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CASE REPORT

# Hepatitis Delta recurrence post-liver transplantation in absence of detectable hepatitis B surface antigen and hepatitis B virus DNA in peripheral blood

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**KEYWORDS**
Hepatitis Delta;
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Therapy

Hepatitis Delta in humans is caused by the Delta agent (HDV), a virusoid classified in the *Incertae sedis* genus Deltavirus of the recently delineated viral realm *Riboviria* (https://talk.ictvonline.org/taxonomy/) [1,2]. This infectious agent is a subviral satellite comprised by a short (≈1,700-nucleotide large) circular RNA with a high degree of self-complementarity and a single gene that can only spread between hepatocytes by borrowing hepatitis B virus (HBV) envelope protein. Therefore, HDV propagation in the liver is dependent on the concurrent presence of hepatitis B virus and the production of hepatitis B surface antigen (HBsAg) in hepatocytes. The prevalence of HDV infection has been deemed to be approximately 5% in patients chronically-infected with HBV, but a recent met-analysis described that this rate may be 14.6%, and 10.6% in populations without risk factors of intravenous drug use or high-risk sexual behaviour [2,3]. Such co-infection is of clinical concern as Delta agent clearance is most often unreached, with only 25–30% of success by 24 weeks of pegylated interferon treatment [4], the only efficient and recommended drug to date. Moreover, HDV increases chronic hepatitis progres-

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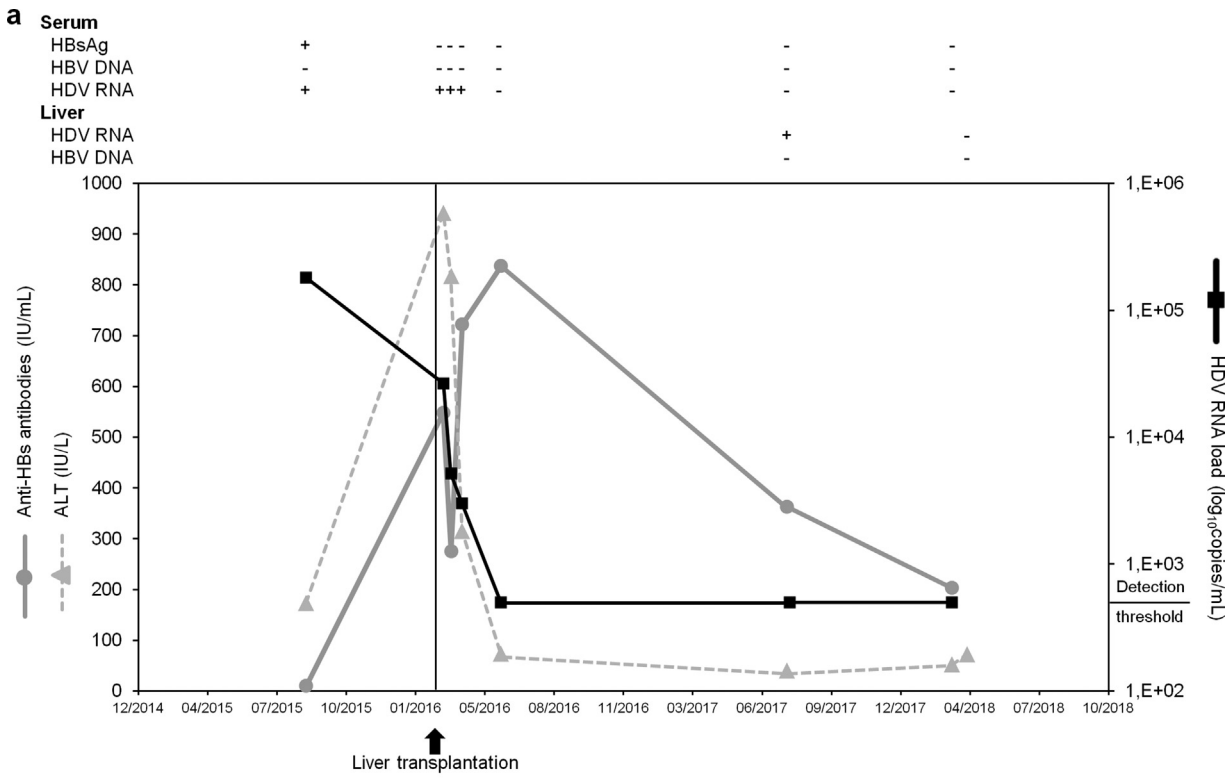


