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A holistic evaluation of patients with chronic Hepatitis D virus (HDV) infection enrolled in the Italian PITER-B and delta cohort

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ABSTRACT

Background and Aims: We aimed to characterize the epidemiologic and comorbidities profiles of patients with chronic Hepatitis D (CHD) followed in clinical practice in Italy and explored their interferon (IFN) eligibility.
Methods: This was a cross-sectional study of the PITER cohort consisting of consecutive HBsAg-positive patients from 59 centers over the period 2019-2023. Multivariable analysis was performed by logistic regression model.
Results: Of 5492 HBsAg-positive enrolled patients, 4152 (75.6%) were screened for HDV, 422 (10.2%) were anti-HDV positive. Compared with HBsAg mono-infected, anti-HDV positive patients were more often younger, non-Italians, with a history of drug use, had elevated alanine transaminase (ALT), cirrhosis, or hepatocellular carcinoma (HCC). Compared with Italians, anti-HDV positive non-Italians were younger (42.2% age $\leq$ 40 years vs. 2.1%; $P < 0.001$), more often females (males 43.0% vs. 68.6%; $P < 0.001$) with less frequent cirrhosis and HCC. HDV-RNA was detected in 63.2% of anti-HDV-positive patients, who were more likely to have elevated ALT, cirrhosis, and HCC. Extrahepatic comorbidities were present in 47.4% of anti-HDV positive patients and could affect the eligibility of IFN-containing therapies in at least 53.0% of patients in care.
Conclusions: CHD affects young, foreign-born patients and older Italians, of whom two-thirds had cirrhosis or HCC. Comorbidities were frequent in both Italians and non-Italians and impacted eligibility for IFN.

Introduction

Chronic infection by the Hepatitis Delta virus (HDV) causes aggressive and difficult-to-treat Hepatitis in Hepatitis B antigen (HBsAg)-positive patients [1]. The epidemiological profiles of chronic Hepatitis D (CHD) have changed in the last decades [2-4] mainly due to Hepatitis B virus (HBV) vaccination campaigns in most countries and increasing immigration from areas where HDV is endemic [5-14].

About two-thirds of HBsAg-positive individuals with anti-HDV have an active infection, shown by the presence of HDV ribonucleic acid (RNA) in blood, which fuels disease progression and requires treatment [15,16]. Unfortunately, only interferon (IFN)-based therapies, which were feasible and effective in a small proportion of cases, were available for these patients for decades. With new antivirals on the horizon, understanding and assessing HDV-infected patients' current epidemiological and clinical profiles is needed to determine eligibility and potential treatment prioritization criteria in line with overall patient morbidity. Accordingly, this study aims to describe the hepatic and extrahepatic morbidity profile of patients enrolled in the Italian Platform for the Study of Viral Hepatitis Therapy (PITER) HBV/HDV cohort. This should also provide HDV screening and care indications in HBsAg-positive patients.

Patients and methods

This cross-sectional, observational study examined patients enrolled in the Italian PITER HBV/HDV cohort [17]. Briefly, consecutive patients, except those enrolled in clinical trials, irrespective of antiviral treatment, seen in 59 centers throughout Italy (40.4% in Northern Italy, 17.3% in Central Italy, and 42.3% in Southern Italy) from November 2019 to February 2023 who were HBsAg positive for at least 6 months, with or without HDV, Hepatitis C virus (HCV) and human immunodeficiency virus (HIV) co-infection were included. Additionally, patients with past HBV infection who were HBsAg negative and those with acute HBV were excluded.

Baseline demographic, clinical, and laboratory characteristics were recorded for each patient at enrolment using a specific electronic case report form (eCRF). Potential liver disease cofactors were recorded (alcohol use, diabetes, elevated body mass index (BMI), HIV and HCV coinfections); alcohol intake was categorized as ongoing, previous, or no use by a cut-off of 3 U/day; diabetes was having fasting glucose ≥126 mg/dL at repeated determinations; and overweight/obesity was a BMI 25-30/>30 kg/m^2. In addition, extrahepatic morbidities were recorded, with special attention to severe, predefined comorbidities that could contraindicate the use of IFN. Each clinical center performed virological and routine analyses, which included testing for HBsAg, Hepatitis B e antigen (HBeAg)/HBe antibody (anti-HBe), anti-HDV/HCV antibodies, and HIV co-infection using commercially available enzyme-immunoassays, and HCV-RNA and HIV-RNA testing by commercial kits. For the aims of this study, the qualitative HDV-RNA test data were collected by each clinical center. The following real-time assays were used: RoboGene HDV RNA Quantification kit 2.0 (Robogene GmbH, Leipzig, Germany; lower limit of detection (LOD) = 6 IU/mL; 35.5% of centers); EurobioPlex HDV qRT-PCR (Eurobio, Les Ulis, France, LOD = 100 IU/mL; 22.6% of centers), Bosphore HDV Quantification-Detection kit (Anatolia Geneworks, Sultanbeyli, Turkey, LOD = 45 cp/mL; 16.1% of centers), RealStar HDV RT-PCR (Altona Diagnostics GmbH, Hamburg Germany, LOD = 9.48 IU/mL; 9.7% of centers), DiaPro HDV RNA quantification kit (Dia.Pro Diagnostic Bioprobes Srl, Sesto San Giovanni (MI), Italy, LOD = 100 cp/mL; 6.4% of centers) and in-house validated assays (LOD = 500 cp/mL; 9.7% of centers).

