DDR Spain Study: Nationwide Reflex Testing and Characterization of Hepatitis Delta Virus Infection

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BACKGROUND

Hepatitis delta virus (HDV) infection in people with hepatitis B virus (HBV) accelerates the progression of liver disease and is associated with a higher risk of cirrhosis and hepatocellular carcinoma. The DDR Spain initiative was designed to harmonize HDV diagnosis across the country through reflex anti-HDV and HDV-RNA testing, while providing a comprehensive description of individuals with HDV in Spain.

METHODS

DDR Spain is a multicenter, nationwide, ambispective, observational study, including a retrospective evaluation of diagnostic practices in 2022–2024 and a prospective arm initiated in 2024. The prospective phase systematically collects clinical, demographic, and virological information from individuals who tested anti-HDV-positive enrolled in participating centers.

RETROSPECTIVE PHASE

Between 2022 and 2024, 65,763 HBsAg-positive individuals were included from 80 centers in 16 out of Spain's 17 Autonomous Communities. Reflex anti-HDV implementation rose from 55.1% in 2022 to 85.8% in 2024 (p<0.05), while anti-HDV prevalence remained stable (5.6%, 4.9%, 5.3%). HDV-RNA coverage increased from 72.6% in 2022 to 91% in 2024(p<0.05), and the proportion of active HDV infection declined from 40.3% to 31% (Figure1). However, regional heterogeneity was detected (Figure 2)

Figure 1. Delta Double Reflex Uptake in Spain (2022–2024).

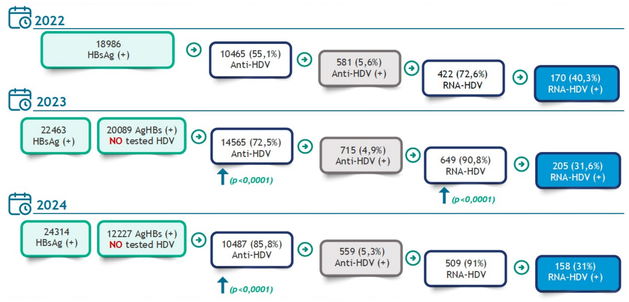
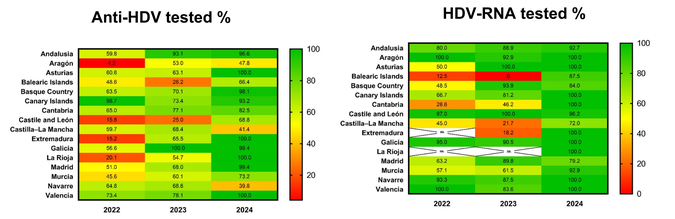


Figure 2. DDR Uptake by Autonomous Communities



This nationwide Spanish study provides a timely assessment of real-world implementation of double reflex diagnostic (DDR) testing for hepatitis D virus (HDV) across Spain. We show that nationwide DDR implementation is feasible and clinically impactful, improving case identification, while also revealing remaining gaps that require diagnostic harmonization and organizational alignment among centers. In parallel, our prospective cohort offers updated and unique data on HDV epidemiology and clinical characteristics in Spain. Notably, a substantial proportion of HDV-infected patients present with advanced liver fibrosis, underscoring the need for earlier detection and streamlined diagnostic pathways.

