

# Implementation of anti-HDV reflex testing among HBsAg-positive individuals increases testing for hepatitis D

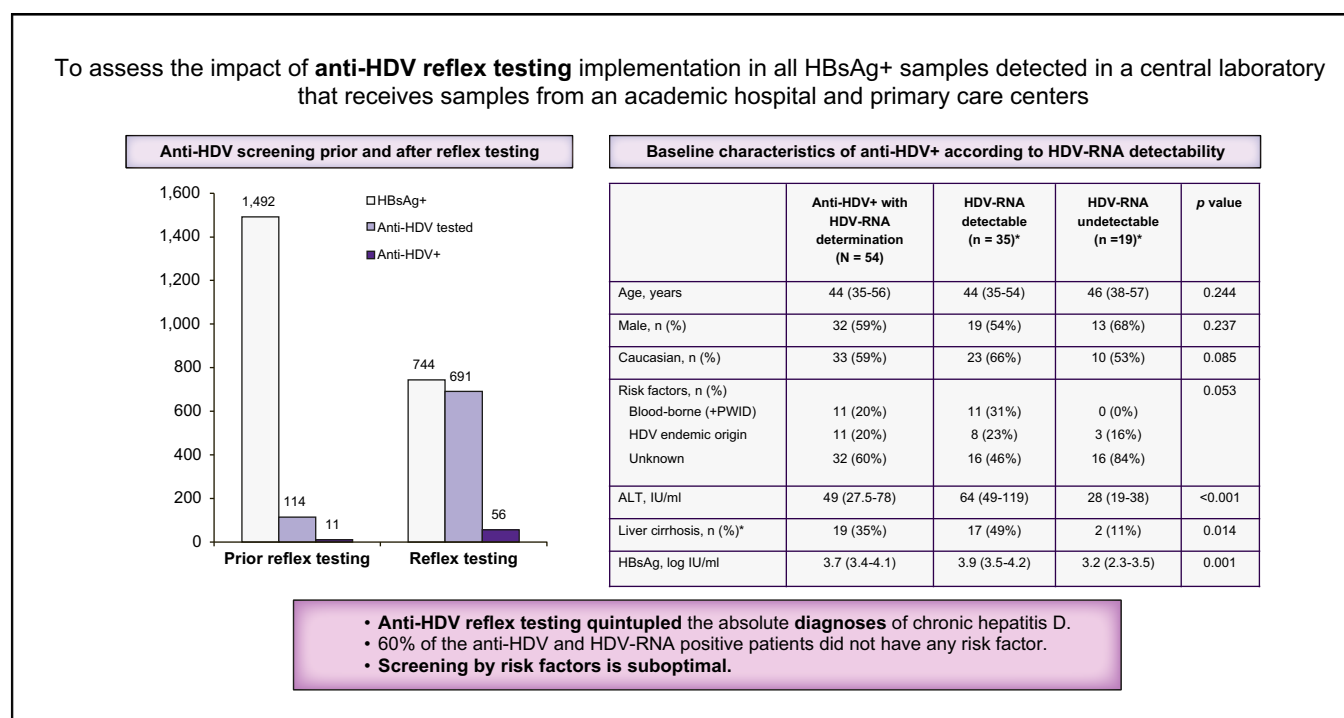
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## Graphical abstract



## Highlights

- HDV testing rates in daily clinical practice are low despite EASL guidelines recommending universal screening.
- Implementation of HDV reflex testing led to a 5-fold increase in the number of HBV cases diagnosed with hepatitis D.
- Risk factors were unknown in 60% of anti-HDV positive cases, supporting systematic anti-HDV reflex testing in all HBsAg-positive patients.
- It would be of great value to assess the cost-effectiveness of anti-HDV reflex testing.

