



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

Loreta A. Kondili^{1,2,*}, Giuseppina Brancaccio³, Maria Elena Tosti¹, Barbara Coco⁴, Maria Giovanna Quaranta¹, Vincenzo Messina⁵, Alessia Ciancio⁶, Filomena Morisco⁷, Valentina Cossiga⁷, Ernesto Claar⁸, Valerio Rosato⁸, Marianna Ciarallo⁹, Irene Cacciola¹⁰, Francesca Romana Ponziani¹¹, Lucia Cerrito¹¹, Roberta Coppola¹², Francesco Longobardi¹², Elisa Biliotti¹³, Alessia Rianda¹³, Francesco Barbaro¹⁴, Nicola Coppola¹⁵, Maria Stanzone¹⁵, Francesco Barchiesi¹⁶, Stefano Fagiuoli¹⁷, Mauro Viganò¹⁷, Marco Massari¹⁸, Francesco Paolo Russo¹⁹, Alberto Ferrarese²⁰, Diletta Laccabue²¹, Vito Di Marco²², Pierluigi Blanc²³, Aldo Marrone²⁴, Giulia Morsica²⁵, Alessandro Federico²⁶, Donatella Ieluzzi²⁷, Alba Rocco²⁸, Francesco Giuseppe Foschi²⁹, Alessandro Soria³⁰, Ivana Maida³¹, Luchino Chessa³², Michele Milella³³, Elena Rosselli Del Turco³⁴, Salvatore Madonia³⁵, Liliana Chemello³⁶, Ivan Gentile³⁷, Pierluigi Toniutto³⁸, Matteo Bassetti³⁹, Lorenzo Surace⁴⁰, Leonardo Baiocchi⁴¹, Adriano Pellicelli⁴², Adriano De Santis⁴³, Massimo Puoti⁴⁴, Elisabetta Degasperi⁴⁵, Grazia Anna Niro⁴⁶, Anna Linda Zignego⁴⁷, Antonio Craxi⁴⁸, Giovanni Raimondo¹⁰, Teresa Antonia Santantonio⁹, Maurizia Rossana Brunetto⁴⁹, Giovanni Battista Gaeta⁵⁰, on behalf of PITER Collaborating Investigators[#]

¹ Center for Global Health, Istituto Superiore di Sanità, Rome, Italy

² UniCamillus-Saint Camillus International University of Health Sciences, Rome, Italy

³ Department of Molecular Medicine, Infectious Diseases, University of Padua, Padua, Italy

⁴ Hepatology Unit, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy

* Corresponding author: Loreta A. Kondili, Center for Global Health, Istituto Superiore di Sanità, Rome, Italy.

E-mail address: loreta.kondili@iss.it (L.A. Kondili).

