Diploma Thesis

Hepatitis D Epidemiology in Styria

submitted by

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under the supervision of

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Univ.-Prof. Dr. med. univ. Rudolf Stauber

Graz, August 24, 2023

I declare that I have written the present diploma thesis fully on my own and without any assistance from third parties.
Furthermore, I confirm that no sources have been used in the preparation of the thesis other than those indicated in the thesis itself.

Graz, August 24, 2023 *Lisa Finster eh*

Contents

Abbreviations

ACLD	Advanced chronic liver disease
ALT	Alanine amino transferase
EMA	European medicines agency
FDA	Food and drug administration
HBV	Hepatitis B virus
HBsAg	Hepatis B antigen
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HIV	Human immunodeficiency virus
IFN α	Interferon alpha
IgG	Immunoglobulin G
NTCP	Sodium taurocholate co-transporting polypeptide
PCR	Polymerase chain reaction
Peg-IFN- α	Pegylated interferon alpha
RNA	Ribonucleic acid
SVR	Sustained virologic response

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Zusammenfassung

Hintergrund und Ziel der Studie:

Hepatitis D gilt als die schwerste Form der infektiösen Hepatitis; weltweit weisen mehr als 15 Millionen Hepatis-B-Antigen (HBsAg)-positive Menschen Antikörper gegen das Hepatitis-D-Virus (HDV) auf. Zur HDV-Prävalenz in der Steiermark liegen keine verlässlichen Daten vor.
Ziel dieser retrospektiven epidemiologischen Analyse war es, Daten zu allen Patient*innen mit Hepatitis D, welche an der Medizinischen Universität Graz betreut wurden, ab dem Jahr 2010 zu analysieren. Die Anzahl dieser Patient*innen wurde mit jener der steirischen HBs-Ag-positiven Patient*innen korreliert.

Materialien und Methoden:

In diese Studie wurden alle Patient*innen mit nachweisbarer Plasma-HDV-RNA eingeschlossen, die im Zeitraum von 01.01.2010 bis 31.12.2022 im Labor für Molekulare Diagnostik der Medizinischen Universität Graz gemeldet wurden. Nach der automatisierten Extraktion der Plasmaproben unter Verwendung der eMAG®-Plattform (bioMerieux) wurden die mit dem RoboGene HDV Quantification Kit 2.0 (RoboGene) in Kombination mit dem LightCycler® 480 II Roche) amplifiziert und quantifiziert.

Ergebnisse:

Insgesamt wurden in der Steiermark 12 HDV-RNA-positive Patient*innen identifiziert, von denen 7 an der Medizinischen Universität Graz betreut wurden. Zwei der 7 Patient*innen verstarben während des Beobachtungszeitraums aufgrund eines fortgeschrittenen chronischen Leberversagens, eine Patientin übersiedelte nach Kärnten. Von den restlichen 4 Patient*innen erhält einer seit Juni 2022 eine Therapie mit Bulevirtide. Die Laborwerte des behandelten Patienten zeigten einen deutlichen Rückgang sowohl der Plasma-Viruslast (HDV-RNA) als auch der Lebertransaminase ALT.

Schlussfolgerung:

Die Zahl der HDV-RNA-positiven Patient*innen, die in der Steiermark leben, ist aktuell gering. Die Mehrheit der HDV-Infizierten leidet an einer fortgeschrittenen Lebererkrankung. Daher ist es besonders wichtig, alle HBsAg-Infizierte auf HDV zu testen, um rechtzeitig eine anti-HDV Therapie einleiten zu können.

Abstract

Background and Aim of the Study:

Hepatitis D is considered to be the most severe form of infectious hepatitis; worldwide more than 15 million hepatis B antigen (HBsAg) carrier show antibodies against hepatitis D virus (HDV). For Styria, there is no reliable data on HDV prevalence available.
The aim of this retrospective epidemiological analysis was to provide data on all outpatients at the Medical University of Graz with hepatitis D from 2010 onwards. The number of these patients was correlated to that of HBsAg-positive Styrian patients.

Materials and Methods:

In this study, all patients with detectable plasma HDV RNA reported at the Molecular Diagnostics Laboratory, Medical University of Graz within the period Jan 01, 2010 through Dec 31, 2022 were included. HDV RNA was extracted from plasma samples on the automated eMAG® platform (bioMerieux) followed by amplification and quantitation on the LightCycler® 480 II (Roche) instrument by using the RoboGene HDV Quantification Kit 2.0 (RoboGene).

Results:

A total of 12 HDV-RNA positive patients living in Styria were identified, with 7 of them being outpatients at Medical University of Graz. Two of these seven patients deceased due to liver failure, another one moved to Carinthia. Of the remaining 4 patients, one is receiving anti-HDV therapy with Bulevirtide from June 2022 onwards. A significant decrease of plasma viral load (HDV-RNA) and aminotransferase ALT was observed.

Conclusion:

Currently, the number of patients positive for HDV RNA living in Styria is low; however, the majority of patients with chronic hepatitis D suffers from advanced chronic liver disease. With the novel therapeutical approaches, it is of paramount importance to test all HbsAg carriers for of HDV coinfection and start anti-HDV therapy as soon as possible.

1 Introduction

Hepatitis D is considered the most severe form of infectious hepatitis. The hepatitis D virus (HDV) is an incomplete RNA virus that occurs only in patients infected with the hepatitis B virus (HBV). HDV needs the HBV surface antigen (HBsAg) for replication [1].

The infection with HDV can occur in an acute or chronic process. Chronic hepatitis D is the most progressive form of infectious hepatitis [2]. It rapidly leads to liver cirrhosis and is associated with an increased risk of hepatocellular carcinoma thus causing considerable mortality worldwide [3].

Hepatitis D virus infects approximately 4.5% of people who are suffering from a chronic hepatitis B infection, this corresponds to an estimated number of 12 million people affected worldwide [4]. However, the estimated number of unreported cases may be significantly higher [5].

1.1 Prevalence and transmission

Hepatitis D generally affects people globally. There are several regions with a high prevalence of HDV infections (Fig. 1). Mongolia has the highest anti-HDV prevalence worldwide among HBsAg-positive people with almost 37%. In countries of Western and Central Africa, in the Republic of Moldova, and in Vietnam, prevalence rates are also high with ratios above >10% [4].

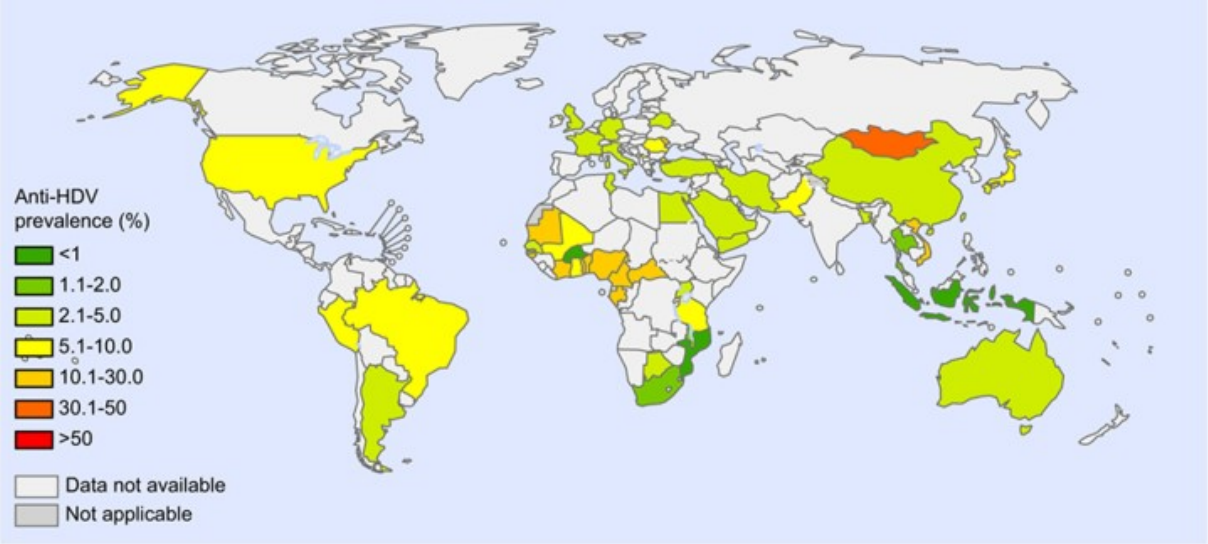


Fig. 1: Prevalence of anti-HDV among HBsAg positive people in the general population. Note that no data are available for Austria [4].

