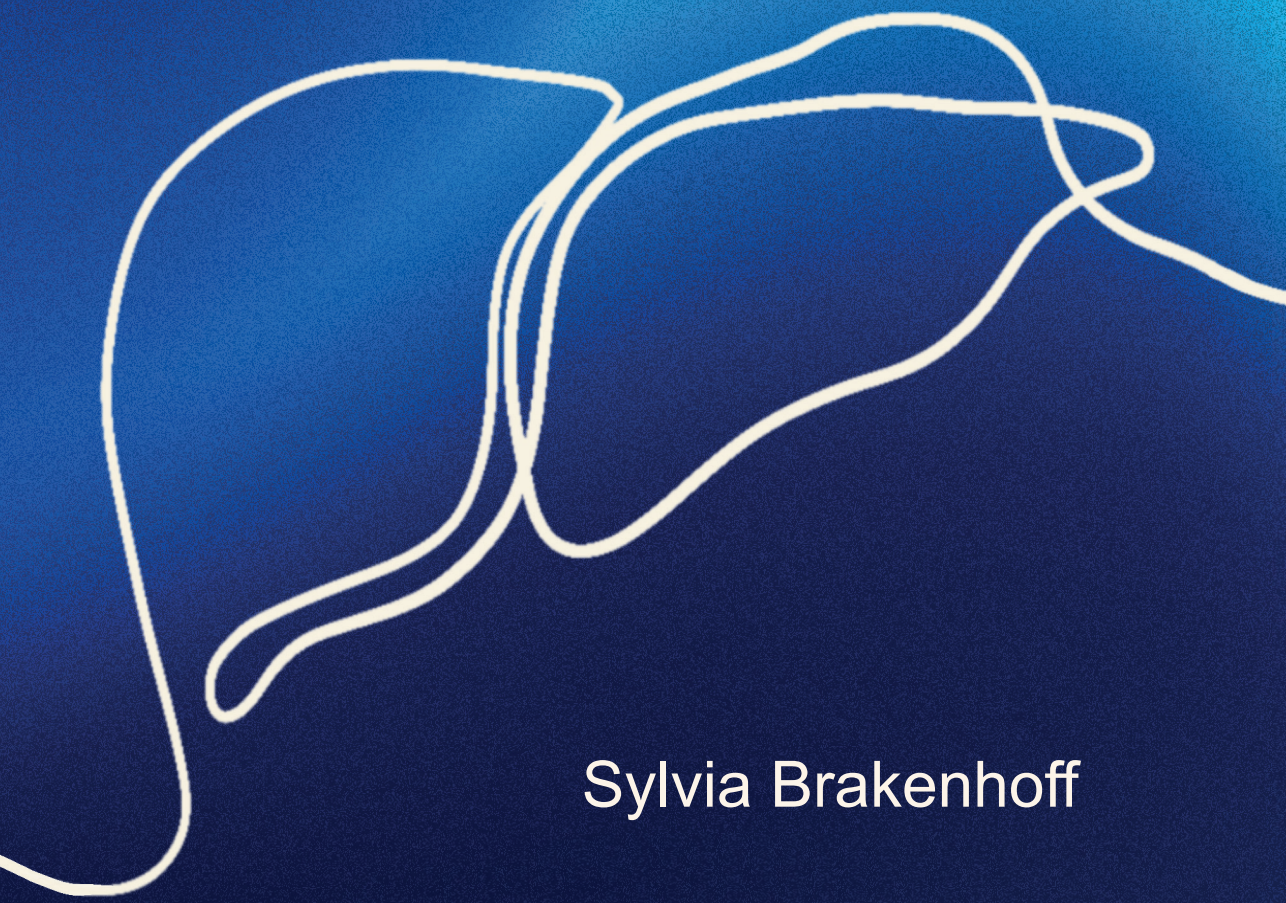
Optimising Care for Patients with Chronic Hepatitis B and C



Sylvia Brakenhoff

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Sylvia Merel Brakenhoff

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Optimising Care for Patients with Chronic Hepatitis B and C

Optimaliseren van de behandeling voor patiënten met chronische hepatitis B en C

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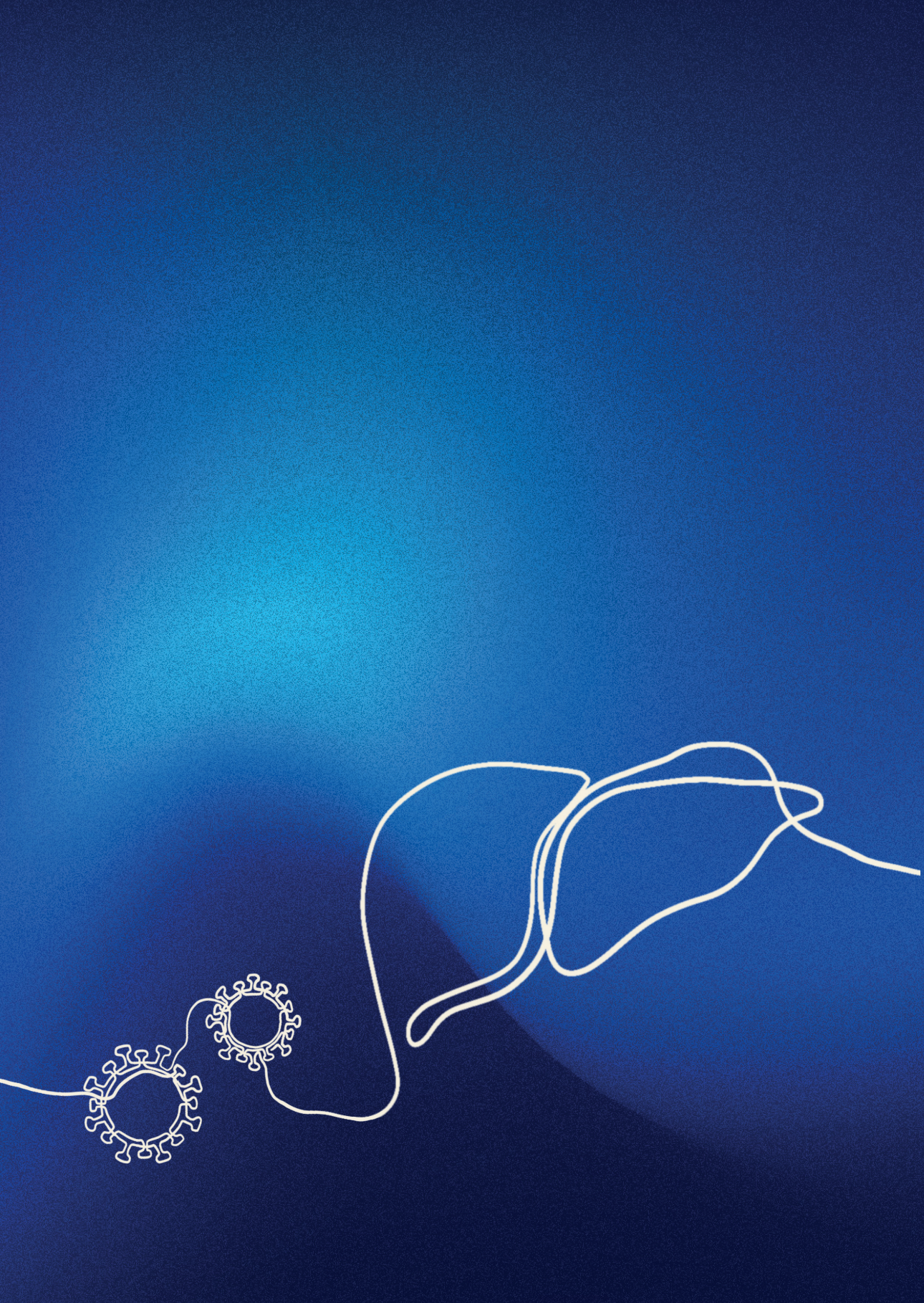
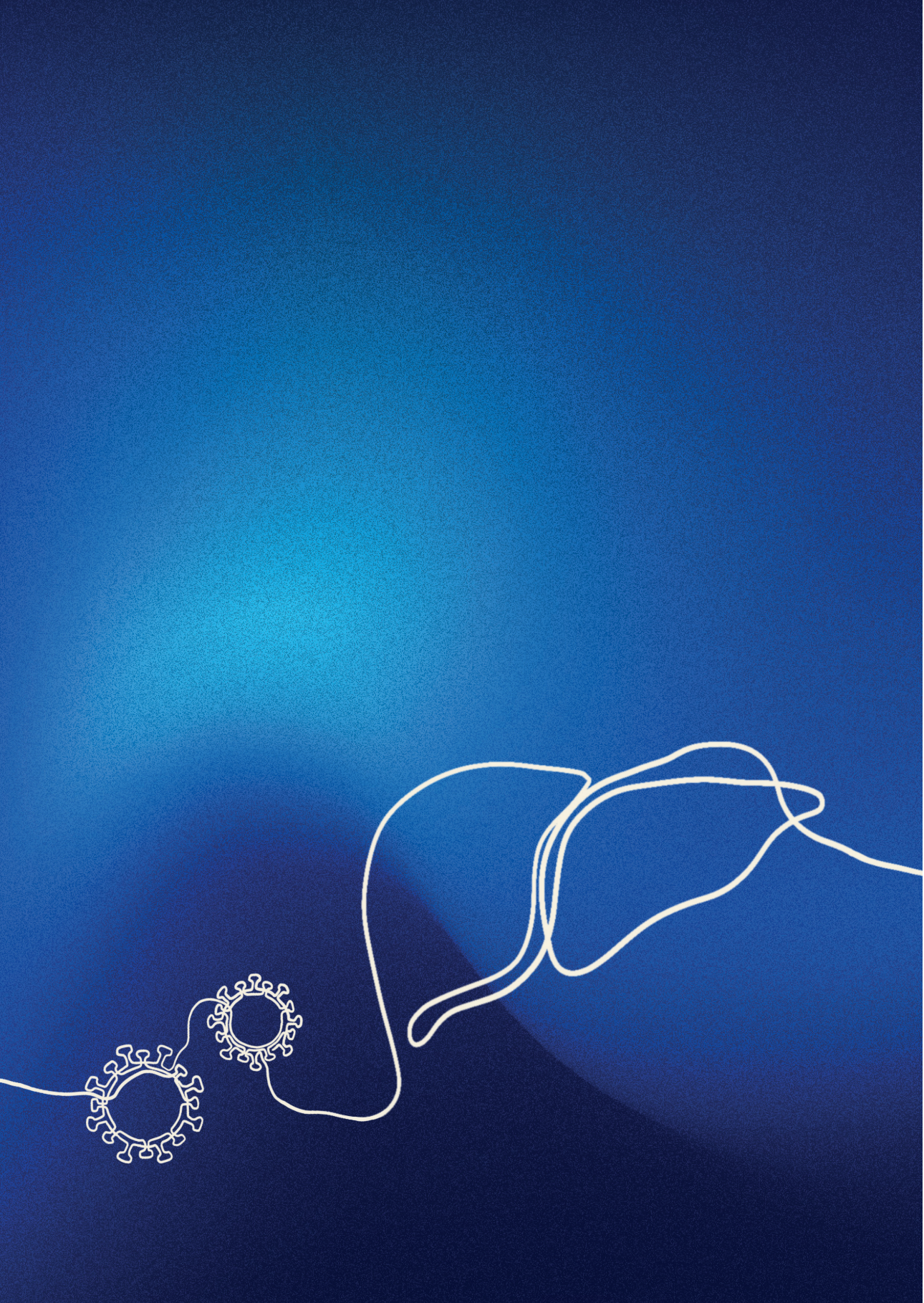


PART I

Introduction



CHAPTER 1

General Introduction and aims of this thesis

Epidemiology

Viral hepatitis is considered a global public health threat. Worldwide, 296 million individuals are infected with the hepatitis B virus (HBV) and 93 million with the hepatitis C virus (HCV).[1,2] The prevalence of hepatitis varies across the globe. The highest prevalence of HBV is observed in East Asia and sub-Saharan Africa, where approximately 5-10% of the population is chronically infected.[1,3] For HCV, the highest prevalence is observed in Central and East Asia, West Africa, and the Eastern of Europe, with a prevalence of up to 3.6%.[3,4]

The Netherlands is considered a low-endemic country, with a prevalence of 0.34% for HBV (~40,000 individuals) and 0.16% for HCV (~28,000 individuals).[5] The highest prevalence has been observed among high-risk groups, including migrants, incarcerated people, people who (have) inject(ed) drugs (PWID) and men who have unsafe sex with men (MSM).[5,6]

Disease burden

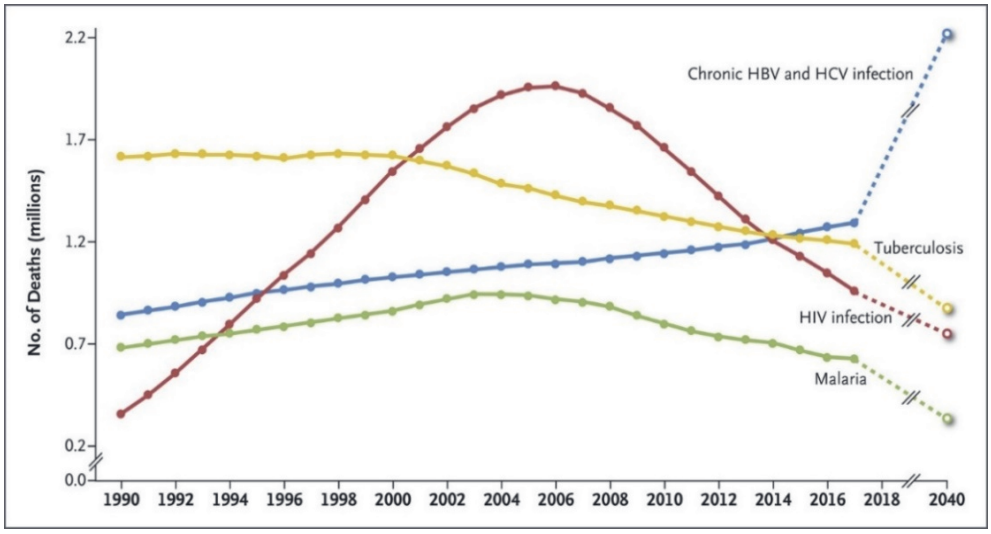
Patients with a chronic viral hepatitis infection are at risk to develop cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC).[1,2] Chronic hepatitis B (CHB) and C (CHC) are leading causes of HCC, as they are together responsible for 73% of HCC cases and 63% of cases with liver cirrhosis.[7,8]

In 2019, approximately 820,000 and 290,000 patients died worldwide due to respectively hepatitis B and C related morbidity.[1,2] Nowadays, the mortality from hepatitis B and C exceed the mortality from Human Immunodeficiency Virus (HIV), tuberculosis, and malaria (Figure 1).[3]

Virology

Viral hepatitis B and C are noncytoplasmatic, blood-borne viruses. Replication takes place in the hepatocytes. Transmission of both HBV and HCV occurs via blood-blood contacts such as needle stick injury, intravenous drug abuse, tattooing, piercing and exposure to infected blood and body fluids. For HBV, one of the most common transmission routes, especially in high-endemic countries, is perinatal transmission.[1,2]

Figure 1. The number of deaths of viral hepatitis as compared with deaths from Human Immunodeficiency Virus (HIV) infection, malaria and tuberculosis



Reproduced with permission from Thomas et al.[9], Copyright Massachusetts Medical Society. Dashed lines are projections from Foreman et al.[10]

Hepatitis B

HBV, first isolated in 1965, is a member of the *Hepadnavirus*. It includes a small enveloped DNA virus. Currently, nine genotypes have been identified (genotype A-I).[11] The distribution of HBV genotypes varies across the world, with the highest prevalence of HBV genotypes A and D in Europe, Africa and India, and HBV genotypes B and C in Asia.[12]

After infection, HBV travels via the blood to the liver. The circulating virion contains an envelope (hepatitis B surface antigen; HBsAg) and a nucleocapsid which contains a core protein (hepatitis B core antigen; HBcAg) and partially double-stranded circular DNA (Figure 2). After viral entry in the hepatocyte, HBsAg is shed and the nucleocapsid enters the nucleus. In the nucleus of the hepatocyte, HBV DNA is incorporated into the host genome.[13,14]

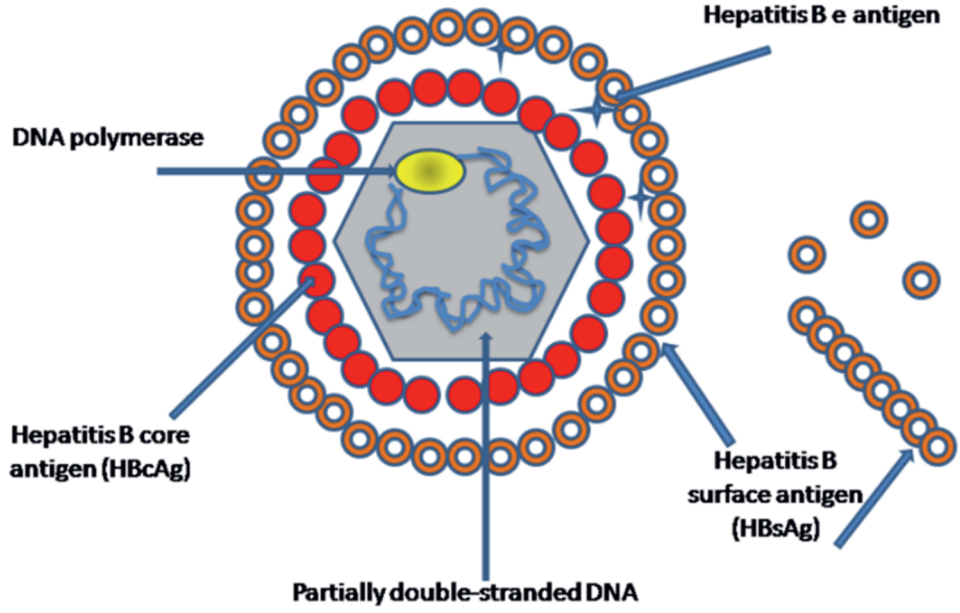
Covalently closed circular DNA (cccDNA) persists in the hepatocytes and acts as a template for all viral particles via transcription of four overlapping open reading frames (ORFs). Each ORF encodes for a polyadenylated RNA subtype, which subsequently serves as a template for the transcription of a number of proteins.[15-17]

Functional cure of hepatitis B is defined as loss of HBsAg.[18] However, cccDNA persists in the liver in the majority of patients, resulting in a major barrier to cure a

CHB infection. These patients remain at risk for HBV reactivation during or after treatment with high-risk immunosuppressive agents.[19,20] Therefore, the ultimate goal is the eradication of intrahepatic cccDNA. Monitoring its kinetics during therapy is therefore desired. However, cccDNA can only be quantified invasively using liver biopsy. Therefore, non-invasive serological markers that correlate with cccDNA are needed to assess the efficacy of anti-viral agents.

Recently, HBV RNA and hepatitis B core-related antigen (HBcrAg) have emerged as such serum biomarkers, as they correlate with transcriptional activity of cccDNA and therefore may reflect intrahepatic replication activity.[21,22]

Figure 2. Simplified representation of the hepatitis B virus (HBV)



Hepatitis C

HCV was first isolated in 1989 as a blood-borne non-A, non-B viral hepatitis.[23] HCV is a single-stranded, positive-sense RNA virus of the family *Flaviviridae*. The viral genome contains an ORF encoding for structural proteins (core, E1 and E2), an ion channel, and non-structural proteins (NS2, NS3, NS4A, NS5A and NS5B).[24] Currently, eight genotypes have been identified (genotype 1-8), with around 90 subtypes (a-k).[25] HCV genotypes 1 and 3 are globally the most prevalent genotypes.[26]

Natural course

An acute infection with HBV or HCV remains asymptomatic in the majority of patients and is often self-limiting. However, the infection becomes chronic if the virus persists for at least six months.