[#] **PITER Collaborating Investigators:** Alessio Aghemo (IRCCS Humanitas Research Hospital IRCCS, Rozzano, Italy); Chiara Baiguera (Niguarda Hospital, Milan, Italy); Pier Maria Battezzati (San Paolo Hospital, University of Milan, Italy); Sara Battistella (University of Padua, Italy); Maria Grazia Bavetta (Villa Sofia-Cervello Hospital, Palermo, Italy); Costanza Bertoni (IRCCS Ospedale San Raffaele and Università Vita-Salute San Raffaele, Milan, Italy); Carolina Boni (Azienda Ospedaliero-Universitaria di Parma, Parma, Italy); Department of Medicine and Surgery, Università degli Studi di Parma, Parma, Italy); Paola Brambilla (ASST Cremona, Cremona, Italy); Antonella Bray (ASL Brindisi, Università di Bari, Italy); Federica Briano (San Paolo Hospital, Savona, Italy); Enrico Carmenini (Azienda Ospedaliera San Camillo Forlanini, Rome, Italy); Francesco Castelli (Spedali Civili and University of Brescia, Brescia, Italy); Luisa Cavalletto (University Hospital of Padua, Italy); Federica Cerini (San Giuseppe Hospital, Milan, Italy); Luciana Chidichimo (SS Annunziata Hospital, Cosenza, Italy); Elisa Colella (Fondazione IRCCS San Gerardo dei Tintori Monza, Italy); Giuliana Cologni (Papa Giovanni XXIII Hospital, Bergamo, Italy); Silvia Como (University of Palermo, Palermo, Italy); Romina Corsini (Azienda Unità Sanitaria Locale, IRCCS di Reggio Emilia, Reggio Emilia, Italy); Chiara Costa ("Magna Graecia" University, Catanzaro, Italy); Rosa Cotugno (Fondazione IRCCS 'Casa Sollievo della Sofferenza', San Giovanni Rotondo, Foggia, Italy); Silvia Cretella (Alma Mater Studiorum Bologna University, Italy); Fernando De Angelis ("Policlinico Umberto I" Hospital, Sapienza University of Rome, Rome, Italy); Pasqualina De Leo (San Paolo Hospital, Savona, Italy); Giovanni Di Perri (University of Turin, Turin, Italy); Elisabetta Falbo (Azienda Sanitaria Provinciale CZ-Distretto del Lametino, Lamezia Terme (CZ), Italy); Luigina Ferrigno (Istituto Superiore di Sanità, Rome, Italy); Ezio Fornasiero (Azienda Sanitaria Universitaria Integrata di Udine, Udine, Italy); Daniela Francisci (AOS Maria della Misericordia, Perugia, Italy); Pietro Gatti (Department of Internal Medicine, ASL Brindisi, Università di Bari, Italy); Pietro Lampertico (Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico; CRC "A. M. and A. Migliavacca" Center for Liver Disease, University of Milan, Milan, Italy); Ilaria Lenci (University of Tor Vergata, Rome, Italy); Anna Licata (University of Palermo, Palermo, Italy); Ivana Maida (University of Sassari, Sassari, Italy); Alfredo Marzano (San Giovanni Battista Hospital, Turin, Italy); Antonio Mastroianni (SS Annunziata Hospital, Cosenza, Italy); Cesare Mazzaro (IRCCS Centro di Riferimento Oncologico (CRO), Aviano, Pordenone, Italy); Monica Monti (Interdepartmental Centre MASVE, University of Florence, Italy); Gerardo Nardone (University of Naples Federico II, Naples, Italy); Laura Ambra Nicolini (Università degli Studi di Genova, Policlinico S. Martino IRCCS, Genova, Italy); Nicola Passigato (University Hospital Borgo Trento, Verona, Italy); Maria Bruna Pasticci (Azienda Ospedaliera "Santa Maria" Terni, Italy); Piera Pierotti (Santa Maria Annunziata Hospital, Florence, Italy); Biagio Pinchera (University of Naples Federico II, Naples, Italy); Teresa Pollicino (University Hospital of Messina); Carmen Porcu (University Hospital, Monserrato, Cagliari, Italy); Giulia Quartini (Azienda Ospedaliera "Santa Maria" Terni, Italy); Gabriele Rancatore (University of Palermo, Palermo, Italy); Mario Romeo (University of Campania Luigi Vanvitelli, Naples, Italy); Maria Grazia Rumi (San Giuseppe Hospital, Milan, Italy); Annalisa Saracino (University of Bari, University Hospital Policlinico, Bari, Italy); Ornella Schioppa (IRCCS Centro di Riferimento Oncologico (CRO), Aviano, Pordenone, Italy); Ilaria Serio (IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy); Roberta Soffredini (Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy); Marco Tizzani (San Giovanni Battista Hospital, Turin, Italy); Matteo Tonnini (IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy); Carlo Torti ("Magna Graecia" University, Catanzaro, Italy); Daniela Valenti (Papa Giovanni XXIII Hospital, Bergamo, Italy); Tata Xhimi (Istituto Superiore di Sanità, Rome, Italy); Serena Zaltron (Spedali Civili and University of Brescia, Brescia, Italy); Alessia Zoncada (ASST Cremona, Cremona, Italy).

