

Antiviral Treatment and Liver-Related Complications in Hepatitis Delta

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Hepatitis delta virus (HDV) is the most severe form of viral hepatitis. Pegylated interferon alfa (PEG-IFN α) is effective in only 25%-30% of patients and is associated with frequent side effects. The aim of this study was to analyze the clinical long-term outcome of hepatitis delta in relation to different antiviral treatment strategies. We studied 136 anti-HDV-positive patients who were followed for at least 6 months in a retrospective single-center cohort (mean time of follow-up, 5.2 years; range, 0.6-18.8). Liver cirrhosis was already present in 62 patients at first presentation. Twenty-nine percent of patients did not receive any antiviral treatment, 38% were treated with interferon alfa (IFN α)-based therapies, and 33% received nucleos(t)ide analogues (NAs) only. Clinical endpoints defined as hepatic decompensation (ascites, encephalopathy, and variceal bleeding), hepatocellular carcinoma, liver transplantation, and liver-related death developed in 55 patients (40%). Patients who received IFN α -based therapies developed clinical endpoints less frequently than those treated with NA ($P = 0.02$; HR, 4.0) or untreated patients ($P = 0.05$; HR, 2.2; 17%, 64%, and 44%), respectively, which was significant in both chi-square and Kaplan-Meier analysis. In addition, considering various clinical and virological parameters, IFN α therapy was independently associated with a more benign clinical long-term outcome in multivariate logistic regression analysis ($P = 0.04$; odds ratio, 0.25; 95% confidence interval, 0.07-0.9). Loss of HDV RNA during follow-up was more frequent in IFN α -treated patients and strongly linked with a lower likelihood to experience liver-related complications. **Conclusion:** IFN α -based antiviral therapy of hepatitis delta was independently associated with a lower likelihood for clinical disease progression. Durable undetectability of HDV RNA is a valid surrogate endpoint in the treatment of hepatitis delta. (HEPATOLOGY 2016;00:000-000).

The hepatitis delta virus (HDV) is an incomplete RNA virus, which requires the helper function of the hepatitis B virus (HBV) envelope for transmission and therefore infects hepatitis B surface antigen (HBsAg)-positive patients only.^(1,2) Approximately 10-20 million individuals are coinfecting with HDV and HBV worldwide.⁽³⁾ Hepatitis delta is the most severe form of viral hepatitis rapidly progressing to liver cirrhosis with consecutive liver-related endpoints like portal hypertension and encephalopathy, resulting in high cumulative rates of liver-related morbidity and mortality.⁽⁴⁻⁹⁾ Recent European

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; APRI, AST platelet ratio index; AST, aspartate transaminase; BEA, Base-line Event-Anticipation score; CHC, chronic hepatitis C; CIs, confidence intervals; gGT, gamma-glutamyl transpeptidase; HBeAg, hepatitis B e antigen; HbsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBV DNA, hepatitis B viral DNA; HCC, hepatocellular carcinoma; HDV, hepatitis delta virus; HDV RNA, hepatitis delta viral RNA; HIV, human immunodeficiency virus; HR, hazard ratio; IFN α , interferon alfa; INR, international normalized ratio; LLN, lower limit of normal; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; NAs, nucleos(t)ide analogues; ORs, odds ratios; PEG-IFN α , pegylated interferon alfa.

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single-center cohort studies reported a progression to liver cirrhosis in 62%-67% of patients,^(4,5) resulting in a hepatocellular carcinoma (HCC) rate of up to 23% after a mean follow-up of 269 months.⁽⁴⁻⁶⁾ Survival rates less than 50% after 15 years were reported in HDV genotype 1-infected patients in Taiwan.⁽¹⁰⁾

Treatment options for hepatitis delta are limited because HDV does not encode for own viral enzymes, but uses host polymerases for replication. HBV polymerase inhibitors are ineffective against HDV,⁽¹¹⁻¹⁴⁾ even though long-term therapy with tenofovir has been suggested to be beneficial in human immunodeficiency virus (HIV)-HBV-HDV triple-infected patients.⁽¹⁵⁾ Therapy with pegylated interferon alfa (PEG-IFN α) leads to a posttreatment week 24 hepatitis delta viral RNA (HDV RNA) response in approximately one quarter of patients.⁽¹⁶⁻¹⁸⁾ However, PEG-IFN α is side-effect prone, and several contraindications have to be considered. Moreover, late HDV-RNA relapses have been described in more than half of the responder patients in the prospective international Hep-Net International Delta Hepatitis Intervention Trial-1.⁽¹⁹⁾ Subsequently, the clinical benefit of PEG-IFN α therapy of hepatitis delta has been questioned, in particular, if HBsAg clearance is not achieved.⁽²⁰⁾

In HBV monoinfection, prolonged suppression of HBV replication by antiviral therapy is clearly associated with reduced frequency of hepatic decompensation and development of HCC.⁽²¹⁻²³⁾ Similarly, sustained virological response to IFN α -based therapy of chronic hepatitis C (CHC) leads to lower liver-associated and overall mortality.⁽²⁴⁻²⁷⁾ In contrast, there are very limited data on the potential clinical long-term effects of antiviral therapies in hepatitis delta. One a small study of patients treated in the early 1990s suggested that

high doses of conventional IFN α may result in a better outcome after a follow-up of 14 years.⁽²⁸⁾ In addition, IFN α -based antiviral therapy was associated with a better survival in retrospective cohort studies,^(5,29) but the number of treated patients was small.

The aim of this study was to evaluate the potential effects of different antiviral treatment strategies of the course of liver disease in a large, well-defined single-center cohort of hepatitis delta patients.