Hepatitis B

During an acute HBV infection, serum aminotransferase activity is elevated. After this first phase, they often normalise but the virus persists in the liver. HBsAg becomes present in serum three weeks after the infection, which is a marker for active viral replication. Four to six weeks after exposure, hepatitis B e antigen (HBeAg) and IgM antibodies to HBcAg (anti-HBc IgM) become detectable in serum. Anti-HBc IgM disappears four to eight months after an acute infection. When the HBV infection is cleared, HBsAg and HBeAg disappear and anti-HBs, anti-HBc and anti-HBe IgG antibodies become detectable in the serum. HBV DNA is detectable as long as HBsAg is present in serum.[27-29]

Among individuals infected with HBV, the age of infection is an important predictor for developing a chronic infection. In case of perinatal transmission, the risk of developing a chronic infection is 95%. In case of infection at adult age, this risk is ~5%.[1,2]

A chronic HBV infection, or carrier state, is defined as HBsAg present in serum for more than six months. Four phases of chronic HBV infection have been reported in the natural course: (1) HBeAg-positive chronic HBV infection (previously known as the “immune tolerant phase”), (2) HBeAg-positive chronic hepatitis B (previously known as the “immune clearance or immuno-active” phase), (3) the HBeAg-negative chronic HBV infection (previously known as the “inactive carrier state”), and (4) HBeAg-negative chronic hepatitis B (previously known as the “resolving/reactivation state”).[18,27-29] The duration of each phase shows great inter-individual variation. This is due to the host’s immune response against the virus. Figure 3 displays the serum markers of a chronic HBV infection.

Figure 3. Serum markers of a chronic HBV infection

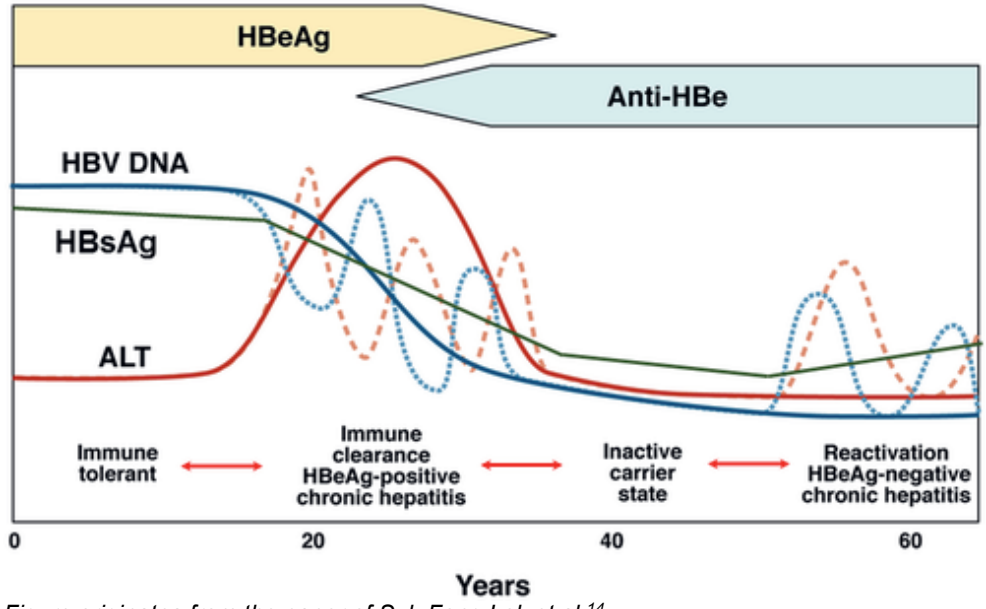


Figure originates from the paper of Suk-Fong Lok et al.[14]

In the first phase, patients are asymptomatic.[27,30] This phase is characterised by high serum HBV DNA levels and normal aminotransferases, together with the presence of HBsAg and HBeAg in serum. In the liver, there is no or minimal necroinflammation or fibrosis. This phase may last for decades and is mostly seen in patients infected at birth. During the symptomatic "immune clearance" phase, a rise in aminotransferase levels and a decrease in HBV DNA levels occur. In the liver, there is moderate to severe necroinflammation and progression to fibrosis can occur. In most patients with a chronic HBV infection, an HBeAg-seroconversion takes place at a certain time point where the HBeAg is cleared and anti-HBe antibodies are formed, leading to a next phase called the "inactive carrier state". This phase is characterised by a low level of HBV DNA in the liver and serum, but in most cases without damaging the liver significantly (and with normal aminotransferases). However, some inactive carriers have intermittent symptomatic phases, which are called exacerbations or flares. During these flares, serum aminotransferase and HBV DNA levels rise. This may lead to clearance of the HBV infection (HBsAg-seroconversion). However, in most cases, the chronic HBV infection persists, and new flares may occur. These flares of HBV infection cause hepatocyte necrosis, which may lead to progressive liver damage: fibrosis and eventually cirrhosis.[27-29]

Hepatitis C

An acute infection with HCV results in a chronic infection in 70% of the individuals. Most of these chronically infected patients remain asymptomatic.[2] However, 20-30% of the patients develop liver cirrhosis over a period of 20-30 years. The natural history is influenced by host-, viral-, and environmental-related factors. Older age at infection, male sex, comorbidities associated with metabolic syndrome (including obesity, liver steatosis and diabetes mellitus), HCV genotype 3, co-infection with HBV or HIV, and alcohol use have been associated with (accelerated) fibrosis progression.[31]

Current treatment options

Viral suppression or eradication halts further progression of the liver disease and improves life expectancy.[32,33] Currently, several therapeutic options are available.

Hepatitis B

Viral suppression is the main goal of therapy, as it is difficult to achieve functional cure (HBsAg loss) with antiviral treatment. Current treatment guidelines recommend pegylated interferon (PEG-IFN) or nucleos(t)ide analogues (NA) for the treatment of chronic HBV infection.[18] PEG-IFN acts as an immune modulator but has only moderate antiviral effects. PEG-IFN can induce long-term immunological control after a finite treatment duration in a proportion of the patients.[34] However, an important limitation of PEG-IFN therapy are the (significant) side effects.[18] Therefore, the indication for PEG-IFN mono-therapy is currently limited.

Nucleos(t)ide analogues (such as entecavir and tenofovir) suppress HBV DNA replication by inhibiting the HBV reverse transcriptase. The effectiveness and use of nucleos(t)ide analogues have been extensively shown as long-term viral suppression can be accomplished in the majority of patients and herewith progression to liver cirrhosis and hepatocellular carcinoma can be delayed or prevented. Another advantage of NA is the favourable safety profile, as it has shown limited side effects and it can be used in every HBV infected patient.[18] Therefore, NA therapy forms nowadays the cornerstone in the treatment of CHB patients.

There is growing interest if serum biomarkers can be used in the prediction of treatment response. **Chapter 2** of this thesis aimed to explore the role of concomitant decline in HBV RNA and viral antigens HBsAg and HBcrAg during antiviral therapy in the prediction of off-treatment response. **Chapter 3** studied the association between serum levels of antibodies to HBcAg (anti-HBc) and other viral antigens, histological inflammatory activity, and response to immunomodulatory therapy.

Hepatitis C

In the last decade, enormous progress has been made in the treatment of patients infected with hepatitis C. Nowadays, virological cure can be achieved in >95% of the patients treated 8-12 weeks with direct-acting antiviral agents (DAAs). Viral cure is defined as a sustained virological response (SVR), which includes undetectable HCV RNA >12 weeks after therapy.[35]

Reactivation of hepatitis B and flares

As previously described, chronic hepatitis B infection is a dynamic disease. Acute elevations in alanine aminotransferases (ALT) can occur during the natural course of a chronic infection, which are also known as flares. Most flares are asymptomatic. However, ALT flares can cause symptomatic hepatitis which can lead to hepatic decompensation and, in rare cases, death.[36]

Flares can be influenced by host-, viral- and therapy-related factors. Host factors include age, sex and ethnicity. ALT flares are more frequently observed in younger adults, but rarely in children. In addition, ALT flares are more frequently observed in men compared to women, and in Asian compared to white individuals.[36] Viral factors include HBeAg-status and HBV genotype, as more flares are observed in HBeAg-positive patients and in patients infected with HBV genotype C (compared to genotype B).[36]

Furthermore, ALT flares can also be iatrogenic: during PEG-IFN therapy, after finite NUC therapy, and provoked by high-risk immunosuppressive or cytotoxic agents such as rituximab or corticosteroid.[36]

It has been debated whether ALT flares are beneficial, as there are thought to represents the attempts of the hosts host's immune system to eliminate the virus.[36] In **Chapter 4**, we aimed to study whether off-treatment ALT flares are associated with favourable virological outcomes.

Finite antiviral therapy – NA cessation

Although NA is very effective in suppressing HBV replication, HBsAg loss is rarely observed during therapy. Therefore, lifelong continuous treatment has been the backbone of antiviral management in CHB patients for many years.[18] However, lifelong therapy is associated with costs, antiviral resistance to NA agents and drug-related side effects. Therefore, a growing interest has risen in finite NA therapy.

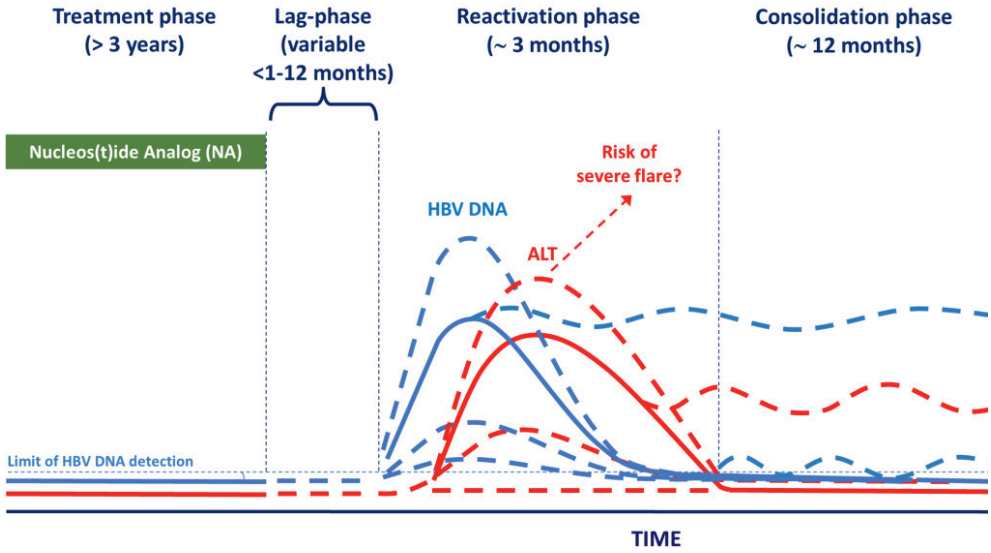
The first reports originated from Asia, where NA therapy is only reimbursed for a limited time period. The former Asian-Pacific (APASL) clinical practice guideline on the management of hepatitis B recommended NA cessation in case of undetectable HBV DNA on three occasions at least six months apart among HBeAg-negative

patients and on two occasions after on-treatment HBeAg seroconversion among patients who were HBeAg-positive at start of NA therapy, regardless whether a patient had liver cirrhosis or not.[37] These studies demonstrated that a proportion of patients achieved durable off-treatment HBV DNA suppression (sustained response) and even functional cure (HBsAg loss).[38-41] Based on these favourable outcomes, the European Association for the study of the Liver (EASL) included in the updated guideline the suggestion that NA could be discontinued. However, they pointed out the possible risks of NA cessation caused by viral relapses and possible concomitant ALT flares. Therefore, NA cessation is discouraged among patients with liver cirrhosis.[18] The American Association for the Study of Liver Diseases (AALSD) remains with its former statement, including that NA may only be stopped if HBsAg loss is achieved.[42]

Following the updated guideline, Western countries commenced NA stop studies.[43,44] These studies showed a typical course after NA cessation, which has been displayed in Figure 4. After NA cessation, ALT and HBV DNA initially remain suppressed (lag phase). The duration of this lag phase differs among patients. However, in the majority of patients a reactivation phase follows. This reactivation phase is marked by a rise in HBV DNA load (viral relapse), which is often followed by ALT elevation (combined relapse or biochemical relapse). These ALT increases can be mild and transient, but severe hepatic flares have been observed during this phase. These flares require immediate re-treatment to prevent further progression to liver decompensation. After this reactivation phase, several outcomes have been described, including a long-term low or undetectable HBV DNA load with normal ALT levels (sustained response). Some patients even achieve HBsAg loss. However, a proportion of patients remain viremic and some also keep elevated ALT levels. The latter patient group require therefore also re-treatment.[45]

However, these studies also pointed out the potential risks, as a number of (fatal) liver decompensation has been described.[46,47] Therefore, NA should only be ceased in a strict selection of HBeAg-negative patients without signs of (advanced) liver fibrosis or cirrhosis, and closely monitored after NA withdrawal and re-treated if necessary. However, no definite consensus has been reached regarding patient selection, off-treatment monitoring plan and re-treatment criteria.

Figure 4. Graphical representation of the typical course of a patient after stopping long-term NA treatment



ALT levels are presented in red and HBV DNA levels in blue. During the first phase (treatment phase) both ALT and HBV DNA levels are low. Figure originates from the paper by Lampertico and Berg.[45]

Chapter 5 of this thesis investigated the safety and off-treatment response among a cohort of patients that ceased NA therapy. This cohort included patients that were selected with strict inclusion criteria and closely monitored. That NA discontinuation is not without risk, has been demonstrated in **chapter 6**, which describes a fatal case of a patient who developed acute liver failure due to hepatitis B reactivation. Therefore, predictors of severe hepatic flares are needed. Therefore, **chapter 4** aimed to assess if serum levels of serum biomarkers HBV RNA, HBsAg and/or HBcrAg at the end-of-treatment can predict ALT flares. These findings can be used to guide decision-making regarding therapy discontinuation and off-treatment follow-up.

Global elimination of viral hepatitis

With the emerging morbidity and mortality caused by viral hepatitis, and available effective antiviral agents and vaccines, the World Health Organization (WHO) has called for action. In 2016, the WHO published its Global Health Sector Strategy, setting the goal of global elimination of viral hepatitis as a public health threat by 2030. This elimination target has been defined as a reduction of 90% in new cases (95% decline in hepatitis B virus infections, 80% decline in hepatitis C virus

infections) and 65% reduction in mortality, compared with incidence and mortality numbers of 2015.[3]

Dutch situation

In response to this WHO report, a National Hepatitis Plan was developed in the Netherlands, focusing on five key pillars of interest: (1) awareness and vaccination, (2) identification of infected persons, (3) diagnostics and treatment, (4) improving care organisation and surveillance, and (5) monitoring the disease landscape.[48]

Awareness and vaccination

In the Netherlands, primary prevention strategies have resulted in a low incidence of viral hepatitis. These strategies include the screening of blood products and harm reduction strategies for PWID and MSM. In addition, HBV vaccination has been offered to high-risk populations, including new-borns (between 1989-2011 to new-born born from HBsAg-positive mothers, and since 2011 to all new-born), travellers to endemic countries, MSM, sex workers, PWID, and healthcare workers.[48]

Identification of infected persons

Another important strategy to reach elimination, is to identify infected persons. Presumably, many individuals with chronic hepatitis are currently still undiagnosed.[3] As nationwide screening has been proven to be less (cost-) effective in low-endemic countries such as the Netherlands,[5,49,50] micro-elimination has been considered the more favourable approach. Micro-elimination is the concept of elimination within populations with a high prevalence. These so-called key populations include migrants, MSM, PWID, and people with inherited bleeding disorders.[5,48]

In addition, it is currently unknown whether diagnosed patients are adequately being managed and treated according to current guidelines.[48] Retrieval of ever-diagnosed patients who are lost to follow-up has been considered another key population for (micro-) elimination. Regional retrieval projects have been performed in the past, which have shown that a substantial part of the ever-diagnosed hepatitis C population did not receive curative treatment and might still be chronically infected.[51-53] **Chapter 7** of this thesis includes a nationwide retrieval project of lost to follow-up chronic hepatitis C patients, which can serve as a blueprint for other low-endemic countries.

Currently, routine screening for hepatitis B is performed among blood/organ/stem cell donors, pregnant women, patients treated with high-risk immunosuppressive/ cytotoxic agents, high-risk groups (HIV-positive patients and MSM), and healthcare workers. Hepatitis C screening is performed among blood/organ/stem cell donors and high-risk groups (MSM and patients with HIV, haemophilia, and haemodialysis).

However, routing screening is currently not performed in migrants, PWID and incarcerated people.[48] Therefore, many patients could remain undetected.

Diagnostics and treatment

An active hepatitis B or C infection is diagnosed using laboratory tests. These tests include respectively the quantification of serum HBsAg and anti-HCV. In case of a positive test result, HBeAg plus HBV DNA and HCV RNA should be performed respectively to determine the disease stage.[54]

Treatment is important to reduce the prevalence, incidence and mortality. In the Netherlands, every patient with a chronic HCV infection is eligible for antiviral treatment.[35,55] For HBV-infected patients, the indication for antiviral therapy is based on viral load, HBeAg-status, the amount of inflammation (ALT levels), fibrosis stage, family history, and the presence of extra-hepatic manifestation of the disease.[18,56] In addition, patients with a chronic or prior hepatitis B infection who are treated with high-risk immunosuppressive or cytotoxic agents, do also have an indication for antiviral therapy to prevent an HBV reactivation.[18,56] However, compliance with the current medical guidelines is currently unknown. In **chapter 8** of this thesis, we evaluated the performance of hepatitis B screening in patients treated with rituximab, as well as whether those patients with a resolved or chronic hepatitis B infection received antiviral therapy as advised.

Improving care organisation and surveillance

In the Netherlands, several disciplines are involved in viral hepatitis care. These include general practitioners (GPs), microbiologists, public health services (in Dutch: GGD), obstetricians (for HBV), physicians in prisons/addiction care/asylum centres, and medical specialists including Hepatologists and infectious disease specialists.

