From the Department of Medicine, Huddinge
Karolinska Institutet, Stockholm, Sweden

# RISK OF HEPATOCELLULAR CARCINOMA, LIVER-RELATED COMPLICATIONS, AND DEATH IN PERSONS LIVING WITH CHRONIC HEPATITIS B AND D

Habiba Kamal, MD
حبيبة كمال


Stockholm 2023

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2023

ISBN 978-91-8017-041-3

# Risk of hepatocellular carcinoma, liver-related complications, and death in persons living with chronic hepatitis B and D

Thesis for Doctoral Degree (Ph.D.)

By

**Habiba Kamal, MD, MSc**

The thesis will be defended in public at B64 Lecture Hall Karolinska University Hospital Huddinge, Stockholm, Sweden, 2023-08-25, 9:00 AM

***Principal Supervisor:***
Adjunct Professor
Soo Aleman, MD, PhD
Karolinska Institutet
Department of Medicine, Huddinge
Division of Infectious Diseases

***Co-supervisor(s):***
Associate Professor
Ann-Sofi Duberg, MD, PhD
Örebro University
Department of Medical Science
Division of Infectious Diseases

Adjunct Professor
Per Stål, MD, PhD
Karolinska Institutet
Department of Medicine, Huddinge
Division of Hepatology

Professor
Heiner Wedemeyer, MD, PhD
Department of Gastroenterology, Hepatology and Endocrinology
Hanover Medical School, Germany

***Opponent:***
Professor
Nina Weiss, MD, PhD
University of Copenhagen
Department of Clinical Medicine
Division of Infectious Diseases

***Examination Board:***
Professor
Lise-lotte Gluud, MD, PhD
University of Copenhagen
Department of Internal Medicine
Division of Hepatology

Associate Professor
Anders Ternhag, MD, PhD
Karolinska Institutet
Department of Medicine, Solna
Division of Infectious Diseases

Associate Professor
Johan Ursing, MD, PhD
Karolinska Institutet
Department of Medicine, Solna
Division of Infectious Diseases

*To heaven's angel, my soul mate and backbone, my beloved sister*

*"Al Shaymaa Kamal"*

*And to the loving memories of Kamal Tolba El-Sayed, Edward Fikry Asheyya, Ebrahim Ahmed Saleh, and Nadia Ahmed Saleh.*

**أهدى هذه الأطروحة العلمية**

**لعروس الجنة الملاك توأم روحى و سندى أختى**

**الشيماء كمال**

**لروحها الطاهرة**

**و للذكرى العطرة**

**لكمال طلبة السيد، إدوارد فكرى إشعيا، إبراهيم أحمد صالح و نادية أحمد صالح**

"...but I have to tell you this: this whole thing is not about heroism. It's about decency. It may seem a ridiculous idea, but the only way to fight the plague is with decency."

The Plague; by Albert Camus (1913-1960)
First published in 1947 in France as *La Peste*.

# Table of Contents

# Popular science summary of the thesis

Invasion of the liver by viruses causes inflammation (hepatitis) as the body's immune system reacts to contain the infection. The most common chronic viral hepatitis are hepatitis B and C affecting millions of people worldwide, causing ill health, loss of productivity, and complications related to scarring (cirrhosis) of the liver. In this thesis, we studied hepatitis Delta virus (HDV), which with hepatitis B virus (HBV) *co-infects* a person without prior HBV infection or *superinfects* a person living with a prior HBV infection. The insufficient awareness of HDV, the common belief that it is a vanishing disease, and the non-standardization of tests led to the underscreening of this virus and underestimated the number of persons living with this disease. In Study I, we showed that persons with HDV replication are at a higher chance of developing cirrhosis, liver-related complications, and need for liver transplantation than those who cleared the virus. A tendency towards more benign disease course in those who responded to interferon (IFN) therapy than those who did not respond. In Study II, we showed that the risk of developing liver cancer in persons with HDV is twice as much as the risk in persons living with chronic hepatitis B (CHB) only, with a higher risk in persons living with human immunodeficiency virus (HIV) and concomitant HBV and HDV infection. In Study III, we demonstrated the high frequency of HDV screening reaching 90% of persons with HBV at Karolinska University Hospital, this could be attributed to standardized clinical routine of managing persons with HBV infection through an initial meeting with specialized nurse in viral hepatitis. We noted also that persons living with human immunodeficiency virus (HIV) had a lower chance of getting screened for HDV. Delayed diagnosis of persons with HDV replication might be a possible factor for worse disease course. In Study IV, we evaluated the risk of developing liver cancer in a nationwide population of African-born Swedish residents with CHB and found that men with concomitant HCV or HDV infection surpass this threshold at ages 20-40 years, younger than the suggested age to conduct screening for liver cancer in some guidelines. We highlighted the need for studies examining the risk of liver cancer according to individual risk factors for more personalized and cost-effective care.

# Swedish summary

Invasion av levern av virus orsakar inflammation (hepatit) eftersom kroppens immunsystem reagerar för att begränsa infektionen. Den vanligaste kroniska virala hepatiten är hepatit B och C som drabbar miljontals människor över hela världen, vilket orsakar ohälsa, produktivitetsförlust och komplikationer relaterade till ärrbildning (cirros) i levern. I denna avhandling studerade vi hepatit Delta-virus (HDV), som bara infekterar samtidigt eller en tidigare person som lever med hepatit B. Fortsatt HDV i kroppen orsakar den mest sällsynta typen av viral hepatit; kronisk hepatit Delta (KHD). Personer som lever med KHD har en snabbare takt mot cirros och möjligen ökad risk för levercancer jämfört med personer med endast kronisk hepatit B (KHB). Den otillräckliga medvetenheten om HDV, den vanliga uppfattningen att det är en försvinnande sjukdom och icke-standardiseringen av tester ledde till ”underscreening” av detta virus och underskattade antalet personer som lever med denna sjukdom. Personer som replikerar HDV har en högre chans att utveckla cirros, leverrelaterade komplikationer och behov av levertransplantation än de som rensade viruset. Risken för att utveckla levercancer hos personer med HDV är dubbelt så stort som risken för personer som lever med endast kronisk hepatit B (KHB), med högre risk hos personer som lever med humant immunbristvirus (HIV) och samtidig HBV- och HDV-infektion. Levercirros är en grogrund för cancerutveckling, men vissa personer med KHB kan utveckla cancer utan skrumplever. Kostnadseffektivitetsanalys för att övervaka personer med KHB utan cirros identifierade en tröskel på 2 per 1000 personer per år för att genomföra halvårsvis ultraljudsundersökning. Ändå är åldern för att påbörja levercancerövervakning hos personer av afrikansk härkomst olika över riktlinjerna på grund av få studier som undersöker denna långsiktiga risk i denna population. Vi utvärderade denna risk i en rikstäckande population av afrikanskfödda svenska invånare med KHB och fann att män med samtidig HCV- eller HDV-infektion överträffar denna tröskel vid 20–40 år, yngre än den föreslagna åldern i vissa riktlinjer. Vi lyfte fram behovet av studier som undersöker risken för levercancer enligt individuella riskfaktorer för mer personlig och kostnadseffektiv vård.

# Arabic summary

## ملخص عربي

تتسبب إلتهابات الكبد الفيروسية المزمنة "ب" و "ج" و "دلتا" فى إعتلال الصحة وفقدان الإنتاجية لدى ملايين الأشخاص في أنحاء العالم ،لا سيما المضاعفات المرتبطة بتليف الكبد.

فى هذه الأطروحة المكونة من أربع ورقات بحثية، قمنا بدراسة التاريخ المرضى لإلتهاب الكبد الفيروسى دلتا (HDV) بالأبحاث (١،٢،٣). حيث يحتاج فيروس "دلتا" فيروس "ب" لحدوث العدوى متسببا فى أندروأشرس أنواع إلتهابات الكبد الفيروسية المزمنة. حيث تتسارع وتيرة تليف الكبد لدى المصابين بفيروس"دلتا" وربما يتزايد خطرالإصابة بسرطان الكبد مقارنة بالأشخاص المصابين فقط بالتهاب الكبد "ب" المزمن.

تتضارب الإحصاءات التى تقدرعدد المصابين بفيروس دلتا حول العالم، متراوحة بين ١٢ إلى٦٠ مليون نسمة.

أدى عدم كفاية الوعي بهذا الفيروس، والإعتقاد السائد بأنه مرض نادر الحدوث لا سيما عدم حساسية الفحوصات، إلى قلة فحص و تشخيص هذا الفيروس وعدم دقة حصرعدد الأشخاص الذين يعيشون بهذا المرض. إرتبطت بعض العوامل مثل نشاط الفيروس، سن المريض ودرجة تليف الكبد بزيادة مخاطر المضاعفات المصاحبة لأمراض الكبد والحاجة إلى زراعة الكبد. كما يرتفع خطر الإصابة بسرطان الكبد إلى الضعف لدى الأشخاص المصابين بفيروس دلتا مقارنة بقرناءهم المصابين فقط بالتهاب الكبد "ب"، مع وجود مخاطر أعلى لدى الأشخاص المصابين بفيروس نقص المناعة البشرية .

تعد أفريقيا وآسيا أكثر القارات المتوطنة بفيروس الكبد "ب". كما أشارت العديد من الدراسات إلى حدوث سرطان الكبد فى الفئات الأصغرعمرا لا سيما حدوث الأورام السرطانية مع عدم وجود تليف للكبد لدى الأشخاص المنحدرين من أصل أفريقي.

و لذلك تختلف الإرشادات الطبية للوقاية الثانوية بسبب قلة الدراسات طويلة الأمد التي تفحص هذا الخطر في هذه المجموعة من االمرضى. قمنا بتقييم خطر الإصابة بسرطان الكبد لدى عدد من السكان السويديين المولودين في أفريقيا والمصابين بـفيروس "ب" ووجدنا أن الرجال المصابين بعدوى فيروس "ج" أو "د" ترتفع لديهم الإصابة بسرطان الكبد بداية من عمر20-40 سنة ، و هى فئة عمرية قد تتخطاها توجيهات الفحص الدورى لسرطان الكبد المقترحة في بعض الإرشادات.

من خلال هذه الأطروحة سلطنا الضوء على الحاجة إلى مزيد من الدراسات الحديثة التي تقيم مخاطر الإصابة بسرطان الكبد وفقًا لعوامل المخاطر الفردية للحصول على رعاية أكثر تخصيصًا وفاعلية من حيث النفع والتكلفة.

# Abstract

Habiba Kamal (2023): Risk of hepatocellular carcinoma, liver-related complications, and death in persons living with hepatitis B and D viruses. Stockholm, 2023.

Chronic hepatitis B virus (HBV) infection affects 257 million individuals and is a leading cause of liver-related morbidity and mortality. Hepatitis D virus (HDV) is a satellite virus, that needs HBV for packing and propagation, hence infecting only individuals with HBV infection. It is estimated that around 9-19 million individuals are living with chronic hepatitis Delta (CHD), hence it is the least common among viral hepatides. CHD demonstrates a severe course of liver disease than CHB. The treatment options are still limited, and approved therapies are at a high price. This thesis aims to characterize the natural course of HBV and HDV infection in a low-endemic setting, predictors of disease progression, and the effect of therapy on the disease course.

In **Study I,** we identified 337 patients with positive anti-HDV antibody from 11 infectious disease clinics in Sweden assembling a nationwide cohort. During a mean follow-up of 6.5 years, HDV RNA replication was significantly associated with a composite outcome of any liver-related decompensation, HCC, and liver transplantation. The response to IFN therapy was suboptimal; 18.8% had a virological response defined as negative or more than 2 log decline of HDV RNA level and a more benign disease course was seen in virological responders compared to non-responders. HDV RNA replication was independently associated with liver decompensation events, undergoing liver transplantation, and a trend toward higher HCC risk.

In **Study II**, we conducted a systematic review and meta-analysis of published peer-reviewed cohort studies examining the risk of HCC in patients with HBV/HDV infection compared to peers with HBV mono-infection. The pooled relative risk was 2.12 (95% confidence interval CI 1.14-3.95), with a particularly higher risk in patients with HIV/HBV/HDV co-infection and substantial heterogeneity between studies.

In **Study III,** we present the HDV cascade of care during three decades at Karolinska University Hospital (KUH) as a secondary care referral facility in the Southern Stockholm region. In 4095 patients with positive HBsAg, (90.4%, n=3703) have undergone an anti-HDV test. Anti-HDV positive was prevalent in (83.7%, n=310) and (65.2%, n=202) patients who had HDV RNA replication. Older age, cirrhosis, and getting a late anti-HDV diagnosis were independently associated with any prevalent liver outcome. Despite the high screening rate reaching 95%, 8% of persons meeting the criteria of the American Association for the Study of the Liver (AASLD) as "high-risk" of infection did not receive any screening test and 28% of persons with cirrhosis received a remote screening test (after two years). Persons with concurrent HIV and HBV infection were less likely to receive a screening test.

In **Study IV**, we analyze a nationwide cohort of African-born Swedish residents with CHB without cirrhosis (n=3865), followed for a mean of 12 years from the date of HBV diagnosis in Sweden to the incidence of HCC. The cohort was compared to individuals without HBV

in 1 to ≤3 on age, sex, and county of residence to persons from the same area of birth (n=8,488) and in 1 to ≤ 10 on age, sex, and county of residence with a cohort from the general population (n=39,278). African-born men with CHB were significantly younger at HCC development compared to women and peers from comparator cohorts. The cost-effectiveness surveillance threshold at 0.2% was exceeded at age 54 years (IR=0.20/100PYs, 95%CI 0.10-0.40) in men and at age 59 years (IR=0.21/100 PYs, 95%CI 0.10-0.45) in women, while at 20-40 years in the presence of concomitant HDV and HCV co-infection in men. The probability of HCC was more pronounced at younger ages in men compared to women. African-born men with CHB had 10.6 times higher risk to develop HCC compared to African-born peers without HBV and a 35.3 times higher risk than the general population. The study provides absolute and relative estimates of HCC development in a nationwide large cohort of African-born first-generation persons with CHB, without cirrhosis at baseline living in a different environmental setting.

To conclude, HDV RNA replication, older age, and cirrhosis in patients with anti-HDV positive are independent predictors of progressive liver disease and need for liver transplantation. Lack of response to IFN therapy might be associated with a worse disease outcome. Based on our pooled analyses, HDV infection is associated with two-times higher risk to develop HCC, compared to HBV mono-infection with a higher risk in persons with triple HIV/HBV/HDV infection. Nine out of 10 patients with CHB received an anti-HDV test at KUH. Delayed HDV diagnosis was independently associated together with older age and cirrhosis with a liver-related outcome. Liver cirrhosis is a fertile ground for cancer development, but some people with CHB can develop cancer without cirrhosis. Cost-effectiveness analysis to surveil persons with CHB without cirrhosis identified a threshold of 2 per 1000 persons per year to conduct a semi-annual ultrasound examination. Nevertheless, the age to start liver cancer surveillance, in persons of African origin is different across guidelines due to few studies examining this risk in this population for a long time. It is unclear if the current 0.2% cost-effectiveness threshold for HCC surveillance in persons without cirrhosis might miss a population of younger patients with co-morbidities who are at increased risk to develop HCC. Our research highlights the need for cost-effectiveness studies in contemporary cohorts of persons living with CHB particularly in African-born men given the substantial number of HCCs occurring at younger ages in this population.

# List of scientific papers

The thesis is based on the following original papers:

I. **Kamal H**, Westman G, Falconer K, Duberg AS, Weiland O, Haverinen S, Wejstål R, Carlsson T, Kampmann C, Larsson SB, Björkman P, Nystedt A, Cardell K, Svensson S, Stenmark S, Wedemeyer H, Aleman S.
Long-Term Study of Hepatitis Delta Virus Infection at Secondary Care Centers: The Impact of Viremia on Liver-Related Outcomes. Hepatology. 2020 Oct;72(4):1177-1190.

II. **Kamal H**, Fornes R, Simin J, Stål P, Duberg AS, Brusselaers N, Aleman S.
Risk of hepatocellular carcinoma in hepatitis B and D virus co-infected patients: A systematic review and meta-analysis of longitudinal studies.
J Viral Hepat. 2021 Oct;28(10):1431-1442.

III. **Habiba Kamal**, Karin Lindahl, Michael Ingre, Caroline Gahrton, Kerstin Karkkonen, Piotr Nowak, Jan Vesterbacka, Per Stål, Heiner Wedemeyer, Ann-Sofi Duberg, Soo Aleman
The cascade of care for patients with chronic hepatitis delta in Southern Stockholm, Sweden for the past 30 years
*Submitted*

IV. **Habiba Kamal**, Michael Ingre, Per Stål, Gabriel Westman, Daniel Bruce, Heiner Wedemeyer, Ann-Sofi Duberg and Soo Aleman
Age and sex-specific risks for hepatocellular carcinoma in African-born persons with chronic hepatitis B and without cirrhosis
*Submitted*

# List of abbreviations

| | |
|---|---|
| AASLD | American Association for the Study of Liver Diseases |
| AAR | Aspartate aminotransferase to Alanine aminotransferase ratio |
| ALT | Alanine aminotransferase |
| Anti-HDV | Antibodies to hepatitis D virus |
| APASL | Asia- Pacific Association for the study of the Liver |
| APRI | Aspartate aminotransferase to platelet ratio index |
| AST | Aspartate aminotransferase |
| ATC | Anatomical Therapeutic Chemical Classification System |
| AUROC | Area under the receiver operating characteristic curve |
| BCLC | Barcelona Clinic Liver Cancer |
| BEA-score | Baseline anticipation score |
| cccDNA | Circular covalent closed DNA |
| CHB | Chronic hepatitis B |
| CHD | Chronic hepatitis D |
| CI | Confidence interval |
| D4FS | Delta-4 Fibrosis Score |
| DFS | Delta Fibrosis Score |
| EASL | European Association for the Study of the Liver |
| ELISA | Enzyme-linked immunosorbent assay |
| F0-F4 | Fibrosis stages 0-4 |
| HBsAg | Hepatitis B surface antigen |
| HBV | Hepatitis B virus |
| HBIG | HBV immunoglobulin |
| HCC | Hepatocellular carcinoma |
| HCV | Hepatitis C virus |
| HDV | Hepatitis D virus |
| HR | Hazard ratio |
| ICD | International Classification of Disease and Related Health Problems |
| IFN | Interferon |
| IQR | Interquartile range |
| IR | Incidence rate |
| IRR | Incidence rate ratio |
| KM | Kaplan-Meier |
| LNF | Lonafarnib |
| LSM | Liver stiffness measurement |
| MAFLD | Metabolic-associated fatty liver disease |
| MRI | Magnetic resonance imaging |
| MSM | Men who have sex with men |
| MVR | Maintained virological response |
| NAFLD | Non-alcoholic fatty liver disease |
| NAP | Nucleic acid polymer |
| NASH | Non-alcoholic steatohepatitis |

| | |
|---|---|
| NANB | Non-A non-B hepatitis |
| NIH | National Institute of Health |
| NOS | New Castle Ottawa Scale |
| NPV | Negative predictive value |
| NTCP | Sodium taurocholate co-transporting polypeptide |
| NUC | Nucleos(t)ides analogues |
| OR | Odds ratio |
| PCR | Polymerase chain reaction |
| PLHIV | People living with HIV |
| PPV | Positive predictive value |
| RCT | Randomized controlled trials |
| RIA | Radioimmunoassay |
| RNA | Ribonucleic acid |
| ROS | Oxygen reactive species |
| SCB | Statistics Sweden |
| SD | Standard deviation |
| SIR | Standardized incidence ratio |
| SSA | Sub-Saharan Africa |
| SVR | Sustained virological response |
| TACE | Trans-arterial chemoembolization |
| US | Ultrasound |
| USA | United States of America |
| VEGF | Vascular endothelial growth factor |
| WHO | World Health Organization |

# 1 Introduction

Chronic viral hepatitis B, C, and D constitute a significant public health burden with substantial morbidity and mortality affecting ~350 million persons globally [1]. Chronic viral hepatitis B and C causes 80% of incident hepatocellular carcinoma (HCC) worldwide [2]. Hepatitis B virus (HBV), and hepatitis C virus (HCV) are independent risk factors for the development of cirrhosis and are among the leading causes of end-stage liver disease and the need for liver transplantation [3].

HCC is one of the devastating sequelae of chronic liver disease and is a major cause of death in patients with cirrhosis [4]. In 2020, HCC was the 6$^{th}$ most common cancer in adults and the 3$^{rd}$ to lung and colorectal cancer as a leading cause of cancer-related death [5]. The worldwide incidence of HCC closely follows regions with prevalent chronic infection with HBV and HCV, such as Eastern Asia and Sub-Saharan Africa [4,6]. As the global prevalence of obesity and metabolic syndrome is rising, new emerging risk factors for HCC are increasingly recognized shifting the traditional landscape of HCC prevalence [7]. HBV is a hepatotropic virus affecting 3.5% of the global population (257 million) [1]. The most affected regions are Western Pacific and Sub-Saharan African regions [1]. Chronic HBV infection is defined as persistent hepatitis B surface antigen (HBsAg) in the serum of affected individuals for more than 6 months [8]. The transmission route is through blood and body fluids. Perinatal transmission and early transmission during childhood are common routes in endemic regions. The natural course of HBV infection comprises different clinical phases with low-grade inflammation alternating with episodes of high-grade inflammation leading to fibrosis and cirrhosis in 25-40% of persons living with chronic HBV (CHB) [9].

Hepatitis D virus (HDV) is a hepatotropic virus that thrives on HBV to establish its infection and propagation [10]. It is estimated that 5-10% of chronic HBV carriers are co-infected with HDV, translating into 9-19 million individuals worldwide [11]. HDV infection can occur as either a superinfection of persons with CHB or as a simultaneous infection of both viruses [12]. Although the least common among viral hepatides, HDV poses numerous challenges in the field of viral hepatitis [13]. The prevalence of HDV is debated and possibly underestimated [14]. This is mostly attributed to low awareness of HDV especially in primary care settings and the absence of reflex screening risking missing cases in both endemic and non-endemic regions [15]. The primary reports on the prevalence of HDV usually involved small, selected populations, together with the historical non-standardization of HDV diagnostic tests across laboratories rendering these estimates uncertain [14]. The existing body of evidence points to a faster progression towards advanced liver disease and associated complications in persons living with chronic HDV infection compared to those with HBV mono-infection [16]. Despite the promising new therapies targeting HDV life cycle (some are approved, others are in the pipeline), "Delta" hepatitis still lacks an effective therapy that can significantly change the natural course for the majority of patients; hence it is usually described as the most aggressive in viral hepatides [17–19].

# 2 Literature Review

## 2.1 Discovery of HBV and HDV

HBV was discovered by geneticist Baruch Blumberg in 1965 when he noticed an immunoprecipitant formed from mixing the serum of a patient with haemophilia and an aboriginal individual [20]. Named the "Australia antigen", it was linked to persons living in crowded institutions and persons with abnormal serum aminotransferases. Hence, the link between the antigen and hepatitis was confirmed [20].

