Universitat Autònoma de Barcelona

IMPACT OF HEPATITIS D VIRAEMIA ON PATIENT LIVER-RELATED OUTCOMES

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DOCTORAL THESIS

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ABBREVIATIONS

A

AASLD	American Association for the Study of Liver Diseases
ALT	Alanine aminotransferase
APASL	Asian-Pacific Association for the Study of the Liver
APRI	Aspartate aminotransferase to platelet ratio index
AST	Aspartate aminotransferase

B

BEA score	Baseline Event Anticipation score

C

CD4+	Cluster of differentiation 4
CD8+	Cluster of differentiation 8
cDNA	Complementary DNA
CHB	Chronic hepatitis B
CHC	Chronic hepatitis C
CHD	Chronic hepatitis D
CLDQ	Chronic liver disease questionnaire
CLIA	Chemoluminiscence immunoassay
cop	copies

D

DLD	Decompensated liver disease

DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates

E

EASL	European Association for the Study of the Liver
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immune assay
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum

F

FACIT-F	Functional Assessment of Chronic Illness Therapy–Fatigue
FIB-4	Fibrosis-4

H

HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDAg	Hepatitis D antigen
HDV	Hepatitis D virus
HIDIT	Hep-Net-International-Delta-Hepatitis-Intervention-Trial
HIV	Human immunodeficiency virus
HLoQ	High limit of quantification

HRQoL Health-related quality of life

I

ICTV	International Committee on Taxonomy of Viruses
IFN	Interferon
INR	International normalized ratio
IQR	Interquartile range
IU	International Unit

L

L	Litre
LLoD	Low limit of detection
LLoQ	Low limit of quantification
log	logarithm

M

ml	milliliter
µl	mircoliter
mRNA	Messenger RNA

N

N	Number
NA	Nucleos(t)ide Analogues
NTCP	Na/Taurocholate cotransporting polypeptide

O

OLT	Orthotopic liver transplantation
ORF	Open reading frame

P

p	p-value
pegIFN	Pegylated interferon
PROs	Patient reported outcomes

R

RNA	Ribonucleic acid
RNP	Ribonucleoprotein
RT-qPCR	Retrotranscriptase quantitative polymerase chain reaction
RUO	Research use only

S

SST	Serum separator tube

W

WHO	World Health Organization
WPAI	Work Productivity and Activity Impairment

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SUMMARY

Hepatitis D virus (HDV) is an hepatotropic and defective RNA virus that requires the presence of hepatitis B virus (HBV) to propagate. HDV approximately affects 20 million people worldwide. This virus causes one of the most severe forms of chronic liver disease, since the risk of developing liver cirrhosis or liver-related outcomes is between two and three times higher than in those mono-infected by HBV.

The impact of active HDV infection, defined as detectable HDV-RNA, in the progression of chronic hepatitis D (CHD) has been scarcely explored in real-world cohorts. The first study of the present doctoral thesis assesses the occurrence of liver-related outcomes in a cohort of CHD patients in relation to changes in HDV-RNA presence, and the impact of interferon treatment. The results show that persistence of positive HDV-RNA is associated with a higher risk of developing liver-related outcomes, while interferon treatment is linked to lower risk of liver-related outcomes, regardless of achieving a virological response.

Spontaneous HDV-RNA fluctuations have been reported during the natural course of CHD. However, the implications of these changes have not been studied in the context of liver-related outcomes occurrence and interaction with other HBV markers. The second study of this thesis aims to evaluate the proportion of HDV-RNA declines in untreated CHD patients, and its possible correlation with HBV markers. The results show that one-quarter of CHD patients have significant HDV-RNA declines during a median follow-up of 8 years. These declines are also associated with a drop in HBsAg and HBV-RNA levels.

Health-related quality of life (HRQoL) has been extensively assessed in patients with chronic hepatitis B and C, but never studied in chronic hepatitis D individuals. The third study evaluates quality of life scores in untreated CHD patients in relation to HDV viraemia, and compares the results with those of CHB patients. Scores show that CHD patients report higher HRQoL impairments than those with CHB, especially in the worry, emotional, physical and activity impairment domains.

RESUM

El virus de l'hepatitis D (HDV) és un virus ARN hepatotròpic i defectiu que requereix la presència del virus de l'hepatitis B (VHB) per a propagar-se. El VHD afecta aproximadament 20 milions de persones arreu del món. Aquest virus provoca una de les formes més greus de malaltia hepàtica crònica, ja que el risc de desenvolupar cirrosi hepàtica o esdeveniments clínics és entre dues i tres vegades més alt que el dels mono-infectats pel VHB.

L'impacte de la infecció activa, descrita com a presència d'ARN-VHD, en la progressió de l'hepatitis crònica D (HCD) ha estat poc explorada. El primer estudi ha estat dissenyat per avaluar el desenvolupament d'esdeveniments clínics en una cohort de pacients amb HCD en relació amb els canvis en la presència d'ARN-VHD i l'impacte del tractament amb interferó. Els resultats mostren que la persistència d'ARN-VHD positiu s'associa amb un major risc de desenvolupar esdeveniments clínics, i que el tractament amb interferó disminueix el risc de presentar-los independentment de la resposta virològica.

Diversos estudis han reportat fluctuacions espontànies de l'ARN-VHD durant el curs natural de la infecció. Tanmateix, la implicació d'aquestes variacions no ha estat estudiada en el context de l'aparició d'esdeveniments clínics i la interacció amb altres marcadors del VHB. El segon estudi ha avaluat la disminució dels nivells d'ARN-VHD en pacients amb HCD no tractats i la seva possible correlació amb marcadors del VHB. Els resultats mostren que un quart dels pacients amb HCD presenten disminucions significatives de l'ARN-VHD durant un seguiment de 8 anys. Aquestes disminucions també s'han vist associades amb un descens dels nivells d'HBsAg i d'ARN-VHB.

La qualitat de vida relacionada amb la salut s'ha avaluat àmpliament en pacients amb hepatitis crònica B (HCB) i C, però mai s'ha estudiat en individus amb hepatitis crònica D. El tercer estudi ha avaluat les puntuacions de qualitat de vida en pacients amb HCD no tractats en relació amb la virèmia del VHD, i

ha comparat els resultats amb els dels pacients amb hepatitis crònica B. Les puntuacions mostren que els pacients amb HCD reporten deterioraments de salut més alts que els que tenen HCB, especialment en els dominis de preocupació, deteriorament emocional, físic i d'activitat.

1. INTRODUCTION

1.1. HEPATITIS DELTA VIRUS

1.1.1. HISTORY

Hepatitis delta virus (HDV) was first identified in 1977 by Dr Mario Rizzetto (Turin, Italy) when a nuclear antigen was described in hepatocytes of patients infected with hepatitis B virus (HBV) with severe liver disease. Firstly, this antigen was thought to be another type of HBV antigen. Since the discovery of HBV in the late 1960's, several odd antigenic structures were observed in HBV-infected patients, most of them dying away during the infection.(1) Further collaboration between Georgetown University and Turin University was established to study this new nuclear antigen, which was named *d-antigen* to distinguish it from other newly found viral particles. Several in vivo experiments were performed in chimpanzees and results showed that *d-antigen* was not coming from HBV but from a separate defective virus that required HBV to produce complete viral particles.(2)(3) Following the already established nomenclature on hepatitis virus, it was named hepatitis delta virus.

Over the following years, research rapidly progressed and HDV was fully cloned and sequenced by 1986.(4) HDV was described as a satellite virus since it needed HBV to replicate despite not having any nucleotide homology with HBV genome. (5) Nowadays, HDV is known to be an unconventional human liver pathogen, not yet described in other animals.(6)

1.1.2. CLASSIFICATION

Hepatitis delta virus has been recognized by the International Committee on Taxonomy of Viruses (ICTV) as the only representative of the *Deltaviridae* family, *Deltavirus* genus. Despite showing structural and replicative similarities to other viroids and virusoids, it was considered distinct enough to be classified as a separate genus.(7)

1.1.3. EPIDEMIOLOGY

In 2019, World Health Organization reported that 296 million people were living with chronic hepatitis B (CHB).(8)

Worldwide prevalence on HDV infection has been more difficult to assess. Mainly for the challenge that is performing large screening programs specific for HBsAg-positive individuals, for the significant sampling bias of the reported studies, and for the non-representative samplings in all areas of the world.(9)

Latest epidemiology reports show an important disparity of results regarding anti-HDV prevalence. A recent study by Stockdale *et al.* reported a 4.5% anti-HDV prevalence amongst HBsAg-positive individuals, globally representing a total of 12 million individuals infected by HDV (Figure 1).(10) Moreover, other high-quality reports from 2018 and 2020 described an anti-HDV global prevalence of 14.57% and 13.02% respectively in HBsAg-positive population, translating to roughly 39 and 35 million infected people worldwide.(11) (12)

Sampling bias is an important hindrance when assessing anti-HDV prevalence. The probability of finding anti-HDV patients in endemic areas is ten times higher in HBsAg-positive cirrhotic patients than in HBsAg-positive asymptomatic blood donors. The target screening groups vary amongst the reported studies, therefore creating a sampling bias responsible for the disparity of results.(9)

Certainly, implementation of vaccination programs against HBV in 1990s impacted on HDV prevalence. In high-income countries, the current epidemiology of HDV relies on ageing patients with advanced liver fibrosis and young immigrants coming from endemic areas.(9) Still, HDV infection is believed to be underestimated due to the lack of screening programs in HBsAg-positive individuals.(13)

Amongst HBsAg-positive people, the countries with higher HDV prevalence rates are Mongolia (36.9%), Republic of Moldova (>10%) and some countries from Occidental Africa (>10%). Moreover, several isolated communities are noted

to have high anti-HDV prevalence such as indigenous Amazonian Amerindian tribes in Bolivia, Brazil, Colombia and Venezuela, indigenous tribes in the Uttar Pradesh region of India, and specific populations in Greenland and Greece. (10)

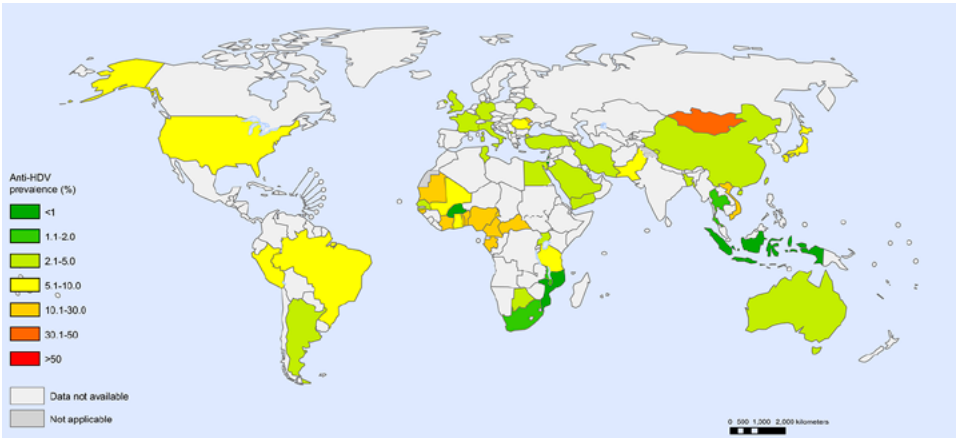

Figure 1. Hepatitis D infection prevalence worldwide. Modified figure. (10)

Eight HDV genotypes have been reported so far. The most prevalent is genotype 1, which predominates globally. Other genotypes are known to be more localized, such as genotype 2 in Asia, genotype 3 in Amazonian Latin America, genotype 4 in Japan and Taiwan and genotypes 5-8 in Africa (Figure 2).(14)

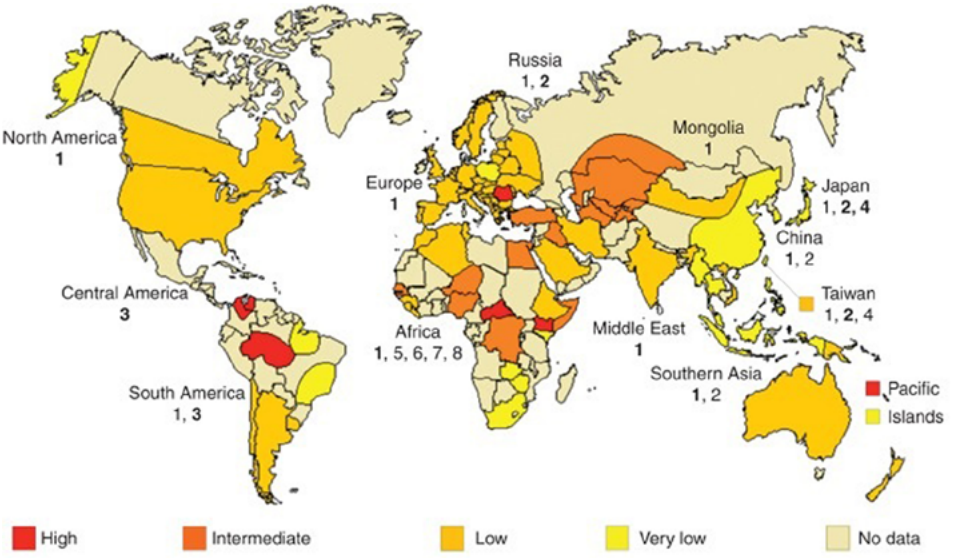

Figure 2. Distribution of the eight HDV genotypes around the globe. (14)