- ⁵ Department of Infectious Diseases, Sant'Anna Hospital, Caserta, Italy
- ⁶ Gastroenterology Unit, Città della Salute e della Scienza of Turin, University Hospital, Turin, Italy
- ⁷ Liver and Biliary System Unit, Department of Clinical Medicine and Surgery, University of Naples, Naples, Italy
- ⁸ Hepatology Unit, Betania Hospital, Naples, Italy
- ⁹ Infectious Diseases Unit, Ospedali Riuniti, Foggia, Italy
- ¹⁰ Department of Internal Medicine, University Hospital of Messina, Messina, Italy
- ¹¹ Liver Unit, Digestive Disease Center, CEMAD Division of Internal Medicine and Gastroenterology, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy
- ¹² Department of Hepatology, Gragnano Hospital, Gragnano (NA), Italy
- ¹³ National Institute for Infectious Diseases, Lazzaro Spallanzani-IRCCS, Rome, Italy
- ¹⁴ Department of Medicine, Infectious Diseases Unit, University Hospital of Padua, Padua, Italy
- ¹⁵ Infectious Diseases Unit, Department of Mental Health and Public Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy
- ¹⁶ Clinical Infectious Diseases, Polytechnic University of Marche, Ancona, Italy
- ¹⁷ Department of Medicine, University of Milan Bicocca & Gastroenterology Hepatology and Transplantation, Papa Giovanni XXIII Hospital, Bergamo, Italy
- ¹⁸ Malattie Infettive, Azienda Sanitaria Locale, IRCCS di Reggio Emilia, Reggio Emilia, Italy
- ¹⁹ Department of Surgery, Oncology and Gastroenterology, Gastroenterology Unit, University of Padua, Padua, Italy
- ²⁰ Gastroenterology Unit, University Hospital Borgo Trento, Verona, Italy
- ²¹ Department of Medicine and Surgery, Università degli Studi di Parma, Parma, Italy.
- ²² Biomedical Department of Internal and Specialistic Medicine University of Palermo, Unit of Gastroenterology and Hepatology, Palermo, Italy
- ²³ Infectious Disease Unit, Santa Maria Annunziata Hospital, Florence, Italy
- ²⁴ Department of Advanced Medical and Surgical Sciences, University of Campania Luigi Vanvitelli, Naples, Italy
- ²⁵ Unit of Infectious Diseases, IRCCS Ospedale San Raffaele, Milan, Italy,
- ²⁶ Hepato-Gastroenterology Division, Department of Precision Medicine, University of Campania Luigi Vanvitelli, Naples, Italy
- ²⁷ Liver Unit, University Hospital of Verona, Verona, Italy
- ²⁸ Department of Clinical Medicine and Surgery, Hepato-Gastroenterology Unit, University of Naples Federico II, Naples, Italy
- ²⁹ Internal Medicine, Hospital of Faenza, A.U.S.L. of Romagna, Italy
- ³⁰ Clinic of Infectious Diseases, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy
- ³¹ Infectious and Tropical Diseases Unit, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy
- ³² Liver Unit, University Hospital, Monserrato, Cagliari, Italy
- ³³ Clinic of Infectious Diseases, University of Bari, University Hospital Policlinico, Bari, Italy
- ³⁴ Infectious Diseases Unit, Department for Integrated Infectious Risk Management, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
- ³⁵ Department of Internal Medicine Villa Sofia-Cervello Hospital, Palermo, Italy
- ³⁶ Department of Medicine-DIMED, Padua University, University Hospital, Padua, Italy
- ³⁷ Department of Clinical Medicine and Surgery, University of Naples Federico II, Naples, Italy
- ³⁸ Hepatology and Liver Transplant Unit, Azienda Sanitaria Universitaria Integrata di Udine, Udine, Italy
- ³⁹ Clinica Malattie Infettive, Università degli Studi di Genova, Policlinico S. Martino IRCCS, Genova, Italy
- ⁴⁰ Ambulatorio di Epatologia e Infettivologia, Azienda Sanitaria Provinciale CZ-Distretto del Lametino, Lamezia Terme (CZ), Italy
- ⁴¹ Hepatology Unit, University of Tor Vergata, Rome, Italy
- ⁴² Hepatology Unit, Azienda Ospedaliera San Camillo Forlanini, Rome, Italy
- ⁴³ Department of Internal Medicine, Policlinico Umberto I Hospital, Sapienza University of Rome, Rome, Italy
- ⁴⁴ Infectious Disease Unit, Niguarda Hospital, Milan, Italy
- ⁴⁵ Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
- ⁴⁶ Division of Gastroenterology and Endoscopy, Fondazione IRCCS 'Casa Sollievo della Sofferenza', San Giovanni Rotondo, Foggia, Italy
- ⁴⁷ Department of Experimental and Clinical Medicine, Interdepartmental Centre MASVE, University of Florence, Italy
- ⁴⁸ Gastroenterology and Hepatology Unit, PROMISE, University of Palermo, Palermo, Italy
- ⁴⁹ Department of Clinical and Experimental Medicine, University Hospital of Pisa, Pisa, Italy
- ⁵⁰ Infectious Disease Unit, University L. Vanvitelli, Naples, Italy