The routes of transmission of HDV are identical to those for HBV with the main route from mother to child during birth and delivery, along with contact with blood or other body fluids. HDV can thus be transmitted through injection-drug use, sex with an infected partner, sharing needles or syringes [2].

Geographic differences concerning transmission have been recorded. HDV is not endemic in northern Europe and in the United States. Infections in these countries are mainly confined to intravenous drug users. HDV transmission disappeared in polytransfused subjects and hemophiliacs as a result of HBV vaccination and blood screening for HBsAg. In areas like the Mediterranean basin, where HDV is endemic, the parenteral route is responsible for most cases of HDV transmission [6]. To prevent and control HDV infection, transmission through HBV must be stopped through hepatitis B immunization, blood safety, safe injection practices in health care settings and using clean needles and syringes [2].

1.2 HDV prevalence in Austria

The HDV prevalence in Austria has been published for the first time in December 2021 [7].

In the period from 2010 to 2020, 347 HBsAg-positive patients tested positive for anti-HDV immunoglobulin G (IgG), 202 of them were tested on plasma HDV RNA (145 were not tested on plasma HDV RNA). Of 202 plasma HDV RNA positives, 126 were confirmed positives by HDV RNA testing, while 76 were found to be HDV RNA-negatives (Fig. 2). The regions of origin were Eastern Europe (38.1%), Central Asia (20.6%), Mediterranean countries (18.3%), Austria (11.9%), Africa (6.3%), the Middle East (4.0%), and North and South America (0.8%) [7].

54.8% of all Austrian patients were male and the average age was 46.3 years [7].

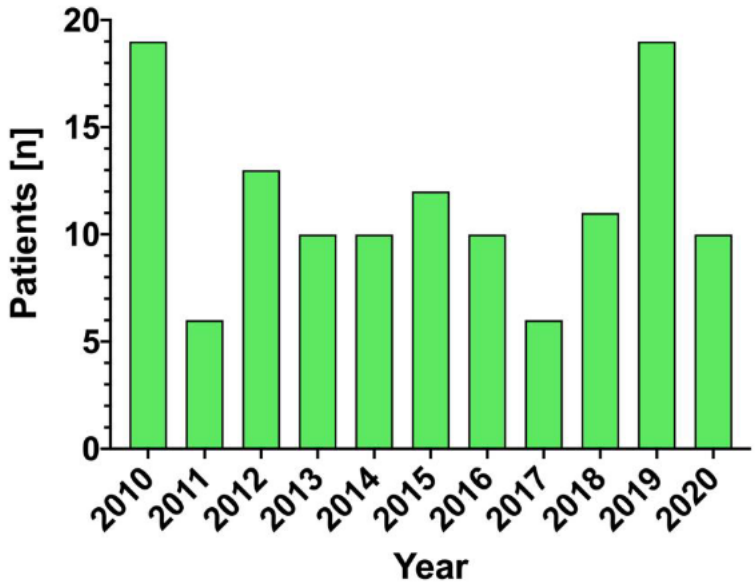


Fig. 2: No. of HDV RNA-positive patients in Austria, 2010-2020 [7].

1.3 Viral structure

HDV is an incomplete RNA virus needing HBV as helper virus being incapable of infecting in the absence of HBV (Fig. 3). HDV is formed by a lipoprotein envelope containing HBsAg on the outside [8]. Small and large hepatitis D antigens and a single stranded RNA molecule are located in the inner nucleocapsid. The small hepatitis D antigen is essential for HDV genome synthesis, the large hepatitis D antigen is required for HDV particle formation inhibiting HDV RNA synthesis [9]. The RNA genome of HDV is unique among animal viruses although it shares common characteristics with some plant viroids, for example, the replication mechanism which uses a host RNA polymerase [10].

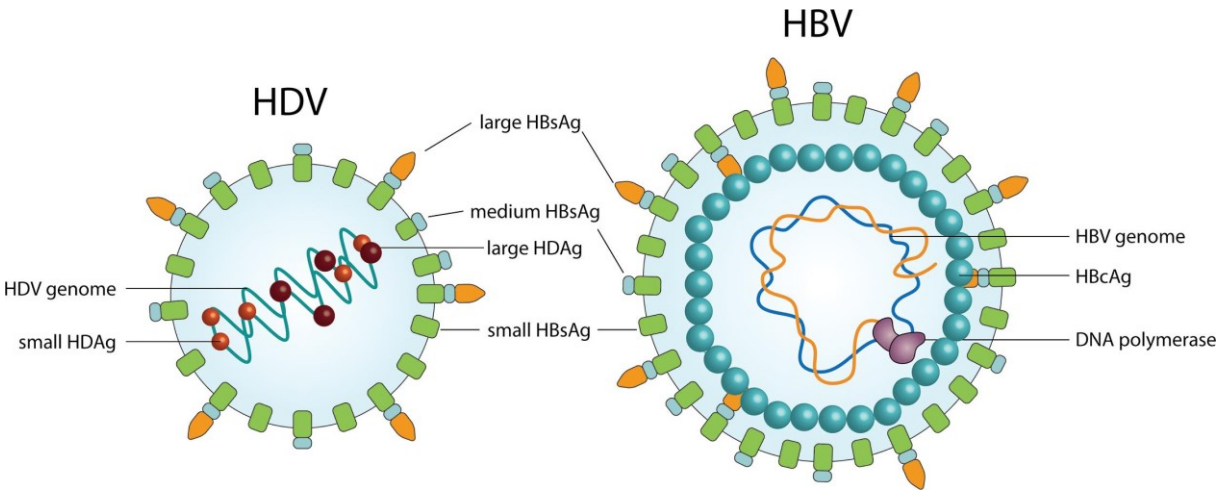


Fig. 3: HBV as a helper virus for HDV [11].

1.4 Genotypes

At least eight HDV genotypes have been found with variable geographical distribution. HDV-1 infection can lead from mild to severe disease and is the most common genotype found worldwide [11,12]. HDV-2 infection is associated with a mild hepatitis [12]. HDV-2 is prevalent in Asia, especially in Japan, Taiwan, and Russi**a** [8]. HDV-3 infection usually leads to severe hepatitis [12]. It occurs in South America, particularly in the Amazon Region of Peru, Venezuela, Columbia, and Brazil [8]. There is only limited information concerning the clinical outcome of the remaining five HDV genotypes [12]. While HDV-4 is found in Japan and Taiwan, genotypes 5,6,7, and 8 mainly occur in Africa [8].

1.5 Acute Hepatitis D

Symptoms of acute hepatitis D are very similar to other acute viral infections of the liver, although they proceed quicker. Unspecific symptoms such as nausea, fatigue, anorexia, and lethargy develop after an incubation period of 3 to 7 weeks. Elevated serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are also typical and sometimes followed by jaundice [13].

Acute hepatitis D is exclusively found together with hepatitis B, either as coinfection or as superinfection [14]. Coinfection usually leads to an acute hepatitis that cannot be separated clinically from ordinary hepatitis B. Acute coinfection rarely progresses to chronicity, only in 2% of cases. In most cases, the outcome is complete recovery, similar to acute hepatitis B [13].

In case of superinfection, HDV infects people with chronic hepatitis B. The outcome of superinfection is variable; it generally causes severe hepatitis. It may present with an aggravation of the existing chronic hepatitis B leading to liver decompensation [13]. Superinfection with HDV progresses to chronicity in 80-100% [14] [15].

In general, acute hepatitis D can range from mild to severe hepatitis followed by fulminant hepatic failure. Fulminant hepatic failure occurs in 1% of HBV/HDV co-infected individuals and in 5% of HDV superinfected individuals [16].

1.6 Chronic Hepatitis D

Chronic hepatitis D is defined as occurrence of HDV infection for over 6 months [3]. It is acquired through superinfection and a very common complication of acute hepatitis D [13]. Chronic hepatitis D is the most severe form of viral hepatitis; people suffering from this disease are at high risk for developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma [17]. Rates of these complications are significantly greater in coinfected patients compared to patients with HBV mono-infection [18]. Coinfected patients go through liver transplantation more often and are at a growing risk of mortality [3].

1.7 Diagnosis

In general, HDV screening is recommended for HBsAg-positive persons, who show a risk for HDV. Furthermore, screening is also suggested in persons born in countries with high HDV endemicity, males who have sex with males, people who have ever injected drugs, individuals infected with HIV or HCV, people with multiple sexual partners or sexually transmitted disease [2] [19]. A study from the United States confirms that HBsAg-positive persons and persons showing risk factors mentioned above, should definitely be screened [20].

