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Autoantibody titers and IgG levels in chronic hepatitis D virus infection

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1 Aims

The principal objective of this thesis is to assess autoantibody titers and immunoglobulin G (IgG) levels in individuals afflicted with chronic hepatitis D virus (CHD) infection. Clinical observations indicate a higher likelihood of nonspecific autoimmune phenomena, characterized by elevated autoantibody titers and IgG levels, in CHD patients in comparison to those with chronic hepatitis B virus (CHB) mono-infection. Although elevated autoantibody titers are well-documented in CHB patients and in those with chronic hepatitis C virus (HCV) infection, investigations into their frequency among CHD-infected individuals are notably lacking.

Here, we juxtapose autoantibody titers in CHB and CHD patients against those in individuals grappling with autoimmune hepatitis (AIH), a condition characterized, among other criteria, by the presence of autoantibodies. We posit that hepatitis virus infections result in a less frequent and less pronounced manifestation of serological autoimmune phenomena when compared to bona fide autoimmune diseases.

2 Introduction

2.1 Autoantibodies in liver diseases

The recognition of autoantibodies in the context of viral infections, particularly hepatitis B virus (HBV) and HCV, has been extensively documented (1). Given the diagnostic challenges associated with autoimmune-mediated liver diseases, which are frequently underdiagnosed, the evaluation of liver-related autoantibodies is imperative in patients exhibiting elevated transaminases or hepatitis. While most of these antibodies lack disease specificity or organ specificity, their identification is essential for the diagnosis of autoimmune hepatitis (AIH) (2). This section delineates the pertinent autoantibodies relevant to this study, representing a subset of the most prevalent autoantibodies in (autoimmune) liver diseases (Table 2.1).

Autoantibody	Target antigen(s)	Liver disease	Conventional method of detection
ANA	Chromatin Histones Centromeres Cyclin A Ribonucleoproteins Double-stranded DNA Single-stranded DNA	AIH-1 PBC PSC HBV HCV HDV HEV DILI NALFD ALD Wilson disease	SKL IFT, HEp-2 IFT
SMA	Microfilaments Intermediate filaments	Same as ANA	SKL IFT
LKM1	Cytochrome P4502D6	AIH-2 HCV	SKL IFT
AMA	PDC-E2	PBC Variant syndromes	SKL IFT
SLA	O-phosphoseryl-tRNA$^{(sec)}$ selenium transferase	AIH HCV	ELISA

Table 2.1 Autoantibodies and their targets in liver diseases (1-3) ANA, antinuclear antibodies; SMA, anti-smooth muscle antibodies; LKM1, anti-liver kidney microsomal antibody type 1; AMA, anti-mitochondrial antibodies; SLA soluble liver antigen-antibody; PDC-E2, E2 subunits of the 2-oxo acid dehydrogenase complexes; AIH, autoimmune hepatitis; AIH-1 or -2, autoimmune hepatitis type 1 or 2; PBC, primary biliary cholangitis; PSC primary sclerosing cholangitis; HBV, viral hepatitis B; HCV, viral hepatitis C; HDV, viral hepatitis D; HEV, viral hepatitis E; DILI, drug-induced liver injury; NAFLD, non-alcoholic fatty liver disease; ALD, alcohol-induced liver disease; SKL IFT, indirect immunofluorescence test testing on rodent stomach, kidney and liver tissue; HEp-2 IFT, indirect immunofluorescence test testing on HEp-2 cells.

Antinuclear antibodies (ANA)

ANAs are autoantibodies targeting self-proteins within cellular nucleus structures. The indirect immunofluorescence test (IFT) using HEp-2 cells, a cell-line substrate from human laryngeal carcinoma, is the standard method for ANA detection (5).
The autoantibodies attached to the HEp-2 cell substrate are visualized using a fluorescence microscope after staining with fluorescein-labeled anti-immunoglobulin antibodies. The adherence of these antibodies to specific nuclear antigens results in distinct fluorescence patterns, correlating with various autoimmune diseases (4). The main ANA staining patterns are homogenous, speckled, nucleolar, and centromere (4) (see Figure 2.1). Quantifying ANAs in serum involves titration through dilution, with screening tests utilizing 1:40 and 1:160 dilutions. Despite their association with AIH, ANAs lack specificity for a particular (liver) disease, as they are also observed in viral hepatitis, drug-induced liver injury (DILI), Wilson disease, alcohol-induced liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), and various extrahepatic autoimmune diseases (2).

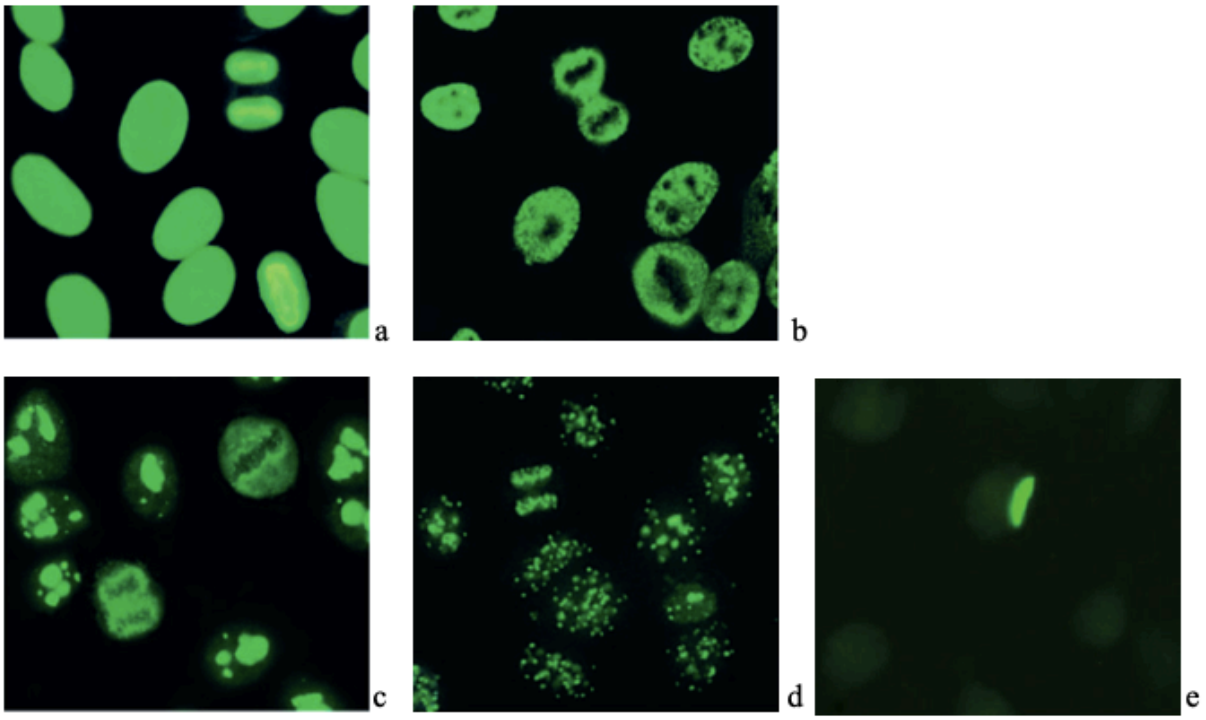

Figure 2.1: ANA immunofluorescence patterns recognized by the EUROPattern Suite including homogenous (a), speckled (b), nucleolar(c), centromeres (d), and none/unspecific (e). (5)

Anti-smooth muscle antibody (Anti-SMA or SMA)

SMA targets subunits of different cytoplasmic filaments. The standard screening method involves IFT on frozen sections of rat liver, kidney, and stomach, displaying distinctive staining patterns (3, 7). In type 1 autoimmune hepatitis (AIH-1), anti-SMAs are prevalent, often co-occurring with ANAs. Higher SMA titers exhibit increased AIH specificity. Moreover, sub-specificity towards f-actin has been associated with AIH (6). However, SMA can also be observed in up to 25% of patients with CHB, and chronic HCV infection. (4, 7-9)

Anti-Liver-Kidney Microsomal Type 1 Antibody (Anti-LKM1)

Anti-LKM1 targets cytochrome P450 CYP2D6 and is associated with type 2 autoimmune hepatitis (AIH-2). Detection involves IFT on frozen rat liver, kidney, and stomach sections, with distinct staining patterns. Anti-LKM1 titers correlate with disease activity in AIH-2 and HCV infection. Molecularly-based immune assays, such as ELISA, can aid in clarification when immunofluorescence patterns are ambiguous (3). In 1983, LKM autoantibodies were also described in CHD patients and later termed LKM-3 (7). These autoantibodies were present in 13-14% of Italian HDV carriers. The major LKM-3 autoantigen was identified as an epitope on family 1 UGTs (UGTl) (8).

Anti-Mitochondrial Antibody (AMA)

AMAs target E2 subunits of 2-oxo acid dehydrogenase complexes (PDC-E2) in the inner mitochondrial membrane. Screening involves IFT on rat kidney and liver sections, akin to anti-LKM1 patterns. A confirmatory test using molecularly based immune assays (ELISA or Western blot) can be useful. Different AMA patterns (M1 to M9) exist, with specific patterns (M2, M4, M8, M9) serving as biomarkers for primary biliary cholangitis (PBC). Positive AMA titers can also occur in AIH, indicating a variant syndrome. However, AMA is occasionally detected in HCV patients (11).

Anti-Soluble Liver Antigen Antibody and Anti-Liver-Pancreas Antigen Antibody (Anti-SLA)

Originally described as individual antibodies targeting the same antigen, anti-Liver-Pancreas (LP) and anti-SLA are now collectively referred to as anti-SLA. The standard diagnostic test involves ELISA or Western blot, as immunofluorescence is ineffective for anti-SLA detection. Anti-SLA is highly specific for AIH-1 and AIH-2, although only a minority of AIH patients exhibit these antibodies (2,3). Notably, anti-SLA is also present in up to 10% of HCV patients (16).

2.2 Hepatitis B virus

The hepatitis B virus is a partially double-stranded hepatotropic DNA virus causing both acute and chronic hepatitis B virus infections (17). Transmission occurs through various means, including blood contact, perinatal transmission, sexual contact, and parenteral routes. Chronic hepatitis B virus infection, characterized by prolonged presence of hepatitis B surface antigen (HBsAg) for over 6 months, often results from early-life acquisition, leading to lifelong infection. In contrast, infections acquired later in life may resolve acutely and rarely result in fulminant liver failure (18).

CHB infection is a significant public health concern, potentially progressing to cirrhosis in up to 40% of untreated cases, with risks of decompensated liver disease and hepatocellular carcinoma (HCC) (19-21). Notably, HBV can exhibit oncogenic potential by integrating into the host genome, contributing to HCC (17).

The management of HBV infection primarily involves the use of reverse transcriptase inhibitors (nucleos(t)ide analogues (NAs)) and, less commonly, pegylated interferon alpha (IFNα). While the complete elimination of HBV remains a challenging treatment goal, achieving HBsAg seroclearance (functional cure) is considered a more realistic objective, especially when initiated early after infection. Vaccination stands out as the most effective preventive measure against HBV (18, 22).

Epidemiology

In 2015, the World Health Organization (WHO) reported that approximately 257 million individuals, accounting for 3.5% of the global population, were chronically infected with HBV. The distribution of CHB varies significantly worldwide, influenced by the availability of screening tools and access to vaccines (9). Central and East Asia, sub-Saharan Africa, and the Pacific regions exhibit the highest prevalence of HBV, primarily through vertical transmission (10).

In Europe, the prevalence varies across regions, with less than 1.5% in Northern Europe, less than 2% in Southern Europe, less than 1% in Western Europe, and less than 5% in Eastern Europe. The prevalence of CHB in European countries has steadily declined over the past 30 years, attributed to the widespread use of the hepatitis B vaccine (9).

Virology

Genotypes

There are nine HBV genotypes (GT), A-I. Epidemiological data suggest that 96 % of CHB infections worldwide are caused by five of the nine genotypes, of which GT C is most common at 26 % (11). It's noteworthy that different HBV GT are associated with distinct outcomes in terms of chronicity, disease progression, and responses to IFNα-treatment (12).

HBV life cycle

The hepatitis B virion is a compact, enveloped DNA virus with an outer lipoprotein envelope and an inner nucleocapsid core containing the viral genome. The genome encodes essential viral proteins, including hepatitis B virus, HBsAg (small, medium, and large), hepatitis B core antigen (HBcAg), hepatitis B e antigen (HBeAg), X protein (a crucial regulator of HBV replication), and HBV polymerase (13).

The HBV life cycle involves binding to the sodium taurocholate co-transporting polypeptide (NTCP) receptor via the pre S1 attachment site (L - HBsAg), internalization in endosomal vesicles, removal of surface proteins, and uncoating of the nucleocapsid. The nucleocapsid is then transported to the nucleus, where the viral DNA in its relaxed circular form (rcDNA) undergoes repair and conversion into closed covalent circular DNA (cccDNA). This cccDNA forms an episomal chromatinized structure, contributing to the persistent nature of HBV within hepatocytes. Integration of HBV DNA into the host genome occurs, conferring oncogenic potential. The conversion to cccDNA is a crucial aspect in determining clinical characteristics such as chronicity, lifelong persistence, carcinogenesis, and the limited efficacy of antiviral treatment. Antiviral therapy can suppress viral replication, but complete eradication is challenging due to the stable cccDNA reservoir (13-15).

Pregenomic RNA (pgRNA) is exported from the nucleus, serving as the template for reverse transcription, leading to the formation of two strands of HBV DNA. The DNA-containing capsid binds to HBV surface proteins, undergoes translocation into the endoplasmic reticulum, exits hepatocytes through the secretory pathway, and is released as a mature virus particle. (see Figure 2.2) (13-15).