Patients and Methods

PATIENTS

All anti-HDV-positive patients referred to Hannover Medical School from 1987 to 2013 were screened. Patients were included in the study if they had detectable HBsAg and either anti-HDV immunoglobulin G antibodies or HDV RNA for at least 6 months. Detailed inclusion criteria can be seen in the article of Calle Serrano et al.⁽⁷⁾ Only patients with follow-up data of at least 6 months were included. Patients were excluded if they had already undergone liver transplantation (LT) or had developed HCC before the first observation. From altogether 386 screened patients, a total of 136 patients fulfilling the inclusion criteria were recruited to the study. Virological parameters for hepatitis B, C, and delta were measured as described.⁽³⁰⁻³²⁾ Sera for retrospective HDV-RNA testing were available for 114 patients. For the other patients, HDV-RNA results were documented in the referral letters, but quantitative values were not considered for further analysis because of the lack of assay standardization between different labs.⁽³³⁾ Seventy-five patients were already part of a previous analysis that applied more-stringent inclusion criteria to define a prognostic

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score.⁽⁷⁾ In the previous study, patients were included if they had an available follow-up of at least 18 months with a minimum of three visits no longer than 2 years between consecutive visits. Because of the extended inclusion criteria, 61 patients were additionally selected. Liver-related clinical endpoints were examined along the follow-up. Liver-related endpoints were defined as hepatic decompensation (ascites, encephalopathy, and variceal bleeding), LT, HCC, or liver-related death. Cirrhosis was diagnosed based on liver histology (F5 and F6 according to the ISHAK score)⁽³⁴⁾ or by transient elastography (≥ 13.0 kilopascals). If these data were not available, presence of cirrhosis was considered if patients had already clinical evidence of hepatic decompensation in the past or if at least two of the following criteria were present: aspartate/alanine aminotransferase (AST/ALT) ratio >1 ; cholinesterase $<$ lower limit of normal (LLN); platelets $<100,000/\text{mL}$; international normalized ratio (INR) >1.5 ; and/or splenomegaly (largest dimension >12 cm).

STATISTICAL ANALYSES

Statistical analyses were performed by using SPSS software (SPSS Inc., Chicago, IL).

All parameters were described as mean \pm SD. *P* values ≤ 0.05 were considered as statistically significant.

Continuous variables were analyzed by *t* tests. For nonparametric parameters Mann-Whitney U tests were used. A chi-square-test was calculated for the comparison of discrete variables. In case of an expected cell count ≤ 5 , Fisher's exact test was used instead. Parameters that were associated with a better clinical long-term outcome in univariate logistic regression models ($P < 0.1$ or rather $P < 0.05$ if there were many parameters univariately associated) were additionally compared in multivariate analysis. Multivariate logistic regression analyses were performed by using the likelihood ratio test for backward selection. Odds ratios (ORs), including their 95% confidence intervals (CIs), were calculated for the logistic regression models. The association of parameters with clinical long-term outcome were also calculated in a time-depending Cox regression model, in which case hazard ratios (HRs) were calculated. Using Kaplan-Meier analysis, we estimated the cumulative event free survival within various groups of therapy. In these groups, significant differences concerning event-free survival were indicated by log-rank tests.

ETHICS

The study was in line with the formalities of the ethic committee of Hannover Medical School (Hannover, Germany). For the measurement of cytokines, chemokines, and angiogenetic factors, the ethic committee of Hannover Medical School had reviewed the experiments and approved this study design (No. 5258).

Results

A cohort of 136 patients was studied with a mean follow-up of 5.2 years (range, 0.6 -18.8). Patient characteristics are shown in Table 1. As expected for a hepatitis delta cohort recruited in central Europe, 70% of patients were born either in Eastern Europe, Central Asian countries, or the Eastern Mediterranean region. Most patients were infected with HDV genotype 1 and HBV genotype D. HBV precore and basal core promotor variants of hepatitis B e antigen (HBeAg)-positive patients are shown in Supporting Table S1. Severity of hepatitis-delta-related liver disease was confirmed, given that 49 (36%) of patients had a platelet count below $100,000/\mu\text{L}$, 39% presented with an AST platelet ratio index (APRI) score of >2 , 38% showed an FIB-4 score of >3.25 , and 28% had evidence of an impaired liver synthesis function with albumin levels of <35 g/dL. Cirrhosis was present in 62 patients (46%) at first presentation, including 12 with a Child-Pugh score of ≥ 7 at inclusion. Nearly three quarters of the patients were classified according to the HDV specific Baseline Event-Anticipation (BEA) score as BEA-B or BEA-C (<http://hepatitis-delta.org/physicians-and-scientists/calculators/>),⁽⁷⁾ indicating a high risk to develop clinical liver-related complication within 5 years.

CLINICAL LONG-TERM OUTCOME

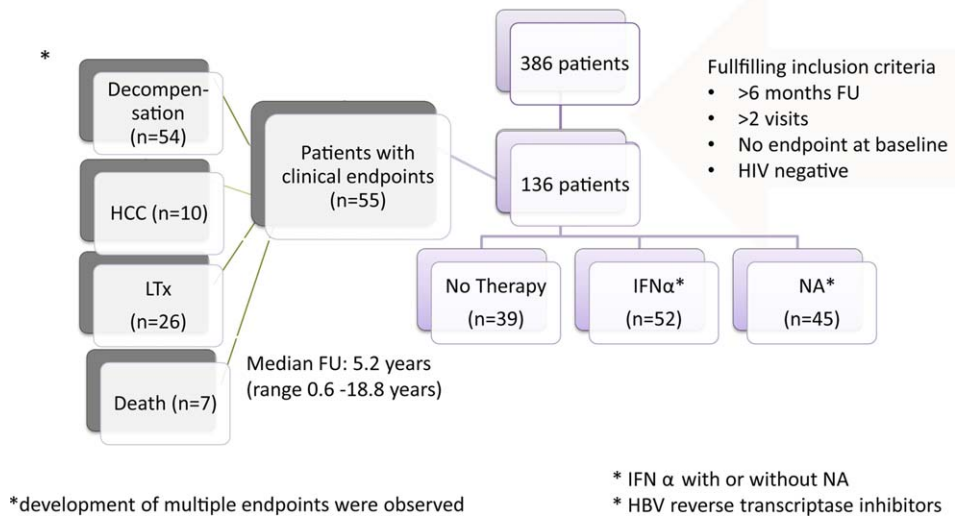
During follow-up, 30 of the 74 patients who were without cirrhosis at inclusion progressed to liver cirrhosis within a mean time of 3.2 years (range, 0.5-11.7) whereas 44 did not develop liver cirrhosis at all. A liver-related clinical endpoint was observed in 55 patients. Decompensation occurred in 54 patients within a mean follow-up of 3 years (range, 0.6-9.8), with ascites being the leading symptom in 27. Esophageal bleeding occurred in 9 patients after a mean time of 2.2 years (range, 0.6-11.7). Encephalopathy developed in 8 patients after 4.4 years (range, 1.3-8.9). Ten