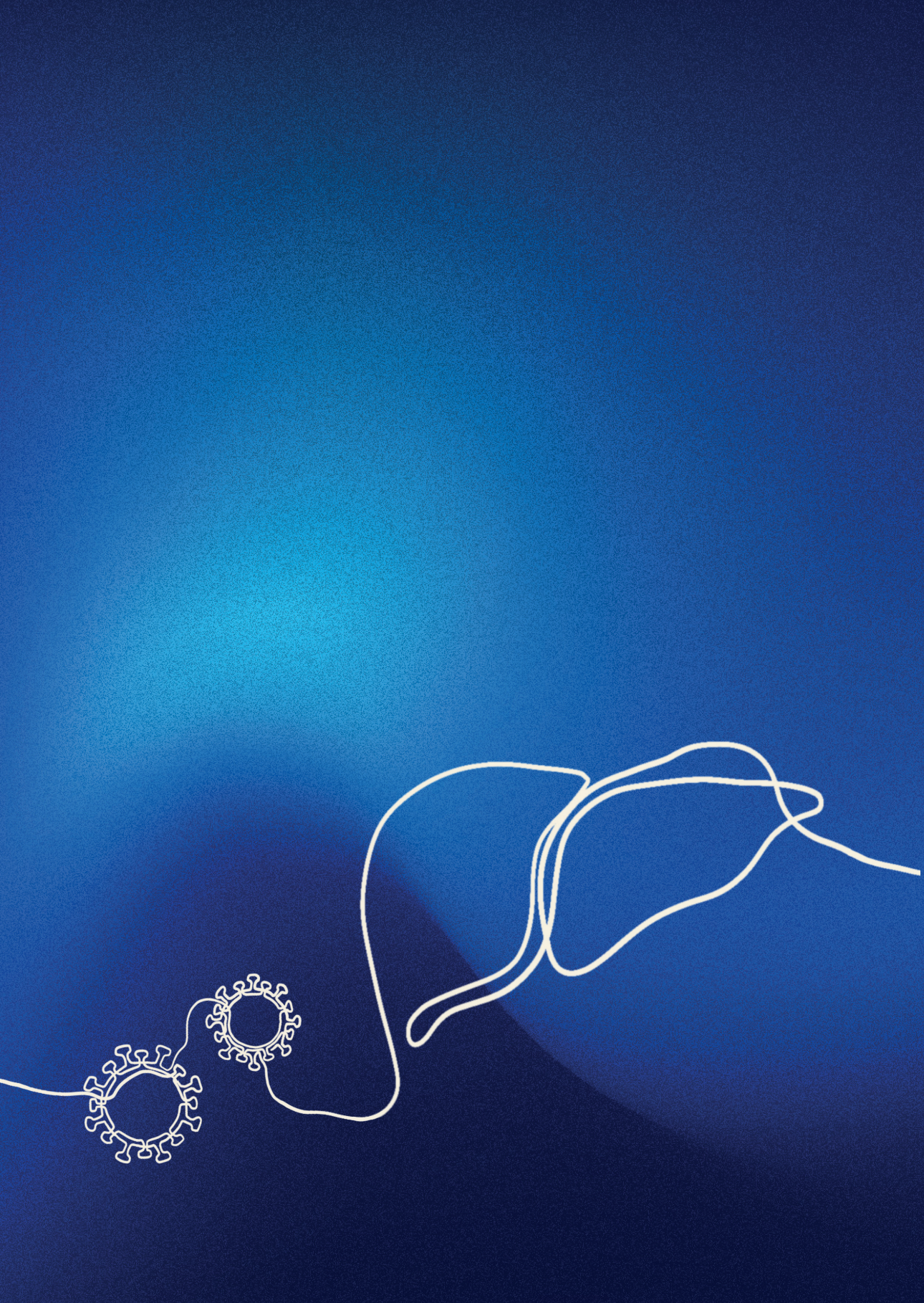
GPs, obstetricians and public health services are involved in the identification and detection of infected patients. The Dutch guideline for GPs advises HCV and HBV screening among high-risk groups or patients with elevated ALT levels. Next, the guideline advises biannual surveillance of chronic hepatitis B patients, and referral of every hepatitis C patient and patients with an active hepatitis B infection (HBeAg-positive, high viral load and/or elevated ALT levels).[54] These patients are referred to an hepatitis treatment centre, a hospital which is certificated for the treatment of viral hepatitis. However, it is currently unknown whether the surveillance, referral and treatment of viral hepatitis patients is performed correctly in primary care or hospitals.

Chapter 9 of this thesis provides insight into the prevalence of hepatitis B and C in primary care, and studied whether the management of these patients was in accordance with the guideline of general practitioners. **Chapter 10** assessed the adherence to medical guidelines in a high-expert academic hospital.

In addition, **chapter 11** is about individualising viral hepatitis care beyond medical guidelines. In this chapter, we evaluated the possibilities of a medical guideline add-on that includes patient-profiled data from high-quality publications on pharmacotherapy of hepatitis C. With this add-on, treatment can be tailored for the individual patient.

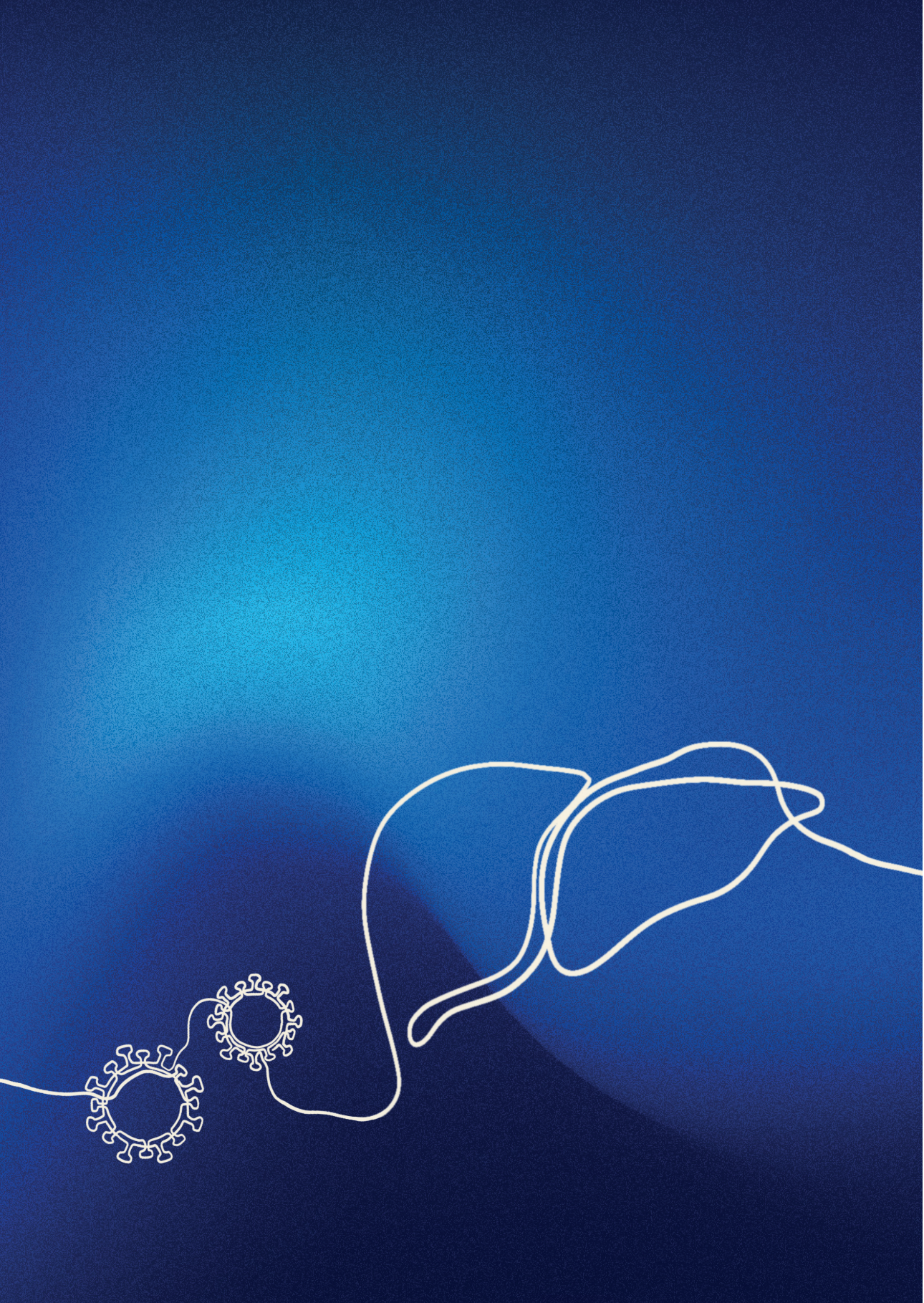
Monitoring the disease landscape

A national registration system including every patient with viral hepatitis is important to monitor the quality of care. However, such a database is not (yet) available. However, a pilot is currently executed for viral hepatitis C in collaboration with the Stichting HIV monitoring (SHM).



PART II

Finite antiviral therapy in chronic hepatitis B patients



CHAPTER 2

Hepatitis B virus RNA decline without concomitant viral antigen decrease is associated with a low probability of sustained response and hepatitis B surface antigen loss

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Alimentary Pharmacology & Therapeutics. 2021 Jan;53(2):314-320.

ABSTRACT

Background & Aim(s): Serum hepatitis B virus (HBV) RNA may reflect intrahepatic HBV replication. Novel antiviral drugs have shown potent HBV RNA decline without concomitant hepatitis B surface antigen (HBsAg) decrease. How this relates to off-treatment response is yet unclear. We aimed to study the degree of on-treatment viral antigen decline among patients with pronounced HBV RNA decrease in relation to off-treatment sustained response and HBsAg loss.

Methods: HBV RNA, HBsAg and hepatitis B core-related antigen (HBcrAg) were quantified in chronic hepatitis B patients who participated in two randomised controlled trials of peginterferon-based therapy. Sustained response (HBV DNA < 2,000 IU/mL) and/or HBsAg loss were assessed in patients with and without on-treatment HBV RNA response (> 2 log HBV RNA decline or > 1 log decline resulting in an undetectable value at on-treatment week 24), stratified by concomitant HBsAg decline (< 0.5/0.5-1/> 1 log).

Results: We enrolled 279 patients; 176 hepatitis B e antigen (HBeAg)-positive, 103 HBeAg-negative. Sustained response was achieved in 20.4% of patients. At on-treatment week 24, HBV RNA response was associated with higher sustained response rates (27.4% versus 13.0% in non-responders, p = 0.004). However, among patients with an HBV RNA response (n = 135), 56.4% did not experience > 0.5 log HBsAg decline. Amongst HBV RNA responders, sustained response was achieved in 47.6% of those with > 1 log HBsAg decline (n = 20/42), versus 16.0% with < 0.5 log decline (n = 12/75, p = 0.001). Similar results were obtained with HBcrAg and when response was defined as HBsAg loss.

Conclusion: In this cohort, many patients with HBV RNA response during peginterferon-based treatment did not experience HBsAg and/or HBcrAg decline. Absence of concomitant decline in these viral antigens was associated with low rates of treatment response and HBsAg loss. Future trials should therefore consider kinetics of combined biomarkers to assess antiviral efficacy.

INTRODUCTION

Chronic hepatitis B (CHB) infection is one of the main causes of end-stage liver disease and hepatocellular carcinoma (HCC).[1] The optimal goal of antiviral treatment is the achievement of an off-treatment sustained response to reduce the incidence of HCC and limit the progression of liver disease.[57] However, this remains difficult to achieve, as covalently closed circular DNA (cccDNA) persists in the hepatocytes.[15,58,59] On-treatment maintained viral suppression is therefore a second-best alternative.

While novel compounds are emerging, current therapeutic options are still limited to nucleos(t)ide analogues (NAs) and pegylated interferon (PEG-IFN). Nucleos(t)ide analogues are well tolerated and suppress HBV replication effectively.[60] Nonetheless, nucleos(t)ide analogues do not directly affect cccDNA[61] and are therefore associated with a limited off-treatment sustained response rate.[38,62] A finite duration of PEG-IFN can result in higher sustained response rates, as it is able to inhibit HBV transcription and reduces the production of viral particles through targeting cccDNA.[63] However, sustained response is only achieved in a limited proportion of patients.[63-65] PEG-IFN therapy is currently experiencing a revival, as it may be more effective when combined with novel antivirals.[66,67]

Eradication of intrahepatic cccDNA is considered to be a crucial step in the clearance of the hepatitis B virus (HBV), and monitoring its kinetics during therapy is highly desirable. However, cccDNA can only accurately be quantified invasively by liver biopsy. Therefore, non-invasive serological markers that correlate with intrahepatic replicative activity of HBV are needed to assess the efficacy of (novel) antiviral agents in CHB patients.

Recently, hepatitis B virus (HBV) RNA has emerged as a potential prognostic biomarker for treatment response, as it correlates with transcriptional activity of cccDNA and therefore may reflect intrahepatic replication activity.[21,68-70] Recent studies suggest that a decline in serum HBV RNA levels during treatment with nucleos(t)ide analogues or PEG-IFN is associated with treatment response, although overall declines during treatment were limited.[71-76] Interestingly, recent phase 1 studies of novel capsid assembly modifiers have shown substantially stronger HBV RNA declines, which has been interpreted as a possible sign of a more potent effect on the intrahepatic HBV reservoir.[77]

However, besides the observed decline in serum HBV RNA during capsid assembly modifiers therapy, little changes in hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) concentrations were observed in these patients.[77] How this relates to long-term off-treatment response is yet unclear.[78] We therefore aimed to study the degree of on-treatment HBsAg and HBcrAg decline among

patients with pronounced HBV RNA decrease, both in relation to off-treatment sustained response and HBsAg loss.

PATIENTS AND METHODS

Study population

For the current study, we enrolled chronic hepatitis B (CHB) patients who participated in two global randomised controlled trials (the 99-01 and PARC study; trial registration numbers NCT00114361 and NCT00146705). Detailed information regarding inclusion criteria and study design have been described elsewhere.[34,79] In short, the 99-01 study enrolled hepatitis B e antigen (HBeAg)-positive patients (n = 266), who were randomised to treatment with PEG-IFN alpha-2b 100 µg/week plus lamivudine 100 mg/day or PEG-IFN plus placebo for 52 weeks.[34] In the PARC study, HBeAg-negative patients (n = 133) were treated with PEG-IFN alpha-2a 180 µg/week monotherapy or PEG-IFN combination therapy with the addition of ribavirin 1000-2000 mg for 48 weeks.[79] Response was assessed at six months after therapy discontinuation (end of follow-up; EOF). For both studies, eligible patients had been HBsAg positive for at least six months, had a serum HBV DNA level of more than 10,000 copies/mL (equals ± 2,000 IU/mL) and an elevated ALT greater than 1.5-2 times the upper limit of normal (ULN) within eight weeks before randomisation. The original study protocols were in line with the ethical guidelines of the 1975 Declaration of Helsinki and approved by the medical ethical committees. Both the 99-01 and PARC studies demonstrated that combination therapy was not superior to PEG-IFN monotherapy, and data were therefore pooled for the current analysis.[34,79] For the current study we selected patients from the original studies if data was available for our primary outcome (sustained response) and a baseline HBV RNA measurement was available.

Serum HBV RNA, HBsAg and HBcrAg quantification

HBV RNA was quantified from serum samples using rapid amplification of complimentary DNA (cDNA)-ends (RACE)-based real-time polymerase chain reaction (PCR) at a central laboratory (University Hospital Leipzig, Germany). The PCR technique has been described previously.[71] The lower limit of detection (LOD) for HBV RNA in this assay was 800 copies/millilitre (c/mL).[80] Quantitative HBsAg levels were assessed using Abbott Architect (Abbott, Abbott Park, IL). The assay's LOD for HBsAg levels was 0.05 IU/mL HBcrAg was quantified using the Lumipulse® *G* HBcrAg assay (Fujirebio Europe). The LOD of HBcrAg measurements was 100 U/mL (2 log U/mL).[81] As HBcrAg partially depends on HBeAg-status and is low or undetectable in most HBeAg-negative patients during therapy,[81] the relationship between HBcrAg and response was assessed in the HBeAg-positive subgroup only.

Endpoints

Primary outcomes were sustained response and HBsAg loss. Sustained response was defined as HBV DNA < 2,000 IU/mL six months after end-of-treatment. HBsAg loss was assessed at end of follow-up and during long term follow-up.[34,74,79] HBV RNA, HBsAg and HBcrAg levels were measured at baseline, on-treatment week 12, on-treatment week 24, end-of-treatment (EOT) and during follow-up. HBV RNA response was defined as HBV RNA decline of either > 2 log or an HBV RNA decline or > 1 log which resulted in HBV RNA level below the LOD. HBsAg decline was categorised as < 0.5 log, 0.5-1 log and > 1 log. HBcrAg decline was categorised as < 1 log, 1-3 log and > 3 log. For this study, we assessed HBV RNA response and concomitant HBsAg or HBcrAg decline at on-treatment week 24. For sensitivity analysis, HBV RNA response at different time points (on-treatment week 12 and EOT) and in different subgroups (HBeAg-status and type of treatment) were also assessed.

Statistical analysis

Statistical analysis was performed using IBM SPSS for Windows version 25.0 (SPSS Inc., Chicago, Illinois, USA). Descriptive data were reported as percentages, means (± standard deviation; SD) and medians (with interquartile range; IQR) when appropriate. Data was tested for significance using chi-squared test, Fisher exact test or student t-test where appropriate. Univariate logistic regression analysis was used to estimate odds ratios (OR) of HBsAg decline for sustained response in patients with HBV RNA response at on-treatment week 24. Differences were considered statistically significant when $p < 0.05$. Graphic representation of the results was performed using Graph Pad Prism version 5 for Windows (GraphPad Software, San Diego, California, USA).

RESULTS

Patient characteristics

This study included 279 patients; 176 HBeAg-positive, 103 HBeAg-negative. Baseline characteristics of these patients are displayed in Table 1. Mean baseline HBV RNA levels were 5.9 log c/mL (± 1.6) in the overall study population. HBV RNA levels were higher in HBeAg-positive patients; 6.8 log c/mL (± 1.2) compared to 4.3 log c/mL (± 0.9) in HBeAg-negative patients. Baseline HBV RNA levels were already below the LOD in 12 patients (4.3%). Mean quantitative HBsAg levels were 4.2 log IU/mL (± 0.7) at baseline. In HBeAg-positive patients mean baseline HBcrAg levels were 8.3 log U/mL (± 0.7). In the overall population, sustained response was achieved in 57 patients (20.4%) and HBsAg loss in 18 patients (6.5%)

Table 1. Patient characteristics

Characteristics	HBeAg-positive (n = 176)	HBeAg-negative (n = 103)
Age at inclusion, years (median, IQR)	32 (23-41)	41 (33-50)
Male (n, %)	135 (76.7)	74 (71.8)
Race (n, %)		
Caucasian	116 (65.9)	98 (95.1)
Asian	43 (24.4)	3 (2.9)
Other	17 (9.7)	2 (1.9)
HBV genotype (n, %)		
A	53 (30.1)	15 (14.6)
B	18 (10.2)	0 (0)
C	35 (19.9)	2 (1.9)
D	64 (36.5)	81 (78.6)
Other	6 (3.4)	5 (4.9)
Study treatment (n, %)		
PEG-IFN mono	86 (48.9)	52 (50.5)
PEG-IFN + LAM	90 (51.1)	NA
PEG-IFN + RBV	NA	51 (49.5)
Laboratory results at baseline		
HBV RNA§ (mean, ±SD)	6.8 (±1.2)	4.3 (±0.9)
HBsAg‡ (mean, ±SD)	4.4 (±0.7)	3.8 (±0.5)
HBV DNA‡ (mean, ±SD)	8.3 (±1.0)	6.0 (±1.2)
HBcrAg† (mean, ±SD)	8.3 (±0.7)	5.1 (±1.2)

HBV, hepatitis B virus; PEG-IFN, peginterferon; LAM, lamivudine; RBV, ribavirin; HBsAg, quantitative hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; IQR, interquartile range; n, number; SD, standard deviation; NA, not applicable.
§ Logarithmic scale, copies/mL
‡ Logarithmic scale, IU/mL
† Logarithmic scale, U/mL

On-treatment HBV RNA response is associated with sustained response and HBsAg loss

HBV RNA data at on-treatment week 24 was available in 258/279 patients. Of those, 135 experienced an HBV RNA response (52.3%) at week 24 of therapy. Patients with an HBV RNA response had significantly higher rates of sustained response and HBsAg loss (Figure 1A). Among 135 patients with an HBV RNA response, sustained response was observed in 37 patients (27.4%) and HBsAg loss in 14 patients (10.4%). In contrast, of the 124 patients that did not experience an HBV RNA response, only 16 patients achieved sustained response (13.0%) and one patient achieved HBsAg loss (0.8%; $p = 0.004$ for sustained response and $p = 0.001$ for HBsAg loss).

Similar results were obtained after stratification by HBeAg-status. Among the HBeAg-positive patients with an HBV RNA response (n = 85, 54.1%), sustained response was observed in 20 (23.5%) and HBsAg loss in ten patients (11.8%). In contrast, of the 72 patients (45.9%) that did not experience an HBV RNA response, only seven (9.7%) achieved sustained response and none (0.0%) achieved HBsAg loss ($p = 0.022$ for sustained response and $p = 0.003$ for HBsAg loss). Among the HBeAg-negative patients with an HBV RNA response (n = 50, 49.5%), 17 patients (34.0%) achieved sustained response and four patients (8.0%) achieved HBsAg loss. In contrast, of the 51 patients (50.5%) without an HBV RNA response, sustained response was observed in only nine patients (17.6%) and HBsAg loss in one patient (2.0%; $p = 0.060$ for sustained response and $p = 0.162$ for HBsAg loss).

