, strong consensus**).

Statement

- Currently, indefinite treatment with HBIG and NA in combination is considered the gold standard, but evidence on HBIG discontinuation after 1–2 years is gradually accumulating (**LoE 4, n.a.**). Further studies, particularly in the setting of clinical trials, are warranted to assess the safety of this approach.

Unlike for CHB, liver failure rather than HCC is the most frequent reason for liver transplantation in CHD and, at least in Europe, in the last 15 years the rate of LT for CHD has overtaken that for CHB, underlying the need for effective treatments for CHD.^{189,190} Since the late '80s, LT has proven to be an essential therapeutic option for patients with CHD, given the good survival rate, despite evidence of intrahepatic recurrence of HDV infection in over 70% of cases.^{191–193} The presence of HDAg in the graft was usually associated with mild histological alterations, such as degenerative lesions of the hepatocytes and steatosis without evidence of progressive liver disease, unless there was recurrence of a florid HBV infection.¹⁹⁴ The available data suggest that a full HBV infection of the graft has to be established to support florid HDV infection and disease. The low HBV viraemia of patients with CHD reduced the risk of HBV/HDV recurrence even when only HBIG was used. Combined prophylaxis with HBIG and NA further improved post-transplant outcomes.^{195–197} Currently, the best prophylactic strategy to prevent post-transplant HDV recurrence is based on long-term administration of HBIG combined with a high genetic barrier NA.^{196,197} The optimal HBIG dose and type of administration have not been conclusively defined and they will vary over time after transplant. After the early post-transplant period, according to the recommendation of the European Liver and Intestine Transplant Association (ELITA), anti-HBs serum levels >100 mIU/ml appear to be enough and HBIG can be given on a fixed schedule or on-demand to maintain the antibody threshold.¹⁹⁶ Among NAs, entecavir and tenofovir alafenamide should be preferred.

As robust data from large studies are lacking for patients with CHD, the optimal prophylaxis for patients transplanted for HBV/HDV coinfection is similar to that for HBV mono-infection.²⁵ Given that HBsAg is necessary for HDV entry into

hepatocytes and to support effective HDV spread in the liver, post-transplant recurrence of HBsAg seropositivity may be detrimental for HBV/HDV transplant patients, although it might be acceptable for HBV-monoinfected patients, given the high efficacy of NAs in inhibiting HBV replication. For this reason, up to now, post-transplant prophylactic approaches associated with a small risk of recurrence of serum HBsAg, such as monoprophyllaxis with a NA, were not considered acceptable for patients transplanted for HBV/HDV coinfection.¹⁹⁶ Thus, the combination of HBIG administration and a high genetic barrier NA currently represents the optimal strategy for prevention of HDV recurrence in HBV/HDV transplant patients.¹⁹⁸

The data supporting the efficacy of the combination of HBIG with a NA in HBV/HDV transplant patients are limited: in a small, historic, retrospective study including 46 HBV/HDV transplant patients,¹⁹⁹ 21 received HBIG alone and 25 received a combination of HBIG and lamivudine. HBIG was always given intramuscularly, to maintain anti-HBs levels >500 mIU/ml during the first 6 months post-transplant and >200 mIU/ml thereafter. There was no HBV/HDV recurrence in either group, but the authors concluded that the combination of HBIG and lamivudine was a more cost-effective approach, because the group receiving NAs required lower amounts of HBIG.¹⁹⁹ In another study including 26 HBV/HDV transplant patients,²⁰⁰ no HBV/HDV recurrence was observed with long-term combination of high-dose HBIG and lamivudine. A larger study assessed 128 HBV/HDV transplant patients who received tenofovir disoproxil fumarate and HBIG at high (5,000 IU during anhepatic phase and 2,000 IU/day) or low (2,000 IU during anhepatic phase and 500 IU/day) intravenous doses for 7 days if they had pre-transplant HBV DNA > or <1,000 copies/ml followed by monthly intravenous HBIG infusions targeting anti-HBs >100 mIU/ml.²⁰¹ There was no HDV recurrence, although HBsAg positivity was observed in 4/128 (3.1%) patients; these data support the relevance of profound inhibition of HBV replication on avoiding recurrence of hepatitis D. In two recent studies in which HBIG was discontinued,^{202,203} HDV recurrence was observed in approximately 6% (2/34 and 1/17) of patients during a median follow-up of 28 and 204 months (1 of 2 patients with HBV/HDV recurrence in the first study had received a graft from an HBsAg-positive donor).²⁰² However, HDV recurrence was not observed after HBIG discontinuation in an additional 64 cases reported by five other groups.^{204–208} At present, due to the small but not negligible risk of HDV recurrence, HBIG cessation, with continuation of prophylaxis with NAs alone or in combination with an entry inhibitor, should be investigated in clinical trials, as recommended by the ELITA position statement and by the transplant expert community.^{123,196}