1.1.4. TRANSMISSION

Hepatitis delta virus is transmitted through infected blood, blood-derived products or sexual contact, like other known hepatic viruses. Transmission from mother to child is possible but rare.(15) In low endemicity populations, those reported to be at higher risk of HDV infection are people who inject intravenous drugs and people with hepatitis C virus (HCV) or human immunodeficiency virus (HIV) infection.(16) The risk of co-infection also appears to be potentially higher in men who have sex with men and commercial sex workers.(15)(17) In high endemicity populations the transmission occurs mainly through intrafamilial, iatrogenic and sexual spread. Iatrogenic transmission was formerly more prevalent in haemodyalisis and blood transfusion recipients. Fortunately, this transmission method has abruptly diminished over the last decades thanks to regulatory and hygienic policies, being only mostly reported in developing countries.(18)

1.1.5. HDV COMPOSITION

Hepatitis delta virus is considered the smallest human virus ever described, being more similar to plant viroids than to other known animal viruses.(19)

HDV is approximately 36 nanometers in diameter and it is composed of a coat of HBV envelope proteins (HBsAg) and a ribonucleoprotein (RNP), formed by a circular RNA genome strand and two isoforms of hepatitis delta antigen (small (S-HDAg) and large (L-HDAg)), as shown in Figure 3.(20)(21)

The viral envelope consists of an external lipidic membrane from the host cell and three HBsAg protein isoforms (small S-HBsAg, median M-HBsAg and large L-HBsAg).(2) S-HBsAg is the most abundant and it is crucial for the HDV entrance into the host cell and virion release.(22)(23) The L-HBsAg is the binding site of hepatocyte's receptor sodium taurocholate cotransporting polypeptide (NTCP), which plays a role in HDV's entry to the host cell.(24) Reports show that HDV can be ensembled only with S-HBsAg but needs from L-HBsAg to become

infective.(25) M-HBsAg has no role in HDV's infectivity.(26)

The HDV genome consists of a small RNA strand of about 1700 nucleotides that fold on itself. About 74% of all the nucleotides are involved in auto-complementary pairing, allowing the genome to adopt a rod-like closed structure. (27) HDV replicates in a double-rolling-circle model, which involves the host RNA polymerase II and generates a complete complementary copy of the genome known as the antigenome.(28) HDV genome has one single open reading frame (ORF) that is transcribed, which encodes for the two antigen delta proteins; large (L-HDAg) and small (S-HDAg).

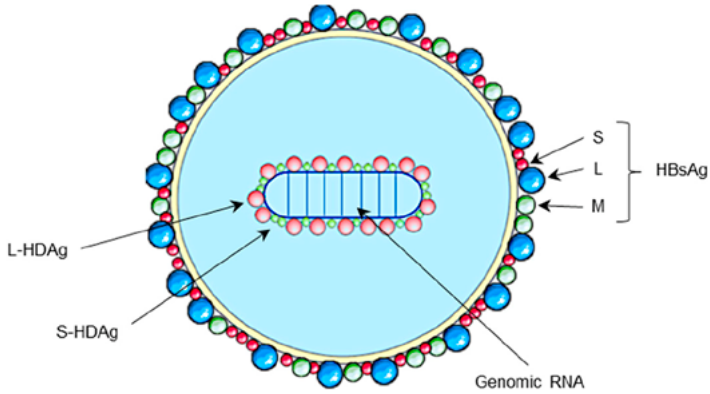

Figure 3. Hepatitis delta virus structure, conformed by a viral envelope (HBsAg proteins) and a ribonucleoprotein (HDAg proteins and genomic RNA). (29)

There are some unique features that characterize HDV beyond its simplicity: the transcription is performed by a host enzyme, it has the property of a ribozyme (it retains genetic information while acting as a catalyst), and it can perform essential RNA editing (it can modify its RNA sequence from the original template).(21)(27)

1.1.6. HDV REPLICATION

HDV is a satellite of HBV since it needs HBsAg proteins to form a fully infective virion.

To replicate, the HDV virion particle attaches to the hepatocyte creating an interaction between L-HBsAg and a membrane receptor of the hepatocyte

(NTCP). The virion enters the cell uncoated and it is targeted into the cell nucleus. HDV's genomic RNA is transcribed in the nucleus to form; 1) antigenomic RNA, which forms the template of the circular genome for the replication of new RNA transcripts, and 2) messenger RNA (mRNA), which contains the open reading frame for protein translation.

Once transcribed, mRNA is exported to the cytoplasm where it is translated in the endoplasmic reticulum (ER) to form new molecules of S-HDAg and L-HDAg. The new antigen molecules return to the nucleus where S-HDAg isoform supports further genome replication, and where both forms of HDAg associate with new transcripts of genomic RNA to form newly full ribonucleoproteins.

Ribonucleoproteins are then exported to the cytoplasm where L-HDAg facilitates association with HBV envelope proteins in the ER. The new viral particles are then exported from the hepatocyte via Golgi apparatus to re-infect further cells (Figure 4).(20)

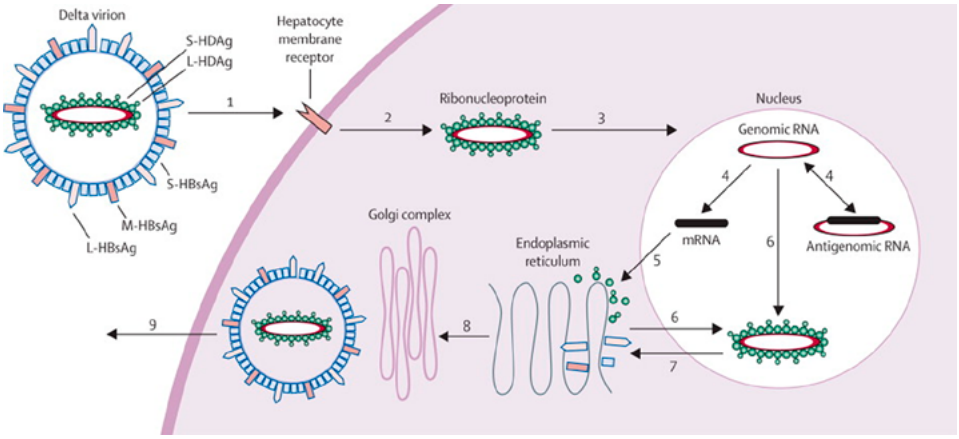

Figure 4. HDV replication cycle, from virion entrance into hepatocyte to exportation of new viral particle. (20)

1.1.7. HBV REPLICATION IN HBV-HDV INFECTION

Hepatitis B virus has a very complex life cycle. Upon infection, HBV's genomic rcDNA (relaxed-circular DNA) is converted to cccDNA (covalently closed circular DNA), which presents highly stable characteristics and acts as the template for transcription of all viral mRNAs. Four classes of HBV-mRNAs are then exported to the cytoplasm for further protein translation and DNA replication.(30)

In HDV infection, the presence of HBV is necessary to complete HDV's life cycle. Despite the imperative requirement of HBV presence, it has been proved that satellite viruses can often overcome its helper replication. In HBV-HDV infection, studies conducted both in experimental models and patients have shown a decrease in HBV replication, with minimal impact on the expression of HBsAg. There is evidence suggesting that integrated HBV-DNA can be a source of HBsAg even without active HBV replication.(16)

The cross-talk between HDV and HBV has been described in a few studies. Firstly, experimental models showed a correlation between the peak of L-HDAg accumulation and low HBV replication, suggesting a direct L-HDAg inhibitory effect over HBV replication.(31)(32)(33) Secondly, HDAg has exhibited a capability to interact with HBV pregenomic-RNA (template for viral DNA reverse transcription and protein synthesis) and selectively destabilize it.(34) Lastly, HDV can induce a strong type I IFN response, which may increase expression of antiviral IFN-stimulated genes that regulate cccDNA and decrease HBV-RNA levels, ultimately contributing to the inhibition of HBV replication.(35) Despite the hypotheses, the action mechanism of HDV inhibition over HBV has not been strictly proved. Moreover, in some cases HBV do not follow the inhibition pattern and can spontaneously fluctuate showing variability of dominances with HDV over time.(36)(37)

1.2. ACUTE HEPATITIS D

HDV infection can be acquired by patients already infected with HBV (super-infection) or through simultaneous infection by both HDV and HBV (co-infection). (38)

Generally, the outcome of HBV-HDV co-infection is benign with acute self-limited hepatitis and few cases of acute liver failure, as reported in acute hepatitis B.(39) When co-infection occurs, a considerable elevation of HBV-DNA, HDV-RNA and ALT levels can appear, sometimes showing a severe symptomatic course. Despite this, there is usually a rapid decrease in all parameters after acute onset resolves (Figure 5). Like in acute HBV mono-infection, a small risk of acute liver failure remains possible (<5%), presenting massive hepatocyte necrosis and need for urgent liver transplantation.(38)

In contrast, most HBV-HDV super-infections evolve to chronicity, usually causing significant worsening of underlying chronic hepatitis B and sometimes liver failure. (38) With initial elevation of HDV-RNA and ALT, HBV-DNA usually persists at low levels (Figure 5). Usually, there are no associated symptoms at early onset.

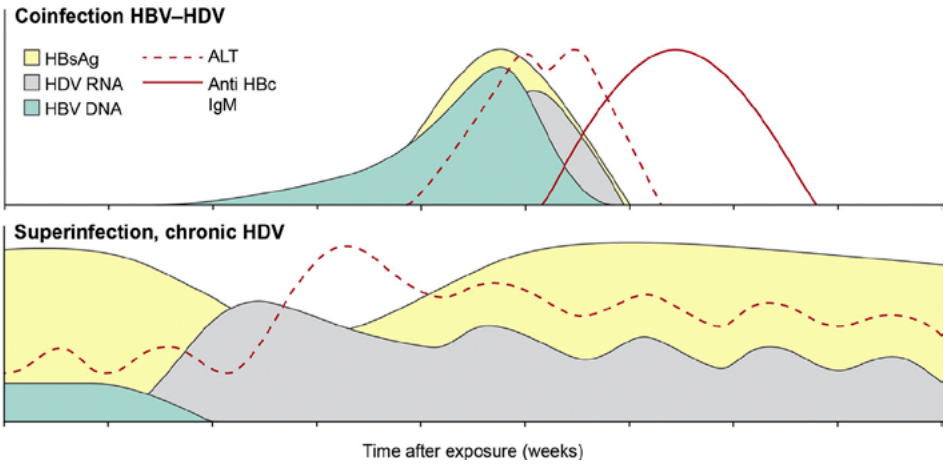

Figure 5. Serological and virological patterns during HBV-HDV co-infection and super-infection. Modified figure. (40)

1.3. CHRONIC HEPATITIS D

Chronic hepatitis D (CHD) is a disease that occurs when hepatitis delta virus remains active in the organism for at least 6 months.(39) CHD is the most severe form of chronic liver disease, often rapidly progressing to liver cirrhosis.(12)

More than fifty years after its discovery, CHD still remains a global health problem that is not fully understood. It has been designated an orphan disease in the European Union and in the US since it has scarcely been adopted by the pharmaceutical industry and since it is far more prevalent in developing countries. (41)

1.3.1. NATURAL HISTORY

The natural history of chronic hepatitis D tends to lead to disease progression and liver cirrhosis when the virus is active (detectable HDV-RNA).(42) Patients with undetectable levels of viraemia have a better prognosis and lower risk of liver cirrhosis and/or liver-related mortality than those detectable.(43)(44) It has been estimated that liver cirrhosis occurs in up to 80% of CHD patients and 15 years earlier than in chronic hepatitis B.(40) The direct linkage of hepatocellular carcinoma (HCC) and CHD has not been established yet. An Italian study reported HCC development in 2.8% of CHD patients (45), but some cohorts prove CHD to be an independent factor of HCC.(46)(47)

During the natural history of the infection, it is known that HDV replicates and fluctuates in spontaneous patterns, also showing changes in the interaction with HBV. In a Spanish study from 2006, viral replication of HBV and HDV was observed to be quite variable during periodic follow-ups, as shown in Figure 6. HDV replication predominated in 54.5% of the samples, HBV predominated in