TABLE 1. Baseline Characteristics and Factors Univariately Differentiating Between the Treatment Groups Based on ANOVA (Continuous Values) and Chi-Square Analysis (Discrete Values)

	Total Cohort	No Therapy	NA	IFN α	P Value
Total	136	39	45	52	
Sex (%)	Male = 92 (67) Female = 44 (33)	Male = 26 (67) Female = 13 (33)	Male = 30 (67) Female = 15 (33)	Male = 36 (69) Female = 16 (31)	>0.9
Age, years, mean \pm SD (range)	37.6 (14.1-61.3)	34.6 (14.1-57.7)	39.8 (18.5-61.3)	38.1 (15.0-59.9)	0.1
Country of origin (%)	Eastern Mediterranean = 49 (36) Eastern Europe = 46 (34) Italy = 8 (6%) other = 33 (24)	Eastern Mediterranean = 11 (28) Eastern Europe = 9 (23) Italy = 3 (8%) other = 16 (41)	Eastern Mediterranean = 20 (44) Eastern Europe = 13 (29) Italy = 3 (7%) other = 9 (20)	Eastern Mediterranean = 18 (35) Eastern Europe = 24 (46) Italy = 2 (4%) other = 8 (15)	0.06
Previous therapy (%)	33 (24)	5 (13)	14 (31)	14 (27)	0.13
AST, \times ULN, mean \pm SD (range)	2.4 \pm 2.1 (0.5-12.3)	1.9 (0.5-7.3)	2.7 (0.7-12.3)	2.5 (0.7-9.8)	0.23
ALT, \times ULN, mean \pm SD (range)	2.6 \pm 3.4 (0.2-32.0)	2.2 (0.2-14.7)	2.7 (0.3-32.0)	2.7 (0.3-7.7)	0.74
AP, \times ULN, mean \pm SD (range)	0.9 \pm 0.6 (0.1-3.9)	1.1 (0.4-3.9)	1.0 (0.1-2.2)	0.9 (0.2-3.0)	0.16
gGT, \times ULN, mean \pm SD (range)	1.4 \pm 2.3 (0.1-20.6)	1.2 (0.2-7.3)	1.8 (0.1-20.6)	1.3 (0.2-10.7)	0.44
Bilirubin, μ mol/L, mean \pm SD (range)	18.6 \pm 23.6 (3.0-174.0)	22.7 (5.0-174.0)	23.1 (5.0-148.0)	11.1 (3.0-25.0)	0.03
Albumin, g/L, mean \pm SD (range)	39.0 \pm 6.6 (22.0-56.0)	41.7 (27.0-56.0)	36.2 (22.0-55.0)	40.0 (33.0-50.0)	<0.01
Platelets, 1,000/ μ L, mean \pm SD (range)]	133.5 \pm 71.4 (16.0-335.0)	128.8 (16.0-323.0)	107.7 (32.0-335.0)	159.8 (35.0-331.0)	<0.01
INR, mean \pm SD (range)	1.15 \pm 0.2 (0.9-2.7)	1.3 (0.9-2.7)	1.3 (0.9-1.9)	1.1 (0.9-1.3)	<0.01
APRI score, mean \pm SD (range)]	2.7 \pm 5.1 (0.2-27.1)	3.3 (0.2-25.0)	3.4 (0.2-27.1)	1.8 (0.3-10.0)	0.12
FIB-4, mean \pm SD (range)]	3.8 \pm 4.5 (0.2-34.7)	4.3 (0.3-34.7)	4.9 (0.3-24.0)	2.4 (0.2-12.0)	0.02
AST/ALT ratio, mean \pm SD (range)	1.0 \pm 0.8 (0.1-6.2)	1.2 (0.4-6.2)	1.2 (0.4-3.6)	0.8 (0.1-2.3)	<0.01
HBsAg levels, log(IU/mL), mean \pm SD (range)]	3.6 \pm 0.1 (2.0-4.4)	3.4 \pm 0.9 (2.1-4.4)	3.7 \pm 0.5 (2.2-4.1)	3.7 \pm 0.7 (2.0-4.3)	0.5
HBV-DNA levels, (log[IU/mL], mean \pm SD (range)	3.1 \pm 1.6 (0.8-7.8)	3.8 \pm 1.9 (0.8-6.3)	3.4 \pm 1.7 (1.4-7.8)	2.6 \pm 1.4 (1.0-6.5)	0.2
HDV-RNA viremia, n patients (%)	81 (75)	17 (61)	34 (81)	30 (79)	0.1
HDV genotype	GT 1 = 92 GT 5 = 2	GT 1 = 20 GT 5 = 0	GT 1 = 32 GT 5 = 2	GT 1 = 38 GT 5 = 0	0.2
HBeAg, n patients (%)	24 (21)	5 (15)	9 (21)	10 (26)	0.5
BEA score, n patients (%)	A = 41 B = 81 C = 14	A = 15 B = 19 C = 5	A = 10 B = 27 C = 8	A = 16 B = 35 C = 1	0.05
MELD, mean \pm SD (range)	9.2 \pm 3.3 (5.0-21.0)	10.2 (5.9-21.0)	9.9 (6.0-18.7)	7.7 (5.0-10.0)	<0.01
Cirrhosis, n patients (%)	62 (46)	17 (44)	30 (67)	15 (29)	<0.01
Child-Pugh classes, n patients (%)	A = 33 (73) B = 12 (27) C = 0 (0)	A = 9 (75) B = 3 (25) C = 0 (0)	A = 13 (62) B = 8 (38) C = 0 (0)	A = 11 (92) B = 1 (8) C = 0 (0)	0.2
Clinical endpoints, n patients (%)	55 (40)	17 (44)	29 (64)	9 (17)	<0.01

Abbreviation: ULN, upper limit of normal.

FIG. 1. Number of patients recruited in the different treatment groups and development of liver related endpoints along follow up (median FU, 5.2 years). Abbreviations: FU, follow-up; LTx, liver transplantation.



patients presented with more than one decompensation event. HCCs were detected in 10 patients after a mean time of 6.1 years (range, 0.6-15.3). LT was necessary in 26 patients after 4.1 years (range, 0.6-13.0). Seven patients died after a mean follow-up of 6.8 years (range, 1.9-16.4), 3 of them with liver-related causes of death; for the other 4, there was no information of the specific cause of death.

