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BRIEF DEFINITIVE REPORT

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Residual low HDV viraemia is associated HDV RNA relapse after PEG-IFNa-based antiviral treatment of hepatitis delta: Results from the HIDIT-II study

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Abstract

The role of low levels of HDV-RNA during and after interferon therapy of hepatitis D is unknown. We re-analysed HDV RNA in 372 samples collected in the HIDIT-2 trial (Wedemeyer et al, Lancet Infectious Diseases 2019) with the Robogene assay (RA; Jena Analytics). Data were compared with the previously reported in-house assay (IA). We detected HDV-RNA in one-third of samples previously classified as undetectable using the highly sensitive RA. Low HDV viraemia detectable at week 48 or week 96 was associated with a high risk for post-treatment relapse, defined as HDV

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RNA positivity in both assays at week 120. HDV RNA relapses occurred in 10/15 (67%) patients with detectable low HDV RNA at week 48 and in 10/13 (77%) patients with low viraemia samples at week 96. In contrast, the post-treatment relapse rate was lower in patients with undetectable HDV RNA in both assays during treatment.

KEYWORDS
HDV, Hepatitis D, PCR, relapse, residual viraemia

1 | INTRODUCTION

Hepatitis B virus (HBV)/hepatitis D virus (HDV) coinfection represents the most severe form of viral hepatitis frequently leading to progressive liver disease. Treatment of HDV infection with pegylated interferon alfa (PEG-IFNa) is currently considered the standard of care. Previous studies demonstrated off-treatment HDV RNA responses in 25%-30% of cases.[1] In interferon alpha-based therapy of hepatitis C virus (HCV) infection, a response-guided treatment approach based on HCV RNA kinetics during therapy was well-established before direct acting antivirals were introduced.[2] This concept required highly sensitive RNA assays, as the detection of residual viraemia was strongly associated with viral relapse after treatment.[3] To what extend this paradigm may also hold true for PEG-IFNa treatment of HDV is unknown.

Virological responses regarding HDV RNA were previously determined with in-house assays with rather high limits of detection and great variability.[4] Thus, misclassification of low viraemic samples as negative was not infrequent. We here aimed to investigate the frequency and impact of low HDV RNA viraemia detectable only with a highly sensitive HDV RNA assay in patients with HBV/HDV coinfection during and after PEG-IFNa treatment.

2 | PATIENTS AND METHODS

In the HIDIT-II study,[5] 120 patients were treated with PEG-IFNa-2a alone or in combination with tenofovir disoproxil for 96 weeks. HDV RNA had been determined at the central virology laboratory using the Cobas TaqMan system with an in-house assay (Roche Diagnostics, Mannheim, Germany) with stored frozen EDTA samples as described before.[6] This in-house assay (IA) had a lower limit of detection (LOD) of 15 copies/mL, as the study was initiated in 2009 when no WHO HDV RNA standard was available. A conversion factor was later determined: 1 copy/mL in this assay equals to 62 IU/mL, amounting to an LOD 930 IU/mL. Quantitative results here were reported above 18 600 IU/mL (300 copies/mL) only.

Samples for re-testing for all time-points [baseline, week 48, week 96 and follow-up week 24 (week 120)] were available for 93 patients. HDV RNA was extracted by manual extraction using the INSTANT Virus RNA/DNA Kit, (Analytik Jena AG, issue 04/2017) and was re-analysed with the RoboGene HDV RNA Quantification assay (RA) Kit 2.0 (Analytik Jena AG, Rev5_09/2017), performed on the LightCycler 480 II (Roche Diagnostics, Mannheim, Germany). Both kits were used according to the manufacturer's protocol. The lower limit of detection (LOD) of the RoboGene assay was 14 IU/mL; quantitative results were here reported above 100 IU/mL only. Liver biopsies were available in 76 (81.7%) patients at baseline and 65 (69.9%) at end of treatment and evaluated according to the HAI score.[7] Cirrhosis was diagnosed in 35 (37.6%) patients. It was either histologically diagnosed or in cases without liver biopsy it was assumed to be present if at least two of the following criteria were fulfilled: ratio of aspartate aminotransferase vs alanine aminotransferase greater than 1, bilirubin levels of greater than 1.5 times upper the limit of normal, albumin levels <35 g/L, platelet counts of less than 100 000/μL, presence of oesophageal varices.

Statistical analysis was performed using the platform Vassarstats (http://vassarstats.net/) and SPSS software (v21, SPSS Inc, Chicago, IL). Chi-square test, Mann-Whitney *U* and Kruskal-Wallis test were performed to test for significant differences between the groups. *P*-values less than .05 were considered significant.