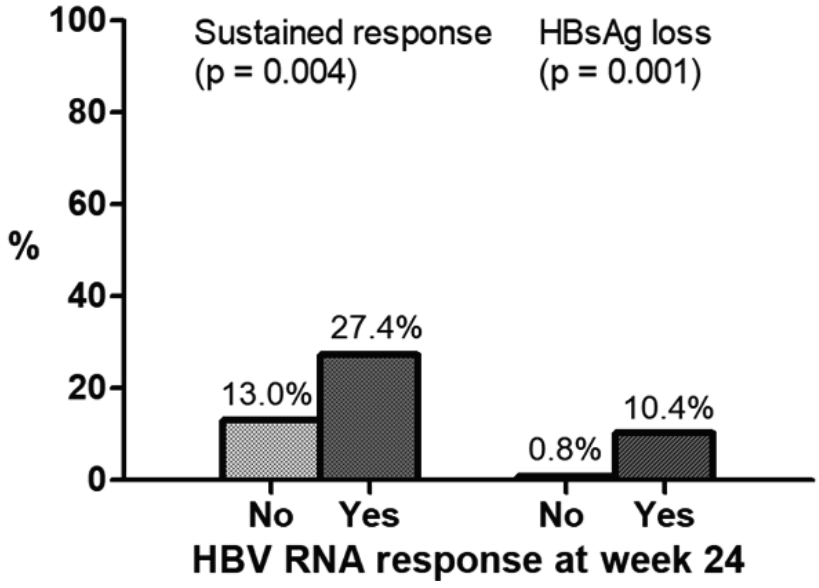
Similar results were obtained when HBV RNA response was assessed at on-treatment week 12 or at end-of-treatment.

Low response rates in the absence of HBsAg decline in patients with HBV RNA response

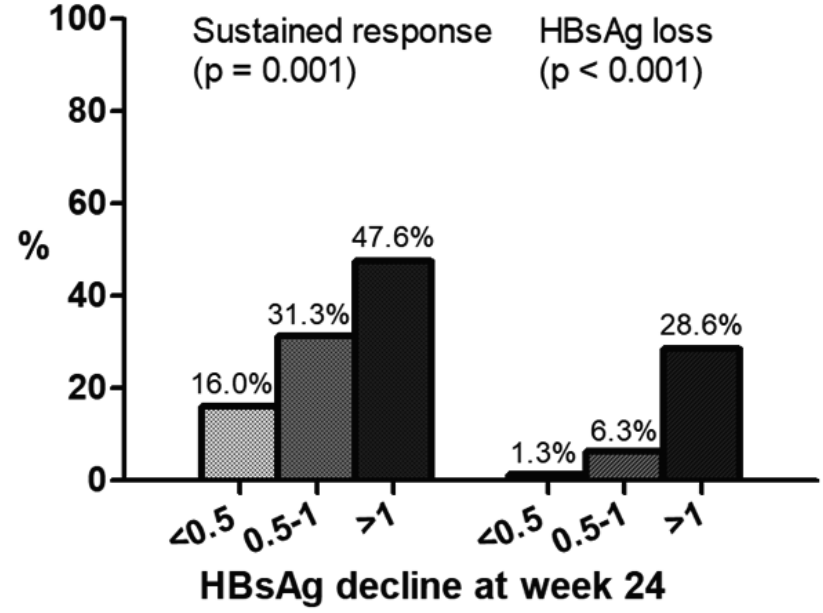
Among the 135 patients with an HBV RNA response at on-treatment week 24, HBsAg data was available in 133 patients. Among patients with an HBV RNA response, a more prominent decline in HBsAg was observed in those who achieved sustained response, compared to those who did not (Figure 2A); mean HBsAg declines were 1.7 versus 0.6 log IU/mL ($p = 0.001$, Figure 1B), with an OR of 1.779 ($p < 0.001$).

Among the 133 HBV RNA responders, 75 patients (56.4%) did not experience at least 0.5 log HBsAg decline at that same time point. Of those 75 patients, only 12 achieved sustained response (16.0%) and one achieved HBsAg loss (1.3%). Of the 42 patients (31.2%) with a concomitant HBsAg decline of more than 1 log, sustained response was achieved in 20 patients (47.6%) and HBsAg loss in 12 patients (28.6%; $p \leq 0.001$).

Figure 1



A)



B)

(A) Rates of sustained response (HBV DNA <2,000 IU/mL) and hepatitis B surface antigen (HBsAg) loss in patients with and without hepatitis B virus (HBV) RNA response at on-treatment week 24 (n=258).

(B) Rates of sustained response and HBsAg loss in subgroup of patients with HBV RNA response at on-treatment week 24 (n=133), stratified by HBsAg decline (<0.5, 0.5-1 or >1 log).

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

Similar results were obtained when data were stratified according to HBeAg-status at baseline. HBV RNA response at on-treatment week 24 was observed in 85/157 HBeAg-positive (54.1%) and in 50/101 HBeAg-negative (49.5%) patients. Among the 85 HBeAg-positive patients with an HBV RNA response, sustained response rates were 10.8% in patients with HBsAg decline of < 0.5 log versus 42.4% in patients with > 1 log HBsAg decline; HBsAg loss rates were 0.0% versus 30.3% ($p = 0.006$ for sustained response and $p < 0.001$ for HBsAg loss).

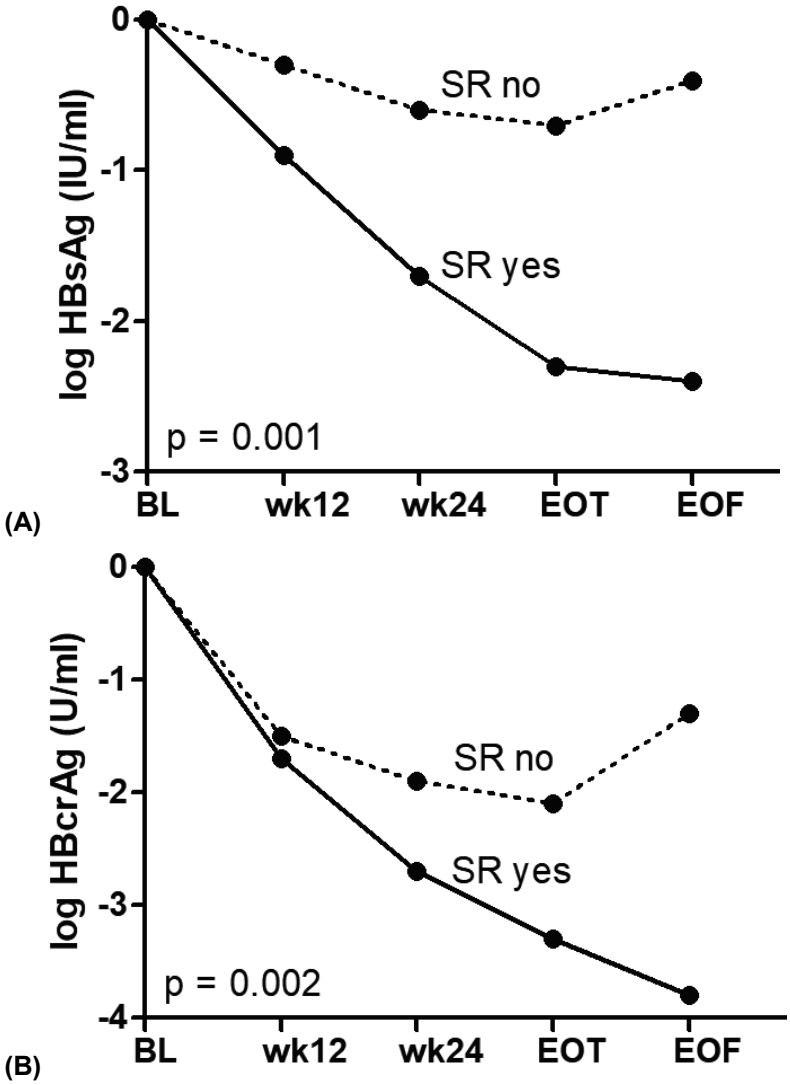
Among the 50 HBeAg-negative patients with an HBV RNA response, sustained response rates were 21.1% (with HBsAg decline < 0.5 log) versus 66.7% (with HBsAg decline > 1 log); and HBsAg loss rates were 2.6% versus 22.2% ($p = 0.002$ for sustained response and $p = 0.037$ for HBsAg loss). Findings were also consistent when analyses were limited to patients treated with PEG-IFN monotherapy; sustained response rates were 18.0% in patients with HBsAg decline < 0.5 log, versus 65.2% in patients with HBsAg decline > 1 log; HBsAg loss rates were 2.0% versus 30.4% ($p \leq 0.001$).

Low response rates in the absence of HBcrAg decline in HBeAg-positive patients with HBV RNA response

In the 157 HBeAg-positive patients HBV RNA response was observed in 85 patients (54.1%) at on-treatment week 24. HBV RNA responders who achieved sustained response showed a more prominent on-treatment decrease in HBcrAg than those who did not (Figure 2B); mean declines were 2.7 versus 1.9 log U/mL at week 24 ($p = 0.002$, Figure 2).

Of the 85 patients with an HBV RNA response at week 24, 79 had HBcrAg data available. A total of 14 patients (17.7%) did not experience at least 1 log HBcrAg decline, of whom only one (7.1%) achieved sustained response and HBsAg loss (Figure 3). Conversely, sustained response was observed in 69.2% (9/13) and HBsAg loss in 53.8% (7/13) of the patients with > 3 log HBcrAg decline ($p < 0.001$ for both sustained response and HBsAg loss).

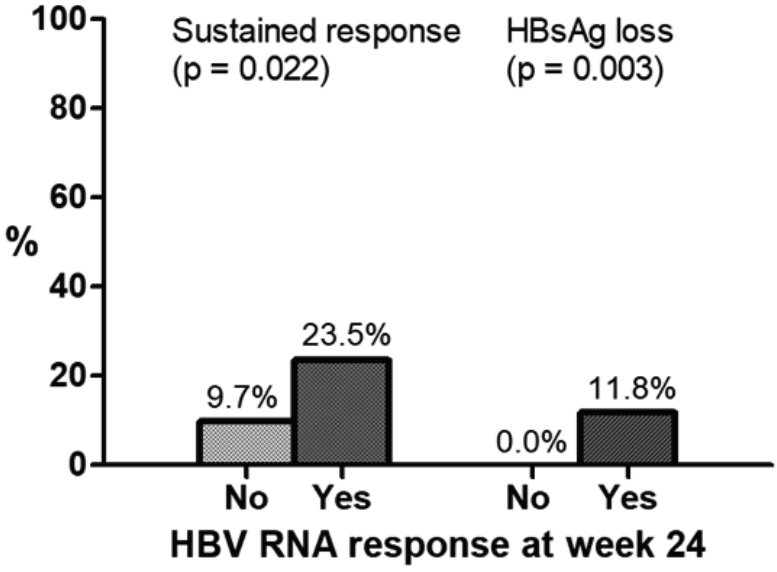
Figure 2



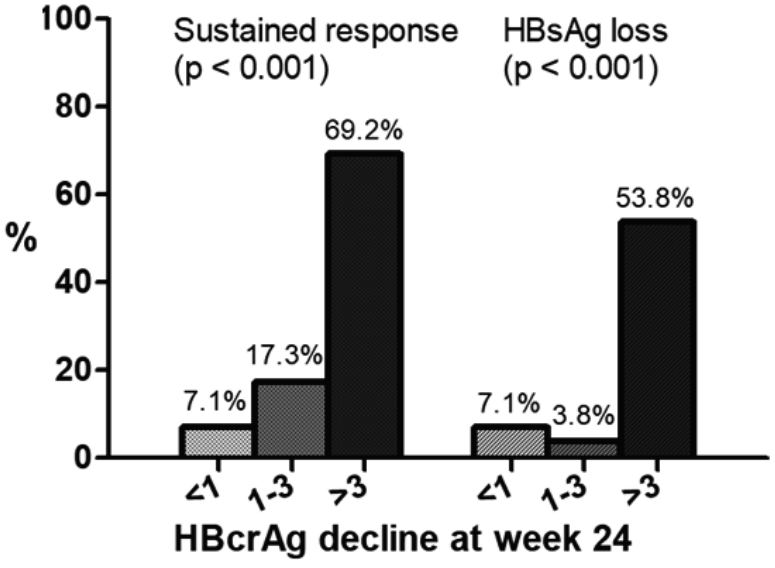
A) Hepatitis B surface antigen (HBsAg) decline during treatment and follow-up in patients with an hepatitis B virus (HBV) RNA response at on-treatment week 24, stratified by patients with and without sustained response (HBV DNA <2,000 IU/mL; SR). B) Hepatitis B core-related antigen (HBcrAg) decline during treatment and follow-up in patients with an HBV RNA response at on-treatment week 24, stratified by patients with and without SR. Assessed in patients with positive hepatitis B e antigen (HBeAg) at baseline. P-value for comparison at week 24.

Abbreviations: HBsAg, hepatitis B surface antigen; HBcrAg; hepatitis B core-related antigen; wk, week; SR, sustained response; BL, baseline; EOT, end-of-treatment; EOF, end of follow-up.

Figure 3



(A)



(B)

A) Rates of sustained response (HBV DNA <2,000 IU/mL) and hepatitis B surface antigen (HBsAg) loss in patients with and without hepatitis B virus (HBV) RNA response at on-treatment week 24. Assessed in patients with positive hepatitis B e antigen (HBeAg) at baseline (n=157). B) Rates of sustained response and HBsAg loss in subgroup of patients with HBV RNA response at on-treatment week 24 (n = 79), stratified by hepatitis B core-related antigen (HBcrAg) decline (<1, 1-3 or >3 log). Assessed in patients with positive HBeAg at baseline.

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen.

DISCUSSION

The current study, a pooled analysis of two randomised trials, confirms that on-treatment HBV RNA decline is associated with higher rates of sustained off-treatment response and HBsAg loss in patients treated with finite PEG-IFN based treatment regimens. However, many patients with HBV RNA decrease did not experience a decline in the viral antigens HBsAg and/or HBcrAg. In our study, treatment response was predominantly observed in patients with both HBV RNA and viral antigen decline, whereas response rates in patients without concomitant viral antigen decrease were very low. These findings suggest that combinations of biomarkers should be used to ascertain a clinically relevant response to antiviral therapy and may have important implications for the interpretation of the antiviral efficacy of novel antiviral agents, such as capsid assembly modifiers.

In the past years, several viral biomarkers have been identified that may be used to estimate the probability of response to antiviral therapy, possibly through correlations with intrahepatic HBV transcriptional activity. Examples include serum levels of viral antigens, such as HBsAg, HBeAg and HBcrAg, and more recently also serum levels of HBV RNA. It is important to note that the intrahepatic cccDNA acts as a template for all of these biomarkers through transcription of four overlapping open reading frames (ORFs). Each ORF encodes for a subtype of polyadenylated RNAs, which subsequently serve as templates for the transcription of a number of proteins.[16,17] Their relative expression may be influenced by many factors, including host immune responses and antiviral therapy. Interpretation of kinetics of a single biomarker may therefore be misleading if changes in other biomarkers are not considered.

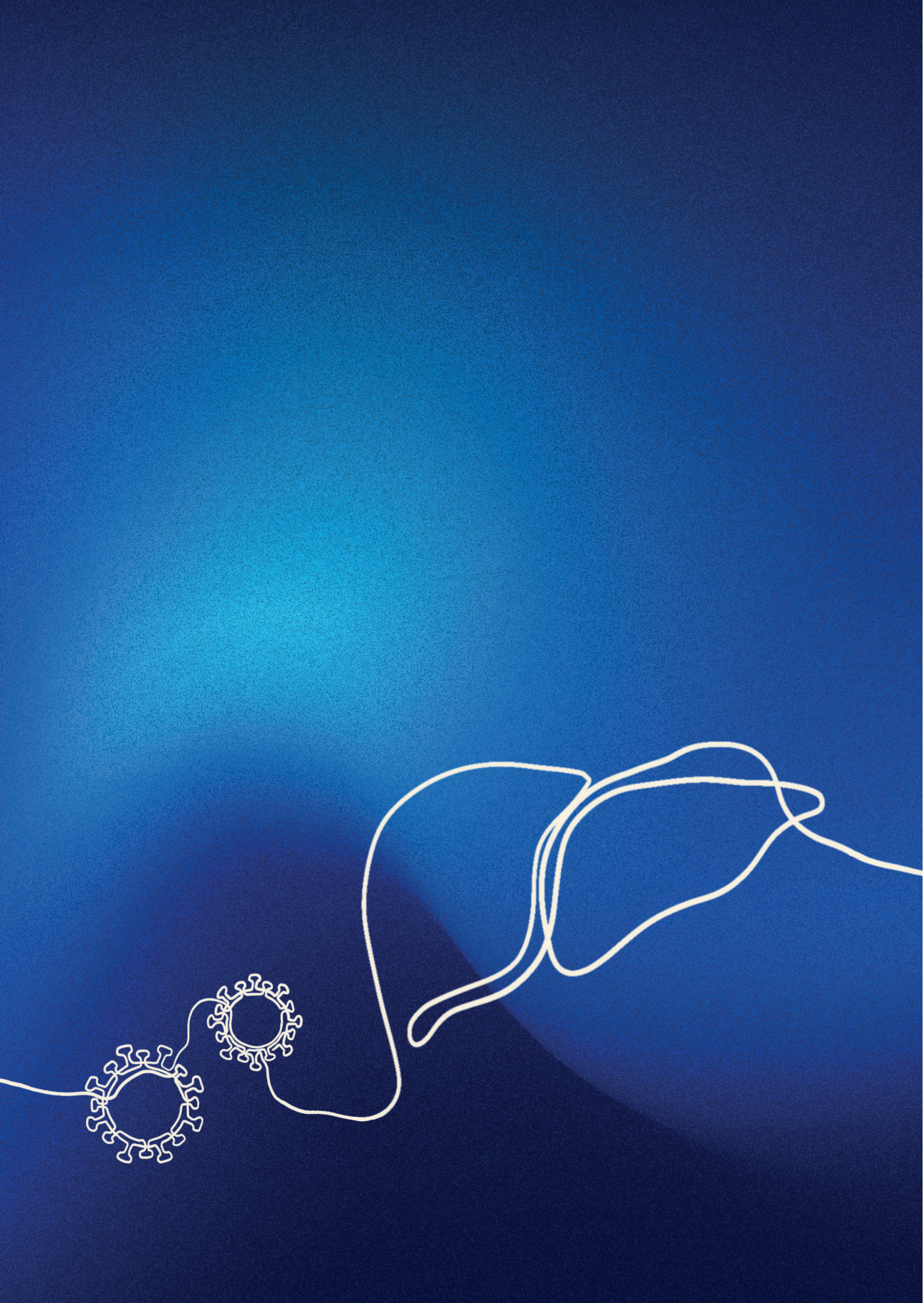
The potential advantage of HBV RNA as a biomarker is based on the assumption that serum HBV RNA levels reflect an early step in the HBV replication process. A decline of HBV RNA levels has therefore been postulated to directly reflect a decrease in HBV transcriptional activity, either through a reduction in the cccDNA template or inhibition of transcriptional activity. This phenomenon is elegantly demonstrated by studies that show potent HBV DNA, but limited HBV RNA, decreases with NA therapy,[72,76] since NA therapy does not influence HBV RNA production nor the cccDNA reservoir.[61] On the other hand, among the few patients experiencing significant HBV RNA declines during treatment with nucleos(t)ide analogues or PEG-IFN, HBV RNA decline was associated with higher rates of off-treatment sustained response.[70,72,75,76]

Interestingly, two preliminary reports of recent studies in patients treated with novel capsid assembly modifiers have demonstrated superior HBV RNA declines.[66,77] Whether these declines also translate to higher rates of off-treatment sustained response remains to be determined as off-treatment data have not yet been reported. Importantly, both studies showed virtually no declines in viral antigens such as HBsAg and HBcrAg, despite the potent effects on HBV RNA. This apparent disconnection between the different markers may be accounted for by the relative short treatment duration of capsid assembly modifiers in experiment trials, as well as the different mode of action of the capsid assembly modifiers, which may have direct effects on HBV RNA production whilst not interfering with viral antigen production. The observed decrease in HBV RNA may therefore not reflect cccDNA decline. Until now, it has been unclear how such a response, i.e. a strong HBV RNA decline but with persistently high levels of viral antigens, relates to the prospect of subsequent treatment response.