HDV was discovered in 1977, in Italy by Rizzetto and colleagues, in a group of patients with HBsAg-positive chronic hepatitis. Liver biopsy from a subset of these patients revealed a new antigen [21]. Further collaboration with the National Institute of Health (NIH) and Georgetown University, in the USA; revealed "Delta agent" as a separate virus from HBV [22].

## 2.2 Molecular structure and replication

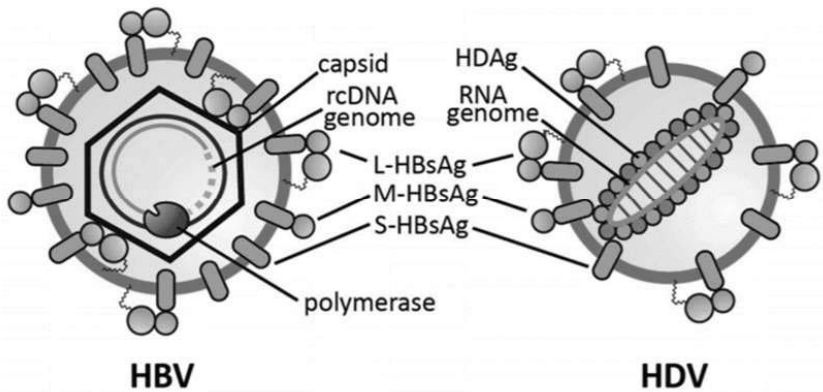


Figure 1. Structure of HBV and HDV virions showing both virions sharing the same envelope proteins. Adapted from Lempp FA. and colleagues [23] under an open access Creative Commons license.

HBV is a partial double-stranded hepatotropic DNA virus that belongs to the Hepadnaviridae family [24,25]. HDV is a satellite virus that does not code for its surface proteins; therefore, needs HBV surface proteins for packing, entry into the hepatocytes, and intrahepatic spread [25,26]. HDV uses HBV surface proteins three forms (small or S-HBsAg, medium or M-HBsAg, and large or L-HBsAg) on which it depends to create its envelope for exit and re-entry into hepatocytes (Figure 1) [25,26].

The genome structure of HDV is a circular, covalently closed, single-stranded RNA molecule [26]. As HDV shares the same viral envelope with HBV, both infect the hepatocytes similarly [26]. First, HDV attaches to the heparan sulfate proteoglycans on the outer surface of the hepatocyte membrane. Then, HDV interacts with the human sodium taurocholate co-transporting peptide (NTCP) receptor as its functional receptor on the hepatocyte membrane for its entry [27]. Upon cellular entry, HDV ribonucleoprotein

is released in the cytoplasm, and replication occurs in the nucleus [28]. HDV does not possess its RNA polymerase; it uses the host cell polymerase to replicate [29].

The regulation of viral replication is mediated by both forms of HDAg; large HDAg (L-HDAg) and small HDAg (S-HDAg). The S-HBsAg can package the HDV ribonucleoprotein and assemble it into virions, and L-HBsAg is needed for infectivity [10]. So, HDV replication is autonomous from HBV, HDV replication can thrive by transmission of HDV RNA through cell division even in the absence of HBV infection [30]. However, the assembly, release, and propagation of HDV virions are entirely dependent on HBsAg. Hence, HDV only infects HBV carriers.

## 2.3 Epidemiology

### 2.3.1 Incidence and global prevalence of HBV

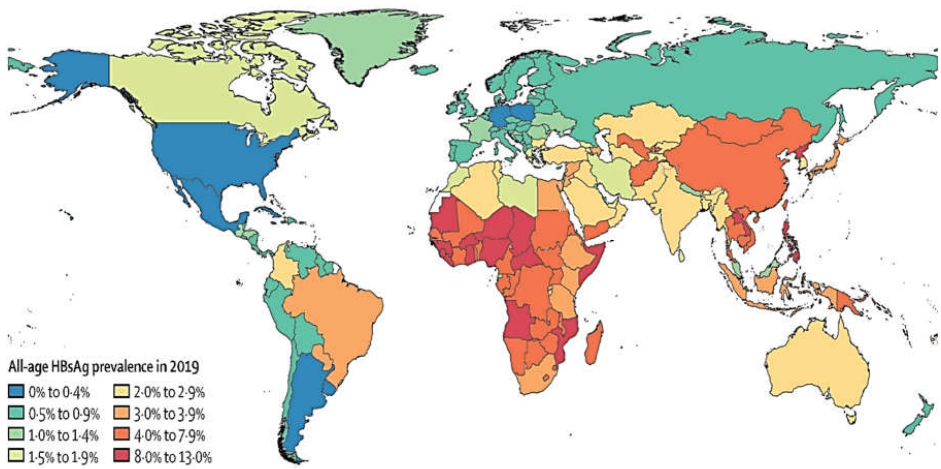


Figure 2. The global prevalence of HBV mono-infection in 2019, adapted from Sheena, Brittney S. and colleagues [31] under an open access Creative Commons license.

According to the WHO estimates, almost 296 million individuals are living with HBV infection in 2019, with a yearly 1.5 (1.1-2.6) million new infections and 820 (450-950) thousand HBV-related deaths mostly attributed to cirrhosis and HCC [32].

The prevalence of HBV is highly variable across the world and has been rigorously reviewed and estimated [1]. The prevalence is high in the West Pacific, and African regions (~7.1, and 6.5% of individuals living with chronic HBV infection, respectively), and moderately high prevalence is noted in the Eastern Mediterranean region (~3%). Areas of low prevalence include Europe (1.1%), and the Americas (1.2%), and of moderate prevalence include South-Eastern Asia (3.1%), where most infections occur in adulthood due to sexual or parenteral routes of transmission (Figure 2) [31].

### 2.3.2 HBV genotypes

HBV has high mutation rates as it lacks the proofreading activity of its reverse transcriptase. The high error rate during viral replication and frequent nucleotide substitutions, give different genotypes and sub-genotypes [33]. So far, ten different genotypes of HBV (A-J) with wide variability in geographical distribution have been recognized [33]. The different genotypes are associated with patterns of transmission, and possibly migration flow in different populations. For instance, genotypes A, D, and E were identified in Southern-Eastern, Northern, and Western African regions, respectively [33]. Genotypes B and C are common in Asia. F, G, and H are mainly located in Southern America [33]. While in Europe, genotype A and D are more frequently identified than genotypes B, C, and E [34]. Genotype I was identified Southeast Asia (Vietnam and Laos), and genotype J in Japan [35]. HBV genotypes were associated with different outcomes in CHB as well as response to IFN therapy [35].

### 2.3.3 Incidence and global prevalence of HDV

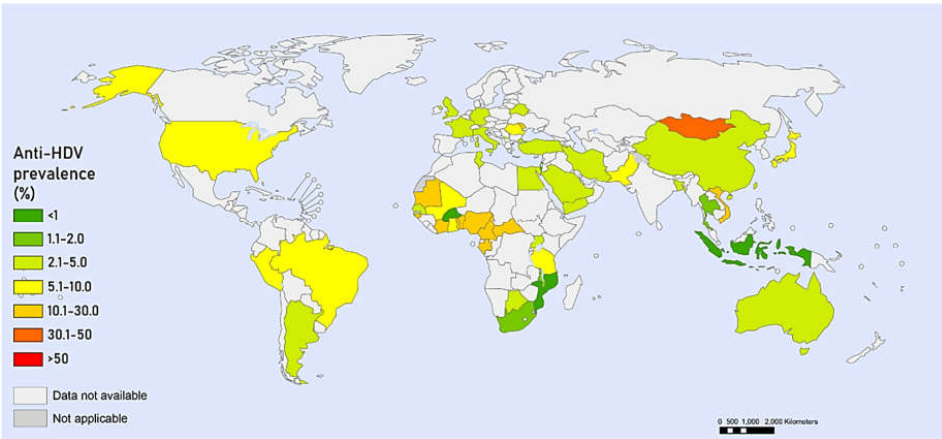


Figure 3. The prevalence of anti-HDV among HBsAg carriers per country. Adapted from Stockdale AJ. and colleagues [11] under an open access Creative Commons license.

The prevalence of HDV is still debated (Table 1) [14,15]. In 2018, a systematic review and meta-analysis by Chen and colleagues estimated ~20% of chronic HBV carriers are co-infected with HDV detected as anti-HDV positive; this corresponds to 60-70 million persons living with HDV infection worldwide [36]. These figures have been revised in a recent meta-analysis agreeing with previously published reports that around 4.5% of persons with positive HBsAg carry anti-HDV positive [37]. To the best available evidence, there are 12 (9 -19) million persons have anti-HDV positive [11].

Table 1. Systematic reviews and meta-analyses on the global prevalence of HDV

| Publication by the first author, year | Number, and duration of studies included | HDV ascertainment | HDV prevalence in positive HBsAg (%) | Prevalence in number |
|---|---|---|---|---|
| Chen et al., 2018 [38] | 182, 1 Jan 1977-31st December 2016 | Anti-HDV positive | 14.57% (95% CI 12.93 to 16.27) | 72 451 000 |
| Stockdale et al., 2020 [11] | 282, 1 January 1998-28 January 2019 | Anti-HDV Ig G Excluded IgM, anti-HDV Ag, initial HDV RNA | 4.5% (95% CI 3.6-5.7) among all HBsAg-positive | 12.0 (8.7–18.7) million |
| Miao et al., 2020 [16] | 634, From inception to February 2019 | Anti-HDV positive | 13.02% (95% CI, 11.96–14.11) among HBV carriers | 48–60 million |

*Abbreviations: HBsAg= hepatitis B surface antigen; HDV= hepatitis D virus; CI=confidence interval*

The geographic distribution of HDV is variable with particularly high prevalence in central Asia especially Mongolia, Moldova [39], Pakistan [40], Iran, Eastern Turkey [41], the Amazon basin [42], and Central Africa (Figure 3) [43]. In Europe, lower prevalence has been reported in Northern Europe, ranging from 1-2% among screened blood donors, to 11% in referral hospitals [11,36,43]. Southern and especially Eastern Europe have been considered areas of high endemicity [11]. In Africa, the prevalence of HDV in the general population in Northern and Sub-Saharan Africa was 5·01% (95% CI: 1·25–8·27) and 8.4 % (95% CI: 4.7-12.8) respectively, while among persons with liver diseases is 20.7% (95% CI:9.9-44.5) and 15.0% (95% CI:6.7-25.6), respectively [43,44].

### 2.3.4 The genotype distribution of HDV

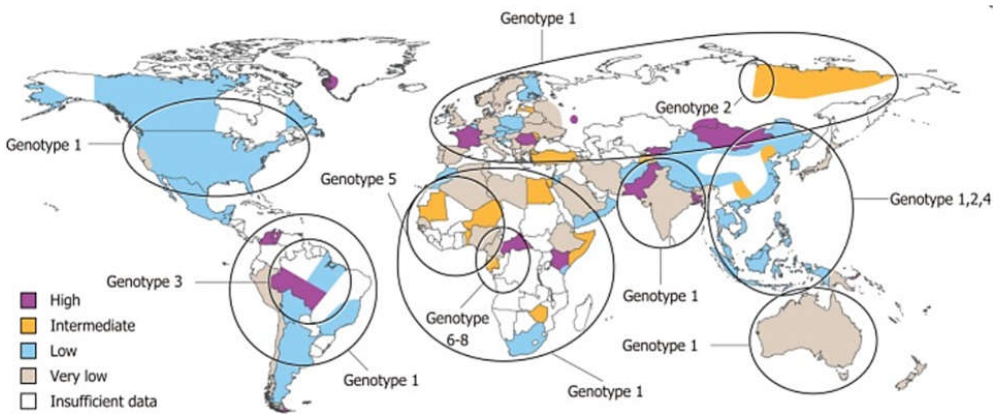


Figure 4. The prevalence of HDV genotypes among HBsAg positive per region. Adapted from Gilman C. and colleagues [45] under an open access Creative Commons license.

Eight genotypes of HDV have been described [46]. Genotype 1 is universally distributed with prevalence in Northern Africa, the Middle East, Europe, and Northern America [14]. Genotype 2 has been found in the Far East and Russia, and genotype 3 in Southern America, and Amazonia [14]. Genotypes 5–8 have been found in Africa. Studies are limited on the natural course of CHD per genotype, with few data suggesting different CHD progression by genotype. For instance, genotype 1 has a variable disease course (Figure 4) [45]. Genotype 2 has been associated with a lower incidence of cirrhosis and decompensation events, and genotype 5 showed a better response to IFN therapy

[45,47,48]. This while HDV genotype 3 has been responsible for epidemics of severe and fulminant hepatitis, which is common in Southern America [49].

### 2.3.5 Modes of transmission of HBV and HDV

HBV is transmitted when a person who is not infected or immune is exposed to infectious blood products or body fluids through the skin (percutaneous) or mucous membranes [50]. In areas of high endemicity, transmission occurs mostly perinatally (vertical), and during childhood (horizontal) mostly in household and siblings contact besides adult transmission which includes horizontal and sexual routes [50]. Before post-exposure prophylaxis, newborns of HBeAg-positive mothers risked 40% to 90% to develop chronic HBV infection [51]. Administration of the HBV vaccine and hepatitis B immunoglobulin (HBIG) at birth followed by the scheduled vaccine have markedly decreased chronic infection in newborns of HBeAg-positive mothers [52]. Close household contacts living with HBV and sexual exposure are sources of transmission for unvaccinated individuals in both endemic and non-endemic regions [53]. Persons with immunosuppression states are at higher risk to develop chronic infection after acute HBV exposure [54]. The transmission of HBV through breastfeeding is debated, mothers living with HBV could breastfeed their infants if they received prophylaxis [55].

Currently, WHO recommends that pregnant women with HBsAg positive should receive tenofovir prophylaxis from the 28$^{th}$ week of pregnancy at least until birth to prevent vertical transmission. Newborns will receive a timely HBV vaccine dose followed by 2 to 3 doses of vaccine [55].

HDV is transmitted as in HBV through contact with blood or body fluids. Mother-to-child transmission is rare but possible [56]. In endemic regions where HBV prevalence is high, HDV infection might occur early in life, leading to a lifelong chronic infection [56].

Some data suggest a higher prevalence of HDV among men and particularly among the 20-39 years age group highlighting the possibility of increased risk via sexual transmission [36,57]. The higher prevalence of HDV in people who use intravenous drugs (PWID) and in persons with HCV or HIV has been consistently demonstrated in studies from different settings, and in pooled analyses possibly due to shared routes of transmission [11,16,58]. Intrafamilial transmission constitutes an important route of transmission in endemic regions [59]. Other possible risk factors to be recognized are nosocomial routes of infections such as cosmetic, and dental procedures, especially in areas of historically high endemicity [60]. In Southern Europe and other endemic regions, the decrease in HBV and HDV prevalence was attributed to the implementation of mass HBV vaccination programs [61], screening of blood products, and improved sanitary and socioeconomic conditions [62]. This decrease in HDV incidence noticed in the nineties in some regions in Europe has been halted possibly due to the rise of immigration from endemic regions [57,63].

## 2.4 HBV and HDV infection in Sweden

Sweden is a low-endemic country for HBV with an estimated prevalence of 0.2% translating to around 20,000-30,000 in a population of 10 million inhabitants [64]. For

HDV, the estimates are around 0.01% translating to around 850 persons [65]. Since 1969, HBV is a notifiable disease by law in Sweden, and initially both the physician and the laboratory report positive HBsAg, as well as positive anti-HDV and HBV DNA tests [66]. Reflex laboratory testing of HDV infection in persons with positive HBsAg is not performed.

The majority of patients with HBV and HDV in Sweden are individuals from high-endemic regions who were infected in the perinatal period or early childhood [67]. The notification rates of HBV infection per 100,000 persons have decreased from 13.02 (95% CI 12.32-13.76) in 2015 to 7.71 (95% CI 7.18-8.27) in 2018 [67]. The great majority of HBV infection is reported as chronic, with an estimated 2.16% (95% CI 1.35-3.43) prevalent liver-related morbidity at the time of diagnosis in 2018 [67]. This is while the incidence of acute HBV infection has decreased for the last decade in Sweden, with most domestic infections occurring mainly among PWID and heterosexual transmission [66].

HBV vaccination is recommended free of charge for all infants and unvaccinated children under 18 years old from an area of intermediate to high HBV endemicity since the year 2015 [68]. In 2019, HBV vaccination coverage reached 97.3% [68]. All pregnant women are offered an HBV test in the national screening program for pregnant women, and all newborns to HBsAg-positive mothers are vaccinated at birth. Women with high HBV DNA replication are offered antiviral treatment and their newborns receive anti-HBV immunoglobulins at birth [69].

Figures from statistical data from the Public Health Agency of Sweden (Folkhälsomyndigheten) show an overall steady incidence of HBV infection diagnosed in Sweden till the year 2011 [66]. The number increased annually from 2011 to 2015; then a decrease has been noted likely attributed to a decrease in migration from high-endemic countries. Regarding HDV, Sweden is among the countries which experienced an annual increase in newly diagnosed persons with HDV since 2005 (Figure 5) [70]. The number of new cases tripled in the time duration 2010-2020 compared to 1997-2009 probably due to the increase in immigration from the Eastern Mediterranean region in the last decade [70]. The number of HDV diagnoses reported from 1997 to December 2022 is estimated to be 866, with most of the infections occurring outside Sweden.

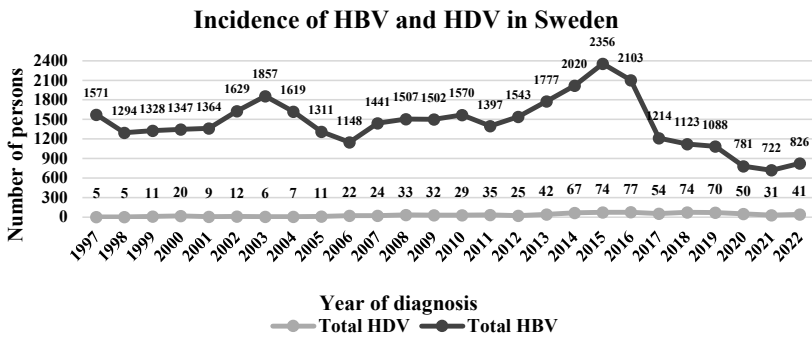


Figure 5. The incidence of hepatitis D virus (HDV) and hepatitis B virus (HBV) infections in Sweden per year of diagnosis. Numbers are adapted from the Public Health Agency of Sweden (Folkhälsomyndigheten) [70].

### 2.4.1 The course of HDV infection in Sweden

In 1986, Lindh G. and colleagues studied a cohort of 280 cases with acute viral hepatitis in Stockholm Sweden, where 63 patients (23%) had non-A non-B hepatitis (NANB), who were more likely PWID (41%) and have a history of travel abroad (29%). At 6 months follow-up, 25% of NANB hepatitis progressed to chronic hepatitis with elevated alanine aminotransferase (ALT), compared to 2% in those with acute HBV infection [71].

In a subsequent analysis, Lindh G. and colleagues revealed that 32% of patients with NANB were anti-HDV positive, concluding that HDV infection occurred in Stockholm at the beginning of the 1970s [72]. Other studies from Gothenburg and Malmö reported the high prevalence of anti-HDV among PWID [73,74]. Similar finding of the preponderance of anti-HDV in PWID was also reported in Norway at the same time [75].

In 2012, Ji J. and colleagues presented a population-based study, where they reported a 4-times higher risk to develop HCC in persons with CHD (SIR=3.90, 95% CI 1.61-7.22) compared to persons with CHB mono-infection [76]. There is a scarcity of studies characterizing the demographics and long-term prognosis of patients with CHD in Sweden, especially in the last decade.

### 2.4.2 The course of HBV infection in Sweden

In a nationwide cohort of 9517 individuals with CHB assembled from 1990-2003, Duberg A. and colleagues reported a standardized mortality ratio (SMR) of 2.3 (95% CI 2.0-2.6) in persons with HBV mono-infection compared to the general population and higher risk noted in those with HBV/HCV co-infection at an SMR of 8.5 (95% CI 7.3-9.8) [77]. Liver cancer was the culprit of 46% of liver-related death in persons with CHB and 33% in those with HBV/HCV co-infection in the same analysis [77]. In 2010, Davíðsdóttir L. and colleagues reported that persons with CHB have increased standardized incidence ratio (SIR) of HCC in older ages than 0-29 years, with the highest relative risk (SIR=54) noted in those aged 50-59 years compared to the general population in a nationwide cohort study of persons with chronic HBV infection in Sweden [78].

Asian-born men with CHB exceeded 0.2% IR of HCC in ages 40-49 years old in a nationwide cohort assembling all persons with CHB [79]. Compared to Western Europeans with CHB, Eastern/Southeastern Asians had 5.50 (95% CI 1.63-18.52) times the risk to develop HCC [79]. This while Sub-Saharan African (SSA) born individuals with CHB showed 2.17 times the risk to develop HCC, yet not statistically significant (95% CI 0.60-7.80) [79]. In the same analysis, HDV infection was significantly associated with HCC development regardless of sex [79].

### 2.4.3 The natural history: HBV infection

Acute infection with HBV ranges from asymptomatic infection to severe acute hepatitis [80]. The average incubation period is 2 months (range 1.5-3 months), with elevation of serum ALT, 1 to 2 weeks before onset of jaundice. The infection is asymptomatic in infants and young children less than 5 years and in immunocompromised adults. In adults and older children symptoms include nausea, malaise, abdominal pain, and subsequent jaundice with darkening of the colour of urine and a change in the colour of stool [80].

Fulminant hepatitis occurs in less than 1.5% of cases. The risk of progressing to chronic infection decreases with the age at infection. Chronic hepatitis develops in 80-90% of infections acquired during infancy, in 20%-60% of those infected during early childhood (less than 5 years), while 95% of infections are resolved in adults with normal immunity [81].

Assessment of a suspected case of HBV infection includes assessment of HBV serological markers together with liver disease severity through biochemical and fibrosis markers [82].

Chronic hepatitis B (CHB) is associated with an increased risk of cirrhosis and HCC, owing to the host immune-mediated response to infection causing chronic liver inflammation [54]. It is estimated that a third of the global cirrhosis cases and half of HCC cases are attributed to CHB, the figures are even higher in highly endemic regions [80,83]. This risk might increase in association to host factors like co-morbidities (diabetes, metabolic syndrome), habits such as alcohol overconsumption, aflatoxin consumption, and smoking, as well as virological factors (genotypes, mutations) [82].

The main features of chronicity (infection and hepatitis) are used in the new European Association for the Study of the Liver (EASL) nomenclature to describe the different phases of chronic HBV infection [82]. Those phases are not necessarily sequential and are described into HBeAg positive chronic infection, HBeAg positive chronic hepatitis, and HBeAg negative chronic infection and chronic hepatitis which have replaced the previously used terms of immune tolerance, immune reactive HBeAg positive and inactive carrier, and HBeAg negative chronic hepatitis, respectively presented in Table 2.