Two expert clinical monitors and one physician were involved in ensuring data quality, which was checked through periodic remote monitoring using specific queries. The presence of liver cirrhosis was defined by liver biopsy (Metavir ≥4 or Ishak score ≥6), transient elastometry (liver stiffness measurement [LSM] >12.5 kPa), or unequivocal laboratory and imaging features.

Statistical analysis

Patients were analyzed according to their HDV status, i.e., the presence of anti-HDV antibodies and detectable HDV-RNA in serum and compared to patients with HBV mono-infection. The comparisons were performed using the Chi-Squared or Fishers̒ Exact test (when necessary) for categorical variables and the Mann-Whitney U test for continuous variables. Chi-squared for trend was used for age and Child-Pugh. The receiver operating characteristic (ROC) analysis was used to detect the cut-off of alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) values that better discriminate between anti-HDV or HDV-RNA positive or negative patients for the presence of cirrhosis. Multivariate logistic regression models explored the independent association between the considered variables and HDV-RNA positivity. The associations are presented by Odds Ratios (OR) and their 95% Confidence Intervals (95% CI). A two-tailed *P*-value < 0.05 was considered statistically significant. The data were analyzed using the STATA Statistical Software, version 16.1 (StataCorp 2019).

Results

Demographic and clinical characteristics of HDV-infected patients

The study enrolled 5492 HBsAg-positive patients, of whom 422 were positive for anti-HDV antibodies, 3730 were negative, and 1340 had not been screened for anti-HDV (Figure 1). The demographic and clinical characteristics of the patients tested for anti-HDV are summarized in Table 1. The overall prevalence of anti-HDV was 422 of 4152 HBsAg positive patients (10.2%; 95% CI 9.3-11.1), ranging from 3.7% in patients older than 70 to 13.6% in those aged 51-60. Compared with HBV mono-infected patients, anti-HDV positive patients were younger (median age 55 vs. 59 years; $P < 0.001$), with a history of intravenous drug use (10.0% vs. 1.7%; $P < 0.001$) and abnormal ALT values (59.4% vs. 15.3%; $P < 0.001$) and more often non-Italian natives (33.6% vs. 22.8%; $P < 0.001$). Among disease co-factors, anti-HCV and anti-HIV antibodies were more often present in anti-HDV positive patients. HCV-RNA was present in only one patient with anti-HDV and eight without. HIV-RNA was detected in two anti-HDV-negative patients, neither on antiretroviral therapy at the time of enrolment. Diabetes and overweight/obesity were more frequent among HBV mono-infected patients. Overall, 76.5% anti-HDV positive patients (N = 323, of whom 77% were cirrhotic) and 67.8% HBV mono-infected patients (N = 2529, of whom 32% were cirrhotic) ($P < 0.001$) were on antiviral treatment (>95% of which consisted of nucleoside analogs [NUCs]).

Among anti-HDV positive patients, cirrhosis was diagnosed by liver biopsy ± elastometry in 23.4% of cases, by elastometry in 36.1%, and by clinical and instrumental data in 40.5% of cases; the proportions were 23.8%, 31.7%, and 44.5%, respectively, for HBV mono-infected patients.

Overall, cirrhosis (70.8% vs. 23.9%; $P < 0.001$) and hepatocellular carcinoma (HCC) (10.2% vs. 2.9%; $P < 0.001$) were more frequent among anti-HDV positive patients. Presence of esophageal varices and ascites, an episode of variceal bleeding, encephalopathy, and portal thrombosis were more frequent in anti-HDV positive patients compared to anti-HDV negative patients (all $P < 0.001$). The multivariable analysis indicated that age, drug use, ALT value, presence of cirrhosis, past IFN therapy, and HIV co-infection remain