## Lay summary

Chronic hepatitis delta (CHD) is a viral disease caused by HDV, which requires the presence of HBV to propagate. HDV infection can cause rapid progression to cirrhosis, among other severe complications. The prevalence of CHD worldwide is controversial, and the infection often goes unrecognised, mainly because of unawareness among physicians. Use of reflex testing in other viral hepatitis has proven to increase detection and linking-to-care of infected patients. Implementation of anti-HDV testing in all HBsAg-positive patients has led to a 5-fold increase in the number of HDV diagnoses in an academic hospital and primary care centres.



# Implementation of anti-HDV reflex testing among HBsAg-positive individuals increases testing for hepatitis D

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**Background & Aims:** Although EASL guidelines recommend anti-HDV testing in all HBsAg-positive individuals, HDV infection remains an underdiagnosed condition. We describe the impact of an HDV screening program by reflex anti-HDV testing in all HBsAg-positive samples and compare the results before and after its implementation.

**Methods:** In total, 2,236 HBsAg-positive determinations were included from January 2018 to December 2021. Only the first sample from each participant was evaluated: 1,492 samples before reflex anti-HDV testing (2018–2020) and 744 samples after (2021). Demographic and clinical characteristics of anti-HDV-positive patients were collected.

**Results:** Before reflex testing, anti-HDV had been tested in 7.6% (114/1492) of HBsAg-positive individuals: 23% (91/390) attended in an academic hospital and only 2% (23/1,102) in primary care centres. After reflex testing was established, 93% (691/744) of HBsAg-positive cases were evaluated for anti-HDV: 91% (533/586) in the academic hospital and 100% (158/158) in primary care. The anti-HDV-positive prevalence was similar before and after reflex testing: 9.6% (11/114) and 8.1% (56/691), respectively. However, the absolute number of anti-HDV-positive patients increased. Most anti-HDV-positive patients were young, HBeAg-negative, Caucasian males. HDV-RNA was detectable in 35 (65%) of 54 tested, HBV-DNA was undetectable in 64%, and alanine aminotransferase levels were normal in 48%.

**Conclusions:** Anti-HDV reflex testing quintupled the absolute number of diagnoses of chronic hepatitis D infection. Before the reflex test, a large percentage of HBsAg-positive individuals had not undergone any anti-HDV determination. Implementation of reflex testing increases the diagnosis of patients with chronic hepatitis D.

**Lay summary:** Chronic hepatitis delta (CHD) is a viral disease caused by HDV, which requires the presence of HBV to propagate. HDV infection can cause rapid progression to cirrhosis, among other severe complications. The prevalence of CHD worldwide is controversial, and the infection often goes unrecognised, mainly because of unawareness among physicians. Use of reflex testing in other viral hepatitis has proven to increase detection and linking-to-care of infected patients. Implementation of anti-HDV testing in all HBsAg-positive patients has led to a 5-fold increase in the number of HDV diagnoses in an academic hospital and primary care centres.

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## Introduction

HDV is a hepatotropic single-stranded RNA virus that requires the presence of HBV to propagate. HDV causes one of the most severe forms of chronic viral hepatitis, being associated with a 2- to 3-fold higher risk of developing liver cirrhosis and hepatocellular carcinoma than infection by HBV alone.<sup>1,2</sup> Implementation of vaccination programs against HBV in the nineties undoubtedly had an impact on HDV prevalence in young populations.<sup>3</sup> In high-income countries, the current burden of

HDV mainly encompasses ageing patients with advanced liver fibrosis and young immigrants from endemic areas.<sup>4</sup> Still, HDV infection is believed to be underestimated owing to a lack of, or suboptimal, screening programs in HBsAg-positive individuals.<sup>5</sup>

Anti-HDV testing in all HBsAg-positive patients is recommended in the 2017 EASL guidelines on the management of HBV infection,<sup>6</sup> as well as the 2016 Asian-Pacific guidelines on the management of chronic hepatitis B (APASL, Asian-Pacific Association for the Study of the Liver).<sup>7</sup> However, the 2018 American Association for the Study of Liver Diseases (AASLD) guidelines recommend anti-HDV testing only in HBsAg-positive individuals who are at risk, such as people who inject drugs, men who have sex with men, individuals at risk of acquiring sexually transmitted diseases, and immigrants from areas where HDV is highly endemic.<sup>8</sup> Although anti-HDV testing in all HBsAg-positive

Keywords: Chronic hepatitis D; Anti-HDV reflex testing; Anti-HDV screening; HDV diagnosis.

