INTRODUCTION

Hepatitis delta virus (HDV) is the smallest virion known to be able to infect humans. Its negative-sense, single-stranded, circular RNA consists of only 1700 base pairs, and the HDV genome encodes for one single antigen, called the hepatitis delta antigen (HDAg). HDAg exists in two different variations, the large (L-HDAg) and the small (S-HDAg) version which only differ by 19 additional amino acids at the c-terminal region of the protein. However, neither the S-HDAg nor the L-HDAg provide a coating for the virus. Therefore, HDV relies on glycoproteins from other viruses for propagation. Until now, it was believed that the only virus that is able to sufficiently envelop HDV is hepatitis B virus (HBV). However, recent studies have shown that hepatitis C virus (HCV) and dengue virus (DENV) can also envelope HDV in vitro cell culture experiments and animal models. However, the clinical relevance of these findings and whether HDV replication occurs in real-world hepatitis B surface antigen (HBsAg)-negative HCV patient cohorts remain unknown. To this aim, we analysed 323 HCV-RNA-positive and HBsAg-negative sera for the presence of HDV-RNA and anti-HDV antibodies (anti-HDV). All 323 (100%) samples were negative for HDV-RNA. Interestingly, 8/316 samples tested positive for anti-HDV. The HBV serology of these eight patients showed a positive result for HBV core antibodies (anti-HBc) indicating a seroconversion of an acute HBV infection in the past. None of the anti-HBc-negative patients were positive for anti-HDV. Our results indicate a distinctly low probability of replicative HDV infection in HCV mono-infected patients in Germany. Current German clinical guidelines rightly recommend performing HDV screening only in HBsAg-positive patients. However, larger studies on this subject should be performed in regions that are endemic for chronic HBV/HDV as well as HCV infections.

KEYWORDS
hepatitis B virus, hepatitis C virus, hepatitis delta virus, viral hepatitis

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Abstract
In vitro cell culture experiments and animal models have demonstrated that hepatitis delta virus (HDV) can theoretically propagate being enveloped by human pathogenic viruses other than hepatitis B virus (HBV), namely hepatitis C virus (HCV) and dengue virus. However, the clinical relevance of these findings and whether HDV replication occurs in real-world hepatitis B surface antigen (HBsAg)-negative HCV patient cohorts remain unknown. To this aim, we analysed 323 HCV-RNA-positive and HBsAg-negative sera for the presence of HDV-RNA and anti-HDV antibodies (anti-HDV). All 323 (100%) samples were negative for HDV-RNA. Interestingly, 8/316 samples tested positive for anti-HDV. The HBV serology of these eight patients showed a positive result for HBV core antibodies (anti-HBc) indicating a seroconversion of an acute HBV infection in the past. None of the anti-HBc-negative patients were positive for anti-HDV. Our results indicate a distinctly low probability of replicative HDV infection in HCV mono-infected patients in Germany. Current German clinical guidelines rightly recommend performing HDV screening only in HBsAg-positive patients. However, larger studies on this subject should be performed in regions that are endemic for chronic HBV/HDV as well as HCV infections.

1SHORT COMMUNICATION

Hepatitis delta virus propagation enabled by hepatitis C virus—Scientifically intriguing, but is it relevant to clinical practice?

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Abbreviations: ALT, alanine transaminase; anti-HBc, anti-hepatitis B core antigen antibodies; anti-HBs, anti-hepatitis B surface antigen antibodies; anti-HDV, anti-hepatitis delta virus antibodies; AST, aspartate transaminase; DENV, dengue virus; GT, genotype; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDAg, hepatitis delta antigen; HDV, hepatitis delta virus; L-HDAg, large hepatitis delta antigen; LOD, lower limit of detection; SDeV, snake delta virus; S-HDAg, small hepatitis delta antigen; ULN, upper limit of normal; γGT, gamma-glutamyltransferase.
HDV is the hepatitis B virus (HBV). Accordingly, HDV has been characterized as an obligate satellite virus of HBV. However, in a recent experimental study, Perez-Vargas et al. demonstrated for the first time that HDV can be propagated and passed in vitro using glycoproteins of other ‘helper’ viruses including hepatitis C virus (HCV) and dengue virus (DENV). Furthermore, both viruses, HCV and DENV, were able to promote the egress of infectious HDV particles in a similar range and pattern as HBV. Perez-Vargas et al. then went on to show that HCV superinfection of HDV mono-infected hepatocytes can further rescue an ongoing HDV infection, leading to reoccurrence of HDV in the supernatant of these cells. Using human liver chimeric mice, we previously demonstrated that HDV replication can persist in the absence of HBV and even be amplified by cell division. In a comparable mouse system, Perez-Vargas et al. could now demonstrate that HCV could also support HDV infection and spreading in vivo.