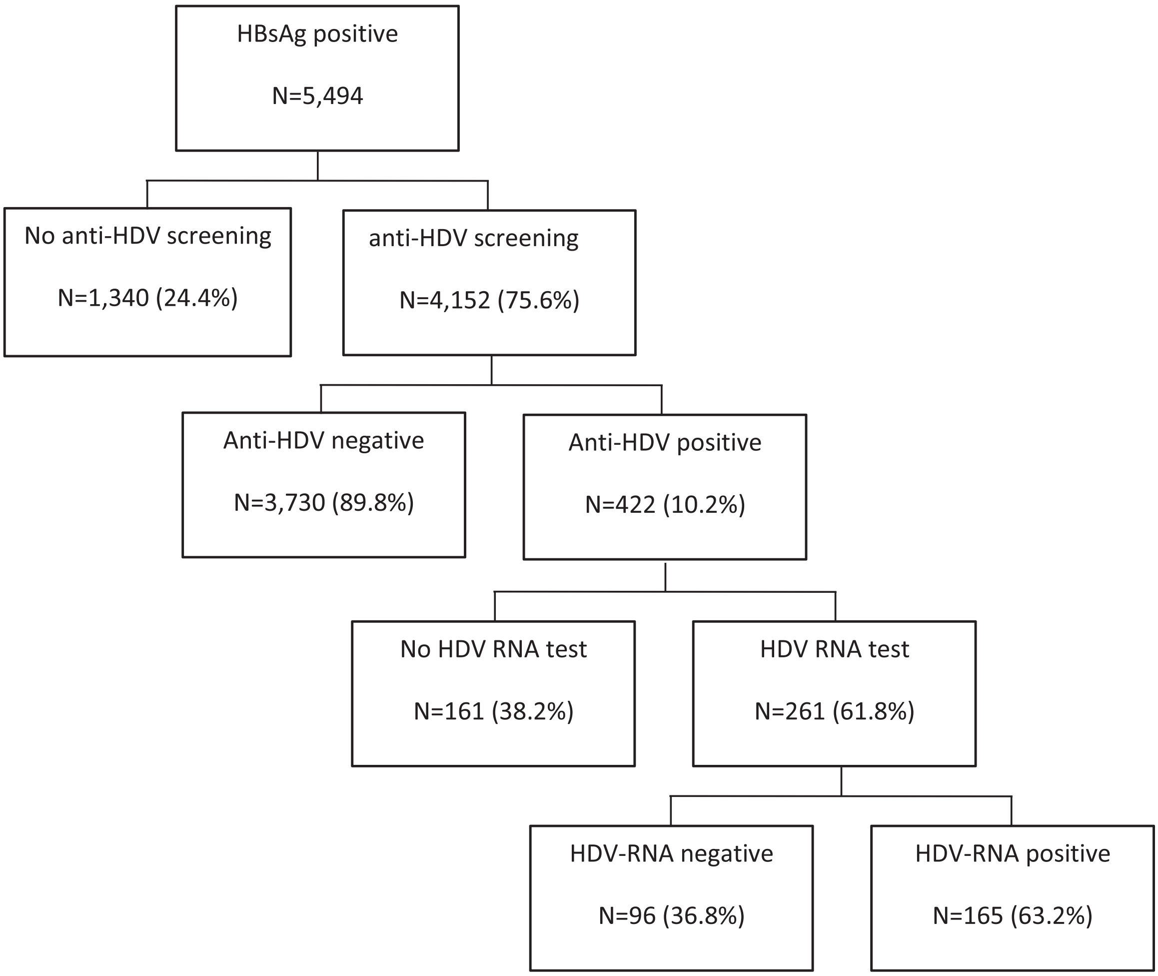

Figure 1. Flow chart of the patients enrolled in the study.

independently associated with anti-HDV positivity (Supplementary Table 1).

The distribution of liver cirrhosis by age and patient origin showed that 75% of patients under the age of 40 years were of non-Italian origin, and 12.0% over 40 years of age (Supplementary Figure 1). Anti-HDV prevalence was 142/992 (14.3%; 95% CI 12.2-16.6) among non-Italian patients and 280/3150 (8.8%; 95% CI 7.9-9.9) among Italians. Non-Italian patients mainly came from Eastern Europe (85.2%; most often Moldova, Romania, and Albania) followed by sub-Saharan Africa (7.7%) and Asia (2.1%); the remaining (N = 7; 4.9%) were from different areas. Anti-HDV positive patients were 56.3% from Northern, 27.3% from Central and 16.2% from-Southern Italy. Non-Italians were primarily in Northern and Central Italy (50.2% of anti-HDV positive patients vs. 11.9% in Southern Italy), younger (42.2% age $\leq$ 40 years vs. 2.1%; $P < 0.001$), females (males 43% vs. 68.6%; $P < 0.001$) less frequently with cirrhosis (61.3% vs 75.7%; $P = 0.002$) and HCC (5.0% vs. 12.8%; $P = 0.014$). Detectable HBV deoxyribonucleic acid (DNA) and a shorter duration of NUC therapy were significantly more frequent in non-Italians (Supplementary Table 2).

Patients not screened for anti-HDV were older Italian natives with normal ALT, less frequently with cirrhosis but more frequently with HCC. Presence of HCV or HIV coinfections, HBeAg positivity and HBV DNA positivity were significantly more frequent in patients not tested for HDV, whereas patients who underwent previous IFN therapy and were on NUC therapy for HBV chronic infection were more frequently tested for anti-HDV (Table 2). In the multivariable model, presence of HCC, HIV co-infection, and past IFN therapy were significantly associated with a lack of anti-HDV testing (Supplementary Table 3).