Antiviral therapies were administered to 97 patients during follow-up. Thirty-eight percent of the patients received IFN α -based therapies (36 PEG-IFN α and 16 conventional non-PEG-IFN α) with (n = 30) or without (n = 22) concomitant or subsequent nucleos(t)ide analogues (NAs), 33% were treated with NAs alone, and 29% did not receive any antiviral therapy (Fig. 1). Mean duration of IFN α -based therapy was 12 months (range, 0.5-36.0). Details of antiviral therapies used are listed in Table 2. Patient characteristics of the three

groups are listed in Table 1. There were no differences in biochemical disease activity as determined by AST, ALT, gamma-glutamyl transpeptidase (gGT), and alkaline phosphatase (AP) levels. Similarly, baseline virological parameters were similar in the three groups, including quantitative HBsAg, HDV-RNA viremia, and hepatitis B viral DNA (HBV DNA) values. NA-treated patients had the most advanced stage of liver disease considering differences in bilirubin, albumin, platelet values, INR as well as AST/ALT ratios, and BEA and Model for End-Stage Liver Disease (MELD) scores (Table 1).

ANTIVIRAL THERAPY AND LIVER-RELATED CLINICAL COMPLICATIONS

Patients who received IFN α -based therapies developed less frequently clinical endpoints compared to patients treated with NA only or untreated patients (IFN α vs. untreated, $P < 0.01$; CI, 0.2-0.9; IFN α vs. NA, $P < 0.01$; CI, 0.1-0.5) in chi-square analysis. In addition, patients treated with NAs alone developed more often clinical endpoints than untreated patients ($P = 0.05$; CI, 0.9-2.1; Table 1). These differences were confirmed in Kaplan-Meier and Cox model analysis, where IFN α -based therapy was also associated with a more-benign clinical outcome in comparison to treatment with NAs ($P = 0.02$; HR, 4.0; 95% CI, 1.9-

TABLE 2. Antiviral Therapy During Follow-up

	Medication	No. of Patients
	Lamivudine	29
	Lamivudine+adefovir	2
	Entecavir	4
	Adefovir	6
	Tenofovir	4
Combination with IFN α	Lamivudine	2
Combination with PEG-IFN α	Adefovir	7
	Tenofovir or placebo	21
IFN α mono	IFN α	14
	PEG-IFN α	8

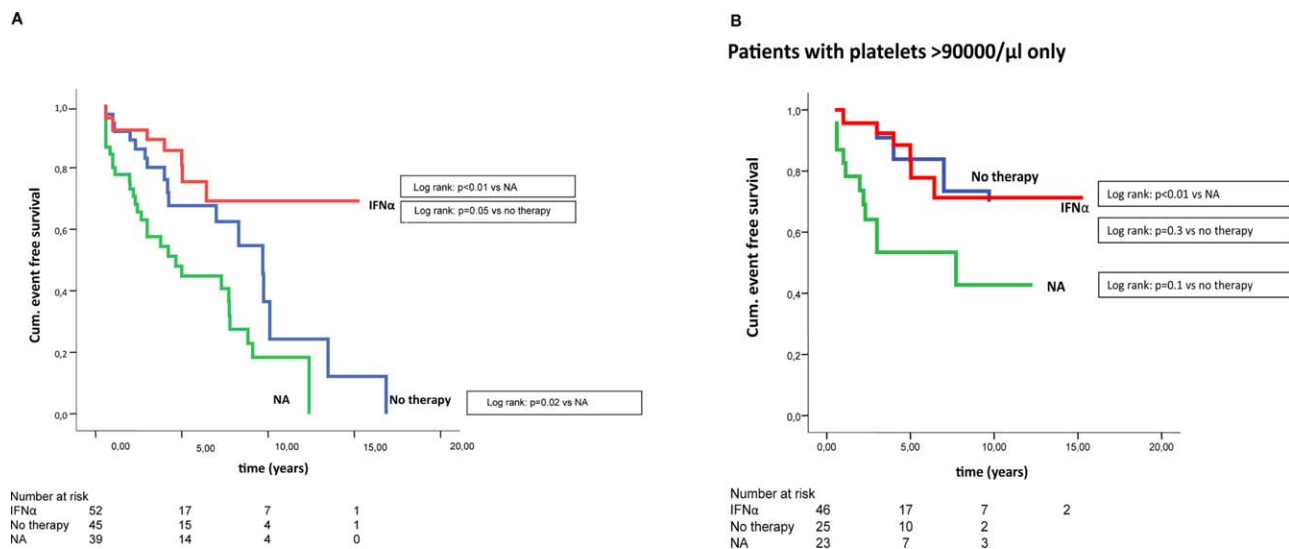


FIG. 2. (A) Cumulative event-free survival of untreated patients and those treated with IFN α or NAs. Patients treated with IFN α developed significantly less liver-related endpoints than those treated with NAs ($P < 0.01$) or untreated patients ($P = 0.05$). (B) Cumulative event-free survival of patients treated with IFN α compared to untreated patients and those treated with NAs, excluding all patients with platelet counts $< 90,000/\mu\text{L}$. Even after excluding patients with more-advanced liver disease, IFN α -based therapy was still significantly associated with a more-benign clinical long-term outcome compared to patients treated with NA ($P < 0.01$). Abbreviation: Cum., cumulative.

8.6) and untreated patients ($P = 0.05$; HR, 2.2; 95% CI, 1.0-5.0; Fig. 2A). Given that patients treated with IFN α had less-advanced liver disease than the other two groups, we compared the clinical course in all three groups for patients with a platelet count of $> 90,000/\mu\text{L}$ only who would have qualified for IFN α therapy. Even after excluding the more-advanced patients, IFN α -treated patients showed an improved outcome compared to NA-treated patients ($P < 0.01$; HR, 0.3; 95% CI, 0.1-0.7), but not to untreated patients ($P = 0.4$; HR, 0.6; 95% CI, 0.2-1.8; Fig. 2C).

We next investigated which specific clinical event accounted for the observed differences between IFN α -treated and untreated or NA-treated patients (Fig. 3A-D). Although there were no differences between the three treatment groups concerning development of HCC ($P = 0.84$; CI, 0.7-1.8) and death ($P = 0.4$; CI, 0.6-2.2; chi-square analysis), both LT ($P < 0.01$; CI, 1.7-3.3) and hepatic decompensation ($P < 0.01$; CI, 1.5-3.6) occurred more often in patients not treated with IFN α compared to NA, which was confirmed by Kaplan-Meier analysis ($P < 0.01$; CI, 4.6-10.9 [decompensation]). Besides, LT ($P < 0.01$; CI, 1.5-3.2) and decompensation ($P = 0.04$; CI, 1.1-2.6) occurred more often in untreated patients compared to IFN α -treated patients in chi-square analysis.