Screening for HDV infection is done with anti-HDV tests searching for anti-HDV immunoglobulin G (IgG); however, these tests are prone to a high false-positive rate [21]. In case of a positive result, confirmatory testing must thus be done by using reverse transcriptase polymerase chain reaction (PCR) to detect an active HDV infection (Fig. 4) [22].

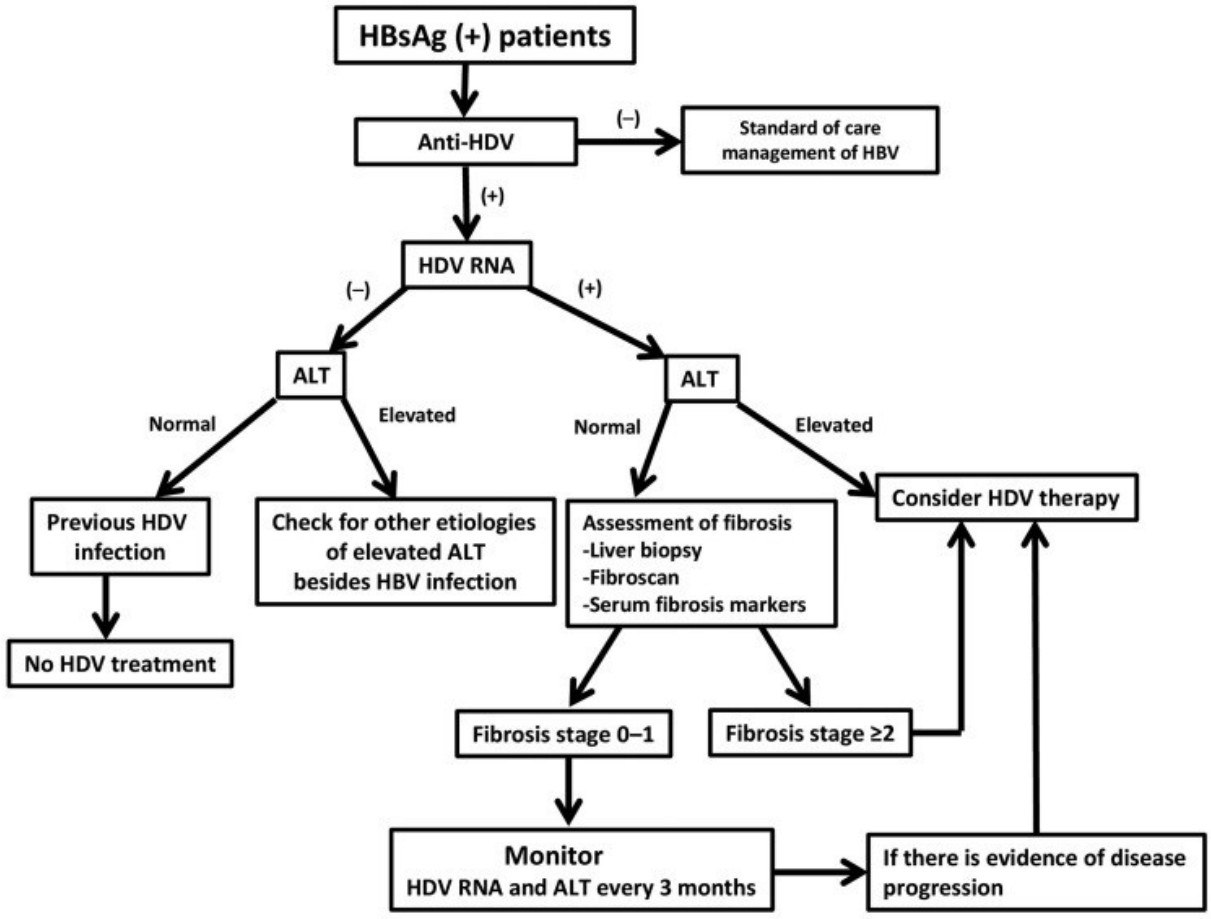


Fig. 4: Algorithm for the the evaluation of active hepatitis D [23].

1.8 HDV and risk of Hepatocellular Carcinoma (HCC)

HCC is a common malignancy of the liver and the sixth most prevalent cancer in the world (Fig. 5). It represents the third leading cause of cancer-related death [23].

80-90% of all HCC cases worldwide are caused by chronic viral hepatitis B, C and D [23].

Since hepatitis D is considered the most severe form of viral hepatitis, the risk of developing cirrhosis, HCC, or liver failure within 5 to 10 years is approximately 70% [24]. The risk of developing HCC is further increased in case of co- or triple-infections with other chronic viral infections. HBV/HDV coinfected patients are at a two-fold increased risk of developing HCC, compared to HBV mono-infected patients [24]. HBV/HIV/HDV triple infected patients are at a 6-fold increased risk of developing HCC, compared to HBV/HIV coinfected patients [24].

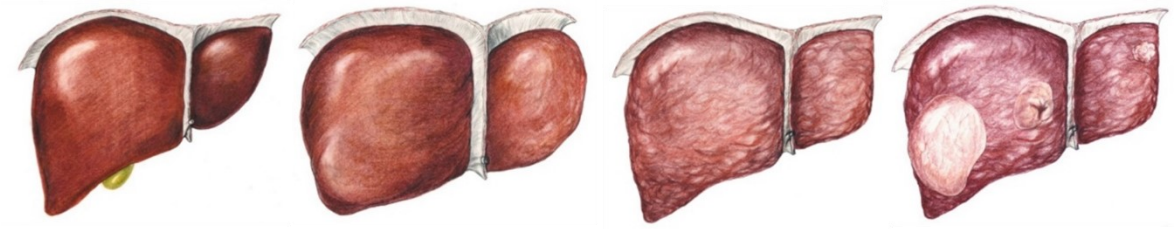


Fig. 5: Stages of HCC development (kindly provided by Harald H. Kessler).

Stages of fibrosis in the HDV RNA-positive Austrian cohort have been described recently [7]. Thirty-six of 60 patients (60%) showed signs of advanced chronic liver disease (ACLD) with fibrosis stages 3 or 4 (Fig. 6, 7).

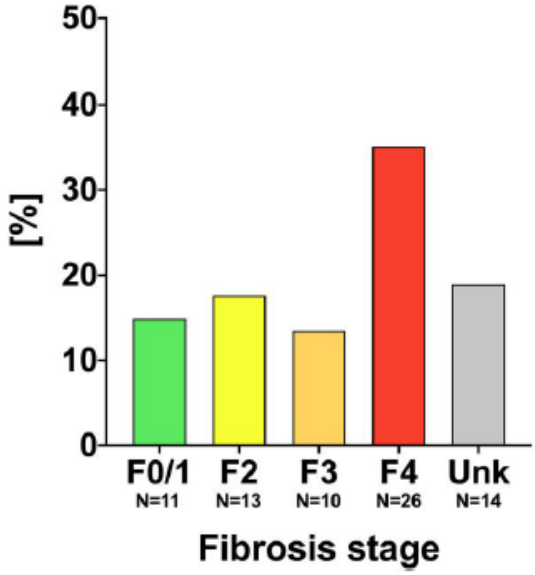


Fig. 6: Distribution of fibrosis stages within the "active" Austrian cohort (n=74) [7].

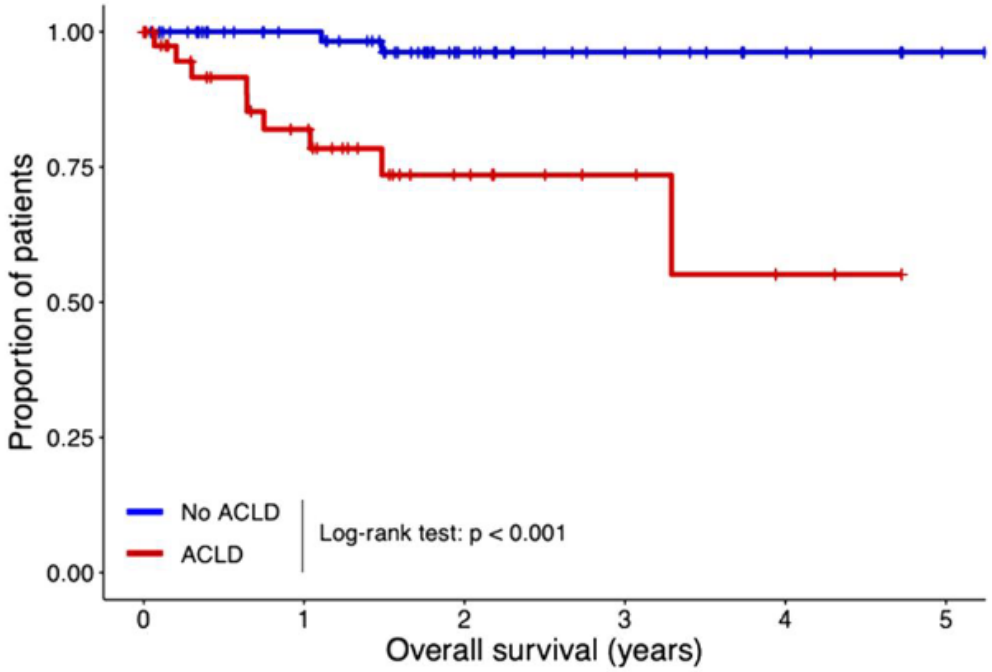


Fig. 7: Overall survival time stratified by the presence/absence of hepatitis D virus-related advanced chronic liver disease at baseline [7].