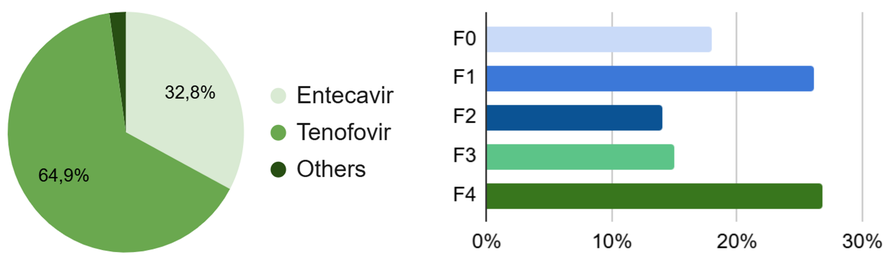
PROSPECTIVE PHASE

By January 2025, **589 individuals** who tested anti-HDV-positive were prospectively recruited. As shown below, median age was 54 years (IQR 42–61), 59% male, and 50% Spanish-born, 19% were persons living with HIV (PLWH) and 20% with HCV. Indicators of advanced fibrosis were notable: 26% had FIB-4 >2.67 and among 247 individuals with transient elastography, mean liver stiffness was 10.4 kPa; 15% had METAVIR F3 and 27% F4. Previous hepatic decompensation was documented in 22% of evaluable cases. Advanced fibrosis was significantly higher for HDV-RNA positive patients [p<0,001 vs HCV-RNA negative (17%)]. Chronic active infection was significantly higher in coinfected PLWH [p<0,05 compared to non-HIV coinfected (25%), and to HCV coinfected (24%)]. Genotype 1 was predominant (90%).

Age(median, IQR)	54 (42 - 61)
Sex (male)	346 (59 %)
Origin(Spain)	292 (50 %)
HIV(Ab-positive)	92/492 (19 %)
HCV(Ab-positive)	105/527 (20 %)
Advanced fibrosis (**FIB-4 > 2.67**)	26 %

HDV-RNA positive	41 %
Median RNA-HDV (log)	4.03 (2.6-5.1)
Genotype 1 (%)	90 %
FIB-4 > 2.67	39 %
PLWH, HDV-RNA positive	49 %
PLWH, **FIB-4 > 2.67**	37%
HCV pos, HDV-RNA positive	53 %
HCV pos, **FIB-4 > 2.67**	38%

Half of patients enrolled in the cohort were under HBV treatment, 65% on tenofovir and most of the rest on entecavir. Median liver stifness, measured through transient elastography was 7.8 kPa (IQR: 5.5-11.7). Fibrosis stage, according to METAVIR is shown below.



PROSPECTIVE PHASE

Chronic active hepatitis (HDV-RNA +) was analyzed using univariate and multivariable models. In multivariate analysis, HBV treatment was associated with a higher risk of active infection, whereas older age, higher platelet count, and higher HBV viral load were associated with a lower risk.

Univariate Analysis (Quantitative variables)	Active infection median (q1-q3)	Non-active infection median (q1-q3)
Age	49 (41-58)	56 (43.75-62)
Platelets (x10^9/µl)	158 (103.75-196)	215 (159-257)
Glucose (mg/dL)	87 (79.25-96.75)	90 (82-101)
AST (GOT) (IU/L)	50 (38–72)	27 (21–36)
ALT (GPT) (IU/L)	53.5 (35.25–79)	23 (18–32)
GGT (IU/L)	49 (27–91.9)	25.5 (17–48)
Total bilirubin (mg/dL)	0.7 (0.5–1.04)	0.6 (0.4–0.85)
Albumin (g/dL)	4.1 (3.7–4.425)	4.3 (4–4.5)
INR	1.11 (1.04–1.21)	1.03 (0.96–1.1)
FIB_4	2 (1.3–4.025)	1.4 (1–2.1)
HBV-DNA (IU/mL)	0 (0–11.5)	10 (0–365)
qHBsAg (IU/mL)	320 (67.035–3,705.84)	59.155 (3–2,247.645)
Transient Elastography (kPa)	9.85 (7.175–13)	6.05 (4.4–8.3)

Univariate Analysis (Qualitative variables)	p-value (X^2)
HBV treatment (yes/no)	5.34E-12
Degree of liver fibrosis (F0-F4)	1.16E-12

Multivariate Analysis	Odd Ratio	IC 95%	p-value
HBV treatment (vs no)	2.307	1.410-3.803	0.001
Age (x10 years)	0.955	0.936-0.973	3.31E-06
Platelets (x10*10^3/µl)	0.920	0.883-0.957	5.08E-05
ADN-VHB (log10 UI/mL + 1)	0.834	0.722-0.956	0.011

To prevent overfitting, the number of variables in the multivariable model was limited; clinically relevant variables such as qHBsAg were excluded due to a marked reduction in sample size.

Significant liver damage defined as FIB-4 >2.67 was analyzed using univariate and multivariable models. In multivariate analysis, HBV treatment, active HDV infection, GGT and total bilirubin were associated with a higher risk of elevated FIB-4, whereas higher hemoglobin levels were associated with a lower risk.