Our study shows to the best of our knowledge for the first time that many patients experiencing HBV RNA decline during treatment with conventional antiviral agents do not experience concomitant decreases in HBsAg and HBcrAg. In this cohort, response rates were extremely low in this group, underscoring the complex interplay between these biomarkers. These findings may have important implications for studies evaluating novel antiviral agents, in particular the capsid assembly modifiers and other agents interfering with HBV RNA production, as it appears that HBV RNA decline itself might not be an adequate predictor of sustained response. We therefore argue that HBV RNA decline should not be used as a primary endpoint for treatment trials.

Strengths of our study include the large and very well characterised patient sample enrolled from two global randomised trials and the availability of data on a wide range of biomarkers. We were also able to assess both sustained response and HBsAg loss. However, despite the large number of patients, subgroup analysis did limit the number of available cases per group. Nevertheless, our findings were consistent when separately analysing patients according to HBeAg-status or treatment regime. However, validation of our findings among these subgroups, as well as confirmation of our hypothesis on the applicability to novel agents, warrants further investigation.

In conclusion, HBV RNA decline without concomitant viral antigen (HBsAg and/or HBcrAg) decline is associated with low off-treatment sustained response rates an HBsAg loss. Combinations of viral markers should be used to accurately assess response to antiviral therapy.



CHAPTER 3

Levels of antibodies to hepatitis B core antigen are associated with liver inflammatory activity and response to peginterferon in patients with chronic hepatitis B

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ABSTRACT

Background & Aim(s): Emerging evidence suggests a pivotal role for B cell responses in the natural history of chronic hepatitis B. Serum levels of antibodies to hepatitis B core antigen (anti-HBc) vary across infection stages, but their role in predicting response to antiviral therapy is uncertain.

Methods: Anti-HBc levels were assessed before peginterferon (PEG-IFN) therapy in chronic hepatitis B patients who either initiated *de novo* PEG-IFN (n = 299; 195 hepatitis B e antigen [HBeAg] positive) or started PEG-IFN as add-on to an existing nucleo(s)tide analogue backbone (n = 91; all HBeAg-positive). Associations were explored between anti-HBc and (1) serum biomarkers, (2) liver histological findings, and (3) treatment response.

Results: We studied 390 patients. The hepatitis B virus (HBV) genotype were A, B, C, and D in 24%, 9%, 16%, and 49%, respectively; 72% of patients were Caucasian. Among currently untreated HBeAg-positive patients, anti-HBc was correlated with HBV DNA, hepatitis B core-related antigen (HBcrAg), hepatitis B surface antigen (HBsAg) and HBV RNA, but not with alanine aminotransferase (ALT). Higher anti-HBc was associated with more severe histological inflammatory activity ($p < 0.001$), irrespective of HBeAg status. After *de novo* PEG-IFN, higher anti-HBc levels were associated with HBeAg loss, sustained response, HBsAg decline and HBsAg-clearance ($p < 0.050$). Among patients treated with add-on PEG-IFN, higher anti-HBc was associated with HBeAg loss ($p = 0.012$).

Conclusion: Serum anti-HBc levels correlate with histological inflammatory activity. Higher anti-HBc levels were associated with favourable treatment outcomes. These findings suggest that anti-HBc could be used to select patients most likely to respond to immunomodulatory therapy.

INTRODUCTION

The natural history of chronic hepatitis B (CHB) infection is marked by distinct clinical phases, which are characterised by different patterns of serum HBeAg status, viral load and transaminase levels reflecting the highly complex host-virus interplay.[82]

The immune system appears to act as a double-edged sword in patients with CHB; in an attempt to clear infected cells it causes liver inflammation and injury that may result in development of liver fibrosis and, ultimately, cirrhosis.[83] Emerging evidence suggests that, besides the innate immune system and virus-specific T cells, B cells play a role in the defence against HBV.[83-85] A recent study showed that that the humoral immune response among CHB patients is mainly mediated by HBcAg-specific memory B cells and not HBsAg-specific B cells. Furthermore, serum levels of antibodies to hepatitis B core antigen (anti-HBc) varied across the different phases in the natural history of chronic hepatitis B (CHB), with higher levels observed during phases with more pronounced liver inflammation.[86] The relationship between serum anti-HBc levels and hepatic inflammation is compelling, as currently used biomarkers (such as alanine aminotransferase [ALT]) correlate rather poorly with histological activity.[87] This is especially relevant in the light of studies suggesting that circulating immune markers may predict response to immunomodulatory therapy.[88,89]

We therefore aimed to study the association between serum levels of anti-HBc and (1) other serum biomarkers, (2) histological inflammatory activity, and (3) response to immunomodulatory therapy in patients with CHB.

PATIENTS AND METHODS

Study population

This study included CHB patients who participated in four global randomised controlled trials (the 99-01, PARC, ARES, and PEGON studies). Trial design and inclusion criteria have been described in detail elsewhere.[34,79,90,91] In short, the 99-01 study included HBeAg-positive patients (n = 266) who were randomised to *de novo* PEG-IFN treatment with either PEG-IFN alpha-2b 100 µg/week alone or in combination with lamivudine for 52 weeks.[34] In the PARC study, HBeAg-negative patients (n = 133) were randomised to *de novo* PEG-IFN treatment with either PEG-IFN alpha-2a 180 µg/week mono-therapy or PEG-IFN plus ribavirin 1000-2000 mg combination therapy for 48 weeks.[79] The ARES study enrolled HBeAg-positive patients (n = 175) who started with entecavir (ETV) 0.5 mg/day monotherapy, and were subsequently randomised to receive either PEG-IFN alpha-2a add-on therapy from week 24 to week 48 (n = 85) or to continue ETV mono-therapy (n = 90).[90] In the PEGON (n = 77), HBeAg-positive patients who have been treated for at least one year with nucleo(s)tide analogue (NA) therapy were enrolled and randomised to

receive 48 weeks of add-on PEG-IFN therapy (n = 39) or to continue NA monotherapy (n = 38).[91]

All patients had CHB defined as HBsAg-positivity for at least six months. For the 99-01, PARC and ARES studies, additional inclusion criteria comprised serum HBV DNA levels of more than 10,000 copies/ml (± 2,000 IU/ml) and ALT ≥ 1.3 times (ARES study) or ≥ 1.5-2 times (99-01 and PARC studies) the upper limit of normal (ULN) at baseline.[34,79,90] Additional inclusion criteria of the PEGON study included serum HBV DNA levels < 2,000 IU/mL and ALT levels < 5 ULN during NA therapy.[91] The original study protocols have been approved by the medical ethical committees and are in line with the Declaration of Helsinki of 1975. All patients provided written consent.

For this study, we selected patients who received *de novo* PEG-IFN (ie. patients from 99-01 and PARC) or add-on PEG-IFN (i.e, the patients enrolled in the add-on PEG-IFN arms from ARES and PEGON) as shown in Supplementary Figure 1.

Biochemistry and virology

Anti-HBc (IgG) was measured at baseline (i.e. before initiation of IFN; pre-treatment levels) and at end of PEG-IFN treatment (EOT levels), using Lumipulse® G CLEIA anti-HBc assay (Fujirebio Europe, lower limit of detection [LLOD] 15 IU/mL). HBsAg was quantified using the Abbott Architect (Abbott Park, IL) with a LLOD of 0.05 IU/mL. For HBV DNA the LLOD was 400 copies/mL (~80 IU/mL; in-house TaqMan PCR assay, Rotterdam, the Netherlands) for 99-01[34], 35 copies/mL (~10 IU/mL; Taqman, Roche Diagnostics, Basel, Switzerland) for PARC[79] and 20 IU/mL (Cobas TaqMan 48, Roche Diagnostics, Basel, Switzerland) for ARES[90] and PEGON[91] participants. HBV RNA (University Hospital Leipzig, Germany) was measured using rapid amplification of complementary DNA (cDNA)-ends (RACE)-based real-time polymerase chain reaction (LLOD 800 copies/mL).[71,80] HBcrAg was quantified using Lumipulse® G HBcrAg assay (Fujirebio Europe) according to the manufacturer's instructions, with a lower limit of quantification (LLOQ) of 1,000 U/mL (3 log) and LLOD of 2 log.[81] Serum interferon-y inducible protein 10 (IP-10) was quantified using ELISA (Alta Analytical Laboratory, San Diego, USA). ALT was quantified using automated techniques at the participating centres.[34,79,90,91]

Liver histology

Pre-treatment liver histology was assessed in patients treated with *de novo* PEG-IFN (i.e. those enrolled in 99-01 or PARC). Liver inflammation was scored using to the histological activity index (HAI, range 0-18).[87,92] HAI scores were categorised as no inflammation (HAI 0-3), mild inflammation (HAI 4-8), and moderate-severe inflammation (HAI 9-18).[42,93] Liver fibrosis classification was based on Ishak fibrosis stage.

Definitions of treatment response

Treatment response was assessed at end of PEG-IFN treatment (EOT) and at six months after PEG-IFN withdrawal (end of follow-up [EOF]; in *de novo* PEG-IFN patients only). On-treatment ALT flares were defined as an increase of serum ALT ≥ 5x ULN during PEG-IFN treatment.[36,42] Outcomes assessed at EOT included HBeAg loss and decline in HBsAg (≥ 1 log from baseline). Outcomes assessed at EOF included sustained response (HBV DNA < 2,000 IU/mL) and HBsAg loss.

Statistical analysis

Analyses were performed in the overall population, and stratified by treatment strategy (*de novo* or add-on PEG-IFN) or baseline HBeAg status. Descriptives are presented as numbers (with percentages), medians (with interquartile range; IQR) and means (± standard deviation; SD). Correlations between pre-treatment anti-HBc levels and age, HAI score, and pre-treatment serum ALT, IP-10, HBV DNA, HBsAg, HBcrAg and HBV RNA levels were assessed using Pearson correlation coefficient in the subset of patients treated with *de novo* PEG-IFN (stratified by HBeAg status). Associations between anti-HBc levels and histology or treatment outcomes were assessed using continuous data (with associations assessed using student t-test, ANOVA, logistic regression and area under the ROC curve; AUROC), and, since no cut-offs are defined in current literature, after categorisation into three groups of equal size (low/intermediate/high).

Multivariable analyses were performed by entering anti-HBc levels (as units of 0.1 log IU/mL) and other potential predictors (including age, sex, HBV genotype A, HBeAg status at baseline, and serum ALT, HBsAg and HBV DNA levels at baseline) into a backward selection based logistic regression model. Differences were considered statistically significant when $p < 0.05$. IBM SPSS for Windows version 25.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Graph Pad Prism version 5 for Windows (GraphPad Software, San Diego, California, USA) was used for graphical representation of the results.

RESULTS

Patient characteristics

In total, we enrolled 390 patients; 299 treated with *de novo* PEG-IFN (195 HBeAg-positive) and 91 treated with add-on PEG-IFN. Patient characteristics are displayed in Table 1. The HBeAg-positive *de novo* PEG-IFN cohort included predominantly Caucasian patients (76.4%), with genotypes A or D (respectively 37.9% and 39.0%). The HBeAg-negative *de novo* PEG-IFN cohort included predominantly Caucasian patients (94.2%), with genotype D (78.8%). The add-on PEG-IFN cohort included predominantly Asians (61.5%), with genotype A/B/C/D in 4.4/23.1/38.5/34.1%.

Table 1. Characteristics of the patients with pre-treatment antibodies against hepatitis B core antigen

	De Novo PEG-IFN, HBeAg-Positive n = 195	*De Novo* PEG-IFN, HBeAg-Negative n = 104	Add-on PEG-IFN, HBeAg-Positive n = 91
Age at inclusion, years (median, IQR)	33 (25-44)	41 (33-49)	30 (24-38)
Male (n, %)	153 (78.5)	75 (72.1)	65 (71.4)
Race (n, %)			
Caucasian	149 (76.4)	98 (94.2)	33 (36.3)
Asian	31 (15.9)	4 (3.8)	56 (61.5)
Other	15 (7.7)	2 (1.9)	2 (2.2)
HBV genotype (n, %)			
A	74 (37.9)	14 (13.5)	4 (4.4)
B	15 (7.7)	0 (0.0)	21 (23.1)
C	23 (11.8)	3 (2.9)	35 (38.5)
D	76 (39.0)	82 (78.8)	31 (34.1)
Other	7 (3.6)	5 (4.8)	-
Pre-treatment Liver inflammation (HAI score; n, %)			
None (HAI 0-3)	37/155 (23.9)	18/98 (18.4)	-
Mild (HAI 4-8)	106/155 (68.4)	72/98 (73.5)	-
Moderate-severe (HAI 9-18)	12/155 (7.7)	8/98 (8.2)	-
Study treatment (n, %)			
PEG-IFN monotherapy	104 (53.3)	51 (49.0)	-
PEG-IFN + LAM	91 (46.7)	-	-
PEG-IFN + RBV	-	53 (51.0)	-
NA + add-on PEG-IFN	-	-	91 (100)
Baseline Laboratory results			
ALT[α] (median, IQR)	130 (89-186)	94 (65-183)	102 (63-169)
Anti-HBc[ǂ] (mean, ±SD)	3.80 (±0.46)	4.16 (±0.39)	2.88 (±0.73)
HBsAg[ǂ] (mean, ±SD)	4.41 (±0.60)	3.86 (±0.50)	3.72 (±0.66)
HBV DNA[ǂ] (mean,±SD)	8.37 (±0.83)	6.08 (±1.21)	2.74 (±1.49)

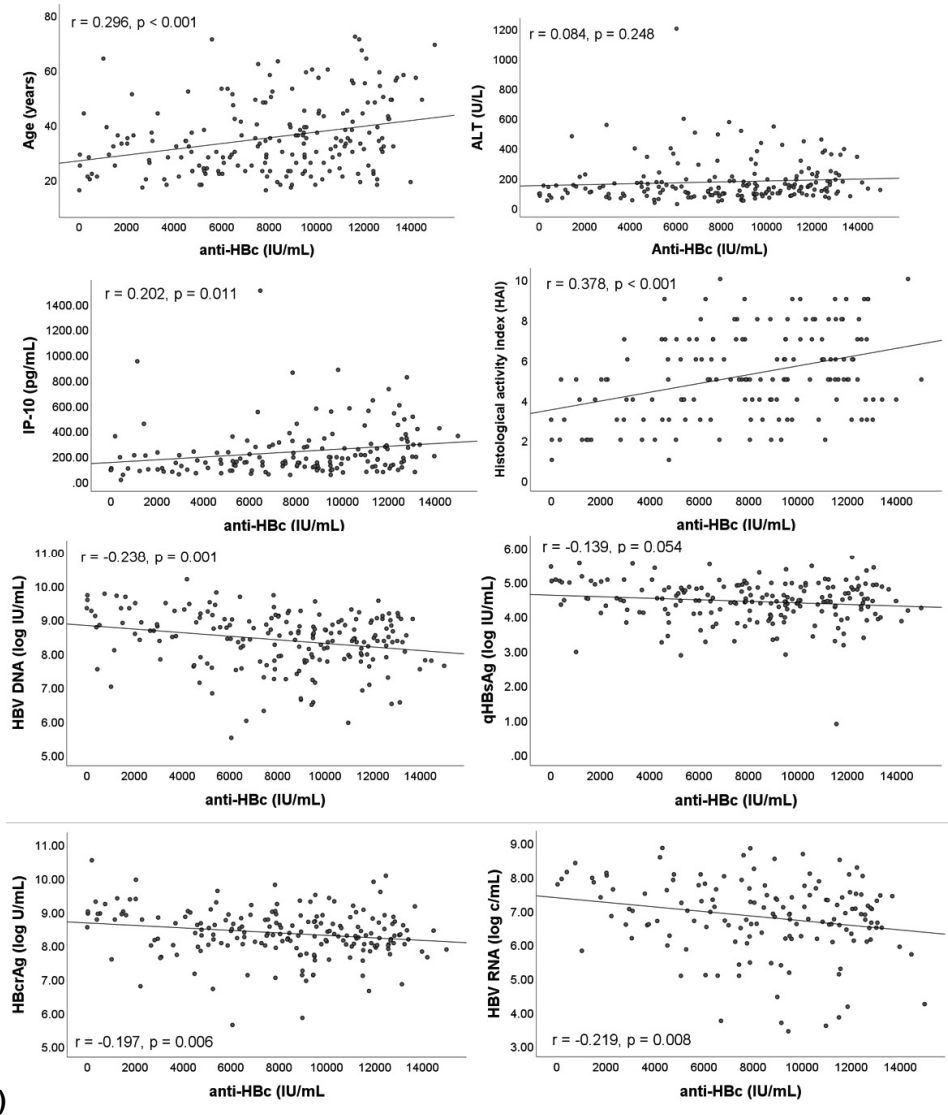
HBV RNA$^{\pi}$ (mean, ±SD)	6.79 (±1.11)	4.38 (±0.98)	4.85 (±1.50)
HBcrAg$^{\Sigma}$ (mean, ±SD)	8.35 (±0.70)	5.00 (±1.42)	8.11 (±0.76)
Treatment response (n, %)			
On-treatment ALT flares$^{\infty}$	102/194 (52.6)	48/103 (46.6)	6/90 (6.7)
HBeAg loss EOT$^{\Omega}$	78 (40.0)	-	16/90 (17.8)
HBsAg decline EOT (≥ 1 log)	53/174 (30.5)	19/102 (18.6)	8/89 (9.0)
Sustained response$^{\beta}$	37/170 (21.8)	25/95 (26.3)	-
HBsAg loss$^{¥}$	16/173 (9.2)	1/100 (1.0)	3 (3.3)

$^{\alpha}$ U/L
‡ Logarithmic scale, IU/mL
$^{\pi}$ Logarithmic scale, copies/mL
$^{\Sigma}$ Logarithmic scale, U/mL
$^{\infty}$On treatment ALT flare is defined as ALT ≥ 5x the upper limit of normal during PEG-IFN therapy.
$^{\Omega}$ HBeAg loss at end-of-treatment in pre-treatment HBeAg-positive patients
$^{\beta}$ Sustained response was defined as HBV DNA < 2,000 IU/mL six months after end of PEG-IFN treatment
$^{¥}$ HBsAg loss was defined as HBsAg clearance at EOF.
Abbreviations: HBV, hepatitis B virus; PEG-IFN, peginterferon; LAM, lamivudine; RBV, ribavirin; NA, nucleos(t)ide analogue; anti-HBc, antibodies to hepatitis B core antigen; HBcrAg, Hepatitis B core-related Antigen; HBeAg, Hepatitis B e Antigen; HAI, histological activity index; HBsAg, quantitative hepatitis B surface antigen; EOF, end of follow-up (i.e. six months after PEG-IFN treatment withdrawal); EOT, end of PEG-IFN treatment; IQR, interquartile range; c/mL, copies/millilitre; IU/mL, international units/millilitre