1.9 Therapy

Patients suffering from chronic HDV infection with detectable HDV RNA and proof of active liver disease must be treated [19]. There is no therapy required for asymptomatic patients who show normal liver enzymes [25]. The general aim of HDV therapy is to achieve suppression of HDV replication, which usually goes along with normalization of ALT levels [19].

Interferon alpha (IFN α) and pegylated interferon alpha (Peg-IFN- α) treatments have been widely used off label, since they are not FDA or EMA approved, as anti-HDV strategy in the last 20-30 years (Table 1). A 48-week course of weekly subcutaneous injections of pegIFNα suppresses HDV replication in approximately 20%–30% of the patients 24 weeks off therapy, although with significant side effects [26]. Peg-IFN-α therapy induces low rates of sustained virologic response (SVR), which means undetectable HDV RNA viral load at least for 6 months after treatment [27,28]. Because IFN-α based therapies show side effects, such as myalgias, exacerbation of psychiatric illness, flulike symptoms or hematologic toxicity, the indication for treatment must be assessed rigorously [29]. With Peg-IFN-α therapy, Peg-IFN-α therapy SVR rates are observed between 23 and 57% [30,31]. Peg-IFN-α therapy is not recommended for patients who suffer from advanced cirrhosis, due to the danger of decompensation [32]. In general, many patients are not eligible for Peg-IFN-α therapy due to the limitations described [33].

Table 1: Interferon therapy options [34].

Drug	Substance	Mode of action	Availability
Peg-IFN- α	Protein	Cytokine, activating innate immune system	Approved for HBV, off-label use for HDV
Peg-IFN-λ1	Protein	Cytokine, activating innate immune system	Phase II

Three novel anti-HDV therapies, the entry inhibitor bulevirtide, the prenylation inhibitor lonafarnib, and the nucleid acid polymer REP-2139 have been developed recently [26] (Table 2, Fig. 8). Monotherapy with these direct-acting-antivirals indicates improved therapeutic outcomes, but they must be used long term. These new treatment options combined with IFNs lead to a decline of the HDV load [34].

Table 2: Novel anti-HDV therapies [34].

Drug	Substance	Mode of action	Availability
Bulevirtide	PreS1 peptide	NTCP binding, blocking HBV/HDV entry	Phase III, CMA by EMA in July 2020
Lonafarnib	Small molecule	Inhibiting L-HDAg prenylation and HDV secretion	Phase III
REP 2139	Nucleic acid polymer	Inhibiting HBsAg/HBV /HDV secretion, possibly also HBV/HDV entry	Phase II

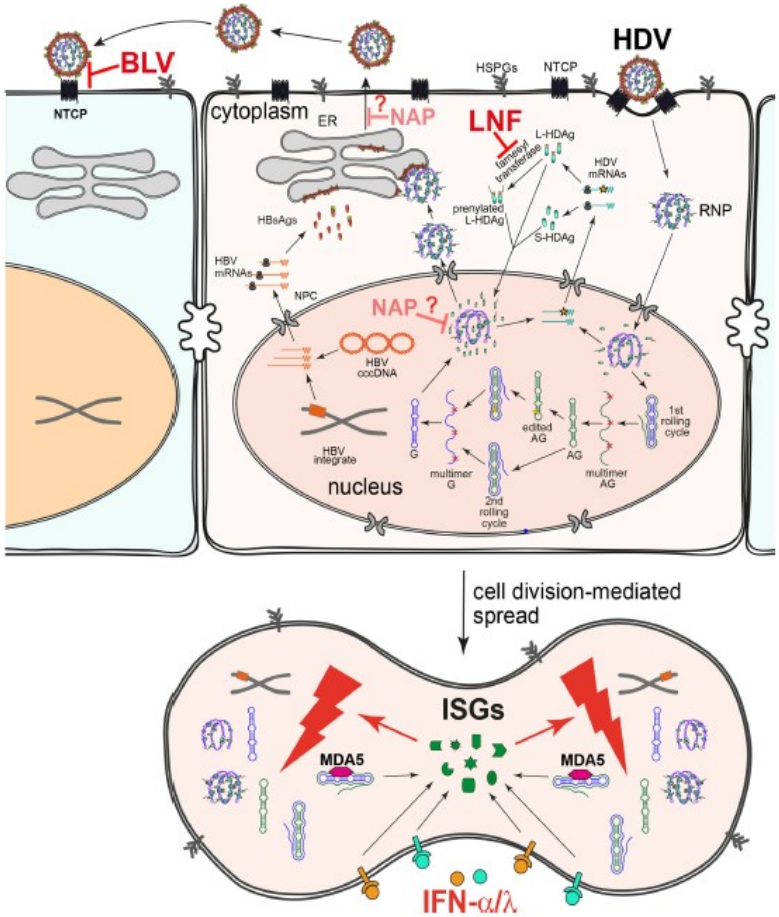


Fig 8: HDV life cycle, spreading pathways, and drug targets [27].

Bulevirtide (BLV) can block virus entry into the hepatocytes by inactivating the sodium taurocholate co-transporting polypeptide (NTPC) [35] (Fig. 9). It is a subcutaneously supplied lipopeptide, which can be applied with or without Peg-IFN-α and was approved in 2020 in the European Union (EU) under the name Hepcludex® [26]. With BLV, a significant reduction of both HDV load and ALT level is achieved [34]. (Table 3)

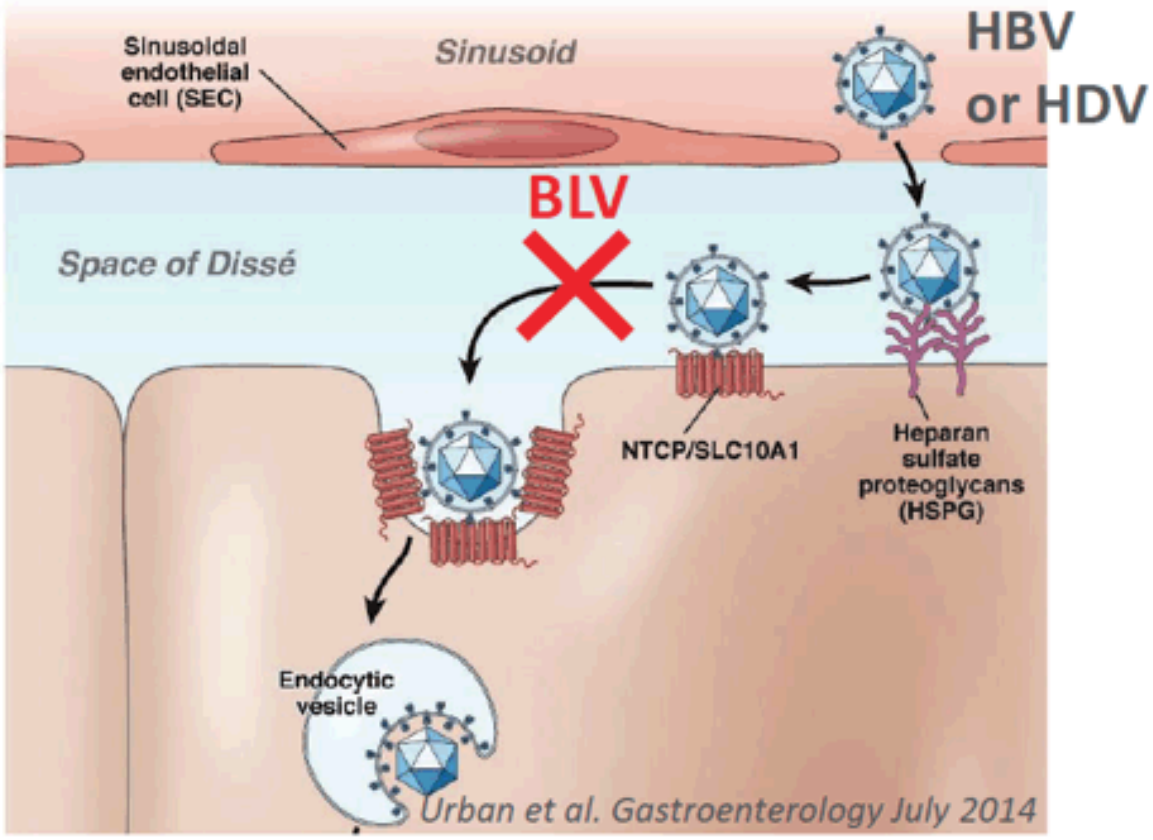


Fig. 9: BLV blocks HBV and HDV entry by binding to the bile acid transporter NTCP in vitro and in vivo [36].