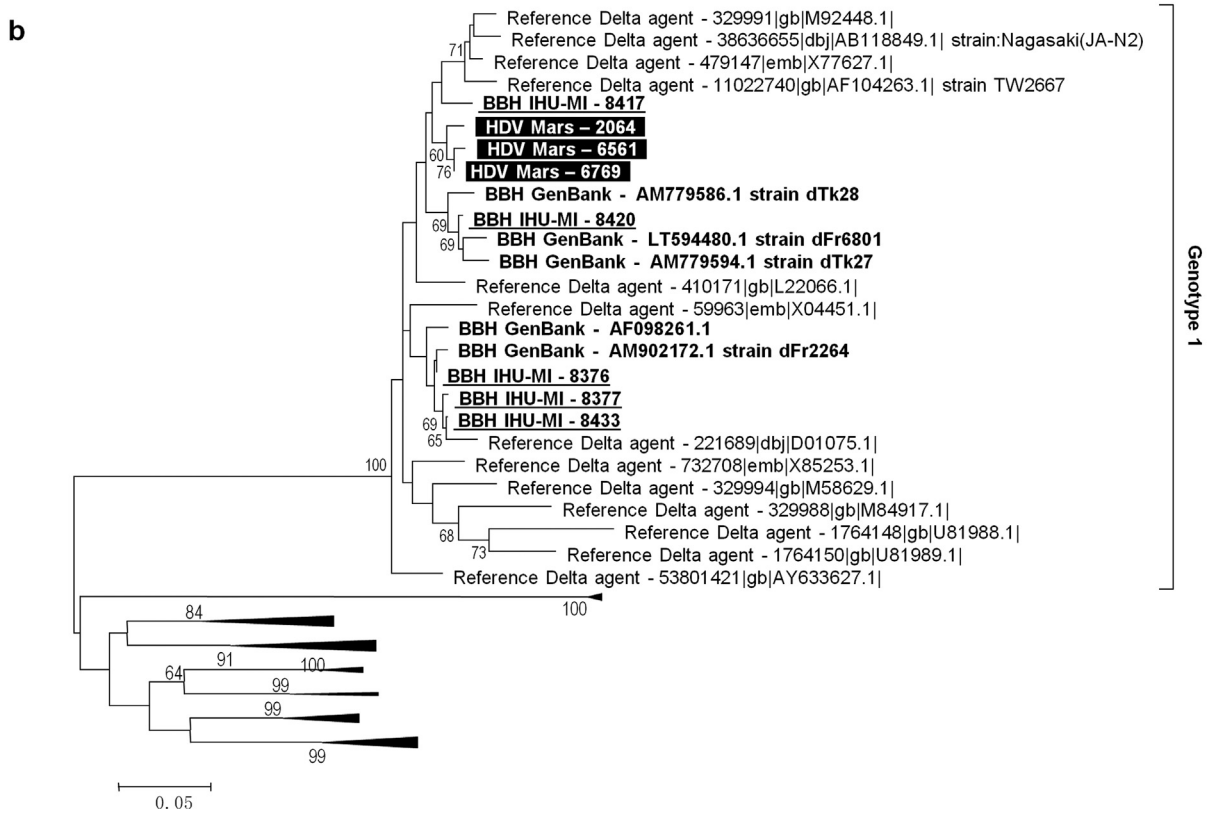


**Figure 1** Evolution of virological and biochemical markers and hepatitis Delta virus phylogenetic tree based on Delta antigen encoding gene. a. Evolution of virological and biochemical markers. ALT: alanine aminotransferase level; HBs: hepatitis B surface; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HDV: Delta agent. b. Hepatitis Delta virus phylogenetic tree based on Delta antigen encoding gene. The fragment of the gene encoding the hepatitis Delta virus (HDV) antigen was 232-nucleotide long and corresponded to nucleotides 906-1137 of the HDV genome GenBank accession no. AF104263.1. HDV sequences obtained by Sanger population from the index case are indicated by a white bold font and a black background. The five sequences with the highest BLAST