Active HDV infection

HDV RNA in plasma was detected in 165 (63.2%; 95% CI 57.1-69.1) of the 261 tested patients. Of the 161 patients (38%) not tested for HDV RNA, altered ALT levels ($P = 0.044$), Child-Pugh B/C cirrhosis ($P = 0.003$) and HIV infection ($P = 0.004$) were significantly more frequent than in tested patients (Supplementary Table 2). Compared with anti-HDV-positive patients with undetectable serum HDV-RNA (Table 3), HDV-RNA-positive patients had a similar median age ($P = 0.9$); however, age-related prevalence was variable ($P = 0.007$), ranging from 55.9% in those aged 61-70 years to 72.1% in those $\leq$40 years and 100% in patients over 70. Cirrhosis was more frequent among HDV-RNA-positive patients (75.8 vs. 63.5%; $P = 0.035$). However, the prevalence of cirrhosis in anti-HDV positive/HDV-RNA negative patients was higher (63.5%) than in HBV mono-infected patients (29.9%; $P < 0.001$). The proportion of HCC cases was higher in HDV RNA-positive patients (15.3% vs. 3.2%; $P = 0.003$), but comparable in RNA-negative patients and HBV mono-infected (3.2% vs. 2.9%; not significant) (Tables 1 and 3). Abnormal ALT values (> 35 IU/L) were observed in 74.4% of the HDV RNA-positive patients compared to 25.0% in those HDV RNA-negative ($P < 0.001$). The odds of being HDV-RNA positive were independently associated with elevated ALT values (Adj. OR 13.58,

Table 1
Epidemiological Characteristics of Enrolled Patients by Anti-HDV Status

	Anti-HDV Positive N = 422	Anti-HDV Negative N = 3730	*P*-Value
Age median (Q1, Q3)	55 (46, 62)	59 (48, 68)	<0.001
≤ 40	66 (15.6)	515 (13.8)	<0.001
41-50	77 (18.2)	589 (15.8)	
51-60	147 (34.8)	932 (25.0)	
61-70	104 (24.6)	960 (25.7)	
>70	28 (6.6)	734 (19.7)	
Males	253 (60.0)	2335 (62.6)	0.287
Non-Italian natives	142 (33.6)	850 (22.8)	<0.001
Injection drug use	35 (10.0)	55 (1.7)	<0.001
ALT median (Q1, Q3)	45 (27-79)	22 (16-30)	<0.001
ALT > 35 IU/L	243 (59.4)	566 (15.3)	<0.001
GGT >50 IU/L	121 (36.3)	344 (11.1)	<0.001
HBeAg positive	25 (6.1)	253 (6.8)	0.582
Cirrhosis	299 (70.8)	892 (23.9)	<0.001
Esophageal varices	88 (20.85)	153 (4.10)	<0.001
Previous bleeding	13 (3.08)	28 (0.75)	<0.001
Ascites	34 (8.06)	58 (1.55)	<0.001
Encephalopathy	13 (3.08)	27 (0.72)	<0.001
Portal thrombosis	10 (2.37)	22 (0.59)	<0.001
Child-Pugh			
A	251 (84.0)	791 (88.7)	0.005
B	41 (13.7)	99 (11.1)	
C	7 (2.3)	2 (0.2)	
HCC	42 (10.2)	106 (2.9)	<0.001
HBV DNA positive	115 (29.1)	1330 (36.5)	0.004
HDV RNA positive (261 tested)	165 (63.2)	—	—
Previous IFN	142 (33.6)	580 (15.5)	<0.001
Potential disease co-factors			
Ongoing alcohol use	65 (18.7)	775 (22.6)	0.130
Past use	51 (14.7)	412 (12.0)	
Diabetes	29 (6.9)	375 (10.0)	0.037
BMI 25-30	109 (25.8)	1173 (31.4)	0.004
BMI ≥ 30	32 (7.6)	384 (10.3)	
anti-HCV positive	39 (10.4)	126 (3.6)	<0.001
anti-HIV positive	17 (4.8)	35 (1.1)	<0.001
Ongoing therapy (>95% NUCs)	323 (76.5)	2529 (67.8)	<0.001
Years of NUC therapy median (Q1, Q3)	4.4 (2.0-8.0)	6.0 (2.7-9.7)	<0.001

ALT: alanine aminotransferase; GGT: gamma glutamyl transpeptidase; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; HCC: hepatocellular carcinoma; HCV: Hepatitis C virus; HDV: Hepatitis D virus; HIV: human immunodeficiency virus; IFN: interferon; NUCs: nucleos(t)ide analogues; Q1: quarter 1; Q3: quarter 3.