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individuals is suggested in the European and Asian-Pacific guidelines, it is believed that many cases remain untested.

The aims of the present study were to determine the anti-HDV testing rates in all HBsAg-positive samples in a large population over a 3-year period, to assess the impact of anti-HDV reflex testing to diagnose HDV in HBsAg-positive cases, and to describe the epidemiology and clinical characteristics of anti-HDV-positive individuals in our setting.

## Materials and methods

### Study design

The study was divided into 2 parts, including a retrospective and a prospective analysis. In the retrospective part, anti-HDV requests were evaluated in all HBsAg-positive samples processed in a central laboratory attending a catchment area of 450,000 inhabitants in the northern part of Barcelona (Spain) from January 2018 to December 2020.

The central laboratory received samples from 1 academic hospital and 17 primary care centres. All HBsAg-positive samples were reviewed by 1 of the investigators to determine whether an anti-HDV test was subsequently requested at any time, and whether HDV-RNA had been determined in anti-HDV-positive cases. A database was constructed to record baseline demographics and the clinical and laboratory characteristics of anti-HDV-positive patients. The overall and annual percentages of anti-HDV testing and HDV-RNA testing were analysed.

In the prospective part of the study, anti-HDV reflex testing was manually added to all HBsAg-positive serum samples over a 1-year period (January–December, 2021). Demographics and clinical characteristics, including HDV-RNA, were recorded in all anti-HDV-positive patients, as was done in the retrospective part.

HBV serological markers (HBsAg and HBeAg) and anti-HCV antibodies were tested using commercial electrochemiluminescent immunoassays on a COBAS 8000 instrument (Roche Diagnostics, Rotkreuz, Switzerland). Anti-HDV antibodies were analysed with the Liaison XL murex Anti-HDV kit (DiaSorin, Saluggia, Italy), and anti-HIV antibodies with the Liaison XL murex HIV Ab/Ag kit (DiaSorin, Saluggia, Italy). HBV-DNA was quantified by real-time PCR with a detection limit of 10 IU/ml (COBAS 6800, Roche Diagnostics, Mannheim, Germany). HDV-RNA was quantified with an in-house 1-step quantitative RT-PCR using the HDV-RNA international standard of the World Health Organization (1st World Health Organization International Standard for Hepatitis D Virus RNA for Nucleic Acid Amplification Technique-based Assays; PEI code number: 7657/12),<sup>9</sup> with a detection limit of 100 IU/ml and a quantification limit of 600 IU/ml. Liver fibrosis stage was assessed by transient elastography: the cut-offs were >7.5 kPa for advanced fibrosis and >13.5 kPa for liver cirrhosis.

### Statistics

Normally distributed quantitative variables were compared with the Student *t* test and expressed as the mean  $\pm$  SD. Variables with a non-normal distribution were analysed with the Mann-Whitney *U* test and expressed as the median and inter-quartile range (IQR). Categorical variables were compared using the Chi-square test or Fisher's exact test when frequencies were <5% and expressed as frequencies and percentages. Results were considered statistically significant at a *p*-value <0.05. All statistical analyses were carried out using IBM SPSS, 20 (SPSS Inc., Armonk, NY, USA).

## Results

### Routine hepatitis D screening

In total, 1492 HBsAg-positive samples were sent to the central laboratory between January 2018 and December 2020 and all were reviewed. Anti-HDV testing had been performed in 114 (7.6%) cases, and 11 (9.6%) were anti-HDV positive.