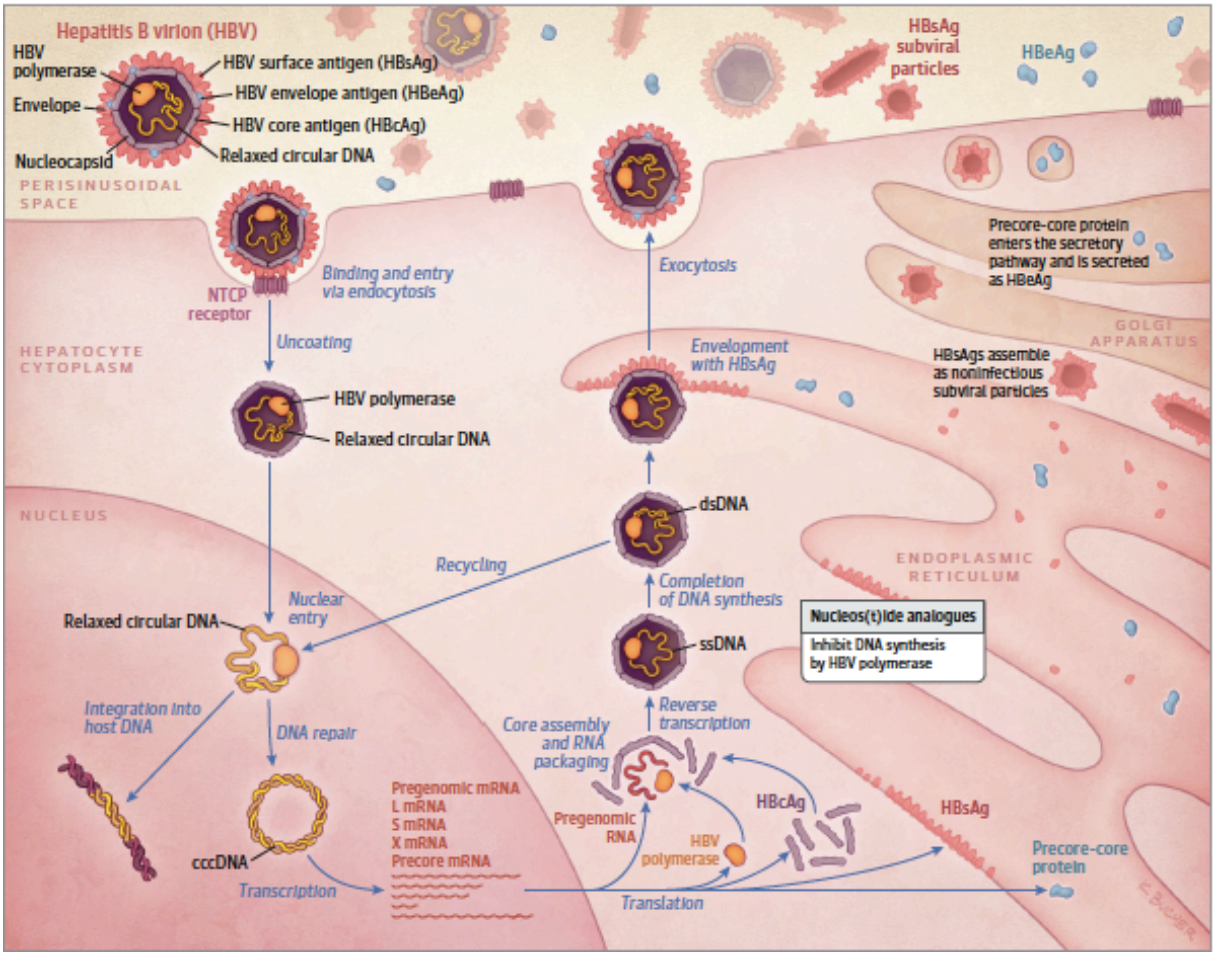

Figure 2.2: Hepatitis B virus and its life cycle (13)

Clinical aspects

Acute hepatitis B infection

Around one-third of adults experiencing acute hepatitis B infection will display symptoms such as fever, fatigue, malaise, abdominal pain, and jaundice. Conversely, the remaining two-thirds of patients remain asymptomatic, potentially allowing the acute infection to go undetected. Among adults with acute HBV infection, only 5-10 % will progress to develop CHB (13).

Chronic hepatitis B infection

CHB infection is defined as the persistence of HBsAg for over 6 months following the initial infection. This condition is marked by the failure to trigger an effective adaptive immune response, leading to the ongoing persistence of the virus. The HBV itself is non-cytopathic, meaning it does not directly harm hepatocytes. However, the recognition of the virus as a

foreign antigen activates the host's immune system, which targets and eliminates infected liver cells, resulting in inflammation and necrosis of liver tissue (13).

CHB infection is a dynamic process shaped by the interplay between HBV replication and the host immune response. It's crucial to differentiate between chronic infection and chronic hepatitis since not all individuals with chronic HBV infection exhibit chronic hepatitis (16).

The natural history of CHB infection is divided into five phases, taking into account the presence of HBeAg, HBV DNA levels, alanine aminotransferase (ALT) values, and the grade of liver inflammation (see Table 2.2) (16).

	HBeAg-positive		**HBeAg-negative**	
	chronic infection	**chronic hepatitis**	**chronic infection**	**chronic hepatitis**
HBsAg	High	High/intermediate	Low	Intermediate
HBeAg	Positive	Positive	Negative	Negative
HBV DNA (IU/ml)	$> 10^7$	$10^4 – 10^7$	< 2000 *	> 2000
ALT	Normal	Elevated	Normal	Elevated **
Liver disease	None/minimal	Moderate/severe	None	Moderate/severe

*Table 2.2: Natural history and assessment of patients with chronic HBV infection based upon HBV and liver disease marker (16). * HBV DNA levels can be between 2000 and 20,000 IU/ml in some patients without signs of chronic hepatitis. ** persistently or intermittently*

In Phase 1, known as HBeAg-positive chronic infection, there is the presence of serum HBeAg and HBV DNA. The minimal host immune response is reflected in normal ALT values, and liver histology does not indicate a higher level of inflammation or fibrosis. This phase is more common and prolonged in vertically infected patients. The likelihood of spontaneous HBeAg loss during this phase is very low. Importantly, individuals in this phase are highly contagious due to the elevated viral load (15, 16).

In Phase 2, termed HBeAg-positive chronic hepatitis, there is the presence of serum HBeAg along with intermediate to high levels of HBV DNA. The immune response targeting infected hepatocytes leads to increased ALT levels, and liver histology shows moderate or severe inflammation and progressive fibrosis. The immune response varies over time, resulting in fluctuating ALT levels. This second phase typically occurs several years after the first and concludes with the loss of HBeAg (13, 15, 16).

Most patients undergoing HBeAg loss achieve seroconversion and experience additional suppression of viral loads, transitioning into the HBeAg-negative infection phase. However, some individuals struggle to control HBV infection and progress to HBeAg-negative chronic hepatitis, a phase that may persist for many years (13, 15, 16).

Phase 3, termed HBeAg-negative chronic infection, is characterized by the presence of serum antibodies to HBeAg (anti-HBe), undetectable or low HBV DNA levels (< 2000 IU/ml), and ALT levels within the normal range. Liver histology shows minimal inflammation and low fibrosis. These patients have a low risk of progression to cirrhosis or HCC if they remain in this phase. However, some patients progress to an HBeAg-negative hepatitis. HBsAg loss and/or seroconversion may occur spontaneously in 1-3 % of cases per year (15, 16). Typically, such patients may have low levels of serum HBsAg (<1000 IU/ml) (17).

Phase 4, known as HBeAg-negative chronic hepatitis, is characterized by negative serum HBeAg usually with detectable anti-HBe and persistent or fluctuating moderate to high levels of serum HBV DNA and persistently or fluctuating elevated ALT values. Liver histology shows inflammation and fibrosis. This phase is associated with low rates of spontaneous disease remission (15, 16).

Phase 5, named occult HBV infection, is characterized by serum-negative HBsAg and positive antibodies to HBcAg (anti-HBc), with or without detectable antibodies to HBsAg (anti-HBs). Patients in this phase have normal ALT values and usually undetectable serum HBV DNA. However, in liver biopsies, HBV DNA can frequently be detected. In these patients, immunosuppression can lead to HBV reactivation. Patients with HBsAg loss before the onset of liver cirrhosis have a minimal risk of progression to cirrhosis or developing HCC and have therefore an improvement in survival (15, 16).

Possible consequences of chronic hepatitis B infection

The risk of developing liver cirrhosis or HCC varies and is influenced by the individual's immune response. However, in untreated patients with chronic hepatitis caused by CHB infection, the 5-year cumulative incidence of cirrhosis ranges from 8-20%. Among individuals with cirrhosis, the 5-year cumulative risk of hepatic decompensation is 20%, and the annual risk of HCC is 2-5% (16).

HBV- associated extrahepatic manifestation

Twenty percent of HBV patients may develop extra-hepatic manifestations, such as vasculitis, polyarthritis nodosa, glomerulonephritis, dermatitis, polyarthralgia, arthritis, lung disease, and aplastic anemia (16, 18). Mixed cryoglobulinemias, positive rheumatoid factors, or high titers of autoantibodies may be found in these patients (16, 19).

Extrahepatic manifestations can be associated with significant morbidity and even mortality. Thus, awareness and recognition of these manifestations are of extraordinary importance to provide an early diagnosis and accurate treatment. The precise pathogenesis of extrahepatic manifestations has not been determined in all details, but it is considered to be immune-mediated (19).

Diagnostics

After HBV infection, it takes four to seven weeks for HBV DNA and HBsAg to become detectable in serum or the liver. Tests typically identify HBsAg, HBeAg, anti-HBs, anti-HBc, anti-HBe, and HBV DNA (15).

The confirmation of HBV infection usually involves detecting HBsAg in serum, which remains measurable even when serum HBV DNA is undetectable in PCR tests. The HBsAg level holds prognostic significance, incorporated into scores predicting the risk of HCC development and indicating the risk of viral rebound after stopping NAs (17).

Positive anti-HBs in HBsAg-negative patients may result from HBV vaccination, recovery from acute hepatitis, or HBsAg seroconversion in chronic HBV infection (15). Detection of HBeAg and anti-HBe is crucial for determining the phase of chronic HBV infection (16). Anti-HBc serves as a marker for acute, chronic, and resolved HBV infection, predicting HBV reactivation in patients undergoing immunomodulatory therapy. Immunoglobulin M (IgM) anti-HBc may be the sole detectable marker in fulminant acute HBV infection but is also present during exacerbation of HBV infection. Therefore, measuring anti-HBc is usually recommended for diagnosing patients suspected of experiencing an acute exacerbation of chronic HBV infection (15).

PCR testing for HBV DNA in serum directly assesses viral replication, guiding therapy decisions based on concentration and monitoring treatment efficacy. Indications for treatment consider factors such as DNA concentration, transaminase levels, fibrosis, comorbidities, extrahepatic manifestations, and the risk of developing liver cirrhosis and/or HCC (20).

Therapy

Serum viral load levels, ALT, and the degree of liver fibrosis determine treatment indications for patients with CHB infection. The primary goal is to prevent disease progression, liver failure, and the development of HCC. Studies have demonstrated a direct correlation between HBV DNA levels, the risk of cirrhosis, and HCC development (20, 21). Consequently, treatment for CHB infections aims for long-term suppression of HBV DNA levels or, ideally, achieving HBsAg loss, indicating profound suppression of HBV replication (15, 16).

In most patients, antiviral treatment leads to the elimination of chronic HBV-induced necro-inflammatory activity, halting the progression of liver fibrosis and reducing the risk of HCC development. Some cases may require therapy to prevent mother-to-child transmission or HBV reactivation. Additionally, patients experiencing HBV-associated extrahepatic manifestations may require treatment (15, 16).

For patients meeting the criteria for therapy, two main treatment options are available: interferon-based therapy and nucleotide analogs.

Interferon-based therapy

Interferons, as mentioned earlier, are cytokines naturally produced by immune system cells in response to viral infections. They exhibit antiviral and immunomodulatory effects, although the precise mechanism of action remains unclear (13).

Therapy with pegylated IFNα is recommended for patients with mild to moderate chronic hepatitis B and selected cases of compensated cirrhosis without portal hypertension, considering the significant adverse effects associated with this treatment.

Pegylated IFNα is administered subcutaneously once a week for 48 weeks, resulting in a sustained off-treatment response in approximately 30% of patients. A treatment response is defined as serum HBV DNA < 2000 IU/ml, along with ALT levels within the normal range. In HBeAg-positive patients, it includes HBeAg loss or seroconversion. Notably, HBsAg loss (functional cure) is achieved in only 10% of treated patients (22).

Nucleotide Analogues

NAs inhibit the RNA-dependent DNA polymerase reverse transcriptase and are typically taken orally once daily for an extended duration, often until HBsAg loss is achieved. These drugs are

well-tolerated, with generally mild to moderate adverse effects reported in clinical trials, including fatigue (3-10%), dizziness (5-10%), and headache (5-15%) (13).

In Europe, six different NAs are approved: lamivudine (LAM), adefovir dipivoxil (ADV), telbivudine (TBV), entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF). They can be classified based on their resistance barrier, with lamivudine, adefovir dipivoxil, and telbivudine having a low barrier, and entecavir, tenofovir disoproxil fumarate, and tenofovir alafenamide having a high barrier (16).

