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Severe Liver-Related Outcomes in Patients With Hepatitis Delta: Results From a Multi-Ethnic Multicenter Long-Term Follow-Up Study

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ABSTRACT

Hepatitis B virus (HBV)–hepatitis delta virus (HDV) coinfection is the most severe form of chronic viral hepatitis, but the factors that determine disease progression and severity are incompletely characterised. This long-term follow-up study aims to identify risk factors for severe liver-related outcomes. In this multicentre national cohort study, data from admission until the last visit between 2001 and 2023 was retrospectively collected from 162 HBV-HDV coinfected patients. The inclusion criteria were HBsAg or HBV DNA positivity, anti-HDV or HDV RNA positivity, and at least one follow-up visit. The median follow-up was 6.2 years (IQR 3.3–10.2). At baseline, 68/152 (44.7%) patients were diagnosed with advanced liver fibrosis. Forty patients (24.7%) had at least one severe liver-related outcome during follow-up. HDV viremia was detectable in 92 patients (64.3%) at last evaluation and was more frequently detectable in patients of European origin ($p < 0.001$). HDV RNA-positive patients had a 4.7-fold higher risk for severe liver-related outcomes ($p < 0.001$) and were more frequently diagnosed with advanced fibrosis at baseline ($p = 0.007$) compared to HDV RNA-negative patients. Multivariate analyses identified HDV RNA positivity, as well as several markers for liver disease severity, such as INR, platelet count, and advanced fibrosis at baseline, and age at admission as independent risk factors for severe liver-related outcomes. In conclusion, almost one in four HBV-HDV coinfected patients developed a severe liver-related outcome during follow-up. Several markers for liver disease severity and HDV RNA positivity were the strongest predictors for outcomes.

Abbreviations: ALT, alanine aminotransferase; Anti-HCV, hepatitis C virus antibodies; Anti-HDV, hepatitis delta virus antibodies; AST, aspartate aminotransferase; BEA, baseline-event-anticipation; CHD, chronic hepatitis delta infection; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis delta virus; HIV, human immunodeficiency virus; HIVAg-Ab, human immunodeficiency virus antigen and antibodies; HR, hazard ratio; IFN, interferon-based treatment; INR, International Normalised Ratio; IQR, interquartile range; KM, Kaplan–Meier; LTx, liver transplantation; MELD, Model for End-Stage Liver Disease; MSM, men who have sex with men; NRC, national reference centre; NUC, nucleos(t)ide analogues; peg-IFN, pegylated interferon; PWID, people who inject drugs.

1 | Introduction

Hepatitis delta virus (HDV) is a defective satellite RNA virus that requires the presence of the hepatitis B virus (HBV) for its viral propagation. Although the disease burden of HDV varies largely across different geographical regions, the overall seroprevalence of HDV antibodies (anti-HDV) in hepatitis B surface antigen (HBsAg) carriers is estimated to be 4.5% (95% CI 3.6–5.7) [1]. Similar rates were reported for Belgium [2]. HDV is more endemic in certain risk groups like people who inject drugs (PWID), recipients of blood transfusions, sex workers, men who have sex with men (MSM), and migrants [3]. The actual HDV seroprevalence is speculated to be even higher, because most seroprevalence studies are clinician-based and are therefore not representative of both the general population and the above-mentioned risk groups [4].

HBV-HDV coinfection is considered to be the most severe form of chronic viral hepatitis, often resulting in a severe and rapidly progressive liver disease [5, 6]. The management of patients with a chronic hepatitis delta infection (CHD) remains challenging. The current therapeutic options include the off-label use of pegylated interferon (peg-IFN) and the first-in-class entry inhibitor bulevirtide (BLV). However, interferon-based treatments (IFN) come with a variable success rate and an unfavourable side effect profile. While data on the long-term efficacy and safety of BLV are lacking, and the optimal treatment duration remains undefined, HDV-suppressive treatment with BLV shows promise in the management of CHD [7]. Additionally, emerging evidence suggests an increased beneficial effect by combining peg-IFN and BLV in a finite treatment duration, with a higher proportion of patients achieving undetectable HDV RNA levels [8]. However, due to the need for daily subcutaneous injections and unavailability in a high number of countries, including Belgium, BLV treatment is not yet widely applied.

Long-term follow-up studies on clinical outcomes are complicated in countries with a low HBV prevalence, such as Europe and the United States of America, as HDV mainly affects underserved migratory populations with interregional mobility and poor access to healthcare due to several cultural barriers [4]. Previously, several cohort studies have been performed in Europe, for example, in Sweden, Italy, Spain, Germany and France [9–14]. However, some of these studies were hampered by a monocentric [11], cross-sectional study design [14], a limited follow-up duration [10] or a low number of events [9, 12]. Furthermore, definitions of cirrhosis or advanced liver fibrosis were often based on clinical or biochemical criteria [9, 13, 14], while liver biopsy or elastography studies are more scarce. Finally, these cohort studies show a substantial heterogeneity in their study designs, inclusion criteria, and used assays, making the intercomparison of outcome data difficult. Added to these roadblocks is the absence of FDA-approved anti-HDV and HDV RNA assays [15]. It is clear that uniform HDV diagnostic assays combined with long-term, large cohort studies are required to better characterise the natural history of HBV-HDV coinfections and determine the risk factors that define the disease progression and severity.

This national multicentre cohort study with centralised HDV RNA assays investigates long-term outcomes of CHD patients and aims to identify risk factors for severe liver-related outcomes.

2 | Patients and Methods

2.1 | Patients

This is a multicentric retrospective cohort study of HBV-HDV coinfected patients identified at the National Reference Centre (NRC) for Viral Hepatitis, which performs (confirmatory) hepatitis delta testing for most of the hospitals and laboratories throughout the country. Eight Belgian hospitals were contacted to participate in the study based on their expertise, the number of samples sent for HDV diagnosis to the NRC, and geographic location to cover the entire country. Inclusion criteria were: (1) HBsAg or HBV DNA positive, (2) anti-HDV or HDV RNA positive, (3) sufficient baseline data, and (4) at least one follow-up visit. After excluding double patients seen at several hospitals and those with insufficient baseline data, a total of 162 individual patients were included (Figure 1).

2.2 | Methods

All relevant data, from admission to the last recorded visit between July 2001 and January 2023, was uniformly collected from medical charts using a dedicated electronic case report form. Demographic data consisted of age at admission, sex, and origin. The origin refers to the continental (geographic) origin of the patient and was based on self-reported country of birth and/or the investigator's opinion of origin. Patients originating from Turkey, Syria, or Iran were allocated to the 'Middle East' group. Laboratory parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), International Normalised Ratio (INR), total bilirubin, creatinine, and thrombocytes and derivatives such as the Model for End Stage Liver Disease (MELD) and baseline-event-anticipation (BEA) score. The MELD score was calculated only in the patients with advanced liver fibrosis at baseline. The BEA score was calculated based on the publication by Calle Serrano et al., which is a point-based risk score based on sex, age, region of origin, INR, thrombocytes, and bilirubin [16]. The following virological parameters were collected: hepatitis B e antigen (HBeAg), HBV DNA, HBsAg, anti-HDV, HDV RNA, hepatitis C antibodies (anti-HCV), and human immunodeficiency virus antigen and antibodies (HIVAg-Ab). Liver disease severity evaluations, consisting of biopsies, radiology reports (ultrasonography, computed tomography, and magnetic resonance imaging scans), liver stiffness measurements (Fibroscan and shear wave elastography), and upper gastrointestinal endoscopy reports, were collected at baseline and throughout follow-up. Advanced liver fibrosis was defined as having either liver histology Metavir $\geq$ F3, liver stiffness measurement $>$ 10.4 kPa, and/or the presence of clinical and radiological criteria for cirrhosis, such as signs of portal hypertension (ascites and oesophageal varices). Patients with a liver histology Metavir $<$ F3, liver stiffness measurement $\leq$ 10.4 kPa, and without any clinical and radiological signs of cirrhosis were regarded as having no advanced liver fibrosis. The liver stiffness measurement cut-off of 10.4 kPa