Anti-HDV determination had been requested in 91 (23%) of 390 samples from the academic hospital, and in only 23 (2%) of 1,102 samples from the primary care centres (*p* <0.001).

The percentage of HBsAg-positive samples tested for anti-HDV was similar across the years: 6.4% (36/564) in 2018, 8.1% (47/578) in 2019, and 8.9% (31/350) in 2020, with most anti-HDV determinations being requested by the academic hospital (32/36, 38/47, and 21/31, respectively).

### Implementation of anti-HDV reflex testing

Reflex anti-HDV testing was established in January 2021, and data were collected from January 2021 to December 2021. In this prospective analysis, 93% (691/744) of HBsAg-positive samples were tested for anti-HDV: 91% (533/586) from the academic hospital and 100% (158/158) from primary care.

The overall prevalence of anti-HDV was 8.1% (56/691). This value was similar to the 9.6% (11/114) prevalence observed before the start of reflex testing (2018–2020). However, the absolute number of patients diagnosed with HDV increased from 11 cases in the 3 years before reflex testing to 56 cases in 2021 (Fig. 1).

### Epidemiological and clinical characteristics of anti-HDV-positive individuals in our setting

HDV-RNA was determined in 54 of the 67 anti-HDV-positive patients, and 35 (65%) of them had detectable HDV-RNA. Among the total of anti-HDV-positive patients, most were young Caucasian males, 48% had normal alanine aminotransferase (ALT) levels, 60% advanced fibrosis, and 34% liver cirrhosis. Nine patients were co-infected with HCV, and 1 had detectable HCV-RNA. Four patients were co-infected with HIV; all were receiving antiretroviral therapy. The main epidemiological, clinical, serological, and virological characteristics of anti-HDV-positive individuals are shown in Table 1. Those with detectable HDV-RNA had

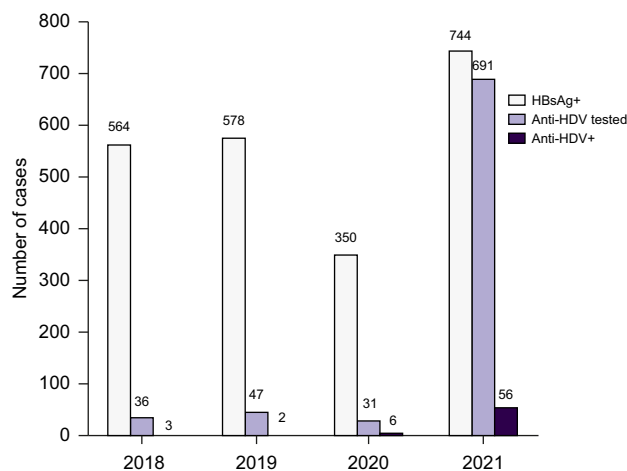


Fig. 1. Number of anti-HDV testing and positive cases among HBsAg-positive samples. Anti-HDV, anti-hepatitis D virus antibodies.

**Table 1. Demographic, serologic, virologic, and clinical data of all anti-HDV-positive cases with HDV-RNA determination.**