From a clinical perspective, these findings may have far-reaching implications, including the potential risk of HDV infection in HBV-negative patients as discussed by Maya and Ploss. Furthermore, these results raise the question of the possibility of HDV being able to infect a broader range of organs or tissues since the glycoproteins on the surface of a virus would determine its tropism. In this context, it is interesting to consider that in snakes, a delta-like virus (SDeV) was recently discovered. Similar to HDV, diverse viral envelope proteins support packaging of and infection by SDeV. Moreover, not only the liver but also the kidney and the brain were affected by SDeV infection, supporting in vivo spreading of SDeV mediated by a non-liver selective ‘helper virus’. Again, this fact is raising the question whether active HDV infection is restricted to infections of the liver.

Still, it is important to keep in mind that while the above-mentioned observations of HDV spreading mediated by HCV were made in vivo in a mouse model in the absence of an adaptive host immune system. An environment far less hostile for any virus infection than the human host. Similar results in a clinical settings are rather improbable. Moreover, in HCV/HDV-infected mice HDV titres were overall lower and declined further at later time points calling in question the longevity of HCV/HDV co-replication and the ability to transmit HDV. In real life, the high titres of hepatitis B surface antigen (HBsAg) during chronic infection make HBV the ideal ‘partner in crime’, and it seems highly unlikely that occult HDV replication has been missed in the absence of HBsAg all these years.

### METHODS

However, in order to rule out present or past HDV replication in HBsAg-negative patients, we analysed HDV-RNA and anti-HDV in clinical specimens of a German patient cohort with active HCV mono-infection. Patients were diagnosed at our centre between 2010 and 2019 and were all HCV-RNA positive. The study was approved by the local ethics committee (WF-036/20). Quantitative HCV testing was performed using the cobas6800 or cobas CAP/CTM (both Roche Diagnostics) with a lower limit of detection (LOD) of 8.46 IU/mL and 15 IU/mL, respectively. HBsAg, anti-hepatitis B surface antigen (anti-HBs) and anti-hepatitis B core antigen (anti-HBc) IgG and

| TABLE 1 | Clinical and virological patient characteristics |
|----------|-----------------------------------------------|
| Age—years | 323  | 52  | 6-92  |
| Sex       |      |     |       |
| F         | 125  | 38.7|       |
| M         | 196  | 60.7|       |
| HCV load—IU/mL | 323  | 301 000 | <15-60 000 000 |
| HCV genotype |      |     |       |
| 1         | 12   | 3.7 |       |
| 1a        | 67   | 20.7|       |
| 1b        | 73   | 22.6|       |
| 2         | 24   | 7.4 |       |
| 3         | 85   | 26.3|       |
| 4         | 18   | 5.6 |       |
| 5         | 2    | 0.6 |       |
| 6         | 2    | 0.6 |       |
| N/A       | 40   | 12.4|       |
| Time since diag.—years | 284  | 5.9  | 0.3-40 |
| AST—ULN   | 238  | 1.2  | 0.1-45.5 |
| ALT—ULN   | 239  | 1.6  | 0.4-37.6 |

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; diag., diagnosis; F, female; HCV, hepatitis C virus; M, male; N/A, no data available; ULN, upper limit of normal.
IgM were determined using the Alinity I system (Abbott) or Centaur XL (Siemens Healthcare).