Table 2. the new terminology of the different phases of chronic HBV infection per EASL guidelines. Adapted from reference [82] under Creative Commons open-access license.

| | **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** | $>10^7$ IU/ml | $10^4$-$10^7$ IU/ml | <2,000 IU/ml | >2,000 IU/ml |
| **ALT** | Normal | Elevated | Normal | Elevated |
| **Liver disease** | None/minimal | Moderate/severe | None | Moderate/severe |
| **Old nomenclature** | Immune tolerant | Immune reactive HBeAg positive | Inactive carrier | HBeAg negative chronic hepatitis |

Owing to the dynamic nature of CHB, some patients may not fit into either of these phases, hence the importance of individualized management [82].

**Phase 1:** is characterized by the presence of serum HBeAg, high replication of HBV DNA, and normal serum ALT level. Patients are highly contagious during this phase due to high HBV DNA replication. In the liver, there is minimal or negligible liver necro-inflammation or fibrosis, but a high level of HBV DNA integration and clonal hepatocytes suggesting that potential initiation of carcinogenesis occurs in this phase. This phase is more prevalent in individuals infected during the perinatal period.

**Phase 2:** Chronic hepatitis with positive HBeAg, is characterized by the presence of serum HBeAg, elevated levels of HBV DNA, and high ALT levels. In the liver, there is moderate or

severe liver necro-inflammation and accelerated progression of fibrosis. This phase is more commonly observed in individuals infected during adulthood. Most patients seroconvert HBeAg, suppress HBV DNA and transit into HBeAg negative infection phase. For certain patients, HBV DNA replication will persist and progression to HBeAg negative chronic hepatitis phase will occur.

**Phase 3**: Formerly referred to as the inactive carrier phase, this phase is characterized by the presence of anti-HBe antibodies, undetectable or low HBV DNA levels, and normal ALT. Some patients may exhibit normal ALT levels and moderate elevation of HBV DNA (<20.000 IU/ml), as well as minimal hepatic necro-inflammation and fibrosis. Patients remaining in this phase have a minimal risk of progression to cirrhosis or HCC. Spontaneous HBsAg loss and/or seroconversion may occur in 1-3% of patients per year, especially in those with low serum HBsAg level.

**Phase 4:** HBeAg-negative CHB is marked by detected anti-HBe, moderate to high elevation of HBV DNA level, and elevated ALT levels. This phase is associated with low rates of spontaneous disease remission.

**Phase 5** is characterized by negative HBsAg and positive anti-HBc antibodies with or without anti-HBs. Individuals in this phase have normal ALT values, and undetectable serum HBV DNA, while HBV circular covalent closed DNA (cccDNA) can be detected in the liver. This phase is known as occult HBV infection. Loss of HBsAg before the onset of cirrhosis is associated with minimal risk of progression to cirrhosis, decompensation, HCC, and better survival [82]. The average HBsAg clearance occurs at 1.3% per year, more frequent in individuals older than 50 years of age [84]. Older age, men, HBeAg negative, low HBsAg (<100 IU/mL) and genotype C were positive predictors of spontaneous HBsAg clearance [85]. Nevertheless, patients with cirrhosis who have negative HBsAg remain at risk of developing HCC. Immunosuppression may exacerbate HBV reactivation in some patients [82].

### 2.4.4 The natural history: HDV infection

The outcome of HDV infection depends on the pattern of infection (Figure 6). HDV superinfection of persons with CHB leads to progression to chronicity in 90% of the patients, whereas simultaneous co-infection of both HBV and HDV often leads to resolved infection (less than 10% chronicity) [86]. A higher risk for acute liver failure exists in the case of acute HBV/HDV infection than acute HBV mono-infection [87].

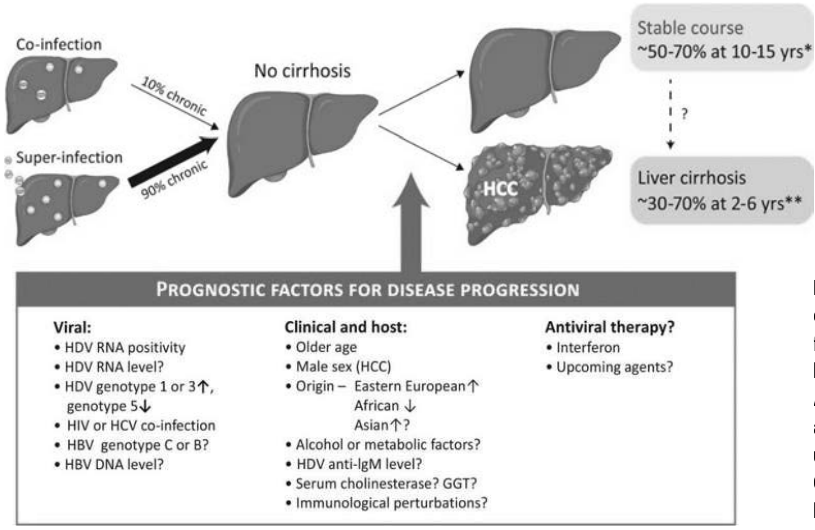


Figure 6. The natural course and prognostic factors of chronic hepatitis Delta (CHD). Adapted from Kamal H. and colleagues [63] under a Creative Common open access license.

### 2.4.5 Liver cirrhosis

In response to chronic inflammation, hepatic stellate cells are activated and produce extracellular matrix components, mostly collagen and growth factors that increase endothelial cell migration, neo-angiogenesis, and fibrosis with subsequent distortion of hepatic architecture [88]. The damaged hepatocytes secrete chemokines that impair immune-mediated tumor suppression. This deranged milieu in cirrhosis promotes tumor development. The secretion of cytokines and growth factors favors the proliferation of tumor cells, suppresses apoptosis and the anti-tumor function of surrounding lymphocytes. Cytotoxic T (CD8+) depletion in mice has been associated with increased HCC and paradoxically has been associated with pro-tumorigenic function in other cases [89].

It is estimated that in untreated persons with CHB who acquired the infection perinatally, around 25% of men and 8% of women will die of cirrhosis complications and/or HCC [84]. In CHD, 60-80% of patients progress to cirrhosis within 5-10 years of diagnosis [17]. Studies on HDV reported a higher propensity toward liver decompensation events and the need for liver transplantation describing CHD course as the most severe among viral hepatitis [90]. The progression to cirrhosis has been estimated to be three times higher in HDV patients, occurring 10 years earlier compared to HBV and HCV patients [90]. The annual incidence of liver decompensation has been estimated to range from 3%-4% [91], and decompensation might be a more common outcome in CHD rather than HCC [19]. Most earlier studies on the natural course of HDV were derived from small cohorts with multiple co-morbidities or from tertiary care centres with a paucity of large cohort studies [17]. In recent years, several studies demonstrated that persistent HDV RNA replication is associated with worse liver-related outcomes, irrespective of HDV RNA or ALT absolute levels [91–93]. Furthermore, it is unclear whether patients with resolved HDV infection or undetected HDV RNA are at higher risk of liver-related complications compared to HBV mono-infected peers.

## 2.5 Hepatocellular carcinoma (HCC)

Hepatocellular carcinoma (HCC) constitutes 90% of primary liver malignancies, followed by cholangiocarcinoma. In 2020, HCC was the 6th most common malignancy in adults and the 3rd to lung and colorectal cancer as a leading cause of cancer-related death [5]. HCC is strongly associated with chronic liver diseases. Most HCC cases (>90%) are due to viral hepatitis, and 50% are attributed to HBV infection, particularly in areas of high endemicity [4]. The incidence of HCC varies across geographical regions according to the prevalent risk factors. HCV is a leading cause of HCC in North America, Europe, and Japan, HBV is by far the leading cause in the majority of Asia and Sub-Saharan Africa [94]. The implementation of HBV vaccination, and effective therapies against HCV have significantly decreased the burden of HCC related to viral hepatitis. Metabolic-associated fatty liver disease (MAFLD) is becoming an increasingly common cause of HCC especially in Western countries [95].

Men have a higher risk to develop HCC than women regardless of age, possibly due to culminating risk factors in men, and the possible protective role of estrogen in women [96]. The factors associated with an increased HCC risk include host factors like older age, sex, family history of liver cancer, geographic region, exposure to toxins such as alcohol overconsumption, smoking, aflatoxin, dietary iron, or co-infection like HIV and metabolic associated as in hemochromatosis, porphyria, and diabetes mellitus.

Cirrhosis is a premalignant condition, persons with cirrhosis have a 2-8% annual incidence of HCC and is the strongest risk factor associated with HCC irrespective of etiology [97]. Despite considerable advancement in screening and diagnosis, HCC incidence and mortality remain high [94]. Based on annual forecasts, HCC-related mortality is expected to exceed 1.3 million deaths in 2040 [98].

The oncogenic potential of HBV in inducing HCC is compelling [99]. Persons with CHB have ~25% increased lifetime risk of developing HCC [100]. HCC can develop in both cirrhotic and noncirrhotic liver in the context of persistent HBV infection [9]. HCC tends to occur earlier in persons with CHB compared to peers with CHC owing to the early onset of infection in HBV [101]. The younger age at HCC has been observed in studies in HBV and HCV endemic regions as well as in non-endemic settings [101,102]. However, knowledge is scarce on the characterization of HCC risk in individuals from endemic areas, especially in Africa. Sub-Saharan Africa has a high prevalence of HCC, but most of the studies conducted were hospital-based case series or prevalence studies [101,103]. There is a paucity of studies on the HCC risk in African-born individuals living in a different environmental setting.

### 2.5.1 HBV carcinogenesis

HBV infection causes insertional mutations, leading to overexpression of telomerase enzyme, activation of potent oncogenes involved in cell cycle control, and eventually promoting cell transformation. Other factors in HBV carcinogenesis involve dysregulation of regulatory proteins HBVx (HBx) modulating molecular pathways involved in cell proliferation such as mitogen-activated protein kinase (MAPK)/Ras/Raf/c-Jun, nuclear factor kappa beta, JAK-STAT, and protein kinase C [104].

HBx activates the beta-catenin signaling pathway, leading to its accumulation. Beta-catenin has been associated with carcinogenesis. HBx induces mitotic abnormalities and genomic instability [104]. HBx leads to the upregulation of angiotensin-2 and vascular endothelial growth factor (VEGF) related to angiogenesis and tumor progression. HBx is also associated with increased oxygen reactive species (ROS) production in the mitochondria, DNA damage, and oxidative stress [104]. In persons with CHB, the risk of HCC might be higher in association with certain HBV genotypes like genotype C, or geographic region as SSA or Southeast Asia, and with concomitant co-morbidities like diabetes or obesity [105]. In Asian patients with HBsAg clearance, the cumulative incidence of HCC at 5 years was 1.5%. The risk for HCC was 2.5 times higher in men and 4.5 times higher in those older than 50 years in the same study [105]. Primary prevention through HBV vaccination and effective viral suppression using anti-viral therapies are the only interventions to prevent and respectively decrease the risk of progressing to cirrhosis and HCC [106].

### 2.5.2 HDV carcinogenesis

Whether the carcinogenic effect of HDV is indirect through the acceleration of cirrhosis or a direct oncogenic effect is unclear. Data suggest that HDV affects pathways involved in cell cycle regulation, DNA damage repair, and replication [107]. HDV causes genetic instability, upregulates genes involved in the control of cell and DNA replication, damage, and repair [89]. L-HDAg enhances transforming growth factor-beta (TGF-beta) and C-Jun pathways implicated in carcinogenesis [89].

### 2.5.3 HCC in Sweden

In Sweden, primary liver cancer is the 13$^{th}$ most common malignancy and the 7$^{th}$ leading cause of cancer-related mortality [5]. The incidence is 5/100.000 in men aged 55 years, peaking to 30/100.000 in men aged 75 years old [108]. The men: women ratio is 3:1 and 75% are older than 65 years at diagnosis [108].

Most primary liver cancers are HCC (80%), while cholangiocarcinoma constitutes 20% [109]. In a recent study analyzing the national register data of liver and biliary tracts tumors between 2009-2016, 55% of HCC were attributed to HCV infection and/or alcohol-related liver disease with approximately 40% having no underlying liver disease [110]. The majority of patients had Barcelona Clinic Liver Cancer (BCLC) stage C and D at diagnosis reflecting a rather advanced disease stage [110]. Curative treatment was received in 36% of patients with HCC in Stockholm according to another Swedish study, where 18% received trans-arterial chemoembolization (TACE) or systemic therapy (Sorafenib) and in 52% best supportive care was offered as main therapy [111].

### 2.5.4 HCC surveillance: an ongoing debate

HCC is asymptomatic in the early stages of the disease; prognosis crucially depends on early diagnosis when curative therapies are feasible. Hence, all liver societies recommend HCC surveillance in individuals at heightened risk of HCC, specifically those with cirrhosis regardless of etiology, and in subpopulations of patients with CHB in the absence of

cirrhosis [112]. In persons with Child C cirrhosis, surveillance is only recommended for those who are on the liver transplantation list, owing to their favorable survival [113]. Persons without cirrhosis who exceed the cost-effectiveness 0.2% surveillance threshold are recommended to undergo regular HCC surveillance [114,115]. Thresholds are based on prior simulation studies, observations from mostly Asian and Caucasian populations, with heterogenous co-morbidities, and not adjusting for competing risks [116,117]. HCC surveillance is a universally conducted biannual ultrasound (US) examination +/- alpha-fetoprotein tumor marker [118].

The annual risk of HCC varies according to the etiology of liver disease with persons with HCV-cirrhosis carrying 3-8% risk and around 2-4% in persons with CHB-cirrhosis [119,120]. Lower estimates of HCC incidence have been shown in persons with metabolic-related cirrhotic liver diseases ranging from 1.1% in a retrospective study including 130 facilities from Veterans Health Administration setting to 3.3% in tertiary referral facilities [121,122]. Persons with alcohol-related liver cirrhosis have an annual incidence of 0.7% in a register-based setting to 2.5% in a tertiary care facility [123,124]. In persons with HCV-related cirrhosis, 1 out of 3 persons developed HCC during a 17-years follow-up study [125]. The choice of the biannual ultrasound examination is based on the mean HCC tumor doubling time estimated at 4 to 6 months and cost-effectiveness analyses [118]. Some local guidelines recommend 3-4 months of HCC surveillance for "extremely high-risk" groups defined as those with Child class B and C [126]. Detection of small HCC was shown to be similar between 3-6 months of surveillance interval [127]. In Sweden, a 6-month ultrasound examination is offered to individuals with cirrhosis and those at higher risk of HCC development [128]. Notably, data pointed to that 20%-70% of NAFLD (non-alcoholic fatty liver diseases)-related HCC occurred in non-cirrhotic liver, the corresponding figure in CHB is 15%-40% [121,129]. The survival benefit of surveillance has been demonstrated in observational studies and pooled analyses, and in some randomized controlled trials (RCTs) exclusively conducted in Asian populations [130–133]. To date, there is no RCT on HCC surveillance in persons with cirrhosis, most likely due to ethical concerns and due to the strong preference towards surveillance among clinicians and patients [134].

Despite these recommendations, data from real-world studies suggest suboptimal surveillance practices where less than 20% of patients receive HCC screening [135]. Factors associated with receiving HCC surveillance include the level of care, the early identification of liver disease stage by the healthcare provider, patient adherence, and patient's race [113]. Nearly 1 in 5 persons who had suboptimal surveillance was attributed to undiagnosed cirrhosis before HCC development according to a US study [136].

### 2.5.5 HCC surveillance in persons with CHB

The American Association for the Study of Liver Diseases (AASLD) in 2021 guidelines, recommends the initiation of HCC surveillance at the age of 40 years for African/North American black individuals with HBV (with no distinction of US-born or not), at age 40 years for Asian men, and age 50 years for Asian women with HBV [115]. The updated 2023 AASLD practice guidance, advised to initiate surveillance early in persons of African origin (regardless of sex) from their twenties [137]. The European Association for the Study of the Liver (EASL) recommends a PAGE-B scoring to assess the risk of HCC, while the Asia

pacific association for the Study of the Liver (APASL) recommends starting surveillance for CHB of African origin at age 20 [119,138].

Several models have been developed to predict HCC in CHB, mostly derived in Asian and Caucasian cohorts and many still lack external validation [139].

Whether HDV exerts an added risk of HCC development compared to HBV mono-infection has been debated since HDV discovery. Studies pointed out that decompensation events rather than HCC might be the most common complication of CHD [19]. On the other hand, multiple cross-sectional studies described different frequencies of HCC in HBV/HDV co-infected patients compared to HBV mono-infection [140,141]. The association was still debated through cohort studies [90,93,142]. Recently published pooled analyses suggested a higher risk of HCC in HDV (Table 1). Male sex, diagnosis of cirrhosis, HDV viremia, and lack of antiviral treatment were the main factors associated with HCC in some reports [143,144].

### 2.5.6 Diagnosis of HCC

In a US study, 75% of HCC were detected through surveillance, while 10%, 6% were incidentally discovered or owing to symptoms, respectively [136]. In a Swedish study, 22% of HCCs were detected through surveillance, in line with a pooled analysis of 59 studies reporting a 28% of HCCs were diagnosed by surveillance [131,145].

Diagnosis of HCC depends on the size and pattern of the liver nodule. Contrast-enhanced CT or magnetic resonance imaging (MRI) showing characteristics of hyperenhancement in the arterial phase and washout in the portal venous phase constitute the main diagnostic hallmark of HCC. Notably, these typical features apply to patients with cirrhosis and CHB [146]. Therefore, equivocal findings might need tighter imaging follow-up, a contrast-enhanced study, and further histopathological assessment by biopsy [115].

### 2.5.7 Survival

Curative therapies include surgical resection offered for patients with early-stage tumors, preserved hepatic function, good performance status, and clinically insignificant portal hypertension [114,115]. Liver transplantation is offered to patients with limited tumor extent with no macrovascular or distant spread. Ablative therapies are usually offered to patients with early-stage cancer, who are not suitable for surgery. Palliative therapies as trans-arterial therapies as chemoembolization are associated with a median survival of 26-40 months [147].

In advanced HCC stages, systemic agents are offered with a median survival of 10-20 months according to the therapeutic agent [146]. The estimated survival time is multifactorial depending on HCC staging and subsequent treatment modality, degree of liver function, and patient status, with overall better 5 years survival in 70% of persons detected with early-stage cancer and a median of 12-16 months in persons diagnosed at late stages [146].

## 2.6 Liver transplantation in HBV and HDV

Patients with acute fulminant hepatitis due to superinfection or co-infection with HDV and patients with CHD progressing to end-stage liver disease and/or developed HCC have the only remaining treatment option of liver transplantation for survival [148].

Patients with CHD have a lower risk of HBV recurrence, and better survival post-liver transplantation compared to patients with chronic HBV mono-infection [149,150].

This might be attributed to the suppression of HBV replication in persons with CHD [150]. Administration of HBIG post-liver transplantation has further improved the outcome and substantially decreased the recurrence of HDV among patients undergoing liver transplantation [151].

## 2.7 Animal model in HDV research

HDV is limited to organisms that support the replication of HBV or Hepadnaviredіea. Animal models that could support HDV replication include chimpanzees, woodchuck, and bats [152]. Moreover, the discovery of HBV/HDV NTCP receptor enabled to study cell lines from humans and different species that can express the NTCP receptor and sustain HDV replication. These models provided useful data that helped to elucidate the characteristics of the HDV virus lifecycle. Many limitations are usually met with the ethical aspect of animal research, consideration of the number of animals to the statistical significance and the genetic variability of these animals.

Other animals used in HDV research were transgenic mice expressing large and small HDAg [153], and humanized mice where engrafting of human hepatocytes in an immune-deficient mouse was done mimicking HBV and HDV infection [153].

## 2.8 Diagnosis of HBV and HDV infection

The diagnosis of acute and chronic HBV infection is based on the detection of serum HBsAg [82]. In chronic infection, repeated detection of HBsAg 6-months apart confirms chronicity [54]. Anti-HBs implies HBsAg seroconversion, or response to HBV vaccine, recovery from acute infection in previously negative HBsAg patients. Anti-HBc (IgM) is a marker of acute infection and helps differentiate the co-infection with HBV and HDV from superinfection. Detection of HBeAg distinguishes phases of chronic hepatitis, and serum HBV DNA replication is tested regularly as a marker for treatment efficacy and HCC risk [82].

Currently, the recommendations regarding screening for HDV infection differ according to issuing scientific societies. EASL recommends HDV screening in all persons with positive HBsAg at least once [82]. While AASLD recommends target screening in groups with risk factors such as migrants from high HDV endemic regions, PWID, men who have sex with men (MSM), patients with HCV or HIV infection, patients with elevated transaminases while HBV DNA is low or undetectable [54]. Despite these recommendations, real-world studies suggest a suboptimal screening for HDV [154–156].

The diagnosis of HDV infection includes markers as anti-HDV antibodies detected by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) [157].

The total serum anti-HDV antibody is used as the initial screening for detecting HDV infection. During acute infection, the anti-HDV IgM subtype appears earlier; however, it does not confirm the diagnosis or help differentiate between acute and chronic infection due to its infrequent detection. Serum HDAg is only detected in the acute phase of HDV infection. Usually, for a short window of time, intrahepatic HDAg is detected by immunohistochemistry, and HDV RNA is detected by in situ hybridization [157].

Detection of HDV RNA by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) confirms HDV infection. Detected HDV RNA, together with a positive anti-HDV antibody, can help differentiate between chronic and previous infections as well as follow-up of virological response to treatment. However, the lack of standardization still poses a challenge for HDV, and PCR results are often not comparable between laboratories in different regions [158]. Several advances in HDV RNA detection have been achieved with currently available in house and reverse transcription PCR assays for HDV RNA quantification [159]. However, high variability in the diagnostic performance of these tests, especially withing different genotypes is still challenging [159]. To date, HDV genotype is not routinely done as its use is limited to research settings.

### 2.8.1 Occult HBV infection, occult HDV

Occult HBV relies on detecting HBV DNA in the liver or by serological tests in individuals with negative HBsAg. The prevalence of occult HBV infection is variable as the diagnosis largely relies on the sensitivity of serological assays. In HBsAg negative blood donors, a pooled analysis estimated the prevalence to be 0.2% (95% CI 0.1%-0.4%) [160]. The current figures vary according to the studied population; 10-45% in persons with HIV, PWID, on hemodialysis, or having HCV infection, and higher prevalence (~60%) in persons with advanced liver diseases and HCC [161]. Literature is scarce on HDV RNA replication in persons with negative anti-HDV. Studies described such findings are mainly case reports in persons with immunosuppression [162].