The main advantage of treatment with potent NAs with a high resistance barrier (e.g. ETV, TDF, TAF) is the expected long-term antiviral efficacy, which results in undetectable HBV DNA in most compliant patients. These drugs can be used safely in all HBV-infected patients and are the only treatment option for several patient subgroups, including patients with decompensated liver disease, liver transplantation, acute hepatitis B, severe chronic HBV exacerbation, and extrahepatic manifestations. Additionally, NAs are the only option for preventing HBV reactivation in patients with immunosuppression (16).

2.3 Hepatitis D virus

The hepatitis D virus is a defective RNA virus first identified in 1977 in Italy by Mario Rizzetto and colleagues who determined a new antigen, named δ, by direct immunofluorescence in patients with HBV infection (23). Moreover, δ antibodies were initially found in the serum of HBV-infected patients with severe hepatitis (23). In the 1980s, Rizzetto et. al. conducted studies in chimpanzees, which showed that HDV was infectious and required HBsAg to complete its life circle (24). Therefore, HDV can occur only as a co-infection with HBV or a super-infection of an HBV carrier. While a co-infection becomes chronic in just 2-10 % of the cases, a superinfection leads to a chronic HBV/HDV infection in over 90 % of the cases (25, 26). HDV infection leads to the most severe form of viral hepatitis, which is associated with higher rates of liver cirrhosis, HCC development, and liver-related mortality compared to patients with HBV mono-infection (25-29).

Nucleos(t)ide analogues do not affect HDV replication. Pegylated IFNα-based therapies were the only treatment option until the summer of 2020 when the first potentially effective drug Bulevirtide (BLV), was approved for HDV treatment by the European Medicines Agency (EMA). BLV is a synthetic N-acylated pre-S1 lipopeptide that blocks the binding of HBsAg-enveloped particles to the sodium taurocholate co-transporting polypeptide (NTCP), the cell entry receptor for both HBV and HDV. BLV shows promising results, long-term data must be awaited. (30)

Epidemiology

Globally, approximately 62-72 million people suffer from CHD (31). This prevalence is nearly twice that of the human immunodeficiency virus (HIV). However, determining the exact global prevalence of HDV is challenging due to varied and non-standardized screening practices and limited access to screening tests in many endemic areas.

HDV prevalence is primarily assessed through the detection of total antibodies to HDV (anti-HDV) among HBsAg carriers (32). It varies significantly by geographic region and is influenced by migration movements and increased access to prophylactic HBV vaccination, which also provides default vaccination against HDV (32, 33).

The highest prevalence of HDV infection with about 60 % of all HBsAg-positive patients is reported in Mongolia, a country with an extraordinarily high rate of HCC (34). Other high-prevalence areas include the Amazon basin (35), West Africa (36, 37), the Mediterranean basin (38), and Eastern Europe (39). It is reported that HDV infection is more common in men than

in women (26, 40). HDV can be transmitted by blood and blood-derived products and sexual contact. Therefore, individuals at high risk for HDV infection are from endemic areas, intravenous drug users, men who have sex with men, and patients with multiple sexual partners (26).

Virology

Genotypes

The HDV genome is a compact circular single-stranded RNA genome (41). Eight heterogeneous HDV genotypes have been described with up to 40 % of sequence divergence (42). GT 1 is distributed worldwide and is the main genotype in Europe and North America. GT 2 and GT 4 are predominant in East Asia, while GT 3 is mainly found in South America. Genotypes 5-8 are primarily endemic in Africa (43) (Figure 2.3). In comparison with GT 1, GT 2 and GT 4 seem to cause milder liver disease, as GT 2 has been associated with a lower incidence of liver cirrhosis, HCC, and decreased mortality than GT 1. GT 3 is associated with a more severe course of acute infections and a higher risk of acute liver failure (29).

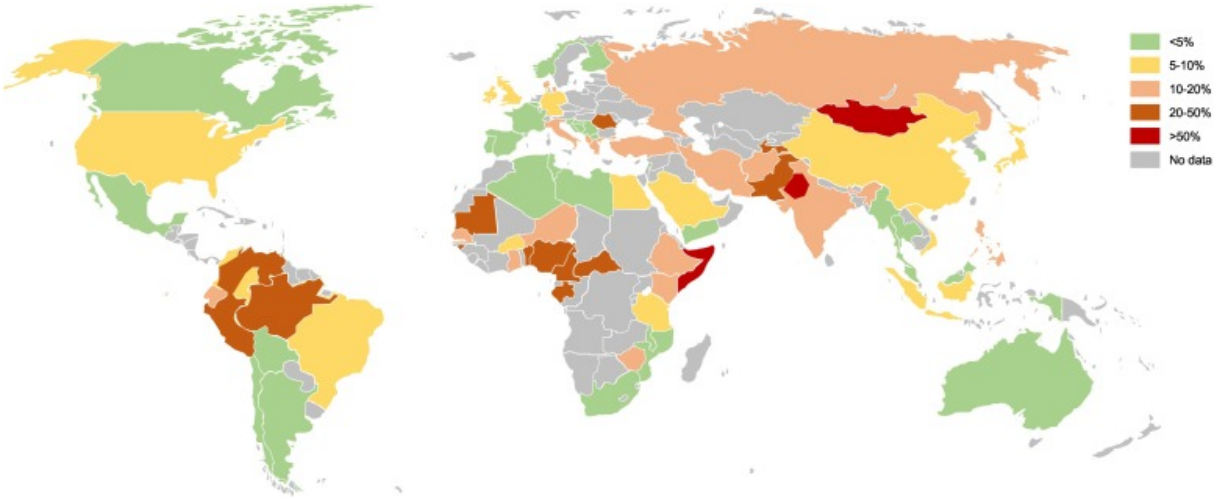

Figure 2.3: Estimated prevalence of HDV infection and the respective GT distribution (45)

HDV life cycle

HDV is the smallest known human RNA virus and is composed of a circular 1700 base pair (BP) containing single-stranded RNA and the hepatitis D antigen forming the HDV ribonucleoprotein (RNP). HDAg is the only protein that is known to be encoded by the HDV genome. It consists of two isoforms: the large and the small HDAg. As HDV is a defective virus and does not code for its surface proteins, it uses three forms of HBV surface proteins (S-HBsAg, M-HBsAg, and L-HBsAg) to envelope (44, 46, 47). The preS1 region of the large HBsAg interacts with the NTCP receptor on the hepatocytes for entry (48). After entering the cell the virion becomes uncoated resulting in an HDAg translocation of the nucleocapsid to the nucleus. The antigen has no RNA polymerase activity. To replicate its genome, the virus hijacks the cellular RNA polymerases of the host, which might then treat the genome as double-stranded DNA because of its folded, rod-like structure. Three forms of RNA are made in the host during replication: circular genomic RNA, circular complementary antigenomic RNA, and linear antigenomic RNA, which is the messenger RNA. The mRNA is exported to the cytoplasm and translated at the endoplasmatic reticulum to form new molecules of hepatitis D antigen. The S-HDAg returns to the nucleus where it supports further genome replication. L-HDAg undergoes prenylation before assembly. Both forms of hepatitis D antigen associate with new transcripts of genomic RNA to form new RNPs. RNPs are exported to the cytoplasm where large HDAg facilitates association with HBV envelope proteins in the ER to form new virus particles. These particles bud through an intermediate compartment and are then exported from the hepatocyte via the trans-Golgi network to re-infect further cells (see Figure 2.4) (49).

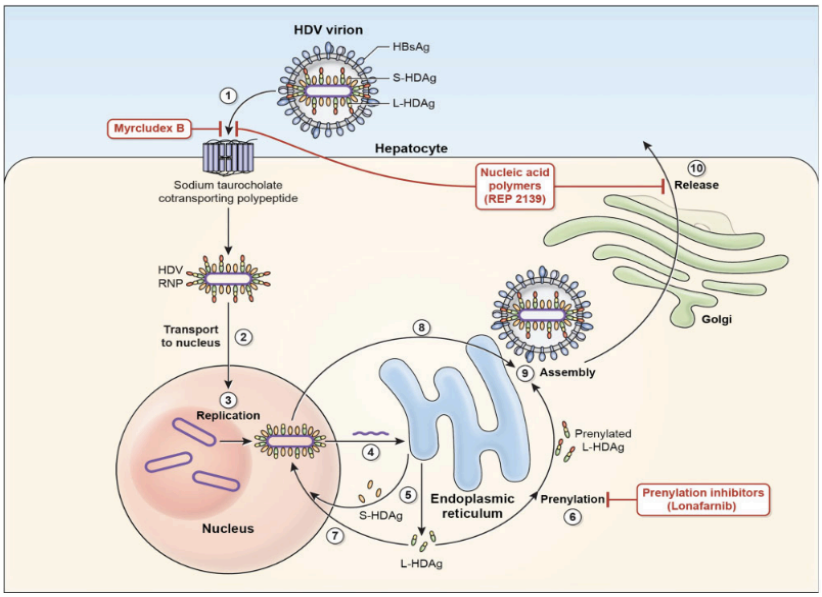

Figure 2.4: Hepatitis D virus and its life cycle (49)

Clinical aspects

Acute hepatitis D infection

Acute HDV starts after an incubation period of 3-7 weeks and may come along with non-specific flu-like symptoms, high levels of transaminases, and jaundice (25). Acute hepatitis due to HDV infection can either appear as a simultaneous co-infection with HBV or as a super-infection in chronically infected HBV patients. While co-infection usually occurs as acute self-limited hepatitis and rarely progresses to a chronic HDV infection, HDV super-infection leads to a chronic HDV infection in 90 % of the cases (47, 50). Clinically, patients with a co-infection and a super-infection may present with the same symptoms, but the acute phase of the HDV super-infection is characterized by more active HDV replication that leads to a suppressed HBV viral load and anti-HBc IgM is usually negative or with low titer in superinfected patients (25, 50).

The severity can vary from mild to fulminant hepatitis, whereas fulminant hepatic failure has been reported to occur in 1 % of HBV/HDV co-infected patients and 5% of HDV super-infected patients (51).

Chronic hepatitis D and its consequences

CHD infection is defined as a persistent HDV infection for more than six months (50). Clinically, patients may be asymptomatic or have non-specific symptoms like fatigue, malaise, and anorexia (25). Most patients will have predominant HDV replication with suppressed HBV replication and are HBeAg negative. It is hypothesized that HDAg suppresses HBV transcription as well as HBV virion release (52). In many patients with predominant HDV replication, hepatitis B viral loads are sometimes even under the level of quantification (28). However, some patients show similar viral loads of HBV and HDV. Rarely, do patients show a predominant HBV replication. Interestingly, these patients are often HBeAg-positive (53).

Patients with a chronic HBV/HDV infection have an accelerated progression to liver cirrhosis and an increased risk of hepatic decompensation, HCC development, and mortality than patients with an HBV mono-infection (27, 53, 54).

Diagnostics

When HDV was first described in 1977, the diagnosis could only be made by immunohistochemical staining of HDAg in liver tissue (23). Over time testing for IgM / IgG anti-HDV became available. Apart from being able to diagnose an HDV infection serologically, these tests also helped to classify an HDV infection into chronic or acute in addition to liver chemistry and clinical symptoms (28, 55). Furthermore, a positive anti-HDV IgM level can be found in acute hepatitis D infection but also during flares of chronic HDV infection (56). It is assumed that anti-HDV IgM levels correlate with disease activity in hepatitis D (57). In the early 1990s, serum HDV RNA PCR techniques were developed that enabled qualitative testing of HDV in HBsAg-positive patients (58, 59). Quantitative testing and therefore measurement of HDV RNA viremia became possible in the early 2000s (60).

Screening strategies for HDV differ between the United States and Europe. Despite an increasing body of evidence pointing at a suboptimal diagnosis of HDV infection, screening in the United States is only advised in patients with specific risk factors including a history of intravenous drug use (IVDU), high-risk sexual behavior, patients from endemic HDV areas, HIV or HCV infection and patients with elevated aminotransferases with low or undetectable HBV DNA. European guidelines, on the other hand, recommend screening all HBsAg-positive patients (31).

In both guidelines, the anti-HDV total antibody is currently recommended as a first screening approach, and if positive HDV RNA PCR should be followed (49).

Prevention

HBV vaccination protects effectively against both HBV and HDV infection. Vaccination campaigns have indeed reduced the reservoir of HBV patients who can potentially be infected with HDV (62).

Currently available antiviral treatments

Pegylated IFNα

Interferon-α is a cytokine endogenously produced by immune system cells in response to viral infections. It prosses antiviral and immunomodulatory effects; however, its precise mechanism of action has not been clarified yet (13). Despite being the only recommended treatment for hepatitis D (16, 63), its efficiency is not satisfying, since 75% of patients treated for 48 weeks showed a relapse 24 weeks after treatment discontinuation or later (64), and a prolonged duration of treatment did not show any additional benefit (61). Additionally, many patients

suffer from considerable side effects like hematology toxicity, nausea, fatigue, psychiatric disorders, and flu-like symptoms. Pegylated IFNα is available as an injectable formulation and needs to be administered once a week.

Nucleoside/nucleotide Analogues

While NAs inhibit HBV replication by interacting with the HBV reverse transcriptase, HBsAg expression is barely affected (65). The tested NAs including famciclovir (66), adefovir (64), ribavirin (67), lamivudine (65), and entecavir (68) showed no effectiveness against HDV.

Bulevirtide former known as Myrcludex B

Bulevirtides molecular structure corresponds to the L-HBsAg and inhibits viral entry by binding to its natural receptor, NTCP, at the membrane of hepatocytes. Data from preclinical studies indicate that the antiviral effect can occur without interference with the bile acid transport function of NTCP (47).