The high genetic barrier NAs (entecavir, tenofovir disoproxil fumarate, tenofovir alafenamide) currently represent the first-line agents for treatment and prevention of HBV.²⁵ However, in the transplant setting, tenofovir disoproxil fumarate may be better avoided, if possible, as it is associated with some risk of nephrotoxicity that could be exacerbated by the concomitant use of calcineurin inhibitors.²⁵

Treatment endpoints

Which parameters should be monitored during and after treatment?

Recommendations

Virologic markers:

- Virological response to treatment of CHD should be determined during and after therapy (**LoE 3, strong recommendation, strong consensus**).
- HDV RNA should be quantified every 6 months during treatment and whenever there is a clinical indication (**LoE 5, strong recommendation, consensus**).
- For pegIFN α -based finite therapy, HDV RNA should be tested at the end of treatment, after 6 and 12 months and yearly thereafter (**LoE 4, strong recommendation, strong consensus**).
- In case of BLV discontinuation, HDV RNA should be tested at the time of treatment discontinuation, after 1, 3, 6, 12 months and yearly thereafter to monitor the relapse of viral replication (**LoE 4, strong recommendation, consensus**).
- HBsAg testing should be performed every year during and after therapy (**LoE 3, strong recommendation, consensus**).
- For pegIFN α -based therapy, quantitative HBsAg may be determined every 6 months during and every 12 months after treatment (**LoE 3, weak recommendation, strong consensus**).
- HBV DNA should be determined every 6 months in all treated patients who are not on NA therapy (**LoE 3, strong recommendation, strong consensus**); in case of BLV discontinuation, more frequent HBV DNA testing may be required (**LoE 5, weak recommendation, strong consensus**).

Biochemical markers:

- Testing for biochemical markers of liver disease activity (*i.e.* aminotransferases), full blood count and, in addition, liver function tests, whenever clinically indicated, should be performed during antiviral treatment (**LoE 3, strong recommendation, strong consensus**).
- Frequency of testing should be at least every 3–6 months, with the timing modulated according to the stage of liver disease and type of treatment (**LoE 3, strong recommendation, strong consensus**).
- For pegIFN α -based finite therapy, testing should be performed at the end of treatment, at least at month 6 and 12 after the end of treatment and yearly thereafter (**LoE 4, strong recommendation, consensus**).
- In case of BLV discontinuation, testing should be performed at the time of treatment discontinuation and at least after 1, 3, 6 and 12 months or more frequently according to clinical need (**LoE 4, strong recommendation, strong consensus**).

Liver imaging:

- Liver stiffness determination may be performed yearly during and after antiviral treatment of CHD (**LoE 5, weak recommendation, strong consensus**).

Histology:

- Liver biopsy should be performed in patients during and/or after antiviral treatment where histological diagnosis would aid clinical management (**LoE 3, strong recommendation, consensus**).

Clinical events:

- Patients with CHD should be monitored during and after treatment for the development of liver-related clinical events (**LoE 3, strong recommendation, strong consensus**).

The aim of CHD treatment is to reduce the progression of chronic liver disease, decreasing the incidence of cirrhosis, hepatic decompensation, HCC, and liver-related mortality.⁴⁵ In addition, treatment is also aimed at improving the quality of life of patients with liver disease.²⁰⁹ In trials, clinical endpoints are difficult to assess; however, in chronic hepatitis B and C it has been shown that they can be achieved by suppressing viral replication.^{25,210} In clinical trials for the treatment of CHD, virologic and biochemical endpoints and their combination have been used as “surrogate” endpoints to assess the effectiveness of treatment.^{45,211,212} However, at present, long-term evidence of their correlation with clinical benefit is limited to IFN α treatment,^{49,50,99,145} while studies for BLV are ongoing.