## 2.9 Clinical scores

To predict the clinical outcomes and the progression of patients with chronic HBV and HDV, some risk scores and non-invasive markers of fibrosis were developed. Several of these scores were based on clinical and laboratory parameters that predict the progression to liver-related outcomes in untreated chronic HBV carriers. Combined scores such as GAG-HCC (guide with age, gender, HBV DNA, co-promoter mutations and cirrhosis)-HCC [163], CU (Chinese University)-HCC [164], and REACH-B (risk estimation for HCC in chronic hepatitis B score) were developed to predict HCC risk in persons with CHB [100]. Most of these scores were developed and validated in Asian patients. A recent PAGE-B (platelet, age, gender) score showed good performance in Caucasian patients [165]. The baseline-event-anticipation score (BEA score) is a commonly used score developed by Calle and colleagues, studying 75 patients with anti-HDV positive through

a median follow-up of 5 years, and the score was validated in two independent cohorts [166]. In BEA score variables such as age>40 years, male sex, eastern Mediterranean region of origin, lower platelets count, elevated serum international normalized ratio (INR), and elevated serum bilirubin are allocated points. The summation of these points categorizes patients by the hazard to develop liver-related outcome (mild, moderate, and severe) as well might help to prioritize patients for IFN treatment [166].

### 2.9.1 Staging of fibrosis in HBV and HDV

#### *2.9.1.1 Liver biopsy*

It is considered the gold standard for assessing the degree of inflammatory activity and fibrosis stage in HBV and HDV and occasionally can be used to confirm the diagnosis of HCC. However, liver biopsy is associated with invasiveness, risk for complications, also sampling, and significant interobserver variability limiting its use to unequivocal cases [167]. Nevertheless, liver biopsy might not be suitable for repeated evaluation of liver disease progression during follow-up. EASL recommends liver biopsy when contributing to the management of the patient, for staging liver CHD stage when clinical signs and imaging evidence of cirrhosis are missing or conflicting and when other autoimmune component is suspected in the disease [159].

#### *2.9.1.2 Liver stiffness measurement (LSM)*

Transient elastography (TE) is a fast, simple tool that replaced liver biopsy in many settings; however, it is operator dependent and challenging to obtain results in cases of severe obesity and ascites [168]. Substantial variability exists when comparing LSM cut-off values for detecting METAVIR F3 or F4 between patients with chronic HBV or HCV, and CHD [168].

The utility of non-invasive tests like LSM in CHD is sparsely studied. Nevertheless, adding indirect parameters of liver inflammation such as ALT to LSM in combined scores might exaggerate hepatic fibrosis values owing to the significant inflammation in persons with CHD [169]. The Delta Fibrosis Score (DFS) comprised variables such as age, serum gamma-glutamyl transferase (GGT), serum albumin, and serum cholinesterase levels was evaluated in 100 patients with CHD [170]. The DFS correlated with an area under the receiver operating curve (AUROC) of 0.87, a sensitivity of 85%, and a positive predictive value (PPV) of 93% to predict advanced fibrosis/cirrhosis F3-F6 [170]. The Delta-4 Fibrosis Score (D4FS) evaluated in 77 patients with CHD included variables such as GGT, platelet count, ALT, and liver stiffness measurement and demonstrated an AUROC of 0.94 in a validation cohort to predict cirrhosis [171]. An exploratory analysis of 100 patients with CHD with available liver biopsy in the D-LIVR study (RCT evaluating the efficacy of Lonafarnib, ritonavir +/- pegIFN therapy versus placebo for the treatment of CHD) was done. Liver stiffness values at a cut-off of 13 kilopascal (kPa), was able to correctly classify cirrhosis in 66.7% with a PPV of 58.3%. Fibrotest (a group of biomarkers associated with fibrosis and cirrhosis) at a cut-off of 0.74, was able to correctly classify the diagnosis of cirrhosis in 74.5% with a PPV of 65.2%. AST to platelet ratio index (APRI test) classified correctly 58% with a cut-off >1 and a PPV of 43.3%, FIB-4 at a cut-off of 3.25 correctly

identified 72% with a PPV of 65.0%. AST to ALT ratio (AAR) correctly identified 67% with a PPV of 57.1% [172].

To date, there are no studies analysing the serial changes and variability of LSM during interferon therapy and whether LSM can predict virological response and clinical outcome [159].

#### *2.9.1.3 HCC surveillance in patients with HBV and HDV*

To date, there are no specific recommendations for patients with HDV infection, and they are included in the guidelines for HBV mono-infection [82,114].

## 2.10 Special populations

### 2.10.1 HBV-HDV-HIV infection

Concurrent infection with HIV and/or HCV is not an infrequent finding among patients with CHD owing to the shared routes of transmission. The WHO estimates that 1% of persons with CHB are also co-infected with HIV translating to 2.7 million individuals globally [173]. Among PLHIV, approximately 10% are also CHB carriers in the US [174]. In the multi-centre analysis EUROSIDA cohort, HDV constituted 14.5% of HBV/HIV patients predominantly in PWID [175]. HBV/HDV/HIV triple-infected patients have higher risks of liver-related death and overall mortality; despite that, 67% were receiving antiretroviral treatment [176]. HCC risk was 9 times higher in triple infected patients compared to HBV/HIV peers, highlighting the burden of HDV in this population group [177]. While effective therapy against HBV and HCV has decreased liver-related complications in patients with HIV. The lack of effective therapy in patients with HDV predisposes those living with HIV to a higher risk of cirrhosis, HCC, and end-stage liver disease [178].

### 2.10.2 HBV-HDV-HCV infection

Several studies demonstrated that in triple HBV/HDV/HCV infection, there is a decrease in HCV replication and suppression of HBV DNA replication in 80% of patients implying that HDV is the dominant virus [179,180]. However, cirrhosis is more prevalent among patients with triple infection HBV/HDV/HCV with a higher tendency towards HCC development [181].

## 2.11 Treatment

### 2.11.1 Treatment of HBV infection

The treatment aims to achieve virological suppression leading to biochemical and histological improvement; ultimately preventing the progression to fibrosis, cirrhosis, and liver-related complications. The decision to start therapy considers the patient's age, risk of HBV transmission, co-morbidities, family history of HCC or cirrhosis, and presence of extrahepatic manifestations. The approved therapies for persons with CHB are broadly classified as immunomodulators (pegylated-IFN-$\alpha$ therapy) and the currently preferred

antiviral agents due to their high barrier to HBV resistance namely Entecavir 0.5 mg daily, Tenofovir disoproxil fumarate 300 mg daily, and Tenofovir alafenamide 25 mg daily [182]. Other anti-HBV therapies with a low barrier to HBV resistance include Lamivudine, Adefovir dipovoxil, and Telbivudine [97].

EASL and AASLD recommend treating all patients with cirrhosis regardless of age, ALT level, or HBV DNA levels [54,97]. Patients without cirrhosis should be offered treatment if HBV DNA levels are above 2000 IU/ml, serum ALT is above the ULN, and liver biopsy shows at least necroinflammation and/or moderate fibrosis. Patients with HBV DNA >20.000 IU/ml and ALT more than 2 times the upper limit of the normal (ULN) can start treatment without a liver biopsy. Per AASLD, persons with ≥F2-≥F3 stages should be treated regardless of ALT or HBeAg status, or HBV DNA level. Persons who are older than 40 years of age should be considered for treatment if persistent ALT>ULN with HBV DNA ≥2000 IU/ml [54]. Treatment of HBV in individuals with HBeAg positive is primarily aimed at those with persistent ALT elevation more than 2 times ULN +/- HBV DNA > 20,000 IU/ml [54].

Weekly pegylated interferon α (pegIFN-α) therapy 180 mcg aims to induce immunological control for finite treatment duration. IFN therapy is associated with ~10% HBsAg loss after 1 year of treatment. A longer duration of treatment might increase the response, but it is intolerable by some patients. Usually, due to its low response and intolerable side effects, IFN therapy is resorted to patients with HBeAg positive, young, with less advanced liver disease stage, with lower HBV DNA levels and higher ALT levels [82].

High-potency NUCs have an excellent safety profile. One to five years of treatment achieve sustained suppression of HBV DNA and normal ALT levels in almost all the patients. Clearance of serum HBsAg is minimally achieved in around 2-3% of patients at an annual rate of 0.3% [82]. These agents are the only option to prevent HBV reactivation in persons with CHB under immunosuppression, also used to prevent HBV transmission in patients with high viremia who are not on anti-HBV therapy [97].

### 2.11.2 Treatment of HDV

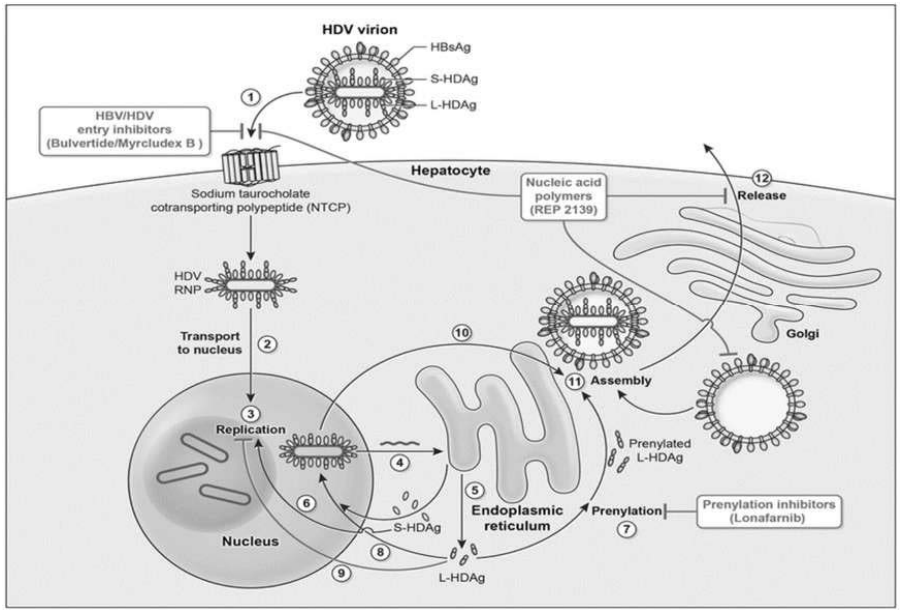


Figure 7. HDV entry into the cells and different therapeutics targeting HDV life cycle. Adapted from Koh C. and colleagues [183] under an open access Creative Commons license.

HDV lacks virus-specific polymerases constituting a challenge for antiviral therapy to target its replication. Furthermore, HDV autonomously replicates from that of HBV which renders therapies that successfully suppress HBV replication ineffective against HDV RNA replication. The aspired endpoint in HDV is HBsAg loss with anti-HBs seroconversion. Eradication of HDV RNA from the liver in persons with HBsAg is an alternative endpoint. A surrogate endpoint can be used as a proxy of HDV RNA eradication from the liver is undetected serum HDV RNA. This later endpoint is used as a marker of treatment efficacy during therapy, at the end of treatment, and 6 months post-treatment discontinuation (off-therapy). As this endpoint is uncommonly achieved /maintained, another secondary endpoint was suggested consisting of at least 2 log IU/ml decline of HDV RNA together with normal ALT. Many therapeutic agents targeting HDV life cycle are under investigation (Figure 7).

#### *2.11.2.1 Interferon (IFN-α) based therapy*

PegIFN-α therapy given for 12-18 months was until recently the only treatment used for patients with CHD with a virological response of 20~30% [184]. Initial evaluation of IFN-α therapy in CHD suggested a better virological response in those treated with higher doses (9 million IU twice weekly) compared to low doses (3 million IU/twice weekly) [185]. However, no patients achieved a sustained virological response at one-year follow-up after therapy [185]. Neither prolonged pegIFN-α for 72 weeks, nor increasing the doses up to 360 mcg/week for prolonged duration resulted in better virological response [186,187]. Treatment with IFN therapy is limited to persons with compensated liver disease and to

compliant patients who can tolerate IFN therapy. The response to pegIFN-α was similar in CHD with early and advanced fibrosis [188]. However, a functional cure defined as HBsAg loss is rarely achieved, and late relapses are common [189]. The combination of pegIFN-α with ribavirin [186] or lamivudine [190] or adefovir[191] or Tenofovir [192]was not associated with a significantly higher virological response.

#### *2.11.2.2 The long-term effects of antiviral therapy on the natural course of chronic hepatitis D*

Studies demonstrated IFN-α based therapy treated patients had a more benign course of CHD compared to non-treated and NUCs treated patients [143]. Improvement in long-term clinical outcome and survival, as well as regression of advanced fibrosis, was observed in patients who received high doses of IFN-α based therapy compared to those who received low doses or no treatment [193]. However, IFN-α based therapy is considered in patients with less advanced liver disease, and late virological relapse was noted in up to 50% of patients at 6 months post-treatment follow-up [189]. This points to the challenge in HDV treatment regarding the clearance of HDV RNA as a surrogate marker of treatment success in patients who remain HBsAg positive. There is a paucity of studies characterizing patients with late relapse. It has been suggested that less advanced liver disease is associated with better response to IFN-α based therapy emphasizing the importance of early diagnosis and treatment of CHD [19]. Undetected HDV RNA at week 24 of treatment start was a predictor of virological response at the end of treatment in one study [194]. Patients who had a decrease in HDV RNA of less than 1 log and no decrease in HBsAg at week 24 of the treatment baseline could be identified as non-responders [194].

#### *2.11.2.3 Novel therapies for HBV and HDV*

Several new therapeutics in HBV carriers to achieve HBsAg sero-clearance with minimal chance of virological rebound are investigated. Some of the promising agents include short interfering RNAs, capsid assembly modifiers/ inhibitors, and viral entry or release inhibitors [182]

Currently, several novel therapies interfering with the life cycle of HDV showed therapeutic potential in phase II and III trials, some has been granted conditional approval. The anticipated aims of these novel agents are to achieve functional cure, or higher virological control and better tolerance; and more patients with advanced disease stage could be treated.

Bulivertide: HDV entry inhibitor which targets the initial HBV and HDV entry into hepatocytes through competitive inhibition of NTCP receptor binding has been approved in Europe in 2020 [195]. Formerly known as Myrcludex, Bulevirtide irreversibly blocks the NTCP hindering the entry of HDV into the hepatocytes. It is administered daily as a subcutaneous injection as monotherapy or in combination with pegIFN-α and /or Tenofovir disoproxil fumarate and demonstrated a good safety profile despite a dose-dependent elevation in bile acids with no clinical implication.

In the MYR202 study, increasing daily doses of Bulevirtide (2, 5, 10 mg) was associated with a 2-log decline or undetectable HDV RNA at the end of treatment in 46%, 47%, and 77% of patients with CHD, where 3% achieved this endpoint in those treated with tenofovir disoproxil fumarate. ALT normalization was noted in 43%, 50%, and 40% of Bulevirtide and 6% in TDF, with no changes in HBsAg. HDV RNA relapse occurred in 60%, 80%, and 83% of end-of-treatment responders and was associated with a moderate elevation in ALT levels [196].

In the MYR203 phase II study, Bulevirtide at variable doses with or without IFN therapy was prolonged to 48 weeks of therapy. At 24 weeks off-treatment (week 72), undetectable HDV RNA was achieved in 53% of those assigned to 2 mg Bulevirtide and pegIFN-α. In the same arm, ALT was normalized in 53.8%. HBsAg loss or more than one log decline at week 72 was noted only in patients with combination therapy of bulevirtide and pegIFN-α with better response in 2mg bulevirtide (40%) than in 5 and 10 mg (in both 13.3% of patients had achieved this endpoint) [197].

Extended therapy with 10 mg Bulevirtide for up to 3 years in two patients with compensated cirrhosis was associated with ALT normalization within 7 months and undetectable HDV RNA before one year of therapy. The biochemical and virological response was maintained for over 3 years despite dose reduction to 5 mg and 2 mg. A patient demonstrated the disappearance of esophageal varices and resolution of features of autoimmune hepatitis associated with HDV infection [198,199]. In 18 Caucasian patients with compensated CHD cirrhosis and clinically significant portal hypertension, 78% had a virological response, ALT decreased to normal values in 83% and combined virological and biochemical response was observed in 67% of patients [200].

Lonafarnib (LNF): HDV assembly inhibitor which interferes with HDV virion assembly and release of infectious particles from infected hepatocytes [201]. When administered orally LNF induced a dose-dependent reduction in HDV RNA levels. Ritonavir (a cytochrome P450 3A4 inhibitor) is combined with LNF to enable lower doses and better gastrointestinal tolerability of LNF while optimizing its antiviral efficacy. PegIFN-α was used to achieve more reduction of HBsAg levels.

In the phase 2 dose-finding LOWR-2 study, HDV RNA was undetected in 5/13 patients assigned to 50 mg twice per day LNF with ritonavir 100 mg twice per day for 24 weeks. LNF 50 mg twice daily with ritonavir 100 mg twice daily achieved a more than 2 log decline or undetected HDV RNA in 7/18 (39%) of patients and ALT normalization in 60% at 24 weeks end of treatment. Adding pegIFN-α to this regimen increased the end-of-treatment virological response to 89% and biochemical response to 78% [202].

REP 2139: although the exact mechanism is unknown; nucleic acid polymer (NAP) blocks the assembly, and release of HBsAg subviral particles. This might further reduce HBsAg needed for HDV assembly [203]. In 12 treatment-naïve patients with non-cirrhotic CHD from Moldova, REP 2139 was given as 500 mg intravenous once per week dose for 15 weeks as monotherapy, followed by 15 weeks of add-on pegIFN-α and 250 mg REP 2139, then followed by 33 weeks of pegIFN-α monotherapy [204]. The study underwent 63

months, and at 6 months after treatment follow-up, 5/12 (42%) cleared HBsAg, 5 (42%) had anti-HBs positive and 7/12 (58%) had undetectable HDV RNA. This virological response was confirmed by extending the off-treatment follow-up to 3.5 years, where 7/11 (63.6%) had undetectable HDV RNA and 4 (36.4%) had HBsAg loss [205].

Pegylated IFN lambda binds to unique type III IFN receptors which are restricted to cells of epithelial origin including the hepatocytes, leading to a better safety profile than type I IFN which are expressed in different tissues [206]. In the LIMT-HDV phase II study, subcutaneous weekly 120 mcg IFN lambda monotherapy versus 180 mcg together with Tenofovir disoproxil fumarate /entecavir in 30 patients with CHD resulted in undetectable HDV RNA, ALT normalization and a combined endpoint of more than 2 log decline and normal ALT was more frequently achieved in the combination regimen. The treatment with IFN lambda was well tolerated, with fewer side effects of elevated transaminases responding to dose reduction [207]. In the LIFT HDV study, 26 patients with CHD received LNF 50 mg twice daily and ritonavir 100 mg twice daily combined with pegylated interferon lambda at weekly doses of 180 mcg for 24 weeks. At the end of treatment, 11/26 (42%) had undetectable HDV RNA, at 24 weeks of therapy 5/26 (19%) maintained HDV RNA <40 IU/ml, but none cleared HBsAg. Dose reduction was needed in 3/26 (11%) of patients and treatment discontinuation in 4/26 (15%) of patients [208].
Other novel therapies under investigation like RNA interference and antisense oligonucleotides decrease HBsAg levels in the absence of IFN therapy and they might help against HDV.

## 2.12 Summary

This review of the literature demonstrates that although four decades have elapsed since HDV discovery, several areas still need to be elucidated. Well-designed studies are needed to characterize the risk factors and long-term outcomes in populations living with CHB and CHD. The possible increased risk of HCC among populations living with HDV and HBV needs to be studied in different populations to implement early diagnosis, therapy, and effective individualized surveillance.

This thesis, therefore, addresses some of these knowledge gaps for a further understanding of the natural course, management, and survival of individuals living with CHD and CHB.

# 3 Research Aims

The overall aim of the thesis is to characterize the long-term liver-related outcomes in persons living with CHD and CHB and identify the predictors associated with HCC development. We also evaluate the cascade of care of HDV, and the factors associated with screening.

Specific objectives:

- To describe the impact of HDV RNA viremia on liver-related events in a nationwide cohort of persons with CHD and the effect of interferon therapy and virological response on the disease course **(Study I).**
- To investigate whether persons with HDV have a higher risk of developing HCC than peers with HBV mono-infection and to identify subgroups at higher risk **(Study II)**.
- To evaluate the cascade of care for persons with HDV at Karolinska University Hospital and identify factors associated with screening and the effect of delayed HDV screening **(Study III)**.
- To characterize the incidence rate and risk for HCC in African-born persons with CHB in relation to age, sex, regions of birth, and co-morbidities. Also, we wanted to compare the risk to matched persons from the same country of birth and the general population without HBV infection **(Study IV).**

# 4 Materials and Methods

## 4.1 Data sources and registers

The quality Register InfCare Hepatitis was used in studies I and III. The Swedish Health Registers were used for paper IV and matching was done by Statistics Sweden. The registers can be linked through a unique personal identification number. Study II used data from published studies.

*The Swedish InfCare hepatitis* founded in 2008, includes demographic data, laboratory and virological parameters, liver stiffness measurements, and treatment type in persons with viral hepatitis B, C, and D [209].

*The Cancer Register (CR)* was founded in 1958, with 95% completeness on new cancer diagnoses [210]. Since 1958, the diagnoses are reported according to the International Statistical Classification of Diseases for Oncology (ICD-7) revision and from 2005 the cancers are coded according to the third revision. Clinicians and pathologists are mandated by law to report cancer diagnoses to this register. The completeness for HCC is lower as its diagnosis relied mainly on histopathological verification and now the diagnosis is mainly via radiological findings [109].

*The National Patient Register* (NPR) was founded in 1964 [211]. It is mandatory to report dates of hospital admission and discharge, surgical procedures, and discharge diagnoses to this register since 1987. Data on outpatient specialist care has been reported since 2001 but not primary care. The reporting on Inpatient care is higher than on outpatient care [212]. Since 1998, reports are coded according to the ICD-10-SE revision [211].

*The Prescribed Drug Register* (PDR) was established in 2005 and contains all dispensed prescription drugs at pharmacies in Sweden. The register has high completeness and validity [213]. The register contains data on patient age, sex, and residence, on prescriber, and prescription such as date, type of ordinance, number of packages, date of purchase, and costs [214]. The prescriptions are coded according to the Anatomical Therapeutic Chemical Classification System (ATC) code [214].

*The Cause of Death Register (DR)* is a highly complete register of all deaths in Sweden since 1952, including data on death reported to the tax authorities [215]. The underlying cause of death based on death certificates is reported in 96% of all deaths. The causes of death were coded according to ICD-9 in 1987-1996, and according to ICD-10 since 1997 [215].