2.4 Autoimmune phenomena in viral hepatitis

Autoimmune phenomena in patients with viral hepatitis can range from autoantibody seropositivity to a clinically manifest autoimmune disease. These phenomena are especially well documented for HBV and HCV infections (69, 70) and have also been described for hepatitis D but with a limited database (69).

In general, autoimmune manifestations are the consequence of an aberrant immune response caused by the recognition of self, and non-self-antigens and viruses are considered to play an important role in triggering autoimmune phenomena in genetically susceptible individuals. Although precise knowledge of the interaction between viruses and the immune system is still limited, several mechanisms have been proposed to explain the breakdown of self-tolerance caused by viral infection (71).

One mechanism is molecular mimicry, a mechanism by which infectious agents trigger an immune response against autoantigens (72) due to structural similarity between epitopes from microorganisms and self-antigens (69). In detail, a susceptible host becomes infected with a pathogen whose antigens are immunologically similar to the antigens of the host, but so different that they elicit an immune response. As a result, the tolerance to the affected autoantigens breaks down, and the pathogen-specific immune response cross-reacts with host antigens. This process is mediated by naive T cells with an autoreactive potential, which becomes activated on exposure to the exogenous peptides. B cells produce antibodies in response to signals from activated helper T cells that can cause tissue damage by activating complement pathways (see Figure 2.5) (72). Another suspected mechanism is bystander activation, in which a nonspecific and overreactive antiviral immune response creates a localized pro-inflammatory environment associated with the release of self-antigens from the injured tissue. These self-antigens are subsequently taken up and presented by antigen-presenting cells (APC) to stimulate the previously non-responsive, but autoreactive T-cells in the pro-inflammatory environment and trigger autoimmunity (71). A related process is called epitope spreading, in which inflammation triggers the release of self-antigens. Uptake and processing of antigen by B cells result in de novo activation of autoreactive B cell clones by the initial autoreactive T cells causing a broadening of the autoreactive B cell response (71). The immune response can be directed against additional different epitopes from the same antigen (intramolecular spreading) or other antigens (intermolecular spreading) (73, 74). Once the

process is established, the initial antigen may no longer be required to sustain the autoimmune response (69).

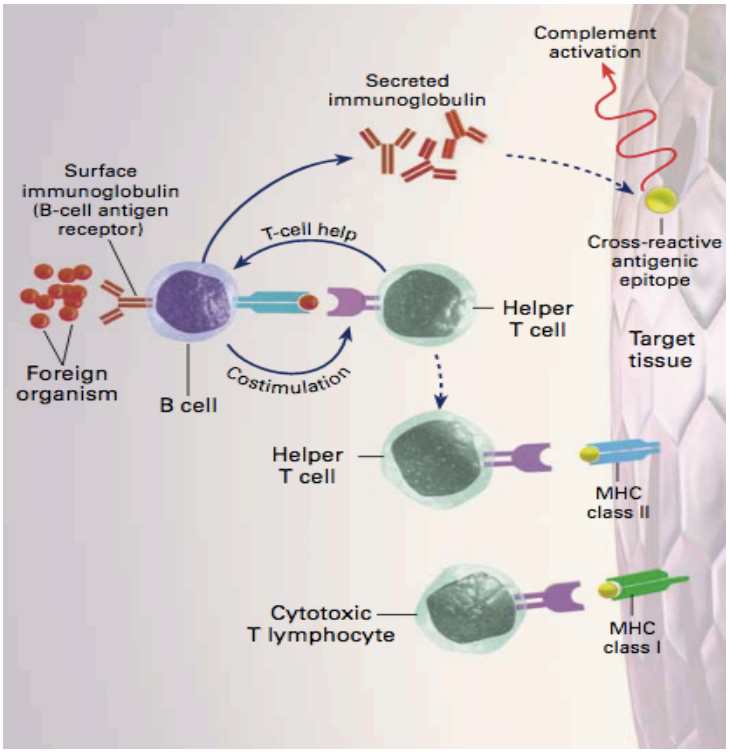

Figure 2.5: Molecular Mimicry (72) An immune response against foreign organisms is triggered by the presentation of peptides by antigen-presenting cells to T cells in combination with appropriate stimulatory signals. If the presented peptides (small red spheres) are similar to host peptides (small green spheres), there will also be an immune response directed against the host peptides. This process is mediated by naive T cells with autoreactive potential, which become activated on exposure to the exogenous peptides. B cells produce antibodies in response to signals from activated helper T cells that can cause tissue damage by activating complement pathways. Cytotoxic T lymphocytes recognize mimicked antigens in association with MHC class I molecules on the surface of target tissues and cause direct tissue damage. Previously cryptic antigens may be released after tissue damage, resulting in an enhanced response. (72)

In 2015, Li et al. (75) investigated the prevalence of non-organ specific antibodies (NOSA) in 325 patients with chronic HBV infection. They reported that 58.2% of the patients showed any positive NOSA, which was significantly higher than in healthy controls (6.7%). In detail, in the HBV cohort, 23,4% showed positive ANA titers (> 1:100) and 3.1% showed positive SMA titers. Other frequently measured antibodies were AMA-M2 (6.8%), anti-PML (11.1%), anti-gp210 (12.6%), and anti-Ro52 (28%). As a possible explanation of the cross-reactivity with ANA and SMA, Gregorio et al. (76) reported similarities in amino acid sequences between HBV-DNA polymerase and putative antigenic targets of ANA and SMA. In 1995, McFarlane et al. reported, that 22 % of their studied hepatitis D patients showed NOSA (77), whereas

further details were not published. However, data about molecular mimicry and hepatitis D are lacking.

Differentiating autoimmune phenomena in viral hepatitis from true autoimmune (liver) disease is clinically of utmost importance, as a concomitantly existing autoimmune liver disease would necessitate immunosuppressive treatment, which bears the risk of dampening control of the virus-induced liver disease. Furthermore, antiviral therapy would need to be considered for virus-related autoimmune phenomena.

2.5 Autoimmune hepatitis

Autoimmune hepatitis (AIH) is an immune-mediated chronic liver disease leading to (necro)inflammation and can cause liver cirrhosis. AIH is characterized by elevated serum transaminase and immunoglobulin G levels, detectable autoantibodies, and characteristic histopathological findings (6, 78). Based on different autoantibody profiles AIH is classified into two subtypes, autoimmune hepatitis type 1 (AIH - 1) and type 2 (AIH-2). The presence of ANA and/or SMA defines AIH – 1, which is with 80-90 % the predominant type of AIH and affects both children and adults. Autoimmune hepatitis type 2 is characterized by the presence of anti-liver cytosol type – 1 (anti-LC1) and/or anti-LKM1 and in rare cases anti-LKM3 antibodies. This subtype occurs much rarer (5-10 %), manifests predominantly at a young age, and is associated with a more severe course than AIH-1. However, apart from these two well-defined AIH subtypes 5-15 % of the patients with AIH present without autoantibodies or develop them during disease after an acute onset and are therefore categorized as seronegative, which leads to major obstacles in a timely diagnosis. However, in general, the majority of patients respond well to immunosuppressive treatment (1, 2, 79, 80).

Additionally, AIH can be associated with cholestatic features, and therefore also meet the diagnostic criteria either of primary biliary cholangitis (PBC) or of primary sclerosing cholangitis (PSC). This phenomenon is commonly termed an overlap syndrome and should be defined as a distinct type of autoimmune liver disease but classified according to the respective predominant autoimmune liver disease as AIH, PBC, or PSC with features of another autoimmune liver disease (79, 80).

Epidemiology

AIH occurs globally in children and adults of all ages, genders, and ethnicities. However, there is a female preponderance as 75-80 % of all patients diagnosed with AIH are women. As mentioned above, AIH differs in the age of disease onset depending on the subtype of AIH. Type 1 AIH affects people of all ages with two peaks, one in adolescence at the age of 10 to 18 years and the other in adulthood around the age of 40 years. Type 2 AIH mainly affects children, including infants, adolescents, and young adults (< 25 years of age). The estimated incidence ranges from 1-3 cases per 100,000 individuals per year varying across countries (79).

Pathogenesis

The precise pathogenesis of AIH still remains unclear. However, it is assumed that it is a combination of alterations in immune tolerance, a genetic predisposition, and environmental triggers, which in sum induce a T-cell-mediated (auto)immune response targeting almost unknown hepatic antigens, which then leads to necroinflammation and fibrotic change (78, 81). It is presumed that molecular mimicry, among other stimuli, may trigger the (auto)immune response and thereby lead to autoimmune hepatitis (82). In support of this hypothesis, exists a case report of a child, who acquired HCV infection after liver transplantation due to alpha - 1 antitrypsin deficiency, then developed anti-LKM1 and was diagnosed with AIH – 2 many years later even though the initial HCV infection had been cleared (79). This unusual clinical course might be explained by the fact that an amino acid sequence of the liver enzyme CYP2D6, which is the target of anti-LKM1 (see above), shows a high level of homology with proteins encoded by HCV and members of the herpesvirus family (82).

Clinical aspects

The presentation of AIH is heterogenous and varies from a chronic insidious course to fulminant hepatic failure. Most patients (30-50%) experience a mild disease onset and progression with unspecific symptoms such as fatigue, headache, anorexia, and nausea. However, many patients (15 - 30%) are entirely asymptomatic and identified by chance when blood samples are taken in another context, for instance in the diagnostic work-up of extrahepatic autoimmune diseases. It is known that 20-50% of patients diagnosed with AIH are affected by other, (extrahepatic) autoimmune diseases, the most common being autoimmune thyroid disease. Due to these mild unspecific or missing symptoms, AIH is diagnosed in advanced stages of fibrosis in around 30% of the patients (79, 80, 83). However, about one-third of the patients with AIH show acute symptoms such as jaundice, severe fatigue, abdominal pain, and impaired liver function as coagulopathy and/or hepatic encephalopathy. These acute symptoms may be due to a new onset or to an acute exacerbation of a pre-existing undiagnosed AIH. This can be differentiated with the help of a liver biopsy, which is recommended anyway in patients with acute, severe symptoms. Histological findings with advanced fibrosis or cirrhosis are suggestive of an exacerbation of a pre-existing AIH. In fulminant liver failure, massive hepatic necrosis is typically seen (79, 80).

Diagnostics

For the reasons described above diagnosis of AIH can be challenging. There is no single test of AIH. Diagnosis is based on a scoring system that was developed in 1993 by the International Autoimmune Hepatitis Group (IAHG). This score was revised in 1999 and for clinical purposes simplified in 2008 (78, 79). The simplified score criteria include autoantibodies, immunoglobulins, histological features, and viral hepatitis status, whereas high levels of IgG and high titers of autoantibodies can bring extra points (see Table 2.3). Histological findings play a pivotal role and are even necessary to establish the diagnosis of AIH. Liver histology also provides information on the inflammatory activity and the extent of liver damage. It is especially helpful to exclude alternative diseases such as hepatotropic viruses, Wilson disease, NAFLD, ALD, or DILI. Characteristic histological findings in AIH include interface hepatitis, portal lymphoplasmacytic infiltrate, rosette formation, and emperipolesis, (see Figure 2.6) (79). The severity is assessed using the Ishak modified hepatic activity index (mHAI), which ranges from 0 to 18 points. In addition to the laboratory and histopathological findings, a structured interview is of course necessary, particularly to evaluate possible contact with harmful substances. For initial risk stratification, further disease, and therapy monitoring, it is highly important to non-invasively assess the extent of liver fibrosis, e.g. via vibration-controlled transient elastography (VCTE), as advanced fibrosis is associated with worse outcomes (80).