Table 3: Retrospective, multicenter, investigator-driven, real-world study of Bulevirtide 2mg/day self-administered for up to 72 weeks in Italian patients with chronic HDV with compensated cirrhosis (N=93) [36].

Parameter	BL	W8	W24	W48	W72
Virological response, %	0	8	67	77	75
Biochemical response (ALT<40 U/L), %	9	33	67	67	81
Combined response (virologic & biochemical), %	0	1	45	56	63
Median bile acids, µmol/l (range)	23	60	37	63	40

Lonafarnib (LNF) is an inhibitor of the farnesyl transferase and stops the prenylation of the large HDAg (L-HDAg) (Fig. 10). Ritonavir, a cytochrome P450/3A4 inhibitor was combined with LNF; this combination allows a reduction of LNF dosage and achieves higher systemic exposure and a better control of side effects. In combination with Peg-IFN-α, LNF leads to a significant decrease of serum HDV RNA as well as HBsAg and viral replication [26,37].

Lonafarnib is also used for the treatment of progeria and progeroid laminopathies. In 2020, lonafarnib received its first approval in the USA to reduce the risk of mortality in Hutchinson-Gilford Progeria Syndrome and for the treatment of processing-deficient progeroid laminopathies [38].

Fig 10: Lonafarnib is an orally active farnesyltransferase inhibitor. (from: https://www.clinicaltrialsarena.com/projects/zokinvy-lonafarnib-for-the-treatment-of-progeria-usa/) (accessed: 31.05.2023)

Nucleid acid polymers are oligonucleotides showing a wide variety of antiviral activity against numerous viruses [26] (Fig. 11). Monotherapy with REP-2055/REP-2139 has been shown to decrease HBsAg and HBV DNA concentrations in serum [39]. In a pilot study, it was reported that REP-2139 also reduced the HDV RNA level in some patients [40].

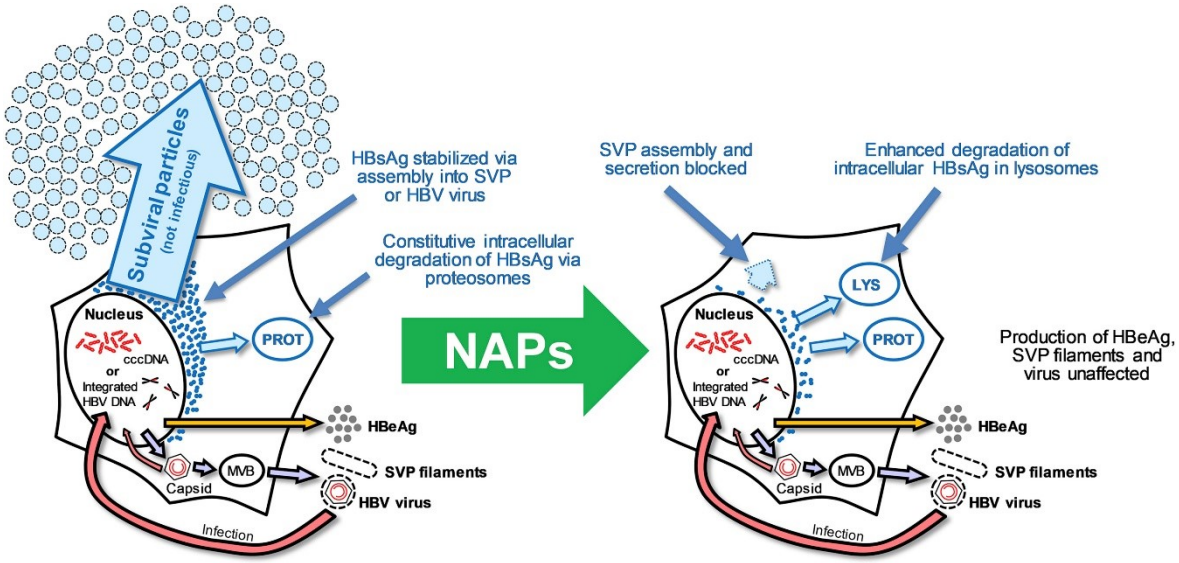


Fig 11: Antiviral effects of NAPs [40].

2 Objectives

The aim of this retrospective epidemiological analysis was to collect data on HDV RNA-positive Styrian patients under medical care at the Medical University of Graz. Furthermore, the need of effective antiviral therapy for these patients was estimated. If therapy had already been started, the efficacy was monitored.

3 Material and methods

3.1 Patients

In this study, all patients with detectable plasma HDV RNA reported at the Molecular Diagnostics Laboratory and under medical care at the Medical University of Graz within the period 01.01.2010 through 31.12.2022 were included.

3.2 Instruments used in this study

3.2.1 The eMAG®

The eMAG® (bioMérieux S.A., Marcy l´Etoile, France) is a fully automated nucleic acid extraction platform. Extractions are performed utilizing the magnetic silica technology. The platform allows DNA and RNA extraction of 48 samples in parallel and distribution of nucleic acid extracts in specially designed tubes at the end of the extraction [41]. Flexible input/elution volumes are also possible within a single run. The internal control and magnetic silica fragments are added automatically. The workflow of the instrument is standardized, and it is separated into two independent sections for 24 samples each, which means that 48 extractions can be done in parallel. It takes 90 minutes for 48 extractions [42].

3.2.2 The LightCycler® 480 II

The LightCycler® 480 II (Roche Molecular Systems, Rotkreuz, Switzerland) is a real-time PCR system including a silver thermal block cycler unit (Fig. 12). The instrument is operated by a separated desktop data station (Fig. 13). The thermal cycling system is able to accurately and rapidly reach and maintain reaction temperatures. It is possible to change between 96- and 384-mulitiwell plate formats. To achieve identical performances with these plates, a new form of thermal block cycler was integrated into the LightCycler® 480. This new thermal block cycler contains Therma-Base technology to ensure ideal heat transfer and distribution to all samples. Generally, Peltier elements are responsible for heating and cooling. The novel Therma-Base unit is located underneath the Peltier elements and is based on evaporation and condensation of a working fluid in a thin vacuum chamber enabling rapid heat distribution and temperature equilibration. There is also a cooling element located below the Therma-Base unit, which includes a maximized inner surface area to facilitate rapid heat absorption. The thermal block cycler can complete one PCR run in less than an hour [43].

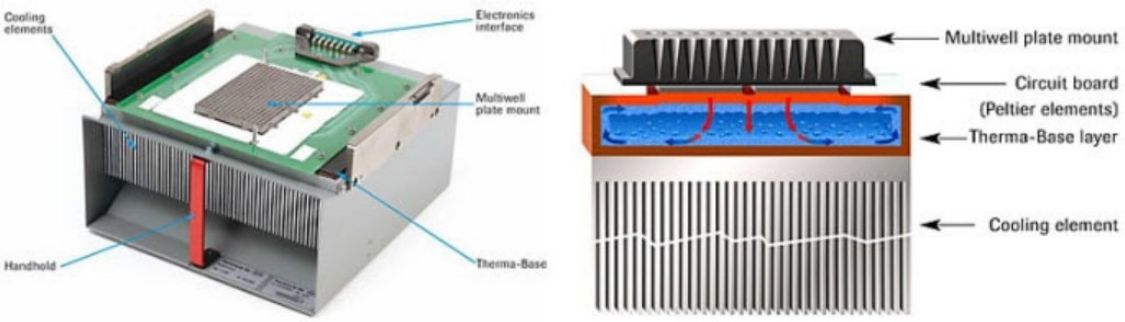


Fig. 12: LightCycler 480 384-well thermal block cycler (left) and cross section showing the integration of the Thermal-Base (right) [42].