	Anti-HDV-positive with HDV-RNA determination (N = 54)	HDV-RNA detectable (n = 35)*	HDV-RNA undetectable (n = 19)*	p value
Age, years	44 (35–56)	44 (35–54)	46 (38–57)	0.244
Male, n (%)	32 (59)	19 (54)	13 (68)	0.237
Ethnicity, n (%)				0.085
Caucasian	33 (59)	23 (66)	10 (53)	
African	15 (28)	7 (20)	8 (42)	
Asian	6 (11)	5 (14)	1 (5)	
Risk factors, n (%)				0.053
Blood-borne (including PWID)	11 (20)	11 (31)	0 (0)	
HDV endemic country	11 (20)	8 (23)	3 (16)	
Unknown	32 (60)	16 (46)	16 (84)	
Platelets, ×10 <sup>9</sup> /L	167 (115.5–217.5)	147 (104–187)	208 (145–251)	0.013
ALT, IU/ml	49 (27.5–78)	64 (49–119)	28 (19–38)	<0.001
Normal ALT, n (%)	23 (43)	8 (23)	15 (79)	<0.001
AST, IU/ml	41 (28–83)	63 (39–115)	28 (23–32)	<0.001
GGT, IU/ml	43 (25.5–75)	56 (28–83)	28 (22–47)	0.003
Non-invasive markers				
Transient elastography, kPa	9.5 (6.1–15)	12 (8–16.4)	7.8 (5.6–11)	0.024
FIB-4	1.7 (1.3–3.9)	2.1 (1.3–5.0)	1.4 (0.9–1.9)	0.028
APRI	0.7 (0.4–1.8)	1.3 (0.6–3.2)	0.4 (0.3–0.6)	<0.001
Advanced fibrosis, n (%)*	17 (32)	10 (29)	7 (37)	0.044
Liver cirrhosis, n (%)*	19 (35)	17 (49)	2 (11)	0.014
HBsAg, log IU/ml	3.7 (3.4–4.1)	3.9 (3.5–4.2)	3.2 (2.3–3.5)	0.001
HBeAg, n (%)	4 (7)	4 (11)	0 (0)	0.193
HBV-DNA, IU/ml	3.1 (2.5–4.5)	2.7 (2.4–4.2)	3 (2.5–4.1)	0.602
HBV-DNA undetectable, n (%)	15 (28)	9 (26)	6 (32)	0.043
HBV-DNA detectable not quantifiable, n (%)	22 (63)	20 (57)	2 (11)	
HBV-DNA detectable, n (%)	17 (31)	6 (17)	11 (58)	<0.001
HDV-RNA, log IU/ml*	4.9 (4.5–5.7)	n.a.	n.a.	n.a.

Quantitative variables are expressed as median and inter-quartile range and qualitative variables as number and percentage.

ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; FIB-4, fibrosis-4; GGT, gamma glutamyl transferase; n.a., not applicable, PWID, people who inject drugs.

\* Determined in 50 patients.

significantly lower platelet levels, higher ALT, aspartate aminotransferase, and gamma glutamyl transferase levels, and more often advanced fibrosis and liver cirrhosis than those with undetectable HDV-RNA. In addition, patients with detectable HDV-RNA showed statistically lower HBsAg levels and had undetectable HBV-DNA more often. In addition, a considerable pool of patients (39%) had detectable but unquantifiable HBV-DNA (<20 IU/ml).

## Discussion

Analysis of laboratory testing from 2018 to 2020 found that screening for HDV infection was low in our setting. Anti-HDV antibodies were tested in only a small proportion of HBsAg-positive samples: <10% in a catchment population of 450,000 inhabitants in Barcelona. Most screening requests (~80%) came from the academic hospital, with very few requests from primary care centres, even though these tests were available. This suggests that awareness of HDV infection is low among primary care physicians. The HDV screening rate was similar over the 3 years analysed except during the COVID-19 pandemic, in which the number of HBsAg-positive samples decreased, particularly during the first wave of the infection, when a lockdown of more than 3 months was declared (15 March–21 June). Significant decreases in HBV and HCV testing during the start of the COVID-19 pandemic have also been reported.<sup>10</sup>

The low HDV screening rate diverged from the 2017 EASL recommendations for the management of hepatitis B, which encourage anti-HDV testing in all HBsAg-positive cases regardless of the presence of risk factors or severity of the liver disease. In a previous oral communication, our group reported that the

HDV testing rate before publication of the EASL guidelines was even lower in our setting, with only a slight increase after the guidelines emerged (7.5% vs. 9.4%, *p* = 0.015).<sup>11</sup>

Better results were seen in the large Bordeaux Metropolis Without Viral Hepatitis programme. The anti-HDV testing rate was 49% in 988 HBsAg-positive patients, and 10% (50/483) of them were anti-HDV positive. HDV-RNA was tested in 39 cases. The higher HDV screening rate in the Bordeaux study compared with that of our review before reflex testing is likely attributable to the existence of an already established programme in Bordeaux for this purpose, whereas HDV testing was requested at the physicians' discretion in our hospital. However, despite the programme, half the HBsAg-positive samples in the Bordeaux study remained untested and potentially undiagnosed.