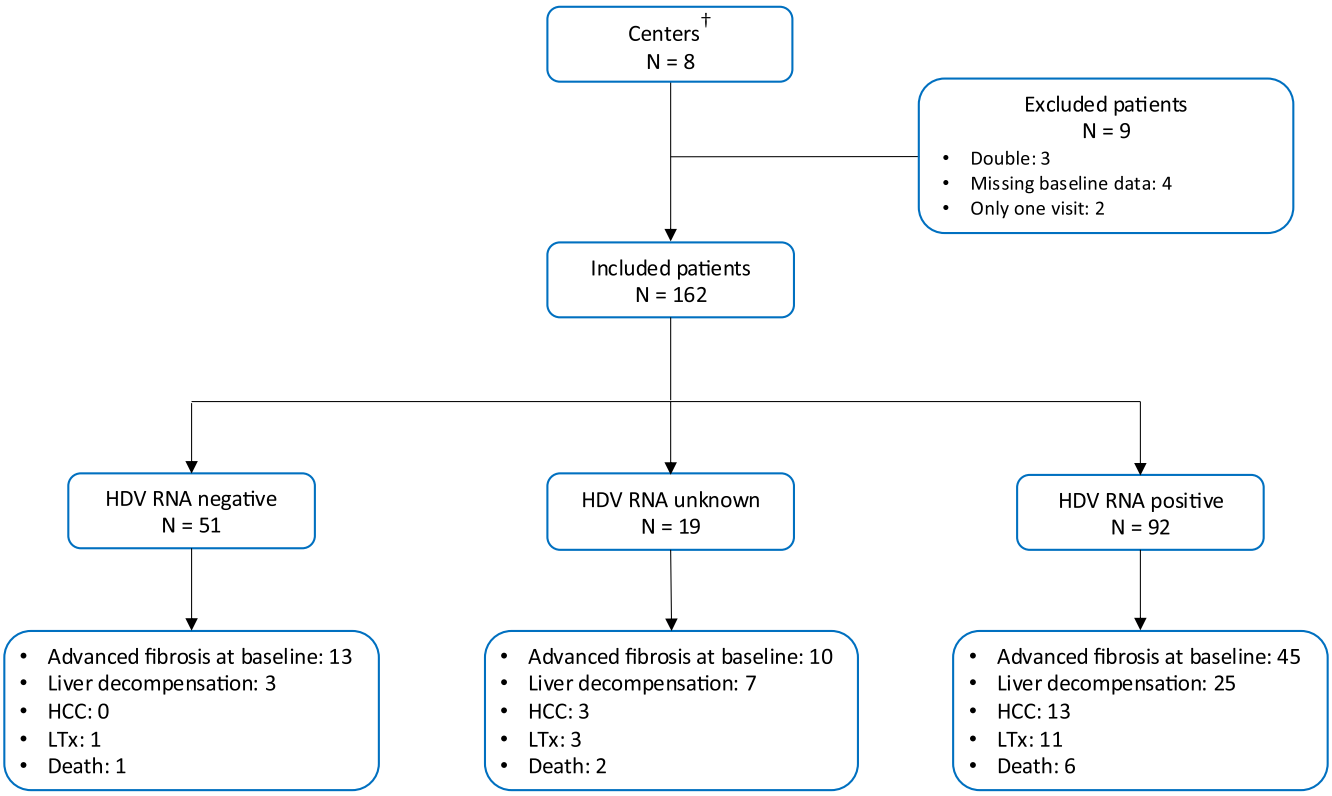

FIGURE 1 | Patient flow chart and overview of severe liver-related outcomes during follow-up according to HDV RNA status. HCC, hepatocellular carcinoma; LTx, liver transplantation; †The participating sites were identified based on the central database at the National Reference Centre.

was selected based on Roulot et al. [17] Baseline was defined as the first available liver evaluation. The median time until first liver evaluation was 0.21 years (IQR 0.02–1.42 years). Follow-up was defined as the time between admission and the date of last visit, liver transplantation, or death. Severe liver-related outcomes were defined as hepatocellular carcinoma (HCC), liver transplantation (LTx), death, or liver decompensation according to their clinical presentation with ascites, hepatic encephalopathy, or variceal bleeding and were recorded during follow-up. Virological parameters related to routine HBV and HDV viremia testing were obtained from the medical charts. Due to the long data collection period and multiple centres involved in our study, various virological assays were used over time. The main advantage is that most HDV RNA analyses were performed centrally in the same laboratories in Belgium. Up until 2015, the University Hospital Ghent used an in-house assay as previously described and externally evaluated in Le Gal et al. [18] From 2015 to June 2018, the University Hospital Ghent quantified the HDV RNA using the RoboGene HDV RNA Quantification Kit 2.0 (Analytik Jena, Jena, Germany) with a lower limit of detection (LLOD) of 14 IU/mL and a lower limit of quantification (LLOQ) of 63 IU/mL. Afterwards, the RealStar HDV RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) with an LLOD of 13.4 IU/mL and LLOQ of 429 IU/mL was used. From January 2015 until October 2018, the NRC used an in-house assay based on the methods described by Kodani et al. [19] Thereafter, HDV RNA was quantified using the Realstar HDV Real-Time PCR kit 1.0, with a LLOD and LLOQ of 13.4 IU/mL and 1000 IU/mL, respectively (Altona Diagnostics, Hamburg, Germany). Patients with a detectable HDV viremia at last evaluation were considered to be HDV RNA positive. Persistent virological response after IFN treatment was defined as persistent undetectable HDV RNA at least 24 weeks after the end of treatment, up until the last analysis. As all data was collected retrospectively and was de-identified, there was no written informed consent obtained from the subjects. The study was approved by the ethics committees of the University Hospital of Antwerp (ref. 17/02/013) and the participating centres. All research was conducted in accordance with both the Declarations of Helsinki and Istanbul.

2.3 | Statistical Analyses

Descriptive results are presented as median (interquartile range (IQR) or range) and numbers (percentage). Two-tailed Fisher's exact test or Chi-squared and Mann–Whitney *U* test; independent samples *t*-test or Kruskal–Wallis test were used for comparing categorical and continuous variables between groups, respectively. Cox proportional hazards regression was used to evaluate the association between baseline characteristics and severe liver-related outcomes. Due to the low number of severe liver-related outcomes in this cohort and, subsequently, the risk for overfitting the model, it was not possible to perform a multivariate Cox proportional hazards regression including all identified variables. As such, in Table 4, three models were selected. The included variables were selected based on the results of the univariate analysis and the available literature. Collinearity was checked by assessing the variance inflation factor of the variables before inclusion in the multivariate models. Hazard ratios (HRs) are presented

with the 95% confidence interval (CI). Time-to-event analyses are presented using the Kaplan–Meier (KM) analysis. All analyses were performed using SPSS version 28.0.1 or 29.0.1 (SPSS Inc., Chicago, IL, USA) or R Statistical Software version 4.3.0 (R Core Team). The figures were created using GraphPad Prism version 10.1.0. Statistical analyses were two-sided with a $p < 0.05$ to be considered significant.