CI 95%: 5.75-32.07) and advanced cirrhosis after adjusting for age, sex, BMI, HCC, GGT, origin, and NUC therapy for more than 2 years (Supplementary Table 3).

ROC analysis showed that an ALT value >35 IU/mL had the best discriminating power, allocating anti-HDV with 82% accuracy (negative predictive value, NPV = 95.0%; positive predictive value, PPV = 30.0%). GGT had a lower discriminatory power (accuracy of 62%). However, GGT with the cutoff value of 43 IU/mL showed a 74% accuracy (NPV = 93.5%; PPV = 24.5%) in detecting the presence or absence of cirrhosis in anti-HDV-positive patients (data not shown).

Extrahepatic comorbidity profile

One or more comorbidities were present in 200 of the 422 anti-HDV positive patients (47.4%), of whom 78 (39.0%) had more than one comorbidity. Comorbidities were present in 142/280 Italian (50.7%) vs 58/142 (40.8%) non-Italian anti-HDV positive patients ($P = 0.05$) and in 1811/2880 (62.9%) Italian vs. 265/850 (31.2%) non-Italians HBV mono-infected patients ($P < 0.001$). The main comorbidities by anti-HDV status by age class are reported in Table 4A. Neuro-psychiatric disorders were more frequent among younger (≤55) HDV patients; in contrast, autoimmune diseases and being overweight were more frequent among older (>55) patients with HBV mono-infection.

Eligibility to interferon (IFN)

An estimate of eligibility for IFN-containing treatments for HDV-RNA positive patients (n = 165) was made by the presence of contraindications due to advanced liver disease or severe extra-hepatic comorbidities as listed in Table 4B. By sequentially eliminating patients with at least one absolute contraindication (40.6%) and patients with relative contraindications (12.7%), 46.7% of the patients could be eligible for IFN.

Discussion

There is a renewed interest in chronic Hepatitis D due to the availability of new treatments. The results of this study are based on a representative nationwide cohort of HBsAg-positive patients with or without HDV infection and highlight important factors. First, the majority of HDV-positive patients have advanced liver disease or established cirrhosis in both the young, non-Italian population and the older Italian patients. Second, comorbidities are frequent, and their role in influencing liver disease management and different antiviral treatment eligibility should be evaluated in clinical practice. Finally, patients with HDV infection have a heterogeneous clinical profile in different countries/areas, which requires an analysis by local studies to address containment measures.

Table 2
Characteristics of Patients by Anti-HDV Tested Status

	Anti-HDV Tested N = 4152	Anti-HDV not Tested N = 1340	*P*-Value
Age median (Q1, Q3)	59 (48-68)	60.5 (50-69)	<0.001
≤ 40	581 (14.0)	149 (11.1)	<0.001
41-50	666 (16.0)	201 (15.0)	
51-60	1079 (26.0)	320 (23.9)	
61-70	1064 (25.6)	377 (28.1)	
>70	762 (18.3)	293 (21.9)	
Males n (%)	2588 (62.3)	825 (61.6)	0.616
Non-Italian natives	992 (23.9)	241 (18.0)	<0.001
Injection drug use	90 (2.5)	21 (2.2)	0.644
ALT median (Q1, Q3)	22 (17-33)	22 (17-30)	0.121
ALT > 35 IU/L	809 (19.6)	192 (16.5)	0.017
GGT > 50	465 (13.6)	137 (13.3)	0.834
Cirrhosis	1191 (28.7)	323 (24.1)	0.001
Child-Pugh			
A	1042 (87.5)	284 (87.9)	0.918
B	140 (11.7)	36 (11.1)	
C	9 (0.8)	3 (0.9)	
HCC	148 (3.7)	88 (6.7)	<0.001
Alcohol			
Ongoing use	840 (22.3)	228 (23.6)	0.011
Past use	463 (12.3)	85 (8.8)	
HBeAg positive	278 (6.8)	115 (8.8)	0.012
Anti-HCV	165 (4.2)	55 (7.2)	<0.001
Anti-HIV	52 (1.4)	29 (4.4)	<0.001
HBV DNA positive	1445 (35.8)	504 (42.1)	<0.001
Previous IFN	722 (17.4)	117 (8.7)	<0.001
Ongoing therapy (>95% NUCs)	2812 (67.7)	846 (63.2)	0.002
Years of NUC therapy median (Q1, Q3)	5.7 (2.3-9.5)	5.9 (2.4-9.7)	0.519

ALT: alanine aminotransferase; GGT: gamma glutamyl transpeptidase; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; HCC: hepatocellular carcinoma; HCV: Hepatitis C virus; HDV: Hepatitis D virus; HIV: human immunodeficiency virus; IFN: interferon; NUCs: nucleos(t)ide analogues; Q1: quarter 1; Q3: quarter 3.