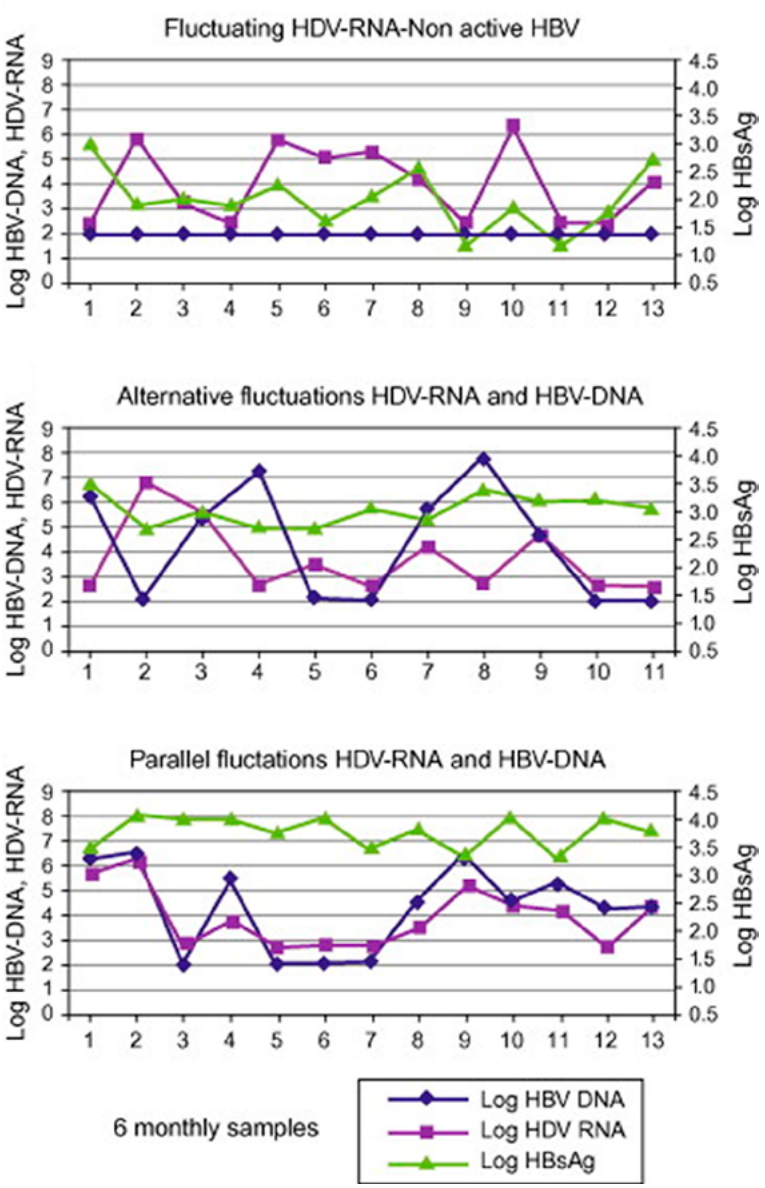

Figure 6. Examples of HDV-RNA and HBV-DNA fluctuating profiles in CHD patients. (36)

30.3% and both HBV and HDV maintained similar levels in 15.2% of the cases. (36)

A white paper written by experts on CHD management proposed a HDV-RNA decline cutoff of 2 or more logarithms (≥2 log10) as a possible surrogate marker

for clinical improvement.(48) No other implications for HDV-RNA fluctuations have been studied.

1.3.2. ANTIVIRAL THERAPIES

For many years, the only approved therapy for chronic hepatitis D was interferon alpha (IFNα) or its pegylated version (pegIFNα).(39)(49)

Despite being the only treatment, it has a rather suboptimal response. It has been described that a variable percentage of patients, ranging from 17% to 47%, achieve undetectable HDV-RNA by end of therapy. However, many of these patients relapse during follow-up.(50)(51) Moreover, IFN treatment has several contraindications that limit its use, and it often shows strong adverse events that condition patient's quality of life.(39)

Since the results of IFN are far from desirable, several investigational drugs are currently in development.

Recently, European Medicines Agency has conditionally approved the use of Bulevirtide on a specific set of patients with CHD. This drug acts as an inhibitor of HBV-HDV entry into the hepatocyte and is currently in phase III of clinical trials.(52) Other drugs under investigation in phase II and phase III include IFN lambda; which targets type III IFN receptors, highly expressed in hepatocytes, Lonafarnib; which acts as a farnseyl-transferase inhibitor, and REP 2139; a nucleic acid polymer.(40)

1.4. DIAGNOSIS OF HDV INFECTION

Recommendations of European Association for the Study of the Liver (EASL) on management of HBV infection from 2017 suggest anti-HDV testing in all HBsAg-positive subjects (39), as well as the Asian-Pacific guidelines from 2016 (APASL, Asian-Pacific Association for the Study of the Liver).(53) On the other

hand, American guidelines from 2018 (AASLD, American Association for the Study of Liver Diseases) only recommend anti-HDV testing in those HBsAg-positive who are at risk (people who inject drugs, men who have sex with men, those at risk for sexually transmitted diseases and immigrants from high HDV endemic areas).(54)

1.4.1. ANTI-HDV

For the initial diagnosis, a serological test for presence of anti-HDV IgG antibodies is performed.

Anti-HDV IgG antibodies are usually determined qualitatively from patient plasma (extracted in EDTA tube) or serum (extracted in serum separator (SST) tube).

The technique is based on chemoluminiscence immunoassay (CLIA). It consists in adding a HDV-specific recombinant antigen to the patient sample, where present anti-HDV antibodies can attach. Then, conjugates of monoclonal antibodies and isoluminol are added, which will react with the HDV antigen - anti-HDV antibody pairings. After adding a chemolumuniscent reactive to the solution, isoluminol will emit light signaling that can be captured through a photomultiplicator. The amount of expressed light will be directly proportional to the quantity of anti-HDV in the sample.(55)

Based on a systematic meta-analysis for HDV prevalence performed in 2016 (10), there are more than 32 commercial kits available for anti-HDV testing, the most frequently used shown in Table 1.

Table 1. Most frequently used anti-HDV IgG determination commercial kits

Manufacturer	Usual assay	Sensitivity/specificity
Abbott Laboratories, North Chicago, USA (56)	EIA	96.8%/99%
Dia. Pro Diagnostic Bioprobes, Italy (57)(58)	ELISA	>98%/>98%
ETI-AB-DELTAK-S, DiaSorin, Saluggia, Italy (59)	CLIA	99.4%/98.9%
Wantai Biological Pharmacy, Beijing, China (60)	ELISA	100%/100%
Organon Teknika, The Netherlands (61)	microELISA	93%/80%

Following a recent study from Chen et al (62), it has been shown that commercial kits can lead to false negatives in patients with low anti-HDV concentrations. The study compares a commercial anti-HDV assay with a novel anti-HDV microarray technique in a hyper-endemic region of Mongolia, resulting in a 7.1 % of false negatives from commercial kit testing. On the contrary, a Taiwanese study compared an anti-HDV commercial kit with a specific recombinant HDAg derived from a local dominant HDV strain, and showed a 2.6% of false anti-HDV positives when using the commercial kit.(63)

Anti-HDV IgM is also another used marker for CHD. After primo-infection, anti-HDV IgM levels are maintained positive at low titles during a limited period of time in case of self-limited infection, with positive persistency at high titles when infection evolves to chronic.(64) However, this technique has limited usefulness in daily clinical practice since it is not commercially available.

1.4.2. HDV-RNA

In order to assess whether HDV infection is active or not, HDV-RNA is quantified in plasma or serum. There are many steps for HDV-RNA quantification, some involving manual techniques and some involving automated tests.

First, nucleic acid extraction is performed. Patient's sample is put in contact with a proteinase and after a high temperature incubation period, HDV-RNA will be released. This step can be conducted manually or through an automated system. A 2018 study from Bremer *et al.* compared some of the most used automated nucleic acid extraction systems (AmpliPrep, MagNA Pure, QIAcube QBK and QIAcube VRK) with a manual extraction protocol and proved that automated systems showed a rather important underestimation of HDV-RNA levels after quantification.(65) In contrast, a similar study from 2015 compared manual and automated nucleic acid extractions for HBV-DNA and HCV-RNA quantification, and proved that automated systems gave higher viraemias in each case.(66)

While manual extraction requires several manipulations of the sample, which increases the risk of cross-contamination and human error, automated extraction guarantees a consistency of the technique and lower risk of cross-contamination.

After nucleic acid extraction, HDV-RNA quantification is performed through RT-qPCR (retrotranscriptase quantitative polymerase chain reaction).

Since about 74% of all nucleotides of HDV-RNA are involved in auto-complementary pairing, hydrogen bonds between nucleotides need to be firmly broken. Therefore, a strong thermal shock is applied, which consists of a high temperature incubation period (~95ºC) followed by a low temperature incubation period (~-80ºC) to prevent the reattachment of the nucleotide pairings.(67) Then, RT-qPCR is performed, whose principles are shown in Figure 7.

In summary, reverse transcriptase will first retro-transcribe RNA to cDNA (complementary DNA) using the free dNTPs (deoxynucleotide triphosphates).

Afterwards, denaturation of the DNA strands will take place during a high temperature incubation period (~72ºC). Primers will then attach to the specific amplification region of the cDNA, and the DNA polymerase will transcribe the same region in exponential cycles. Short DNA probes, which are attached to a fluorescent reporter, will bind in-between the target sequence of the amplification region. When the DNA polymerase reaches the probe, it will be cleaved and it will emit a fluorescent signal that can be detected after each cycle.(68)

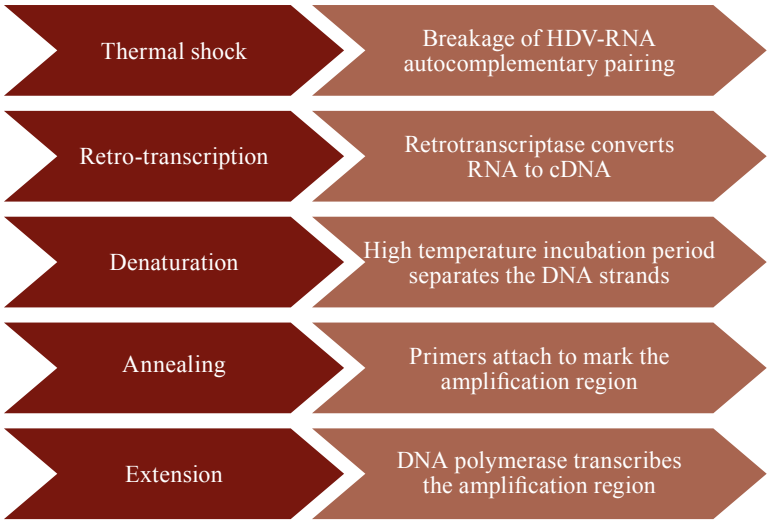

Figure 7. Principles of one-step RT-qPCR for HDV-RNA quantification.

There are many available commercial kits for HDV-RNA quantification (Table 2), with different instrument applications, prime and probe targets, limits of detection and quantification, sensitivities and specificities.

In all cases, an HDV-RNA standard is needed in order to have a known concentration to part form. In-house techniques use a self-validated standard, usually being either in vitro transcribed HDV-RNA or a DNA plasmid containing HDV target sequences.(69) In-house standards can be highly accurate but they hardly allow reproducibility of results amongst other laboratories.