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sion towards liver cirrhosis and cancer as compared to HBV mono-infection [5]. The HDV dependence on HBsAg means that HDV RNA is not detected in serum in case of concurrent HBsAg and HBV DNA negativity [6]. In this view, liver graft is a particular setting for HDV-co-infected chronic hepatitis B patients as liver transplant recipients receive both a HBV polymerase inhibiting drug and anti-HBs immunoglobulins, which prevents HBV recurrence and is associated with HBsAg negativity [7]. We describe here a case of protracted HDV RNA positivity despite HBsAg and HBV DNA negativity following liver transplantation.

The patient was a 32-year-old cirrhotic man chronically-infected with HBV and HDV who underwent liver transplantation in February 2016. He had been infected with HBV through materno-foetal transmission; transmission route of HDV was not documented and HDV infection had been diagnosed in 2012. He was diagnosed with cirrhosis in 2012 primarily based on ruptured oesophageal varices. Since then, he received tenofovir disoproxil fumarate (245 mg/d), but he was never treated for his hepatitis Delta. Before graft, HBV DNA load was below the detection threshold of 10 IU/mL (RealTime assay; AbbottDiagnostics, Mannheim, Germany) and HDV RNA load was 5.3 $\log_{10}$copies/mL as determined using an in-house qPCR assay targeting the delta antigen coding-gene (forward-primer: 5′-ACCTCCAGAGGACCCCTTC (nucleotides 300-318 in reference to genome GenBank Accession no. AF104263.1); reverse-primer: 5′-CTAGCCCCGTTGCTYTCTTT (411-430); probe: FAM-CCATAGCGATRGGAGGAGATGCTAGGAG-TAMRA (354-381)); estimated detection limit, 500 copies/mL; linearity range up to 7.0 $\log_{10}$copies/mL. HBsAg was strongly positive (serum-to-threshold ratio, 4,883; Architect assay, Abbott Diagnostics, Wiesbaden, Germany) and alanine aminotransferase level was 173 IU/L (Fig. 1a). After orthotopic liver transplantation, the patient received ciclosporine, mycophenolate mofetil, as well as tenofovir disoproxil fumarate and human anti-HBs immunoglobulins (10,000 IU every three weeks). Temporary postoperative ascitic decompensation occurred 11 days post-graft. Then, acute cytolysis was observed 29 days post-graft with an alanine aminotransferase level that raised up to 950 IU/L. Liver biopsy showed evidence of acute rejection (Banff score: 4) and mild lobular hepatitis was also noted. Outcome was favorable within days after an increase of the immunosuppressive therapy. Neither HBsAg nor HBV DNA were detected in the patient's serum and the anti-HBs antibody titer was 548 IU/L. However, HDV RNA load was 4.4 $\log_{10}$ in serum and it remained detectable until at least 8 weeks post-graft. The presence of HBV DNA in the liver at that time point cannot be excluded, but a liver biopsy sample was not available for virological testing. HDV sequence retrieved from the serum using an in-house Sanger population sequencing procedure (first round: forward-primer: 5′-TCGGATGCCCAGGTCGGA (849-866), reverse-primer: 5′-ATGAGCCRTYCCGAKTCGA (1577-1595); second round: forward-primer: 5′-ATGCCGACCCGAAGAGGAA (888-906), reverse-primer: 5′-AGGAYGAMAATCCCTGGC (1445-1462)) was of genotype 1 (Fig. 1b). Sequences recovered pre- and post-transplantation were > 99% identical and were clustered together in the phylogenetic analysis apart from their best match in the NCBI GenBank and our local nucleotide sequence databases, which strongly suggested HDV recurrence. HDV RNA was eventually negative in serum 16 weeks post-graft. Nevertheless, HDV RNA was detected in a liver biopsy specimen collected 16 months after transplantation, whereas it was no longer detected in peripheral blood. HDV Ag was not tested in the available liver biopsy samples as this marker is not assessed in our laboratory. No HDV RNA quantification was performed at that time but cycle threshold (Ct) for detection was 25. HDV RNA detection was confirmed on the liver biopsy specimen following another nucleic acid extraction. In contrast, HBV DNA was not detected at this time point neither in the serum (using the DxN VERIS assay, Beckman Coulter, Brea, CA, USA), nor in the liver biopsy sample as assessed by conventional PCR [8].