A study performed in a London centre with clinic-led anti-HDV testing reviewed the clinical and laboratory data of 168 HBsAg-positive patients and reported a 40% (67/168) prevalence of anti-HDV-positive status.<sup>12</sup> These reports, along with our findings, highlight the need for health policy initiatives aimed at improving HDV screening, especially relevant since the arrival of new HDV drugs.

There was a considerable difference in the diagnostic yield in our setting following implementation of HDV reflex testing, which led to a 5-fold increase in the number of HBV cases diagnosed with hepatitis D. The increase was similar in both the academic hospital and primary care centres. Reflex testing has been evaluated in other viral infections such as hepatitis C, particularly for viraemia detection. With this approach, additional blood drawn for HCV-RNA testing and an appointment for the results can be avoided in anti-HCV-positive cases.<sup>13</sup> A study



in the emergency department of an academic hospital showed that reflex testing enables detection of active HCV infection in a single sample.<sup>14</sup> The authors reported a 0.7% prevalence of active HCV infection, a value almost 3-fold higher than that seen in the general population. Anti-HDV reflex testing has been evaluated in a single centre in London, but the results obtained were not compared with prior routine testing.<sup>12</sup>

The profile of our anti-HDV-positive patients is in line with the findings from other European cohorts.<sup>15–17</sup> A considerable number were migrants from countries where HDV infection is endemic (20%) or patients with blood-borne risk factors (20%). Risk factors were unknown in the remaining 60%, which further supports the rationality of systematic anti-HDV reflex testing in all HBsAg-positive patients, rather than testing according to risk factors. In addition, those with detectable HDV-RNA showed higher ALT levels and more often had cirrhosis,<sup>18</sup> and viraemic

patients had lower HBV-DNA levels, supporting the theory of HDV suppression over HBV.<sup>19</sup>

The main limitation of our study is the scarce data regarding risk factors for hepatitis D in HBsAg-positive cases, making it difficult to assess whether HDV testing was mainly requested in high-risk groups, as recommended in the AASLD guidelines. However, the increase in the number of HDV cases detected by reflex testing suggests that not all patients with HDV risk factors had been routinely screened. Finally, it would be of value to assess the cost-effectiveness of establishing reflex testing for this purpose.

In summary, HDV testing rates in daily clinical practice are low despite EASL guidelines recommending universal screening. HDV infection is associated with significant liver-related mortality; hence, the importance of establishing anti-HDV reflex testing algorithms for an early HDV diagnosis.

### Abbreviations

AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; APASL, Asian-Pacific Association for the Study of the Liver; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; FIB-4, fibrosis-4; GGT, gamma glutamyl transferase; PWID, people who inject drugs.

### Financial support

This study received support in part from the Instituto de Salud Carlos III (PI20/01692).

### Conflicts of interest

MB has served as a speaker and advisory board member for Gilead, Roche, and Arbutus. RE has served as a speaker and advisory board member for Gilead. MRB has served as a speaker for Abbvie and Gilead.

Please refer to the accompanying ICMJE disclosure forms for further details.

### Authors' contributions

Guarantor of the article and responsibility for the integrity of the work as a whole, from inception to published article: MB. Study design: MB, RE, FRF. Collected the clinical and laboratory data: AP, AR, EV, JV, ABD. Performed laboratory testing: AR, BP. Carried out the analysis and interpretation of data: MRB, MB, AP, AR. Drafted the manuscript: MB, AP. Reviewed the manuscript: MB, MRB, RE, FRF. Approved the final version of the article: all authors.

### Data availability statement

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

### Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhepr.2022.100547>.

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