In 2013, the World Health Organization (WHO) created the first Standard for Hepatitis D Virus RNA for Nucleic Acid Amplification Techniques-based assays

(PEI code number: 7657/12) in need of finding a standard consensus. Fifteen laboratories from nine countries participated in the study.(70) The material with highest HDV-RNA concentration (>7 log10 copies/mL) determined by the RoboGene assay was chosen as the potential candidate for the WHO standard. Two candidate preparation replicates (S1 and S2) were analyzed, alongside an individual clinical plasma sample. After reproducibility check amongst the participant centers, no differences in mean estimates for S1 and S2 were observed. The candidate standard (S1/S2) was assigned a HDV-RNA unitage of 575,000 IU/ml or 5.76 log10IU/ml.(71)

After implementation of the WHO international standard, many studies validating the standard were performed. A recent European multicenter study from Stelzl *et al.* aimed to improve the comparability of quantitative results reported by different laboratories. The RoboGene HDV-RNA Quantification Kit 2.0 was used along with the first WHO international standard, and both manual and automated acid nucleic extraction protocols were considered. Results showed that any modification of a validated protocol required determination of protocol-specific correction factors and that the first WHO international standard maximized the correct harmonization of quantitative results.(72)

Another recent study from Bremer *et al.* reported that one third of formerly classified HDV-undetectable patients after pegIFN treatment, had in fact positive HDV viraemia when retesting samples with the RoboGene kit instead of an in-house PCR assay.(73)

In 2016, Le Gal *et al.* compared RT-qPCR results for HDV-RNA quantification using the WHO international standard and more than 50% of participant laboratories failed to detect at least one sample. Discrepancies were attributed to primers and probes mismatches. Therefore, despite the WHO international standard, differences between in-house qPCRs and commercial kits can be observed in HDV-RNA quantification.(74)

Table 2. Compilation of available HDV-RNA quantification commercial kits

Commercial kit	Manufacturer	RT-qPCR	Primer/ probe location	LLOD	LLOQ – HLOQ	Standard (genotype)	Genotype	Sensitivity / Specificity
RoboGene® HDV quantification kit	Roboscreen, Leipzig, Germany	One-step	L-HDAg	6-14 IU/ml	14 to 4·109 IU/ml	RNA (HDV-1)	1-8	95% / 100%
Lightmix HDV kit	Roche Life Science, Berlin, Germany	Two-step	HDAg	100 IU/ml	2.75 to 7 log cop/ml	DNA (HDV-1)	1	ND
DiaPro HDV RNA quantification kit	DiaSorin, Saluggia, Italy	Two-step	HDAg	100 cop/ml	2 to 7 log cop/ml	DNA (HDV-1)	1-8	ND
RealStar® HDV RT-PCR Kit (RUO)	Altona Diagnostics, Hamburg, Germany	One-step	HDAg	9.48·10-3 IU/µl	4·10-2 to 4·105 IU/µl	RNA (HDV-1)	1-8	95% / 100%
HDV genesig® standard kit	Primerdesign Ltd, Camberley, UK	One-step	HDAg	100 cop/ml	ND	RNA	1-8	ND
HDV Real Time RT-PCR Kit	LifeRiver Diagnostics, Shanghai, China	One-step	ND	5·103 cop/ml	1·104 to 1·108 cop/ml	DNA	ND	ND
Eurobioplex HDV kit (RUO)	Eurobio, Les Ulis, France	One-step	HDAg	1 log IU/ml	2.75 to 8.5 log IU/ml	RNA	1-8	97.7%/ 93.4%

2. HYPOTHESIS

2. HYPOTHESIS

Chronic hepatitis D is a neglected disease frequently forgotten amongst physicians. It is estimated that more than 12 million people worldwide are infected by HDV, potentially developing liver cirrhosis and eventually hepatocellular carcinoma.

To date, a few studies have described the natural history of chronic hepatitis D, but there are still some topics to be addressed.

This doctoral thesis encompasses three studies focused on the natural course of chronic hepatitis D.

The first study assesses whether persistence of HDV-RNA has an impact on liver-related outcomes in patients with chronic hepatitis D.

The second study is designed to search for spontaneous HDV-RNA changes in untreated patients, and highlights the importance of using a standardized method to quantify HDV-RNA.

The third study pretends to determine the health-related quality of life of patients with chronic hepatitis D compared to those with chronic hepatitis B.

The following hypotheses are proposed:

- Active HDV viraemia could be associated with a higher risk of progression to liver cirrhosis, hepatocellular carcinoma and liver-related decompensation and mortality.
- Spontaneous fluctuations of HDV-RNA $\geq$2 log10 may happen during the natural history of HDV infection, and could impact on the development of liver-related outcomes.
- Changes on HDV-RNA levels may be associated with variations on HBsAg values.
- Quality of life scores may differ in subjects with HDV infection in comparison

with those with HBV mono-infection and could be related to the presence of HDV viraemia.

3. AIMS

3. AIMS

The main aim of the present doctoral thesis is to study the evolution of HDV-RNA during the natural history of the infection, and assess its potential role in the development of liver-related outcomes and the quality of life of patients with chronic hepatitis D.

The secondary aims are:

- To evaluate whether persistently positive HDV viraemia has a role in the development of liver-related outcomes.
- To determine if HDV-RNA spontaneously fluctuates during the natural course of the disease and if those fluctuations have a role in liver-related outcomes.
- To establish a relation between HDV-RNA changes and other HBV markers such as quantitative HBsAg and HBV-RNA.
- To assess if chronic hepatitis D patients have worse quality of life scores than those with chronic hepatitis B and to study whether HDV viraemia impacts on the results.

4. COMPENDIUM OF ARTICLES

4.1. METHODOLOGY SUMMARY

The first study was a multicentre collaboration including all anti-HDV positive adult patients with compensated chronic liver disease. Demographical, clinical and laboratory data were collected every 6 months during routine visits for all patients. Demographics included sex, age, ethnicity, country of birth and risk factors. Laboratory parameters consisted of platelet counts, prothrombin time, international normalised ratio (INR), bilirubin, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase, gamma-glutamyltransferase, alkaline phosphatase and albumin. Serological and virological determinations included hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B e antibodies (anti-HBe), anti-HDV, anti-HCV and anti-HIV antibodies, as well as HBV-DNA and HDV-RNA.

The baseline-event-anticipation score was calculated as described in the original cohort.(75) This indicator encompasses parameters such as sex, age, region of origin, coagulation factor levels and bilirubin levels, adding one point for each factor to predict the exposure and risk of developing a clinical event in a chronic hepatitis delta patient. The score is divided into three risk groups: mild (< 2 points), moderate (2-5 points) and severe (> 5 points).

Clinical outcomes were defined as: progression to liver cirrhosis, hepatic decompensation such as ascites, hepatic encephalopathy and/or oesophageal haemorrhages; development of hepatocellular carcinoma, liver transplantation and/or liver-related death.

The second study consisted on a multicenter retrospective project. Adult patients with compensated liver disease, detectable HDV-RNA, adequate follow-up of >3 years, and at least 1 sample per year were included. Those who tested positive

for hepatitis C virus (HCV) or HIV antibodies, or those who had received IFN therapy 3 years prior to inclusion in the study were excluded.

Demographic, clinical, and laboratory data were collected from the patients' medical records. Demographics included sex, age, ethnicity, country of birth, and risk factors. Laboratory parameters were recorded, including platelet count, prothrombin time, INR, bilirubin, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, and albumin. Liver cirrhosis was diagnosed by liver biopsy (fibrosis stage ≥F5 according to the Ishak score),

and on the combination of transient elastography (≥13.0 kPa) and clinical data (low platelets levels and abnormal liver ultrasound). Serological and virological determinations included hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B e antibodies (anti-HBe), anti-HDV, anti-HCV, and anti-HIV antibodies, as well as HBV-DNA, HBV-RNA and HDV-RNA. These parameters were tested during routine visits and were part of the clinical work-up for each patient.

Serum HDV-RNA was re-tested in a central laboratory by an in-house one-step quantitative RT-PCR, using the WHO (World Health Orgenization) international standard.

The third study was unicentric. Patients with CHD completed 3 PROs questionnaires (Chronic Liver Disease Questionnaire (CLDQ), Functional Assessment of Chronic Illness Therapy–Fatigue (FACIT-F), and Work Productivity and Activity Impairment (WPAI)). Higher scores of CLDQ and FACIT-F indicate better HRQoL (Health-related quality of life) while higher scores of WPAI indicate worse HRQoL. Results were harmonized and compared with those of patients with CHB. In addition, demographics and analytical parameters were collected at time of study entry.

4.2. ARTICLE 1

Long-term clinical outcomes in patients with chronic hepatitis delta: the role of persistent viraemia

Adriana Palom | Sergio Rodríguez-Tajes | Carmen A. Navascués | Javier García-Samaniego | Mar Riveiro-Barciela | Sabela Lens | Manuel Rodríguez | Rafael Esteban | Maria Buti

Palom A, Rodríguez-Tajes S, Navascués CA, *et al.* Long-term clinical outcomes in patients with chronic hepatitis delta: the role of persistent viraemia. Aliment Pharmacol Ther. 2020;51(1):158-166.

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4.3. ARTICLE 2

One-quarter of chronic hepatitis D patients reach HDV-RNA decline or undetectability during the natural course of the disease

Adriana Palom | Sara Sopena | Mar Riveiro-Barciela | Angela Carvalho-Gomes | Antonio Madejón | Sergio Rodriguez-Tajes | Luisa Roade | María García-Eliz | Javier García-Samaniego | Sabela Lens | Marina Berenguer-Hayme | Francisco Rodríguez-Frías | Helena Hernandez-Évole | Ana Isabel Gil-García | Ana Barreira | Rafael Esteban | Maria Buti

Palom A, Sopena S, Riveiro-Barciela M, *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(4):462–469

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5. OVERALL SUMMARY OF RESULTS

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The first study assessed the epidemiological and clinical characteristics of a chronic hepatitis D cohort and their clinical outcomes in relation to the persistence or absence of HDV-RNA. It also evaluated the impact of IFN therapy in a real-world cohort of chronic hepatitis D subjects and the validation of the baseline-event-anticipation (BEA) score.(76)

A total of 2888 HBsAg-positive patients were tested for anti-HDV, of whom 151 (5.2%) were positive. A total of 118 patients with adequate follow-up were included. Most were men (58%), Caucasian (79%), with a median age of 49 years (IQR, 35-54 years), and 35 (30%) had liver cirrhosis at the time of inclusion. HDV-RNA was detected in 86 (73%) patients, who were younger, had lower HBV-DNA levels and had more often liver cirrhosis than those with undetectable HDV-RNA.

Patients were followed for a median period of 8 years (IQR, 3-15 years). During follow-up, 28 (24%) subjects experienced at least one clinical event.

5.1. CLINICAL OUTCOMES IN RELATION TO HDV-RNA DETECTABILITY

Overall, patients with initially detectable HDV-RNA showed a trend towards higher rates of liver cirrhosis development and liver decompensation in comparison to patients with undetectable HDV-RNA (31% vs 0%, p=0.002 and 28% vs 3%, p=0.019, respectively).

After follow-up, among the 86 patients with initially detectable HDV-RNA, 65 (76%) still had persistently detectable HDV-RNA (median, 5 log IU/mL; range, 4.1–6 log IU/mL) and the other 21 (24%) became undetectable within a median period of 9 years (IQR, 3–12 years).

Patients who had persistently positive levels of HDV-RNA were slightly more likely to have liver cirrhosis than those who later became HDV-RNA undetectable

(36.9% vs 19%, p=0.10). However, the rates of liver decompensation (29.2% vs 28.6%, p=0.59), liver-related deaths (6.2% vs 9.5%, p=0.45) and need for liver transplantation (10.8% vs 4.8%, p=0.37) were similar between persistently HDV-RNA-positive patients and those who became undetectable during follow-up.

5.2. CLINICAL OUTCOMES IN RELATION TO IFN THERAPY

Overall, 31 HDV-RNA positive subjects received IFN therapy at standard doses: 19 before study inclusion and 12 during the study. The median duration of IFN therapy was 48 weeks (IQR, 24–96 weeks), decided by the attending physician.

Among these patients, 13 (42%) were non-responders (HDV-RNA positive), 8 (26%) achieved undetectable HDV-RNA but then relapsed, and 10 (32%) achieved persistently undetectable HDV-RNA for a median of 1.5 years (IQR, 1–5 years). The percentage of patients with liver cirrhosis was similar between those who received IFN and those who did not (46% vs 43%, p=0.49). IFN treatment was associated with a lower rate of liver decompensation (12.9% vs 38.2%, p=0.011) and a trend towards HDV-RNA negativization by the end of follow-up (32.3% vs 20%, p=0.156).

5.3. VALIDATION OF BEA SCORE IN THE PREDICTION OF CLINICAL OUTCOMES

The baseline-event-anticipation score at inclusion showed an average index of 2.36 (moderate risk). The score was higher in patients with detectable HDV-RNA (2.59, moderate risk) compared to those undetectable (1.71, mild risk) (p=0.002), suggesting a higher risk of developing clinical outcomes.

The risk of liver decompensation during follow-up varied according to BEA score. At least one clinical event was experienced by 6% of patients in the mild risk group, 25% in the moderate risk group and 80% in the severe risk group (p<0.001).

The second study focused on the evaluation of spontaneous HDV-RNA fluctuations during the natural course of CHD infection. A HDV-RNA decline of ≥2 log10 was proposed as a possible surrogate marker for clinical improvement. Changes in serum HDV-RNA concentrations were assessed in untreated CHD subjects using a sensible technique of quantification and the WHO international standard. Moreover, these fluctuations were analyzed in corrleation with other HBV markers.

In total, 323 samples from 56 patients were included. The majority were male, middle-aged, Caucasian and HBeAg-negative. Twenty-four (43%) had liver cirrhosis and 36 (64%) were receiving nucleos(t)ide analogues (NAs). At inclusion, 13 (23%) patients had normal ALT levels, 40 (71%) detectable HBV-DNA, and 11 (20%) detectable HBV-RNA.