FACTORS ASSOCIATED WITH THE DEVELOPMENT OF LIVER-RELATED CLINICAL EVENTS

Factors associated with the development of clinical events in univariate and multivariate logistic regression analysis are shown in Table 3. Of note, biochemical disease activity, as determined by ALT ($P = 0.2$; CI, 0.9-1.0) and AST levels ($P = 0.3$; CI, 0.9-1.0), presence of HDV RNA at baseline ($P = 0.4$; CI, 0.3-1.6), quantitative HBsAg levels ($P = 0.3$; CI, 1.0-1.0), or HBV-DNA values ($P = 0.2$; CI, 0.9-2.0) were not associated with the clinical long-term outcome. Backward logistic regression model analysis revealed that quantitative platelet count ($P < 0.01$; CI, 1.0-1.0), albumin levels ($P = 0.02$; CI, 0.8-0.9), a positive HBeAg status ($P = 0.04$; CI, 0.02-0.9), and IFN α -based therapy ($P = 0.04$; CI, 0.07-0.9) were independently associated with a favorable outcome in multivariate analysis (Table 3). The OR for patients treated with IFN α to develop a liver-related complication was 0.25. There were no differences in the clinical long-term outcome between patients who had received IFN α monotherapy versus those who were treated with IFN α /NA combination therapy ($P = 0.4$; OR, 1.9) or

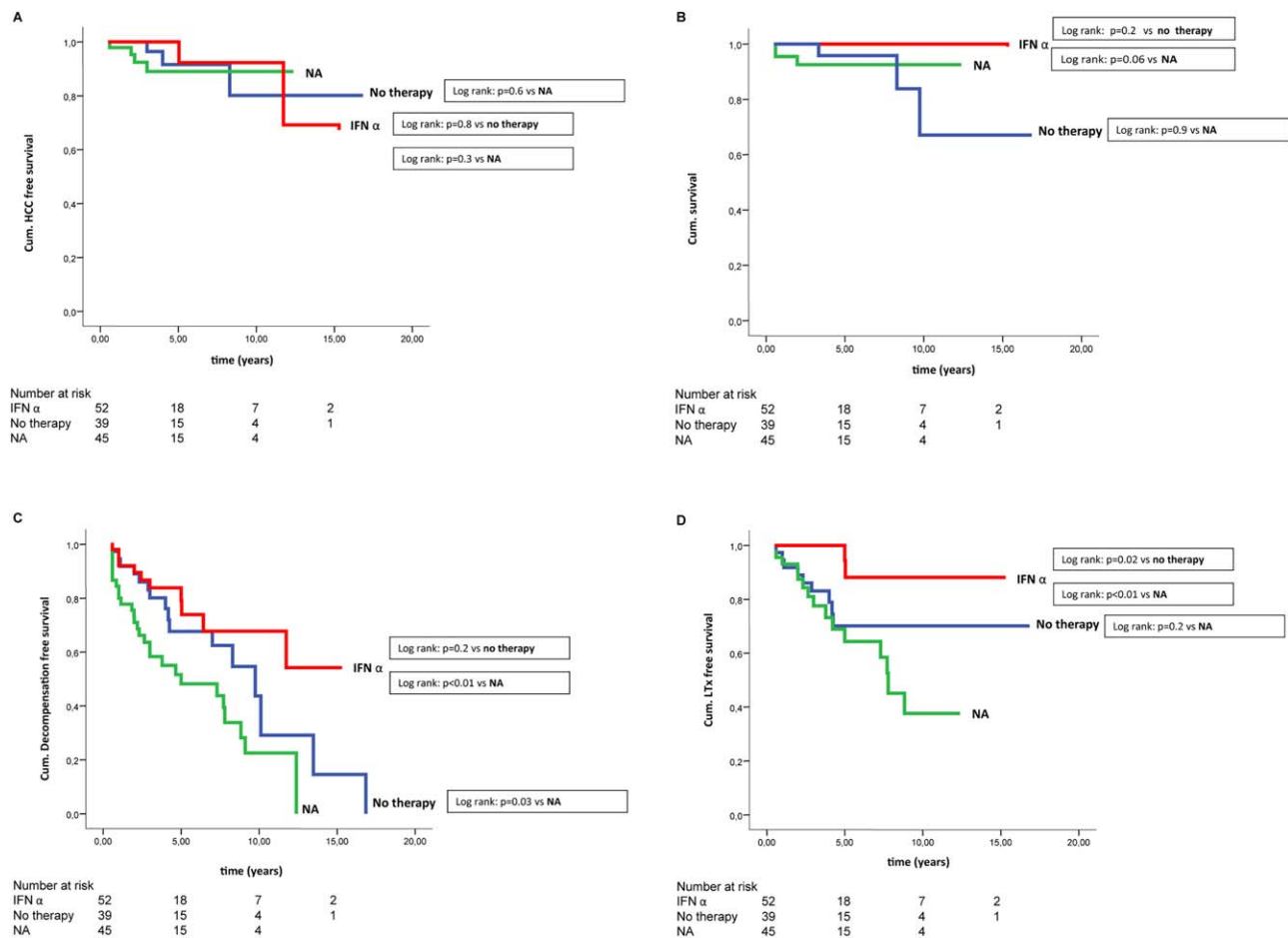


FIG. 3. (A) Cumulative HCC-free survival of patients treated with IFN α compared to untreated patients and those treated with NAs. There were no differences in the development of HCC along follow-up over the three groups. (B) Cumulative death-free survival of patients treated with IFN α compared to untreated patients and patients treated with NAs. There were no differences of death rates along follow-up over the three groups. (C) Cumulative decompensation-free survival of patients treated with IFN α compared to untreated patients and those treated with NAs. Patients treated with IFN α -based therapy developed less frequently decompensation compared to NA-treated patients ($P < 0.01$). (D) Cumulative liver transplantation free survival of patients treated with IFN α compared to untreated patients and patients treated with NA. In patients treated with IFN α -based therapy liver transplantation was less often necessary compared to patients treated with NA ($p < 0.01$) or untreated patients ($p = 0.02$). Abbreviation: Cum., cumulative.

sequential therapy ($P = 0.8$; OR, 0.9) in a univariate logistic regression model.