As previously discussed, the data on IFN α in CHD suffer many limitations owing to study design and the major changes in the diagnostic performance of the assays used to monitor the virologic (HDV RNA) responses.⁴² Nevertheless, several studies investigated the association between the achievement of specific virologic endpoints and survival or development of liver-related events (Table 3). Briefly, survival is improved in case of HBsAg loss,^{120,145} while clearance of serum HDV RNA at 24 weeks or at any point post-treatment has been associated with decreased liver-related complications (liver-related death, liver transplantation, liver cancer and hepatic decompensation) in 10 year follow-up.⁵⁰ Taking into account that the assay used to measure HDV RNA in the latter study had a sensitivity of about 900 IU/ml, this finding suggests that the achievement of low HDV RNA levels (<1,000 IU/ml) could be associated with a benign CHD outcome.⁴⁵ In agreement with this hypothesis, there is data suggesting that combined ALT normalisation and significant reduction (≥ 2 log) of HDV RNA obtained during IFN α treatment and maintained thereafter was associated with improved long-term clinical outcomes.⁴⁹

In recent years, both the FDA and the EASL-AASLD HBV treatment endpoints conference synthesised these data into specific endpoints for clinical trials for CHD treatment with novel antivirals, focusing on the two major treatment strategies^{211,212}: maintenance treatment and finite treatment (Table 4). Despite the lack of robust validation in clinical practice, such endpoints can reasonably be used in the clinical management of patients with CHD in real life.

Table 3. Virologic endpoints inferred from IFN α or pegylated IFN α treatments.

Endpoint	Parameter	Rate of occurrence with IFN α treatment	Clinical benefit
Ideal	HBsAg loss*	2.5% (0-25%) ¹¹⁹	Yes ¹²⁰
Desirable	Undetectable HDV RNA - 24 weeks after EOT - for 2 years after EOT - 8.9 years after EOT	29% (24-34%) ¹¹⁹ 50% ¹¹⁵ 36.6% ⁵⁰	Yes ^{115,120,145}
Acceptable	≥ 2 log HDV RNA decline at EOT, maintained thereafter	n.a. ⁴⁹ 10/14 patients with normal ALT at EOT, maintained in 7/12 (58.3%) after 12 years	Yes ⁴⁹

ALT, alanine aminotransferase; HBsAg, HBV surface antigen; HDV, hepatitis D virus; EOT, end of treatment; IFN α , interferon- α ; n.a., not assessable.
*24 weeks post-treatment.

Table 4. Primary endpoints for clinical trials of new anti-HDV treatments.

	Maintenance treatment	Finite treatment
FDA Developing drugs for CHD treatment (October 2019)	Surrogate endpoint likely to predict clinical benefit: ≥ 2 log reduction in HDV RNA and ALT normalisation (acceptable)	Undetectable HDV RNA and ALT normalisation (the timing of assessment according to treatment strategy)
EASL-AASLD HBV treatment endpoints conference (March 2019)	≥ 2 log reduction in HDV RNA (might suffice)	Undetectable HDV RNA at 6 months after end of treatment ALT normalisation (desired) HBsAg loss (ideal)

ALT, alanine aminotransferase; CHD, chronic hepatitis D; HBsAg, HBV surface antigen; HDV, hepatitis D virus.

Monitoring of virologic markers

HDV RNA

Reducing HDV replication is a primary goal of treatment of HDV infection; therefore, viral load should be regularly determined during treatment using well-standardised, validated real-time molecular assays.⁴² As a ≥ 2 log HDV RNA decline is a conditional criterion to define response to treatment, quantitative HDV RNA monitoring in sequential serum samples should be performed in the same laboratory and with the same assay to avoid inter-laboratory variations and to minimise inter-assay variability.^{42,45} Testing every 6 months seems to be reasonable to monitor response to therapy and its maintenance during prolonged treatment. Additional testing can be performed whenever clinically required (e.g. to rule out a viral breakthrough in case of ALT flares in a patient with previous evidence of response) or in clinical protocols to further investigate the on-treatment HDV RNA kinetics and their correlation with response. At present, robust data to tailor treatment (either with BLV monotherapy or pegIFN α) according to on-therapy HDV RNA kinetics are missing, even if a study suggested a correlation between week-24 HDV RNA levels and virologic response at 6 months post-treatment in pegIFN α -treated patients.⁶⁴ Likewise, futility rules to discontinue either pegIFN α or BLV treatment are missing, even if the predictive role of a <1 log HDV RNA decline after 24 or 48 weeks of therapy is under investigation.^{64,165,213}