Cases of seemingly ''isolated'' post-liver transplant HDV recurrence have been described before the advent of PCR assays that dramatically increased the sensitivity of virus detection [9—11]. However, it turned out thereafter that despite the serum samples of these patients tested negative for HBsAg, they tested positive for HBV DNA by molecular biology assays and HDV particles had an HBsAg-comprised envelope. Mederacke et al. reported a series of 26 patients co-infected with HBV and HDV who underwent liver transplantation [12]. They observed that HDV RNA became undetectable in serum in all cases after 1-10 days in parallel with HBsAg decay. Nonetheless, they also described HDV antigen positive detection in the liver of 6 patients up to 19 months post-graft in absence of HBsAg in serum and of HBV DNA in the liver. It is worthy to note that in this study none of the patients exhibited HDV RNA detection in serum in the absence of HBV markers, which contrasts with the present case. Here, a transient and residual HBsAg synthesis, even if undetected in the serum, may have been sufficient to assemble HDV viral particles and allow their release into the peripheral blood. Alternatively, the detected HDV RNA may have originated from non-enveloped HDV ribonucleoproteins

scores recovered from the NBCI GenBank nucleotide sequence database (http://www.ncbi.nlm.nih.gov/nucleotide/), indicated by BBH for best blast hit and a black bold font, or from the IHU Méditerranée Infection sequence database, indicated by BBH IHU-MI and a black bold and underlined font, were incorporated in the phylogeny reconstruction, in addition to reference sequences for HDV genotypes. Nucleotide alignments were performed using the MUSCLE software (http://www.ebi.ac.uk/Tools/msa/muscle/). The evolutionary history was inferred in the MEGA6 software (http://www.megasoftware.net/) using the Neighbor-Joining method and the Kimura 2-parameter method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree; the scale bars indicate the number of nucleotide substitutions per site. Bootstrap values > 50% are labeled on the tree.

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released during hepatocyte lysis. It could not be determined if there were HDV particles or only non-enveloped HDV RNA in the peripheral blood, but plasma viral titers rather suggest the first hypothesis. *In vitro* studies have shown the ability of the HDV genome to replicate in hepatocytes in the absence of HBV [2]. This may release HDV RNA into the bloodstream until HDV is cleared because it is unable to infect new hepatocytes in the absence of HBsAg [13]. Thus, Samuel et al. studied 61 liver-transplant recipients who received continuous anti-HBs passive immunoprophylaxis and were not reinfected with HBV, and they detected HDV RNA in the serum or HDV antigen in the liver from 88% of these patients during the year following liver graft and in no more than 5% therafter [14]. More recently, Giersch et al. demonstrated that the HDV genome persists during cell division *in vivo*, and that HDV transmission between dividing cells can be HBsAg-independent and can maintain and increase intrahepatic HDV infection [15]. Post-transplantation, there is a period of considerable cell division, and this phase could be at the origin of such HDV propagation. In addition, mono-infection of human liver progenitor HepaRG cells with HDV was associated with a strong HDV replication [16]. Overall, the present case highlights that in the tranplant setting, HDV viremia can be deconnected from the presence of HBV DNA and HBsAg in peripheral blood. This needs to be considered for the interpretation of virological tests and questions on the clinical impact of such HDV 'only' recurrence.