### RESULTS AND DISCUSSION

In total, 323 patients were analysed. All patients had an ongoing chronic HCV infection with a median viral load of 301,000 IU/mL (range < 15-60,000,000 IU/mL). The median age was 52 years (range 6-92 years) and the predominant HCV genotype (GT) was GT 1 (152/323) followed by GT 3 (85/323). The median time since diagnosis was 5.9 years (range 0.3-40 years). In most patients, the level of transaminases was increased with aspartate transaminase at a median level of 1.2 upper limit of normal (ULN) (range 0.1-45.5 ULN) and alanine transaminase of 1.6 ULN (range 0.4-37.6 ULN). For a summary of the clinical and virological patient characteristics, see Table 1.

All patients were HBsAg negative but in 88/323 (27.24%) of patients, anti-HBc was positive indicating a past but currently immunologically controlled HBV infection.\(^6\) All of the 323 HCV mono-infected patients analysed were HDV-RNA negative in a broad reactive quantitative HDV polymerase chain reaction with

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**FIGURE 1** Results of quantitative HCV and HDV testing (cobas 6800 or cobas CAP/CTM, both Roche) and anti-HDV, anti-HBc and anti-HBs test results (Alinity I system, Abbott or Centaur XL, Siemens Healthcare) displayed in a flowchart. Abbreviations: anti-HBc, anti-hepatitis B core antigen antibodies; anti-HBs, anti-hepatitis B surface antigen antibodies; anti-HDV, anti-hepatitis delta virus antibodies; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; N/A, no data available; PCR, polymerase chain reaction.
a LOD of <10 IU/mL. The HDV assay was automated on the open channel of the cobas6800 system, and the primer and probes were based on the publication of Coller et al. Interestingly, 8/316 (2.53%) patients were anti-HDV positive tested with the Liaison XL (DiaSorin, Saluggia, Italia; n = 9/316 sera were diluted 1:2-1:10 with dilution buffer [Abbott] for anti-HDV detection). Comparing the anti-HDV concentration of these eight patients to 38 HBV/HDV co-/superinfected patients diagnosed at our centre in the last 12 months revealed a median concentration with 3.25 AU/mL (range: 1.12-8.64 AU/mL) and 8.53 AU/mL (range: 1.22-9.82 AU/mL), respectively. All of the eight HCV patients with positive anti-HDV were also tested positive for anti-HBc (median: 6.88 S/CO; range: 3.85-7.88 S/CO). Next, testing for anti-HBs antibodies demonstrated that 4/8 (50%) were anti-HBs positive (median: 19.5 mIU/mL; range: 12.31-80.98 mIU/mL) whereas 4/8 (50%) of the eight anti-HDV-positive patients tested negative for anti-HBs (as defined by titres below 10 mIU/mL). Furthermore, clinical and epidemiological information concerning the eight anti-HDV-positive patients showed that 6/8 (75%) were Caucasian, 2/8 (25%) non-Caucasian and 2/8 (25%) had a history of intravenous drug abuse. For 2/8 patients, laboratory information on aspartate transaminase (AST), alanine transaminase (ALT) and gamma-glutamyltransferase (γGT) were missing. The remaining six patients demonstrated elevated levels of AST and ALT with a median of 52 U/L and 50.5 U/L, respectively. The γGT levels were increased in 3/6 patients (50%; median: 293 U/L). In contrast, there was not a single patient in whom HDV antibodies were detected and in whom anti-HBc was negative (n = 225; Fisher’s exact test P < .0001, see Figure 1). Interestingly, at the time of revising this manuscript a new study did show for the first time HDV/HCV coinfection in 2/160 HCV-RNA–positive patients from Venezuela. To summarize our findings, we cannot exclude that patients had transient production with recombinant HCV/HDV particles, but while we are awaiting additional studies in endemic regions for HCV and HDV like Turkey or Mongolia, we conclude that the risk of HDV infection in HCV mono-infected patients in Germany is distinctly remote. These results confirm the current clinical guidelines to perform HDV testing only in HBsAg or HBV-DNA-positive patients.

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CONFLICT OF INTEREST

The authors declare they have no competing financial interest.

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