3 | RESULTS

We tested 372 samples collected during the HIDIT-II study with both HDV RNA assays. Concordant results were observed in 336 samples with 259 (69.6%) samples testing positive with the RA and the IA and 77 (20.7%) samples yielding negative results with both methods. Discordant results between the two assays were evident in 36 (9.6%) samples. Of note, 31% of the samples previously classified as negative with the IA were HDV RNA positive when re-tested with the RA.

HDV RNA was undetectable by both methods in 25 (27%), 29 (31%) and 22 (24%) patients at treatment weeks 48 and 96 and week 120, respectively, as shown in Figure 1A. Low viraemia, defined as HDV RNA detection in the RA with a negative test result in the IA was evident in 15 (16%), 13 (14%) and 7 (8%) patients at these time points as shown in Figure 1B, with HDV RNA levels below 100 IU/mL in most cases. Importantly, low HDV viraemia detectable at week 48 or week 96 was associated with a high risk for post-treatment relapse, defined as positivity in both assays at week 120. HDV RNA relapses occurred in 10/15 (67%) patients with detectable low HDV RNA at week 48 and in 10/13 (77%) patients with low viraemia

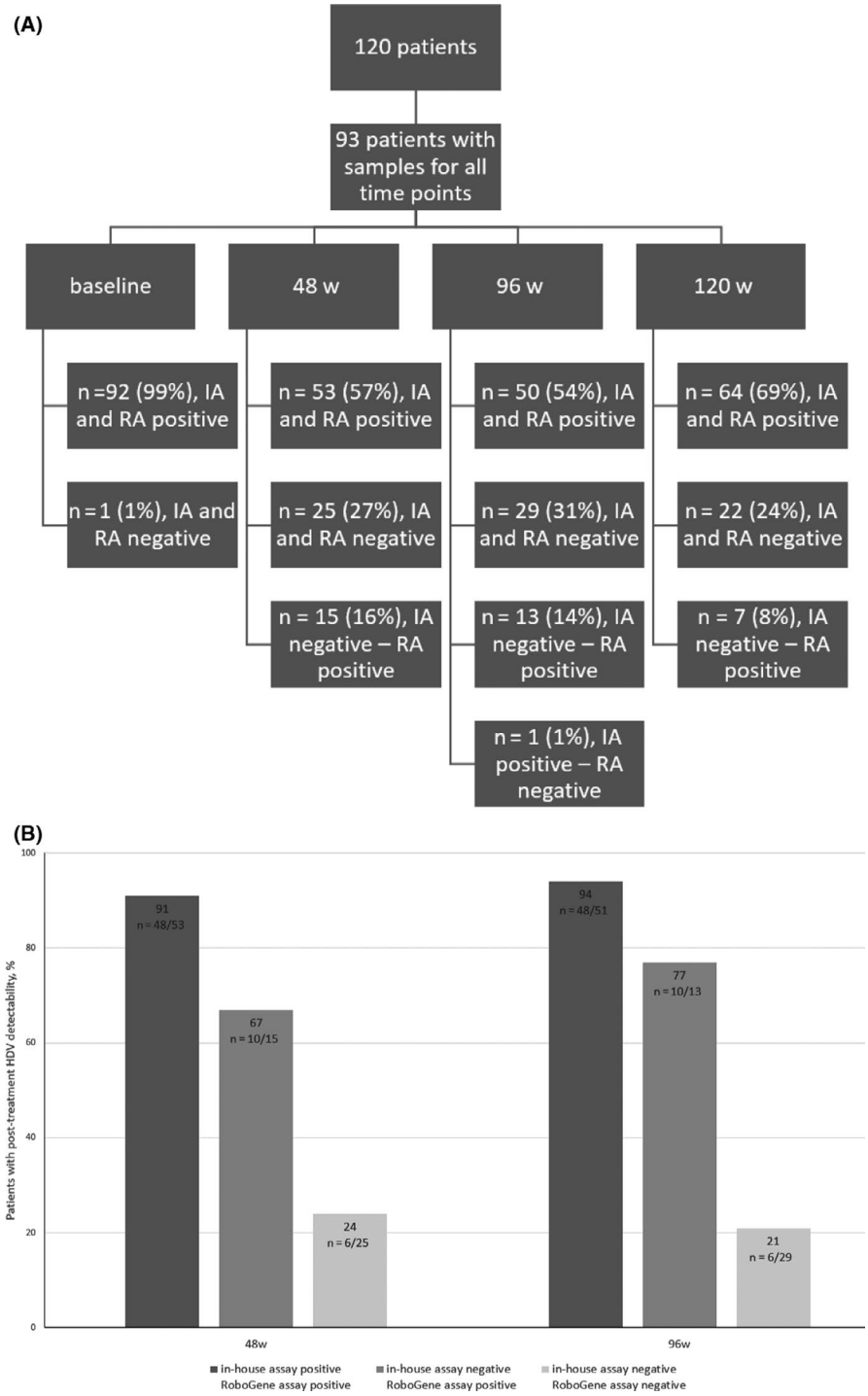

FIGURE 1 HDV RNA status of our patient cohort during and after treatment with PEG-IFNa (A). Percentage of patients with post-treatment HDV detectability stratified according to their HDV RNA status at 48 and 96 weeks of therapy (B). w, week; IA, in-house assay; RA, RoboGene assay