Fig. 13: Light Cycler at Molecular Diagnostics Laboratory, Medical University of Graz

3.2.3 The RoboGene HDV Quantification Kit 2.0

The RoboGene® HDV RNA Quantification Kit 2.0 (AJ Roboscreen GmbH, Leipzig, Germany) is a Conformité Europeéne (CE) / in vitro diagnostics (IVD)-labeled assay proposed for real-time PCR quantification of HDV RNA in serum samples or human EDTA plasma. It utilizes the manual INSTANT Virus RNA/DNA Kit (AJ Innuscreen GmbH, Berlin, Germany) in combination with the LightCycler® 480 II instrument (Roche Molecular Systems, Rotkreuz, Switzerland), the ABI 7500 Fast (Applied Biosystems, Darmstadt, Germany), or the Rotor-Gene™ 3000/6000/Q (Qiagen, Hilden, Germany) reporting results in IU/mL. There are two kit editions obtainable: low profile strips 0.1 ml for LightCycler® 480 II (Roche) and ABI 7500 Fast (Applied Biosystems) real-time PCR systems and regular profile tubes 0.2 ml for application on Rotor-Gene™ (Qiagen). In this study, the LightCycler® 480 was used for HDV RNA quantification. The assay is designed for patients with chronic HDV infection, who show symptoms and other laboratory markers of the disease. The test is used to assess viral response to antiviral treatment as measured by changes in HDV RNA levels in EDTA plasma and serum [44].

The RoboGene® HDV RNA Quantification Kit 2.0 is able to detect all genotypes of HDV by using probes and primers specific for a subsequence of the HDV antigen. Through amplification of the included quantification standard strip in parallel, determination of the specimen concentration is completed. There is always a synthetic internal control (IC) included to monitor the entire process from RNA extraction to the real-time PCR. The IC serves to avoid false-negative results. Different fluorescent reporter dyes at specific wavelengths are used to detect amplification of HDV RNA in samples and standards and of internal control RNA [44].

The RoboGene® HDV RNA Quantification Kit 2.0 is built on the TaqMan® PCR technology (Fig. 14). It combines amplification with fluorescence-based online detection of the nucleic acid of interest. This assay includes a conventional set of target-specific primers in combination with a fluorescence-labelled oligonucleotide probe, complementary to the desired target sequence. The Taq DNA polymerase displays a 5’→ 3’ exonuclease activity, which cleaves the probe and displaces the

fluorescent dye from the quencher. The fluorescence signal increases, which is proportional to the target amplification during each PCR cycle [44].

Fig.14: RoboGene HDV® RNA Quantification Kit 2.0 (from:https://www.roboscreen.com/products/viral-pathogens/robogene-hdv-rna-quantification-kit-20/) (accessed 14.01.2023) [44].

4 Results

In this study, 12 patients with detectable plasma HDV RNA were found in Styria at the Molecular Diagnostics Laboratory, Medical University of Graz from 01.01.2010 through 31.12.2022. Two of them deceased in 2019. Currently (as of 31.03.2023), four patients are under medical care at the Medical University of Graz. One of them has started with anti-HDV therapy in June 2022. Results obtained from the 7 HDV-RNA positive outpatients at Medical University of Graz are shown on the following pages.

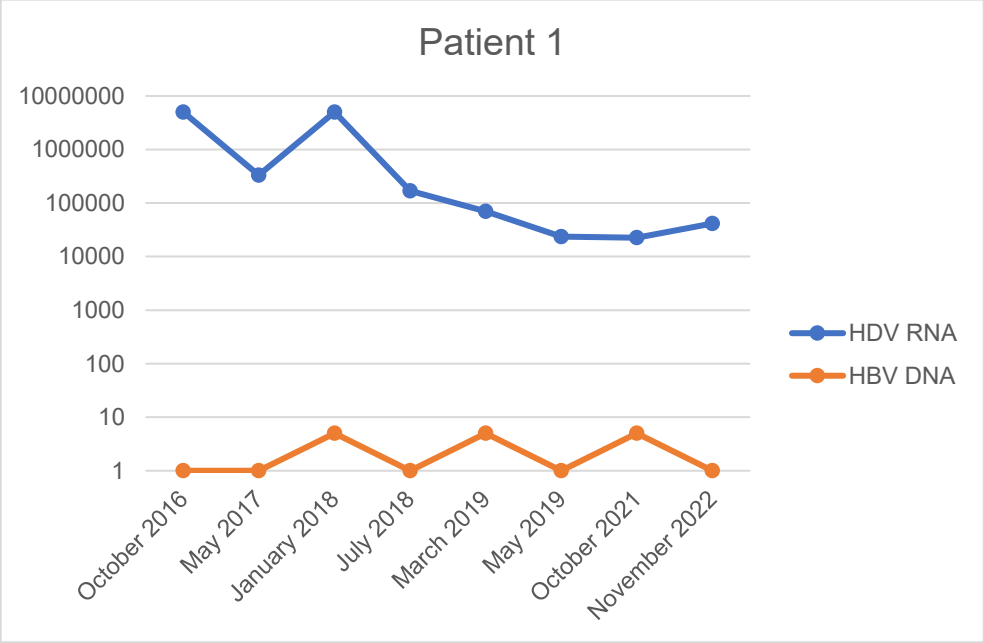
4.1 Patient 1

This female patient was born in 1968 and the country of origin is the Dominican Republic. Her first consultation at LKH Graz took place in June 2012. The graph below shows all HDV-RNA and HBV-DNA results available.

Additionally, ALT levels were documented. Her first level reported in June 2012 was 176 U/L. Her last reported level in November 2022 was 21 U/l. The last Fibroscan™ was done in 2012, resulting in 21.8 kPa.

The patient had no significant signs of ascites or hepatic encephalopathy, but small varices were found, and she showed signs of cirrhosis (Child-Pugh-Score: Child A). The patient is undergoing a therapy against HBV with Viread 245mg per day from November 2013 onwards.

The patient suffers from alcohol abuse. If alcohol abstinence could be achieved, the initiation of Bulevirtide should be considered.

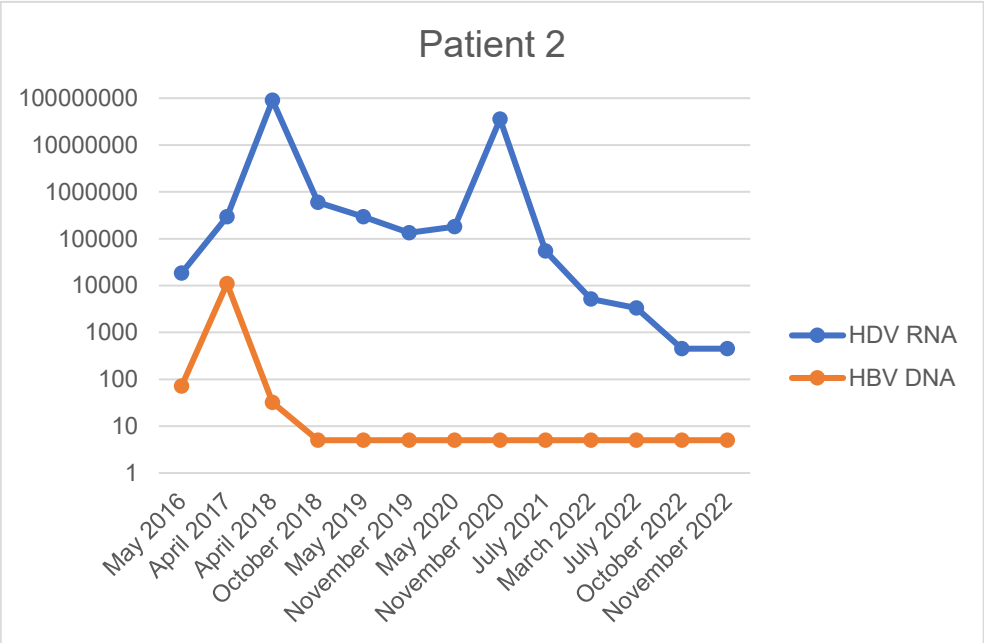


4.2 Patient 2

This male patient was born in 1979 and the country of origin is Turkey. His first consultation at LKH Graz took place in November 2015. The graph below shows all documented HDV-RNA and HBV-DNA results available.

Additionally, ALT levels were documented. His first reported level in November 2015 was 459 U/l. His last reported level in March 2023 was 57 U/l. The last Fibroscan™ was done in May 2019, resulting in 14.5 kPa. A liver biopsy was performed in March 2022 according to the fibrosis score (Ishak) 4 out of 6 points were assigned, which equals severe fibrosis (F3).

The patient has no significant signs of ascites or hepatic encephalopathy, and no varices were found. The patient is undergoing a therapy against HBV with Baraclude 1mg per day from October 2015 onwards. The therapy against HDV with Bulevirtide 2mg, supplied as a daily subcutaneous injection, started in June 2022.