The overall prevalence of anti-HDV (10.2%) is similar to that in other Italian studies [2,8,18]; and in health care settings in other countries, except for some hyperendemic areas in which prevalences are higher [19]; minor differences may be due to the increasing proportion of non-Italian patients included in the various studies performed in different years [20] and to the heterogeneity of the enrolment criteria [21,22]. Of note, anti-HDV prevalence was 3.9% among those aged >70 years (almost all Italian natives), while ranging from 11-13% in the other age groups. Most Italian patients who acquired HDV during the HBV/HDV epidemic 40-50 years ago did not survive, and thus, we are currently observing long-term survivors with a slower-progressing disease [23]. The present data confirm previous observations of the cohort of patients in care in Italy, which consists of younger, predominantly female non-Italian patients of childbearing age, potentially more subject to HBV and HDV screening, and an older cohort of primarily male Italian patients. This epidemiological pattern is common in Mediterranean Europe [7,8,12], while in Northern Europe, the United Kingdom (UK), and the United States (US), Delta infection largely prevails among definite ethnic or behavioral high-risk groups [5,6,9-11,14]. In this study, non-Italian patients were more commonly from Eastern Europe, which differs from other European countries where migrants are primarily from Sub-Saharan Africa [5–8], supporting the need for local epidemiological monitoring to establish targeted care measures.

Testing for anti-HDV in HBsAg-positive persons is a global challenge [3,24]. In our context, where 24% of the HBsAg-positive patients remained unscreened, non-Italian natives were more likely to have received an anti-HDV screening test than Italians (Table 2). Thus, country of origin seems to have been recognized as a risk factor, as recently suggested in the US [25], where local risk factors guide HDV screening. In contrast, patients who tested positive for HBeAg, HBV DNA, anti-HCV, anti-HIV, or those with an HCC diagnosis were more likely to remain unscreened for HDV infection. We can speculate that finding a potential explanation for progressive liver disease diverted attention from anti-HDV screening. Reflex testing, automatic testing of all HBsAg-positive individuals for anti-HDV, has been discussed by the European Clinical Practice Guidelines on HDV [26] and was the object of caution. Reflex testing in subjects with a new HBsAg diagnosis would ensure that physicians, particularly those unfamiliar with HDV, provide HDV testing, though a cost-benefit analysis should be evaluated in different epidemiological contexts [26].

As found in other studies [12], more than one-third of patients with anti-HDV in the present study had not been tested for HDV-RNA. This may be due to the lack of standardized testing assays within the centers. However, untested patients had more advanced liver disease, which might have discouraged further investigation in the absence of an effective antiviral therapy or liver transplant indication (Supplementary Table 2).

Elevated ALT levels in patients under NUC therapy were found to be significantly correlated with Hepatitis Delta infection, and a cut-off of 35 IU/L showed an 82% accuracy in predicting anti-HDV status. This was also found in a Greek cohort where HDV viremia was commonly detected, but not exclusively, in anti-HDV-positive patients with elevated ALT and advanced liver disease [12]. Screening of all HBsAg positive persons, according to EASL recommendations [26], should be adopted to overcome late HDV diagnosis. Findings from the present study also support the concept that GGT plays a valuable role in accurately defining advanced fibrosis and supports its inclusion in fibrosis defining scores [27].

In our cohort, 299/422 (71%) of the patients with anti-HDV had cirrhosis, which was 25% of all HBsAg-positive cirrhosis cases enrolled in the study. Considering that 17% of the cirrhosis cases in Italy are estimated to be HBsAg-positive [28], HDV is implicated in 4% of all cases. About one-third of anti-HDV-positive cirrhosis patients had undetectable HDV-RNA at enrolment, keeping