3 | Results

3.1 | Patient Characteristics

In total, 162 patients were included. Baseline characteristics are presented in Table 1. The median follow-up was 6.2 years (IQR 3.3–10.2), and the median age at baseline was 36.4 years (IQR 28.8–43.8). Patients were predominantly male (63.0%) and were mainly of European or African origin (42.6% and 39.5%, respectively).

Of 143 patients with at least one HDV RNA result, 92 (64.3%) were HDV RNA positive at last evaluation. As shown in Table 1, there was no significant difference in age ($p = 0.26$), gender ($p = 0.29$), or follow-up duration ($p = 0.26$) between the HDV RNA-positive and HDV RNA-negative patients. However, laboratory values at baseline varied substantially: HDV RNA-positive patients had a higher AST ($p < 0.001$), ALT ($p < 0.001$) and INR ($p = 0.006$), and lower thrombocytes ($p = 0.007$) compared to the HDV RNA-negative group. Significantly more patients were diagnosed with a past or ongoing hepatitis C virus (HCV) and/or human immunodeficiency virus (HIV) infection ($p = 0.013$ and $p = 0.047$, respectively) in the HDV RNA-positive group. There was no significant difference in treatment history with nucleos(t)ide analogues (NUC) or IFN ($p = 0.70$ and $p = 0.49$, respectively). Those who had a detectable HDV RNA were more frequently diagnosed with advanced liver fibrosis at baseline compared to those with an undetectable HDV RNA (51.1% vs. 27.1%, respectively, $p = 0.007$).

Interestingly, 91.2% of the patients of European origin were HDV RNA positive at the last evaluation. This is significantly higher ($p < 0.001$) than patients of African, Asian and Middle-Eastern descent (Table S1, 52.5%, 37.5% and 27.3%, respectively).

3.2 | Advanced Liver Fibrosis at Baseline

A total of 152 (93.8%) patients had a liver fibrosis assessment available at baseline (Table 2). Strikingly, 68 patients (44.7%) were diagnosed with advanced liver fibrosis at baseline, either by liver biopsy Metavir ≥ F3 (50.0%), liver stiffness > 10.4 kPa (30.9%), or based on clinical and radiological signs (19.1%). Eighty-four (55.3%) patients did not have advanced liver fibrosis at baseline, that is, liver biopsy Metavir < F3 (45.8%) or liver stiffness ≤ 10.4 kPa (54.3%).

Patients without advanced liver fibrosis at baseline were younger ($p < 0.001$), had lower initial AST ($p < 0.001$), INR ($p < 0.001$) and bilirubin values ($p < 0.001$) and higher thrombocytes ($p < 0.001$) compared to patients with advanced liver fibrosis at baseline (Table 2). Moreover, those without advanced liver fibrosis were less frequently HDV RNA positive at last evaluation (55.1% vs. 77.6%, respectively, $p = 0.007$).

3.3 | Severe Liver-Related Outcomes During Follow-Up

During follow-up, 40 (24.7%) patients had at least one severe liver-related outcome (Table 3). In total, 35 patients developed at least one episode of liver decompensation, 16 patients were diagnosed with HCC, 15 patients underwent liver transplantation, and 9 deaths were recorded, resulting in a 5- and 10-year cumulative severe liver outcome probability of 22.0% and 31.0%, respectively. The median age at time of outcome was 50.0 years (IQR 41.7–55.0). Ascites was observed in 32 patients, ranking it the most frequent liver decompensation event, followed by hepatic encephalopathy in 16 patients and variceal bleeding in 5 patients. Notably, 12 patients had a liver decompensation at presentation, and 2 HCCs were diagnosed within 2 months after admission, stressing the sometimes late presentation and long-term pauci-clinical course of CHD.

3.4 | Severe Liver-Related Outcomes in Patients With Advanced Fibrosis at Baseline

Thirty-four (89.5%) of the 40 patients that had a severe liver-related outcome were diagnosed with advanced liver fibrosis at baseline (Table 3). Importantly, 35.3% (12/34) of the outcomes were diagnosed at admission. Those with advanced liver fibrosis at baseline developed a severe liver-related outcome in a median of 0.8 (IQR 0.0–4.9) years, resulting in a 5-year cumulative outcome probability of 42% compared to 4% in those without advanced liver fibrosis at baseline (Figure 2A, $p < 0.001$). In addition, patients diagnosed with advanced fibrosis at baseline more frequently developed liver decompensation (Figure 2C, $p < 0.001$) and HCC (Figure 2E, $p = 0.009$) and had a poorer liver transplant-free (Figure S1A, $p < 0.001$) and overall survival (Figure S1C, $p = 0.006$).

3.5 | Comparison of Outcomes Based on HDV RNA Status

In total, 33 (82.5%) of the 40 patients with a severe liver-related outcome had at least one HDV RNA result available. Of those, 29 (87.9%) were HDV RNA positive at last evaluation. Patients with a positive HDV RNA had a higher chance of developing any severe liver-related outcome compared to those with a negative result (Figure 2B, $p = 0.001$), including higher risks for liver decompensation (Figure 2D, $p = 0.002$), HCC (Figure 2F, $p = 0.003$), and need for liver transplantation (Figure S1B, $p = 0.027$). There was no significant difference in overall survival between the groups (Figure S1D, $p = 0.21$). Cumulative severe liver-related outcome probabilities at 5 and 10 years were 26% versus 7% and 40% versus 11% in HDV RNA-positive vs. HDV RNA-negative patients respectively.

TABLE 1 | Baseline characteristics of the total cohort and according to the hepatitis delta virus RNA status.