Autoantibodies		
ANA or SMA	≥ 1:40	+1
ANA or SMA	≥ 1:80	+2
Anti-LKM1	≥ 1:40	+2
Anti-SLA	Positive	+2
Absent autoantibodies		0
Immunoglobulin level		
IgG	> UNL	+1
	> 1.1 ULN	+2
	Normal	0
Histological findings		
Morphological features of AIH	Compatible	+1
	Typical	+2
	Incompatible	0
Viral hepatitis		
	Absent	+2
	Present	0
Pretreatment aggregate score		
	Definite diagnosis	≥ 7 points
	Probable diagnosis	6 points

Table 2.3 Simplified diagnostic criteria (SDC) (84)

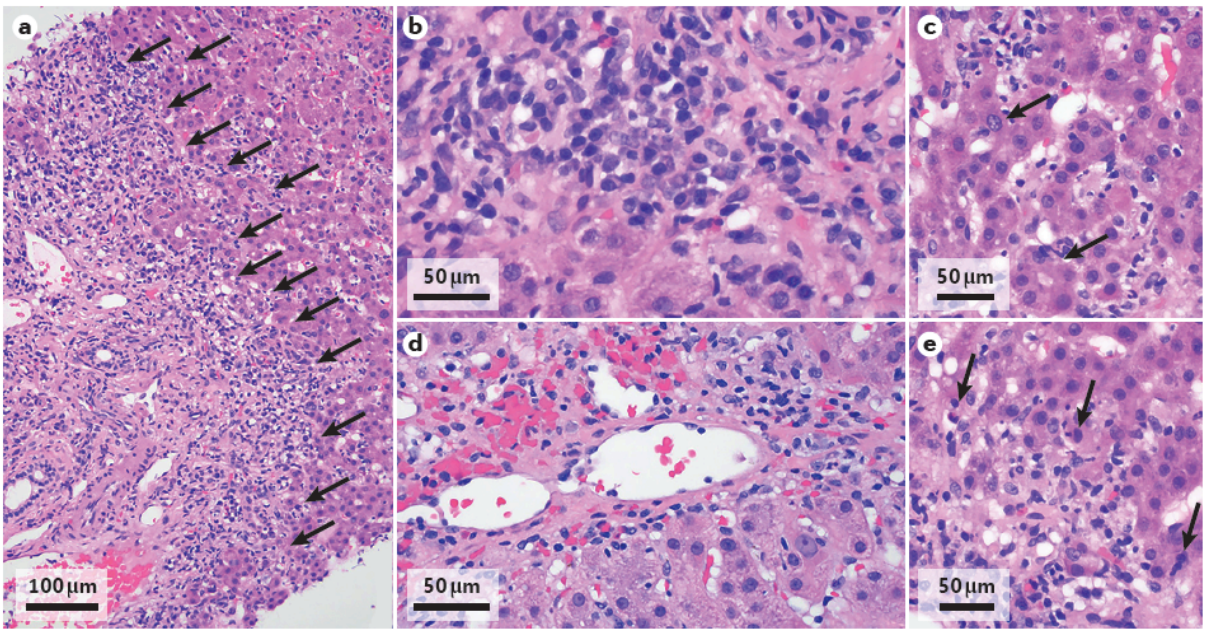

Figure 2.6: Histopathology of AIH. a: chronic AIH with interface hepatitis – lymphoplasmacytic portal inflammation extending into the lobule (arrows). b: chronic AIH with an inflammatory infiltrate consisting of plasma cells, which exhibit prominent pale staining of Golgi adjacent to nuclei. c: chronic AIH with rosettes (arrows) of regenerating hepatocytes. d: acute AIH with perivenulitis of central vein and central zonal necrosis. e: Hepatocyte emperipolesis (presence of an intact cell in the cytoplasm of another cell; arrows) showing a lymphocyte within the cytoplasm of a hepatocyte with a displacement of the nucleus and early phase of apoptosis in AIH. All slides are hematoxylin and eosin-stained. (79)

Therapy

Apart from a few exceptions, patients with AIH should always be treated with immunosuppressive agents (79). The treatment aims to achieve biochemical remission, which is defined as normal serum transaminase and IgG levels. In children with AIH-2, negative or low-titer antibodies are also part of the definition of remission, since anti-LKM1, anti-LC1, and SMA titers correlate with disease activity in this group (3). Biochemical remission correlates with histological remission, which then decreases the risk of disease relapse and progression (85).

Standard treatment of AIH includes predniso(lo)ne and azathioprine, which is effective in the majority of the patients (80-90 %). Corticosteroids are the drugs of choice to achieve remission and azathioprine (a purine analogue) is the drug of choice to maintain remission. In the initial phase, to achieve a rapid response, therapy with predniso(lo)ne (0.5 -1 mg/kg/day) should be started. AIH almost always responds to steroid therapy within 2-3 weeks and if it does not, the diagnosis should be questioned. Due to the numerous systemic side effects of steroids, azathioprine should be added, as a steroid-sparing agent, two weeks after the start of steroid therapy. The delayed azathioprine initiation is due to the potential hepatotoxicity of azathioprine, especially in jaundice patients, and it allows to differentiate azathioprine hepatotoxicity (in 2%) from steroid non-response (79, 80). The predniso(lo)ne dose can then be reduced quickly, under strict transaminase control, to a target dose of 5-10 mg daily. In the further course, the aim is to completely discontinue steroid therapy and treat the patient only with azathioprine. A treatment withdrawal should be undertaken at the earliest after three years and just in patients, who achieved a stable remission for at least the past two years. This trial needs to be undertaken under close clinical and biochemical monitoring since about 20 % of the patients experience a relapse and therefore immunosuppressive treatment needs to be initiated again (79, 80). However, if standard treatment fails, either because of intolerance or insufficient response to the treatment, a disease progression has to be expected. In this case, alternative therapeutic approaches are required (80). Patients, who are intolerant to azathioprine can be switched to 6-mercaptopurine (6-MP), an azathioprine-metabolite, which gets enzymatically converted to the final metabolite of azathioprine, 6-methylmercaptopurine (6 - MMP). Another alternative off-label treatment, which also interacts with purine metabolism, is Mycophenolate mofetil (MMF). It has been shown that 50-75 % of azathioprine-intolerant patients tolerate 6-MP or MMF (80). However, these alternative treatments are rarely effective in patients, who do not achieve full remission on azathioprine. In non-responders to azathioprine, 6-thioguanine (6-TG) levels should be checked to assess both compliance and

aberrant pharmacodynamics since it is a biologically active metabolite. If there is insufficient response despite adequate 6-TG levels, there are some second-line drugs, which have been reported to be effective steroid-sparing agents. Calcineurin inhibitors, such as cyclosporine and tacrolimus, as well as biologicals, in particular, infliximab (anti-TNF) and rituximab (anti-CD20), were successfully used (79, 80, 83).

3 Patients and methods

3.1 Study population

Autoantibody titer and IgG levels were studied in three different cohorts consisting of 40 CHD patients infection, 70 patients with CHB infection, and 46 patients diagnosed with AIH. All patients were treated at specialized outpatient departments of the University Medical Center Hamburg-Eppendorf. The studied data were collected as part of the clinical routine visits between 2010 and 2019 and analyzed retrospectively. While serological markers and PCR analysis confirmed the diagnosis of viral hepatitis, the diagnosis of AIH was secured by serological and histopathological findings according to the EASL clinical practice guidelines (78).

Inclusion criteria for participants in the HDV cohort were positive HBsAg status as well as positive anti-HDV immunoglobulin result and not necessarily an existing viremia. Due to published data from Giersch et al. (86), and Heidrich et al. (87), it can be assumed that HDV persists intrahepatically in HDV PCR negative patients, for example after interferon treatment, and can convert to a viremic infection even in patients with low HBV replication time. In this present study, almost half of the CHD patients show negative HDV PCR at the time of inclusion (19/40).

Patients receiving Interferon therapy within the last six months before inclusion, with a history of malignancy or infection with viral hepatitis C or HIV were excluded. The study was approved by the local ethics committee (WF-035/17). Autoantibody titers, available in 39 patients of the HDV cohort, in 69 patients of the HBV cohort, and in 46 patients of the AIH cohort as well as IgG levels, available in 37 patients of the HDV cohort, in 61 patients of the HBV cohort and 44 patients of the AIH cohort, were analyzed retrospectively (see Figure 3.1).

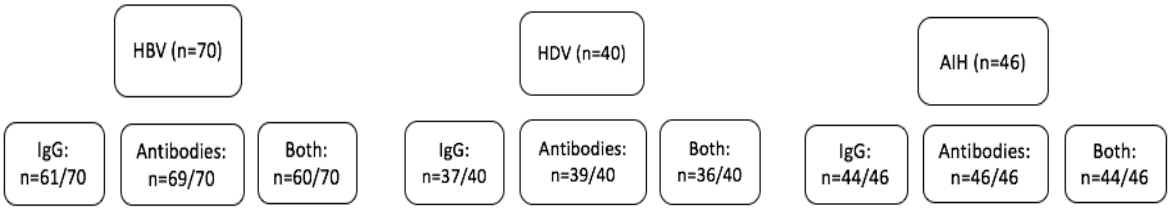

Figure 3.1: Available data in the three cohorts
HBV, viral hepatitis B; HDV, viral hepatitis D; AIH, autoimmune hepatitis; IgG, immunoglobulin G

3.2 Autoantibody diagnostics

Testing for autoantibodies was performed by trained personnel in the Institute of Immunology at the University Medical Center Hamburg-Eppendorf. ANA, SMA, LKM-1, and AMA were assessed by immunofluorescence testing. HEp-2 Cells (human epithelioma cells) and tissue slides (Euroimmun, Lübeck, Germany) were incubated according to the manufacturer´s description, and interpretation was performed manually using the Eurostar microscope (Euroimmun, Lübeck Germany). SLA was tested by ELISA using enzyme immunoassays from Euroimmun, Germany (6, 88). According to our internal laboratory standards we, regarded ANA, SMA, and LKM-1 as positive at a titer of $> 1:80$, AMA as positive at titers of $\geq 1:40$, and SLA were regarded as positive if ≥ 20 U/ml.

3.3 Statistics

All data were collected in a Microsoft® Excel® (Version 12.3.6 for Mac) file and imported into IBM *SPSS* (Version 24.0 for Mac, Armonk, NY, USA, 2016) for statistical analysis.
Data are presented as absolute numbers and percentages or as median and interquartile-range (IQR) Nominal variables were compared using the Chi-Square test, metric variables were analyzed using the Mann-Whitney-U-test and median variables were compared using the unpaired t-test. Spearman´s rho was used for correlation analysis. The distribution of NOSA was also graphically represented in a pie chart. ANA and SMA titers were additionally presented in a stacked bar chart.

4 Results

4.1 Baseline characteristics of the study population

The baseline characteristics of the three cohorts are described in Table 4.1. The three cohorts differed in age, as the youngest patients were found in the CHB cohort with a median age of 37 years, followed by the CHD cohort with a median age of 45 years and the AIH cohort with a median age of 54 years. In the CHD cohort were significantly more men than in the AIH cohort (60% vs 33%).

Transaminases (AST and ALT) were significantly higher in the AIH cohort than in the cohorts of viral hepatitis ($p < 0.01$). However, transaminases were significantly higher in CHD patients infection than in patients with CHB infection ($p < 0.01$).

In the CHD cohort seven patients (17%) were under treatment with NAs and had median hepatitis D and B viral loads below the detection limit (HDV: 0-12200 U/ml; HBV: 0 - 200 U/ml); 17 patients (43%) underwent treatment with IFN and had a median hepatitis D viral load of 32000 (870-220000) U/ml and a median hepatitis B viral load of 70 (6 – 790) U/ml; 16 patients (40%) were therapy naïve and had a median hepatitis D viral load under detection limit (0-300 U/ml) and a median hepatitis B viral load of 378 (35–3490) U/ml. In the CHB cohort, 11 patients (16%) were under treatment with NAs and had a median viral load under the detection limit (0-456 U/ml); one patient (1%) underwent treatment with IFN alpha and had a viral load of 130 U/ml; 58 patients (83%) were therapy naïve and had a median viral load of 2400 (422-20250) U/ml.

There was no statistical difference in the liver stiffness values assessed by transient elastography between the three different cohorts.

Liver biopsies to assess the mHAI score were available in 34 patients with AIH, 12 patients with CHD, and 3 patients with HBV. The median mHAI score was higher in AIH patients (9/18, IQR: 4-16) compared to patients with CHD (6.5/18, IQR 2-10) and CHB (4.5/18, IQR 3-6), although this difference did not reach statistical significance. However, it is important to note that the statement regarding group comparability is limited due to the substantial difference in group sizes.

	AIH (n=46)	HDV (n=40)	CHB (n=70)
Age (years)	54 (37-71)	45 (37-54)	37 (30-47)
Sex (female:male)	31:15	16:24	40:30
AST (U/l)	202 (68-801)	40 (26-92)	22 (18-31)
ALT (U/l)	278 (76-758)	60 (32-117)	31 (19-47)
Viral load (U/ml) in patients currently treated with NAs	-	n=7 HDV: 0 (0-12200) HBV: 0 (0-200)	n=11 0 (0-456)
Viral load (U/ml) in patients with a history of IFN treatment	-	n=17 HDV: 32000 (870–220000) HBV: 70 (6-790)	n=58 2400 (422-20250)
Viral load (U/ml) in therapy naïve patients	-	n=16 HDV: 0 (0-300) HBV: 378 (35-3490)	n=1 130
Transient elastography (kPA)	n=27 8.2 (6.1-17.9)	n=36 7.7 (6.2-14.0)	n=40 5.3 (4.3-6.5)
mHAI Score (0-18 points)	n=34 9 (4-16)	n=12 6.5 (2-10)	n=3 4.5 (3-6)

Table 4.1: Baseline characteristics of the study population. Values are medians with interquartile range (IQR)

4.2 Non-organ specific autoantibody distribution in all cohorts

The distribution of non-organ specific autoantibodies (NOSA) including anti-nuclear antibodies (ANA), smooth muscle actin antibodies (SMA), type 1 liver-kidney microsomal antibodies (LKM-1), anti-mitochondrial antibodies (AMA), soluble liver antigen antibodies (SLA) are described below (Table 4.2).

In a group-wise comparison, positive NOSA titers were more frequently found in the CHD cohort as compared to the CHB cohort (CHD 69 % vs CHB 43 %, $p < 0.05$). However, positive NOSA titers were significantly less frequent in CHD patients infection as compared to patients diagnosed with AIH (CHD 69 % vs AIH 96 %, $p < 0.01$). Neither patients with viral hepatitis nor patients with AIH had positive LKM-1 or AMA titers. SLA was detected in one patient (3 %) diagnosed with HDV and in eight patients (17 %) diagnosed with AIH. However, no patient with CHB had positive SLA.