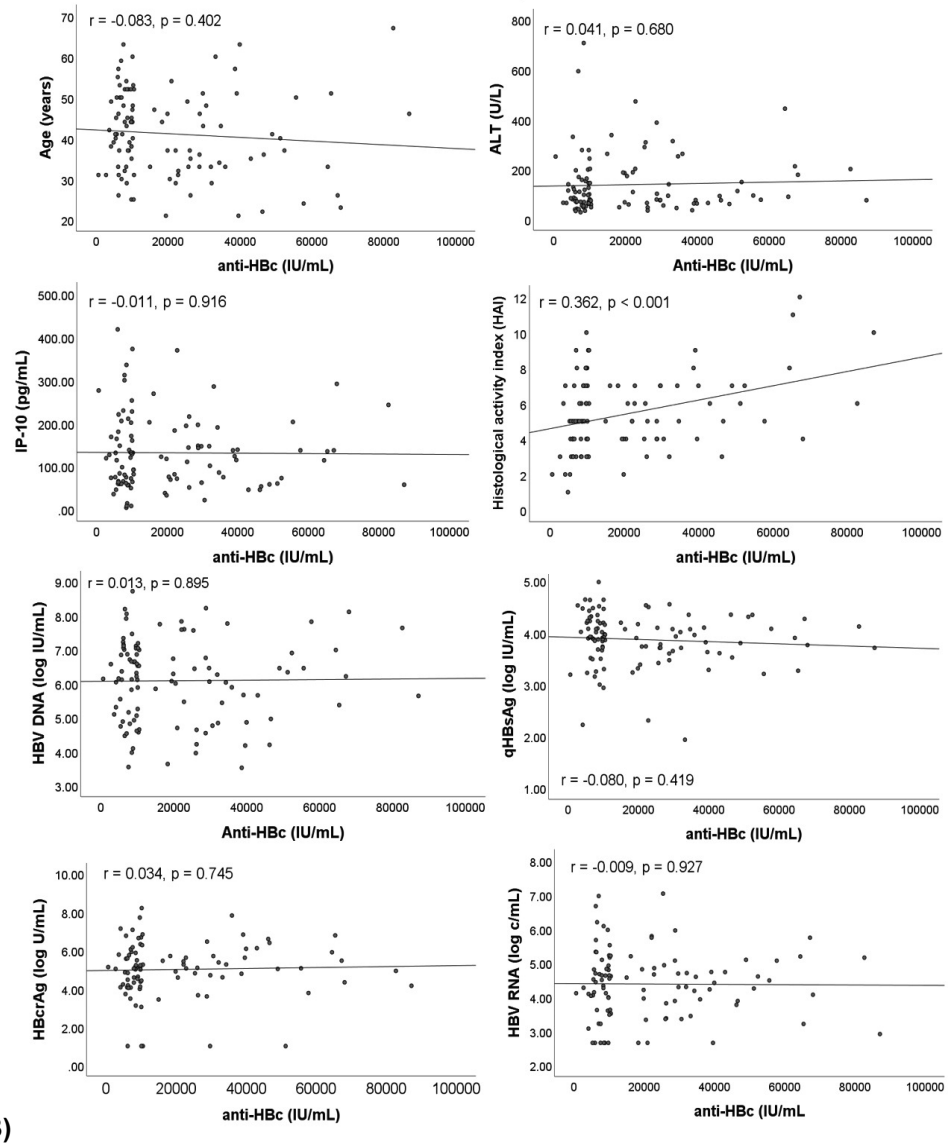
Anti-HBc levels correlate with age, serum IP-10 and markers of viral replication, but not with ALT

Among untreated HBeAg-positive patients, positive correlations were observed for anti-HBc levels with age and pre-treatment serum IP-10 levels, but not with ALT. Negative correlations were observed with markers of viral replication including with HBV DNA, HBcrAg, HBsAg and HBV RNA levels (Figure 1A). Serum anti-HBc levels did not correlate with any of the serum biomarkers in untreated HBeAg-negative patients (Figure 1B). Mean anti-HBc levels varied significantly across HBV genotype. Anti-HBc levels were highest among patients with HBV genotype A and lowest among patients with HBV genotype D: 3.98 log vs 3.61 log IU/mL ($p < 0.001$) among HBeAg-positive and 4.44 log vs 4.16 log IU/mL ($p = 0.036$) among HBeAg-negative patients (Supplementary Figure 2).

Figure 1. Correlation between anti-HBc levels with age, ALT, histological activity index and markers of viral replication among HBeAg-positive (A) and HBeAg-negative (B) patients



A)



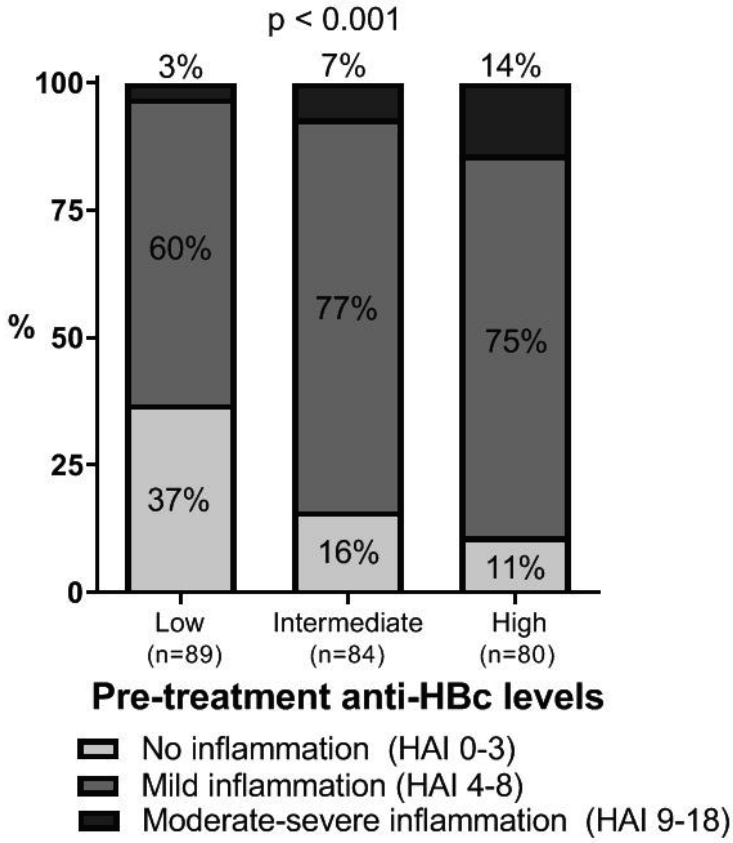
B)

Abbreviations: anti-HBc, antibodies to hepatitis B core antigen; ALT, alanine aminotransferase; c/ml, copies/millilitre; IU/mL, international units/millilitre; HAI, histological activity index; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; HBV hepatitis B virus; IP-10, interferon-y inducible protein 10.

Serum anti-HBc levels correlate with intrahepatic inflammatory activity

Among the 253 patients with pre-treatment liver histology data available, anti-HBc levels correlated with the severity of inflammatory activity (r = 0.38 for HBeAg-positive and r = 0.36 for HBeAg-negative patients, p < 0.001, Figure 1). Among the 89 patients with the lowest pre-treatment anti-HBc levels, only 3 patients (3.4%) had moderate to severe inflammation (HAI 9-18) compared to 11/80 (13.8%) with the highest anti-HBc levels (p < 0.001, Figure 2; AUROC 0.666, 95% CI 0.550 – 0.781, p = 0.014). Similar results were obtained in multivariable logistic regression (aOR for moderate-severe inflammation: 1.24, 95% CI 1.04 – 1.48, p = 0.015).

Figure 2. Relationship between anti-HBc levels and intrahepatic inflammatory activity



Liver inflammation was defined as no inflammation (HAI 0-3), mild inflammation (HAI 4-8), and moderate-severe inflammation (HAI 9-18). Anti-HBc levels were categorised as low, intermediate or high (<3.82/3.82-4.0/≥4.0 log IU/mL for HBeAg-positive and <3.95/3.95-4.40/≥4.40 log IU/mL for HBeAg-negative patients) to create 3 groups of equal size.
Abbreviations: anti-HBc, antibodies to hepatitis B core antigen; HAI, histology histological activity index; EOT, end of PEG-IFN treatment; IU/mL, international units/millilitre

Serum anti-HBc levels decrease during PEG-IFN based antiviral therapy

Baseline anti-HBc levels were higher in untreated patients (i.e., the *de novo* PEG-IFN patients, 3.93 log IU/mL (± 0.47)) when compared to patients on NA therapy (i.e. add-on PEG-IFN patients, 2.88 log IU/mL (± 0.73); $p < 0.001$). Furthermore, PEG-IFN therapy significantly reduced serum anti-HBc levels: mean declines from baseline to EOT were 0.25 log (± 0.36) among HBeAg-positive patients treated with *de novo* PEG-IFN, 0.47 log (± 0.41) in HBeAg-negative patients treated with *de novo* PEG-IFN, and 0.29 log (± 0.28) among patients who received add-on PEG-IFN ($p < 0.001$).

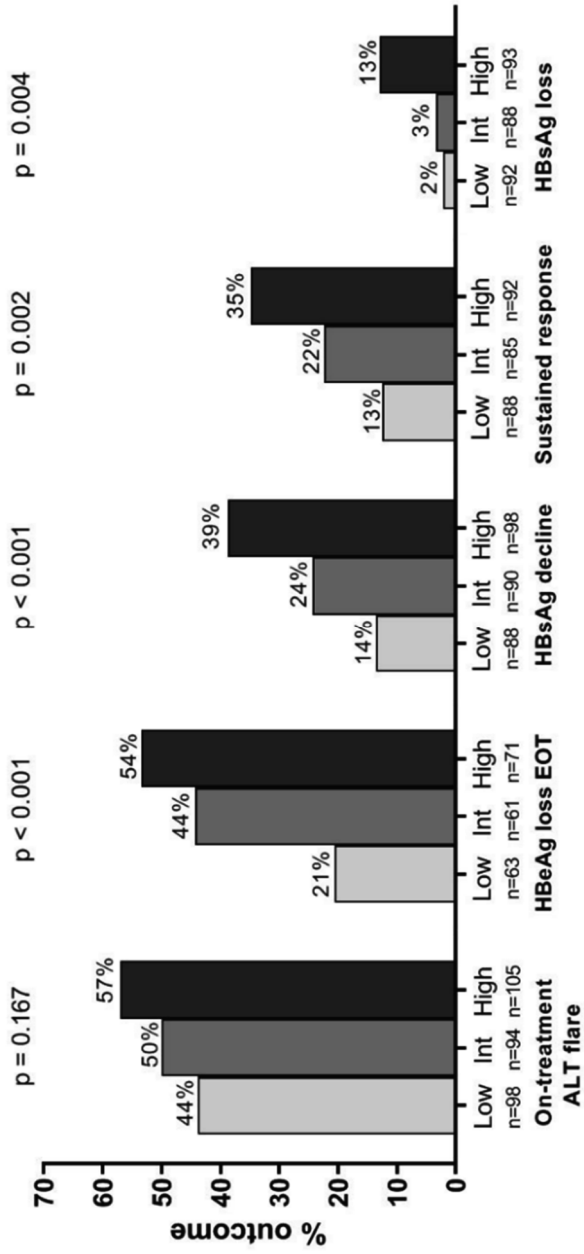
Higher pre-treatment anti-HBc levels are associated with favourable treatment outcomes

De Novo PEG-IFN

Pre-treatment anti-HBc levels were higher in patients with favourable outcomes after PEG-IFN therapy (Figure 3 and 4). Patients with the highest anti-HBc levels achieved sustained response in 35% and HBsAg loss in 13%, compared to 13% and 2% among patients with the lowest anti-HBc levels ($p \leq 0.004$; Figure 3). Interestingly, HBeAg-positive patients with on-treatment ALT flares had higher pre-treatment anti-HBc levels (Figure 4).

The association between higher anti-HBc levels and favourable treatment outcomes were generally consistent after stratification by HBeAg status, although associations were less pronounced in the smaller HBeAg-negative subset (Supplementary Figure 3). Consistent results were obtained in multivariable analysis (Table 2).

Figure 3. Treatment outcome according to pre-treatment anti-HBc level

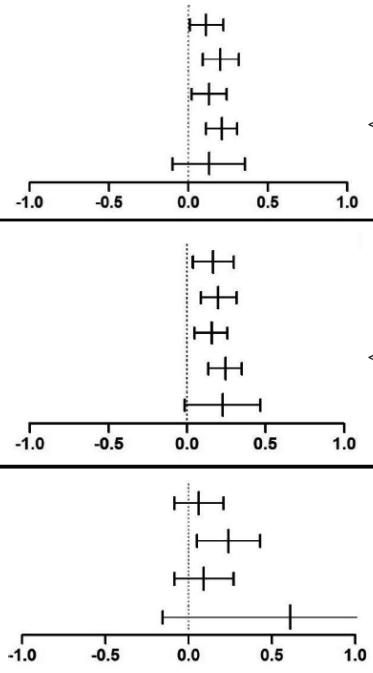


Anti-HBc levels were categorised as low, intermediate or high (<3.82/3.82-4.0/≥4.0 log IU/mL for HBeAg-positive and <3.95/3.95-4.40/≥4.40 log IU/mL for HBeAg-negative patients) to create 3 groups of equal size. An on-treatment ALT flare was defined as an increase of serum ALT ≥ 5x ULN during PEG-IFN treatment. HBsAg decline was defined as a decline ≥ 1 log at EOT. Sustained response was defined as HBV DNA levels of < 2,000 IU/mL six months after end of PEG-IFN treatment. HBsAg loss was defined as HBsAg clearance at EOF.

Abbreviations: EOT, end of PEG-IFN treatment; EOF, six months after PEG-IFN treatment withdrawal; ALT, alanine amino-transferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PEG-IFN, peg-interferon; int, intermediate; IU/mL, international units/millilitre

Figure 4. Pre-treatment anti-HBc levels according to treatment response

	%	Mean anti-HBc (log IU/mL)		Mean difference (log IU/mL)	p-value
		No event	Event		
All patients					
On-treatment ALT flare	50.5	3.87 (±0.55)	3.98 (±0.36)		0.039
HBeAg loss EOT	40.0	3.72 (±0.53)	3.92 (±0.27)		0.001
HBsAg decline EOT (1 log)	26.1	3.91 (±0.45)	4.05 (±0.33)		0.023
Sustained response	23.4	3.88 (±0.51)	4.08 (±0.30)		<0.001
HBsAg loss	6.2	3.92 (±0.49)	4.05 (±0.23)		0.279
HBeAg-positive patients					
On-treatment flare	52.6	3.71 (±0.59)	3.88 (±0.28)		0.012
HBeAg loss EOT	40.0	3.72 (±0.53)	3.92 (±0.27)		0.001
HBsAg decline EOT (1 log)	30.5	3.78 (±0.43)	3.93 (±0.24)		0.003
Sustained response	21.8	3.74 (±0.52)	3.99 (±0.17)		<0.001
HBsAg loss	9.2	3.77 (±0.49)	4.00 (±0.14)		0.060
HBeAg-negative patients					
On-treatment flare	48.0	4.13 (±0.36)	4.19 (±0.42)		0.442
HBsAg decline EOT (1 log)	18.6	4.11 (±0.38)	4.36 (±0.37)		0.013
Sustained response	26.3	4.14 (±0.39)	4.23 (±0.38)		0.312
HBsAg loss	1.0	4.15 (±0.38)	4.76		0.117



An ALT flare was defined as an increase of serum ALT ≥ 5x ULN during PEG-IFN treatment. HBsAg decline was defined as a decline of ≥ 1 log at EOT. Sustained response was defined as HBV DNA levels of < 2,000 IU/mL six months after end of PEG-IFN treatment.
Abbreviations: anti-HBc, antibodies to hepatitis B core antigen; EOT, end of PEG-IFN treatment; EOF, end of follow-up (i.e. six months after PEG-IFN treatment withdrawal); ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PEG-IFN, peginterferon; IU/mL, international units/millilitre.

Table 2. Association between antibodies to hepatitis B core antigen and treatment outcomes in multivariable analysis in patients receiving *de novo* peginterferon

	All			HBeAg-positive			HBeAg-negative		
	aOR	*95% CI*	*P-value*	*aOR*	*95% CI*	*P-value*	*aOR*	*95% CI*	*P-value*
On-treatment ALT flare	1.09	1.02 – 1.17	0.014	1.12	1.02 – 1.23	0.016	1.05	0.92 – 1.20	0.497
HBsAg decline EOT	1.18	1.07 – 1.31	0.001	1.14	1.00 – 1.31	0.058	1.19	1.03 – 1.37	0.017
HBeAg loss EOT	1.13	1.00 – 1.28	0.049	1.13	1.00 – 1.28	0.049	-	-	-
Sustained response	1.13	1.04 – 1.23	0.006	1.30	1.01 – 1.66	0.040	1.09	0.96 – 1.24	0.177
HBsAg loss	1.37	0.95 – 1.98	0.091	1.27	0.86 – 1.88	0.227	-*	-	-

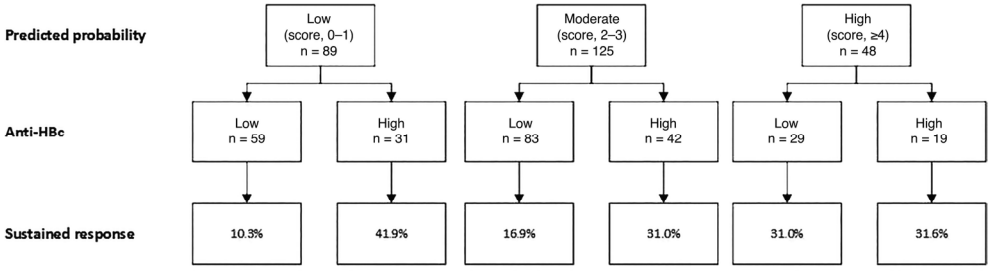
*On-treatment ALT flare was defined as an ALT level ≥ 5x ULN during PEG-IFN treatment. HBsAg decline was defined as a decline of ≥ 1 log 6 months after the EOT. Sustained response was defined as HBV DNA levels of < 2,000 IU/mL six months after end of PEG-IFN treatment. HBsAg loss was defined as loss of HBsAg at any time during treatment or off-treatment follow-up. *Insufficient number of events for multivariable analysis.*
Abbreviations: anti-HBc, antibodies to hepatitis B core antigen; EOT, end of PEG-IFN treatment; ALT; alanine aminotransferase; aOR, adjusted odds ratio; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PEG-IFN, peginterferon.

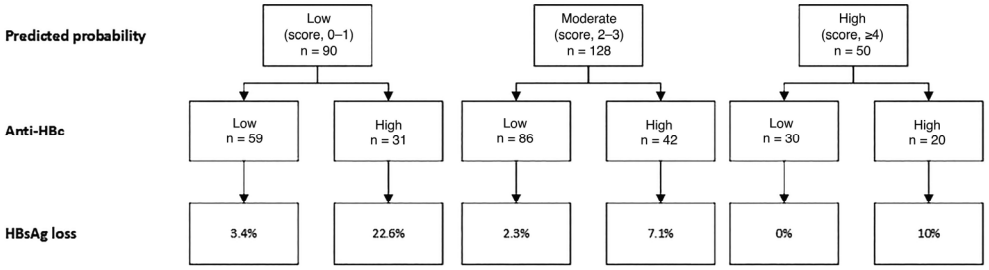
Add-on PEG-IFN

Among patients treated with add-on PEG-IFN, anti-HBc levels were significantly higher in patients with than in patients without subsequent HBeAg loss (3.12 log versus 2.84 log IU/mL, p = 0.012). Anti-HBc levels did not predict on-treatment HBsAg decline. None of the patients in the PEG-IFN add-on cohort achieved HBsAg loss.

In addition, findings were consistent when anti-HBc levels were included in the baseline scoring system of Lampertico *et al*.[94] Among patients with a predicted low (score 0–1) or moderate (score 2–3) probability to response, but high levels of anti-HBc (≥ 4.0 log among HBeAg-positive and ≥ 4.40 log among HBeAg-negative patients) were associated with a higher probability of sustained response and HBsAg loss (Figure 5).

Figure 5. Treatment outcome according to pretreatment levels of antibodies against hepatitis B core antigen (anti-HBc) and predicted probability.





The predicted probability was based on the baseline prediction model including age, sex, hepatitis B surface antigen (HBsAg), hepatitis B virus (HBV) DNA, and alanine aminotransferase levels [20]. Anti-HBc levels were categorised as low versus high; <4.0 versus ≥4.0 log for hepatitis B e antigen (HBeAg)–positive patients and <4.40 versus ≥4.40 log for HBeAg-negative patients. Sustained response was defined as an HBV DNA level <2000 IU/mL 6 months after the end of peginterferon treatment; HBsAg loss, as loss of HBsAg 6 months after the end of treatment.