## Ethical statement

All data have been generated as part of the routine work at Assistance Publique—Hôpitaux de Marseille (Marseille university hospitals), and this study results from routine standard clinical management. This study has been approved by our institution's ethics committee (N°2019-001).

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## Author contributions

Conceived and designed the experiments: PC, PP. Contributed materials/analysis tools: all authors. Analyzed the data: PC, PP, SA. Wrote the paper: PP, PC.

## Disclosure of interest

The authors declare that they have no competing interest.

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## References

[1] Rizzetto M, Alavian SM. Hepatitis delta: the rediscovery. Clin Liver Dis 2013;17:475—87.
[2] Lempp FA, Ni Y, Urban S. Hepatitis delta virus: insights into a peculiar pathogen and novel treatment options. Nat Rev Gastroenterol Hepatol 2016;13:580—9.
[3] Chen HY, Shen DT, Ji DZ, Han PC, Zhang WM, Ma JF, et al. Prevalence And burden of hepatitis D virus infection in the global population: a systematic review and meta-analysis. Gut 2018 [Gutjnl-316601].
[4] Heidrich B, Yurdaydin C, Kabacam G, Ratsch BA, Zachou K, Bremer B, et al. Late HDV RNA Relapse after peginterferon alpha-based therapy of chronic hepatitis delta. Hepatology 2014;60:87—97.
[5] Alvarado-Mora MV, Locarnini S, Rizzetto M, Pinho Jr. An update on HDV: Virology, pathogenesis and treatment. Antivir Ther 2013;18:541—8.
[6] Noureddin M, Gish R. Hepatitis delta: epidemiology, diagnosis and management 36 years after discovery. Curr Gastroenterol Rep 2014;16:365.
[7] European Association for the Study of the Liver. Easl Clinical Practice Guidelines: Management Of Chronic Hepatitis, B., Virus, Infection. J Hepatol 2012;57:167—85.
[8] Petit PR, Borentain P, Aherfi S, Benezech A, Botta-Fridlund D, Tamalet C, et al. Nucleotide And amino acid diversity of hepatitis b virus surface antigen in acute infections, southeastern France. Clin Res Hepatol Gastroenterol 2018;42:E32—4.
[9] Ottobrelli A, Marzano A, Smedile A, Recchia S, Salizzoni M, Cornu C, et al. Patterns of hepatitis delta virus reinfection and disease in liver transplantation. Gastroenterology 1991;101:1649—55.
[10] Smedile A, Rizzetto M, Gerin JL. Advances in hepatitis D virus biology and disease. Prog Liver Dis 1994;12:157—75.
[11] Smedile A, Casey JL, Cote PJ, Durazzo M, Lavezzo B, Purcell RH, et al. Hepatitis D viremia following orthotopic liver transplantation involves a typical HDV virion with a hepatitis b surface antigen envelope. Hepatology 1998;27:1723—9.
[12] Mederacke I, Filmann N, Yurdaydin C, Bremer B, Puls F, Zacher BJ, et al. Rapid early HDV RNA decline in the peripheral blood but prolonged intrahepatic hepatitis delta antigen persistence after liver transplantation. J Hepatol 2012;56:115—22.
[13] Roche B, Samuel D. Liver transplantation In delta virus infection. Semin Liver Dis 2012;32:245—55.
[14] Samuel D, Zignego AL, Reynes M, Feray C, Arulnaden JL, David MF, et al. Long-term clinical and virological outcome after liver transplantation for cirrhosis caused by chronic delta hepatitis. Hepatology 1995;21:333—9.
[15] Giersch K, Allweiss L, Volz T, Helbig M, Bierwolf J, Lohse AW, et al. Hepatitis delta co-infection in humanized mice leads to pronounced induction of innate immune responses in comparison to HBV mono-infection. J Hepatol 2015;63:346—53.
[16] Alfaiate D, Lucifora J, Abeywickrama-Samarakoon N, Michelet M, Testoni B, Cortay JC, et al. HDV RNA Replication is associated with HBV repression and interferon-stimulated genes induction in super-infected hepatocytes. Antiviral Res 2016;136:19—31.