Baseline characteristics HDV RNA status		Total cohort[†] N = 162		HDV RNA negative N = 51		HDV RNA positive N = 92	p
	N =		N =		N =		
Age at admission – years (IQR)	162	36.4 (28.8 – 43.8)	51	35.7 (28.4 – 43.1)	92	35.9 (27.8 – 42.3)	0.26[a]
Male – *n* (%)	162	102 (63.0)	51	35 (68.6)	92	55 (59.8)	0.29[b]
Origin – *n* (%)							
Africa	162	64 (39.5)	51	28 (54.9)	92	31 (33.7)	**< 0.001**[c]
Asia		16 (9.9)		10 (19.6)		6 (6.5)	
Europe		69 (42.6)		5 (9.8)		52 (56.5)	
Middle-East		13 (8.0)		8 (15.7)		3 (3.3)	
Follow-up – years (IQR)	162	6.2 (3.3 – 10.2)	51	6.5 (3.8 – 11.8)	92	6.4 (2.5 – 9.6)	0.26[d]
Laboratory – (range)							
AST (U/L)	162	51 (9 – 957)	51	37 (9 – 140)	92	64 (27 – 957)	**< 0.001**[d]
ALT (U/L)	162	59 (13 – 2211)	51	41 (13 – 205)	92	72 (16 – 2211)	**< 0.001**[d]
INR	152	1.1 (0.9 – 2.6)	46	1.1 (0.9 – 2.5)	89	1.1 (0.2 – 2.6)	**0.006**[d]
Bilirubin (mg/dL)	162	0.6 (0.2 – 18.8)	51	0.6 (0.3 – 8.9)	92	0.6 (0.2 – 18.8)	0.39[d]
Thrombocytes (10^9/L)	161	165 (8 – 333)	51	180 (55 – 326)	92	156 (8 – 333)	**0.007**[d]
MELD (IQR)	68	8.44 (7.29 – 11.65)	13	7.40 (6.98 – 10.04)	45	8.66 (7.40 – 11.46)	**0.22**[d]
MELD ≤ 10; *n* (%)		46 (67.6)		10 (76.9)		31 (68.9)	
MELD > 10 and ≤ 15; *n* (%)		13 (19.1)		1 (7.7)		9 (20.0)	
MELD > 15 and ≤ 25; *n* (%)		7 (10.3)		1 (7.7)		4 (8.9)	
MELD > 25; *n* (%)		2 (2.9)		1 (7.7)		1 (2.2)	
Hepatitis B virus – *n* (%)							
HBeAg positive	156	24 (15.4)	49	6 (12.2)	90	16 (17.8)	0.39[b]
HBV DNA positive	149	100 (67.1)	45	37 (82.2)	89	53 (59.6)	**0.008**[b]
HBV DNA > 2000 IU/mL	149	31 (20.8)	45	15 (33.3)	89	12 (13.5)	**0.007**[b]
HDV RNA positive – *n* (%)	143	92 (64.3)	51	0 (0.0)	92	92 (100.0)	
Coinfection – *n* (%)							
Anti-HCV positive	133	13 (9.8)	44	0 (0.0)	71	10 (14.1)	**0.013**[c]
HIVAg-Ab positive	124	9 (6.5)	39	0 (0.0)	69	7 (10.1)	**0.047**[c]
Treatment history – *n* (%)							
NUC experienced	162	69 (42.6)	51	21 (41.2)	92	41 (44.6)	0.70[b]
IFN experienced	162	46 (28.4)	51	13 (25.5)	92	29 (31.5)	0.49[b]
IFN persistent response	43	10 (23.3)	10	10 (100.0)	29	0 (0.0)	
Advanced liver fibrosis – *n* (%)	152	68 (44.7)	48	13 (27.1)	88	45 (51.1)	**0.007**[b]
Liver biopsy Metavir ≥ F3		34 (50.0)		9 (69.2)		21 (46.7)	
Liver stiffness measurement > 10.4 kPa		21 (30.9)		3 (23.1)		15 (33.3)	
Clinical and radiological signs		13 (19.1)		1 (7.7)		9 (20.0)	

(Continues)

TABLE 1 | (Continued)

Baseline characteristics HDV RNA status	Total cohort† N = 162		HDV RNA negative N = 51		HDV RNA positive N = 92		p
	N =		N =		N =		
BEA score – *n* (%)							
BEA-A	152	78 (51.3)	46	25 (54.3)	90	48 (53.3)	0.44[c]
BEA-B		66 (43.3)		18 (39.1)		40 (44.4)	
BEA-C		8 (5.3)		3 (6.5)		2 (2.2)	

Abbreviations: Anti-HCV, hepatitis C virus antibodies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BEA, baseline-event-anticipation score; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HDV, hepatitis delta virus; HIVAg-Ab, human immunodeficiency virus antigen and antibodies; IFN, interferon-based treatment; INR, International Normalized Ratio; IQR, interquartile range; MELD, Model for End-Stage Liver Disease; NUC, nucleos(t)ide analogue. All bold values have a significant *p* value < 0.05.
[a]Independent samples t-test.
[b]Chi-square.
[c]Two tailed Fisher's exact test.
[d]Mann-Whitney U.
[†]Including 19 HDV patients with unknown HDV RNA status.

3.6 | Comparison of Outcomes Based on Antiviral Treatment

IFN was administered to 46 (28.4%) of the 162 patients (Table 1). Of the 43 (93.5%) patients who had follow-up HDV RNA results available, 10 (23.3%) achieved persistent virological response. Nine patients who received IFN developed at least one severe liver-related outcome during their respective follow-up. Noteworthy, one of the outcomes was observed in a patient who became HDV RNA negative after IFN treatment. This patient was diagnosed with cirrhosis and portal hypertension and deceased at the age of 50.8 years, 4 years after entering follow-up. The death was recorded 4 months after the end of IFN treatment, precluding the evaluation of persistent virological response. Univariate Cox regression indicated no association between prior IFN treatment and severe liver-related outcomes during follow-up (HR = 0.564; 95% CI 0.267–1.190; $p = 0.13$; Table 4). Furthermore, there was no statistical difference in outcome between patients who had a persistent virological response after IFN and those who did not (HR = 0.031; 95% CI 0.00–19.373; $p = 0.29$; Table 4).

Sixty-nine (42.6%) of the 162 patients received NUC treatment (Table 1). NUC treatment was more frequently initiated in patients with advanced liver fibrosis ($p = 0.03$; Table 2). This is in line with the prevailing Belgian reimbursement criteria, as NUC treatment is reimbursed in patients with cirrhosis, regardless of the HBV DNA and/or ALT levels. Although univariate Cox regression showed a trend towards worse prognosis for patients receiving NUC treatment (HR = 1.869; 95% CI 0.998–3.500; $p = 0.051$; Table 4), this reflects the advanced liver disease in these patients, increasing their likelihood of receiving NUC treatment, rather than a direct effect of the NUC treatment itself.

Finally, we analysed whether the quality of CHD patient management in Belgium improved over the years by investigating a time effect in outcomes for patients entering follow-up either before 2010 ($n = 44$; 27.7%), between 2010 and 2015 ($n = 52$; 32.1%), or after 2015 ($n = 66$; 40.7%). There was no association between the period of entry in follow-up and the frequency of observed severe liver-related outcomes ($p = 0.26$).

3.7 | Factors Associated With Severe Liver-Related Outcomes

Univariate Cox regression analysis (Table 4) identified three additional variable categories that were associated with the occurrence of severe liver-related outcomes, apart from HDV RNA positivity at last evaluation (HR = 4.699; 95% CI 1.648–13.396; $p = 0.004$). Firstly, the risk of having a severe liver-related outcome increased with the age at admission (HR = 1.095; 95% CI 1.063–1.129; $p < 0.001$). Secondly, several variables for liver disease severity, consisting of INR (HR = 18.309; 95% CI 8.276–40.504; $p < 0.001$), total bilirubin values (HR = 1.230; 95% CI 1.141–1.326; $p < 0.001$), lower thrombocytes (HR = 0.978; 95% CI 0.971–0.984; $p < 0.001$) and the presence of advanced liver fibrosis at baseline (HR = 12.326; 95% CI 4.371–34.758; $p < 0.001$), were significantly associated with the occurrence of a severe liver-related outcome during follow-up. Thirdly, HIVAg-Ab positivity also significantly increased the risk of having an outcome (HR 3.857; 95% CI 1.590–9.351; $p = 0.003$).

Three multivariate models were selected to investigate the interdependency of identified risk factors for severe liver-related outcomes. The first multivariate Cox regression model included three variables: advanced liver fibrosis at baseline, age at admission, and HDV RNA positivity. The second model investigated INR, thrombocytes, and HDV RNA positivity. In the final model, HDV RNA positivity, HIVAg-Ab positivity, and BEA score were included. All multivariate models (Table 4) demonstrate that HDV RNA positivity at last evaluation remains independently associated with severe liver-related outcomes, irrespective of the included variables. In addition, several markers for liver disease severity (advanced liver fibrosis at baseline, INR, thrombocytes, and BEA score) and age at admission were independently associated with outcomes, but not HIV coinfection.

4 | Discussion

This multicentric retrospective real-world cohort study, including 162 patients from 8 Belgian hospitals with clinical follow-up

TABLE 2 | Baseline characteristics of patients with and without advanced liver fibrosis at baseline.