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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.

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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/m². 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 Fisher's 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 \pm 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

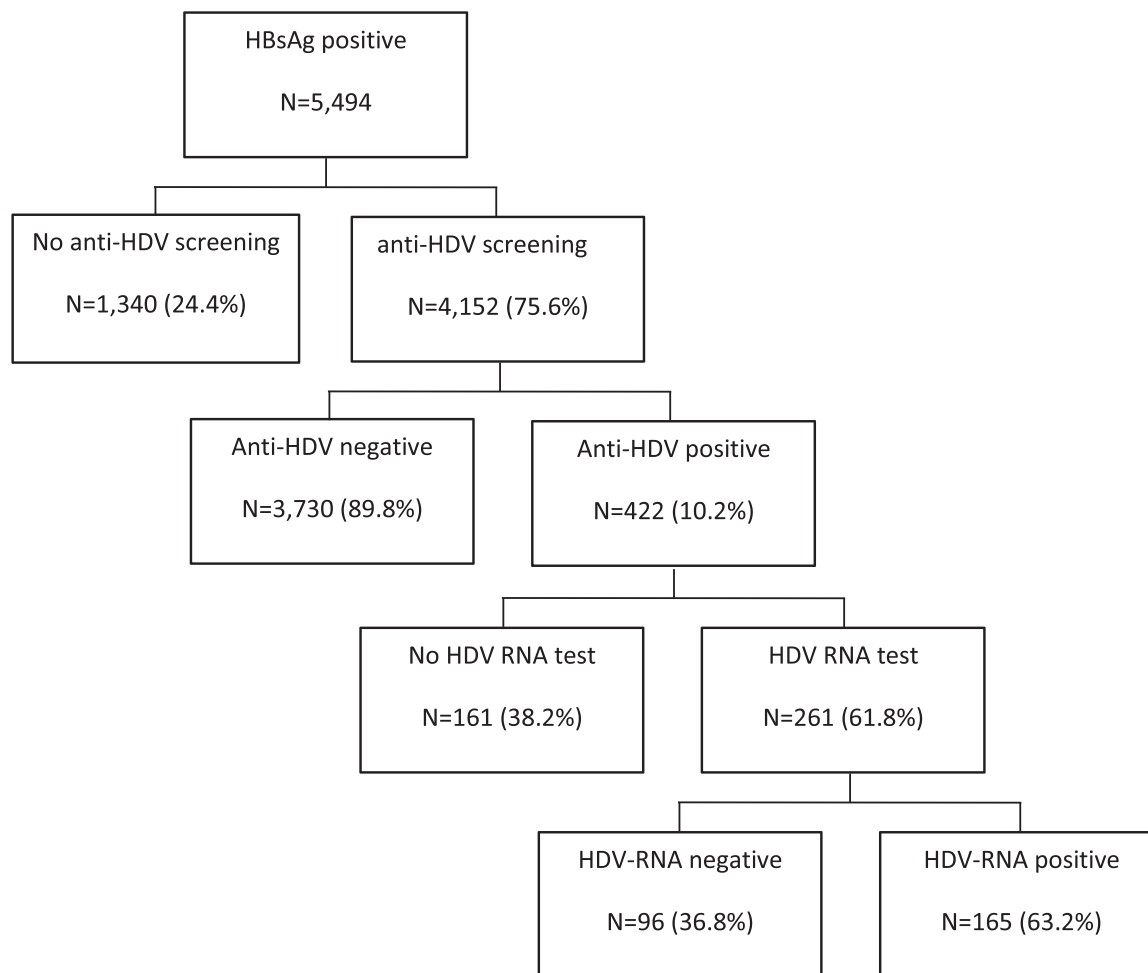


Figure 1. Flow chart of the patients enrolled in the study.

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 extrahepatic 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 infec-

tion. 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)	3 (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		P-Value	Age > 55 Years		P-Value
	Anti-HDV Positive N = 215 N (%)	Anti-HDV Negative N = 1516 N (%)		Anti-HDV Positive N = 207 N (%)	Anti-HDV Negative N = 2214 N (%)	
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 × 10³/μL*); Portal thrombosis; Autoimmune diseases (*hepatitis, LES, rheumatoid arthritis, thyroiditis*); Psychiatric disturbances; Ischemic heart disease; Ischemic brain disease; IBD; Celiac disease; Psoriasis; 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 (≤ 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 coinfecting 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](https://doi.org/10.1016/j.ijid.2024.107115).

References

- [1] Farci P, Niro G. Clinical features of Hepatitis D. *Semin Liver Dis* 2012;32:228–36. doi:10.1055/s-0032-1323628.
- [2] Gaeta GB, Stroffolini T, Chiamonte M, Ascione T, Stornaiuolo G, Lobello S, et al. Chronic hepatitis D: a vanishing disease? An Italian multicenter study. *Hepatology* 2000;32:824–7. doi:10.1053/jhep.2000.17711.