## 4.2 Summary of study methods

Table 3. The design, setting, population, outcomes, and statistical analyses of included studies.

| Study | Design/Setting | Population | Outcome | Statistical analysis |
|---|---|---|---|---|
| **I** | Retrospective Nationwide cohort | Persons with positive anti-HDV from 11 infectious diseases clinic | Incidence of the composite outcome of liver-related events | Cox proportional hazards model. Event-free survival by the Kaplan-Meier method |
| **II** | Systematic review and meta-analysis of longitudinal cohorts | Persons with HBV/HDV coinfection compared to persons with HBV mono-infection | Incidence of HCC | The pooled relative risk of HCC. Meta-regression analysis |
| **III** | Retrospective cohort secondary referral facility | All patients with positive HBsAg at KUH. Patients with positive anti-HDV and HDV RNA replication are identified. | Prevalence and factors associated with HDV screening | Logistic regression model |
| **IV** | Retrospective Register-based matched nationwide cohort | All African-born persons with positive HBsAg in Sweden from 1990-2015, matched comparators from the same country of birth and the general population. | The incidence rate of HCC | Incidence rate and incidence rate ratio by Poisson regression |

*Abbreviations: KUH=Karolinska University Hospital; HBV=hepatitis B virus; HDV= hepatitis D virus; HBsAg= hepatitis B surface antigen; HCC=hepatocellular carcinoma.*

## 4.3 Study design, setting, populations, and outcome measures

The summary of methods used in this thesis is presented in Table 3.

### 4.3.1 Study I

Research question: *what is the impact of HDV RNA replication in persons with CHB?*

For this purpose, a nationwide retrospective cohort of (n=426) persons with anti-HDV positive antibody cared for at 11 infectious diseases departments across Sweden was studied. The patients were identified retrospectively through their consecutive attendance at the departments from the year 2000/2005, using the ICD-code B18.0. InfCare Hepatitis, which is a nationwide viral hepatitis database was also used to further identify persons with anti-HDV positive. All patients' records were manually checked to verify and collect data. We excluded persons with prior HCV or HIV coinfection, prior liver cancer, prior liver transplantation, less than 6 months follow-up, and mislabeled HDV infection. Also, persons who developed HCC or underwent liver transplantation within 6 months of starting follow-up were excluded. The cohort included a total of 337 patients with positive anti-HDV. The diagnosis of cirrhosis was based on liver stiffness value >13.1 kPa, radiologic findings consistent with cirrhosis, or the clinical assessment of the treating physician. Cirrhosis diagnosis was noted at baseline and at follow-up. The patients were followed from the date of the positive anti-HDV test to the first date of the following according to the outcome of interest: progression to cirrhosis, experiencing a decompensation event, HCC, liver transplantation, death, or end of follow-up at 31rst December 2018. Also, we specified a composite outcome variable combining HCC, liver

transplantation, and death to overcome the few outcomes when analyzed separately. Patients were classified according to HDV RNA replication (at least two tests of HDV RNA) into positive and negative groups. Factors such as age, sex, HDV RNA viremia, and IFN therapy were assessed in association with liver-related outcomes. Persons who had undetected HDV RNA at 24 and/or 48 weeks after IFN therapy termination were deemed virological responders and if detected were considered non-responders.

### 4.3.2 Study II

Research question: *do persons with HBV-HDV co-infection have a higher risk of developing HCC compared to persons with HBV mono-infection?*

Therefore, we conducted a systematic review of the literature and meta-analysis. Bibliometric databases were searched from inception until May 2020 for longitudinal cohort studies reporting on the incidence of HCC in persons with HBV-HDV co-infection and peers with HBV mono-infection. We excluded animal studies, studies missing a control group, and cross-sectional reports as it is unclear if HDV infection (exposure) preceded the outcome of interest (HCC). Published articles were scrutinized and reviewed for the final selection of eligible studies. In total, 12 studies met the inclusion criteria reporting on 6099 patients with HBV/HDV co-infection and 57620 patients with HBV mono-infection. The characteristics of the studies and study participants were extracted, and the quality of included studies was assessed by Newcastle-Ottawa Scale (NOS). We pooled the studies according to study design, the inclusion of PLHIV, the exclusion of individuals with HCV co-infection, and studies adjusting for confounders.

### 4.3.3 Study III

Research question: *How many, who, and when get HDV screening tests among persons with positive HBsAg at Karolinska University Hospital (KUH)?*

In this retrospective cohort study, we identified all persons with positive HBsAg who attended the Department of Infectious Diseases, KUH from 1992 to 31st October 2022 (n=4095). KUH is a secondary referral center, that receives referrals from primary care and other healthcare units covering the Southern Stockholm region. Patients with positive HBsAg were consecutively added through a collection of their records to a database and since 2008 records were transferred to the quality and research register of InfCare Hepatitis. The demographics, virological, laboratory parameters, and markers of advanced liver disease of patients at HBV diagnosis were collected. We assessed the prevalence, the time to get an HDV screening test as well as the predictors of getting a screening in this cohort. We further assessed the factors associated with getting an early screening test defined as less than 2 years after HBV diagnosis. We chose this time period as some studies pointed to the rapid progression in persons with anti-HDV positive test as early as two years [16]. In persons with HDV RNA replication, we assessed if delayed testing is associated with the development of a liver-related complication.

### 4.3.4 Study IV

Research question: *what is the incidence rate and risk for HCC in African-born persons with non-cirrhotic CHB in Sweden?*

In this retrospective register-based cohort study with prospectively collected data, we characterized the incidence rate and factors associated with HCC in African-born individuals with CHB, without cirrhosis at HBV diagnosis (n=3,865). The cohort was constructed from the identification of persons with positive HBsAg notification to the National Surveillance Register of SmiNet at the Public Health Agency between 1990 and December 31, 2015. Only African-born individuals were included. Each person with CHB was matched on age, sex, and country of residence with up to 3 persons from the same country of birth without HBV infection (n=8,488) and on age, sex, and county of residence with up to 10 persons from the general population (n=39,278). Controls were obtained from Statistics Sweden. The outcomes and demographics of study participants were ascertained from the National Patient Register (PR), the Causes of Death Register (DR), and the Cancer Registry (CR). We excluded individuals with chronic liver diseases, those with cancer except non-melanoma skin cancer, and those with cirrhosis prior to and within 6 months of the study. Study participants were followed 6 months from the date of HBV notification (used as the same date for matched comparators) to the incidence of HCC ascertained from the CR and DR. This lag was to avoid HCC being present in the participant before the start of follow-up. Study participants were followed-up until 31st December 2019.

## 4.4 Statistical analysis

In all studies continuous variables were presented as mean (standard deviation, SD) when normally distributed, and median (IQR, $25^{th}$-$75^{th}$ quartile range) when skewed. Student-t and Mann-Whitney tests were used for the comparison of normal and skewed continuous variables, respectively. Categorical variables were presented as proportions and were compared using the Chi-Square test or Fisher's exact test, whenever appropriate.

**In Study I**, the incidence rate of the outcome of interest was calculated by dividing the number of events by the total sum of follow-up in the studied group. The cumulative event-free survival for the composite outcome of any liver-related event, of HCC, of decompensation, and of liver-related death was performed using Kaplan-Meier (KM) method. Kaplan-Meier curves were stratified by HDV RNA replication, presence of cirrhosis at baseline, and for the composite outcome by virological response and fibrosis stages. A pairwise comparison of the overall survival between subgroups was performed using a Log-rank test. Cox proportional hazards regression model yielding hazards ratio (95% CI) was performed to characterize the risk for any liver-related event by HDV RNA replication (presence vs absence), cirrhosis at baseline (presence vs absence), and age tested on a continuous scale in all study participants (n=337). Also, the cumulative event-the free survival by the virological response to IFN therapy and by fibrosis stage was illustrated using KM curves. Univariable and multivariable Cox proportional hazards

regression model was performed to test the association of baseline characteristics such as sex, Asian origin, diabetes, and cirrhosis in patients with HDV RNA replication (n=233) with the composite outcome of any liver-related event.

**In Study II,** the crude numbers of persons who developed HCC in persons with HBV/HDV co-infection and persons with HBV mono-infection were pooled in a Maentel-Haenszel random-effects Model. As HCC is considered a rare outcome, odds, and hazard ratios were considered equal. Univariable random-effects meta-regression was used to adjust for potential sources of confounding at the study level such as design, NOS score, average follow-up time, and year of publication. Meta-regression analysis was performed when data is available from more than 8 studies. The heterogeneity between studies was assessed with Higgins $I^2$ statistics, where $I^2$ cut-offs of >75%, >50% and <50% were considered indicative of high, moderate, and low heterogeneity, respectively. Funnel plots were generated to visualize for publication bias and Egger's regression test was used to assess for significant small-study effect.

**In Study III**, a univariable and multivariable logistic regression model yielding odds ratio with 95% CI was used to test the association of baseline characteristics with getting an anti-HDV screening test in 4095 patients with positive HBsAg and getting a positive anti-HDV test in 3703 patients who underwent screening. Also, in 202 patients with HDV RNA replication, the association of baseline parameters and delayed HDV screening was tested using the same model. Variables that were significant in the univariable model were re-introduced in the multivariable model. Whenever multiple surrogates of advanced liver disease were significant in the univariable model, only cirrhosis was introduced in the multivariable model to avoid multicollinearity.

**In Study IV**, a generalized linear model was applied to data to calculate the incidence rates and incidence rate ratios (IRR), using a log-link function together with a Poisson error distribution and an offset with the logarithm of follow-up time constrained to a coefficient of one. The model aimed to calculate the gender-specific age when the incidence rate would cross the surveillance threshold. To that end, a base model was fitted with HCC as the dependent variable and independent variables indicating gender and age on a continuous scale (centered around the mean) together with the interaction age*gender. The base model was used to calculate the gender-specific age when IR crossed above the surveillance threshold proposed at 0.2% and was also used to test if adding co-infections like HCV, HDV, and HIV and co-morbidity like diabetes and region of origin could add to the prediction. The incidence rate of HCC in African -born persons with CHB was compared to comparator cohorts and estimated the relative risk in persons younger than 40 years of age.

### 4.4.1 Sensitivity and subgroup analyses

In **Study I**, to mitigate survival bias (persons with severe disease tend to die early and persons with less severe disease tend to live and progress slowly) we considered analyzing persons newly diagnosed with HDV from 2000/2005.

In **Study II**, multiple subgroup analysis was performed by study design, coinfections, and region of origin.

In **Study III**, to mitigate survival bias, an analysis restricted to persons with an HBV/HDV diagnosis from the year 1992 was performed.
In **Study IV**, subgrouping on sex, and birth region was done.

Data management and statistical analyses were conducted using SPSS IBM Statistics (versions 22.0 and 28.0) in papers I, III, and IV. R version software was used in papers II and IV. SAS® (version 9.4, SAS Institute Inc., Cary, NC, USA) was used in paper IV. All P-values were two-tailed and statistical significance was set to P-value <0.05.

## 4.5 Ethical considerations

The studies constituting this thesis are epidemiological studies on human subjects so concerns might naturally arise from handling sensitive data, research integrity, and active consent of study subjects. The studies are addressing exposure to a viral infection and the outcomes related to this infection, in a particular minority group which might induce stigma. We believe the studies were conducted within the frame of the ethical guidelines, in concordance with the principle of no harm.

Ethical permits were granted in all studies (I, II, and IV) according to the Helsinki Declaration. Informed consent was obtained from study participants in study I and III. We did not exclude deceased persons, where informed consent was not obtained as this might introduce selection bias favoring survivors (survivorship bias). The studies were not interventional, so they did not affect the care of patients or pose extra costs or procedures on the patients. Naturally, there is an element of breach of a person's integrity as during reviewing patient charts other information is disclosed to us. Therefore, all personal identifications were coded during handling and analyzing the data and only data relevant to the aims of the study were collected (studies I and III). Study IV involved register-based data from the National Board of Health and Welfare and linked our data to other National Health registers, therefore informed consent was waived. The final data set contained pseudonymized information, replacing the personal identity number with a serial code, and the National Board of Health and Welfare temporarily holds the key to these codes.

Cognitive bias is a systematic thinking error that arises from judgment influenced by prior memories, experiences, or even personal traits. As the studies involve a predominantly migrant population from historical endemic regions of viral hepatitis, the possibility of stigma and discrimination could not be ruled out. The same applies to subgroups of patients who also have higher risk of exposure to viral hepatitis like PWID. It is important to note that this work aims to increase knowledge and understanding of the natural course of viral hepatitis B, and D which affects 1 in 5 individuals on earth, ultimately to assess and improve care for all persons affected irrespective of ethnicity or region or other affiliations. In this context, studying a smaller proportion of patients for the benefit of a larger group outweighs the harm. Studying risks is fundamental in medical research. Across different medical disciplines, certain diseases might be associated with or likely

more prevalent in certain groups than others. The aspired positive contribution of the current studies to the continuum of care for patients with viral hepatitis might alleviate such nuance of relating certain groups or ethnicity to certain conditions. Nevertheless, any potential breach of personal integrity by conducting these studies is very minimal and early adjusted for in the process of conducting the studies. However, we wished for patients' representatives to be involved in the study design for adding other perspectives that might be overlooked.

# 5 Results

## 5.1 Study I

We identified 337 patients with positive anti-HDV antibody followed for a mean of 6.5 years (SD 5.5). The baseline characteristics of study participants subgrouped by HDV RNA replication are presented in Table 4. Patients with HDV RNA positive were younger compared to persons without HDV RNA replication (mean age 36.3 vs 42.8 years). Most patients originated from Asia (n=151, 44.8%), and the majority were having HDV RNA replication (n=121, 80.1%). Despite the younger age at HDV diagnosis in persons with HDV RNA replication, 29.6 % had cirrhosis vs 8.8% in the group with HDV RNA negative (P<0.001). The parameters of advanced liver disease (lower thrombocyte count, higher APRI score, higher FIB4 score) were significantly prevalent in the HDV RNA positive group compared to peers with negative HDV RNA replication (all P values <0.05). A similar prevalence of diabetes and alcohol overconsumption in both groups could be noted.

Table 4. Baseline characteristics of all anti-HDV positive patients, and by HDV RNA viremic status. P-values of univariable analyses are shown. Reprinted from manuscript [92] under an open access Creative Commons license

| Characteristic | All† | HDV RNA positive | HDV RNA negative | *P* Value |
|---|---|---|---|---|
| | (n=337) | (n=233) | (n=91) | |
| Age, years, mean ± SD | 38.01±12.5 | 36.3±11.8 | 42.8±13.1 | <0.001* |
| Sex men, n (%) | 182 (54) | 119 (51.1) | 55 (60.4) | 0.12 |
| Patients region of origin, n (%) | | | | <0.001* |
| Europe | 61 (18.1) | 40 (17.2) | 17 (18.7) | |
| Asia | 151 (44.8) | 121 (51.9) | 25 (27.5) | |
| Africa | 61 (18.1) | 31 (13.3) | 27 (29.7) | |
| East Mediterranean | 63 (18.7) | 41 (17.6) | 21 (23.1) | |
| HBeAg pos, n patients (%) | 53/310 (17.1) | 42/218 (19.3) | 7/80 (8.8) | 0.03* |
| HBV DNA >2000 IU/ml, n patients (%) | 74/283 (26) | 48/204 (23.5) | 22/69 (31.9) | 0.16 |
| HBV DNA level, $log^{10}$ IU/ml, median (IQR)‡ | 1.8 (1.1-3.2) | 1.7 (1.0-3.1) | 2.4 (1.3-3.5) | 0.68 |
| HBsAg levels, $log^{10}$ IU/ml, median (IQR)# | 4.01(3.3-4.2) | 4.1(3.9-4.2) | 2.6 (1.4-3.35) | <0.01* |
| HDV RNA level, $log^{10}$ copies/ml, median (IQR)& | *na* | 6.9 (5.4-7.9) | *na* | |
| Diabetes mellitus, n patients (%) | 29 (8.6) | 17 (7.3) | 11 (12.2) | 0.15 |
| Alcohol overconsumption, n patients (%) | 42 (12.6) | 24 (10.4) | 13 (14.3) | 0.21 |
| Albumin, g/L, median (IQR) | 38 (35-41) | 37 (35-41) | 38 (36-41) | 0.05 |
| ALT, ×ULN, median (IQR) | 0.89 (0.47-1.5) | 1.1 (0.61-1.7) | 0.43 (0.27-0.94) | 0.03* |
| AST ×ULN, median (IQR) | 1.0 (0.69-1.7) | 1.2 (0.84-2.0) | 0.66 (0.53-0.9) | 0.07 |
| Thrombocytes, $10^9$/L, median (IQR) | 184 (139-233) | 173 (128-221.1) | 201 (165-266.3) | <0.001* |
| APRI score, median (IQR) | 0.62 (0.33-1.3) | 0.74 (0.42-1.6) | 0.32 (0.21-0.61) | 0.001* |
| Liver stiffness values, kpa, median (IQR)¶ | 8.3 (5.6-13.1) | 9.3 (6-14.1) | 4.9 (3.8-8.8) | 0.006* |
| FIB-4, median (IQR)§ | 1.3 (0.83-2.7) | 1.4 (0.83-2.7) | 1.2 (0.81-1.8) | 0.007* |
| Cirrhosis at baseline, n patients (%) | 79 (23.4) | 69 (29.6) | 8 (8.8) | <0.001* |
| Child Pugh classes, n patients (%), n=79 | | | | |
| A | 68 (86.1) | 60 (86.9) | 7 (87.5) | 0.13 |
| B | 4 (5.1) | 4 (5.8) | - | |
| C | 7 (8.9) | 5 (7.2) | 1 (12.5) | |
| BEA score, n patients (%), n=300 | | n=214 | n=78 | 0.04* |
| A | 151 (50.3) | 114 (53.2) | 32 (41) | |
| B | 141 (47) | 93 (43.4) | 45 (57.6) | |
| C | 8 (2.6) | 7 (3.2) | 1 (1.2) | |

*Abbreviations: HDV, hepatitis D virus; SD, standard deviation; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; na, not applicable; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal; BEA, baseline anticipation score.* †*= also included 14 patients with unknown HDV RNA status.* ‡*=available in 310 patients* #*= available in 132 patients.* &*=available in 163 patients.* ¶*= available in 136 patients.* §= available in 284 patients. *P-values < 0.05 are marked with **

During follow-up, 43 (12.8%) patients with anti-HDV positive developed any liver-event, 13 (3.9%) developed HCC, 37 (11.0%) experienced a decompensation event and 28 (8.3%) had liver transplantation or liver-related death. Cirrhosis was associated with 11 times (95% CI 5.7-21.3) the risk to develop any liver-related event and a year-older carried a 5% higher risk (95% CI 1.04-1.08). Patients with HDV RNA replication were associated with 3.8 times the risk (95% CI 1.5-9.8) to develop any liver-related event, a trend towards increased HCC risk (HR=2.6, 95% CI 0.6-11.8), 4.3 times the risk (95% CI 1.5-12.2) to develop

a decompensation event, and 7.4 times the risk (95% CI 1.7-31.8) to experience a liver-related death or liver transplantation.

The cumulative event free survival for different outcomes of interest comparing patients with HDV RNA positive with HDV RNA negative is shown in Figure 8.

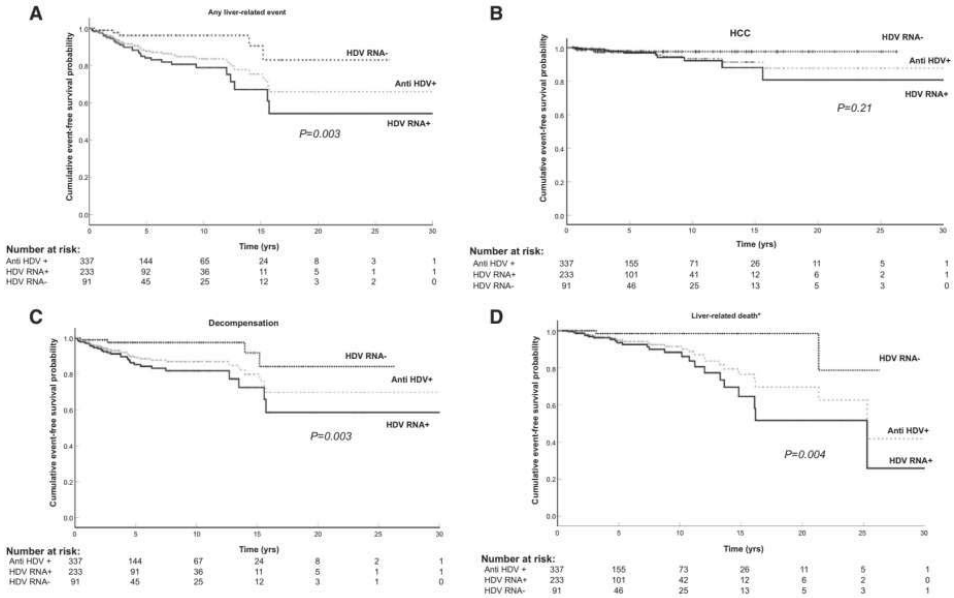


Figure 8. Kaplan-Meier curves showing the cumulative event-free survival of different liver-related outcomes comparing patients with HDV RNA positive with HDV RNA negative. A Pairwise Log-rank test is shown.
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In 233 patients with HDV RNA replication, in multivariable analyses, cirrhosis remained the strongest predictor of developing a liver-event (adjusted hazard ratio aHR=13.6, 95% CI 3.7-49.3) and Asian origin (aHR=6.4, 95% CI 2.0-20.3). Of note, in this group men had a similar risk as women to develop a liver-event (HR=1.6, 95% CI 0.82-3.2), IFN therapy regardless of virological response was not associated with a better outcome (HR=0.83, 95% CI 0.43-1.61) with a trend towards negative association.

One hundred eight (46.4%) patients received IFN therapy for a median duration of 8.7 months (IQR, 6.0-13.7), of whom 57 (52.8%) had overlapping or simultaneous NUC therapies. In total, 19 (18.8%) patients had HDV RNA undetected at 24 and/or 48 weeks after IFN therapy and were considered virological responders. Only one patient lost HBsAg in virological responders vs none in non-responders. In virological responder 2 (10.5%) developed a liver event contrasted to 15 (18.5%) in the non-responder group. The difference was not statistically significant shown in Figure 9.