VIROLOGICAL RESPONSES AND CLINICAL OUTCOME

Given that IFN α -based antiviral therapy was associated with a beneficial clinical outcome of hepatitis delta, the next question to be addressed was whether the primary virological endpoints of antiviral therapy, HDV-RNA loss and HBsAg loss, could be linked to frequencies of clinical events.

HDV-RNA loss during follow-up, defined as negative HDV RNA at the last available visit, occurred in 33 patients. Undetectable HDV RNA at the last available visit was evident in 44% of IFN α -treated patients, 19% of those treated with NA, and in 21% of untreated patients (IFN α vs. NA, $P < 0.01$; CI, 1.3-3.0; IFN α vs. untreated, $P = 0.03$; CI, 1.1-2.3; NA vs. untreated, $P = 0.4$; CI, 0.6-1.5; Fig. 4A). Overall, 19 patients treated with IFN α -based therapy lost HDV RNA. In most of the patients ($n = 12$), loss of HDV RNA occurred during therapy. Only 7 patients lost HDV RNA after therapy was stopped, 5 of them

TABLE 3. Parameters Associated With the Clinical Long-Term Outcome in Univariate and Multivariate Analysis

Parameter	Significance (Univariate)	Significance (Multivariate)*
AST/ALT ratio (linear)	$P < 0.01$; OR, 3.2; 95% CI, 1.6-6.5	Not significant
INR (linear)	$P < 0.01$; OR, 42.4; 95% CI, 4.9-363.7	Not significant
gGT (linear)	$P = 0.02$; OR, 1.0; 95% CI, 1.0-1.1	Not significant
Cirrhosis (yes vs. no)	$P < 0.01$; OR, 9.9; 95% CI, 4.4-21.9	Not significant
Age (linear)	$P < 0.01$; OR, 1.0; 95% CI, 1.0-1.1	Not significant
NA therapy (yes vs. no)	$P < 0.01$; OR, 1.0; 95% CI, 2.1-9.5	Not significant
Albumin (linear)	$P < 0.01$; OR, 0.8; 95% CI, 0.7-0.9	$P = 0.02$; OR, 0.9; 95% CI, 0.8-0.9
Platelet count (linear)	$P < 0.01$; OR, 1.0; 95% CI, 1.0-1.0	$P < 0.01$; OR, 1.0; 95% CI, 1.0-1.0
HBsAg (positive vs. negative)	$P = 0.01$; OR, 0.2; 95% CI, 0.07-0.7	$P = 0.04$; OR, 0.1; 95% CI, 0.02-0.9
IFN α -based therapy (yes vs. no)	$P < 0.01$; OR, 0.17; 95% CI, 0.08-0.4	$P = 0.04$; OR, 0.25; 95% CI, 0.07-0.9

Nonsignificant parameters included in the analysis were bilirubin, AST, ALT, AP, and cholinesterase.

*All parameters with $P < 0.05$ were considered for multivariate analysis.

within 1 year posttherapy. HDV-RNA loss was associated with a beneficial clinical long-term outcome ($P < 0.01$; CI, 0.2-0.8; chi-square analysis and in Cox model [$P = 0.03$; HR, 2.3; 95% CI, 1.0-4.6]). A severe long-term outcome for HDV-positive patients was confirmed in Kaplan-Meier analysis ($P < 0.01$; CI, 1.8-8.2). Of note, patients with only transiently negative HDV RNA followed by subsequent reappearance of HDV RNA showed a similar clinical course to untreated or NA-treated patients (Fig. 4B).

HBsAg loss during follow-up was observed in 10 patients; 8 were treated with IFN α -based therapies, 1 patient received NA only, and 1 cleared HBsAg spontaneously ($P = 0.03$). HBsAg occurred in 3 patients during therapy and 5 lost HBsAg after the end of therapy, 2 of them within the first year of follow-up. Only 1 patient who lost HBsAg developed a clinical endpoint (decompensation attributed to ascites), and this patient was treated with tenofovir whereas none of the patients who cleared HBsAg after IFN α therapy developed liver-related clinical complications ($P < 0.01$; Fig. 4C). HBsAg loss was associated with a beneficial clinical long-term outcome in Fisher's exact test ($P =$

0.04; CI, 0.2-1.6). Besides, Kaplan-Meier analysis indicated a favorable effect of HBsAg loss. Because of the small number of patients undergoing HBsAg loss, the analysis was not significant ($P = 0.08$), but a clear trend was evident (Fig. 4D).

HBV DNA became undetectable in 50 patients during follow-up. There were no significant differences regarding HBV-DNA loss and development of clinical endpoints ($P = 0.1$; CI, 0.9-2.1). However, viral loads fluctuated over time in several cases and NA therapies had been interrupted in some cases.

By analyzing the virological parameters during follow-up in univariate and multivariate logistic regression models, only development of undetectable HDV RNA ($P = 0.02$; OR, 0.3; 95% CI, 0.1-0.8) was independently associated with a benign clinical long-term outcome (Supporting Table S2).

Discussion

To what extent antiviral therapy improves the clinical outcome of hepatitis delta is a current topic of controversial discussion. In a large, single-center cohort, we here confirm the particular severity of hepatitis delta with high cumulative rates of liver-related complications, and we provide evidence that IFN α treatment can improve the clinical long-term outcome. Moreover, prolonged loss of HDV RNA was clearly associated with a more benign clinical course—even in the absence of HBsAg clearance.

Suppression of viral replication by antiviral therapies in patients with both HBV and hepatitis C virus infection were associated with less liver inflammation and a reduction of fibrosis progression or even fibrosis regression. This has subsequently been linked to improved clinical long-term outcomes.⁽²¹⁻²⁷⁾ Our study suggests that similar clinical effects can also be observed in HDV-infected patients being treated with IFN α . This information is of major clinical importance given that IFN α treatment may cause severe side effects and because the overall clinical benefit of IFN α has been questioned for hepatitis C.⁽³⁵⁾ Still, it is widely accepted that IFN α -based therapy has improved the course of liver disease in the majority of successfully treated patients with CHC⁽³⁶⁾ as well as chronic hepatitis B.⁽³⁷⁾ In contrast, robust data on the clinical effects of interferon of hepatitis delta were limited.