5.4. HDV-RNA CHANGES DURING FOLLOW-UP IN UNTREATED SUBJECTS

Overall, there was a significant decline in HDV-RNA levels from baseline (5.3 log10IU/mL) to the last determination (4.3 log10IU/mL) ($p<0.001$) during a mean follow-up of 5.6 (3-16) years. Amongst the 56 patients, 14 (25%) had at least one ≥2 log10 HDV-RNA decline between consecutive samples, including 11 (20%) in whom HDV-RNA became undetectable. In the other 42 (75%) patients, HDV-RNA levels remained unchanged.

5.5. BIOCHEMICAL, SEROLOGICAL, VIROLOGICAL AND CLINICAL OUTCOMES DURING FOLLOW-UP

Overall, ALT levels decreased during follow-up and became normal in 21 (38%) patients at the last follow-up determination. HBsAg levels also decreased during follow-up. The HBsAg drop from baseline to last follow-up determination was larger in patients with a ≥2 $\log_{10}$ HDV-RNA decline (−0.7 ± 1.1 log IU/mL) than in

those without (−0.9 ± 1.2 vs −0.1 ± 0.9, p=0.018). HBsAg loss was documented in 4 (7%) patients; all with ≥2 log10 HDV-RNA decline and persistently normal ALT levels. In those without NA therapy, HBV-DNA levels significantly decreased overtime, regardless of the ≥2 log10 HDV-RNA decline. HBV-RNA levels showed a decreasing trend only in patients with a ≥2 log_{10} HDV-RNA decline.

Twelve patients (21%) experienced at least one clinical event during follow-up. There were no differences in clinical outcomes between patients with and without a ≥2 log_{10} HDV-RNA decline.

The third study addressed patient-reported outcome (PRO) tools in untreated CHD patients and compared them with those obtained for patients with CHB.

In total, 125 questionnaires were administered to 43 CHD patients and 82 CHB patients at the inclusion of the study. In both groups, the majority of patients were male, Caucasian, with a mean age of 50.2±14.2 years and employed. Patients with CHD had significantly higher ALT and AST levels (p<0.0001), and higher levels of hepatic elastography (p<0.0001), APRI (p<0.0001), and FIB-4 (p=0.0037) than those with CHB.

5.6. PATIENT-REPORTED OUTCOMES IN PATIENTS WITH CHD AND CHB

All included patients completed the Spanish versions of CLDQ, FACIT-F, and WPAI.

At study inclusion, the mean CLDQ total score was 5.77 ± 1.06 in patients with CHD and 5.88 ± 0.94 in patients with CHB (p=0.73). Patients with CHD had a significantly worse worry score (p=0.0118) than patients with CHB.

The total FACIT-F score on a scale of 0–160 was 131.4 ±24.5 in patients with CHD and 128.1 ± 25.2 in patients with CHB (p=0.3285). Patients with CHD showed a better functional well-being score in comparison with CHB subjects (p=0.0281).

The results of the WPAI questionnaire showed that subjects with CHD had a higher activity impairment score than those with CHB (p=0.0029). Regarding employment status, those with full time or part time jobs (N=80) had significantly better HRQoL scores than those who were unemployed (N=45) in the physical, social, and fatigue domains of the CLDQ, as well as the abdominal, activity, fatigue, systemic, and worry domains of FACIT-F. When studying the housewife (N=18) vs. non-housewife (N=107) population, data showed better HRQoL in the housewife group (physical, emotional and fatigue domains of CLDQ, and abdominal, activity, fatigue, systemic and worry domains of FACIT-F).

5.7. PATIENT-REPORTED OUTCOMES IN RELATION TO PRESENCE OF HDV-RNA

Within the CHD group (N=43), PROs were analyzed in relation to the presence of hepatitis D viraemia. Twenty-six (60%) patients had persistently detectable HDV-RNA and 17 (40%) patients had undetectable HDV-RNA. There were no differences in the overall scores for the 3 questionnaires based on the presence or absence of HDV-RNA (p>0.10 in all domains).

PROs were also evaluated in relation to the HDV viraemia levels. Patients with high levels (≥2,000 IU/ml) of baseline HDV-RNA (N=19, 44%) proved to have higher activity impairment on the WPAI questionnaire than those with low HDV-RNA levels (<2,000 IU/ml) (p=0.024).

5.8. PATIENT-REPORTED OUTCOMES IN RELATION TO LIVER CIRRHOSIS

Within the CHD group, patients with and without liver cirrhosis at baseline were compared. Results showed that patients with CHD and liver cirrhosis (N=14) had a worse CLDQ systemic score than those without liver cirrhosis (N=29) (p=0.0442).

6. OVERALL SUMMARY OF THE DISCUSSION

6. OVERALL SUMMARY OF THE DISCUSSION

The first study was performed in a contemporary Spanish cohort of chronic hepatitis D (CHD) subjects. It assessed anti-HDV prevalence and appearance of liver-related outcomes according to the presence/absence of HDV-RNA.

The prevalence of CHD is still controversial and difficult to assess due to the disparity in screening criteria around the globe and to the huge sampling bias amongst the literature. Studies show inconsistencies in the targeted subjects. For instance, there is a tenfold increase in HDV-positive subjects in a pool of known cirrhotic HBsAg-positive patients compared to a pool of HBsAg-positive asymptomatic blood donors.(9) Moreover, in low endemic countries such as Spain and Italy, autochthonous anti-HDV prevalence decreases every year and mainly affects older subjects.(77)(78) In contrast, anti-HDV prevalence remains stable or even rises in young subjects from endemic areas with no accessibility to HBV vaccination.(79)(80) This contributes to the variability in the results.

Our first study was performed in all anti-HDV positive patients from four Spanish academic hospitals with consecutive serum samples, irrespective of referral origin, transmission method or liver fibrosis status. The overall anti-HDV prevalence amongst HBsAg-positive subjects was 5.2%. This is in line with other recent Spanish and European epidemiology reports conducted in similar settings. (77)(81)(82)

However, this prevalence is lower than that obtained in previous reports. A Spanish study by Navascués *et al*. reported an anti-HDV prevalence of 7.1% in 1986-1992. (79) In addition, Sagnelli *et al.* described a 8.3% prevalence in an Italian cohort in 1997.(83) Since the HBV vaccine implementation, anti-HDV prevalence has progressively decreased in European countries, only slightly increasing recently due to immigration and globalization. Other factors such as harm reduction programs for people who inject drugs might have also had an impact on such decrease.(84)

In our study, in all anti-HDV positive subjects, HDV viraemia was assessed by presence of HDV-RNA. A 73% had detectable HDV-RNA levels, which is comparable to recent findings in other Caucasian cohorts.(85)(86) At study entry, subjects with detectable HDV-RNA were more often cirrhotic than those with undetectable HDV-RNA, confirming the severity of chronic hepatitis D. Subjects with initial detectable HDV-RNA presented more often progression to liver cirrhosis, liver decompensation, liver transplantation and liver-related death than those with undetectable HDV-RNA.

Romeo *et al.* reported that HDV viraemia is correlated with faster progression to liver cirrhosis.(87) Other previous studies already reported worse prognosis in patients with detectable HDV-RNA compared to those with undetectable HDV-RNA,(88)(89) even in non-cirrhotics.(90)

The subjects in our cohort had a mortality rate of 0.44 per 1000 person-month and a liver-related death and liver transplantation rate of 1.32 per 1000 person-month. Some fairly recent cohorts reported higher mortality rates in patients with CHD: 2.92 per 1000 person-month in Brancaccio *et al.*(91) and 3.67 per 1000 person-month in Kushner *et al.*(46). Unlike in our cohort, these studies included patients with severe co-morbidities such as HIV and HCV co-infection, and with decompensated liver disease, factors strongly associated with higher mortality rates.(47)

Table 3 shows data from several relevant contemporary studies with long-term follow-up cohorts of patients with CHD.

Table 3. Compilation of data from contemporary long-term cohorts of chronic hepatitis D subjects.

Author, year of publication	N patients	Male	Cirrhosis	Therapy	Detectable HDV-RNA	Detectable HBV-DNA	Follow-up (years)	DLD	HCC	OLT	Death events
Romeo R, *et al.* 2009 (39)	299	230 (77%)	104 (35%)	90 IFN, 12 NA	145 (48%)	67 (22%)	19.4	88 (29%)	46 (15%)	29 (10%)	63 (21%)
Niro GA, *et al.* 2010 (86)	188	132 (70%)	127	90 IFN, 6 NA	175 (93%)	61 (32%)	7.8	42 (22%)	17 (9%)	38 (20%)	11 (6%)
Calle Serrano B, *et al.* 2014 (87)	75	52 (69%)	(68%)	24 IFN, 25 NA	59 (78%)	25 (33%)	2-16	32 (43%)	4 (5%)	15 (20%)	5 (7%)
Kushner T, *et al.* 2015 (40)	73	71 (97%)	37	7 IFN, 30 NA	73 (100%)	50 (68%)	14	8.0 per 1,000 person-years	23.0 per 1,000 person-years	N/A	44 per 1,000 person-years
Béguelin C, *et al.* 2016 (41)	104	79 (76%)	(49%)	0	104 (100%)	N/A	8.71	N/A	23 (22%)	N/A	N/A

Wranke A, *et al.* 2017 (79)	136	92 (68%)	62 (46%)	52 IFN, 45 NA	81 (60%)	N/A	5.2	54 (40%)	10 (7%)	26 (19%)	7 (5%)
Coghill S, *et al.* 2018 (82)	549	340 (62%)	109 (20%)	182 NA	179 (33%)	N/A	19	N/A	34 (6%)	18 (3%)	0 (0%)
Palom A, *et al.* 2019 (88)	118	68 (58%)	35 (30%)	21 IFN, 53 NA	86 (73%)	90 (76%)	5.6	28 (24%)	8 (7%)	9 (8%)	6 (5%)
Roulot D, *et al.* 2020 (35)	1112	767 (69%)	312 (28%)	584 IFN	659/748 (88%)	N/A	3.0	267 (24%)	100 (9%)	153 (14%)	38 (3%)
Kamal H, *et al.* 2020 (80)	337	182 (54%)	79 (23%)	108 IFN, 30 NA	233 (69%)	74/283 (26%)	6.5	37 (11%)	13 (4%)	28 (8%)	

In our analysis, detectable HDV-RNA did not correlate with higher risk of developing hepatocellular carcinoma (HCC). These results are in line with a recent German study.(92) The lack of association could indicate that HCC in anti-HDV positive patients is caused by a rapid progression to liver cirrhosis itself and the underlying HBV infection, and not by HDV viral activity at baseline. Therefore, patients with undetectable HDV-RNA and progressive liver disease might have an underestimated risk of developing HCC.

During follow-up, HDV-RNA became undetectable in 21 patients, adding to the 32 initially non-viraemic patients. Those who became HDV-RNA undetectable during follow-up were less likely to develop liver cirrhosis than patients who exhibited persistently positive HDV viraemia. However, no differences were observed in relation to liver decompensation, liver transplantation and liver-related death rates. The already high percentage of patients with baseline liver cirrhosis could explain this lack of association.

In patients with CHD, HBV-DNA is found at very low or even undetectable levels due to the inhibitory effect of HDV on HBV. Giersch *et al.* proved the existence of this effect in vivo and in vitro at certain periods of the co-infection.(93) In addition, a high proportion of patients with CHD usually receive NAs therapy. For this reason, in our study, the majority of patients showed low levels of HBV-DNA, which had no effect on progression to cirrhosis, development of clinical outcomes, HCC, need for liver transplantation or liver-related death.

Interferon (IFN) therapy is associated with transient or, in some cases, even maintained virological response, defined by undetectable HDV-RNA, which correlates with better disease prognosis and reduction of clinical outcomes. Farci *et al.* described that patients who received IFN therapy had lower risk of developing clinical outcomes and/or liver-related death, irrespective of cirrhosis status.(94) Wranke *et al.* reported that IFN-based therapy is associated with a lower likelihood of clinical disease progression, and they also validated HDV-RNA undetectability as a surrogate endpoint for CHD treatment.(85) Yurdaydin

et al. described the favorable effect of IFN-induced maintained viral response on the natural course of the disease.(43)

In 2011, the Hep-Net-International-Delta-Hepatitis-Intervention-Trial-1 (HIDIT-1) was created. It consisted of a randomized controlled clinical trial with Adefovir/ placebo ± pegIFNα for 48 weeks. One-quarter of patients who were on pegIFNα, regardless of Adefovir or placebo showed maintained HDV-RNA clearance.(95) In 2014, they re-tested the participants after a median of 4.5 years. More than 50% of the patients who had undetectable HDV-RNA after the HIDIT-1 study, relapsed.(50)

In 2020, a 10-year follow-up article on the clinical outcomes of the participants from HIDIT-1 was published. Seventeen out of 60 (28%) patients presented clinical complications during follow-up, mainly those with poor response to therapy (detectable HDV-RNA) and baseline cirrhosis.(96)

The role of IFN in our study was difficult to assess due to the small number of treated patients. Despite the limitations, our results showed a reduction of clinical outcomes in IFN-treated patients, even when not achieving undetectable HDV-RNA levels.