Univariate Analysis (Quantitative variables)	**fib4>2,67** median (q1-q3)	**fib≤2,67** median (q1-q3)
Hemoglobin (g/dL)	13.7 (12.3-14.6)	14.5 (13.4-15.45)
GGT (IU/L)	62 (38-112)	28 (19-50)
Total bilirubin (mg/dL)	1 (0.665-1.56)	0.54 (0.4-0.775)
Albumin (g/dL)	3.8 (3.26-4.4)	4.3 (4.0075-4.5)
INR	1.18 (1.09-1.38)	1.03 (0.97-1.1)
HDV-RNA (UI/mL)	673 (0-43850)	0 (0-1297.5)
HBV-DNA (IU/mL)	0 (0-35)	10 (0-190)
qHBsAg (IU/mL)	2477 (178.83-9499.7)	237 (3-3713.8)
Transient Elastography (kPa)	12.6 (8.8-19.2)	7.1 (5.02-9.6)

Univariate Analysis (Qualitative variables)	p-value (X^2)
HBV treatment (yes/no)	2,44E-10
Degree of liver fibrosis (F0-F4)	< 2,2E-16
Previous liver decompensation (yes/no)	1,27E-15
Active infection (yes/no)	2,548E-08

Multivariate Analysis	Odd Ratio	IC 95%	p-value
HBV treatment (vs no)	3,270	1,800-5,940	1,02E-04
Active infection (vs no)	2,100	1,220-3,600	0,007
Hemoglobin (x1 g/dL)	0,849	0,737-0,978	0,024
GGT (x10 IU/L)	1,040	1,010-1,080	0,009
Total bilirubin (x0.5 mg/dL)	2,060	1,530-2,790	2,59E-06

Consistent with FIB-4 findings, advanced fibrosis (METAVIR F3–F4) was further analyzed using univariate and multivariable models. In multivariate analysis, HBV treatment, active infection, and GGT remained significantly associated to a higher risk of advanced fibrosis, while platelet count was associated to a lower risk.

Univariate Analysis (Quantitative variables)	**F3-F4** median (q1-q3)	**F0-F1-F2** median (q1-q3)
Platelets (x10^9/µl)	135 (91–190,5)	214,5 (163,75–245,5)
AST (GOT) (IU/L)	50 (36–85)	29 (22–42)
ALT (GPT) (IU/L)	51 (29–84)	43,75 (20–43,75)
GGT (IU/L)	60,5 (36,5–106)	25 (17–45,75)
Total bilirubin (mg/dL)	0,815 (0,58–1,21)	0,6 (0,41–0,8)
Albumin (g/dL)	3,98 (3,4–4,3)	4,3 (4,055–4,53)
INR	1,13 (1,04–1,23)	1,03 (0,97–1,13)
FIB_4	2,8 (1,775–6,025)	1,4 (1–2)
HDV-RNA (UI/mL)	1605 (0–24375)	880 (0–880)
HBV-DNA (IU/mL)	0 (0–10)	208 (0–208)
Transient Elastography (kPa)	13,65 (10,8–18,9)	6,2 (4,505–7,6)

Univariate Analysis (Qualitative variables)	p-value (X^2)
HBV treatment (yes/no)	6,99E-07
Previous liver decompensation (yes/no)	1,13E-06
Active infection (yes/no)	1,20E-10

Multivariate Analysis	Odd Ratio	IC 95%	p-value
HBV treatment (vs no)	2,300	1,120-4,730	0,024
Active infection (vs no)	2,730	1,390-5360	0,0035
GGT (x10 IU/L)	1,120	1,040-1,210	0,00259
Platelets (x10*10^3/µl)	0,920	0,877-0,965	0,000673

CONCLUSIONS

DDR Spain represents the largest effort to implement standardized HDV diagnosis nationwide. The project demonstrates marked progress in reflex testing and HDV-RNA coverage but underscores persistent regional disparities, reinforcing the urgent need for harmonized screening strategies, professional training, and increased awareness to optimize HDV detection and management. The prospective cohort provides a detailed view of current HDV epidemiology in Spain, highlighting the important load of hepatic illness in this population

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