In hepatitis delta, a study performed in the early 1990s investigated high (9 million units) or low (3 million units) doses of IFN α and showed that patients

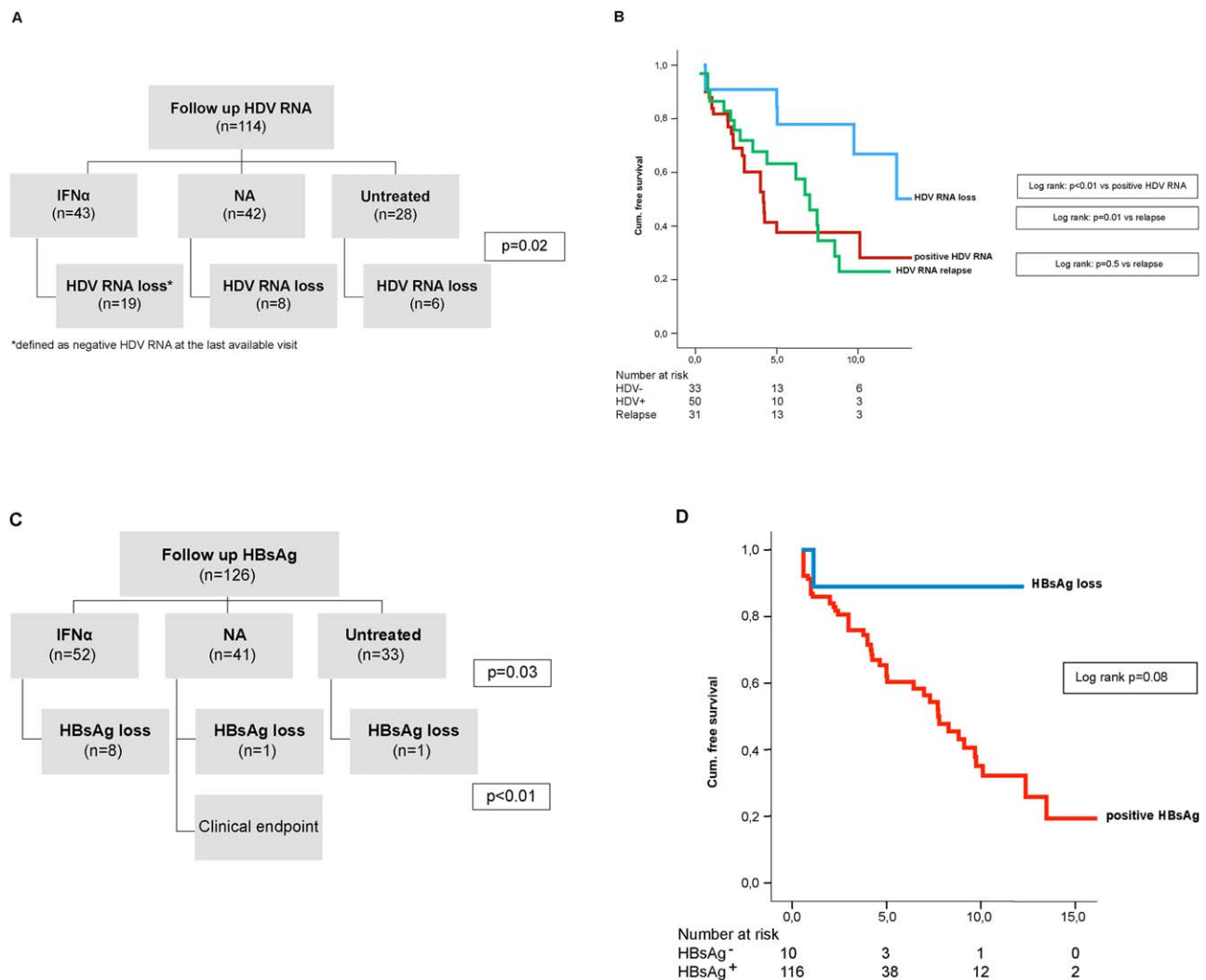


FIG. 4. (A) HDV-RNA undetectability according to the three treatment groups along follow-up. A significant difference in the achievement of HDV-RNA loss along the groups was observed ($P = 0.02$). Undetectable HDV RNA was most often evident in patients treated with IFN α (44%). (B) Cumulative event-free survival from the beginning of observation until end of follow-up of patients with undetectable HDV RNA, HDV-RNA relapse, and patients with positive HDV RNA. HDV-RNA loss was significantly associated with a beneficial clinical outcome compared to patients with positive HDV RNA ($P < 0.01$) or those with transient HDV-RNA loss ($P = 0.01$). (C) Loss of HBsAg according to the three treatment groups. Undetectable HBsAg was significantly associated with IFN α -based therapy compared to those treated with NAs or untreated patients ($P = 0.03$). Only 1 patient with negative HBsAg developed a liver-related clinical endpoint, and this patient was treated with tenofovir ($P < 0.01$). (D) Cumulative event-free survival from the beginning of observation until end of follow-up of patients with negative HBsAg and those with positive HBsAg. Kaplan-Meier analysis indicated a clear trend of a beneficial clinical outcome of patients with undetectable HBsAg ($P = 0.08$). Abbreviation: Cum., cumulative.

receiving the high IFN α dose had a better overall survival after up to 14 years of follow-up.⁽²⁸⁾ However, the number of patients included in this study was relatively small (only 12 patients per arm), and even high doses of IFN α did not lead to complete suppression of HDV RNA. Similarly, the HepNet-Greece cohort showed an improved clinical outcome of patients

treated with IFN α -based therapies, but the number of patients with a longer follow-up was again rather small.⁽²⁹⁾ Lack of antiviral therapy has also been associated with a worse course of liver disease in one Italian single-center study,⁽⁵⁾ which, however, was not the case in another study from Milan.⁽⁴⁾ Summarizing the previous studies, it can be stated that distinct

treatments and responses to therapy were not investigated in a larger cohort with long-term follow-up of more than 5 years. Our study adds therefore important evidence to the field that IFN α -based therapies improve the outcome of hepatitis delta. The effect was very pronounced with an OR of 0.25 in multivariate analysis, a magnitude that would be in line with the risk reduction observed in hepatitis C patients treated successfully with IFN α -based therapies.⁽²⁶⁾