Calle-Serrano *et al.* proposed a baseline-event-anticipation (BEA) score to predict the risk of experiencing liver-related morbidity or mortality in CHD patients. It considers parameters such as age, sex, region of origin, bilirubin, platelets and INR. The score classifies the patient in one of three categories, derived from a hazard ratio analysis in 3 European cohorts; mild risk (BEA-A), moderate risk (BEA-B), and severe risk (BEA-C).(76) In our cohort we assessed the BEA-score in all anti-HDV positive subjects; 6% of mild risk, 25% of moderate risk, and 80% of severe risk patients developed clinical outcomes, which is in line with the results of the original cohorts. This score could be a useful resource for clinical outcome prediction in patients with CHD.

For now, persistent HDV-RNA levels and/or presence of liver cirrhosis are determinant to assess the likelihood of developing clinical outcomes.

In the second study, we evaluated whether untreated patients with CHD showed spontaneous changes in HDV-RNA levels.

Because of the limited access to HDV-RNA testing in some countries, several research groups have explored possible surrogate markers of treatment efficacy. A white paper written by experts on HDV management proposed the following as surrogate markers: undetectable serum HDV-RNA 6 months after stopping treatment and/or a decline in HDV-RNA of 2 or more logarithms (≥ 2 $\log_{10}$) by the end of therapy.(48)

In our study, patients were stratified into two groups: those who presented a HDV-RNA ≥ 2 $\log_{10}$ decline between consecutive samples at any time during follow-up and those who did not. In total, a quarter of CHD patients showed at least one ≥ 2 log10 decline during a 5.6-year follow-up. This decline was strongly associated with HDV-RNA negativization: HDV-RNA became undetectable in 78% of patients with ≥ 2 $\log_{10}$ decline. By the end of follow-up, normal ALT levels were achieved in 64% of patients with HDV-RNA ≥ 2 $\log_{10}$ decline ($p=0.067$) and only in 29% of those without decline ($p=0.204$).

We also observed an association between HDV-RNA ≥ 2 $\log_{10}$ decline and HBsAg decrease. During follow-up, HBsAg showed a decreasing tendency in all patients, however, the drop from baseline to last follow-up was significantly larger in patients with HDV-RNA ≥ 2 $\log_{10}$ decline than in those without decline ($p=0.039$). HBV-RNA also decreased in patients with HDV-RNA ≥ 2 $\log_{10}$ decline ($p=0.066$).

Our cohort includes a high percentage of patients with liver cirrhosis and normal ALT levels; these patients are more likely to have been exposed to a longer length of infection. Thus, this could explain the spontaneous HDV-RNA decline. Spontaneous declines in HBsAg and HBV-DNA have been reported in other long follow-up studies in patients with chronic hepatitis B (CHB). Low or even undetectable HBV-DNA levels have been observed in patients with long-standing infection or advanced liver disease, particularly in those HBeAg-negative with

liver cirrhosis.(97)(98) A similar phenomenon could occur in CHD patients, as described in other cohorts.(99)(37)

In our study, presence of HDV-RNA ≥2 $\log_{10}$ decline did not have an impact on clinical outcomes. Overall, 21% of the patients experienced at least one clinical outcome, irrespective of presence or absence of HDV-RNA ≥2 $\log_{10}$ decline, mainly due to the high rate of patients with baseline liver cirrhosis.

Most studies on the natural history of HDV infection are retrospective analyses, in which HDV-RNA is determined qualitatively or quantitatively by in-house low sensitivity techniques. Since the implementation of the WHO international standard for HDV-RNA quantification, many laboratories have changed their quantification methodology, but this method is still far from being the gold standard. Many hospitals still use in-house techniques, limiting the reproducibility and reliability of results and making it difficult to conduct large multicenter studies.

In 2019, the HIDIT-II study was initiated in another randomized controlled trial with pegIFNα for 96 weeks plus Tenofovir or placebo. In total, 40% of the patients reached undetectable HDV-RNA levels after 96 weeks, as determined by in-house PCR assays.(100) In 2021, the last samples of those patients who were HDV-undetectable at the end of thr HIDIT-II study were re-analyzed. The samples were retested using a commercial kit and the WHO international standard for quantification of HDV-RNA, and results showed that one third of the patients previously classified under 'undetectable' had, in fact, detectable viraemia. Moreover, low HDV viraemia was associated with higher rate of post-treatment relapse, which occurred in 67% of patients with low detectable viraemia at the end of treatment.(73) This highlights the importance of using reliable and reproducible methods of quantification for HDV-RNA.

Chronic hepatitis D is a low prevalence disease; multicentre studies are needed to create relevant cohorts for long-term follow-up analyses. In order to standardize results, it is optimal to perform HDV-RNA quantification with a known

commercial kit (both acid nucleic extraction and RT-qPCR procedures) with replicates, if possible. In addition, the WHO international standard should also be used to harmonize results for further collaborations with other centers.

Our study presents some limitations. First, most patients were white-Europeans infected by HDV genotype 1. Therefore, these results cannot be extrapolated to other non-white populations. Second, a longer follow-up would be needed to assess the impact of HDV-RNA declines on clinical outcomes.

Nonetheless, these findings could have implications on treatment endpoint assessments. The fact that HDV-RNA spontaneously declines ≥ 2 $\log_{10}$ might prompt a revaluation of end-of-treatment endpoints with new emerging therapies for CHD.

The third study evaluated quality of life scores in untreated CHD patients and compared the results with those of CHB patients.

Patient-reported outcomes (PROs) are widely used tools to evaluate health-related problems and life impairment. They have extensively been used in patients with chronic hepatitis C (CHC), with poorer results in patients with cirrhosis than in patients with mild fibrosis.(101) Moreover, PROs results have been reported to improve during and after direct-action antiviral treatment.(102) Younossi *et al.* reported that CHB and CHC patients had worse PROs scores when they had detectable viraemia, than after viral suppression or sustained virological response, when there was a significant improvement.(103) Quality of life in patients with CHD has scarcely been assessed.

In our study, patients with CHD had higher impairments than patients with CHB, particularly in the worry, emotional, physical and activity impairment domains. Within CHD patients, those with HDV-RNA>2000 IU/ml had a significant impairment on the activity domain compared those with low or undetectable HDV viraemia.

With new drugs arising, it is fundamental to monitor the health-related quality of life of CHD patients.

6.1. LIMITATIONS

Chronic hepatitis D is a rather low prevalence disease, hence the difficulty to gather large sample populations. The cohorts analyzed in this doctoral thesis are small. The reported data is in line with other contemporary cohorts, but larger populations would need to be studied to increase the statistical power and to validate the results.

The studied population is not fully representative. Most of the subjects are Caucasian, European and mainly infected by HDV genotype 1. Moreover, the baseline characteristics of the studied population are diverse, highlighting the heterogeneity of the infection in real-life practice but also possibly biasing the results. For instance, almost half of the studied subjects have liver cirrhosis, naturally incrementing the risk of developing a clinical event.

The subjects included in the studies are all enrolled in academic centers with active liver transplantation programs, which may imply that patients attended in these centers have more severe CHD.

7. CONCLUSIONS

7. CONCLUSIONS

1. The first study reports a HDV prevalence of 5.2% among the HBsAg-positive population, with a 73% rate of active HDV infection (detectable HDV-RNA).

2. Patients with persistent HDV viraemia present higher risk of developing liver cirrhosis and clinical outcomes.

3. The second study shows that a quarter of CHD patients achieve a spontaneous HDV-RNA ≥ 2 $\log_{10}$ decline during the natural course of the infection.

4. HDV-RNA decrease of $\geq 2\log_{10}$ is associated with HDV-RNA negativization, normal ALT values and HBsAg loss, though this fluctuation has no impact on the development of clinical outcomes.

5. The last study describes that quality of life results recorded in patients with CHD are more impaired than those observed in CHB, while HDV viraemia does not seem to impact on PROs unless levels of HDV-RNA are high.

8. FUTURE LINES

8.1. POTENTIAL ROLE OF HUMAN LEUKOCYTE ANTIGEN EPITOPES

In addition to HDV viraemia, the host's immune response plays an equally important role in the natural course of the infection. The interaction between HDV and the immune system has been scarcely studied. Similarly to what has been described for HIV and other viruses, Roggendorf *et al.* proposed certain human leukocyte antigen (HLA) epitopes as immune-modulators of the host response against HDV.(104) While some HLA type I aleles have been linked to viral evasion towards immune control, HLA B27 has been suggested as a possible indicator that facilitates infection clearance. Further studies in large cohorts are needed to confirm these findings.

8.2. MASSIVE HDV-RNA SEQUENCING

One of the main characteristics of viral genomes is the high evolutionary rate in which genetic variability is generated. Therefore, variant genomes can be found within the same host, product of high mutation rates.(105) These variants are named quasispecies and they arise dynamically and in relative frequency, influenced by internal factors such as host's immune response, and/or external factors such as antiviral therapy.(106)

During HBV-HDV infection it has been reported that innate immune response is strongly activated, however, it seems not to impair HDV replication while it inhibits HBV.(107) Concerning adaptive immune response, Nisini *et al.* described CD4+ T cell responses towards specific HDAg epitopes, particularly in patients with low levels of HDV viraemia.(108) Grabowski *et al.* also reported that adaptive HDV-specific immune responses contribute to the control of HDV infection.(109) Karimzadeh *et al.* reported that some HDAg epitopes were only partially recognized by CD8+ T cells isolated from patients; these could be mutations that allow HDV to escape the immune response, resulting in persistent infection.(110)

It tempting to hypothesize that adaptive response might adjust and recognize predominant quasispecies. As new mutants arise, immune system has to adapt constantly. This could explain why HDV-RNA spontaneously fluctuates during the natural course of the infection, because host's response requires a period of time to recognize new HDAg mutants.

It could be of great interest to perform massive sequencing of HDV-RNA in patient's serum and observe if there is a pattern of the same mutant epitopes that correlates to HDV viraemia levels.

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10. APPENDIX

10.1. ARTICLE 3

Chronic hepatitis D associated with worse patient-reported outcomes than chronic hepatitis B

Maria Buti | Maria Stepanova | Adriana Palom | Mar Riveiro-Barciela | Fatema Nader | Luisa Roade | Rafael Esteban | Zobair Younossi

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Chronic hepatitis D associated with worse patient-reported outcomes than chronic hepatitis B

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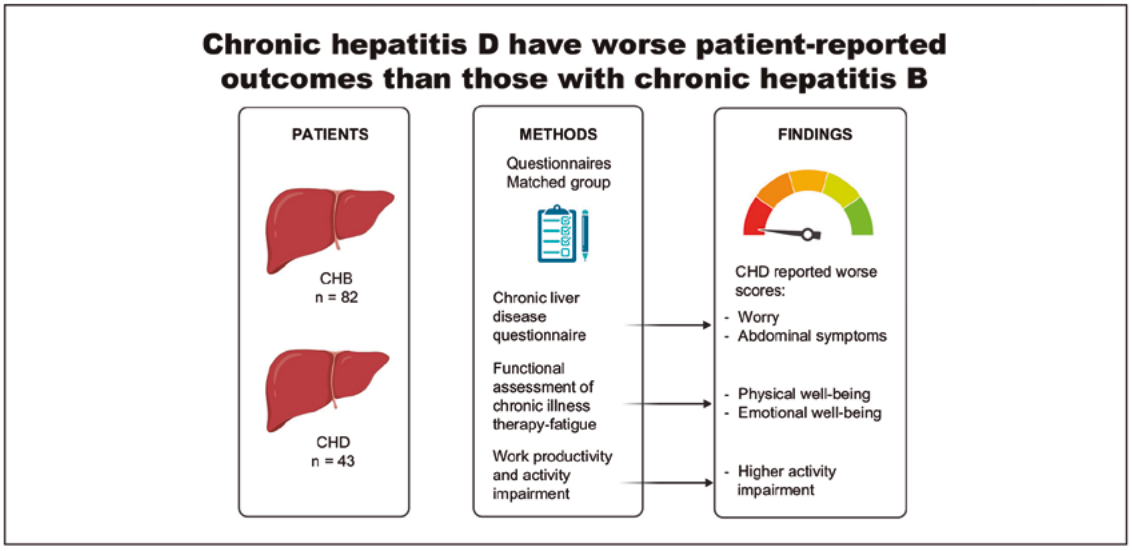
Graphical abstract



Highlights

- Patient-reported outcomes (PROs) have been studied in patients with chronic hepatitis B and C.
- There are no data on PROs for chronic hepatitis D.
- Several scores of health-related quality of life are worse in patients with chronic hepatitis D than in those with chronic hepatitis B.
- PROs are useful for evaluation of quality of life in clinical trials during and after treatment.