Baseline characteristics Advanced liver fibrosis at baseline	Total cohort[†] N = 162		No advanced liver fibrosis at baseline[‡] N = 84		Advanced liver fibrosis at baseline[§] N = 68		p
	N =		N =		N =		
Liver fibrosis assessment – *n* (%)							
Biopsy Metavir							
0 – 1	152	18 (11.8)	84	18 (21.4)	68	0 (0.0)	
2		20 (13.2)		20 (23.8)		0 (0.0)	
3		13 (8.6)		0 (0.0)		13 (19.1)	
4		21 (13.8)		0 (0.0)		21 (30.9)	
Liver stiffness							
< 7 kPa		33 (21.7)		33 (39.3)		0 (0.0)	
≥ 7 and ≤ 10.4 kPa		13 (8.6)		13 (15.5)		0 (0.0)	
> 10.4		21 (13.8)		0 (0.0)		21 (30.9)	
Clinical and radiological signs of cirrhosis		13 (8.6)		0 (0.0)		13 (19.1)	
Age at admission – years (IQR)	162	36.4 (28.8 – 43.8)	84	33.1 (26.5 – 40.0)	68	40.2 (31.0 – 47.9)	**< 0.001**[a]
Male – *n* (%)	162	102 (63.0)	84	49 (58.3)	68	45 (66.2)	0.32[b]
Origin – *n* (%)							
Africa	162	64 (39.5)	84	37 (44.0)	68	21 (30.9)	0.17[c]
Asia		16 (9.9)		10 (11.9)		5 (7.4)	
Europe		69 (42.6)		30 (35.7)		36 (52.9)	
Middle-East		13 (8.0)		7 (8.3)		6 (8.8)	
Follow-up – years (IQR)	162	6.2 (3.3 – 10.2)	84	6.8 (3.8 – 10.9)	68	6.2 (3.2 – 10.0)	0.45[d]
Laboratory – (range)							
AST (U/L)	162	51 (9 – 957)	84	40 (9 – 957)	68	74 (21 – 410)	**< 0.001**[d]
ALT (U/L)	162	59 (13 – 2211)	84	55 (13 – 2211)	68	71 (13 – 367)	0.057[d]
INR	152	1.1 (0.9 – 2.6)	77	1.1 (0.9 – 1.5)	68	1.2 (1.0 – 2.6)	**< 0.001**[d]
Bilirubin (mg/dL)	162	0.6 (0.2 – 18.8)	84	0.5 (0.2 – 9.7)	68	0.8 (0.3 – 18.8)	**< 0.001**[d]
Thrombocytes (10^9/L)	161	165 (8 – 333)	83	190 (79 – 333)	68	119 (26 – 300)	**< 0.001**[d]
MELD (IQR)					68	8.44 (7.29 – 11.65)	
MELD ≤ 10; *n* (%)						46 (67.6)	
MELD > 10 and ≤ 15; *n* (%)						13 (19.1)	
MELD > 15 and ≤ 25; *n* (%)						7 (10.3)	
MELD > 25; *n* (%)						2 (2.9)	

(Continues)

TABLE 2 | (Continued)

Baseline characteristics Advanced liver fibrosis at baseline	Total cohort[†] N = 162		No advanced liver fibrosis at baseline[‡] N = 84		Advanced liver fibrosis at baseline[§] N = 68		p
	N =		N =		N =		
Hepatitis B virus – *n* (%)							
HBeAg positive	156	24 (15.4)	81	11 (13.6)	66	12 (18.2)	0.45[b]
HBV DNA positive	149	100 (67.1)	76	56 (73.7)	64	37 (57.8)	**0.048**[b]
HBV DNA > 2000 IU/mL	149	31 (20.8)	76	16 (21.1)	64	14 (21.9)	0.91
HDV RNA positive – *n* (%)	143	92 (64.3)	78	43 (55.1)	58	45 (77.6)	**0.007**[b]
Coinfection – *n* (%)							
Anti-HCV positive	133	13 (9.8)	67	5 (7.5)	56	7 (12.5)	0.35[c]
HIVAg-Ab positive	124	9 (6.5)	60	3 (5.0)	55	4 (7.3)	0.71[c]
Treatment history – *n* (%)							
NUC experienced	162	69 (42.6)	84	31 (36.9)	68	37 (54.4)	**0.03**[b]
IFN experienced	162	46 (28.4)	84	19 (22.6)	68	24 (35.3)	0.09[b]
IFN persistent response	43	10 (23.3)	19	5 (26.3)	22	4 (18.2)	0.71[b]
BEA score – *n* (%)							
BEA-A	152	78 (51.3)	77	52 (67.5)	68	25 (36.8)	**< 0.001**[c]
BEA-B		66 (43.3)		25 (32.5)		36 (52.9)	
BEA-C		8 (5.3)		0 (0.0)		7 (10.3)	

Abbreviations: Anti-HCV, hepatitis C virus antibodies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BEA, baseline-event-anticipation score; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HDV, hepatitis delta virus; HIVAg-Ab, human immunodeficiency virus antigen and antibodies; IFN, interferon-based treatment; INR, International Normalized Ratio; IQR, interquartile range; MELD, Model for End-Stage Liver Disease. All bold values have a significant *p* value < 0.05.
[a]Independent samples t-test.
[b]Chi-square.
[c]Two tailed Fisher's exact test.
[d]Mann-Whitney U.
[†]Including 10 HDV patients without liver fibrosis assessment available.
[‡]No advanced liver fibrosis is defined as liver biopsy Metavir <F3, liver stiffness ≤ 10.4 kPa and no clinical and radiological signs for cirrhosis.
[§]Advanced liver fibrosis is defined as liver biopsy Metavir ≥F3, liver stiffness > 10.4 kPa and/or clinical and radiological signs for cirrhosis.

spanning over two decades, shows that severe liver-related outcomes, including liver decompensation, HCC, liver transplantation, and death, are frequent in patients with a chronic hepatitis delta infection. HDV RNA-positive patients were more frequently diagnosed with advanced liver fibrosis at baseline and have a 4.7-fold higher risk for severe liver-related outcomes than those with undetectable HDV RNA viremia. HDV RNA positivity remained independently associated with outcomes in all studied multivariate models. Not unexpectedly, additional risk factors for severe liver-related outcomes included age and variables reflecting liver disease severity, such as INR and thrombocytopenia.

At baseline, almost 45% of the cohort was diagnosed with advanced liver fibrosis, at a median age of 40.6 years. Having advanced liver fibrosis at baseline was associated with a higher risk for any type of severe liver-related outcome, including liver decompensation, HCC, liver transplantation, and death, resulting in an alarming 5-year cumulative incidence rate of 42%. Strikingly, more than one third of the observed severe liver-related outcomes in these patients were diagnosed at admission, emphasising the protracted subclinical progression and, subsequently, delayed presentation and diagnosis of CHD.

A drawback in the evaluation of liver disease progression for HBV-HDV coinfections is the difficulty of defining optimal cut-offs of non-invasive markers of liver fibrosis. Several groups have made efforts in determining a cut-off for advanced liver fibrosis in cohorts with paired liver biopsy and liver elastography evaluations. For example, a German group recently defined 10.2 kPa (sensitivity 55% and specificity 86%) as a cut-off for the exclusion of advanced liver fibrosis in a cohort of 144 CHD patients [20]. In this study, the recently proposed cut-off by Roulot et al., who found 10.4 kPa to reflect advanced liver fibrosis in 230 HDV

TABLE 3 | Baseline characteristics according to severe liver-related outcome status during follow-up.