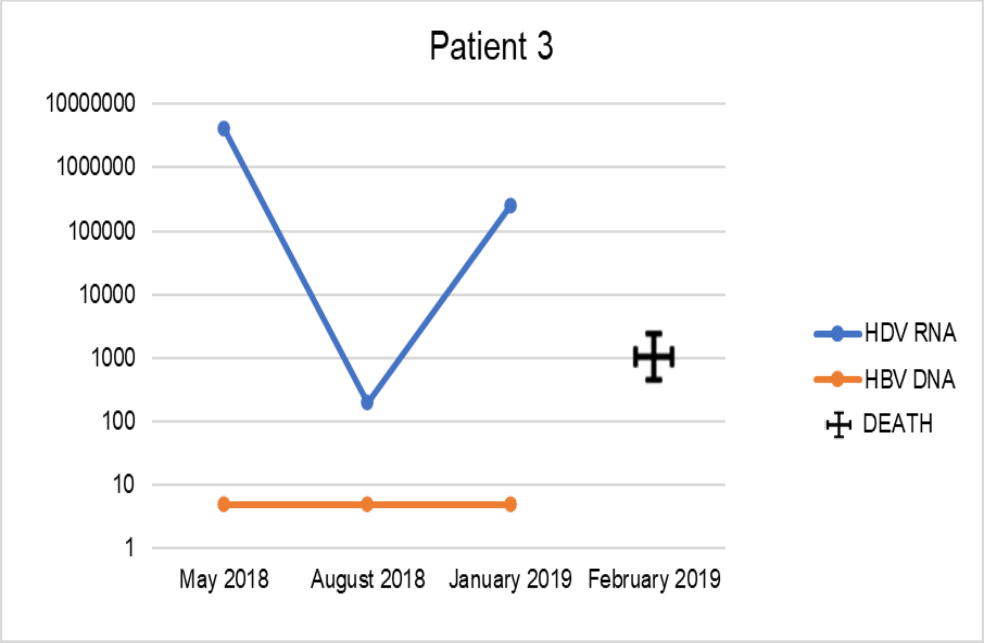


4.3 Patient 3

Patient 3 is female, born in 1961 and the country of origin is Turkey. Her first consultation at LKH Graz took place in May 2018. The graph below shows all documented HDV-RNA and HBV-DNA results available.

Additionally, ALT levels were documented. Her first reported level in May 2018 was 53 U/l. Her last reported level in January 2019 was 648 U/l. There is no Fibroscan™ result available.

The patient had significant signs of ascites and hepatic encephalopathy, varices were found, and she showed signs of cirrhosis (Child-Pugh-Score: Child C). The patient was undergoing a therapy against HBV with Viread 245mg per day from January 2019 onwards. The patient died in February 2019 due to advanced liver failure.

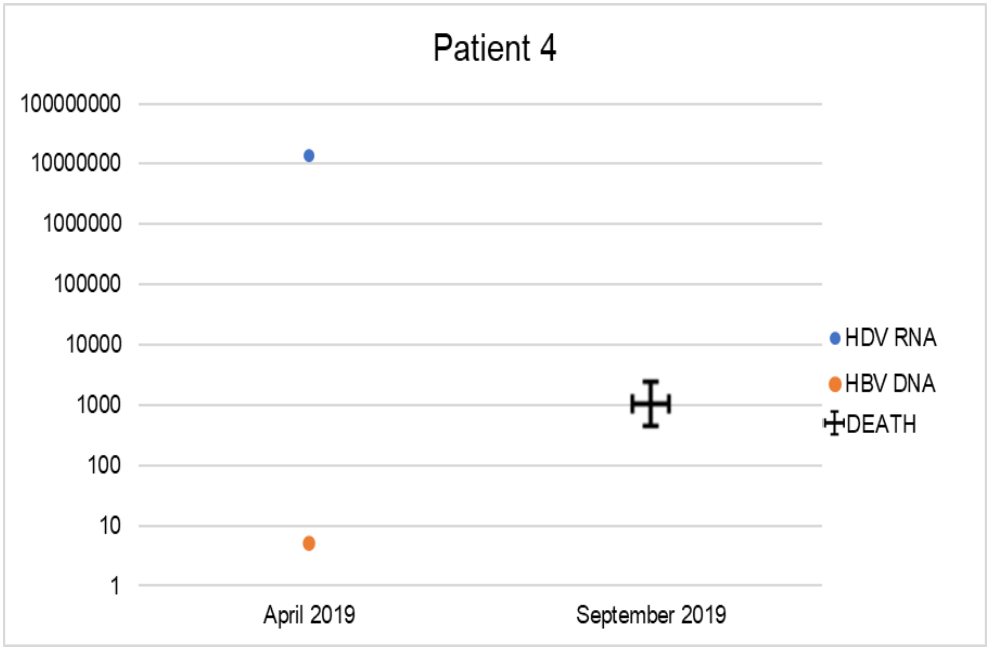


4.4 Patient 4

Patient 4 is male, born in 1974 and the country of origin is Georgia. His first consultation at LKH Graz took place in April 2019. The graph below shows all documented HDV-RNA and HBV-DNA results available.

Additionally, ALT levels were documented. His first reported level in April 2019 was 56 U/l. His last reported level in September 2019 was 15 U/l. There is no Fibroscan™ result available.

The patient had significant signs of ascites, hepatic encephalopathy, varices were found, and he showed sign of cirrhosis (Child-Pugh-Score: Child C). The patient was undergoing a therapy against HBV with Baraclude 0.5mg per day from April 2019 onwards. The patient died in September 2019 due to advanced liver failure.

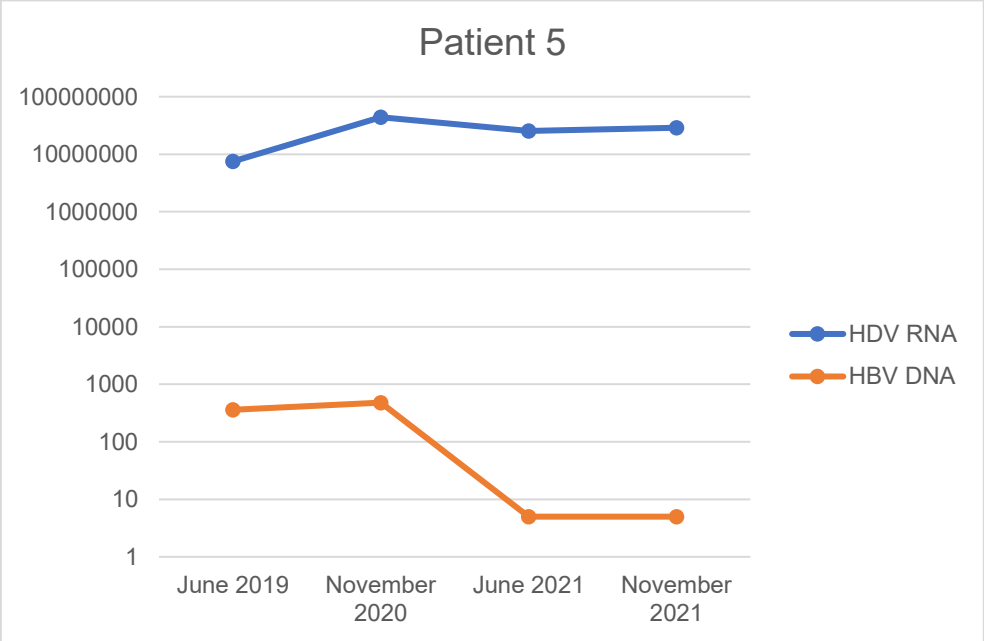


4.5 Patient 5

Patient 5 is female, born in 1981 and the country of origin is Mongolia. Her first consultation at LKH Graz took place in June 2019.The graph below shows all documented HDV-RNA and HBV-DNA results available.

Additionally, ALT levels were documented. Her first ALT level was reported in June 2019 and showed 39 U/l. Her last ALT level was reported in November 2021 and showed 59 U/l. The last Fibroscan™ was done in November 2021, resulting in 8,1 kPa. A liver biopsy was performed in January 2022, according to the fibrosis score (Ishak) 2 out of 6 points were assigned, which equals mild to moderate fibrosis (F1-F2), although a higher stage of fibrosis could not be excluded.

The patient had no significant signs of ascites or hepatic encephalopathy, and no varices were found. The patient is undergoing a therapy against HBV with Baraclude 0.5mg from December 2020 onwards. Shortly after the consultation at the University Hospital Graz in November 2021, the patient moved to Carinthia.