Lay summary

Chronic hepatitis D (CHD) is a viral disease that causes rapid evolution to liver cirrhosis, amongst other severe complications, when compared to patients with chronic hepatitis B (CHB). Health-related quality of life in chronic hepatitis C and CHB has been reported widely, but no studies have been performed on patient-reported outcomes in patients with CHD. Results showed that CHD patients reported worse outcomes in psychological domains such as worry and emotional well-being, as well as in physical domains such as abdominal symptoms, physical well-being, and activity impairment in comparison with patients with CHB.

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Research Article JHEP|Reports

Chronic hepatitis D associated with worse patient-reported outcomes than chronic hepatitis B

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Background & Aims: Health-related quality of life (HRQoL) determined by patient-reported outcomes (PROs) is impaired in chronic hepatitis B (CHB) and C patients, but there are no data regarding patients with chronic hepatitis D (CHD). The aim of this study was to assess PRO scores in untreated patients with CHD and compare them with those obtained for patients with CHB.

Methods: Patients with CHD completed 3 PRO instruments (Chronic Liver Disease Questionnaire [CLDQ], Functional Assessment of Chronic Illness Therapy–Fatigue [FACIT-F], and Work Productivity and Activity Impairment [WPAI]), and the results were compared with those of patients mono-infected with CHB.

Results: In total, 125 patients were included: 43 with CHD and 82 with CHB. Overall, baseline PROs showed differences between both groups. Several assessments, such as the worry score from CLDQ ($p = 0.0118$), functional well-being from FACIT-F ($p = 0.0281$), and activity impairment from WPAI ($p = 0.0029$) showed a significant trend to worse scores in patients with CHD than with CHB. In addition, the linear regression model supports the finding that having CHD as opposed to having CHB was a predictor of a higher worry score (CLDQ) and a higher activity impairment (WPAI).

Conclusions: In this first assessment in CHD, PROs recorded in patients with CHD showed a significant impairment in some domains of HRQoL questionnaires in comparison with those with CHB. Studies in larger cohorts with lengthier follow-up are needed to fully assess patient-reported quality of life over the course of CHD.

Lay summary: Chronic hepatitis D (CHD) is a viral disease that causes rapid evolution to liver cirrhosis, amongst other severe complications, when compared to patients with chronic hepatitis B (CHB). Health-related quality of life in chronic hepatitis C and CHB has been reported widely, but no studies have been performed on patient-reported outcomes in patients with CHD. Results showed that CHD patients reported worse outcomes in psychological domains such as worry and emotional well-being, as well as in physical domains such as abdominal symptoms, physical well-being, and activity impairment in comparison with patients with CHB.

Introduction

Chronic liver diseases, particularly liver cirrhosis and hepatocellular carcinoma, have been associated with reductions in health-related quality of life (HRQoL) and poorer patient-reported outcomes (PROs).[1–3] Regardless of the aetiology of liver disease, patients with chronic viral hepatitis, alcoholic liver disease, or fatty liver disease can have impaired HRQoL, and this poses a significant economic burden on society.[4,5]

Health-related outcomes and PROs have both been extensively investigated in patients with chronic hepatitis C (CHC).[6,7] Several studies performed in different countries showed that PRO scores are impaired during the natural course of HCV infection and are poorer in patients with liver cirrhosis than in those with mild fibrosis.[8–10] Hepatitis C therapy based on interferon demonstrated a decline in quality of life during therapy[11] and a later improvement when HCV elimination was achieved.[12,13] Therapy based on oral direct-acting antivirals (DAAs) has dramatically changed the management of CHC patients, providing effective treatment with improvements in HRQoL during and after therapy.[14,15] A recent study found that chronic hepatitis B (CHB) and CHC patients had worse PRO scores when they tested positive for viraemia, than after viral suppression or sustained virological response, when there was a substantial improvement.[16]

Chronic hepatitis D (CHD) is the most severe form of chronic viral disease,[17] often leading to liver cirrhosis.[18,19] HDV requires the simultaneous presence of HBV to be infectious and fully

Keywords: Viral hepatitis; Health-related quality of life; Chronic Liver Disease Questionnaire; Functional Assessment of Chronic Illness Therapy–Fatigue; Work Productivity Activity Impairment.
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express its pathogenicity and, therefore, HDV infection always occurs in the presence of HBV.[20] Worldwide, 5% of chronically infected HBV patients are also infected with HDV, which yields an estimated 20 million people with HDV infection.[21,22] Current therapy for patients with CHD is interferon alpha (IFNα) or pegylated interferon alpha (peg-IFNα).[23,24] Recently, a new drug, bulevirtide, a first class entry inhibitor has obtained conditional authorisation by the European Medicines Agency (EMA) to treat hepatitis D.[25]

Individuals with CHD are 2–3 times more prone to develop liver cirrhosis than those who are mono-infected by HBV.[26,27] In addition, among anti-HDV-positive patients, those with active HDV infection are more likely to develop liver cirrhosis and clinical events than those who have persistently undetectable HDV viraemia.[28] PROs have not been previously evaluated in patients with CHD, despite the severity of this liver disease.

CHD patients are a heterogeneous population, not only in terms of liver damage, but also regarding their virologic profile, as a result of the interactions between HDV and HBV.[29] The majority of patients with CHD have HDV replication and simultaneous suppression of HBV replication either spontaneously or induced by nucleos(t)ide analogues (NAs) treatment. However, a small percentage of them have both HDV and HBV replication or even spontaneously clear HDV replication.[23] The impact of the virologic profile can influence PROs as it occurs in patients with CHB and CHC.

The aims of this study were to compare PROs between patients with CHD and CHB, and also to the impact of the detection of HDV-RNA on PROs.

Patients and methods

Patients

In this study, consecutive HBsAg-positive patients with anti-HDV antibodies were prospectively enrolled in Hospital Vall d'Hebron (Barcelona, Spain) from January 2018 to December 2019. The inclusion criteria were age over 18 years, HBsAg-positive and with presence of anti-HDV (with or without HDV-RNA) for more than 6 months, lack of significant comorbidities or other extrahepatic manifestations, no antiviral therapy other than NAs for CHB, and a willingness and ability to answer questionnaires in the Spanish language.[30] Patients were excluded if they had previous hepatic decompensation, hepatocellular carcinoma, co-infection with HIV or HCV, or other major conditions that could affect quality of life assessment.[31] A homogenous group of CHB patients testing negative to anti-HDV antibodies and meeting the same inclusion and exclusion criteria was used as the comparator.

In both groups, demographic, educational level, and employment status were collected, as well as clinical and laboratory data (platelet levels, alanine aminotransferase [ALT], aspartate aminotransferase [AST], HBeAg, HDV-RNA, HBV-DNA, liver stiffness measurements, hepatitis B treatment received, clinical events, and history of mental illness). HDV-RNA was quantified using an in-house one-step RT-qPCR. The World Health Organization international standard of quantification was used (with a lower limit of detection [LLOD] of 100 IU/ml and a lower limit of quantification [LLOQ] of 575 IU/ml). HBV-DNA was quantified by a commercial real-time RT-PCR technique with an LLOD of 10 IU/ml and an LLOQ of 20 IU/ml. Liver cirrhosis was defined by clinical and hepatic ultrasound findings, non-invasive markers (fibrosis-4 [FIB-4], AST to platelet ratio index [APRI]), and hepatic elastography (>13.5 kPa) or liver biopsy showing a fibrosis stage ≥F5 according to the Ishak score.

This study was approved by the Vall d'Hebron Hospital ethics committee (PR[AG]247/2018), and was conducted in compliance with the principles of the Declaration of Helsinki, good clinical practice guidelines, and local regulatory requirements. Patients gave oral consent once the study was explained, and all data were anonymised.

PROs were assessed during the patients' regular visits to the hospital. All instruments were given to the patients to complete on their own just before the visit. To avoid bias in the answers, the site staff and patients were blinded to the most recent analytical results at the time PROs were filled out.

Methods

The PRO questionnaires used were the Chronic Liver Disease Questionnaire (CLDQ), the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) and the Work Productivity and Activity Impairment (WPAI).[32]

The CLDQ, the first liver-specific instrument developed, includes 29 items in the following domains: Abdominal Symptoms, Fatigue, Systemic Symptoms, Activity, Emotional Function, and Worry. The CLDQ responses are rated on a scale of 1–7, ranging from having a problem or experiencing a symptom 'all of the time' to 'none of the time', respectively. Thus, higher scores indicate better HRQOL. The original CLDQ was shown to have construct validity in studies on chronic liver diseases.[33]

The FACIT-F questionnaire is a fatigue-specific PRO instrument that includes 4 well-being domains (physical, emotional, social, and functional), and a fatigue subscale.[34] It is designed as a scale of 0–160, where the higher the score, the higher the HRQoL.

The WPAI–Specific Health Problem is used to evaluate impairment in patients' daily activities and work productivity associated with a specific health problem (in this study, HDV or HBV infection). The Work Productivity Impairment domain is a sum of the Absenteeism (lost hours of work) and Presenteeism (self-reported decreased productivity while working) domains; it is assessed only in employed patients. The Activity Impairment domain focuses on impairment in daily activities other than work and is assessed in all participants regardless of their employment status.[35] The sum of specific health problem impairment and impairment attributable to other health reasons is equal to impairment attributable to all health reasons. WPAI outcomes are expressed as impairment percentages, with higher numbers indicating greater impairment and less productivity.

Statistical analysis

Continuous variables are expressed as the mean ± SD, and categorical variables as n (%). In pairwise comparisons of clinical parameters and PRO scores between groups of interest (*e.g.* patients with HDV *vs.* patients with HBV mono-infection, or HDV patients with and without detectable viraemia), the chi-square test and the Mann-Whitney *U* test were used for categorical and continuous parameters, respectively. As mono-infected CHB patients were different from CHD patients by a number of clinical parameters potentially confounding PRO scores, we also utilised a case-control design via selecting matched CHB controls for CHD patients. Matching was done using a propensity score which included age, sex, history of depression or mood disorders, ALT, AST, and platelet count; the propensity scores were then fed to a maximum weight bipartite matching algorithm for

Table 1. Demographic, serologic, virologic, and clinical data of the study cohort.

	Chronic hepatitis D	Chronic hepatitis B	
	No. cases = 43	No. cases = 82	*p* value
Age, years	47.3 ± 11	51.8 ± 15.3	0.1892
Male sex, n (%)	27 (63)	49 (59.8)	0.7413
Caucasian, n (%)	34 (79)	58 (70.7)	0.3151
Primary education, n (%)	22 (51)	45 (56)	0.6404
Secondary education or higher, n (%)	21 (49)	36 (44)	0.6403
Employed, n (%)	32 (74)	48 (58.5)	0.0789
Housewives, n (%)	9 (21)	9 (11)	0.1396
BMI	26.1 (±3.8)	32.0 (±47.6)	0.6651
ALT, IU/ml	68.4 (±69.0)	29.4 (±28.9)	<0.0001
AST, IU/ml	61.1 (±57.1)	28.5 (±17.9)	<0.0001
Platelets, $\times10^9$/L	179.8 (±75.1)	219.9 (±66.5)	0.0061
Hepatic elastography, kPa	10 (6.1-18.1)	4.8 (4.2-5.9)	<0.0001
APRI	1.20 (±1.60)	0.409 (±0.568)	<0.0001
FIB-4	2.61 (±2.95)	1.62 (±1.78)	0.0037
HDV RNA positive, n (%)	26 (60)	0 (0)	-
HBV DNA positive, n (%)	11 (26)	41 (50)	0.0085
HBeAg, n (%)	4 (11)	5 (6)	0.3893
NA treatment, n (%)	27 (63)	32 (39)	0.0115
History anxiety or panic disorder, n (%)	15 (35)	25 (30.5)	0.6167
History depression or mood disorder, n (%)	3 (7)	10 (12.2)	0.3639

All continuous variables are described as median ± IQR, all categorical variables are described as n (%). The Chi-square test and Mann-Whitney *U* test were used for categorical and continuous parameters, respectively. Values of $p \leq 0.05$ were considered statistically significant.
ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; FIB-4, fibrosis-4; NA, nucleos(t)ide analogues.

1:1 matching. Values of $p \leq 0.05$ were considered statistically significant and $p < 0.10$ were considered to indicate a trend (owing to the small sample size).

Results

Baseline characteristics of patients with CHD and CHB

In total, 125 questionnaires were administered to 43 CHD patients and 82 CHB patients at the inclusion of the study. Baseline demographics, educational status, clinical characteristics, and HDV and HBV serologic and virologic markers are shown in Table 1.

In both groups the majority of patients were male, Caucasians, with a mean age of 50.2±14.2 years, and employed. Patients with primary and secondary or higher educational levels were around 50% in both CHD and CHB groups.

Patients with CHD patients had significantly higher ALT and AST levels ($p < 0.0001$), and higher levels of hepatic elastography ($p < 0.0001$), APRI ($p < 0.0001$), and FIB-4 ($p = 0.0037$) than those with CHB mono-infection.