	AIH (n=46)	CHD (n=39)	CHB (n=69)
Any NOSA	44 (96 %)**[1,**3]	27 (69 %)**[1,*2]	30 (43 %)*[2,**3]
ANA	41 (89 %)*[1,**3]	26 (67 %)*[1,*2]	30 (43 %)*[2,**3]
SMA	23 (50 %)**[1,**3]	6 (15 %)**[1,*2]	2 (3 %)*[2,**3]
LKM-1	0	0	0
AMA	0	0	0
SLA	8 (17 %)*[1]	1 (3 %)*[1]	0

Table 4.2: Non-organ specific autoantibody distribution in all cohorts. Significance was tested with the Chi-square test. [1]= CHD compared to AIH, [2]= CHD compared to CHB, [3]= CHB compared to AIH p < 0.05, **p < 0.01*

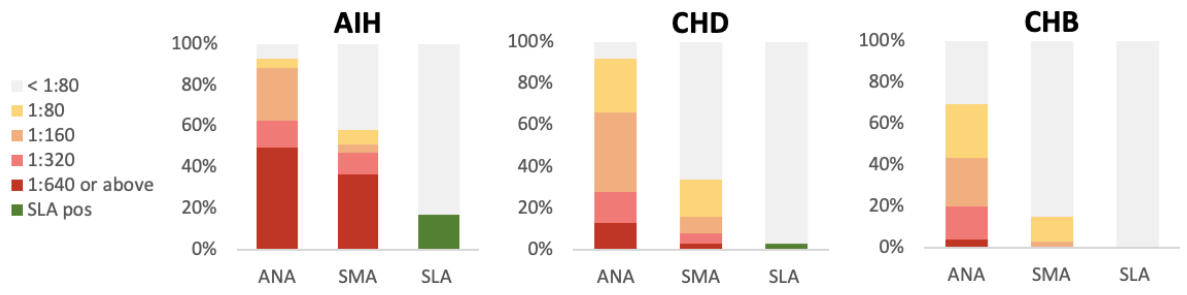

Figure 4.1: NOSA distribution in all cohorts, presented in a stacked bar chart

To determine the frequency of combined positive autoantibodies in all cohorts, the following autoantibody combinations were analyzed, ANA+/SMA-/SLA-, ANA-/SMA+/SLA-, ANA- /SMA-/SLA+, ANA+/SMA+/SLA-, ANA+/SMA-/SLA+, ANA– /SMA+/SLA+ and ANA+/SMA+/SLA+ (see Table 4.3 and Figure 4.2 - 4.4).

Patients with CHB had almost exclusively elevated ANA titers with negative SMA and negative SLA titers. In patients with CHD and AIH, positive ANA titers were also combined with positive SMA titers. However, CHD patients showed this combination significantly less frequently than patients with AIH (HDV 13% vs AIH 35%, $p < 0.05$).
All patients with CHB had negative SLA titers. Just one CHD patient had a positive SLA titer combined with a negative ANA titer and negative SMA titer (3%). However, in AIH patients positive SLA titer occurred more frequently than in patients with CHD; in detail, four patients (9%) had a combination of positive ANA titer and positive SMA titer, three patients (7%) with positive ANA titer and one patient (2%) with positive SMA titer.

	AIH (n=46)	**CHD (n=39)**	**CHB (n=69)**
ANA+ / SMA- / SLA-	18 (39 %)	21 (54 %)	28 (46 %)
ANA- / SMA+ / SLA-	2 (4%)	0	0
ANA- / SMA- / SLA+	0	1 (3)	0
ANA+ / SMA+ / SLA+	4 (9%)	0	0
ANA+ / SMA+ / SLA-	16 (35 %)*[1],**[3]	5 (13 %)*[1,2]	2 (3%) *[2],**[3]
ANA+ /SMA- /SLA+	3 (7%)	0	0
ANA- /SMA+ /SLA+	1 (2%)	0	0
No Autoantibodies	2 (4 %)**[1]**[3]	12 (31 %)**[1,2]	39 (57 %)*[2]**[3]

Table 4.3 Autoantibody distribution in all cohorts. Significance was tested with the Chi-square test.
*[1]= CHD compared to AIH, [2]= CHD compared to CHB, [3]= CHB compared to AIH. * p < 0.05, **p < 0.01*

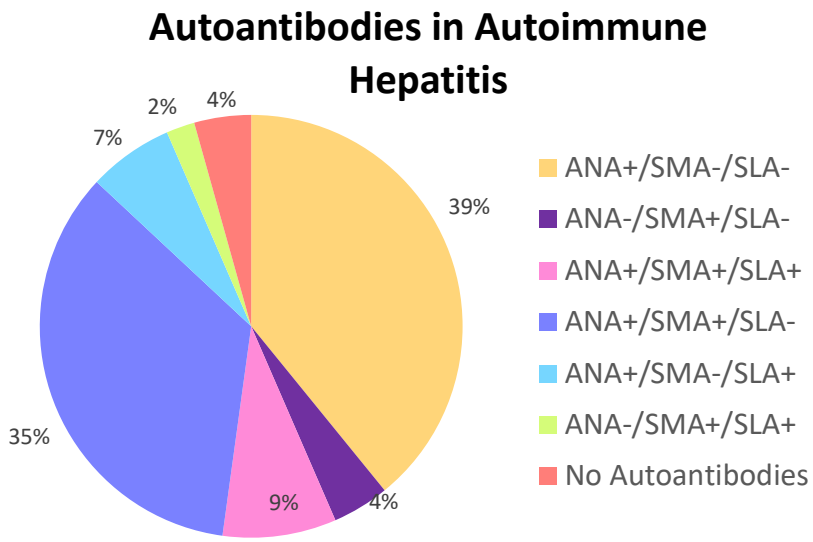

Figure 4.2 Distribution of NOSA in patients with AIH

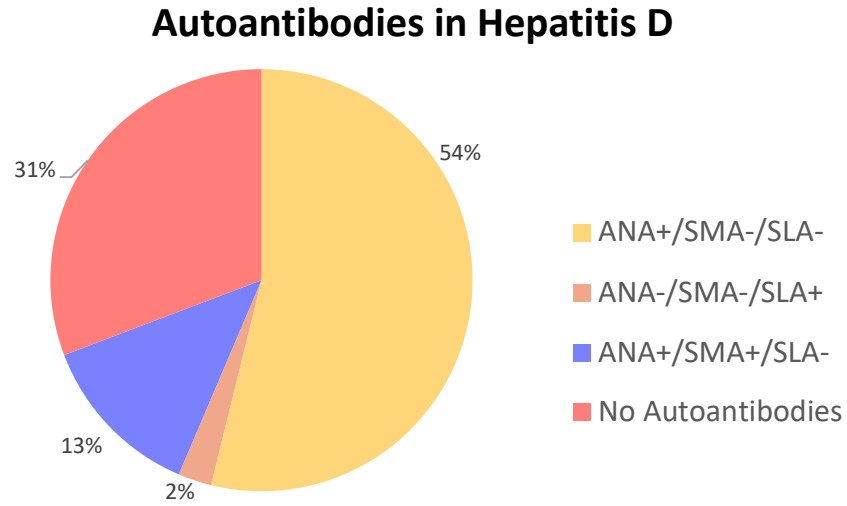

Figure 4.3 Distribution of NOSA in patients with CHD

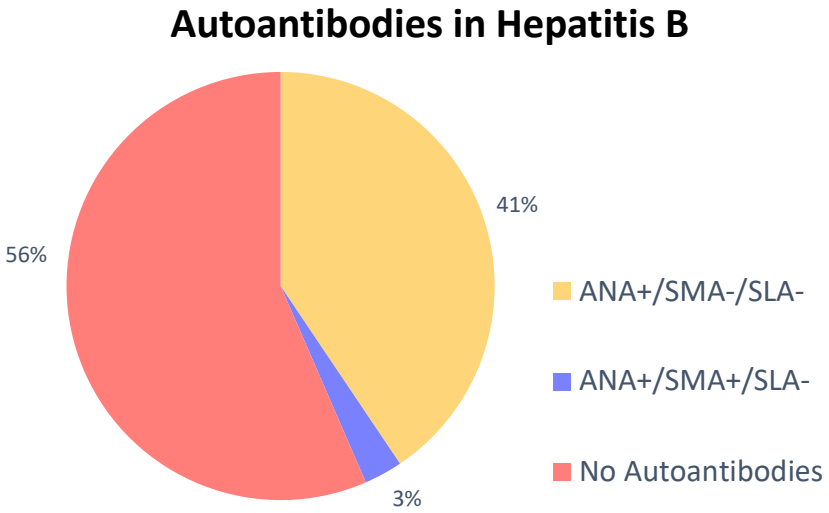

Figure 4.4:Distribution of NOSA in patients with CHB

4.3 Levels of ANA titers in all cohorts

In the following section, the levels of ANA titers in the three different cohorts were investigated. Details are described in Table 4.4.

Positive ANA titers (< 1:80) were more frequent in patients with AIH and CHD than in patients with CHB. Whenever, positive ANA titers (>1:80) were more frequent in patients with AIH than in patients with CHD (AIH 89% vs. CDH 67%, p <0.05). Furthermore, significantly more patients with AIH had ANA titers of ≥ 1:320 compared to patients with CHD (AIH 63% vs CHD 28 %, p < 0.01). Although patients with CHD infection had more frequently positive ANA titers than those with CHB infection (CHD 67% vs CHB 43%, p < 0.05), they did not have more often high ANA titers ≥ 1:320 (CHD 28% vs CHB 20%) (see Figure 4.5).

	AIH (n=46)	CHD (n=39)	CHB (n=69)
ANA <1:80	3 (7 %)**[3]	3 (8 %)**[2]	21 (30 %)**[2,]**[3]
ANA 1:80	2 (4.5 %)**[1]**[3]	10 (26 %)**[1]	18 (26 %)**[3]
ANA 1:160	12 (26 %)	15 (38 %)	16 (23 %)
ANA 1:320	6 (13 %)	6 (15 %)	11 (16 %)
ANA 1:640	6 (13 %)	3 (8 %)	3 (4 %)
ANA 1:1280	8 (17 %)	2 (5 %)	0
ANA 1: 2560	2 (4.5 %)	0	0
ANA 1:5120	7 (15 %)	0	0

Table 4.4: Levels of ANA titer in all cohorts. Significance was tested with the Chi-square test.
*[1]= CHD compared to AIH, [2]= CHD compared to CHB, [3]= CHB compared to AIH. * p < 0.05, **p < 0.01*

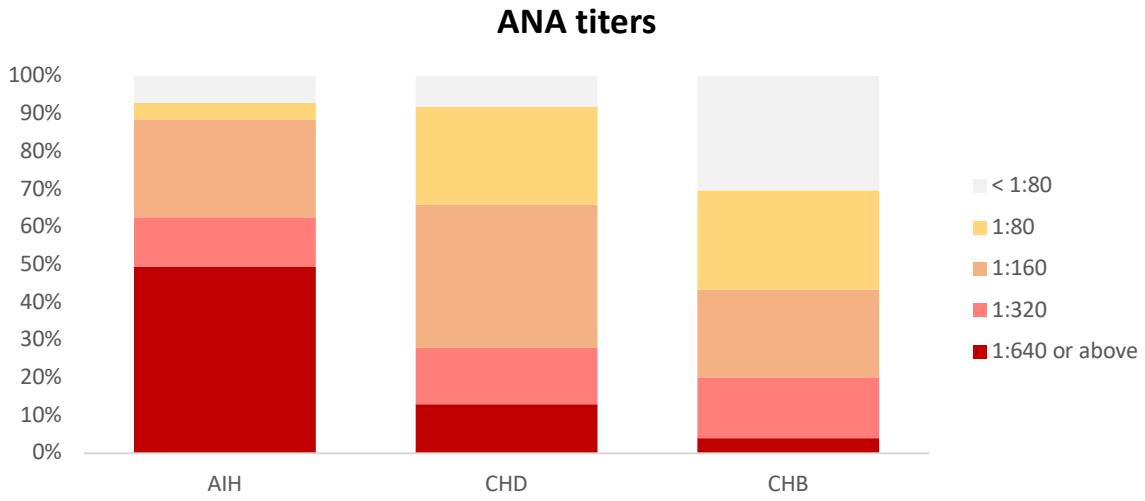

Figure 4.5 ANA titers in all cohorts, presented in a stacked bar chart

4.4 ANA patterns

As previously mentioned, the adherence of fluorescein-labeled anti-immunoglobulin antibodies to specific nuclear antigens leads to distinct fluorescence patterns. This section assessed ANA patterns in all cohorts, with further details provided in Table 4.5.