After treatment discontinuation, regular HDV RNA testing is recommended at different timepoints, according to the type of treatment: 6 and 12 months after the end of pegIFN α treatment and yearly thereafter, because late relapses after pegIFN α have been reported, even after 5-8 years.^{50,120} After discontinuation of BLV monotherapy, viral load should be tested earlier and

more frequently because of the risk of reappearance of viral replication that could be associated with an exacerbation of hepatitis potentially requiring the reintroduction of BLV treatment.¹⁵³

HBsAg

Besides a reduction of HDV RNA, another goal of pegIFN α -based treatment is loss of HBsAg.^{45,212,214} Therefore, HBsAg should be tested during and after pegIFN α because HBsAg clearance may occur years after treatment discontinuation.¹¹⁵ In this setting, treatment duration may also be personalised based on HBsAg kinetics; treatment could potentially be extended beyond 48 weeks in those individuals demonstrating a continuous HBsAg decline.^{63,64,173,214} In contrast, HBsAg serum levels do not change during BLV treatment.¹⁵³ Regular HBsAg monitoring of HBsAg during BLV monotherapy is therefore not needed, although yearly testing may still be considered as spontaneous HBsAg declines have been reported.^{17,54}

HBV DNA

HBV replication may contribute to disease progression in HDV infection. If patients are not on NAs, monitoring of HBV DNA should be performed every 6 months, as HBV/HDV dominance patterns can change over time and during pegIFN α -based antiviral treatment.^{56,73} During BLV treatment, there is no evidence of HBV reactivation, rather a slight reduction of HBV DNA was reported in BLV-treated patients who were not treated with NAs.¹²² Since such on-therapy partial inhibition of HBV replication might favour a rebound, in case of BLV discontinuation, HBV DNA monitoring after BLV discontinuation is recommended in patients who are not on NA treatment.

New HBV markers

The role of monitoring serum HBcrAg and HBV RNA levels during antiviral treatment in CHD is under evaluation; thus, their testing is not currently recommended in clinical practice.⁶⁶

Monitoring of biochemical markers

Aminotransferases

The monitoring of disease activity is mandatory during treatment to evaluate whether a decline of aminotransferase levels parallels the inhibition of viral replication or to catch ALT flares that may occur during or after pegIFN α -based treatment, and may require treatment adaptation, in a timely manner.²⁴ In case of pegIFN α treatment, testing for aminotransferases should be performed every 4 weeks during the first 12 weeks and every 6–8 weeks thereafter. After the end of pegIFN α treatment, testing is recommended at post-treatment weeks 24 and 48, additional controls could be performed at week 4, 8 and 12 according to disease stage. Less frequent testing may be sufficient during BLV monotherapy: every 12 weeks seems to be reasonable. Conversely, in case of BLV discontinuation, monitoring may be required in the first 6 months to identify a possible ALT flare due to the recurrence of HDV replication (that may require the reintroduction of treatment).¹⁵³

Liver function tests

Liver function tests should be performed every 3–6 months, although more frequent testing may be required in patients with cirrhosis or aminotransferase flares.

Complete blood count

A complete blood count should be conducted according to the standard schedule in patients treated with pegIFN α ,²⁵ and at least every 3 months in patients on BLV monotherapy. Individualised monitoring schedules may be required in patients with cirrhosis.

Liver imaging

Liver stiffness

There is no data on the diagnostic and predictive value of liver stiffness measurements during and after pegIFN α -based treatment of CHD. Due to the increased activation of immune cells induced by IFN α , liver stiffness values may even increase during treatment. Liver stiffness values have been shown to decline after 24 and 48 weeks of BLV treatment in most patients, possibly because of the reduction of intrahepatic necro-inflammation.^{122,153} However, the clinical significance of this finding is uncertain. Testing liver stiffness values after treatment – e.g. yearly – may yield useful clinical information on disease progression and potentially influence decisions on re-treatment.