samples at week 96. In contrast, the post-treatment relapse rate was lower in patients with undetectable HDV RNA in both assays during treatment. Only 6 of 25 (24%) patients who had undetectable HDV RNA at week 48 relapsed [$P = .136$, Odds Ratio (OR) 2.78, 95% Confidence Interval (CI) 0.84-9.2] and only 6 of 29 (21%) patients who tested HDV RNA negative in both assays at week 96 ($P = .038$, OR 3.72, 95% CI 1.11-12.41).

Both methods (IA and RA) could predict HDV RNA undetectability after therapy (week 120) reasonably well. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the prediction of HDV undetectability with both assays at week 120 was 95% (75-100), 73 (61-83), 0.5 (0.4-0.7), 1 (0.9-1) and 78% for the IA assay at week 48, 82% (59-94), 90% (80-96), 0.7 (0.5-0.9), 0.9 (0.9-1) and 88% for the RA assay at week 48, 95% (75-100), 70% (58-80), 0.5 (0.3-0.7), 1 (0.9-1), 76% for the IA assay at week 96, 91% (69-98), 86 (75-93), 0.7 (0.5-0.8), 1 (0.9-1), 87% for the RA at week 96.

We next investigated the potential impact of low HDV viraemia on liver disease activity. We here analysed post-treatment samples only as PEG-IFNa treatment per se may alter ALT levels. Of note, low

HDV viraemia at week 120 was not associated in most cases with biochemical hepatitis, as only two in seven (28.6%) patients had elevated ALT levels (71 and 97 IU/mL). In the group with undetectable HDV RNA in both assays the percentage of patients with elevated ALT levels was similar (4/22 patients; 18.2%; $P = .612$, OR 0.56, 95% CI 0.08-3.97). In contrast, ALT was elevated in 75% of cases (48/64 patients) with detectable HDV RNA in both assays. The difference was statistically significant when comparing the group with viraemia detectable with both methods and the group without viraemia ($P < .001$, OR = 13.5, 95% CI 3.98-45.83), or the group with low viraemia ($P = .021$, OR 7.5, 95% CI 1.32-42.51). Any ALT flares resolved spontaneously and required transient dose adjustments of PEG-IFNa only in few cases. Patients with undetectable HDV RNA with the RA assay post-treatment had lower HBV DNA [10 (0-35.75) IU/mL vs 30 (20-325) IU/mL, $P = .008$] compared to patients with detectable HDV RNA.

HDV RNA was undetectable in 13 (37.1%) cirrhotic patients at week 48, in 16 (45.7%) at week 96 and in 13 (37.1%) at week 120. The HDV viral load at baseline, at week 48 and 96 did not differ significantly between patients with and without cirrhosis at baseline. Cirrhosis was identified as a risk factor for HDV viraemia detectable with both methods after end of treatment, at week 120, with cirrhotics being at a higher risk compared to non-cirrhotics ($P = .012$, OR = 3.69, 95% CI 1.35-10.13). No such effect was seen for week 48 or 96. HAI score, including the score for necroinflammatory activity and fibrosis at baseline and end of treatment, were not significantly different amongst viraemic, low viraemic or non-viraemic patients.

4 | DISCUSSION

Re-testing of HIDIT-II samples with a very sensitive commercial HDV RNA assay identified that one-third of samples previously classified as undetectable were actually HDV RNA positive. Low level HDV viraemia could be detected frequently in our cohort during and after treatment, making its potential clinical significance all the more important. Indeed, we found that detection of low HDV viraemia during treatment with PEG-IFNa was associated with a high risk for post-treatment virological relapse. The phenomenon of low viraemia in conjunction with virological failure has not been evaluated before in a setting of HBV/HDV coinfection. However, it has been investigated in other viral infections. Persistent low level HIV viraemia while on antiretroviral therapy has been shown to be an independent risk factor for virological failure.[8] In addition, the presence of minimal residual viraemia in HCV infected patients treated with PEG-IFNa was associated with an increased risk for relapse.[3,9] Our findings confirm this association for the first time for interferon-based treatment of HDV infection as the risk of post-treatment relapse increased by about three-fold if HDV RNA was detectable in the highly sensitive assay. Thus, these results are of major clinical relevance.