Notably, the pattern of ANA antibodies differed significantly between patients with AIH and viral hepatitis. AIH patients were the only ones who had nucleolar patterns. Furthermore, AIH patients displayed significantly more homogeneous ANA patterns than the other cohorts (AIH 22 % vs CHD 2.5 %, $p < 0.01$; AIH 22 % vs CHB 4 %, $p < 0.01$). Patients with viral hepatitis demonstrated significantly more often unspecific ANA patterns than patients with AIH (AIH 15 % vs CHD 37.5 %, $p < 0.05$; AIH 15 % vs. CHB 55 %, $p < 0.01$)

	AIH (n=46)	CHD (n=40)	CHB (n=69)
Nucleolar	4 (9 %)	0	0
Fine speckled	23 (50 %)	23 (57.5 %)	27 (39 %)
Coarse speckled	0	0	1 (1 %)
Homogeneous	10 (22 %)**[1,**3]	1 (2.5 %)**[1]	3 (4 %)**[3]
Other	2 (4 %)	1 (2.5 %)	0
None/Unspecific	7 (15 %)*[1,**3]	15 (37.5 %)*[1]	38 (55 %)**[3]

Table 4.5 ANA patterns in all cohorts. Significance was tested with the Chi-square test.
*[1]= CHD compared to AIH, [2]= CHD compared to CHB, [3]= CHB compared to AIH. * p < 0.05, **p < 0.01*

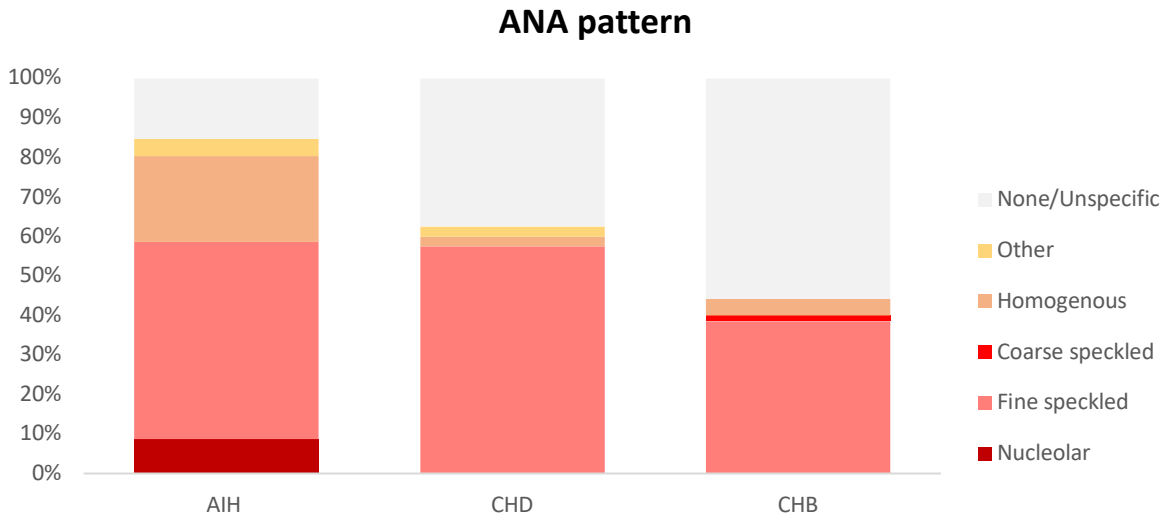

Figure 4.6 ANA patterns in all cohorts, presented in a stocked bar chart

4.5 Levels of SMA titers in all cohorts

In the following section, the level of SMA titers in the three different cohorts are demonstrated. Details are described in Table 4.6 and Figure 4.7.

In general, positive SMA titers (>1:80) were most frequently observed in patients with AIH (CHD 16 % vs CHB 3 % vs AIH 50 %). Moreover, 91 % of the patients with positive SMA titers in the AIH cohort (21 out of 23) had a high SMA titer of ≥ 1:320. Patients with CHD infection tended to have more frequent positive SMA titer than patients with CHB infection, but not at a statistically significant level (CHD 16 % vs CHB 3 %, p=0.055).

	AIH (n=46)	CHD (n=39)	CHB (n=69)
SMA <1:40	17 (37 %)**[3]	22 (56 %)**[2]	56 (81 %)**[2],**[3]
SMA 1:40	3 (4 %)	4 (10 %)	3 (4 %)
SMA 1:80	3 (7 %)	7 (18 %)	8 (12 %)
SMA 1:160	2 (4 %)	3 (8 %)	2 (3 %)
SMA 1:320	5 (10 %)	2 (5 %)	0
SMA 1:640	6 (13 %)	1 (3 %)	0
SMA 1:1280	6 (13 %)	0	0
SMA 1:2560	3 (7 %)	0	0
SMA 1:5120	1 (2 %)	0	0

Table 4.6: Levels of SMA titers in all cohorts. Significance was tested with the Chi-square test.
*[1]= CHD compared to AIH, [2]= CHD compared to CHB, [3]= CHB compared to AIH. * p < 0.05, **p < 0.01*

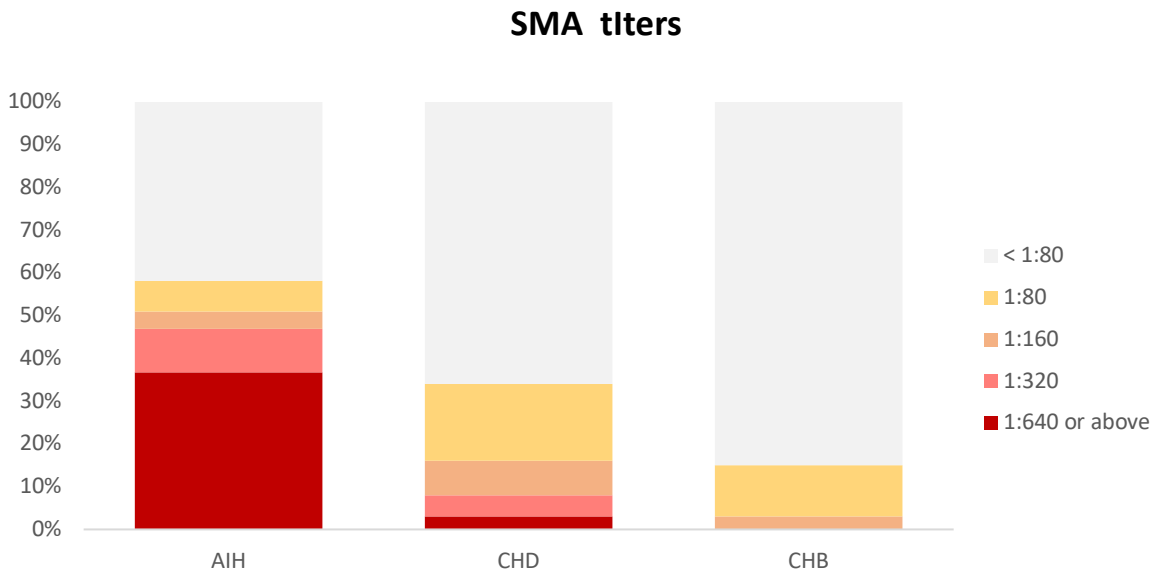

Figure 4.7 SMA titers in all cohorts, presented in a stacked bar chart

4.6 IgG levels in all cohorts

IgG levels of all cohorts are described in Table 4.7.

	AIH (n=44)	CHD (n=37)	CHB (n=61)
IgG (g/l)	19.5 (15.5-27.4)	16.9 (12.3-22.4)	12.7 (10.2-14.3)
Elevated IgG level (> 16 g/l)	32 (73%)*[1],**[3]	20 (54 %)*[1],**[2]	8 (12 %)**[2],**[3]

Table 4.7: IgG levels in all cohorts. *Values are medians with IQR. Significance was tested with an unpaired t-test.*
*[1]= CHD compared to AIH, [2]= CHD compared to CHB, [3]= CHB compared to AIH. * p < 0.05, **p < 0.01*

In the AIH cohort, IgG levels were measured in 44 out of 46 patients with a median IgG level of 19.5 g/l (15.5-27.4), and in 32 of 44 patients (73%) IgG levels were elevated.
In the CHD cohort, IgG levels were measured in 37 out of 40 patients with a median level of 16.9 g/l (12.3-22.4). Elevated IgG levels were found in 20 patients (54%).
In the CHB cohort IgG levels were determined in 61 out of 70 patients. The median IgG level was 12.7 g/l (10.2-14.3). Eight patients (12%) had elevated IgG levels.
Patients with CHD infection had statistically higher IgG levels as compared to patients with CHB infection ($p < 0.01$), but lower IgG levels than patients with AIH ($p < 0.05$) (see Figure 4.8).

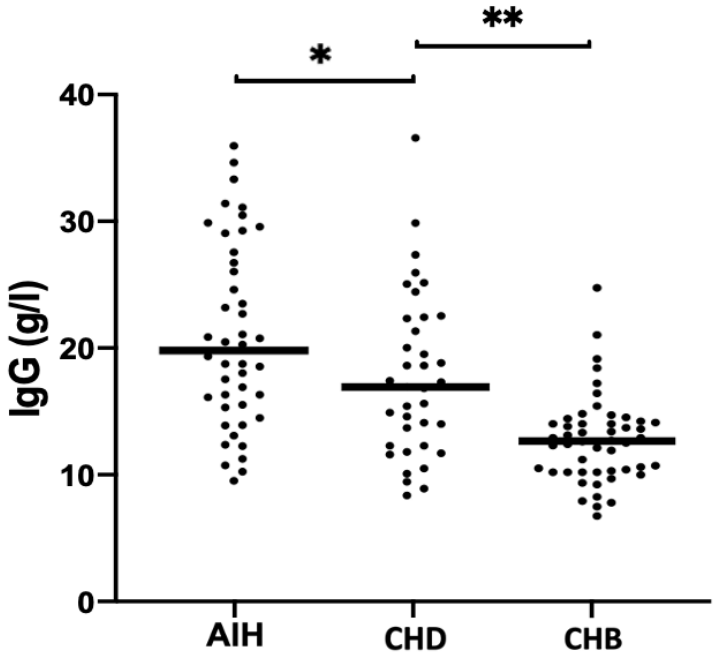

Figure 4.8: IgG levels in all cohorts. Significance was tested with unpaired t-test: p < 0.05, ** p < 0.01*

4.7 Correlation of laboratory parameters with ANA and SMA titer in all cohorts

In Table 4.8 Spearman´s rho correlation coefficients of ANA and SMA titers with laboratory parameters are described.

In patients with CHD was a weak significant correlation between ANA titers and AST as well as a weak significant correlation between ANA titers and IgG levels.

In patients with AIH, a weak correlation between SMA titers and IgG levels could be observed.

Parameter	**Group**		
ANA titers with	**AIH (n=46)**	**CHD (n=39)**	**CHB (n=70)**
ALT	-0.144	0.118	-0.122
AST	-0.156	0.361*	-0.096
IgG level	0.071 (n=44)	0.419 (n=36)*	-0.087 (n=61)
HBV viral load	-	-0.107	0.079
HDV viral load	-	-0.131	-
Age	-0.093	-0.032	0.285
SMA titers with			
ALT	0.187	0.133	-0.041
AST	0.183	0.182	0.063
IgG level	0.342 (n=44)*	0.098 (n=36)	-0.067 (n=61)
HBV viral load	-	-0.183	0.100
HDV viral load	-	0.165	-
Age	-0.202	-0.188	-0.085

Table 4.8: Spearman correlation analysis of ANA titers and SMA titers with laboratory parameters in all cohorts. * $p < 0.05$, ** $p < 0.01$

4.8 Correlation of laboratory parameters with IgG levels in all cohorts

In Table 4.9 Spearman´s rho correlation coefficients of laboratory parameters with IgG are described.

In patients with AIH, a weak significant correlation between IgG level and AST could be detected.

Patients with CHD infection showed a moderate correlation between IgG levels and transaminases (ALT and AST). Furthermore, in CHD patients a correlation between IgG levels and HDV viral load could be detected, while there was no correlation between IgG levels and HBV viral load.

Parameter			
IgG level with	**AIH (n=44)**	**CHD (n=36)**	**CHB (n=61)**
ALT	0.217	0.510**	0.064
AST	0.330*	0.771**	-0.022
HBV viral load	-	-0.158	0.160
HDV viral load	-	0.389*	-
Age	-0.029	-0.209	-0.160

Table 4.9: Spearman correlation analysis of IgG with laboratory parameters in all cohorts. * $p < 0.05$, ** $p < 0.01$

4.9 Hepatitis D subgroup analysis

As mentioned above, 19 CHD patients (48 %) had a hepatitis D viral load below the lower detection limit in the PCR test.

IgG levels

IgG levels in patients with detectable HDV RNA (PCR+), undetectable HDV RNA (PCR-), and CHB mono-infection are described in Table 4.10. Patients with detectable HDV RNA had significantly higher IgG levels than patients with undetectable HDV RNA (p >0.01). Patients with undetectable HDV RNA had similar IgG levels compared to patients with CHB.

	HDV PCR + (n=20)	HDV PCR – (n=19)	CHB (n=61)
IgG (U/l)	20.7 (17.3-25.1)**[1,**2]	13.7 (11.7-15.5)**[1]	12.7 (10.2-14.3)**[2]

Table 4.10: IgG levels in CHD patients PCR+, HDV PCR- and CHB. Values are medians with interquartile range (IQR). Significance was tested with the Mann- Whitney- U test. [1]= HDV PCR+ compared to HDV PCR-, [2]= HDV PCR+ compared to CHB, [3]= HDV PCR- compared to CHB. * p > 0.05, ** p > 0.01

ANA titers

Table 4.11 and Table 4.12 show the frequency of positive ANA titers at different levels in patients with detectable HDV RNA (PCR+) and undetectable HDV RNA (PCR-). Patients with detectable HDV RNA did not show more frequent ANA titers > 1:80 as compared to patients with undetectable HDV RNA. Furthermore, patients with detectable HDV RNA showed similar frequent high ANA titers of ≥ 1:320 as patients with undetectable HDV RNA and patients with CHB infection.

	HDV PCR + (n=20)	HDV PCR – (n=19)	CHB (n=69)
ANA titer ≤ 1:80	7 (35 %)	6 (32 %)	39 (57 %)
ANA titer > 1:80	13 (65 %)	13 (68 %)	30 (43 %)

Table 4.11: Frequency of positive ANA titers in CHD patients PCR+, HDV PCR- and CHB. *Significance was tested with the Chi-square test. [1]= HDV PCR+ compared to HDV PCR-, [2]= HDV PCR+ compared to CHB, [3]= HDV PCR- compared to CHB. * p > 0.05 ** p > 0.01*

	HDV PCR + (n=20)	HDV PCR – (n=19)	CHB (n=69)
ANA titer < 1:320	13 (65 %)	15 (79 %)	55 (80 %)
ANA titer ≥ 1:320	7 (35 %)	4 (21 %)	14 (20 %)

Table 4.12: Frequency of ANA titers ≥1:320 in CHD patients PCR+, HDV PCR- and HBV. *Significance was tested with the Chi-square test. [1]= HDV PCR+ compared to HDV PCR-, [2]= HDV PCR+ compared to CHB, [3]= HDV PCR- compared to CHB. * p > 0.05 ** p > 0.01*

SMA titers

Table 4.13 and Table 4.14 show the frequency of positive SMA titers at different levels in patients with detectable HDV RNA (PCR+), undetectable HDV RNA (PCR-), and patients with CHB mono-infection. Patients with detectable HDV RNA (PCR+) tended to have more frequent SMA titers >1:80 and high SMA titers ≥1:320 as compared to patients with undetectable HDV RNA (PCR-), but not at a statistically significant level (>1:80: p=0.088; ≥1:320: p=0.079).