Table 3
Characteristics of Patients by HDV-RNA Status

	HDV RNA Positive N = 165 (63.2%)	HDV RNA Negative N = 96	*P*-Value
Age median (Q1,Q3)	55 (44-63)	55 (46-62)	0.868
≤40	31 (18.8)	12 (12.5)	0.007
41-50	33 (20.0)	18 (18.7)	
51-60	47 (28.5)	36 (37.5)	
61-70	38 (23.0)	30 (31.2)	
>70	16 (9.7)	0 (0.0)	
Males n (%)	91 (55.2)	59 (61.5)	0.320
Non-Italian natives	63 (38.2)	27 (28.1)	0.099
Injection drug use	15 (10.3)	7 (8.2)	0.600
ALT median (Q1,Q3)	52.5 (35-79)	24 (19-36.5)	<0.001
ALT > 35 IU/L	116 (74.4)	24 (25.0)	<0.001
GGT>50 IU/mL	54 (44.6)	18 (20.4)	<0.001
Cirrhosis	125 (75.8)	61 (63.5)	0.035
Child-Pugh			
A	106 (84.8)	59 (96.7)	0.028
B	18 (14.4)	2 (3.3)	
C	1 (0.8)	0 (0.0)	
Complications of cirrhosis*	45 (36.0)	14 (22.9)	0.073
HCC	25 (15.3)	3 (3.2)	0.003
HBV DNA positive	45 (29.0)	27 (28.7)	0.958
PLT < 150,000	64 (41.3)	51 (53.7)	0.056
MELD >19	2 (1.9)	1 (2.0)	>0.999
Previous IFN	63 (38.2)	40 (41.7)	0.579
Potential disease co-factors			
Ongoing alcohol use	19 (13.6)	19 (21.8)	0.175
Past use	20 (14.3)	15 (17.2)	
Diabetes	12 (7.3)	8 (8.3)	0.811
BMI 25-30	38 (23.0)	30 (31.2)	0.007
BMI ≥30	9 (5.4)	14 (14.6)	
Anti-HCV positive	13 (8.4)	9 (10.2)	0.647
Anti-HIV positive	3 (2.2)	2 (2.4)	>0.999
Ongoing therapy (>95% NUCs)	130 (78.8)	66 (68.8)	0.071
Years of NUC therapy median (Q1,Q3)	4.0 (2.0-7.2)	5.5 (1.2-9.2)	0.220

*Including: presence of esophageal varices; history of bleeding; portal thrombosis; previous/present ascites; and encephalopathy.
ALT: alanine aminotransferase; HBV: Hepatitis B virus; HDV: Hepatitis D virus; HBeAg: Hepatitis B e antigen; HCC: hepatocellular carcinoma; HCV: Hepatitis C virus; HIV: human immunodeficiency virus; GGT: gamma glutamyl transpeptidase; IFN: interferon; MELD: model for end-stage liver disease; NUCs: nucleos(t)ide analogues; PLT: platelets; Q1: quarter 1; Q3: quarter 3.

Table 4
A. Comorbidities by Age Class in Patients With and Without Anti-HDV Antibodies.

	Age ≤ 55 years			Age > 55 Years		
	Anti-HDV Positive N = 215 N (%)	Anti-HDV Negative N = 1516 N (%)	*P*-Value	Anti-HDV Positive N = 207 N (%)	Anti-HDV Negative N = 2214 N (%)	*P*-Value
Autoimmune diseases	12 (5.6)	127 (8.4)	*0.158*	11 (5.3)	301 (13.6)	*0.001*
Hypertension	19 (8.8)	106 (7.0)	*0.328*	51 (24.6)	685 (30.9)	*0.059*
Other cardio-vascular diseases	2 (0.9)	143 (2.8)	*0.100*	16 (7.7)	247 (11.2)	*0.130*
Diabetes	3 (1.4)	49 (3.2)	*0.140*	26 (12.6)	326 (14.7)	*0.398*
BMI ≥ 25	63 (29.3)	524 (34.6)	*0.127*	78 (37.7)	1033 (46.7)	*0.013*
Neuro/psychiatric Disturbances	17 (7.9)	66 (4.3)	*0.022*	8 (3.9)	142 (6.4)	*0.146*
Solid tumors (non-HCC)	6 (2.8)	59 (3.9)	*0.427*	15 (7.2)	243 (11.0)	*0.096*
Blood tumors	7 (3.3)	60 (4.0)	*0.618*	16 (7.7)	191 (8.6)	*0.659*
Kidney diseases	10 (4.6)	53 (3.5)	*0.397*	11 (5.3)	177 (8.0)	*0.168*
Digestive diseases	5 (2.3)	17 (1.1)	*0.180*	8 (1.5)	34 (3.9)	*0.023*
Other (respiratory, skin)	9 (4.2)	56 (3.7)	*0.722*	9 (4.3)	138 (6.2)	*0.277*

BMI: body mass index; HCC: hepatocellular carcinoma; HDV-RNA: hepatitis D virus ribonucleic acid; Peg-IFN: peg-interferon.

B. Eligibility to peg-interferon (IFN) of HDV-RNA positive patients (n = 165) estimated by the presence of liver-related contraindications and extra-hepatic comorbidities

	Absolute Contraindications+	Relative Contraindications++	Eligible to peg-IFN
HDV RNA Positive N (%)	67 (40.6%)	21 (12.7%)	77 (46.7%)*

+ Absolute contraindications: Child B/C cirrhosis; Child A cirrhosis with portal hypertension *(ascites, esophageal varices, platelets <100 × 10^3/μL)*; Portal thrombosis; Autoimmune diseases *(hepatitis, LES, rheumatoid arthritis, thyroiditis)*; Psychiatric disturbances; Ischemic heart disease; Ischemic brain disease; IBD; Celiac disease; Psoriasi; Solid tumors under chemotherapy.
++ Relative Contraindications: Age ≥70 years; Renal failure grade 4-5; Thalassemia trait; NH lymphoma.
* Among these patients, 32 (41.6%) had received IFN therapy.

with the natural history of HDV cirrhosis as shown in recent studies [29,30]. Interestingly, in a cohort of anti-HDV-positive patients whose frozen sera were tested for HDV-RNA (94% HDV-RNA positive at baseline), 46% lost HDV-RNA during a follow-up of about 15 years [31]. The prevalence of HCC was higher in patients with anti-HDV compared with those with HBV mono-infection, as observed in other studies [32] or meta-analyses [33], underlying the potential association of HDV infection with HCC. This data reinforces the need to evaluate the role of an effective antiviral therapy to reduce HCC development risk.