Ultrasound

Ultrasound imaging of the liver should be part of regular HCC surveillance and should be systematically used to monitor the

progression of liver disease by studying blood flow in the portal vein and spleen size, and screening for ascites.¹⁰⁶

Histology

Liver biopsy

Liver histology may be useful to investigate causes of unexplained ALT flares during treatment, since IFN α -based treatment may cause autoimmune events in HDV infection that may require immunosuppressive treatment with corticosteroids.¹²⁴ Regular follow-up liver biopsies are not recommended outside of clinical trials/protocols as their clinical benefit is limited.

Clinical events

Patients should be monitored to evaluate disease progression (development of cirrhosis, hepatic decompensation, and HCC) according to relevant CPGs.^{25,106,210}

Future treatment options

In recent years, a better understanding of the HDV life cycle and its interplay with the host hepatocyte^{1,3,4,9} has led to the identification of new therapeutic targets in host-mediated functions essential for HDV infection, such as the NTCP receptor, which is required for viral entry, and the farnesyl transferase enzyme, which mediates prenylation of the large delta antigen protein that is essential for HDV virion morphogenesis.^{10,150,215,216} Furthermore, given the central role of HBsAg in the production of mature virions and HDV spread within the liver, newly developed drugs that interfere with HBsAg production might represent additional therapeutic tools against HDV. In a phase II, non-randomised study, patients with CHD were treated with nucleic acid polymers (NAPs), amphipathic oligonucleotides that interact with the hydrophobic surface of the HBsAg and selectively destabilise the assembly and/or secretion of subviral particles, leading to degradation of intracellular HBsAg by the lysosomal pathway.²¹⁷ Twelve patients initially received NAP REP 2139 (500 mg intravenously/once weekly) monotherapy for 15 weeks, followed by a lower dose (250 mg) in combination with pegIFN α for an additional 15 weeks, followed by pegIFN monotherapy for 33 weeks. At end of therapy, 9/12 (75%) treated patients had undetectable HDV RNA, which was maintained in 7/11 (64%) at 1 and 3.5 years of follow-up. Interestingly, HBsAg loss was reported in five patients at 1 year and in four at 3.5 years (45% and 36%, respectively).^{65,218} ALT flares occurred during pegIFN α treatment in 5/12 (42%) patients, mainly in those whose HBsAg levels declined <1 IU/ml during the first 3 months of NAP monotherapy.²¹⁸ The results were long lasting in most of the responders, although larger studies are required to confirm these preliminary findings and address the safety of this treatment. HBV gene expression, including mRNA for HBsAg, can be targeted with small-interfering RNAs (JNJ-3989, VIR-2218 and RG6346) and antisense oligonucleotides (bepirovirsen and RO7062931), which have been shown to induce a

significant decline of HBsAg serum levels in HBV-monoinfected patients.^{219–223} These inhibitors might be combined with other molecules, like engineered antibodies against HBV, namely VIR-3434, that not only inhibit HBV entry, but also favour the clearance of HBsAg and activate dendritic cells.²²⁴ VIR-3434 is currently under investigation in phase II clinical studies with VIR-2218 in HDV. Two other drugs, namely, LNF, a farnesyl transferase inhibitor, and pegIFN λ , are in a more advanced phase of clinical investigation. PegIFN λ , while activating the same intracellular signalling pathway and retaining the same biological activity as IFN α , differs because it recognises a different heterodimeric receptor complex that is largely restricted to cells of epithelial origin (liver, lung and gut).^{225,226} In a phase II (LIMT-1) trial, 33 patients were treated with pegIFN λ (120 or 180 μ g subcutaneously once weekly for 48 weeks) and a dose-dependent response was observed at the end of treatment, when 7/14 (50%) of the patients treated with 180 μ g had a >2 log HDV RNA decline or negative HDV RNA compared to 4/19 (21%) patients receiving 120 μ g. Five of the 14 patients (36%) and three of 19 (16%) treated with 180 or 120 μ g pegIFN λ maintained undetectable HDV RNA 24 weeks after the end of therapy. PegIFN λ has also been used in combination with LNF for 24 weeks: 11 of 22 (50%) patients had undetectable HDV RNA at the end of treatment and 23% maintained the response 24 weeks post-treatment discontinuation. Systemic side effects were lower with pegIFN λ than IFN α , even if some patients experienced flu-like symptoms; hyperbilirubinaemia with or without liver enzyme elevation was reported in 24% of patients, mainly of Pakistani origin.²²⁷ A phase III (LIMT-2) trial of pegIFN λ 180 μ g for 48 weeks with 24 weeks of post-treatment follow-up is ongoing.