- [3] Stockdale AJ, Kreuels B, Henrion MYR, Giorgi E, Kyomuhangi I, de Martel C, et al. The global prevalence of hepatitis D virus infection: systematic review and meta-analysis. *J Hepatol* 2020;**73**:523. doi:10.1016/j.jhep.2020.04.008.
- [4] Asselah T, Rizzetto M. Hepatitis D virus infection. *New Engl J Med* 2023;**389**:58–70. doi:10.1056/nejmra2212151.
- [5] Heidrich B, Deterding K, Tillmann HL, Raupach R, Manns MP, Wedemeyer H. Virological and clinical characteristics of delta hepatitis in Central Europe. *J Viral Hepat* 2009;**16**:883–94. doi:10.1111/j.1365-2893.2009.01144.x.
- [6] Cross TJS, Rizzi P, Horner M, Jolly A, Hussain MJ, Smith HM, et al. The increasing prevalence of hepatitis delta virus (HDV) infection in South London. *J Med Virol* 2008;**80**:277–82. doi:10.1002/jmv.21078.
- [7] Aguilera A, Trastoy R, Rodríguez-Calviño J, Manso T, De Mendoza C, Soriano V. Prevalence and incidence of hepatitis delta in patients with chronic hepatitis B in Spain. *Eur J Gastroenterol Hepatol* 2018;**30**:1060–2. doi:10.1097/MEG.0000000000001163.
- [8] Brancaccio G, Nardi A, Madonia S, Fasano M, Verucchi G, Massari M, et al. The present profile of chronic hepatitis B virus infection highlights future challenges: an analysis of the Multicenter Italian MASTER-B cohort. *Digest Liver Disease* 2019;**51**:438–42. doi:10.1016/j.dld.2018.09.008.
- [9] Patel EU, Thio CL, Boon D, Thomas DL, Tobian AAR. Prevalence of Hepatitis B and Hepatitis D virus infections in the United States, 2011–2016. *Clinical Infectious Diseases* 2019;**69**:709–12. doi:10.1093/cid/ciz001.
- [10] Roulot D, Briclher S, Layese R, BenAbdesselam Z, Zoulim F, Thibault V, et al. Origin, HDV genotype and persistent viremia determine outcome and treatment response in patients with chronic hepatitis delta. *J Hepatol* 2020;**73**:1046–62. doi:10.1016/j.jhep.2020.06.038.
- [11] Kamal H, Westman G, Falconer K, Duberg AS, Weiland O, Haverinen S, et al. Long-term study of Hepatitis delta virus infection at secondary care centers: the impact of viremia on liver-related outcomes. *Hepatology* 2020;**72**:1177–90. doi:10.1002/hep.31214.
- [12] Papatheodoridis G, Mimidis K, Manolakopoulos S, Triantos C, Vlachogiannakos I, Veretanos C, et al. HERACLIS-HDV cohort for the factors of underdiagnosis and prevalence of hepatitis D virus infection in HBsAg-positive patients. *Liver International* 2023;**43**:1879–89. doi:10.1111/liv.15638.
- [13] Wranke A, Pinheiro Borzacov LM, Parana R, Lobato C, Hamid S, Ceausu E, et al. Clinical and virological heterogeneity of hepatitis delta in different regions world-wide: the Hepatitis Delta International Network (HDIN). *Liver International* 2018;**38**:842–50. doi:10.1111/liv.13604.