We here found that, in particular, the frequency of hepatic decompensation and need for LT was associated with a reduction induced by IFN α -based therapy whereas there was no difference between different treatment approaches regarding development of HCC. It is discussed controversially whether HDV infection increases risk for HCC compared to HBV monoinfection.⁽²⁾ Overall, incidence of HCCs was rather low in our cohort, supporting the assumption that HDV does display only minor additional oncogenic effects—if at all—beyond promoting fibrosis progression and earlier cirrhosis development. In line with this hypothesis, loss of HDV RNA by IFN α did not influence HCC development in this cohort. Of note, similar to our findings, an Italian single-center study could also not identify any beneficial effect of IFN α on development of HCCs.⁽⁴⁾

This study does not argue against the use of NAs in hepatitis delta. The decision to use NAs in very advanced hepatitis B/D coinfection has frequently been made because no other treatment options were available. European guidelines recommend NAs in decompensated hepatitis B even if low levels of HBV DNA are detectable.⁽³⁸⁾ We would agree with this recommendation given that the underlying hepatitis B disease should be treated also in HBV/HDV coinfection. However, more studies are needed if NAs also have secondary effects on HDV infection as suggested by one group.⁽¹⁵⁾

As for any retrospective study, a potential selection bias needs to be considered, which is of particular importance when different treatment regimens are compared. Antiviral therapies were based on the clinical presentation of individual patients and decisions to initiate treatment may have changed over time. Obviously, IFN α therapy should have been started mainly in patients with compensated liver disease whereas nucleos(t)ide and nucleotide analogues were frequently administered to patients with advanced stages of liver cirrhosis without other treatment options to suppress low levels of remaining HBV replication. This strategy was actually supported by European⁽³⁸⁾ and national

HBV guidelines.⁽³⁹⁾ However, and importantly, IFN α therapy remained an independent factor associated with a more-benign outcome, even when other parameters of advanced liver disease were considered in the multivariate analysis. We could not convincingly answer the question of whether sequential or combination therapies with PEG-IFN α and HBV polymerase inhibitors provide a benefit for hepatitis delta patients attributed to limited number of patients in the different subgroups. Still, comparing patients receiving both types of antiviral drugs with IFN α monotherapy did not reveal any significant differences concerning the clinical long-term outcome.

In this hepatitis delta cohort, independent factors associated with disease progression—in addition to the absence of IFN α -based therapies—were low platelet counts, low albumin levels, and a negative HBeAg status. Platelet counts and albumin levels are well-established parameters indicating portal hypertension and an impaired synthesis function of the liver, respectively, which both have already been linked with a worse clinical course of hepatitis delta.⁽⁷⁾ The finding that a negative result for the HBeAg was also associated with the development of clinical complications in multivariate analysis may be surprising. We already suggested previously that HBeAg-positive hepatitis delta patients have slightly better course of liver disease, even though this difference did not reach formal statistical significance.⁽⁴⁰⁾ A possible explanation for this observation could be the reciprocal interaction between HBV and HDV replication.^(2,41) However, HDV-RNA levels are not different in HBeAg-positive or -negative hepatitis delta patients.⁽⁴⁰⁾ More mechanistic studies are therefore needed to explain this clinical observation. Another option could be differences in baseline characteristics given that HBeAg-positive patients were younger ($P < 0.01$) and had higher platelet counts ($P = 0.03$) as compared to HBeAg-negative patients. Still, considering this variable, HBeAg status remained an independent factor of long-term outcome.

In contrast to another recent report,⁽⁸⁾ baseline HDV-RNA status was not associated with development of liver-related endpoints—neither in univariate nor in multivariate analysis. It has to be considered that the number of patients with available quantitative HDV-RNA levels was small because of the retrospective nature of this study. On the other hand, clearance of HDV RNA during follow-up was clearly an independent parameter associated with a favorable clinical outcome. Overall, only 8 of 33 patients achieving a

prolonged HDV-RNA loss showed disease progression. If HDV-RNA loss was only transient and relapses occurred, the potential clinical benefit was lost (Fig. 4b). Thus, our data add evidence to the assumption that treatment-induced undetectable HDV RNA is a valid surrogate for an improved clinical long-term outcome in hepatitis delta. This information is important for future clinical trials exploring novel antiviral therapies against HDV. Still, it will be important to define to what extent HDV-RNA suppression, or even loss in the absence of HBsAg clearance, is improving the course of liver disease. Until then, HBsAg loss remains the ultimate goal of hepatitis delta treatment, which was associated with a better outcome also in this cohort (Fig. 4D). Unfortunately, and in line with several previous reports, the virological endpoint, HBsAg loss, was reached only by less than 10% of patients despite the long follow-up of up to almost 19 years. Thus, this study again highlights that alternative treatment strategies are urgently needed for hepatitis delta.

Our study had obvious limitations. Although we evaluated a rather large, single-center cohort, the overall number of patients in distinct subgroups was limited. Serum samples for retesting of virological parameters with improved assays were not always available, and storage conditions and time may have influenced test results. This also disabled us from studying quantitative HDV-RNA levels given that this information was available only for a limited number of cases and because no reliable values could be obtained for the remaining patients. As discussed above, the finding of an improved outcome in IFN α -treated patients may be biased by the fact that IFN α treatment could not be administered to individuals with more-advanced liver disease.⁽¹⁷⁾ Thus, the effect of IFN α -based therapy on clinical long-term outcome needs to be evaluated in prospective studies. A long-term follow-up of the HIDIT-1⁽¹⁶⁾ and HIDIT-2⁽⁴²⁾ trials is ongoing. The findings need also to be confirmed for other patients infected with other HBV and HDV genotypes than HBV genotype D and HDV genotype 1. Moreover, HDV RNA has recently been detected also in salivary glands in HBsAg-negative patients with Sjogren's syndrome.⁽⁴³⁾ To what extent antiviral therapies may have an effect on HDV RNA in extrahepatic tissues required further investigation.

In summary, we show that IFN α -based antiviral therapy of hepatitis delta was independently associated with a lower likelihood of clinical disease progression compared to untreated patients or to those treated with NAs. Moreover, durable undetectability of HDV RNA is likely a valid surrogate endpoint in the

treatment of hepatitis delta, indicating a favorable clinical long-term outcome.

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Supporting Information

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