DISCUSSION

There is emerging evidence suggesting that B cells play a pivotal role in the natural history of CHB.[83,86,95] In the current study, higher serum anti-HBc levels correlated with other immune markers, such as IP-10, and were associated with more severe liver inflammation on liver biopsy. Furthermore, higher pre-treatment anti-HBc levels were associated with favourable responses to PEG-IFN therapy. These findings suggest that serum anti-HBc levels could be a valuable new serum biomarker to monitor immune activity in patients with CHB.

During an acute HBV infection, the innate immune response is triggered first, followed by activation of the adaptive immune system. This generally leads to functional cure (i.e. HBsAg loss) among adults.[83,96] However, among CHB patients in whom functional cure is not achieved, alterations in both innate and adaptive immune responses are observed.[83] The important role for B cells in the immune control over HBV has been demonstrated in clinical practice through the risk for HBV reactivation among patients treated with B cell depleting agents such as rituximab, and by detailed analysis of their phenotype and function *ex vivo*.[83,85,86,97,98] B cells secrete antibodies targeted against various antigens including antibodies to HBsAg (anti-HBs), HBeAg (anti-HBe) and HBcAg (anti-HBc). A previous study showed that serum levels of anti-HBc vary across the natural history of CHB, with higher levels observed in disease states with more active inflammation. In our cohort, serum anti-HBc levels correlated with other immune markers, such as serum levels of IP-10, and higher serum levels of anti-HBc were also associated with more severe hepatic inflammation on liver biopsy. Higher anti-HBc levels were also associated with lower levels of markers of viral replication and cccDNA transcriptional activity, such as HBV DNA, HBV RNA, HBcrAg and HBsAg.[21,22,99] Taken together, these findings highlight an association between B cell activation and control over HBV replication. The observed associations with intrahepatic inflammation suggest that there may also be an important clinical diagnostic application for anti-HBc assessment, as currently used biomarkers (such as ALT) correlate poorly with liver histology.[87] High serum anti-HBc levels may be reflective of having increased degrees of liver inflammatory activity, which could potentially influence decision making regarding initiation of antiviral therapy or performing liver biopsy. [100,101]

Another interesting observation in our study was that antiviral therapy reduced serum anti-HBc levels. One year of PEG-IFN therapy was associated with a significant decline in serum anti-HBc levels, and patients currently on NA therapy had the lowest anti-HBc levels in the cohort. These findings are in line with previous studies which showed a more profound on-treatment decline in anti-HBc levels among HBeAg-positive patients treated with NAs than with PEG-IFN.[86,89] Thus, antiviral agents seem to impact anti-HBc levels although the exact mechanism is unclear and may differ for PEG-IFN versus NAs. Previous studies hint that PEG-IFN therapy might

influence the number of B cells or B cell function directly or via bone marrow suppression.[102,103] Whether NA have a direct effect on B cell production or function is uncertain, but the observed effects on anti-HBc levels may also be due to the rapid decline in viral load.[104]

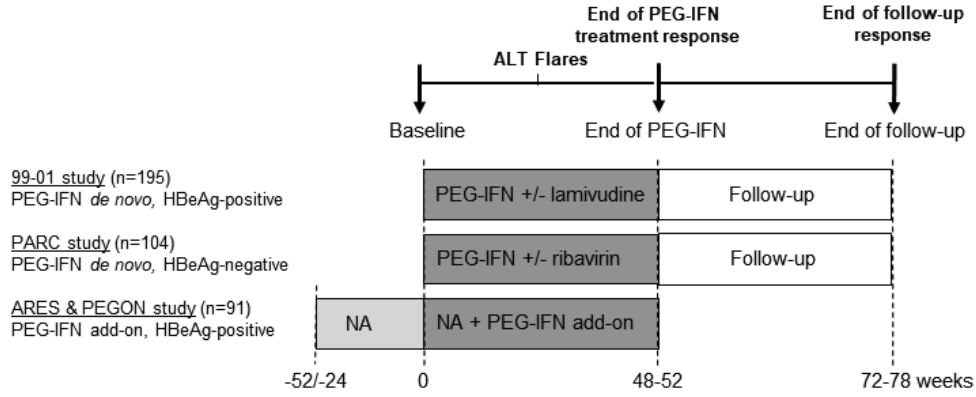
In our cohort, higher levels of anti-HBc were associated with a higher probability of favourable outcomes after treatment with PEG-IFN. Among patients treated with *de novo* PEG-IFN, findings were consistent for multiple endpoints, including HBeAg clearance, sustained HBV DNA suppression, HBsAg decline and HBsAg loss. In the subset of patients treated with add-on PEG-IFN, higher anti-HBc levels also predicted on-treatment HBeAg clearance. These findings are in line with a previous Asian study, comprising HBeAg-positive patients treated with PEG-IFN or NA therapy, which demonstrated that anti-HBc levels of 4.4 log IU/mL were associated with an increased chance of HBeAg seroconversion at EOT.[89] Interestingly, in our study, higher pre-treatment anti-HBc levels were also associated with a higher chance of on-treatment ALT flares, which previous studies have shown to be pivotal in achieving sustained response and HBsAg loss with immunomodulators.[105] When seen in the light of the associations between anti-HBc levels and intrahepatic inflammatory activity, our findings provide further support for the hypothesis that the pre-treatment immune status is an important determinant of response to immunomodulatory therapy. This hypothesis warrants further exploration, especially in studies involving novel immunomodulatory agents.

Our study has several potential limitations. Although our cohort is relatively large and enrolled patients from four randomised controlled trials, stratification by HBeAg status resulted in limited numbers of subjects and events per subgroup, increasing the risk of type 2 statistical error. However, the association between higher anti-HBc levels and favourable outcomes after antiviral therapy was consistent across sub-cohorts, supporting the robustness of our findings (Figure 4, Supplementary Figure 3). Furthermore, the anti-HBc assay we applied assessed only IgG anti-HBc, and whether there is a difference in diagnostic performance with assays that also measure IgM anti-HBc is yet unclear. Also, it is important to note that our *de novo* PEG-IFN studies enrolled predominantly Caucasians, whereas the add-on studies enrolled predominantly Asian patients. External validation of our findings in cohorts with other ethnicities/genotypes is therefore warranted.

In conclusion, our study shows that serum anti-HBc levels correlate with intrahepatic inflammatory activity. Higher serum anti-HBc levels are associated with favourable outcomes after PEG-IFN therapy. These findings provide further support for the importance of B cells in control of HBV infection and suggest that assessment of anti-HBc levels may have important clinical applications.

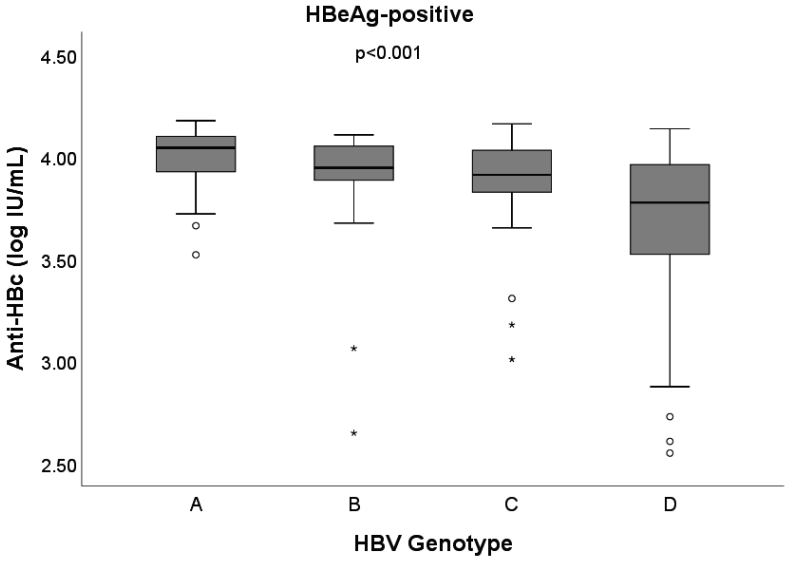
SUPPLEMENTARY FIGURES

Supplementary Figure 1. Graphical representation of the included studies

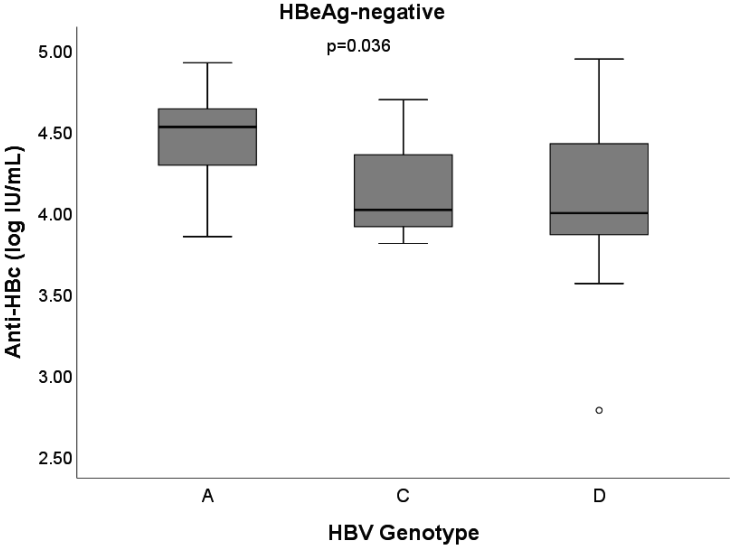


End-of-treatment response was evaluated at end of PEG-IFN treatment and included HBeAg loss and HBsAg decline EOT (≥ 1 log decline). End of follow-up (EOF) response was evaluated six months after PEG-IFN cessation, and included sustained response (HBV DNA < 2,000 IU/mL), and HBsAg loss. On-treatment ALT flares were defined as an elevation of serum ALT levels of ≥ 5x ULN during PEG-IFN treatment.
Abbreviations: PEG-IFN, peginterferon; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; NA, nucleos(t)ide analogues

Supplementary Figure 2. Pre-treatment anti-HBc levels among HBeAg-positive (A) and –negative (B) patients treated with de novo PEG-IFN therapy, stratified by HBV genotype



A)

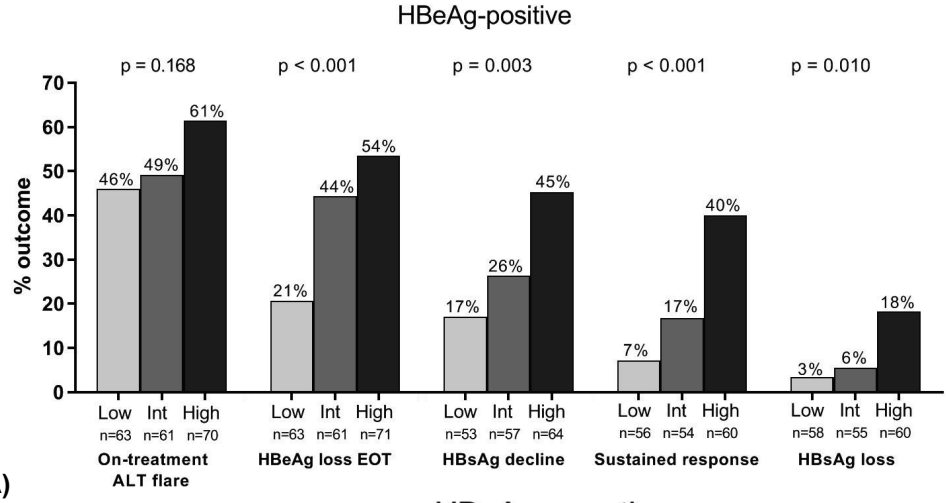


B)

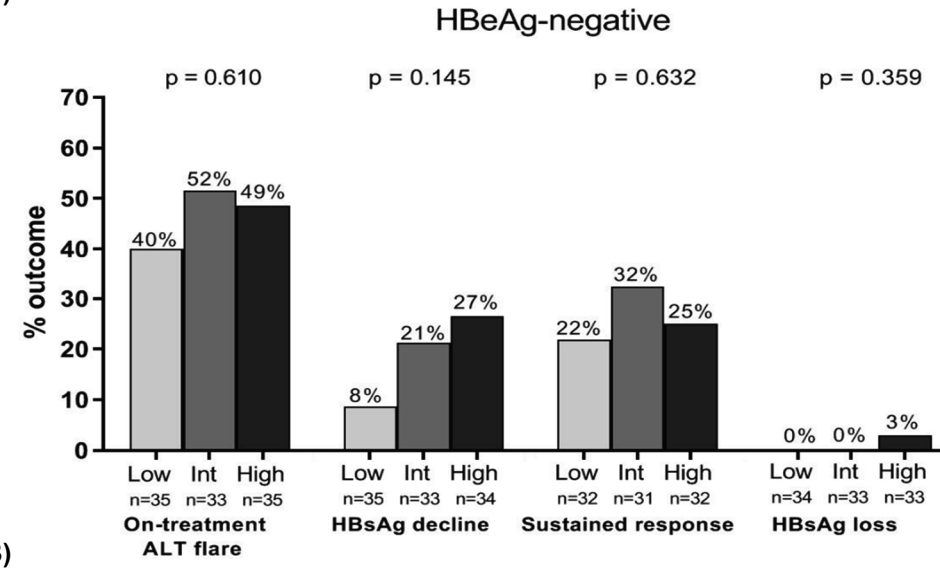
Outliners below 2.5 log were not shown, including 4 HBeAg positive patients with serum levels of 0.70 log (HBV genotype D), two patients with 1.48 log (HBV Genotype D), and 2.29 log (HBV Genotype A).

Abbreviations: anti-HBc, antibodies to hepatitis B core antigen; HBeAg, hepatitis B e antigen; PEG-IFN, peginterferon; HBV hepatitis B virus; IU/mL, international units/millilitre.

Supplementary Figure 3. Treatment outcome according to pre-treatment anti-HBc level among HBeAg-positive (A) and HBeAg-negative (B) patients

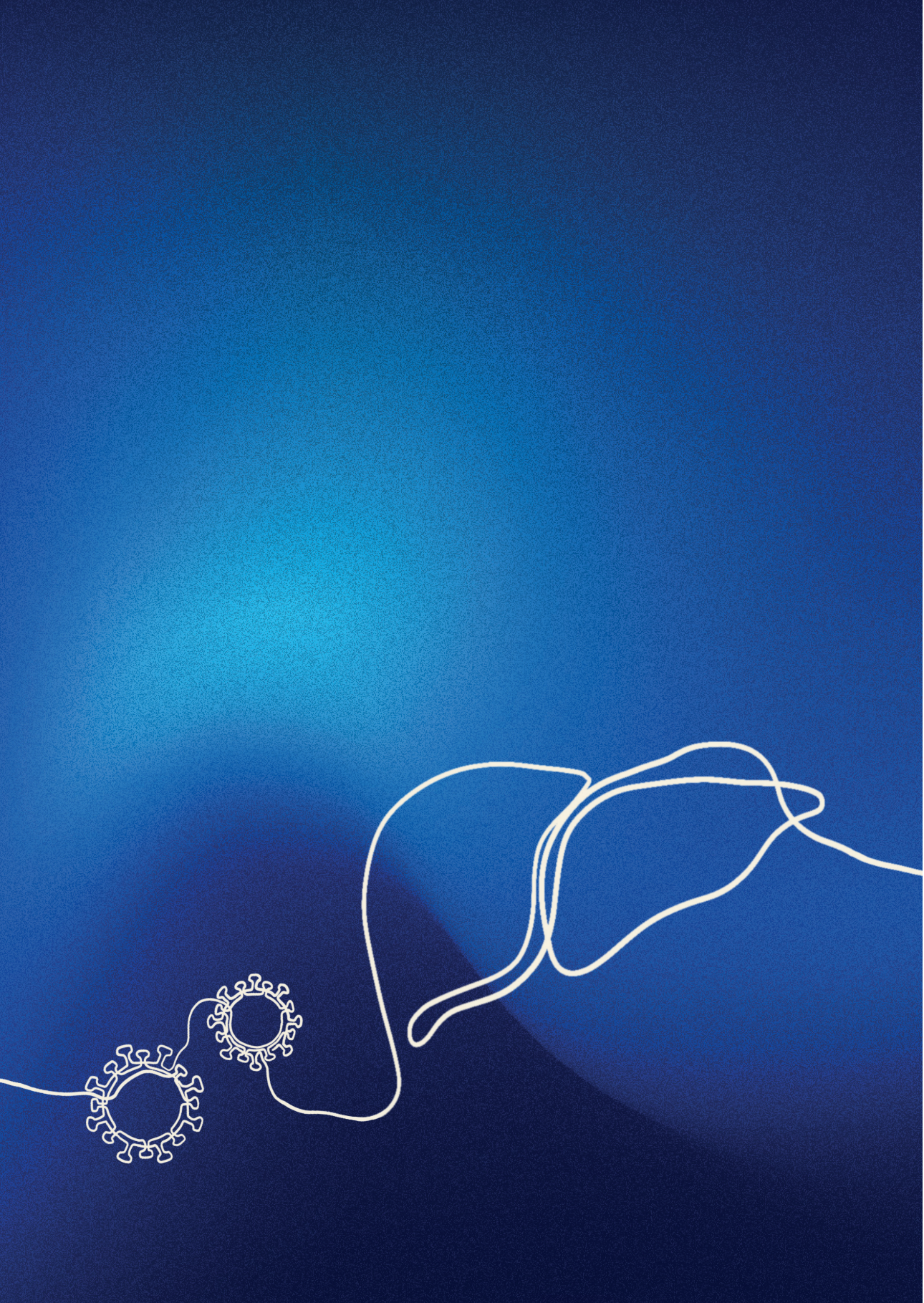


A)



B)

Anti-HBc levels were categorised as low, intermediate or high (<3.82/3.82-4.0/≥4.0 log IU/mL for HBeAg-positive and <3.95/3.95-4.40/≥4.40 log IU/mL for HBeAg-negative patients) to create 3 groups of equal size. An on-treatment ALT flare was defined as an increase of serum ALT ≥ 5x ULN during PEG-IFN treatment. HBsAg decline was defined as a decline ≥ 1 log at EOT. Sustained response was defined as HBV DNA levels of < 2,000 IU/mL six months after end of PEG-IFN treatment. HBsAg loss was defined as HBsAg clearance at EOF.
Abbreviations: EOT, end of PEG-IFN treatment; EOF, six months after PEG-IFN treatment withdrawal; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PEG-IFN, peginterferon; int, intermediate; IU/mL, international units/millilitre.



CHAPTER 4

End-of-treatment HBsAg, HBcrAg and HBV RNA levels predict the risk of off-treatment ALT flares in chronic hepatitis B patients

Sylvia M. Brakenhoff, Robert J. de Knegt, Margo J.H. van Campenhout, Annemiek A. van der Eijk, Willem P. Brouwer, Florian van Bömmel, André Boonstra, Bettina E. Hansen, Thomas Berg, Harry L.A. Janssen, Robert A. de Man, and Milan J. Sonneveld

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ABSTRACT

Background & Aim(s): Since ALT flares after therapy withdrawal are associated with adverse outcomes, risk stratification is of major importance. We aimed to study whether off-treatment flares are related with virological outcomes, and if serum levels of novel biomarkers at end-of-treatment (EOT) can predict flares.

Methods: Chronic hepatitis B patients who participated in three global randomised trials of peginterferon-based therapy were studied (99-01, PARC, ARES). HBV RNA, HBsAg and HBcrAg were quantified at EOT. Associations between EOT biomarker levels and flares were assessed as continuous data and after categorisation. Flares were defined as ALT ≥ 5x ULN during six months after therapy cessation.

Results: We included 344 patients; 230 HBeAg-positive and 114 HBeAg-negative. Patients were predominantly Caucasian (77.0%) and had genotype A/B/C/D in 23.3/7.3/13.4/52.3%. Flares were observed in 122 patients (35.5%). Flares were associated with lower rates of sustained response (3.5% vs 26.8% among patients with and without a flare; $p < 0.001$). Higher HBsAg (OR 1.586, 95% CI 1.231 – 2.043), HBV RNA (OR 1.695, 95%CI 1.371 – 2.094) and HBcrAg (OR 1.518, 95% CI 1.324 –1.740) levels were associated with higher risk of flares ($p < 0.001$). Combinations of biomarkers further improved risk stratification, especially HBsAg + HBV RNA. Findings were consistent in multivariate analysis adjusted for potential predictors including HBeAg-status and EOT-response (HBV DNA < 200 IU/mL).