	HDV PCR + (n=20)	**HDV PCR – (n=19)**	**CHB (n=69)**
SMA titer ≤ 1:80	15 (75 %)	18 (95 %)	67 (97 %)
SMA titer >1:80	5 (25 %)	1 (5 %)	2 (3 %)

Table 4.13: Frequency of positive SMA titers in CHD patients PCR+, HDV PCR- and CHB. *Significance was tested with the Chi-square test. [1]= HDV PCR+ compared to HDV PCR-, [2]= HDV PCR+ compared to CHB, [3]= HDV PCR- compared to CHB. * p > 0.05 ** p > 0.01*

	HDV PCR + (n=20)	**HDV PCR – (n=19)**	**CHB (n=69)**
SMA titer < 1:320	17 (85 %)	19 (100 %)	69 (100 %)
SMA titer ≥ 1:320	3 (15 %)	0 (0 %)	0 (0%)

*Table 4.14: Frequency of SMA titers ≥1:320 in CHD patients PCR+, HDV PCR- and CHB. Significance was tested with the Chi-square test. [1]= HDV PCR+ compared to HDV PCR-, [2]= HDV PCR+ compared to CHB, [3]= HDV PCR- compared to CHB. * p > 0.05 ** p > 0.01*

Transaminases

As shown in Table 4.15 patients with detectable hepatitis D virus (PCR+) had significantly higher transaminase levels than patients with undetectable hepatitis D virus (PCR-). However, patients with undetectable HDV RNA had similar transaminase levels as patients with CHB.

	HDV PCR + (n=21)	**HDV PCR – (n=19)**	**CHB (n=70)**
AST (U/l)	64 (44-99)**[1],**[2]	26 (20-34)**[1]	22 (18-31)**[2]
ALT (U/l)	95 (62-138)**[1],**[2]	32 (20-48)**[1]	29 (19-47)**[2]

Table 4.15: Transaminase levels in CHD patients PCR+, HDV PCR- and CHB. Values are medians with interquartile range (IQR). Significance was tested with the Mann- Whitney- U test.
[1]= HDV PCR+ compared to HDV PCR-, [2]= HDV PCR+ compared to CHB, [3]= HDV PCR- compared to CHB. p > 0.05, ** p > 0.01*

5 Discussion

Infections caused by hepatotropic viruses are known to be linked with various immunopathological manifestations. There is a well-established association between infection and autoimmunity, especially in chronic cases involving hepatitis B and hepatitis C viruses (69). However, there is limited data available on the frequency and pattern of NOSA concerning CHD and its relation to disease or treatment status (77, 89, 90).
In individuals with chronic HDV infection, there is an accumulation of perforin-positive cytotoxic CD4+ T cells, a factor implicated in exacerbating the severity of HDV-related liver infection (91-93). Furthermore, HDV infection has been shown to enhance the antiviral state of hepatocytes, leading to increased chemokine production and antigen presentation (94, 95). While liver damage resulting from chronic HDV infection is primarily considered to be mediated by the immune system (51, 92), the role of autoantibodies in this context remains poorly understood. (90)

This current study, published in 2023 (90), conducted a cross-sectional analysis of available NOSA titers and IgG levels in 42 patients diagnosed with CHD. A control group of 116 individuals was included, comprising 70 patients with CHB and 46 patients diagnosed with AIH. By comparing patients with CHD to those with HBV mono-infection, differences in autoimmune phenomena, represented by autoantibody titers and IgG levels, were linked to the additional presence of HDV infection.

However, the study has some limitations, including its retrospective design, the lack of multicenter validation, and a relatively small sample size. Furthermore, the three cohorts were not adequately matched for age, sex, or the extent of liver damage.

The present analysis focused on a specific set of autoantibodies typically assessed in the routine examination of elevated liver enzymes, predominantly aligned with the autoantibody profile of AIH, PSC, and PBC. (90) Additionally, the study predominantly included patients with HDV genotype 1 infection, which is the most prevalent genotype in Europe (96). However, recent research has highlighted variations in spreading kinetics, treatment outcomes, and disease courses among different HDV genotypes (97, 98). Therefore, it was of interest to investigate whether the autoantibody profile differs across these different HDV genotypes (90).

The existing data regarding the frequency of autoantibodies in patients with CHD is mainly historical, marked by diverse methodologies, limited standardization, and the absence of IFN α treatment. In our present study, patients with CHD exhibited a notably higher propensity to display positive/high NOSA titers compared to patients with CHB (CHD 69% vs. CHB 43%; $p < 0.01$), but less likely than patients with AIH (CHD 69% vs. AIH 96%; $p < 0.01$) (90). The prevalence of positive NOSA titers in patients with AIH observed in our study corresponds to the findings in the existing literature (79). McFarlane et al. reported in 1995 that approximately 20% of CHD patients ($n = 27$) exhibited positive NOSA titers, a prevalence similar to that observed in patients with HBV mono-infection (77). In a study involving 325 Chinese patients diagnosed with CHB, 58.2% demonstrated positive NOSA titers at the same cut-off value employed in our analysis, significantly higher than healthy controls (6.7%) (75). Following our results, ANAs were one of the most predominant NOSA in CHB patients from the Chinese cohort (75). However, it is imperative to note that the database for ANAs in CHD is limited. Diverging from our results, Zauli et al. in 1986 observed a markedly lower prevalence of ANAs in patients with CHD compared to CHB patients (at 9 %) (99).

Notably, patients with AIH and viral hepatitis exhibited significant differences in their ANA patterns. AIH patients displayed significantly more homogeneous ANA patterns than the other cohorts. Furthermore, patients with viral hepatitis demonstrated significantly more often unspecific ANA patterns than patients with AIH (90).

Patients diagnosed with AIH were notably more likely to have positive SMA titers, particularly at high levels (≥ 1:320), in comparison to patients with CHD or CHB. Nevertheless, 8% of patients with CHD also exhibited SMA titers, a phenomenon absent in CHB patients. While patients with HBV predominantly displayed isolated elevated ANA titers, patients with HDV and AIH exhibited combined elevated ANA and SMA titers (90). In a previous study, the prevalence of SMA in CHD did not significantly differ from that observed in patients with CHB mono-infection (99). In the current study, SMA titers correlated with liver stiffness values specifically in CHD patients, while such correlation was not evident in the other cohorts (90). Further extensive studies are needed to validate whether elevated SMA titers are indeed linked to the activity or prognosis of HDV liver disease.

SLA is regarded as the most specific marker for AIH among all AIH-related antibodies. The association between the detection of SLA and the severity of AIH remains controversial (1, 2).

In rare cases, HCV patients with positive LKM-1 antibodies progress to LKM-1 positive autoimmune hepatitis (100). In the present study, no patient exhibited LKM-1 antibodies. While the presence of LKM-1 is a characteristic feature of AIH-2, it´s crucial to highlight that only patients diagnosed with AIH-1 were included in the current study (90). In this study, some CHD patients displayed elevated mHAI scores. The inquiry into whether HDV infection, similar to what has been described in chronic HCV infection, can indeed act as a trigger for AIH remains incompletely elucidated.

Elevated serum IgG levels are recognized as a distinctive feature of AIH, identified in up to 85% of AIH patients, and constitute a component of the diagnostic scoring system for AIH (103). In our study, elevated IgG levels were observed in 73% of AIH patients, 12% of CHB patients, and 54% of CHD patients (90). A prior study by Hartl et al. suggested a correlation between ANA and SMA titers and IgG levels in AIH patients. However, IgG levels were not found to be associated with the extent of liver fibrosis or intrahepatic inflammatory activity (103). In our present study, we identified a significant correlation between IgG and ANA titers in CHD patients, as well as a correlation between SMA and IgG in AIH patients. Moreover, a significant correlation emerged between IgG levels and transaminases, suggesting an association between IgG levels and intrahepatic inflammatory activity in both CHD and AIH patients. Furthermore, within the CHD cohort, elevated IgG levels and HDV viral loads correlated with higher liver stiffness values, and patients with HDV viremia demonstrated significantly higher IgG levels than those without detectable viremia. (90)
Interestingly, in a recently reported case of CHD exhibiting clinical and histological features resembling AIH, the administration of the HBV/HDV entry inhibitor bulevirtide resulted in a rapid normalization of immunoglobulin levels (104). Subsequent studies are essential to assess whether such a therapeutic approach could substantially decrease immunoglobulin levels. The existing literature lacks evidence supporting the utilization of immunomodulating agents, such as steroids, for the treatment of CHD patients with elevated NOSA titers (105, 106).

High NOSA titers may function as potential predictors of autoimmune disease development, persisting even after achieving sustained HDV control (107). Interestingly, Arbuckle et al. found that ANAs with a dilution titer of 1:120 were present in 78% of lupus erythematosus patients examined 3–9 years before clinical manifestation (108). In the CHD cohort, one patient presented with cryoglobulins and received a diagnosis of membranoproliferative glomerulonephritis (90). Prospective studies are crucial to investigating the presence of elevated

autoantibody titers in HDV cohorts, assessing their significance as early predictors of autoimmune disease or as isolated para-infectious phenomena. Furthermore, it is essential to examine the prevalence of HDV infection in other autoimmune diseases. Subsequent research should examine the prevalence of additional clinical symptoms such as arthralgias, sicca symptoms, and autoantibodies typical of other autoimmune diseases, including anti-SSA and anti-SSB in Sjögren's syndrome, as well as cryoglobulins, which have been recurrently found in patients with chronic hepatitis B, C, and E infection (88).

Various mechanisms are believed to contribute to the immunopathology observed in chronic viral infections, including molecular mimicry, impairment of regulatory T-cell activity, and polyclonal activation of B lymphocytes (69, 109). In particular, the polyclonal activation of B cells seems to provide a plausible explanation for the observed correlation between IgG levels and HDV disease activity and severity. The persistence of elevated NOSA titers in non-viremic HDV patients may be ascribed to the development of enduring dysfunctional atypical memory B cells (109, 110). Nevertheless, a more precise understanding of the role of B cells in CHD requires further elucidation through future studies.

In summary, autoantibodies are often identified as a para-infectious feature in CHD patients, yet their clinical significance remains unclear.

6 Conclusion

Our study shows that CHD patients are significantly more likely to have positive NOSA titers and elevated IgG levels than patients with HBV, but significantly less likely than patients with AIH. However, given the substantial presence of autoantibodies and elevated IgG levels detected in CHD patients, our findings reemphasize the importance of completely ruling out the diagnosis of hepatitis virus infection before initiating immunosuppressive treatment for suspected AIH.

Little is known about the clinical relevance of elevated autoantibodies in CHD patients, but it is of great interest whether they are an early predictor of autoimmune disease or rather a singular parainfectious phenomenon. To our knowledge, there are no reports on the development of NOSA titers during CHD. In our study, we investigated that CHD patients with detectable hepatitis D virus replication showed no difference in the level of their ANA/SMA titers as compared to patients without detectable virus replication. Therefore, it can be concluded that the elevated NOSA titers persist even after virus replication has ended in CHD patients. With current therapeutic options, hepatitis D virus infection is expected to persist in hepatocytes, but perhaps this will change with future therapies.

7 Zusammenfassung

Unsere Untersuchungen zeigen, dass Patient*innen mit CHD signifikant häufiger positive NOSA-Titer und erhöhte IgG-Spiegel aufweisen als Patient*innen mit CHB, aber signifikant seltener als Patient*innen mit AIH. Angesichts der erheblichen Präsenz von Autoantikörpern und erhöhten IgG-Spiegel bei Patient*innen mit CHD unterstreichen unsre Ergebnisse erneut, wie wichtig es ist, eine Virushepatitis-Infektion auszuschließen, bevor eine immunsuppressive Behandlung bei Verdacht auf AIH eingeleitet wird.

Über die klinische Relevanz erhöhter Autoantikörper bei CHD-Patient*innen ist wenig bekannt. Es ist von großem Interesse zu eruieren, ob sie ein frühzeitiger Prädiktor für eine Autoimmunerkrankung oder nur ein rein parainfektiöses Phänomen sind. Unseres Wissens nach gibt es keine Berichte über die Entwicklung von Autoantikörper-Titern im Verlauf einer HDV Infektion. Wir konnten jedoch in unserer Untersuchung zeigen, dass CHD-Patient*innen mit nachweisbarer Hepatitis-D-Virusreplikation keinen Unterschied in der Höhe ihrer ANA/SMA - Titer im Vergleich zu Patient*innen ohne nachweisbare Virusreplikation aufwiesen. Daraus lässt sich schließen, dass die erhöhten SMA/ANA-Titer bei CHD-Patient*innen auch nach sistierter Virusreplikation bestehen bleiben. Allerdings, ist davon auszugehen, dass unter den aktuell zur Verfügung stehenden Therapieoptionen Hepatitis-D-Viren in den Hepatozyten persistieren. Vielleicht wird sich dies jedoch mit zukünftigen Therapieoptionen ändern.

8 Appendix

Tables

Figures

9 References

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11 Lebenslauf

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12 Eidesstattliche Erklärung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

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Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....