Baseline characteristics **Severe liver-related outcomes during follow-up**		**No outcome** ***N*** **= 122**		**Outcome** ***N*** **= 40**
	N **=**		***N*** **=**	
Age at admission – years (IQR)	122	32.9 (27.4 – 39.8)	40	46.1 (40.6 – 51.6)
Male – *n* (%)	122	77 (63.1)	40	25 (62.5)
Origin – *n* (%)				
Africa	122	50 (41.0)	40	14 (35.0)
Asia		14 (11.5)		2 (5.0)
Europe		48 (39.3)		21 (52.5)
Middle-East		10 (8.2)		3 (7.5)
Follow-up – years (IQR)	122	6.6 (3.8 – 10.9)	40	4.3 (1.1 – 8.1)
Laboratory – (range)				
AST (U/L)	122	43 (9 – 957)	40	77 (33 – 410)
ALT (U/L)	122	58 (13 – 2211)	40	62 (22 – 367)
INR	112	1.1 (0.9 – 1.5)	40	1.3 (1.0 – 2.6)
Bilirubin (mg/dL)	122	0.5 (0.2 – 9.7)	40	1.0 (0.4 – 18.8)
Thrombocytes (10^9/L)	121	181 (68 – 333)	40	84 (8 – 215)
MELD (IQR)	34	7.43 (6.95 – 8.68)	34	10.85 (8.31 – 12.99)
MELD ≤ 10; *n* (%)		30 (88.2)		16 (47.1)
MELD > 10 and ≤ 15; *n* (%)		2 (5.9)		11 (32.4)
MELD > 15 and ≤ 25; *n* (%)		2 (5.9)		5 (14.7)
MELD > 25; *n* (%)		0 (0.0)		2 (5.9)
Hepatitis B virus – *n* (%)				
HBeAg positive	117	16 (13.7)	39	8 (20.5)
HBV DNA positive	112	76 (67.9)	37	24 (64.9)
HBV DNA > 2000 IU/mL	112	23 (20.5)	37	8 (21.6)
HDV RNA positive – *n* (%)	110	63 (57.3)	33	29 (87.9)
Coinfection – *n* (%)				
Anti-HCV positive	98	9 (9.2)	35	4 (11.4)
HIVAg-Ab positive	90	2 (2.2)	34	6 (17.6)
Treatment history – *n* (%)				
NUC experienced	122	46 (37.7)	40	23 (57.5)
IFN experienced	122	37 (30.3)	40	9 (22.5)
IFN persistent response	34	10 (29.4)	9	0 (0.0)
Advanced liver fibrosis at baseline – *n* (%)	114	34 (29.8)	38	34 (89.5)
Liver biopsy Metavir ≥F3		21 (61.8)		14 (41.2)
Liver stiffness measurement > 10.4 kPa		9 (26.5)		12 (35.3)
Clinical and radiological signs		4 (11.8)		9 (26.5)

(Continues)

TABLE 3 | (Continued)

Baseline characteristics Severe liver-related outcomes during follow-up	No outcome *N* = 122		Outcome *N* = 40	
	N =		*N* =	
Liver fibrosis assessment at baseline – *n* (%)				
Biopsy Metavir	114		38	
0 – 1		17 (14.9)		1 (2.6)
2		18 (15.8)		2 (5.3)
3		11 (9.7)		2 (5.3)
4		10 (8.8)		11 (29.0)
Liver stiffness				
< 7 kPa		32 (28.1)		1 (2.6)
≥ 7 and ≤ 10.4 kPa		13 (11.4)		0 (0.0)
> 10.4 kPa		9 (7.9)		12 (31.6)
Clinical and radiological signs of cirrhosis		4 (3.5)		9 (23.7)
BEA-score – *n* (%)				
BEA-A	112	72 (64.5)	40	6 (15.0)
BEA-B		39 (34.8)		27 (67.5)
BEA-C		1 (0.9)		7 (17.5)

Abbreviations: Anti-HCV, hepatitis C virus antibodies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BEA, baseline-event-anticipation score; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HDV, hepatitis delta virus; HIVAg-Ab, human immunodeficiency virus antigen and antibodies; IFN, interferon-based treatment; INR, International Normalized Ratio; IQR, interquartile range; MELD, Model for End-Stage Liver Disease; NUC, nucleos(t)ide analogue;.

viremic patients with a sensitivity of 70.2% and specificity of 83.5%, was applied [17]. It cannot be excluded that some patients allocated to the advanced liver fibrosis group in fact had lower degrees of fibrosis, although liver fibrosis stage was diagnosed by liver biopsy in almost half of our cohort (72/152 (47.3%)).

Some earlier national cohort studies only included patients from specialised tertiary care centres, which are prone to referral and selection bias [11–13]. In addition, as they were monocentric or performed in a limited number of centres, it is questionable whether these studies are representative of their national situation. Furthermore, the generalisation of the findings is also complicated by their cross-sectional study design [14] or limited follow-up duration [10].

Apart from the long-term follow-up, the main strengths of our cohort are that uniform methods for HDV RNA evaluation were used throughout the study and that all data was collected in a systematic and uniform manner at the participating sites. In addition, thanks to the national aspect of the study, it is ensured that all patients had access to the same clinical management and follow-up, irrespective of their place of residence or origin. Moreover, due to the low number of patients treated with IFN and the fact that bulevirtide is not yet available in Belgium, this real-world cohort study resembles a natural history study of CHD.

Although these are the first-ever results from Belgium, it is defensible that these results also illustrate the situation in other European countries, as they generally have the same anti-HDV seroprevalence, representation of origins, and clinical management. Nonetheless, it is important to note that the severity may be overestimated in this study because of its retrospective nature. Unfortunately, systematic repetitive HDV RNA assessments were not available for a large proportion of our cohort, limiting our ability to investigate the role of fluctuating HDV RNA levels on the disease progression and outcomes in the long term. Previously it has been shown that the HDV genotype influences the progression of fibrosis and liver disease outcomes [10, 21]. As HDV genotyping was not available at the Belgian NRC, we could

FIGURE 2 | Severe liver-related outcomes during follow-up. (A) Overall cumulative incidence of severe liver-related outcomes in patients with and without advanced liver fibrosis at baseline. (B) Overall cumulative incidence of severe liver-related outcomes according to HDV RNA status at last evaluation. (C) Cumulative incidence of liver decompensation in patients with and without advanced liver fibrosis at baseline. (D) Cumulative incidence of liver decompensation according to HDV RNA status at last evaluation. (E) Cumulative incidence of hepatocellular carcinoma in patients with and without advanced liver fibrosis at baseline. (F) Cumulative incidence of hepatocellular carcinoma according to HDV RNA status at last evaluation. HCC, hepatocellular carcinoma; HDV, hepatitis delta virus.