This study is the first describing the hepatic and extrahepatic morbidity profile as well as cofactors for liver disease progression in patients with Hepatitis D in a representative sample population. In the current setting, 47.4% of patients with HDV infection had one or more comorbidities. No significant difference was observed in the presence of comorbidities between Italian and non-Italian HDV positive patients, despite the younger age of the latter. In contrast, comorbidities were more present in Italian HBV mono-infected patients vs. non-Italians, probably due to the older age of the Italians natives. In general, the presence of comorbidities addresses the need for a multidisciplinary approach for these patients and more should be studied on the role of HDV related liver disease management and progression. Of note, there were no differences between anti-HDV positive or negative patients in the younger cohort ($\leq$55) except for a higher frequency of psychiatric disorders in anti-HDV positive persons, which may be related to drug use. Among older patients, there is a higher presence of comorbidities in both HBV mono-infected and HDV coinfected patients, however, there was a lower prevalence among anti-HDV positive patients, which was significant for overweight/obese, autoimmune disorders and digestive disease. The prevalence of co-morbidities was markedly lower than that reported in US [14], once again underlying the difference in epidemiology and risk factors for Hepatitis D.

Peg-IFN, in combination with new anti-HDV drugs, is currently being studied as a potential new therapy for patients with chronic Hepatitis D. However, the presence of comorbidities could make patients ineligible for IFN containing therapies. In the present study, where more than 70% of the patients had cirrhosis, peg-IFN may not be a therapeutic option in about 53% of the patients; in addition, factors related to adherence and quality of life were not considered, thus, more patients than the proportion estimated in this study may not be eligible for IFN-based therapies if other factors beyond comorbidities are considered.

The study population includes consecutive HBsAg-positive patients from many centers across Italy, including those managing a few patients, which makes the sample representative of the patient population in care. The policy of the Italian Health Service also improves representativeness by covering all residents in Italy, which overcomes the limits of studies performed in countries with an insurance-based system. A limitation of this study is the heterogeneity of the methods used for HDV-RNA testing, which might have different linear ranges or detection limits; this made it difficult to explore the relationship between viral load and liver disease stage. An additional bias could be the heterogeneity of low detection limits of the tests, which might have led to missing very low levels of HDV-RNA in some instances; however, this event seems marginal since the vast majority of the patients were tested using assays having an acceptable LOD. In patients with cirrhosis, a high proportion of anti-HDV-positive patients might have lost HDV-RNA, as confirmed by other studies [29,30,31]. In addition, the prevalence and characteristics of HDV-RNA-positive patients in this study align with other studies [12,34]. Finally, the present uncertainty in the interpretation of liver stiffness measures and other noninvasive methods for significant fibrosis in HDV patients [26] might have misdiagnosed cirrhosis in the subgroup of patients who were not diagnosed by liver biopsy or by the presence of clear clinical signs.

In conclusion, this study depicts a comprehensive epidemiological and clinical profile of patients with HDV infection in a European Mediterranean country. The morbidity pattern of patients with CHD is complex, and a holistic clinical approach is required to address present and near-future needs for their care.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval

The study was conducted following the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. The study protocol was approved by the Ethics Committee of the Istituto Superiore di Sanità on 24th July 2019, and by the local Ethics Committees of each clinical center. Patients' data were evaluated through pseudonymous analysis, using codes generated by the electronic case report forms. Written informed consent was obtained from all patients to participate in the PITER study.

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Author's contribution

Conceptualization: LAK, GBG, GB, BC, MGQ. Data curation: MGQ, MET. Formal analysis: MET. Supervision: LAK. Validation: LAK, GB, BC, MET, MGQ. Patients' recruitment, clinical assessments, data collection: GB, BC, VM, AC, FM, VC, EC, VR, MC, IC, FRP, LC, RC, FL, EB, AR, FBarb, NC, MS, FBarc, ST, MV, MM, FPR, AF, DL, VDM PB, AM, GM, AF, DI, AR, FGF, AS, IM, LC, MMi, ERDT, SM, LC, IG, PT, MB, LS, LB, AP, ADS, MP, ED, GAN, ALZ, AC, GR, TAS. Writing-original draft: LAK, GBG, MRB. Writing-review and editing: LAK, GBG, GB, BC, MRB, MET, MGQ. All the authors have read and approved the final manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.107115.

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