LNF, an oral drug originally developed as an anticancer treatment because it interferes with cell cycle regulation, inhibits farnesyltransferase activity in the setting of HDV infection and blocks the farnesylation of the L-HDAg that is mandatory for HDV virion assembly.^{215,216} At present, over 500 patients have been treated in investigational trials where LNF was given either as oral monotherapy or in combination with pegIFN α or pegIFN λ . The phase I LOWR HDV-1 study showed that increasing doses (100–200 mg) of LNF were associated with stronger HDV RNA decline, but more severe

adverse events, mainly diarrhoea, nausea, vomiting, anorexia and weight loss, were observed at higher doses. The addition of ritonavir (RTV) (100 mg QD), which inhibits the major LNF-metabolising enzyme, cytochrome P450 3A4, has enabled the use of lower LNF doses, significantly reducing adverse events, while retaining antiviral efficacy.²²⁸ The optimal LNF/RTV schedule was investigated in the LOWR HDV-2 trial where different doses of LNF (25, 50, 75 and 100 mg twice daily or 100 or 150 mg once a day) plus RTV were given as monotherapy or in combination with pegIFN α for 24 weeks: a ≥ 2 log reduction of HDV RNA or undetectable HDV RNA at the end of treatment was achieved in 6 of 13 patients (46%) in the all-oral combination of LNF 50 mg BID+RTV, and in 8 of 9 (89%) patients treated with LNF (50 or 25 mg BID + RTV) and pegIFN α . Grade 2 and 3 gastrointestinal adverse events occurred in 49% and 22% of patients treated with high and low LNF doses, respectively.²²⁹ In a large ongoing phase III trial, 400 patients were randomised to receive LNF/RTV 50 mg as all-oral therapy or LNF/RTV 50 mg+pegIFN α or pegIFN α or placebo for 48 weeks: a combined response at the end of therapy (≥ 2 log decline or undetectable HDV RNA + ALT normalisation) was achieved in 10.1% of the patients on all-oral LNF: 19.2% of patients receiving the combination compared to 9.6% and 1.9% of patients treated with pegIFN α monotherapy or placebo, respectively. Histological improvement (≥ 2 points of hepatic activity index without fibrosis worsening) was reported in 33%, 53%, 38% and 27% of patients in the four treatment groups.²³⁰ While the 24 week-post treatment data are awaited, LNF, mainly in combination with pegIFN α , appears to be a candidate for the finite therapy of patients with CHD.

A better understanding of the dynamics of HDV and HBV infection in individual patients receiving different antiviral treatments will prompt the optimisation of CHD therapies in the future, guiding the most appropriate combination of drugs with complementary activities. At present, because of its unconventional nature, direct targeting of HDV ribozyme activity remains a major challenge. However, the availability of adequate *in vitro* HDV infection models and well characterised HDV isolates, will enable the identification of candidate sites to be inactivated by gene silencing techniques.²³¹

Appendix. Delphi round consensus on the statements and recommendations of the present CPGs.