Importantly, low HDV viraemia post-treatment at week 120 was not associated with biochemical hepatitis in most cases. The percentage of patients with elevated ALT was similar in patients with low viraemia and patients without viraemia. That being said, the long-term HDV kinetics in low level viraemia patients are unknown and so are the potential consequences of a persistent low level HDV viraemia. In this context, it has to be noted that HDV viraemia may fluctuate overtime.[10] Moreover, a distinct viral load cut-off being associated with a higher risk of liver disease progression has yet to be determined. A study from Milan suggested that the risk to develop cirrhosis was higher in patients with an HDV viral load above 600 000 copies/mL[11] while other studies could not find associations between HDV viral load and the level of liver fibrosis.[12]

Our study highlights that reliable measurement of HDV viral load is crucial for an optimal patient management. Previous experiments have indicated that the diagnostic accuracy of in-house but also commercial HDV viral load quantification assays varied considerably, leading sometimes to failure to detect HDV RNA in HDV RNA positive samples.[4] Several factors have been known to influence HDV viral load quantification including the RNA extraction method[13] and the PCR assay used.[4] We here show the importance of using a very sensitive assay for an accurate evaluation of treatment response.

A limitation of our study is the absence of long-term follow-up and the retrospective nature re-testing stored samples. Nevertheless, it is the first study to describe the phenomenon of low viraemia in HDV infected patients during and after interferon-based treatment and possibly, to determine the value of response-guided therapy in HDV infection. However, prolonged therapy beyond week 48 did not seem to reduce the frequency of low viraemia as a similar percentage of patients had still detectable HDV RNA at week 48 and 96. Our results are of clinical significance, since low viraemia during treatment is associated with a higher risk for relapse. Future trials should use sensitive assays to determine treatment response. The clinical meaning of long-term off-treatment low viraemia requires further investigation.

CONFLICTS OF INTEREST

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REFERENCES

1. Heidrich B, Manns MP, Wedemeyer H. Treatment options for hepatitis delta virus infection. *Curr Infect Dis Rep*. 2013;15(1):31-38.
2. European Association for Study of Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol*. 2014;60(2):392-420.
3. Wiegand J, Neumann K, Bohm S, et al. Importance of minimal residual viremia for relapse prediction in patients with chronic hepatitis C genotype 1 infection. *Clin Infect Dis*. 2011;53(11):1111-1114.
4. Le Gal F, Brichler S, Sahli R, Chevret S, Gordien E. First international external quality assessment for hepatitis delta virus RNA quantification in plasma. *Hepatology*. 2016;64(5):1483-1494.
5. Wedemeyer H, Yurdaydin C, Hardtke S, et al. Peginterferon alfa-2a plus tenofovir disoproxil fumarate for hepatitis D (HIDIT-II): a randomised, placebo controlled, phase 2 trial. *Lancet Infect Dis*. 2019;19(3):275-286.
6. Mederacke I, Bremer B, Heidrich B, et al. Establishment of a novel quantitative hepatitis D virus (HDV) RNA assay using the Cobas TaqMan platform to study HDV RNA kinetics. *J Clin Microbiol*. 2010;48(6):2022.
7. Brunt EM. Grading and staging the histopathological lesions of chronic hepatitis: the Knodell histology activity index and beyond. *Hepatology*. 2000;31(1):241-246.
8. Joya C, Won SH, Schofield C, et al. Persistent low-level viremia while on antiretroviral therapy is an independent risk factor for virologic failure. *Clin Infect Dis*. 2019;69(12):2145-2152.
9. Maasoumy B, Cobb B, Bremer B, et al. Detection of low HCV viraemia by repeated HCV RNA testing predicts treatment failure to triple therapy with telaprevir. *Aliment Pharmacol Ther*. 2014;39(1):85-92.
10. Schaper M, Rodriguez-Frias F, Jardi R, et al. Quantitative longitudinal evaluations of hepatitis delta virus RNA and hepatitis B virus DNA shows a dynamic, complex replicative profile in chronic hepatitis B and D. *J Hepatol*. 2010;52(5):658-664.
11. Romeo R, Foglieni B, Casazza G, Spreafico M, Colombo M, Prati D. High serum levels of HDV RNA are predictors of cirrhosis and liver cancer in patients with chronic hepatitis delta. *PLoS One*. 2014;9(3):e92062.
12. Zachou K, Yurdaydin C, Drebber U, et al. Quantitative HBsAg and HDV-RNA levels in chronic delta hepatitis. *Liver Int*. 2010;30(3):430-437.
13. Bremer B, Anastasiou OE, Ciesek S, Wedemeyer H. Automated nucleic acid isolation methods for HDV viral load quantification can lead to viral load underestimation. *Antivir Ther*. 2019;24(2):117-123.

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