Patient-reported outcomes in patients with CHD and CHB

All patients included completed the Spanish versions of the CLDQ, FACIT-F, and WPAI. The total time needed to fill out the questionnaires ranged from 8 to 15 min.

At study inclusion, the mean CLDQ total score was 5.77 ± 1.06 in patients with CHD and 5.88 ± 0.94 in patients with CHB ($p = 0.73$). Patients with CHD had a significantly worse worry score ($p = 0.0118$) than patients with CHB. There were no other differences in the CLDQ scores for the various domains between these groups.

Table 2. Demographic, serologic, virologic, and clinical data of the matched CHD *vs.* CHB cohort.

	Chronic hepatitis D	Chronic hepatitis B	
	No. cases = 35	No. cases = 35	*p* value
Age, years	47.2 ± 12.0	52.0 ± 17.1	0.3351
Male sex, n (%)	22 (62.9)	26 (74.3)	0.3031
Caucasian, n (%)	28 (80.0)	24 (68.6)	0.2740
Employed, n (%)	25 (71.4)	21 (60.0)	0.3138
BMI	25.5 ± 3.3	39.4 ± 73.4	0.1323
ALT, IU/mL	46.3 ± 33.5	40.8 ± 40.7	0.1747
AST, IU/mL	42.9 ± 25.5	36.5 ± 25.0	0.1153
Platelets, $\times10^9$/L	178.7 ± 77.3	206.5 ± 54.7	0.0941
APRI	0.932 ± 1.308	0.584 ± 0.829	0.0896
FIB-4	2.48 ± 3.14	1.95 ± 2.45	0.3627
HDV RNA positive, n (%)	19 (54.2)	0 (0)	-
HBV DNA positive, n (%)	10 (28.6)	17 (48.6)	0.0856
HBeAg, n (%)	2 (6.9)	4 (11.8)	0.5118
NA treatment, n (%)	21 (60)	15 (42.9)	0.1513
History anxiety or panic disorder, n (%)	13 (37.1)	9 (25.7)	0.3031
History depression or mood disorder, n (%)	2 (5.7)	2 (5.7)	1.0000

All continuous variables are described as median ± IQR, all categorical variables are described by number and percentage. The Chi-square test and Mann-Whitney *U* test were used for categorical and continuous parameters, respectively. Values of $p \leq 0.05$ were considered statistically significant.
ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; FIB-4, fibrosis-4; NA, nucleos(t)ide analogues.

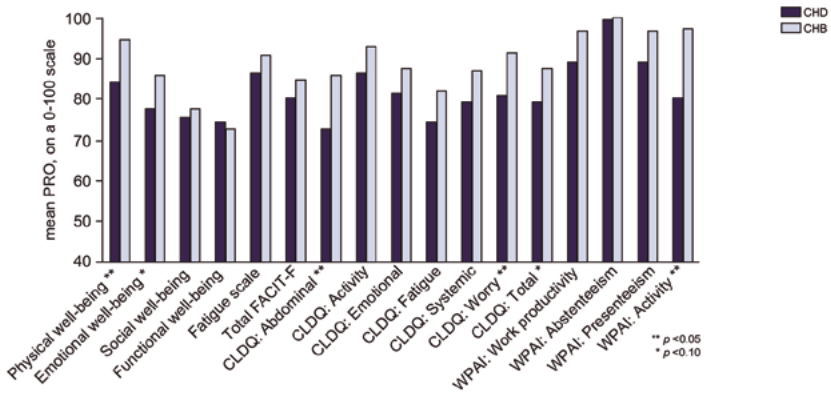

Fig. 1. Patient-reported outcomes comparing patients with chronic hepatitis D and chronic hepatitis B from a baseline characteristic matched cohort. All parameters were normalised to a scale of 0–100 for a comprehensible comparison. The Student *t* test and Mann-Whitney *U* test were used for parametric and non-parametric categories, respectively. Values of $p \leq 0.05$ were considered statistically significant. CHB, chronic hepatitis B; CHD, chronic hepatitis D; CLDQ, Chronic Liver Disease Questionnaire; EWB, emotional well-being; FACIT-F, Functional Assessment of Chronic Illness Therapy–Fatigue; FS, fatigue scale; FWB, functional well-being; PWB, physical well-being; SWB, social well-being; WPAI, Work Productivity Activity Impairment.

The total FACIT-F score on a scale of 0–160 was 131.4 ±24.5 in patients with CHD and 128.1 ± 25.2 in patients with CHB ($p = 0.3285$). A better functional well-being score was observed in CHD than in CHB ($p = 0.0281$), with no other significant differences in other domains. The results of the WPAI questionnaire showed that patients with CHD had a higher activity impairment score than patients with CHB ($p = 0.0029$). No other differences were observed in other domains (Table S1).

In multivariate analysis, a generalised linear regression model showed that having CHD as opposed to having CHB, was an independent predictor of a worse worry score (beta = -0.34, $p = 0.0394$) and high activity impairment (beta = +0.13, $p = 0.0010$).

Regarding educational levels in all patients, results showed that patients with secondary or higher education (N = 57) had better HRQoL than those with none or primary educational level (N = 67) in the abdominal, activity, emotional, fatigue, and systemic domains of the CLDQ, as well as in all domains of the FACIT-F.

Regarding employment status, those with full time or part time jobs (N = 80) had significantly higher HRQoL scores than those who were unemployed (N = 45) in the physical, social, and fatigue domains of the CLDQ, as well as the abdominal, activity, fatigue, systemic, and worry domains of FACIT-F. When studying the housewife (N = 18) *vs.* non-housewife (N = 107) population, data revealed better HRQoL in the housewife group (physical, emotional, and fatigue domains of CLDQ, and abdominal, activity, fatigue, systemic, and worry domains of FACIT-F).

Very similar reproducible results were observed when studying the CHD group and CHB group separately.

Patient-reported outcomes in CHD- and CHB-matched patients

Of all patients with CHD who were included, n = 35 had a matched control with CHB. In this analysis again the majority of patients with CHD were male, Caucasian, and employed. Neither ALT, AST, and platelet levels nor APRI and FIB-4 scores were significantly different between cases and controls (all $p > 0.05$; Table 2).

The mean CLDQ total score was 5.76 ± 1.06 in those patients with CHD and 6.27 ± 0.53 in controls with CHB ($p = 0.07$). Patients with CHD had a significantly worse worry score ($p = 0.0021$) and more abdominal symptoms ($p = 0.0364$) than patients with CHB. There were no other differences in other CLDQ domains.

The total FACIT-F score on a scale of 0–160 was 129.4 ± 24.5 in patients with CHD and 136.8 ± 14.2 in patients with CHB ($p = 0.4416$). Poorer physical well-being ($p = 0.0036$) and emotional well-being ($p = 0.0541$) was observed in CHD than in CHB, with no other significant differences in other domains. The results of the WPAI questionnaire showed that patients with CHD had a higher activity impairment score than patients with CHB ($p = 0.0008$). No other differences were observed in other domains (Fig. 1 and Table S2).

Patient-reported outcomes in relation to the presence of hepatitis D viraemia

Within the CHD group (N = 43), PROs were analysed in relation to the presence of hepatitis D viraemia. Twenty-six (60%) patients had persistently detectable HDV-RNA (active infection) and 17 (40%) patients had undetectable HDV-RNA. There were no differences in the overall scores for the 3 questionnaires relative to those with persistently undetectable HDV-RNA (all $p > 0.10$).

PROs were also evaluated in relation to the HDV viraemia levels. Patients with high levels (≥2,000 IU/ml) of baseline HDV-RNA (N = 19, 44%) proved to have higher activity impairment on the WPAI questionnaire than those with low HDV-RNA levels (<2,000 IU/ml) ($p = 0.024$).

Patient-reported outcomes in relation to liver cirrhosis

No patients with CHB presented liver cirrhosis at baseline or at 1 year of follow-up. Patients with CHD with and without liver cirrhosis at baseline were compared. Results showed that patients with CHD and liver cirrhosis (N = 14) had a worse CLDQ

systemic score than those without liver cirrhosis (N = 29), $p = 0.0442$.

Discussion

This is, to our knowledge, the first study reporting HRQoL through the evaluation of CLDQ, FACIT-F, and WPAI questionnaires in patients with CHD. Overall, the study shows that patients with CHD have higher HRQoL impairments than patients with CHB, especially in the worry, emotional, physical, and activity impairment domains.

Accurate HRQoL assessment can provide valuable information for clinical practice and designing public health policies.[36,37] PROs evaluated using CLDQ, FACIT-F, and WPAI tools have been widely validated for CHC and CHB. However, there are no data for CHD. It is well known that the individual and subjective nature of a patient's perception, together with social and cultural influences, make HRQoL difficult to measure.[38–40]

The CLDQ has been designed as a liver-disease-specific questionnaire, that is reliable, reproducible, easy to use, and has been well-validated in patients with CHC or CHB[16,41] and is anchored by a 2-week recall period.

Fatigue is a common symptom in chronic liver disease, and various questionnaires can be used to evaluate it. FACIT-F has been widely used in hepatitis C patients and it is easy to perform.[30] In studies comparing PROs related to fatigue in patients with CHB or CHC and in the general population, poorer scores were found in patients with chronic viral disease, with no differences between the 2 conditions.[42] In our study, there was a tendency for patients with CHD to have a poorer well-being score, which has also been shown in studies with CHB.

Overall, PRO scores are reported to be poorer in patients with CHC than with CHB, which could be explained by a weaker systemic and differentiated inflammatory impact of HBV infection.[43,44] Similarities in the systemic effects of HDV and HBV chronic infection could justify the smaller differences in HRQoL found here.

An intriguing finding of our study is that the presence of hepatitis D viraemia did not impact in PROs as has been seen in patients with CHB, where suppression of viral replication has been associated with better PROs scores. Only among patients with HDV-RNA levels >2,000 IU/ml, a significant impairment of activity has been observed in relation with those with low or undetectable HDV-RNA levels. In our patients with CHD, HBV-DNA levels were very low or suppressed because of the inhibiting effect of HDV over HBV and/or NAs treatment, as has been previously reported in several studies.[23] In CHC patients, an improvement has been described in HRQoL after DAA treatment and subsequent elimination of hepatitis C.[45,46] The lack of an effective and safe curative treatment for either CHD or CHB could also contribute to the absence of differences between the 2 groups.

Significant differences between educational level, employment status, and housewife population results are in line with those observed in other general population studies[47–49] and even in chronic disease populations.[50] No associations were observed in CHD or CHB groups separately.

Our study has some limitations. First, the number of patients with CHD included is small owing to the limitations in the inclusion criteria, no comorbidities, no previous therapy with interferon, and compensated liver disease. Second, all patients were enrolled in an academic centre with an active liver transplant program that probably had patients with more severe hepatitis D than those observed in other centres.

New therapies for hepatitis D infection are emerging, such as lonafarnib, REP2139, other drugs used for hepatitis B, and combinations of drugs with different mechanisms of action.[51] These drugs have the potential effect to eliminate hepatitis D with different safety profiles. Therefore, it will be fundamental to monitor PROs during the course of these new therapies.

In conclusion, PROs estimating HRQoL in patients with CHD showed to be more impaired than those observed in CHB. HDV viraemia does not seem to impact on PROs unless the levels of HDV-RNA are high. This first results in HRQoL in patients with CHD need to be validated in a larger cohort of patients.

Abbreviations

ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CHD, chronic hepatitis D; CLDQ, Chronic Liver Disease Questionnaire; DAA, direct-acting antivirals; EMA, European medicines agency; FACIT-F, Functional Assessment of Chronic Illness Therapy–Fatigue; FIB-4, Fibrosis-4; HRQoL, health-related quality of life; IFN, interferon; LLOD, lower limit of detection; LLOQ, lower limit of quantification; NAs, nucleos(t)ide analogues; pegIFN, pegylated interferon; PROs, patient-reported outcomes; WPAI, Work Productivity and Activity Impairment.

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Conflicts of interest

MB and RE have served as a speaker and advisory board member for Gilead, Roche, and Arbutus. MRB has served as a speaker for AbbVie and Gilead. ZY has received research funds from Intercept, Merck, BMS, and Siemens, and has served as consultant for Gilead, Terns, Viking, Intercept, Merck, Abbvie, Novartis, BMS, Shionogi, and Siemens.

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

Authors' contributions

Guarantor of the article and responsibility for the integrity of the work as a whole, from inception to published article: MB. Designed the study: MB, FN, ZY. Collected the clinical and laboratory data: LR, AP. Carried out the analysis and interpretation of data: MS, MRB. Drafted the manuscript: LR, AP. Reviewed the manuscript: MB, MRB, ZY, FN. Approved the final version of the article: all authors

Data availability statement

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

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2021.100280.

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