Recommendation/statement	Consensus
Screening for anti-HDV antibodies should be performed with a validated assay at least once in all HBsAg-positive individuals (LoE 3, strong recommendation).	100%
Re-testing for anti-HDV antibodies should be performed in HBsAg-positive individuals whenever clinically indicated (e.g., in case of aminotransferase flares, or acute decompensation of chronic liver disease) (LoE 3, strong recommendation), and may be performed yearly in those remaining at risk of infection (LoE 5, weak recommendation).	100%
HDV RNA should be tested in all anti-HDV-positive individuals using a standardised and sensitive reverse-transcription PCR assay to diagnose active HDV infection (LoE 2, strong recommendation).	96%
In patients with acute hepatitis, anti-HBc IgM should be used to distinguish individuals with HBV/HDV coinfection from HBsAg-positive individuals superinfected with HDV (LoE 3; strong recommendation).	85%
HBV e antigen (HBeAg)/anti-HBe status and HBV DNA levels should be tested because the presence of active HBV infection may worsen the outcome of hepatitis D (LoE 3; strong recommendation).	89%
Fully published data on the use of NITs in patients with CHD are currently limited and the correlation with liver histology is missing in a significant proportion of cases (LoE 4).	96%
Liver biopsy is recommended whenever it may contribute to the patient's management or for grading and staging liver disease when clinical signs or indirect evidence (by imaging techniques) of cirrhosis are absent (LoE 3; strong recommendation).	87%
NITs may be used to assess advanced liver disease, but specific cut-off values are not well established (LoE 5, weak recommendation).	97%
Factors that should be considered to identify patients with CHD at higher risk of liver disease progression include elevated aminotransferases and GGT levels, advanced stage of liver disease, persistence of HDV viraemia, high serum HBV DNA levels and viral coinfections. Cofactors of chronic liver injury, such as alcohol abuse, obesity and diabetes, should also be considered (LoE 4, strong recommendation).	100%
HCC surveillance should be performed with abdominal ultrasound every 6 months in patients with CHD with advanced fibrosis or cirrhosis, regardless of anti-HDV therapy (LoE 3, strong recommendation).	100%
Patients with CHD should receive regular work-up for liver disease at least every 6-12 months (LoE 3, strong recommendation).	96%
Virological parameters measured as part of the clinical work-up should ideally include quantitative assays for HBsAg, HBV DNA and HDV RNA (LoE 5, strong recommendation).	92%
All patients with CHD should be considered for antiviral treatment (LoE 3, strong recommendation).	92%
Patients with decompensated cirrhosis should be evaluated for liver transplantation (LoE 3, strong recommendation).	100%
Patients with HCC may be considered for antiviral treatment on an individualised basis (LoE 5, weak recommendation).	96%
IFN α has been used since the '90s for the treatment of CHD. Mono- and multicentre studies have been conducted with IFN α , with only two randomised phase II studies published. ^{117,124} Nevertheless, long-term data on clinical benefit and safety are available (LoE 2).	96%
All patients with CHD and compensated liver disease, irrespective of whether they have cirrhosis or not, should be considered for treatment with PegIFN α (LoE 2, strong recommendation).	92%
PegIFN α for 48 weeks should be the preferred treatment schedule (LoE 3, strong recommendation).	86%
Personalised treatment durations may be considered based on HDV RNA and HBsAg kinetics and treatment tolerability (LoE 3, weak recommendation).	96%
Despite the lack of data on long-term efficacy and safety, or on the optimal duration of BLV treatment, preliminary results from phase II studies (with BLV given as monotherapy or in combination with pegIFN α), on-treatment data from a phase III trial of BLV monotherapy and real-life studies suggest consideration of BLV as a treatment option for CHD whenever available (LoE 3).	93%
All patients with CHD and compensated liver disease should be considered for treatment with BLV (LoE 3, strong recommendation).	80%
The optimal dose and duration of treatment have not yet been defined (LoE 5). Until further data become available, long-term treatment with BLV, 2 mg once daily, may be considered (LoE 5, weak recommendation).	85%
The combination of pegIFN α and BLV may be considered in patients without pegIFN α intolerance or contraindications (LoE 5, weak recommendation).	88%
NAs should be given in patients with decompensated cirrhosis irrespective of the presence of detectable HBV DNA (LoE 5, strong recommendation).	96%
NAs should be given in patients with compensated cirrhosis and detectable HBV DNA (LoE 5, strong recommendation).	100%
NAs should be given in patients without cirrhosis if HBV DNA levels are higher than 2,000 IU/ml (LoE 5, strong recommendation).	96%
Patients who have undergone liver transplantation for CHD should receive hepatitis B immunoglobulin (HBIG) combined with a high genetic barrier NA after transplantation (LoE 3, strong recommendation).	100%
During the early post-transplant period the optimal HBIG dose has not been defined and varies among centres. Most experienced centres give HBIG at 10,000 IU intravenously in the anhepatic phase, followed by 600–1,000 IU intramuscularly/intravenously daily for 7 days, then weekly for 3 weeks, and then monthly until month 3-6 (LoE 3).	n.a.
After the early post-transplant period (6 months), HBIG should be administered at the dose that maintains anti-HBs serum levels >100 mIU/ml (LoE 3, strong recommendation).	100%
Currently, indefinite treatment with HBIG and NA in combination is considered the gold standard, but evidence on HBIG discontinuation after 1-2 years is gradually accumulating (LoE 4). Further studies, particularly in the setting of clinical trials, are warranted to assess the safety of this approach.	n.a.
Virological response to treatment of CHD should be determined during and after therapy (LoE 3, strong recommendation).	100%
HDV RNA should be quantified every 6 months during treatment and whenever there is a clinical indication (LoE 5, strong recommendation).	89%
For pegIFN α -based finite therapy, HDV RNA should be tested at the end of treatment, after 6 and 12 months and yearly thereafter (LoE 4, strong recommendation).	96%
In case of BLV discontinuation, HDV RNA should be tested at the time of treatment discontinuation, after 1, 3, 6, 12 months and yearly thereafter to monitor the relapse of viral replication (LoE 4, strong recommendation).	93%
HBsAg testing should be performed every year during and after therapy (LoE 3, strong recommendation).	85%
For pegIFN α -based therapy, quantitative HBsAg may be determined every 6 months during and every 12 months after treatment (LoE 3, weak recommendation).	96%