- [14] Gish RG, Jacobson IM, Lim JK, Waters-Banker C, Kaushik A, Kim C, et al. Prevalence and characteristics of hepatitis delta virus infection in patients with hepatitis B in the United States: an analysis of the all-payer claims database. *Hepatology* 2024;**79**:1117–28. doi:10.1097/HEP.0000000000000687.
- [15] Romeo R, Del Ninno E, Rumi M, Russo A, Sangiovanni A, de Franchis R, et al. A 28-year study of the course of hepatitis δ infection: a risk factor for cirrhosis and hepatocellular carcinoma. *Gastroenterology* 2009;**136**:1629–38. doi:10.1053/j.gastro.2009.01.052.
- [16] Palom A, Rodríguez-Tajes S, Navascués CA, García-Samaniego J, Riveiro-Barciela M, Lens S, et al. Long-term clinical outcomes in patients with chronic hepatitis delta: the role of persistent viraemia. *Aliment Pharmacol Ther* 2020;**51**:158–66. doi:10.1111/apt.15521.
- [17] Brancaccio G, Coco B, Nardi A, Quaranta MG, Tosti ME, Ferrigno L, et al. Trends in chronic hepatitis B virus infection in Italy over a 10-year period: Clues from the nationwide PITER and MASTER cohorts toward elimination. *Int J Infect Diseases* 2023;**129**:266–73. doi:10.1016/j.ijid.2023.02.006.
- [18] Ricco G, Coco B, Colombatto P, Oliveri F, Cavallone D, Blevé P, et al. Highly dynamic changes of regional HBV epidemiology over two decades. *Digest Liver Disease* 2023;**55**:519–26. doi:10.1016/j.dld.2022.11.003.
- [19] Polaris Observatory Collaborators Adjusted estimate of the prevalence of hepatitis delta virus in 25 countries and territories. *J Hepatol* 2024;**80**:232–42. doi:10.1016/j.jhep.2023.10.043.
- [20] Stroffolini T, Almasio PL, Sagnelli E, Mele A, Gaeta GB, Andreone P, et al. Evolving clinical landscape of chronic hepatitis B: a multicenter Italian study. *J Med Virol* 2009;**81**:1999–2006. doi:10.1002/jmv.21643.
- [21] Fasano M, Milella M, Carbonara S, Tundo P, Minniti S, Buccoliero G, et al. Apulian infectious diseases network: survey on the prevalence of delta infection among chronic HBV carriers in Apulia. *Front Public Health* 2023;**26**:1247454. doi:10.3389/fpubh.2023.1247454.
- [22] Salpini R, Piermatteo L, Torre G, D'Anna S, Khan S, Duca L, et al. Prevalence of hepatitis D virus infection in Central Italy has remained stable across the last 2 decades with dominance of subgenotypes 1 and characterized by elevated viral replication. *Int J Infect Dis* 2024;**138**:1–9. doi:10.1016/j.ijid.2023.11.005.
- [23] Rizzetto M, Hamid S, Negro F. The changing context of Hepatitis D. *J Hepatol* 2021;**74**:1200–11. doi:10.1016/j.jhep.2021.01.014.