During a median follow-up of 25.4 months (IQR, 24-92) after treatment ends, 6 of 19 (31.5%) virological responders had detected HDV RNA (relapsed)

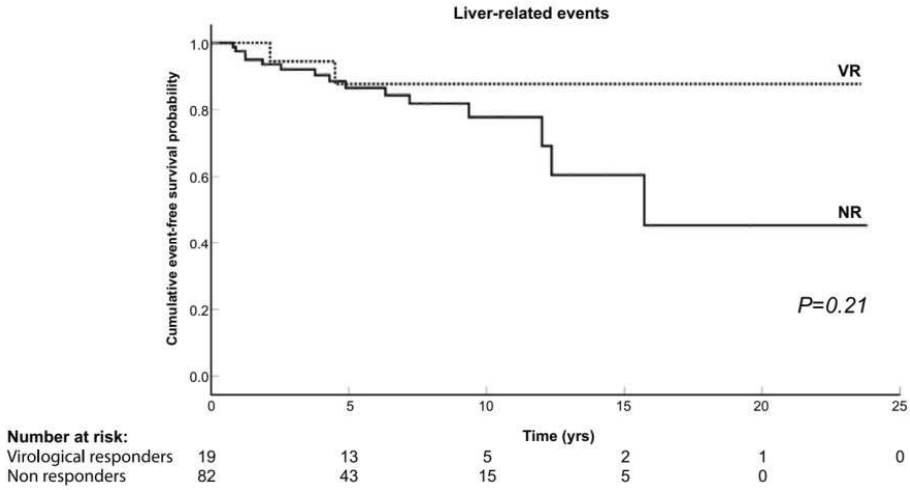


Figure 9. Kaplan Meier curves showing event-free survival of liver-related outcomes in virological responders compared to non-responders. A Pairwise Log-rank test is shown. VR= virological responder defined a negative HDV RNA 24/48 weeks after IFN therapy cessation. NR=non-responder.
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## 5.2 Study II

A total of 12 studies were included in the systematic review and meta-analysis. The studies included overall 63719 persons with CHB, and 6099 (9.7%) were with HBV/HDV coinfection. All study participants were recruited from hospitals. Studies were mostly from Europe, five studies involved nationwide cohorts, six involved hospital-based single centers and one was a multicenter study. Patients were enrolled from 1991 to 2019. The diagnosis of HDV infection was based on the detection of anti-HDV antibody in all studies, only 4 studies provided HDV RNA testing for some patients. The diagnosis of HCC was based on ICD codes in 4 studies, and on radiological, laboratory, and clinical findings in 8 studies. Studies reported a mean duration of 6.5 years of follow-up (range, 2.8-12.3). Three studies enrolled Asian populations, 4 studies enrolled Caucasian populations and 5 studies had mixed populations. In persons with HBV/HDV coinfection, the mean age was 40.4 years, and men constituted 54.7% to 97.0% of participants. In persons with HBV mono-infection, the mean age was 45.9 years and men constituted 58.4% to 96.4% of participants. The prevalence of cirrhosis was 57.2% in persons with HBV/HDV co-infection and 15.5% in persons with HBV mono-infection shown in Table 5.

Table 5. Patients' level characteristics in studies included in the meta-analysis. Reprinted from manuscript [216] under an open access Creative Commons license.

| Study | Total number of participants | Group | Sample size | Age, years, median or mean | Men, % | HDV RNA, % | Population source, % | | | | Cirrhosis*, % | HIV % | HCV % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Cau. | Asi, | Afr. | other | | | |
| **Colombo et al, 1991** [217] | 447 | HBV/HDV<br>HBV | 24<br>46 | Overall<br>55 | Overall<br>68.9 | ND | 100 | - | - | - | 100<br>100 | ND | Separate analysis |
| **Tamura et al, 1993** [218] | 1,127 | HBV/HDV<br>HBV | 69<br>1,058 | 72%<br>(20-49)<br>ND | ND<br>ND | ND | - | 100 | - | - | 12<br>4 | ND | ND |
| **Fattovich et al, 2000** [219] | 200 | HBV/HDV<br>HBV | 39<br>161 | 34<br>49 | 76.9<br>88.8 | ND | 100 | - | - | - | 100<br>100 | Excl. | ND |
| **Caturelli et al, 2003** [220] | 402 | HBV/HDV<br>HBV | 100<br>94 | 46<br>55 | 56<br>70.2 | ND | 100 | - | - | - | 100<br>100 | ND | Separate analysis |
| **Liaw et al, 2004** [221] | 192 | HBV/HDV<br>HBV | 64<br>128 | 34<br>41 | 90.0<br>77.4 | ND | - | 100 | - | - | ND<br>ND | ND | ND |
| **Sheng et al, 2007** [222] | 104 | HBV/HDV<br>HBV | 26<br>78 | 35<br>34 | 96.2<br>96.2 | 36.8 | - | 100 | - | - | ND<br>ND | 100 | 4.8 |
| **Ji et al, 2012** [76] | 9,160 | HBV/HDV<br>HBV | 327<br>8,510 | 34<br>ND | 54.7<br>ND | ND | ND | ND | ND | ND | ND<br>ND | Excl. | Excl. |
| **Manesis et al, 2013** [223] | 2,137 | HBV/HDV<br>HBV | 74<br>1,885 | 43<br>47 | 62.2<br>67.4 | ND | 34<br>90.8 | ND | ND | -<br>4.5 | 17.0<br>7.1 | Excl. | Excl. |
| **Kushner et al. 2015** [155] | 25,603 | HBV/HDV<br>HBV | 73<br>1,935 | 51<br>52 | 97.2<br>96.4 | ND | 51<br>50 | 5.5<br>5.1 | 36<br>34 | 8.1<br>11.3 | 30<br>15 | ND | ND |
| **Beguelin et al, 2017** [224] | 727 | HBV/HDV<br>HBV | 104<br>623 | 34<br>36 | 77.0<br>78.2 | 70.2 | 87<br>68.4 | -<br>6 | 13<br>21 | -<br>4.5 | ND | 100 | ND |
| **Mallet et al, 2017** [129] | 48,189 | HBV/HDV<br>HBV | 5143<br>43,046 | Overall<br>44 | 60.1<br>58.4 | ND | ND | ND | ND | ND | ND | 16.1<br>11.4 | Overall<br>15.1 |
| **Brancaccio et al, 2019** [225] | 112 | HBV/HDV<br>HBV | 56<br>56 | 52<br>52 | 73.2<br>73.2 | 100 | 100 | - | - | - | 80.4<br>82.1 | Excl. | Excl. |

*Abbreviations: HDV= Hepatitis delta virus; HBV=hepatitis B virus; HIV=human immunodeficiency virus; HCV=hepatitis C virus; RNA=ribonucleoprotein; Cau. =Caucasian; Asi.=Asian; Afr.=African; ND= not determined. *=cirrhosis diagnosis at study enrollment*

The quality of studies ranged from 6 to 9 per NOS score. Two studies were matched on age, sex, and liver functions, two studies adjusted for the date of enrollment and overall, there was sparse data on the loss to follow-up.

In persons with HBV/HDV coinfection, 9.7% developed HCC compared to 5.1% in peers with HBV mono-infection. The pooled relative risk was 2.12 times increased risk of HCC (95% CI 1.14-3.95) with moderate statistical heterogeneity (Figure 10). In studies providing adjusted estimates the pooled relative risk was 2.50 (95% CI 1.37-4.57) with moderate statistical heterogeneity ($I^2$=70%).

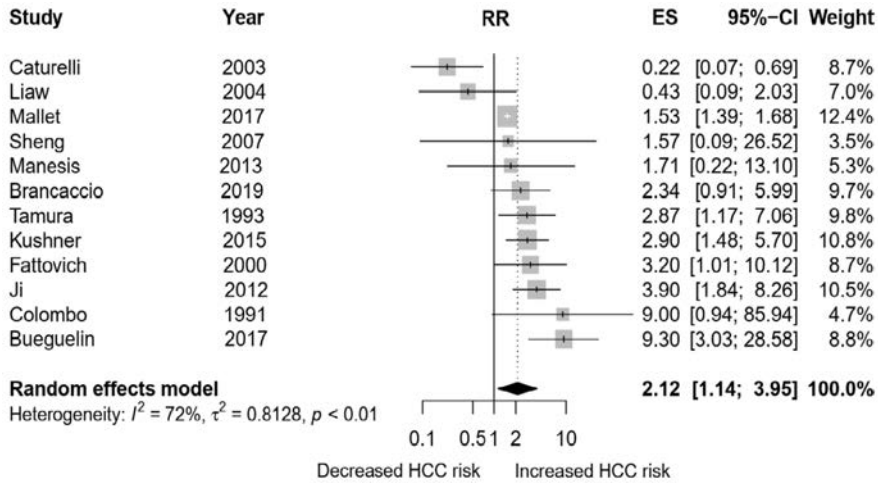



Figure 10. The pooled relative risk of HCC across the studies included in the meta-analysis. Reprinted from manuscript [216] under an open access Creative Commons license.

The funnel plot shown below in Figure 11 did not demonstrate asymmetry and Egger's test did not indicate a small study effect.

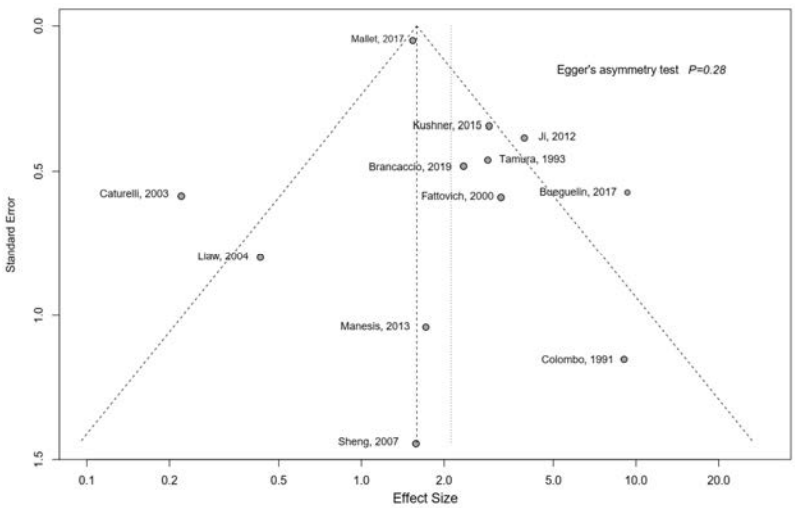



Figure 11. Funnel plot of included studies. Reprinted from manuscript [216] under an open access Creative Commons license.

Table 6. The pooled relative risk by different subgroup analyses. Reprinted from manuscript [216] under an open access Creative Commons license.

| All investigated determinants | | Studies (n) | ES | 95% CI | $I^2$% | Heterogeneity |
|---|---|---|---|---|---|---|
| **Study design** | All studies | 12 | 2.12 | 1.14-3.95 | 72 | Moderate |
| | Prospective | 6 | 3.56 | 1.76-7.22 | 5 | Low |
| | Retrospective | 6 | 1.43 | 0.57-3.62 | 80 | High |
| | Excluding *Mallet et al.** [129] | 11 | 2.21 | 1.11-4.41 | 67.6 | Moderate |
| **Adjustment or matching** | Only adjusted/matched studies | 8 | 2.50 | 1.37-4.57 | 70 | Moderate |
| | Studies with no adjustment | 4 | 1.54 | 0.33-7.10 | 80 | High |
| **Disease stage** | All patient with cirrhosis | 4 | 1.73 | 0.39-7.79 | 81 | High |
| | Different disease stages | 8 | 2.44 | 1.31-4.54 | 70 | Moderate |
| **HIV or HCV population** | Only HIV population | 2 | 5.85 | 1.13-30.36 | 24 | Low |
| | Without HIV population | 4 | 3.05 | 1.79-5.21 | 0 | Low |
| | Without HCV population | 3 | 2.99 | 1.61-5.58 | 0 | Low |
| **Population source** | Asian population | 3 | 1.39 | 0.41-4.76 | 54 | Moderate |
| | Caucasian population | 4 | 1.73 | 0.39-7.79 | 81 | High |
| | Heterogeneous population | 5 | 2.93 | 1.55-5.51 | 79 | High |

**Largest sample size among studies*

In subgroup analyses, HBV/HDV was associated with increased risk in prospective studies (pooled RR=3.6, 95% CI 1.8-7.2) with low heterogeneity ($I^2$=5%). An increased risk in studies with HBV/HDV/HIV co-infection could be demonstrated (pooled RR =5.9, 95% CI 1.13-30.4) with low heterogeneity ($I^2$=24%). Studies on Asian and Caucasian populations with HBV/HDV co-infection did not show a significant positive association with HCC development (Table 6).

Univariable meta-regression analysis did not point to confounding by study level characteristics such as study design, NOS scale, publication year, and follow-up time.

## 5.3 Study III

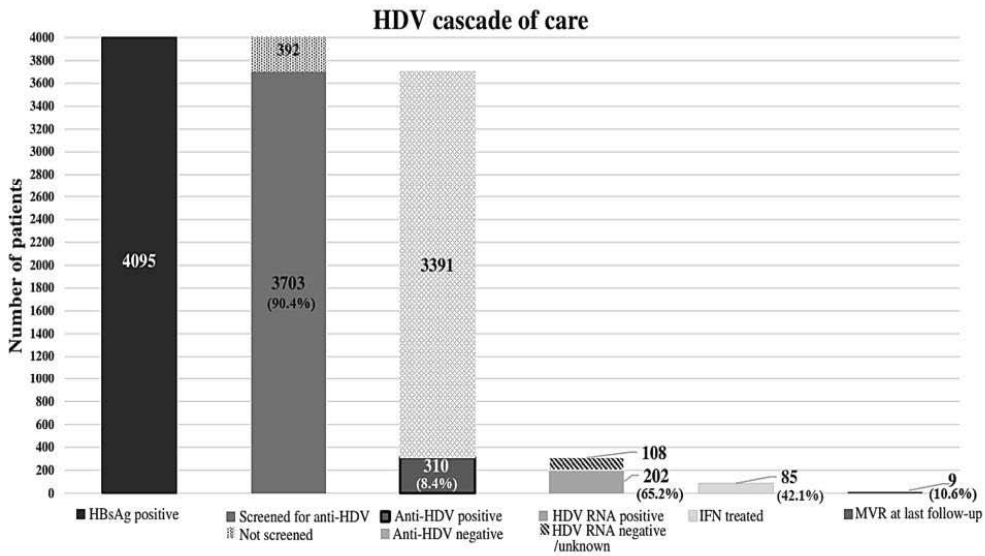
We identified 4095 patients with positive HBsAg cared for at KUH, among whom 3703 (90.4%) had an anti-HDV screening test. In 2367 (64.0%), the screening test was performed less than one year from HBV diagnosis within a median of 1.8 months (IQR 0.0-57.1). There was no difference in sex and age between those screened and unscreened for anti-HDV. Persons with HBV/HIV were more prevalent among those unscreened (8.7% vs 2.1%, p<0.001), and persons reporting a known route of transmission were more prevalent in the unscreened group compared to the screened one (63.8% vs 41.7%, p<0.001). When applying AASLD target screening for high-risk, screened persons were more likely to meet the "high-risk" criteria compared to unscreened (83.4% vs 68.1%, p<0.001). Unscreened persons had higher serum AST, HBsAg, and APRI score compared to the screened group as shown in Table 7.

Table 7. Baseline characteristics of patients diagnosed with positive HBsAg grouped by ever receiving an anti-HDV screening test

| Parameter, proportions per column | All HBsAg positive | HDV tested | No HDV test | P-value |
|---|---|---|---|---|
| Number, (%) | 4095 (100) | 3703 (90.4) | 392 (9.6) | <0.001 |
| Men, n (%) | 2292 (56.0) | 2067 (55.8) | 225 (57.4) | 0.55 |
| Women, n (%) | 1803 (44.0) | 1636 (44.2) | 167 (42.6) | 0.63 |
| Age at HBV diagnosis, median (IQR) | 32.8 (25.3-42.2) | 32.8 (25.4-42.3) | 32.2 (24.2-42.0) | 0.11 |
| Age at HDV testing, median (IQR) | 36.3 (28.9-45.7) | 36.3 (28.9-45.7) | na | na |
| **Area of origin, n (%) per row** | | | | 0.004 |
| America/South America | 35 (100) | 29 (82.9) | 6 (17.1) | |
| Africa | 965 (100) | 875 (90.7) | 90 (9.3) | |
| Asia | 1934 (100) | 1777 (91.9) | 157 (8.1) | |
| Europe | 599 (100) | 523 (87.3) | 76 (12.7) | |
| Eastern Mediterenean ¥ | 511 (100) | 468 (91.6) | 43 (8.4) | |
| Not available (missing) | 562 (100) | 499 (92.0) | 63 (11.1) | |
| **Co-infection/known risk factor, n (%) per column** | | | | |
| Co-infection with HCV | 66/4073 (1.6) | 57/3684 (1.5) | 9/389 (2.3) | 0.26 |
| Co-infection with HIV | 132/3754 (3.2) | 102/3411 (2.1) | 30/343 (8.7) | <0.001 |
| Eligible per AASLD criteria¤ | 3357 (82.0) | 3090 (83.4) | 267 (68.1) | <0.001 |
| Any risk factor for infection excluding endemic region ¤ | 520/1210 (43.0) | 476/1141 (41.7) | 44/69 (63.8) | <0.001 |
| **Laboratory and virological parameters** | | | | |
| Haemoglobin, g/dl, median (IQR) | 14.3 (12.8–15.5) | 14.3 (12.9–15.5) | 14.5 (13.0–15.4) | 0.90 |
| ALT ukat/l, median (IQR) | 0.50 (0.35-0.80) | 0.49 (0.35-0.79) | 0.53 (0.37-0.87) | 0.04 |
| ALT> upper limit of normal, n (%) | 426/2447 (17.4) | 403/2328 (17.3) | 23/119 (19.3) | 0.57 |
| ALT> 2 times upper limit of normal, (%) | 138/2447 (5.6) | 129/2328 (5.5) | 9/119 (7.6) | 0.35 |
| AST ukat/l, median (IQR) | 0.48 (0.39-0.64) | 0.47 (0.38-0.62) | 0.54 (0.44-0.88) | <0.001 |
| Albumin g/dl, median (IQR) | 39 (36–42) | 39 (36–42) | 40 (37–43) | 0.20 |
| Platelets ($\times 10^3/mm^3$), median (IQR) | 225 (190–263) | 224 (187–263) | 230 (180.7–282.3) | 0.17 |
| HBV DNA, log10 IU/ml | 2.9 (1.8–3.7) | 2.7 (1.9–3.5) | 2.8 (1.8–3.7) | 0.14 |
| HBsAg, log10 IU/ml | 3.5 (2.7–4.0) | 3.5 (2.7–4.0) | 3.6 (1.3–4.4) | <0.001 |
| HBeAg positive | 602/3974 (15.1) | 539/3617 (14.9) | 63/357 (17.6) | 0.19 |
| Serum HDV RNA IU/ml& | na | 6.2 (4.3-7.2) | na | na |
| **Fibrosis parameters, n (%)** | | | | |
| Liver stiffness*, Kpa, median (IQR) | 5.30 (4.30-6.90) | 5.40 (4.30-6.30) | 5.50 (5.5–11.0) | 0.30 |
| Liver stiffness≥12.5, n (%) | 102/1940 (5.3) | 99/1915 (5.2) | 3/25 (12.0) | 0.14 |
| APRI score, median (IQR) | 0.29 (0.22-0.41)) | 0.29 (0.22-0.39) | 0.29 (0.23-0.43) | <0.001 |
| APRI score ≥1.5, n (%) | 103/3490 (3.0) | 88/3220 (2.7) | 15/270 (5.6) | 0.004 |
| FIB4, median (IQR) | 0.96 (0.71-1.36) | 0.95 (0.71-1.35) | 1.10 (0.83-1.53) | 0.23 |
| FIB4 score ≥3.25, n (%) | 116/3186 (3.6) | 103/2957 (3.5) | 13/229 (5.7) | 0.09 |
| **Cirrhosis diagnosis, n (%)** | 270/4073 (6.6) | 250/3684 (6.8) | 20/389 (5.1) | 0.22 |
| **Time to HDV testing** | | | | |
| Time to HDV testing from HBV test, month, median (IQR) | 1.8 (0.0–57.1) | 1.8 (0.0–57.1) | na | n |
| Access to HDV testing, less than one year from, n (%) per column | 2367 (57.8) | 2367 (64.0) | na | n |

*All values are within 3 months of HBV diagnosis; ¤=Per American Association for the Study of the Liver eligible criteria for screening. including history of blood transfusion or exposure to blood products, family member with HBV infection, possible sexual contact, intravenous drug use. *= values within 6-12 months of diagnosis including corresponding values of persons who undergone liver biopsy;&available in 154 patients, HBsAg=hepatitis B surface antigen ;¥Eastern Mediterranean includes countries from Europe, Asian and Africa (not added to the total number per region);HBV=hepatitis B virus; SD=standard deviation; HDV=hepatitis Delta virus; HCV= hepatitis C virus; HIV=human immunodeficiency virus; IQR=interquartile range; ALT=alanine aminotransferase; AST=aspartate aminotransferase; na=not applicable; APRI=AST to Platelet Ratio=(AST level (IU/L)/ AST (upper limit of normal (IU/L))/platelet count (109/L) ×100; FIB-4= age(yrs.)×AST level(U/L)/(platelet count [109/L]×√(ALT[U/L]) numbers are rounded to the 2nd decimal.*

Among patients who had a screening test, 310 (8.4%) were anti-HDV positive, in 202 (65.2%) HDV RNA replication was detected, 99 (31.9%) had undetectable HDV RNA and 9 (2.9%) have not been tested for HDV RNA. Eighty-five (42.1%) received an IFN therapy, 68 (80%) completed ≥one year therapy where 9/85 (10.6%) achieved negative HDV RNA from baseline (Figure 12).




*Abbreviations: HBsAg= hepatitis B surface antigen; HDV=hepatitis Delta virus; IFN=interferon, MVR=maintained virological response defined as negative HDV RNA at 6 months post-treatment end and the last follow-up. X-axis represents the cascade of tests/treatment; Y-axis represents the number of patients.*

Figure 12. The cascade of care of HDV

Factors significantly associated with more odds to get a screening test were Asian origin (OR=1.37, 95% CI 1.10-1.72), diagnosis after the year 2012 (OR=6.34, 95% CI 4.80-8.38), while HIV co-infection (OR=0.32, 95% CI 0.21-0.49) and known route of transmission (OR=0.41, 95% CI 0.25-0.67) were associated with less odds to get screened. In multivariable analysis, only HIV coinfection remained significant (aOR=0.10, 95% CI 0.01-0.95). The strongest predictors associated with getting an anti-HDV positive test were cirrhosis (aOR=9.35, 95% CI 4.87-17.95), HCV co-infection (aOR=9.24, 95% CI 3.16-26.99), while being of Asian origin and getting diagnosed after the year 2012 were associated with 2.34 and 1.95 respectively the odds of getting a positive anti-HDV test.

We assessed the factors associated with a liver-related outcome, in multivariable analysis older age, cirrhosis, and delayed HDV screening tests were significantly associated with higher odds of a liver-event as shown in Table 8.