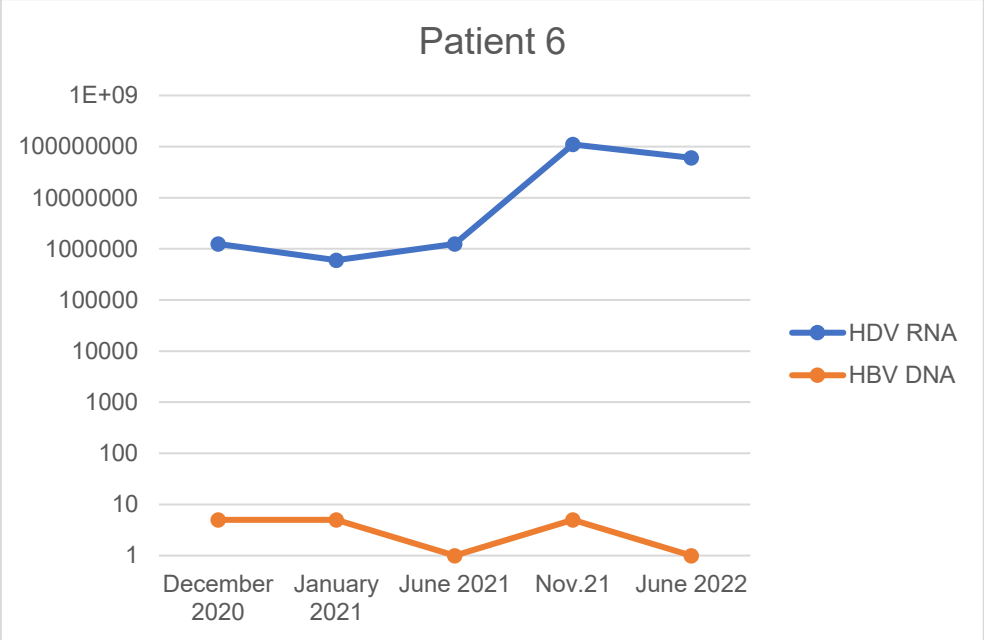


4.6 Patient 6

Patient 6 is female, born in 1965 and the country of origin is Romania. Her first consultation at LKH Graz took place in December 2020. The graph below shows all documented HDV-RNA and HBV-DNA results until 31.12.2022.

Additionally, ALT levels were documented. Her first reported level in December 2020 was 254 U/l. Her last reported level in June 2022 was 114 U/l. The last Fibroscan™ was done in December 2021, resulting in 16.6 kPa.

The patient had no significant signs of ascites or hepatic encephalopathy, and no varices were found. The patient is undergoing a therapy against HBV with Tenofovir 245mg from December 2015 onwards. Her last consultation at LKH Graz took place in July 2022. In this patient, therapy with Bulevirtide was approved and discussed with her in detail. The patient then asked for a cooling-off period, but she did not attend any further appointments.

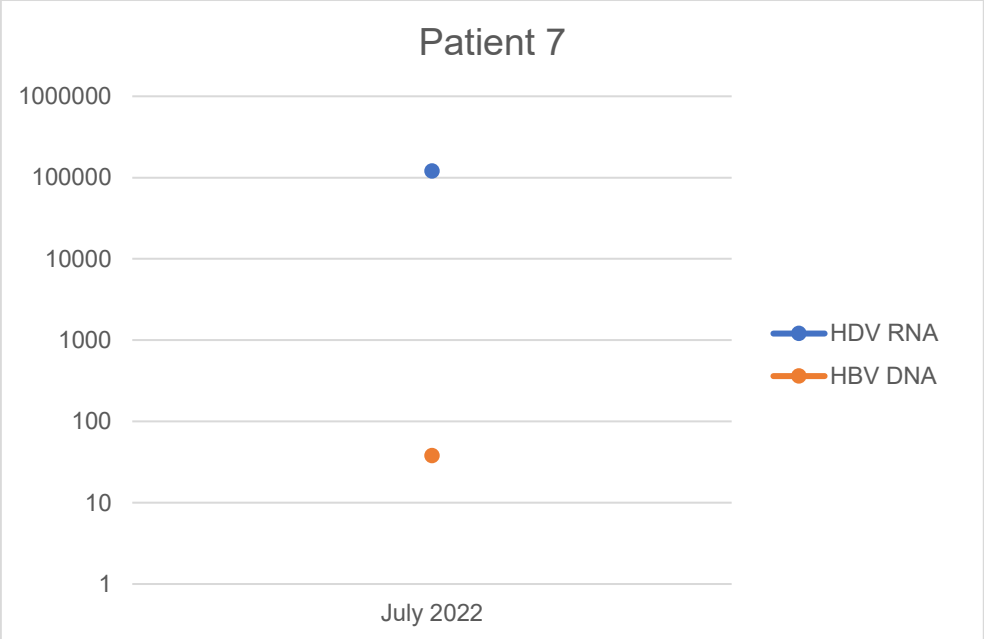


4.7 Patient 7

Patient 7 is male, born in 1977 and the country of origin is Georgia. His first consultation at LKH Graz took place in July 2022. The graphic below shows all HDV-RNA and HBV-DNA results available.

Additionally, ALT levels were documented. His first reported level in July 2022 was 175 U/l. His last reported level in August 2022 was 46 U/l. There is no Fibroscan™ result available.

The patient had no significant signs of ascites or hepatic encephalopathy, and small varices were found. The patient is undergoing a therapy against HBV with Viread 245mg per day from August 2022 onwards. His last consultation at LKH Graz took place in October 2022. Further testing to evaluate Bulevirtide therapy was scheduled in subsequent months, but the patient did not attend these appointments.



5 Discussion

The number of patients living in Styria suffering from HDV infection (HDV RNA-positive) is low; only 12 patients were found to be positive for HDV RNA. In other parts of Austria, the number of seropositive patients (anti-HDV positive) seems to be considerably higher [7]. About 40% of anti-HDV positive patients have never been tested on HDV viremia. Due to this number, a high amount of Austrian viremic patients has yet to be identified [7].

Moreover, all HBsAg positive patients must be screened for anti-HDV at least once [45]. According to the Austrian Federal Health Ministry, there are 42.000 HBV patients living in Austria [46]. As all anti-HDV positive patients were related to this number, the HDV-coinfection rate was reported to be less than 1% [7]. Since there is a lack of testing, the number of HBV/HDV co-infected patients should be much higher [7].

Since HDV is the most severe form of viral hepatitis, it is even more important to identify all HBV/HDV co-infected patients. The risk of developing ACLD and/or HCC is increased in these patients [24]. The majority of HDV RNA-positives investigated in this study suffered from ACLD. In the Austrian cohort, more than 50% of co-infected patients showed signs of ACLD with fibrosis stages 3 or 4 [7].

In this study, all 7 patients are receiving or have received therapy against HBV. In Styria, only one patient is treated with Bulevirtide against HDV currently (data as of March 31, 2023). Bulevirtide is a novel NTPC inhibitor and was approved from the European Medicines Agency (EMA) in 2020. It is administered subcutaneously at a single dose of 2mg/day [26]. Bulevirtide showed promising results in Phase II and Phase III clinical trials and in initial real-world data [47–49]. Administration of Bulevirtide, both as monotherapy and as combination therapy with PegIFNα showed a significant decline in HDV RNA and ALT levels in a substantial proportion of patients, it also expands the variety of therapy options for HDV patients [49]. Furthermore, an increase in bile acids was observed in treated patients; however, no symptoms were observed [49]. In Austria, the determination of bile acids is usually not included in the routine laboratory investigation; due to technical challenges, this parameter is done only at very few centers.

Two other drugs, LNF and REP2139Ca, have also achieved promising results in clinical trials (Phase III), but are not yet approved for HDV therapy [26].

It would have been important to diagnose the patients included in this study earlier. This would have allowed an earlier start of therapy. There might be a number of further patients with urgent need for anti-HDV treatment. This underlines the need for testing all HBsAg-positives for HDV-RNA at the earliest time.

Treatment with Bulevirtide requires a high level of adherence as it must be injected subcutaneously daily. Besides alcohol or drug abuse, lack of speaking German, has a major impact on adherence. Non-native patients usually require more time for training how to administer this drug and for monitoring. Access to hospitals is also generally more difficult.

In conclusion, the number of patients positive for HDV RNA living in Styria is low currently; however, the majority of patients with chronic hepatitis D suffers from advanced chronic liver disease. With the novel therapeutical approaches, it is of paramount importance to test all HbsAg carriers for presence of HDV coinfection and start anti-HDV therapy as soon as possible.

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