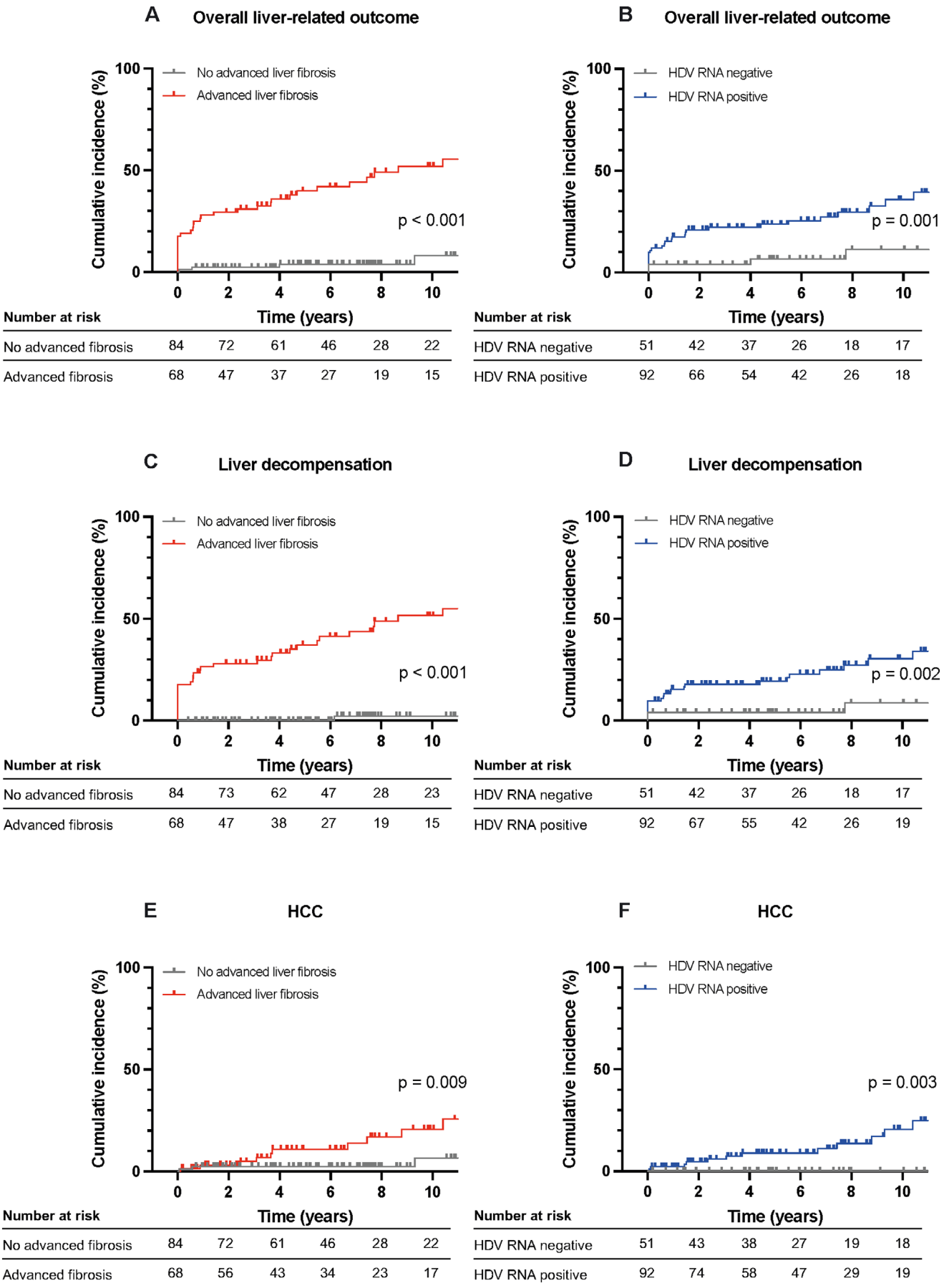

FIGURE 2 | Legend on previous page.

TABLE 4 | Factors associated with severe liver-related outcomes.

Development of severe liver-related outcome	Univariate Cox regression		Multivariate Cox regression Model 1		Multivariate Cox regression Model 2		Multivariate Cox regression Model 3	
	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
Age at admission (years)	1.095 (1.063 – 1.129)	**< 0.001**	1.073 (1.036 – 1.111)	**< 0.001**				
Male	0.860 (0.451 – 1.638)	0.65						
Origin	1.112 (0.831 – 1.488)	0.47						
Laboratory								
AST (U/L)	1.002 (1.000 – 1.003)	0.06						
ALT (U/L)	1.000 (0.998 – 1.002)	0.69						
INR	18.309 (8.276 – 40.504)	**< 0.001**			7.065 (2.556 – 19.533)	**< 0.001**		
Bilirubin (mg/dL)	1.230 (1.141 – 1.326)	**< 0.001**						
Thrombocytes (10^9/L)	0.978 (0.971 – 0.984)	**< 0.001**			0.983 (0.976 – 0.990)	**< 0.001**		
MELD	1.114 (1.039 – 1.195)	**0.002**						
Hepatitis B virus								
HBeAg positive	1.577 (0.724 – 3.437)	0.25						
HBV DNA positive	0.834 (0.424 – 1.640)	0.60						
HBV DNA > 2000 IU/mL	1.117 (0.510 – 2.449)	0.78						

(Continues)

TABLE 4 | (Continued)

Development of severe liver-related outcome	Univariate Cox regression		Multivariate Cox regression Model 1		Multivariate Cox regression Model 2		Multivariate Cox regression Model 3	
	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
HDV RNA positive	4.699 (1.648 – 13.396)	**0.004**	3.024 (1.027 – 8.910)	**0.045**	3.543 (1.207 – 10.399)	**0.021**	6.719 (1.879 – 24.026)	**0.003**
Coinfection								
Anti-HCV positive	1.178 (0.414 – 3.352)	0.76						
HIVAg-Ab positive	3.857 (1.590 – 9.351)	**0.003**					1.170 (0.392 – 3.494)	0.78
Treatment history								
NUC experienced	1.869 (0.998 – 3.500)	0.051						
IFN experienced	0.564 (0.267 – 1.190)	0.13						
IFN persistent response	0.031 (0.000 – 19.373)	0.29						
Advanced liver fibrosis at baseline	12.326 (4.371 – 34.758)	**< 0.001**	8.041 (2.345 – 27.568)	**< 0.001**				
BEA-score								
BEA-A	REF						REF	
BEA-B	6.852 (2.824 – 16.625)	**< 0.001**					5.610 (2.077 – 15.153)	**< 0.001**
BEA-C	22.305 (7.394 – 67.292)	**< 0.001**					24.059 (5.502 – 105.20)	**< 0.001**

Note: Uni- and multivariate Cox proportional hazards regression. All bold values have a significant p value < 0.05.

Abbreviations: Anti-HCV, hepatitis C virus antibodies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BEA, baseline-event-anticipation score; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HDV, hepatitis delta virus; HIVAg-Ab, human immunodeficiency virus antigen and antibodies; IFN, interferon-based treatment; INR, International Normalized Ratio; MELD, Model for End-Stage Liver Disease; NUC, nucleos(t)ide analogue.

not ascertain the impact of HDV genotypes in our cohort. Lastly, potential covariates like metabolic comorbidities, alcohol consumption, diabetes, etc., were not systematically collected and could not be included or corrected for in the models.

Our study underlines the severity of CHD and illustrates why chronic HBV-HDV coinfection is considered to be the most severe form of viral hepatitis. The often late CHD presentation with advanced fibrosis at diagnosis in almost half of patients calls for active case finding and HBV screening with HDV reflex testing in those that are HBsAg positive [7]. Given the multiethnic origins of CHD in Belgium, we especially encourage targeted screening in persons migrating from high HBV endemic countries by point-of-care tests, which have been shown to result in high linkage to care [22, 23]. Importantly, our study also exemplifies that the clinical management of CHD patients did not substantially change between 2001 and 2023 in Belgium. Indeed the outcome of CHD patients was not influenced by their year of diagnosis. Neither NUC treatment nor IFN treatment seemed to have an impact on the outcome of Belgian CHD patients, substantiating the persisting lack of valuable treatment options for CHD in Belgium.

The recently approved treatment BLV holds promise for long-term HDV suppressive management. Nevertheless, the field continues to await definitive curative treatments with finite treatment durations to effectively eradicate both HBV and HDV in these patients.