Table 8. Logistic regression analysis of variables associated with developing any liver-related event defined as HCC, decompensation event, liver transplantation, and/or liver-related death (n=53) in patients with HDV RNA replication (n=202)

| | **Univariable** | | **Multivariable** | |
|---|---|---|---|---|
| **Parameter** | **OR, 95% CI** | **P- value** | **aOR, 95% CI** | **P-value** |
| Age at HDV diagnosis (continuous scale) | 1.07 (1.04-1.10) | <0.001* | **1.04 (1.00-1.07)** | **0.05** |
| Male vs female | 1.69 (0.88-3.24) | 0.11 | | |
| Asian vs non-Asian | 1.55 (0.66-3.62) | 0.32 | | |
| HCV co-infection | 0.83 (0.29-2.38) | 0.73 | | |
| HIV co-infection | 2.18 (0.35-13.49) | 0.40 | | |
| Known route of transmission | 0.84 (0.32-2.19) | 0.73 | | |
| Diagnosis of cirrhosis | 33.00 (11.18-97.40) | <0.001* | **34.0 (10.94-105.66)** | **<0.001** |
| IFN exposed vs non-exposed | 2.13 (1.11-4.9) | 0.02* | 0.96 (0.39-2.39) | 0.93 |
| Maintained virological response | 1.37 (0.51-3.70) | 0.54 | | |
| Reference anti-HDV screening < 2 years after HBV diagnosis | -- | -- | -- | |
| Anti-HDV screening 2-<5 years | 2.48 (0.75-8.26) | 0.14 | 3.46 (0.62-19.47) | 0.25 |
| Anti-HDV screening ≥ 5 years | 3.47 (1.35-8.96) | 0.01* | **7.58 (1.82-31.58)** | **0.01** |

**In univariable analyses variables were tested individually. Age, cirrhosis, IFN treatment, and HDV screening test > 2 years were introduced in the multivariable model yielding adjusted odds ratio. Abbreviations: HBV=hepatitis B virus; HDV=hepatitis D virus; HCV=hepatitis C virus; HIV=human immunodeficiency virus; IFN=interferon; OR=odds ratio; aOR=adjusted odds ratio; CI=confidence interval.*

## 5.4 Study IV

In total 3865 African-born persons with CHB, 58.6% were men. The mean age at HBV notification was 32.1 (SD 11.1) with similar mean age in men and women (32.5 vs 31.4, p=0.19). Most persons with CHB were diagnosed after the year 2000 (77.7%). Women were less likely to have>10 years of education than men (p<0.001). The majority of the cohort was born in Eastern Africa (64.1), followed by Western Africa. Men had more prevalent HCV co-infection, alcohol overconsumption, drug misuse, diabetes, and anti-viral therapies (all p values <0.05), while women had more prevalent obesity (1.6% vs 0.1%, p<0.001). Overall, 19.3% of men had any coinfection and/or diabetes versus 15.5% of women (p=0.002).
The study flow chart in Figure 13 and Table 9 show the distribution of African countries/ regions and baseline characteristics of the cohort.

The study included a cohort of 8488 persons matched on age, sex, county of residence, and same country of birth, and a cohort of 39267 persons matched on age, sex, and county of residence from the general population, both cohorts without HBV infection.

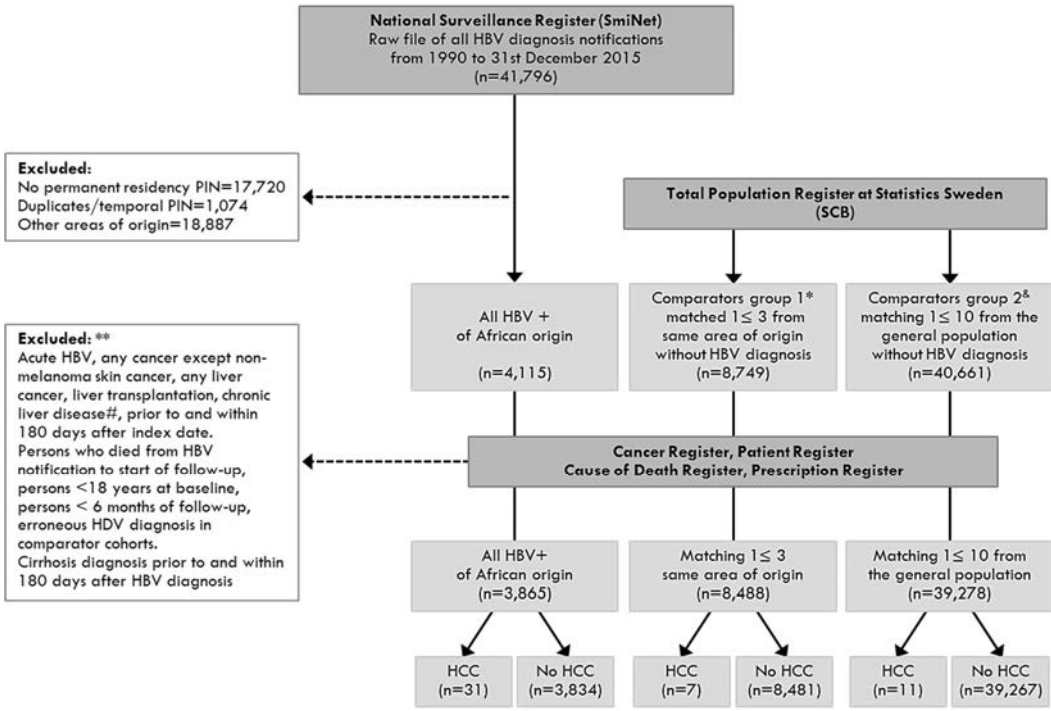



Figure 13. Flow chart of the study participants

Table 9. Baseline characteristics of African-born persons with chronic hepatitis B (CHB) and without cirrhosis, living in Sweden. The characteristics for all and grouped by sex are shown.

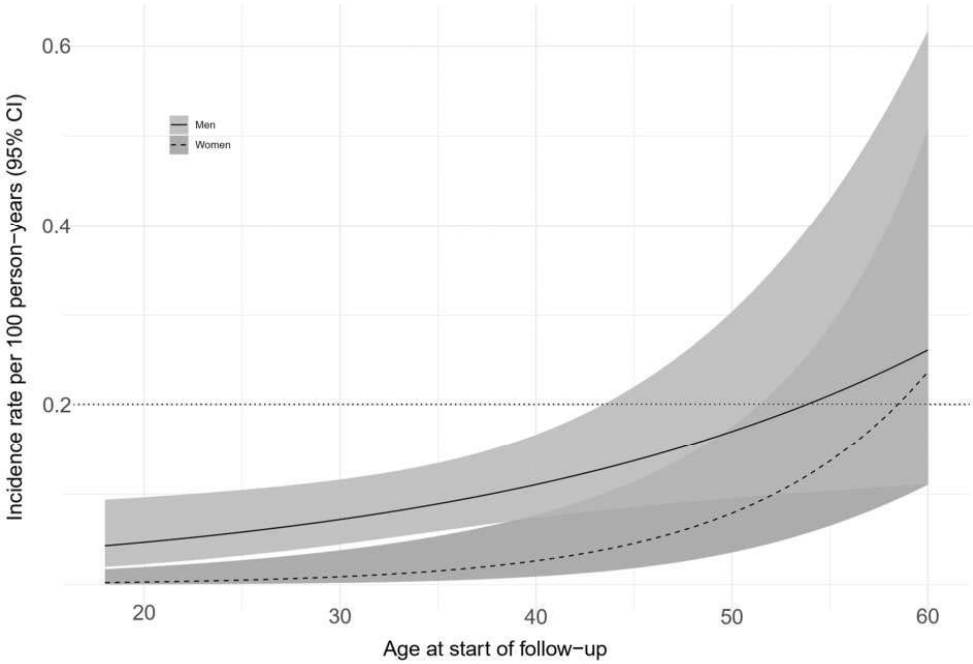
| Characteristics | All (n, %) | Men (n, %) | Women (n, %) | P-value |
|---|---|---|---|---|
| **Total** | 3865 | 2266 (58.6) | 1574 (40.7) | |
| **Age at start of follow-up (years)** | | | | |
| All, mean (SD) | 32.1 (11.2) | 32.5 (11.2) | 31.4 (11.2) | 0.19 |
| **Age groups** | | | | <0.001 |
| 18–29 | 1805 (46.7) | 994 (43.9) | 811 (50.7) | |
| 30–39 | 1252(32.4) | 715 (31.6) | 537 (33.6) | |
| 40–49 | 531 (13.7) | 398 (17.6) | 133 (8.3) | |
| >50 | 277 (7.2) | 159 (7.0) | 118 (7.4) | |
| **HBV diagnosis date (years)** | | | | <0.001 |
| 1990- <2000 | 861 (22.3) | 469 (20.7) | 392 (24.5) | |
| 2000- <2010 | 1554 (40.2) | 895 (39.5) | 659 (41.2) | |
| 2010–2015 | 1450 (37.5) | 902 (39.8) | 548 (34.3) | |
| **Education level (years in school)** | | | | <0.001 |
| ≤9 | 1395 (36.1) | 778 (34.3) | 617 (38.6) | |
| 10–12 | 1246 (32.2) | 756 (33.4) | 490 (30.6) | |
| >13 | 858 (22.2) | 567 (25.0) | 291 (18.2) | |
| Missing | 366 (9.5) | 165 (7.3) | 201 (12.6) | |
| **African region of birth** | | | | 0.90 |
| Northern | 261 (6.8) | 152 (6.7) | 109 (6.8) | |
| Eastern | 2478 (64.1) | 1455 (64.2) | 1023 (64.0) | |
| Middle | 240 (6.2) | 152 (6.7) | 94 (5.9) | |
| Western | 874 (22.6) | 505 (22.3) | 369 (23.1) | |
| Southern | 12 (0.3) | 8 (0.4) | 4 (0.3) | |
| **Co-morbidities** | | | | |
| HCV co-infection | 194 (5.0) | 130 (5.7) | 64 (4.0) | 0.02 |
| HDV co-infection | 105 (2.7) | 63 (2.8) | 42 (2.6) | 0.77 |
| HIV co-infection | 126 (3.3) | 75 (3.3) | 51 (3.2) | 0.84 |
| Alcohol overconsumption | 93 (2.4) | 75 (3.3) | 18 (1.1) | <0.001 |
| Diabetes mellitus | 326 (8.4) | 221 (9.8) | 105 (6.6) | <0.001 |
| Obesity | 28 (0.7) | 3 (0.1) | 25 (1.6) | <0.001 |
| Drug misuse | 60 (1.6) | 47 (2.1) | 13 (0.8) | 0.002 |
| Co-morbidity* | 686 (17.7) | 438 (19.3) | 248 (15.5) | 0.002 |
| **Co-medications** | | | | |
| Interferon therapy | 39 (1.0) | 32 (1.4) | 7 (0.4) | 0.003 |
| Nucleos(t)ide analogues | 260 (6.7) | 178 (7.9) | 82 (5.1) | <0.001 |
| Statin | 224 (5.8) | 157 (6.9) | 67 (4.2) | <0.001 |
| Aspirin | 135 (3.5) | 81 (3.6) | 54 (3.4) | 0.74 |
| **Follow-up time (years)** | | | | |
| Mean (SD) | 12.4 (6.7) | 12.0 (6.6) | 13.1 (6.8) | 0.51 |

*Abbreviations: CHB=chronic HBV; SD= standard deviation; HCV=hepatitis C virus; HDV=hepatitis Delta virus; *=HIV and or HCV and/or HDV and/or diabetes.*

During a median follow-up (IQR) of 11.1 (6.6-17.6) years, corresponding to 48,066 person-years, 31 (0.8%) persons developed HCC. Most were men (n=24, 77.4%) and overall, 52% did not have cirrhosis diagnosis at HCC diagnosis. The mean age at HCC diagnosis was 51.4 (±16.6) years, with significant younger age in men than in women (46.8 vs 67.0 years; p=0.03). In 10 (42%) men, HCC was diagnosed younger than 40 years old, in whom 5 (50%) did not have cirrhosis at HCC diagnosis, and 4 (40%) had any co-morbidity. For the 5

(50%) who had cirrhosis at HCC, all received a diagnosis of cirrhosis less than 3 months before HCC diagnosis.

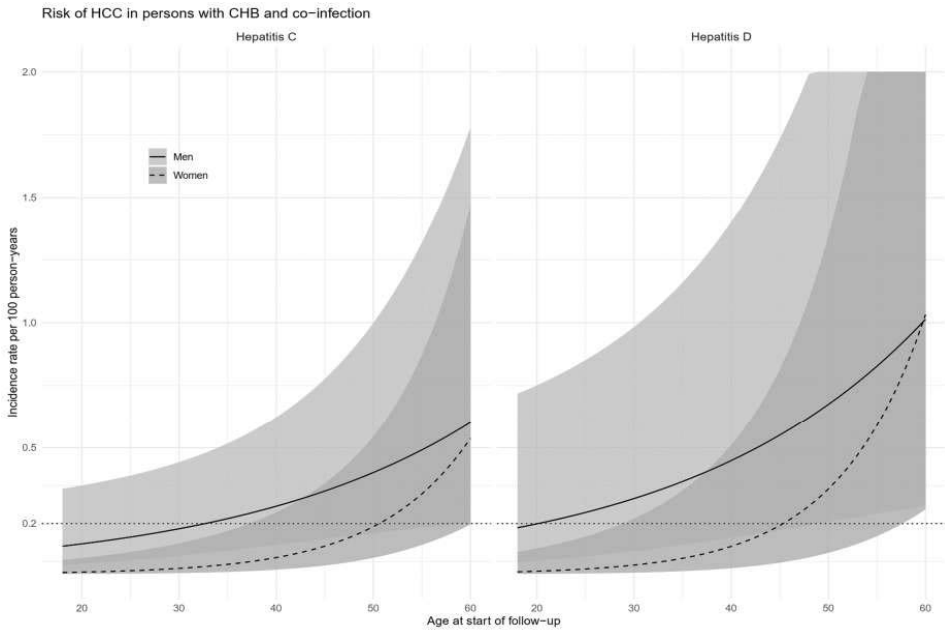
The IR of HCC in men was 0.09/100PYs (0.06-0.13), while in women was 0.03/100PYs (0.01-0.07). The proposed 0.2% cost-effectiveness surveillance threshold was exceeded in men at age 54 years (IR=0.20/100PYs, 95% CI 0.10-0.40) and in women at age 59 years (IR=0.21/100PYs, 0.10-0.45) as shown in Figure 14. Men compared to women had an incidence rate ratio of 2.66 (95% CI 1.21-6.68) as shown in Table 10. Men demonstrated an increased risk in younger age than older age compared to women as suggested by the interaction between male sex and age yielding an IRR of 0.94 (95% CI 0.88-0.99) and suggested by Figure 14 where from around age 45 the risk is women is accelerating more than in men.




*X-axis represents the age at baseline on a contiuous scale. Y-axis represents the incidence rate of HCC per 100 persons per year. The dotted horizontal line marks an incidence rate 0.2% of cost-effective HCC surveillance in individuals without cirrhosis. The 0.2% IR was exceeded in men at age 54 years (IR=0.20/100PYs, 95%CI 0.10-0.40) and in women at age 59 years (IR=0.21/100 PYs, 95%CI 0.10-0.45). Age*sex interaction was IRR (0.94, 95% CI 0.88-0.99) suggesting that the increased risk was more pronounced in younger age in men and attenuated with older age compared to women. Abbreviations: HCC= hepatocellular carcinoma; CHB=chronic hepatitis B; CI=confidence interval.*

Figure 14. The incidence rates of hepatocellular carcinoma (HCC) per 100 person-year in African-born persons with CHB and without cirrhosis at baseline, by sex.

In men with HBV/HCV co-infection, the surveillance threshold was exceeded at age 33 years (IR=0.20/100PYs, 95% CI 0.08-0.48) and in women at age 51 years (IR=0.20/100PYs, 0.07-0.60). In men with HBV/HDV co-infection, the threshold was crossed at 20 years-old (IR=0.21/100PYs, 95% CI 0.05-0.75) and at 46 years-old in women (IR=0.20/100PYs, 95% CI 0.05-0.90) as shown in Figure 15.




*In persons with HBV/HCV co-infection, the 0.2% surveillance threshold was crossed at age 33 years old in men (IR=0.20/100PYs, 95% CI 0.08-0.48) and in women at age 51 years-old (IR=0.20/100PYs,95% CI 0.07-0.60). In persons with HBV/HDV co-infection, the threshold was crossed at 20 years-old in men (IR=0.21/100PYs, 95% CI 0.05-0.75) and at 46 years-old in women (IR=0.20/100PYs, 95% CI 0.05-0.90).*

Figure 15. The incidence rates of hepatocellular carcinoma (HCC) per 100 person-year in African-born persons with CHB and without baseline cirrhosis by HCV and HDV coinfection.

Middle Africans showed younger age at HCC diagnosis (Figure 16) and when comparing the IRR across the different African regions of birth, having Eastern Africans as a reference, Middle Africans had 3.8- times the risk (95% CI 1.08-10.65), Northern Africans had 1.73 (95% CI 0.47-5.38) and Western Africans had 1.77 (95% CI 0.66-4.32) shown in Table 10.

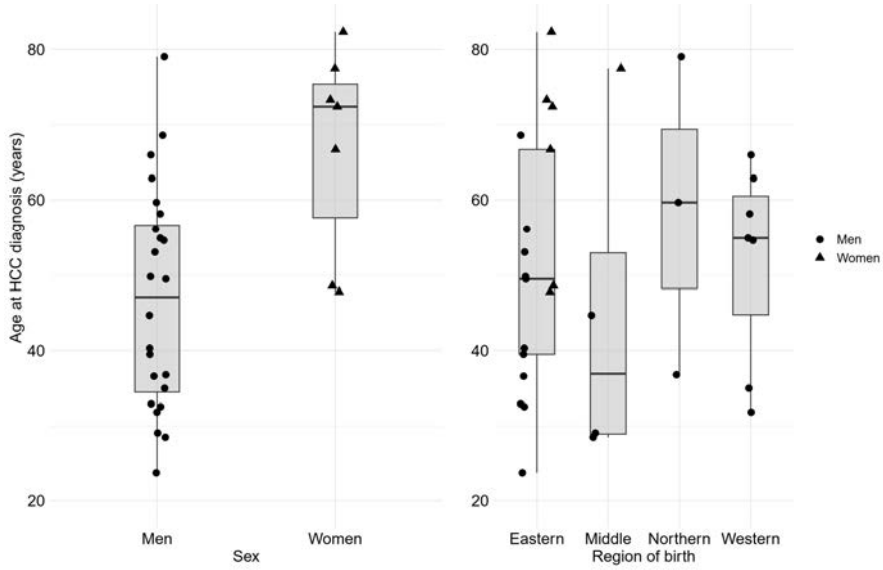



Figure 16. Box plot with dots showing the median age at HCC diagnosis by sex and region of birth.

Table 10. Incidence rate ratios (IRR) for HCC derived from Poisson regression models presenting the association of variables in a chronic hepatitis B cohort of persons with African origin.

| | Univariable | | | Base model (multivariable) | | | Adjusted estimates[#] | | |
|---|---|---|---|---|---|---|---|---|---|
| **Predictors** | **IRR** | **95% CI** | **p** | **IRR** | **95% CI** | **p** | **IRR** | **95% CI** | **p** |
| **Base Model** | | | | | | | | | |
| Age (centered) | 1.07 | 1.04-1.09 | 0* | 1.12 | 1.07-1.18 | 0* | - | - | - |
| Men | 2.66 | 1.21-6.68 | 0.02* | 7.13 | 2.08-42.31 | 0.01* | - | - | - |
| Age * men | - | - | - | 0.94 | 0.88–0.99 | 0.03* | - | - | - |
| **Co-morbidities** | | | | | | | | | |
| HCV co-infection | 4 | 1.49-9.14 | 0.002* | - | - | - | **2.75** | **1.01-6.37** | **0.03*** |
| HDV co-infection | 4.27 | 1.02-12 | 0.02* | - | - | - | **4.47** | **1.06-12.84** | **0.02*** |
| HIV co-infection | 1.99 | 0.32-6.6 | 0.3 | - | - | - | 2.01 | 0.32-6.73 | 0.3 |
| Diabetes mellitus | 1.81 | 0.61-4.33 | 0.2 | - | - | - | 0.97 | 0.32-2.43 | >0.9 |
| **African region of origin (vs Eastern)** | | | | | | | | | |
| Middle | 2.73 | 0.79-7.39 | 0.07* | - | - | - | **3.82** | **1.08-10.65** | **0.02*** |
| Northern | 1.6 | 0.37-4.76 | 0.5 | - | - | - | 1.73 | 0.47-5.38 | 0.4 |
| Western | 1.15 | 0.44-2.66 | 0.8 | - | - | - | 1.77 | 0.66-4.32 | 0.2 |

*Abbreviations: CI= Confidence Intervals; p=p-value. #Adjusted estimates are univariable estimates adjusted for the Base Model. *P-values < 0.05.*

African-born men with CHB had 10.6 times the risk to develop HCC compared to comparators from the same country of birth and 35.3 times the risk to develop HCC compared to the general population. African-born persons without HBV infection had a 3.0 times higher risk (95% CI 1.16-7.74) compared to the general population.

# 6 Discussion

## 6.1 Methodological considerations

This thesis is based on observational studies (I, III, and IV) and a pooled analysis (II). All studies included a descriptive part of the population of interest, at the first healthcare encounter or notification (Studies I, III, IV) and enrollment (Study II). Retrospective data was used for the eligibility (inclusion and exclusion) of study participants and the descriptive part at baseline. A prospective part of the analysis involves analyzing the effect of exposure on the outcome of interest to test whether an association might be present. The term baseline is used to mark the beginning of follow-up and represents a time point of interest such as the date of notification or treatment start…etc. That is common to all study participants. Our studies were based on real-world data that can be generalized to similar settings and levels of care and can further be used in simulation analyses. Randomized Controlled Trials with large enough study subjects provide higher grades of evidence compared to observational studies as confounders are annihilated by randomization. However, the ethical aspects, generalizability, and time to conduct such trials might offer observational studies more feasibility and practicality to generate and test hypotheses in research. In all studies, by design, care was taken that the outcome of interest follows the exposure of interest, to determine temporality.

In **Study I**, we collected data retrospectively on a cohort of persons with anti-HDV positive assembled from a multi-infectious diseases' clinic. We assessed the association of HDV RNA replication with the prospective incidence of a composite of liver-related outcomes. Our results provided new findings of less severe disease outcomes for persons with HDV when a large cohort from 2ndry care clinics is studied.

In **Study II**, individual studies are considered as study subjects. The study included only cohort studies with estimates on HCC incidence occurring in persons with HBV/HDV coinfection and peers with HBV mono-infection. We examined the baseline characteristics of study populations, and we conducted subgroup analyses on study characteristics.

In **Study III**, we described the prevalence of screening of HDV in persons with positive HBsAg from 1992 to 2022, and subsequently classified persons per their every HDV screening. We tested the association of baseline characteristics to the odds of receiving a screening test, and the time to receive a screening test to prevalent liver-related outcomes.

In **Study IV**, we analyzed a cohort with retrospectively collected data and matched the cohort using a register -based on baseline characteristics. We followed these cohorts prospectively for the development of the outcome of interest (HCC). Such design enables studying rare outcomes, allowing the presentation of absolute and relative risks. As age and sex were associated with the incidence of HCC, an interaction term with age*sex was introduced in the regression model.