- [24] Wong RJ, Kaufman HW, Niles JK, Chen C, Yang Z, Kapoor H, et al. Low performance of hepatitis delta virus testing among 2 national cohorts of chronic Hepatitis B patients in the United States. *Am J Gastroenterol* 2022;**117**:2067–70. doi:10.14309/ajg.0000000000001947.
- [25] Terrault NA, Ghany MG. Enhanced screening for Hepatitis D in the USA: overcoming the delta blues. *Dig Dis Sci* 2021;**66**:2483–5. doi:10.1007/s10620-020-06584-w.
- [26] Brunetto MR, Ricco G, Negro F, Wedemeyer H, Yurdaydin C, Asselah T, et al. EASL clinical practice guidelines on hepatitis delta virus. *J Hepatol* 2023;**79**:433–60. doi:10.1016/j.jhep.2023.05.001.
- [27] Da BL, Surana P, Kleiner DE, Heller T, Koh C. The Delta-4 fibrosis score (D4FS): a novel fibrosis score in chronic hepatitis D. *Antiviral Res* 2020;**174**:104691. doi:10.1016/j.antiviral.2019.104691.
- [28] Stroffolini T, Sagnelli E, Gaeta GB, Sagnelli C, Andriulli A, Brancaccio G, et al. Characteristics of liver cirrhosis in Italy: evidence for a decreasing role of HCV aetiology. *Eur J Intern Med* 2017;**38**:68–72. doi:10.1016/j.ejim.2016.10.012.
- [29] Caviglia GP, Martini S, Ciancio A, Niro GA, Olivero A, Fontana R, et al. The hepatitis D virus in Italy. A vanishing infection, not yet a vanished disease. *J Adv Res* 2021;**33**:183–7. doi:10.1016/j.jare.2021.02.009.
- [30] Palom A, Sopena S, Riveiro-Barciela M, Carvalho-Gomes A, Madejón A, Rodríguez-Tajes S, et al. One-quarter of chronic hepatitis D patients reach HDV-RNA decline or undetectability during the natural course of the disease. *Aliment Pharmacol Ther* 2021;**54**:462–9. doi:10.1111/apt.16485.
- [31] Mangia A, Squillante M M, Fraticelli F, Cavorsi M C, Paroni G, Zaffarano L, et al. HDV RNA levels and progression of hepatitis delta infection: a 14 year follow up experience in Italy. *Cells* 2023;**12**:1413. doi:10.3390/cells12101413.
- [32] Brancaccio G, Fasano M, Grossi A, Santantonio TA, Gaeta GB. Clinical outcomes in patients with hepatitis D, cirrhosis and persistent hepatitis B virus replication, and receiving longterm tenofovir or entecavir. *Aliment Pharmacol Ther* 2019;**49**:1071–6. doi:10.1111/apt.15188.
- [33] Alfaiate D, Clément S, Gomes D, Goossens N, Negro F. Chronic hepatitis D and hepatocellular carcinoma: a systematic review and meta-analysis of observational studies. *J Hepatol* 2020;**73**:533–9. doi:10.1016/j.jhep.2020.02.030.
- [34] d'Arminio Monforte A, Tavelli A, Salpini R, Piermatteo L, D'Anna S, Carrara S, et al. Determinants of worse liver-related outcome according to <sc>HDV</sc>infection among <sc>HBsAg</sc>positive persons living with <sc>HIV</sc> : data from the <sc>ICONA</sc>cohort. *Liver Int* 2024;**44**:603–13. doi:10.1111/liv.15804.