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Conflicts of Interest

C.M. received a consulting fee from Gilead. T.V.W. received grants from Gilead and BMS, served as a consultant for Janssen, Gilead, and AbbVie, and received speaker fees from Gilead. All other authors declare no conflicts of interest. Please refer to the accompanying ICMJE disclosure forms for further details.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. A. J. Stockdale, B. Kreuels, M. Y. R. Henrion, et al., "The Global Prevalence of Hepatitis D Virus Infection: Systematic Review and Meta-Analysis," *Journal of Hepatology* 73, no. 3 (2020): 523–532, https://doi.org/10.1016/j.jhep.2020.04.008.

2. E. Ho, P. Deltenre, M. Nkuize, J. Delwaide, I. Colle, and P. Michielsen, "Coinfection of Hepatitis B and Hepatitis Delta Virus in Belgium: A Multicenter BASL Study. Prospective Epidemiology and Comparison With HBV Mono-Infection," *Journal of Medical Virology* 85, no. 9 (2013): 1513–1517, https://doi.org/10.1002/jmv.23653.

3. H.-Y. Chen, D.-T. Shen, D.-Z. Ji, et al., "Prevalence and Burden of Hepatitis D Virus Infection in the Global Population: A Systematic Review and Meta-Analysis," *Gut* 68, no. 3 (2019): 512–521, https://doi.org/10.1136/gutjnl-2018-316,601.

4. M. Rizzetto, S. Hamid, and F. Negro, "The Changing Context of Hepatitis D," *Journal of Hepatology* 74, no. 5 (2021): 1200–1211, https://doi.org/10.1016/j.jhep.2021.01.014.

5. H. Wedemeyer and M. P. Manns, "Epidemiology, Pathogenesis and Management of Hepatitis D: Update and Challenges Ahead," *Nature Reviews Gastroenterology & Hepatology* 7, no. 1 (2010): 31–40, https://doi.org/10.1038/nrgastro.2009.205.

6. T. Asselah and M. Rizzetto, "Hepatitis D Virus Infection," *New England Journal of Medicine* 389, no. 1 (2023): 58–70, https://doi.org/10.1056/NEJMra2212151.

7. M. R. Brunetto, G. Ricco, F. Negro, et al., "EASL Clinical Practice Guidelines on Hepatitis Delta Virus," *Journal of Hepatology* 79, no. 2 (2023): 433–460, https://doi.org/10.1016/j.jhep.2023.05.001.

8. T. Asselah, V. Chulanov, P. Lampertico, et al., "Bulevirtide Combined With Pegylated Interferon for Chronic Hepatitis D," *New England Journal of Medicine* 391, no. 2 (2024): 133–143, https://doi.org/10.1056/NEJMoa2314134.

9. H. Kamal, G. Westman, K. Falconer, et al., "Long-Term Study of Hepatitis Delta Virus Infection at Secondary Care Centers: The Impact of Viremia on Liver-Related Outcomes," *Hepatology* 72, no. 4 (2020): 1177–1190, https://doi.org/10.1002/hep.31214.

10. D. Roulot, S. Brichler, R. Layese, et al., "Origin, HDV Genotype and Persistent Viremia Determine Outcome and Treatment Response in Patients With Chronic Hepatitis Delta," *Journal of Hepatology* 73, no. 5 (2020): 1046–1062, https://doi.org/10.1016/j.jhep.2020.06.038.

11. A. Wranke, B. C. Serrano, B. Heidrich, et al., "Antiviral Treatment and Liver-Related Complications in Hepatitis Delta," *Hepatology* 65, no. 2 (2017): 414–425.

12. A. Palom, S. Rodríguez-Tajes, C. A. Navascués, et al., "Long-Term Clinical Outcomes in Patients With Chronic Hepatitis Delta: The Role of Persistent Viraemia," *Alimentary Pharmacology & Therapeutics* 51, no. 1 (2020): 158–166, https://doi.org/10.1111/apt.15521.

13. G. A. Niro, A. Smedile, A. M. Ippolito, et al., "Outcome of Chronic Delta Hepatitis in Italy: A Long-Term Cohort Study," *Journal of Hepatology* 53, no. 5 (2010): 834–840, https://doi.org/10.1016/j.jhep.2010.06.008.

14. A. Wranke, C. Lobato, E. Ceausu, et al., "Long-Term Outcome of Hepatitis Delta in Different Regions World-Wide: Results of the Hepatitis Delta International Network," *Liver International* 44, no. 9 (2024): 2442–2457, https://doi.org/10.1111/liv.16006.

15. T. Vanwolleghem, P. A. Armstrong, M. Buti, et al., "The Elimination of Hepatitis D as a Public Health Problem: Needs and Challenges," *Journal of Viral Hepatitis* 31 (2024): 47–50, https://doi.org/10.1111/jvh.13891.

16. B. Calle Serrano, A. Großhennig, M. Homs, et al., "Development and Evaluation of a Baseline-Event-Anticipation Score for Hepatitis Delta," *Journal of Viral Hepatitis* 21, no. 11 (2014): e154–e163, https://doi.org/10.1111/jvh.12251.

17. D. Roulot, S. Brichler, L. Richard, et al., "Liver Stiffness Measurement to Detect Severe Fibrosis and Cirrhosis in Patients With Chronic Hepatitis D Infection," *Hepatology* 78, no. S1 (2023): S66–S67, https://doi.org/10.1097/hep.0000000000000580.

18. F. Le Gal, S. Brichler, R. Sahli, S. Chevret, and E. Gordien, "First International External Quality Assessment for Hepatitis Delta Virus RNA Quantification in Plasma," *Hepatology* 64, no. 5 (2016): 1483–1494, https://doi.org/10.1002/hep.28772.

19. M. Kodani, A. Martin, T. Mixson-Hayden, J. Drobeniuc, R. R. Gish, and S. Kamili, "One-Step Real-Time PCR Assay for Detection and Quantitation of Hepatitis D Virus RNA," *Journal of Virological Methods* 193, no. 2 (2013): 531–535, https://doi.org/10.1016/j.jviromet.2013.07.033.

20. L. Sandmann, K. Port, P. Lampertico, et al., "WED-171—Liver Stiffness Measurement as a Non-Invasive Method for the Diagnosis of Liver Cirrhosis in Patients With Chronic Hepatitis D Virus Infection," *Journal of Hepatology* 78 (2023): S1101–S1102, https://doi.org/10.1016/S0168-8278(23)03213-0.

21. M. Spaan, I. Carey, M. Bruce, et al., "Hepatitis Delta Genotype 5 Is Associated With Favourable Disease Outcome and Better Response to Treatment Compared to Genotype 1," *Journal of Hepatology* 72, no. 6 (2020): 1097–1104, https://doi.org/10.1016/j.jhep.2019.12.028.

22. E. Ho, A. Vanderlinden, L. Govaerts, et al., "Opportunistic Screening Using Point-Of-Care Testing Leads to Successful Linkage to Care of HBV-Infected Migrant Populations in a Low Endemic Country," *Journal of Virus Eradication* 10, no. 1 (2024): 100369, https://doi.org/10.1016/j.jve.2024.100369.

23. E. Ho, P. Michielsen, P. Van Damme, M. Ieven, I. Veldhuijzen, and T. Vanwolleghem, "Point-Of-Care Tests for Hepatitis B Are Associated With A Higher Linkage to Care and Lower Cost Compared to Venepuncture Sampling During Outreach Screenings in an Asian Migrant Population," *Annals of Global Health* 86, no. 1 (2020): 2848, https://doi.org/10.5334/aogh.2848.

Supporting Information

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