## 6.2 Bias in research

Bias can result in over-or underestimating the effect of exposure, leading to distorted associations and wrong conclusions. Bias can occur in any phase during the research. Awareness of the potential types of bias is important to better interpret the results and minimize the effect of bias.

### 6.2.1 Measurement (information) bias

This type of bias is faced when the study variables (exposures or outcomes) are systematically measured or classified differently between exposed and non-exposed individuals. Research involving self-reporting, qualitative research, and retrospective studies are prone to this bias.

### 6.2.2 Misclassification bias

This is another type of information bias that involves misclassifying the exposure or outcome leading to wrong interpretations. Random misclassification means the wrong classification occurred at hazards (not differential) in both groups, usually leading the association towards the null. When misclassification is more prevalent in one study group rather than the other, it can over or underestimate the association. As the data in our studies were collected uniformly from validated healthcare registers, we avoided these types of bias.

### 6.2.3 Selection bias

Is a type of systematic error occurring when the difference in the outcome is due to factors related to the outcome regardless of the exposure studied. In study I, we avoided the selection bias towards severe outcomes in prior studies on the natural course of HDV recruiting from tertiary care referral centers.

### 6.2.4 Sampling or ascertainment bias

When the sample of individuals in our study is not representative of the population of interest, limiting the external validity and generalizability of our findings.

### 6.2.5 Attrition bias

When there is systematic drop out of the study, and those who drop out are different in characteristics from those who remain in the study.

### 6.2.6 Survivor ship bias

When selecting a subgroup by certain outcome ignoring to compare them to controls with same exposure and failed the outcome. For example, analyzing only those who survived an event, those who completed IFN therapy, those who had maintained virological response.

### 6.2.7 Surveillance bias

Also known as detection bias is faced when subjects are more likely to have the outcome of interest detected as they receive screening or testing due to other medical conditions tested. This type of bias can be minimized when a control group is selected to receive the same tests and screening, or by excluding those who had the outcome of interest diagnosed with the period of testing. We adjusted for this type of bias in studies I and IV, by excluding those who had the outcome of interest (HCC or composite outcome) within 6 months of baseline.

### 6.2.8 Lead-time and length-time bias

Overestimation of the survival duration is faced in lead-time and length-time bias. The former is the rule in screening research, when adding the survival time from diagnosis through screening and comparing it to the group having the disease diagnosed when symptomatic. The latter is faced when slowly growing tumors are more likely to be detected by screening, and owing to their slow rate of progression, they have longer survival periods. Lead-time bias is the rule in screening research, and adjusting for it is performed through deducing this added time from the screened group.

## 6.3 Confounders

A variable that is associated with the exposure (making it more likely) and independently associated with the outcome, erroneously creating, or masking an association between the outcome and the exposure. We can statistically adjust for confounders through randomization, matching on key characteristics, restriction, subgrouping, and adjusting for the possible known confounders in the regression models. However, observational studies will always have unmeasured or residual confounders. Age, sex, cirrhosis, and HDV RNA replication were included in the Cox regression model in Study I. A univariable meta-regression model was conducted in study II to explore which baseline variable is associated with the outcome. In study III, multivariable logistic regression models adjusted for variables like delayed testing, cirrhosis, and age which might affect the screening as well as liver-related outcomes. In study IV, age and sex were introduced in an interaction term in the base model, and the association of coinfection HCV, HDV, HIV, and DM were tested in a multivariable model. Many variables act as confounders and mediators in the causal pathway of liver disease and the few numbers of outcomes significantly limited multiple adjustments with plausible confounders.

## 6.4 Generalizability

Internal validity refers to the extent our observed findings represent the truth in the population we are studying, without the fallacy of a systematic error. The types of bias mentioned above can threaten the internal validity if not addressed in the study. When the study procures good internal validity, the findings could be applied to the larger

population providing external validity. Whether these findings can be extrapolated into other settings refers to generalizability.

In **Study I,** we sought eleven infectious diseases clinics from different Swedish settings constructing a nationwide cohort capturing >70% of the population of interest (persons with positive anti-HDV). We believe that this cohort is representative of the population living with HDV in Sweden. In **Study III**, the findings can be extrapolated to similar settings, probably with similar healthcare reimbursement and local guidelines. In **study IV**, a nationwide cohort is assembled from validated healthcare registers, so the confidence in the internal validity of our findings is high. It is challenging to extrapolate findings in migrant populations that might have different outcomes based on their ethnicity. The patterns of immigration are different, and certain populations, even age groups among the same ethnic populations prefer certain destinations, nevertheless the change in lifestyle and other risk factors/barriers that might be more pertinent in some groups than others in host countries merit research of its own.

## 6.5 Interpretations of findings

### 6.5.1 Study I

Our findings are in line with several studies demonstrating the association of HDV with liver-related complications, irrespective of ethnicity or genotype. Most of the studies addressing the long-term follow-up of CHD were mostly conducted in tertiary care hospitals with a predilection to more advanced liver disease. Nevertheless, the distinction between patients with HDV RNA replication was rarely made. Fattovich and colleagues studied 39 patients with positive anti-HDV contrasting them to 161 patients with HBV mono-infection [219]. Over a median follow-up of 6.6 years, persons with anti-HDV positive had 3 times the risk to develop HCC [219]. Niro and colleagues reported that persistent HDV RNA viremia was the only prognostic factor of liver failure and mortality in 175 patients of Caucasian origin [226]. Wranke A. and colleagues showed that in a predominantly Eastern Mediterranean/ European cohort of CHD, 63% remained free of cirrhosis after a mean of 3.2 years of follow-up [143]. Paloma A. and colleagues reported that 28% of persons with HDV RNA replication developed a decompensation during a median 8 years of follow-up [93].

A more benign disease course in persons with genotype 5 was shown by Spaan M. and colleagues in a rather young cohort of persons with CHD from SSA [227]. Romeo R. and colleagues suggested that an HDV RNA level of 600,000 copies/ml had an AUROC of 0.73 in patients with chronic hepatitis and is associated with a higher risk of cirrhosis [228]. Our study presents a less aggressive disease course in persons with HDV RNA viremia than previously thought. Indeed, HDV RNA replication was associated with 7.40 times the risk of liver-related death/or liver transplantation. The IR of any liver-related event, respectively HCC was 2.8/100PYs and 0.73/100PYs. The cumulative event-free survival for all patients with HDV RNA viremia was 58%, and 78% of those without cirrhosis at baseline, remained free of cirrhosis after a mean follow-up of 6.3 years. This suggests that cirrhosis is the strongest factor for worse liver outcomes, than HDV RNA viremia.

Cirrhosis was independently associated with 14 times higher risk for a liver event in those with HDV RNA replication.

We attributed the more benign disease course in our study to several factors such as the enrolled study cohort from secondary care centers, rather than tertiary care setting avoiding **referral bias**. Other possible factors are the young age of our cohort (mean age 38.0 (12.5)), the rather lower prevalence of alcohol overconsumption, diabetes, and the exclusion of persons with HCV and HIV co-infection who might have severe disease course. Nevertheless, 46.3% of the cohort was exposed to IFN therapy during follow-up, with a possible beneficial immunomodulatory role. As it is a retrospective cohort study, we cannot rule out possible unmeasured confounders or mediators that might be related to the course of liver disease in this population. Family history of cirrhosis and/or liver cancer, low socioeconomic status, smoking, dietary aflatoxin, and the effect of concomitant co-morbidities such as metabolic syndrome was not assessed owing to incomplete or missing data. The degree of iteration in our study is assumed to be less than 10% of the cohort, and at least 6 months of follow-up was eligibility in the study. Our study was constituted of 80% of migrant populations, 45% from Asia. It might be **generalizable** to other low-endemic settings of HBV, taking into account the difference in population characteristics. Studies on HDV from endemic regions, mostly cross-sectional, pointed to an increased prevalence of cirrhosis and HCC but did not provide absolute incidence rates. Whether migrant populations might be selectively healthier with better outcomes than those in endemic regions needs further research in the context of HDV.

### 6.5.2 Study II

Our pooled analysis included 64 000 persons from 12 studies, showing a two-fold increased risk for HCC was in line with a published meta-analysis with different eligibility criteria. The direct oncogenic role of HDV is debated, with reports suggesting that HDV affects pathways involved in cell cycle regulation and DNA damage repair and replication [107]. Published cohorts show prevalent cirrhosis in 20-40% of individuals with HDV at diagnosis [229,230]. Stockdale and colleagues found that 20% of HCC in CHB is attributed to HDV infection [11]. HDV is underdiagnosed, hence larger and unrecognized risk of HDV on the incidence of HCC might be plausible, especially in endemic regions of HBV and HDV [231]. For instance, a study from Mongolia reported that 80.3% of patients with liver cancer had CHD [231]. Our analysis pointed to a higher risk for HCC in studies in exclusive or enrolling PWHIV, also confirmed in other published meta-analyses [16,232]. This increased risk might be associated with the dominant effect of HDV in HBV/HDV/HIV infection, especially in those with low CD4 T-cells [233]. Another explanation is that PWHIV is treated with NUCs containing regimen, successfully suppressing HBV DNA replication, hence the increased relative risk in those with HIV/HBV/HDV triple infection [234].

In a multi-European center study enrolling 2793 persons with HBV/HIV infection, over a median follow-up of 10.8 years and adjusting for competing risks, HDV infection was associated with 80% increased risk of overall mortality, 3.1-fold the risk of a liver-related cause of death and 6.3-fold the risk to develop HCC [235].

Some limitations were encountered when conducting this study. In the screening phase of search results, many studies were **mislabeled** as case-control, or longitudinal in design. Most of the studies based their diagnosis on an anti-HDV positive test, rather than confirmatory HDV RNA for more than 6 months as a criterion for chronic infection, so **ascertainment bias** could not be ruled out. The analysis did not involve individual data levels on factors that affect HCC risk such as family history of liver cancer, habits, and co-morbidities. We could not assess the influence of anti-viral therapies against HBV and HDV, as only one study reported such data [225]. Adjustment for **lead-time bias** was poorly handled in the included studies. The increased risk of HCC could not be quantified in persons with cirrhosis. The **generalizability** of our analysis might also be limited in African persons with CHD due to the absence of longitudinal data in this population.

### 6.5.3 Study III

An overall screening rate of 90% for HDV, achieving 97.9% in 2010-2020. This screening rate is higher than estimates from European cohorts [229,236] and from American studies [155,156]**.**

The practice of HDV screening is highly variable, and the suboptimal screening encompasses low- and middle-income settings to high income-settings[54,237]. HDV screening has been associated with the level of care (specialized hepatology vs primary care), and time of screening with more screening rates in recent studies and in recently diagnosed patients with HBV [156,236]**.**

We resort this elevated rate of screening to the coordinated routine performed in persons with CHB at KUH. The first patient's visit involves a history taking, contact tracing for family members and sexual partner(s), and a package of blood tests that include a complete blood count, serological tests for HBV, anti-HDV testing, and a baseline liver stiffness measurement. At the physician visit, the patient has baseline investigations for further assessment for treatment eligibility or other management according to the patient's liver stage. The suboptimal screening for HDV is reported in studies in PWHIV, was also encountered in our study. This might reflect a lower awareness of HDV, and the rather non-emphasis on HDV screening in HIV guidelines despite the progressive disease course reported in persons with HBV/HDV/HIV co-infection [235,238]**.**

Our data along with other emerging studies and consensus reports might lean towards adopting automated reflex testing as a solution for more and early diagnosis of populations with CHD. Whether adopting automated reflex screening would translate into better care for patients with HDV is unclear. In our study, delayed HDV screening more than 5 years of HBV diagnosis was significantly associated with 7.6 times the odds (95% CI 1.8-31.6) to experience a liver-related outcome compared to those who received a screening test less than two years. It is important to note that identifying persons with HDV is a step, that would presumably lead to further assessment of treatment need/eligibility and assessment of liver disease stage. To date, there are no specific guidelines for treating persons with CHD. IFN therapy was used as an off-label therapy. Persons with advanced fibrosis and early compensated cirrhosis are offered IFN therapy according to local recommendations and background NUCs if HBV DNA is >2000/IU/ml or if cirrhosis regardless of HBV DNA level.

Data on IFN therapy in HDV suggest better tolerability than in HCV. The difference in population characteristics and the lack of Ribavirin administration offers better tolerance in persons with CHD.

The limitation of our analysis is the **missing data** on the risk factors in a substantial number of patients. This might even argue against risk-based screening as most patients do not mention/recall a route of transmission or fulfill the criteria for high-risk screening. As with retrospective designed studies, **unmeasured confounding** is encountered, as data is lacking on variables that might affect screening and liver disease progression such as other co-morbidities, and barriers to care.

### 6.5.4 Study IV

In our prospective analysis of this unique cohort, we demonstrated a low incidence rate of HCC in 3865 African-born individuals with CHB, during a mean follow-up of 12.0 years. Nevertheless, features of younger age and advanced disease stage at HCC diagnosis in men could be shown, where the mean age of diagnosis in men was 46.8 (SD 14.7), while in women was 67.0 (SD 13.7), with 42% (10/24) of men who developed HCC younger than 40 at diagnosis. Half of men younger than 40 years of age did not have a record of cirrhosis and those who had cirrhosis were all diagnosed less than 3 months before HCC diagnosis. These features are in line with African reports, where 44% of CHB carriers of SSA diagnosed with HCC were younger than 40 contrasted to 6% in Northern-African peers [101], with prior case series and pooled data from 14 African centers [239–241].

This young age at HCC diagnosis in men might reflect the rather young age at baseline; 75.5% of men were younger than 40. Of note, women also had a young age at baseline with 84.3% younger than 40. Yet, men had an IRR of 7.13 (95%CI 2.08-42.3) than women. A similar age range for persons with CHB was reported from a recent US cross-sectional report with predominantly participants from Eastern Africa [242]. The heterogeneity of age-specific HCC rates encountered might be attributed to differences in the age of HBV infection, with higher risks among patients acquiring the infection during childhood, HBV DNA level, genotypes, and concomitant risk factors [243,244]. Nevertheless, the sex disparity seen in our study could be attributed as noted cumulatively in the literature to the higher frequency of co-infections, alcohol overconsumption, and drug misuse observed in men versus women with CHB in our cohort.

In a French case series, Sub-Saharan Africans with HCC related to HBV had the lowest surveillance rate (46%) compared to Northern Africans (73%), Asians (69%), and European peers (76%) [245]. In a US study, patients of SSA origin tended to present with metastasizing HCC regardless of their socioeconomic status, with a poor 5-year survival of 11.5% in another report [246]. The lower incidence of HCC and rather better survival in this cohort (5-year survival 41.2%) could be attributed to the younger age of persons with CHB (median 30.6-year-old), the lower frequency of cirrhosis as well as co-morbidities and possibly different healthcare reimbursement and social security system compared to the above-mentioned cohorts. One cannot rule out the possible selection bias toward "healthier", able to migrate individuals [247].

Co-infections with HCV, HDV, and HIV have been associated with a higher risk to develop HCC in CHB carriers [216]. In our cohort, HCV and HDV co-infections were

associated with increased HCC risk. HBV/HCV co-infection might be more prevalent in PWID with possible late linkage to care, low treatment uptake, and suboptimal adherence, factors that are also described among immigrant populations and strongly correlate with the incidence of HCC [248].

African-born individuals without HBV infection showed an increased risk of HCC compared to the general population. It is unclear whether some individuals have possibly missed HBV diagnosis. In other retrospective analyses of patients with heterogenous HCC etiologies, African Americans had 1.93 (1.29-2.89) higher odds of unrecognized cirrhosis compared to white peers [249]. The higher prevalence of DM (8.8%) and possibly metabolic-associated liver disease compared to the general population might also be a contributor among this group. Most evidence supporting surveillance of HCC is derived from studies on Asian and Caucasian predominant populations [100,250].

The decision to conduct surveillance relies on cost-effectiveness and improvement of survival, especially in patients with Child A and B scores (advanced compensated liver disease) [251]. HBV is an oncogenic virus, and ~ 20% of HCC develops in the non-cirrhotic liver in CHB carriers [252]. In a French study, with the majority of participants (60%) from Africa, 14% of HBV-related HCC developed in the non-cirrhotic liver, with significantly lower age among patients without cirrhosis compared to those with cirrhosis (51 vs 58 respectively) [245]. Similarly, in a Korean study, young patients <30 years of age with HCC, of whom 92% were attributed to HBV infection tended to present with advanced disease stage with similar survival to older counterparts suggesting a late diagnosis in this group [253]. On another aspect, surveillance; especially in low-risk populations might undermine the quality of life, increasing psychological and financial harm [254]. It remains to be determined, whether the current HCC surveillance is targeting a later stage of the liver disease spectrum or might miss a subgroup of younger African men with CHB who are at increased risk for HCC without existing cirrhosis [240]. But important to note, that screening practices can be affected by the level of care [255], clear "recommendations" and knowledge of these recommendations with data suggesting a lower screening rate encountered in HBV infection compared to HCV infection [256,257].

The study had several limitations, including the possibility of **unmeasured confounders** related to the non-randomized design. Others are related to the nature of this population-based analysis, as we did not assess the role of viral kinetics, fibrosis markers, genetic variants, and other risk factors like smoking, family history of liver cancer, and maternal HBV infection that might affect HCC development. Our findings might be generalized to other low-endemic settings, noting the heterogeneity of populations with CHB migrating to the West. We accounted for **surveillance bias** of diagnosing HCC in patients presenting with liver-related morbidity or mortality by starting the follow-up 6 months after baseline and excluding all deaths, liver cancer, and liver transplantation.

# 7 Conclusions

Based on the studies in this thesis we demonstrated that:

- Persons with HDV RNA replication have a worse disease course, especially in older age and with cirrhosis. Chronic hepatitis Delta is associated with a 2-fold higher risk of developing HCC compared to HBV mono-infection. This highlights the importance of early diagnosis of persons with HDV RNA replication who are at increased risk of liver disease progression for early treatment and care.
- Our study confirms the benefit of Interferon therapy in persons with CHD as a trend towards a more benign disease course was associated with virological response to IFN therapy.
- The awareness of HDV infection has improved, yet more emphasis is needed in different settings of care. Early screening for HDV is an important step in the continuum of care and might be associated with a better disease outcome.
- African-born men with CHB without cirrhosis and with concomitant HCV and HDV infections might need to start surveillance at younger age, owing to the frequently seen younger onset of HCC in this population. The need for contemporary cohorts on the incidence of HCC in CHB, especially in those with other co-infections and co-morbidities which might amplify the risk of HCC. Cost-effectiveness analysis is needed to customize HCC surveillance in different populations according to the risks of HCC development.

# 8 Points of Perspective

Throughout this thesis, we highlighted that the burden of chronic hepatitis Delta has been previously overlooked due to insufficient awareness, scarcity of studies from endemic regions, and lack of effective therapies. As we showed in ***Study I***, HDV RNA viremia, had more prevalent cirrhosis at diagnosis and had a significant association with liver-related complications. Persons who responded to IFN therapy were less likely to develop a liver-related event, even if not achieving a maintained virological response, this highlights the importance of further studies on newer agents with and without IFN therapy on the natural course of HDV. In ***Study II***, the increased risk of HCC suggested by our pooled analysis would motivate us to explore other factors implicated in this risk such as the microbiome changes in HBV and HDV. In ***Study III***, we demonstrated a high rate of HDV screening reaching 98% in the last decade since 2010, nevertheless a shorter time to screening. This might be explained by improved awareness, coordinated teamwork in managing persons with viral hepatitis, and enhanced diagnostic capabilities available in a high-income setting. The increased awareness about HDV among different settings of care calls for more studies on the incidence, and risks of liver diseases especially in countries with high-endemicity of HDV. Studies exploring the barriers to care in populations with HDV, the array of co-morbidities, and the patients' related outcomes from real-world cohorts would help understand better the needs of this population. In ***Study IV***, we demonstrated that African-born men with CHB have an increased risk for HCC at younger ages and that they exceed the 0.2% surveillance threshold in 20-30 years with concurrent comorbidities. The late diagnosis and especially in those younger than 40 years-old calls to study the drivers of HCC in this population, in a low-endemic setting. Large-scale prospective studies on the incidence and progression of liver-related outcomes in contemporary cohorts are important for future personalized, cost-effective HCC surveillance, especially in populations without cirrhosis.

# 9 Acknowledgments

As a woman, a mother, a physician, and a migrant I am grateful for this important eye-opening experience in my life; as I discovered many aspects in me and in this world that *I was not aware of* and certainly, they will guide my path forward.

My thanks to **the people I used their data** in this thesis, where these studies were never made possible without their contribution. I hope that this thesis will add to the knowledge and will help to alleviate the burden of viral hepatitis.

***My thanks and sincere gratitude go to:***

My principal supervisor **Soo Aleman**, for giving me this exceptional opportunity to learn, and work among her team, for her support, and greatest contribution to my learning experience.
**Ola Weiland** for teaching me how to write in focus, how to present, compare and interpret my findings.
My co-supervisors **Ann-Sofi Duberg**, **Per Stål,** and **Heiner Wedemeyer** for their insights, discussions, deep knowledge, and scientific contribution to my work.
**Heidi Holmström,** for her kindness, her understanding and all the help with patients consents in study III.
**Sonja Saario,** for her kindness and for teaching me how to perform fibroscan.
**Kerktin Karkkonen,** for teaching me a lot, for her generosity, her deep knowledge, and her empathy in caring for persons living with viral hepatitis.
**Susanne Cederberg and Annika Olsson**, for the great help in patients consents, and answering my questions about InCare hepatitis.
**All colleagues (past and current staff) at I53**, for being such a kind family, and helping me in many things in life and work.

**Mohammed Saqr**, for the life-saving R resources.
**Ammar Barakat**, my mentor (2018-2019) for the importance of learning Swedish.

**Maria Axelsson**, my mentor (2021) for her generosity, encouragement, enthusiasm, and knowledge.
**John Wahren and Ellinor Schmidt,** for their kindness, organizing and giving me along with other colleagues such a beautiful learning experience in"Utbildning *i svenska för läkare och sjuksköterskor med examen från länder utanför EU"* and for never giving up in spite of COVID pandemic.
**Anna-Mia Ekström** and **Nele Brusselaers** for teaching me a lot about writing and thinking outside of the box.
**Karolin Falconer and Gabriel Westman,** for all the discussions, statistical help and suggestions in study I.
**Michael Ingre**, for his deep knowledge in statistics and the statistical help that encompasses studies III, and IV.
**All co-authors,** in the studies for their feedback and comments.
**Leila Relander,** for her generosity and meticulous work helping me to produce the figures and organizing the Kappa.
**KI librarians,** for discussing and conducting the search in study II.
**Amira Ahmed El-Sayed** for her kindness, knowledge, and 2 decades of inspiration (and many more to come).
**Romina Fornes,** for being Romina Fornes, a *Wolverine*.
**Amal Al Azmi** for remembering me with her grace and kindness every Ramadan.
**Mama Mimi, Carmen, Layla** and **all my family**, please forgive me, I love you.

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