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Recommendation/statement	Consensus
HBV DNA should be determined every 6 months in all treated patients who are not on NA therapy (LoE 3, strong recommendation); in case of BLV discontinuation, more frequent HBV DNA testing may be required (LoE 5, weak recommendation).	96%
Testing for biochemical markers of liver disease activity (i.e. aminotransferases), full blood count and, in addition, liver function tests, whenever clinically indicated, should be performed during antiviral treatment (LoE 3, strong recommendation).	100%
Frequency of testing should be at least every 3-6 months, with the timing modulated according to the stage of liver disease and type of treatment (LoE 3, strong recommendation).	100%
For pegIFN α -based finite therapy, testing should be performed at the end of treatment, at least at month 6 and 12 after the end of treatment and yearly thereafter (LoE 4, strong recommendation).	92%
In case of BLV discontinuation, testing should be performed at the time of treatment discontinuation and at least after 1, 3, 6 and 12 months or more frequently according to clinical need (LoE 4, strong recommendation).	96%
Liver stiffness determination may be performed yearly during and after antiviral treatment of CHD (LoE 5, weak recommendation).	100%
Liver biopsy should be performed in patients during and/or after antiviral treatment where histological diagnosis would aid clinical management (LoE 3, strong recommendation).	88%
Patients with CHD should be monitored during and after treatment for the development of liver-related clinical events (LoE 3, strong recommendation).	100%

Two statements have been added following the comments received from the EASL GB and were not included in the Delphi survey, therefore consensus cannot be provided and is marked n.a.

Abbreviations

AASLD, American Association for the Study of Liver Diseases; AUROC, area under the receiver operating characteristic curve; BLV, bulevirtide; CHD, chronic hepatitis D; CPGs, Clinical Practice Guidelines; CSPH, clinically significant portal hypertension; HDAG, HDV antigen; EASL, European Association for the Study of the Liver; ELITA, European Liver and Intestine Transplant Association; GGT, gamma-glutamyltransferase; HBcrAg, HBV core-related antigen; HBeAg, HBV e antigen; HBIG, HBV immunoglobulin; HBsAg, HBV surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; IFN α , interferon- α ; LKM, liver-kidney microsomal; LNF, lonafermib; LT, liver transplantation; NA, nucleos(t)ide analogue; NAPs, nucleic acid polymers; NITs, non-invasive tests; NPV, negative predictive value; NTCP, sodium taurocholate cotransporting peptide; OCEBM, Oxford Centre for Evidence-based Medicine; OR, odds ratio; pegIFN α / λ , pegylated interferon- α / λ ; PPV, positive predictive value; PWID, people who inject drugs; RTV, ritonavir; TE, transient elastography.

Conflict of interest

Please refer to the accompanying EASL disclosure forms for further details.

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Supplementary data

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Author names in bold designate shared co-first authorship

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