Conclusion: Off-treatment ALT flares were not associated with favourable virological outcomes. Higher EOT serum HBsAg, HBcrAg and HBV RNA were associated with a higher risk of flares after therapy withdrawal. These findings can be used to guide decision-making regarding therapy discontinuation and off-treatment follow-up.

INTRODUCTION

First-line treatment options for chronic hepatitis B (CHB) patients comprise pegylated-interferon (PEG-IFN) and nucleos(t)ide analogues (NAs). The main goal of antiviral treatment is off-treatment sustained suppression of viral replication or HBsAg loss, thereby limiting hepatic complications, such as progression to liver cirrhosis and hepatocellular carcinoma (HCC).[57] Unfortunately, treatment withdrawal in HBsAg-positive patients may result in viral rebound, which has been associated to the occurrence of ALT flares.[43,44] Whether such ALT flares are beneficial or harmful for the host is a contentious issue because they have been associated with increased sustained response rates as well as a higher risk of subsequent liver decompensation.[36,106]

Given the potential risk of adverse outcomes with off-treatment ALT flares, risk stratification is of major importance. The occurrence of ALT flares has been reported to be associated with both host-related characteristics, such as age and sex, and viral factors, such as HBeAg-status and end-of-treatment HBV DNA levels.[36,105] The recently identified serum biomarkers hepatitis B virus (HBV) RNA and hepatitis B core-related antigen (HBcrAg), and hepatitis B surface antigen (HBsAg) reflect covalently closed circular DNA (cccDNA) transcriptional activity, and consequently, intrahepatic viral replication.[21,22,70] These factors could therefore also be related to the risk of off-treatment flares.

In this study, we therefore aimed to study (1) the relationship between off-treatment ALT flares and virological outcomes and (2) whether EOT levels of HBsAg, HBcrAg and HBV RNA can be used to predict the risk of ALT flares.

PATIENTS AND METHODS

Study population

In this study we included chronic hepatitis B (CHB) patients who participated in three global randomised controlled trials (the 99-01, PARC and ARES studies). Trial design and inclusion criteria have been described in detail elsewhere.[34,79,90] In short, in the 99-01 study HBeAg-positive patients (n = 266) were randomised to receive either 100 µg/week PEG-IFN alpha-2b mono-therapy or PEG-IFN plus 100 mg/day lamivudine combination-therapy for 52 weeks.[34] The PARC study enrolled HBeAg-negative patients (n = 133), who were randomised for 180 µg/week PEG-IFN alpha-2a monotherapy or PEG-IFN plus 1000-2000 mg ribavirin combination therapy for 48 weeks.[79] In the ARES study, HBeAg-positive patients (n = 175) started with 0.5 mg/day entecavir (ETV) monotherapy and were subsequently randomised to either PEG-IFN alpha-2a add-on therapy from week 24 to week 48 (n = 85) or continuing ETV (n = 90). Responders (defined as HBeAg loss and HBV DNA < 200 IU/ml at

week 48) continued with ETV consolidation therapy until week 72, after which treatment was ceased.[90] For this study, we included only those patients with PEG-IFN add-on therapy, who received ETV consolidation therapy until week 72 (i.e. those who discontinued therapy). The original study protocols have been approved by the medical ethical committees and are in line with the Declaration of Helsinki of 1975.

For the current analysis, only patients with EOT data on at least one biomarker (HBsAg, HBcrAg and/or HBsAg) were eligible for enrolment. Since the risk of an off-treatment flare is negligible in patients with HBsAg loss, we excluded patients who were HBsAg negative at EOT (Figure 1).

Endpoints

An off-treatment flare was defined as an increase of serum ALT five times the ULN within six months after EOT.[36,42] The time point of a flare was defined at the peak value of the ALT rise. If a patient experienced more than one flare, the first one was used for classification. An early flare was defined as a flare that occurred within 12 weeks after EOT, whereas a late flare was defined as a flare that occurred beyond 12 weeks after EOT. Sustained response (SR) was defined as HBV DNA < 2,000 IU/mL six months after treatment cessation. HBsAg loss was assessed at end of follow-up and during long term follow-up.[34,74,79] EOT response was defined as patients who had suppressed HBV DNA (< 200 IU/mL) levels at EOT, in line with the original ARES study protocol.[90]

Laboratory measurements

Serum HBV RNA, HBsAg, HBcrAg were measured at EOT and during follow-up. HBV DNA and ALT were quantified at EOT and, in most patients, every four weeks during the six months follow-up period. HBV RNA was quantified using rapid amplification of complementary DNA (cDNA)-ends (RACE)-based real-time polymerase chain reaction (University Hospital Leipzig, Germany). This technique has been described in detail elsewhere.[71] The assays' lower limit of detection (LOD) was 800 copies/millilitre (c/mL).[80] Quantification of HBsAg was performed using Abbott Architect (Abbott, Abbott Park, IL), with a LOD of 0.05 IU/mL. HBcrAg was quantified using Lumipulse® G HBcrAg-assay (Fujirebio Europe) according to the manufacturer's instructions, with a lower limit of quantification (LOQ) of 1000 U/mL (3 log) and lower limit of detection of 2 log.[81] The LOD for HBV DNA was 400 copies/mL (~80 IU/mL, in-house TaqMan PCR assay, Rotterdam, the Netherlands) for 99-01[34], 35 copies/mL (Taqman, Roche Diagnostics, Basel, Switzerland) for PARC[79] and 20 IU/mL (Cobas TaqMan 48, Roche Diagnostics, Basel, Switzerland) for ARES[90] participants. ALT, prothrombin time and bilirubin tests were performed by automated techniques at the participating centres.[34,79,90]

Statistical analysis

Descriptive data were described as numbers (with percentages), medians (with interquartile range; IQR) and means (± standard deviation; SD). Associations between biomarker levels and ALT flares were assessed using continuous data (with associations assessed using logistic regression and AUROC) and after categorisation (< 3 log versus > 3 log for HBsAg, undetectable versus detectable for HBV RNA, and for HBcrAg < 3 log versus > 3 log for HBeAg-negative and < 6 log versus > 6 log for HBeAg-positive patients; based on mean levels at EOT). HBsAg and HBcrAg were also combined by calculating the previously reported SCALE-B score, calculated as 35*HBsAg (log IU/mL) + 20*HBcrAg (log U/mL) + 2*age (year) + ALT (U/L). This scoring system has been developed to predict the risk of a clinical relapse in patients with finite NA therapy.[107] For this score HBcrAg levels of 2 log were recoded to 1 log, in compliance with the original report.[107]

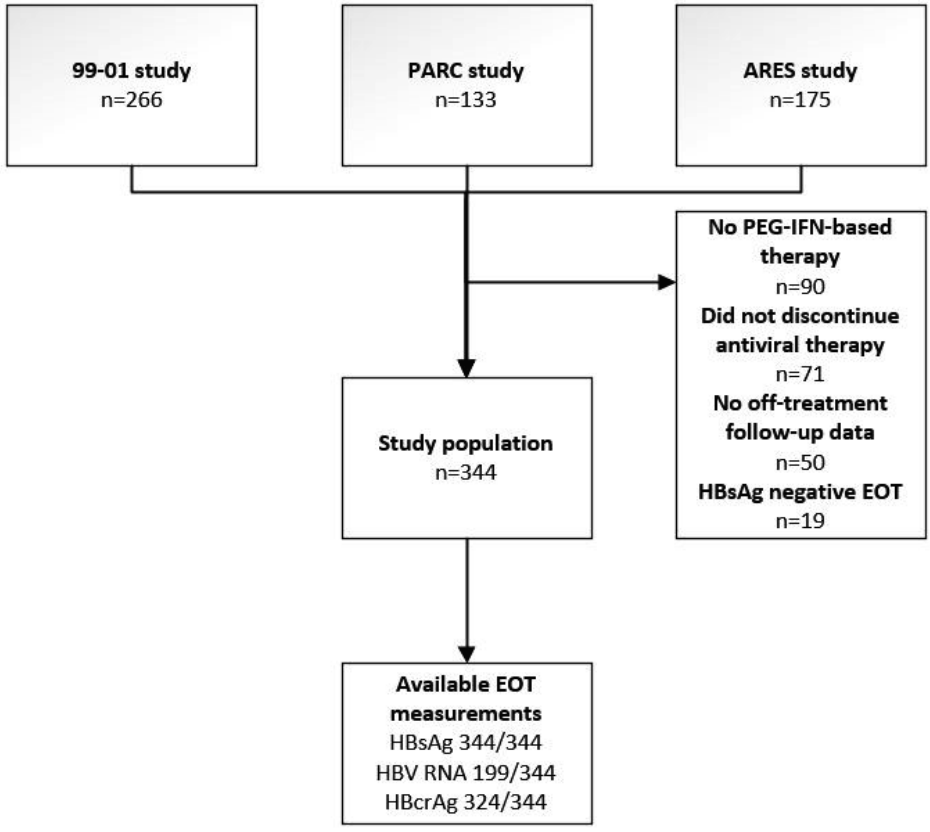
Associations between novel biomarkers and off-treatment outcomes (flares and SR) were also assessed using multivariate logistic regression, for which each biomarker was entered into a model comprising age, sex, HBV genotype A, EOT response, ALT at EOT, and HBeAg-status at baseline. Differences were considered statistically significant when $p < 0.05$. IBM SPSS for Windows version 25.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. GraphPad Prism version 5 for Windows (GraphPad Software, San Diego, California, USA) was used for graphical representation of the results.

RESULTS

Patient characteristics

In total, 344 patients were included (Figure 1, Table 1); 230 HBeAg-positive and 114 HBeAg-negative. An EOT response was observed in 147 patients (57.1%).

Figure 1. Flowchart



Abbreviations: ALT, alanine aminotransferase; EOT, end-of-treatment; HBV, hepatitis B virus; HBcrAg, Hepatitis B core-related Antigen; HBeAg, HBsAg, quantitative hepatitis B surface antigen; PEG-IFN, peginterferon.

Table 1. Patient characteristics

Characteristics	Flare∞ (n = 122)	No flare∞ (n = 222)	*P*-value
Age at inclusion, years (median, IQR)	35 (28-44)	34 (26-46)	0.930
Male (n, %)	86 (70.5)	173 (77.9)	0.126
Race (n, %)			
Caucasian	92 (75.4)	173 (77.9)	0.588
Asian	22 (18.0)	40 (18.0)	
Other	8 (6.6)	9 (4.1)	
HBV genotype (n, %)			
A	20 (16.4)	60 (27.0)	0.232
B	8 (6.6)	17 (7.7)	
C	18 (14.8)	28 (12.6)	
D	71 (58.2)	109 (49.1)	
Other	5 (4.1)	8 (3.6)	
Study treatment (n, %)			
PEG-IFN mono	61 (50.0)	105 (47.3)	0.108
PEG-IFN + LAM	42 (34.4)	65 (29.3)	
PEG-IFN + RBV	18 (14.8)	39 (17.6)	
PEG-IFN + ETV	1 (0.8)	13 (5.9)	
Laboratory results at end-of-treatment			
HBeAg status EOT, positive (n, %)	74 (60.7)	67 (30.2)	< 0.001
ALT (median, IQR) $^{\phi}$	1.7 (1.0 – 2.7)	1.1 (0.8 – 1.7)	< 0.001
HBV DNA‡ (mean, ±SD)	5.1 (±2.7)	3.1 (±2.4)	< 0.001
HBV RNA§ (mean, ±SD)	4.4 (±1.6)	3.2 (±1.3)	< 0.001
HBsAg‡ (mean, ±SD)	3.8 (±0.8)	3.4 (±1.3)	< 0.001
HBcrAg† (mean, ±SD)	6.4 (±1.7)	4.9 (±2.0)	< 0.001
Treatment response			
EOT response$^{\alpha}$	30 (24.6)	117 (52.9)	< 0.001
Sustained response$^{\beta}$ (n, %)	4/113 (3.5)	53/198 (26.8)	< 0.001
HBsAg loss	0 (0)	6/209 (2.9)	0.061

$^{\phi}$ times the upper limit of normal (ULN), U/L
§ Logarithmic scale, c/mL
‡ Logarithmic scale, IU/mL

† Logarithmic scale, U/mL
¥ HBV DNA < 200 IU/ml at end-of-treatment
α EOT response is defined as HBV DNA < 200 IU/mL at end-of-treatment
β Sustained response is defined as HBV DNA < 2,000 IU/mL six months after end-of-treatment
∞Flare is defined as ALT > 5x the upper limit of normal after end-of-treatment.
Abbreviations: HBV, hepatitis B virus; PEG-IFN, peginterferon; LAM, lamivudine; RBV, ribavirin; ETV, entacavir; HBcrAg, Hepatitis B core-related Antigen; HBeAg, Hepatitis B e Antigen; HBsAg, quantitative hepatitis B surface antigen; EOT, end-of-treatment; IQR, interquartile range.

Off-treatment ALT flare characteristics

An off-treatment ALT flare was observed in 122 patients (35.5%); median ALT level at the peak of the flare was 9.4 x the ULN (IQR 6.9 – 16.4). In 74 patients (60.7%) the peak of the flare occurred ≥ 12 weeks after EOT (i.e. a late flare; median 12 weeks after EOT, IQR 8–20). Among the 122 patients with an off-treatment ALT flare, 38 patients (31.1%) experienced concomitant bilirubin elevation, with a median of 24 µmol/mL (range 11 – 152). A bilirubin level > 50 µmol/mL was observed in seven patients (5.7%). Prothrombin time elevation was observed in 20 patients (range 1.01 – 10.4x ULN). None of the patients developed encephalopathy.

Off-treatment ALT flares are associated with lower rates of sustained response and HBsAg loss

Occurrence of an off-treatment flare was associated with a lower probability of SR; 4/113 patients (3.5%) with a flare achieved SR compared to 53/198 (26.8%) patients without a flare (OR 0.100, 95% CI 0.035 – 0.286, p < 0.001). Findings were consistent among the subgroup of patients with an EOT response; 0% of the patients with an off-treatment flare achieved SR compared to 40.0% without a flare (p < 0.001). Similarly, occurrence of a flare was not associated with a more pronounced off-treatment HBsAg decline, and none of the patients with an off-treatment ALT flare achieved HBsAg loss during follow-up (Table 1).

Associations between viral biomarkers and off-treatment ALT flares

<u>HBsAg</u>

Higher HBsAg levels at EOT were associated with a higher risk of off-treatment ALT flares (OR 1.586, 95% CI 1.231 – 2.043, p < 0.001; AUROC 0.608, 95% CI 0.548 – 0.668, p = 0.001). Among the 261 patients with HBsAg levels of > 3 log, 107 patients (41.0%) experienced a flare. In contrast, only 15/83 patients (18.1%) with HBsAg levels of < 3 log experienced a flare (p < 0.001, Figure 2).

Conversely, higher HBsAg levels at EOT were associated with a lower risk of SR (OR 0.480, 95% CI 0.371 – 0.621, p < 0.001; AUROC 0.237, 95% CI 0.171 – 0.304,

p < 0.001). SR was achieved in 25/234 (10.7%) versus 32/77 (41.6%) patients with HBsAg levels of > 3 log versus < 3 log at EOT (p < 0.001, Figure 3). HBsAg loss was exclusively observed in patients with HBsAg levels of < 3 log (7.3% versus 0.0% in patients with HBsAg > 3 log, p < 0.001).

Findings were consistent in multivariate analysis adjusting for other potential predictors (Table 2), in a subgroup of patients with an EOT response (Supplementary Figure 1), and when data was stratified on HBeAg-status and treatment regime (Supplementary Figure 2 – 5).

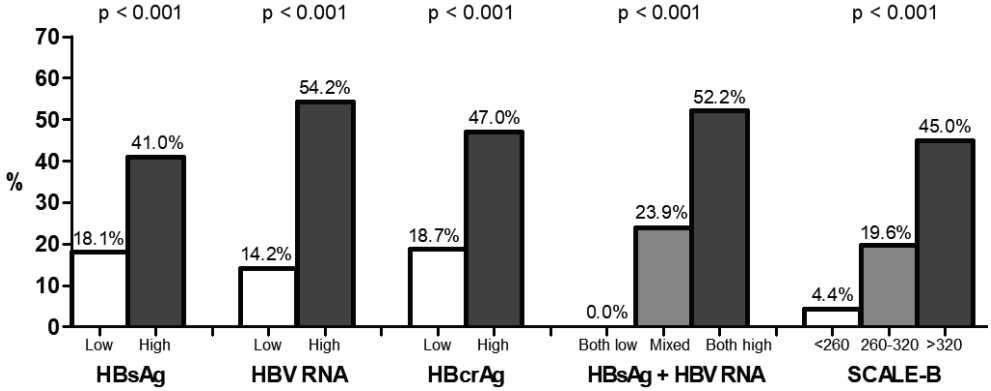
HBV RNA

Higher HBV RNA levels at EOT were associated with an increased risk of off-treatment ALT flares (OR 1.695, 95% CI 1.371 – 2.094, p < 0.001; AUROC 0.726, 95% CI 0.645 – 0.807, p < 0.001). Amongst the 72 patients with detectable HBV RNA levels, 39 patients (54.2%) experienced a flare compared to 18/127 patients (14.2%) with undetectable HBV RNA levels (p < 0.001, Figure 2).

Conversely, higher HBV RNA levels at EOT were associated with a lower risk of SR (OR 0.119, 95% CI 0.017 – 0.807, p = 0.029; AUROC 0.278, 95% CI 0.202 – 0.354, p < 0.001). SR was achieved in 2/68 (2.9%) patients with detectable HBV RNA, versus 35/107 (32.7%) patients with undetectable HBV RNA levels at EOT (p < 0.001, Figure 3). HBsAg loss was exclusively observed in patients with undetectable HBV RNA levels (2.5% versus 0.0% in patients with detectable levels, p = 0.184).

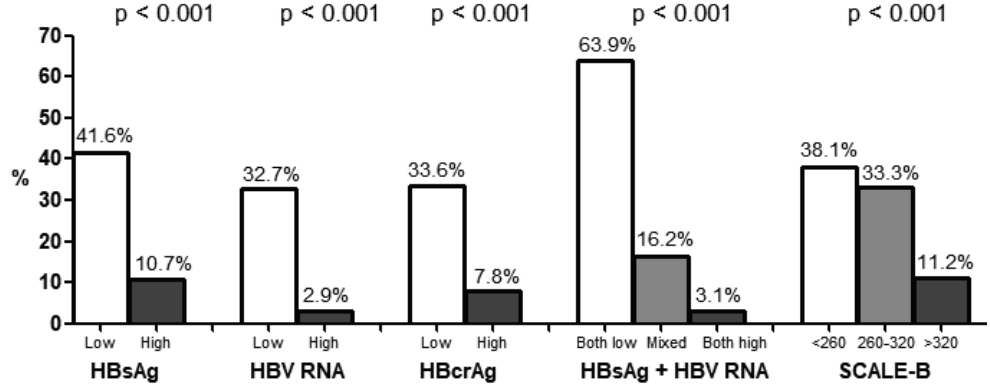
Findings were consistent in multivariate analysis adjusting for other potential predictors (Table 2), in a subgroup of patients with an EOT response (Supplementary Figure 1), and when data was stratified on HBeAg-status and treatment regime (Supplementary Figure 2 – 5).

Figure 2. Rates of off-treatment ALT flares (ALT ≥ 5x ULN) in the overall cohort, according to HBsAg, HBcrAg and HBV RNA levels at end-of-treatment



Biomarker levels were categorised as low versus high for HBsAg (< 3 log versus > 3 log), HBV RNA (undetectable versus detectable) and for HBcrAg (< 3 log versus > 3 log for HBeAg-negative and < 6 log versus > 6 log for HBeAg-positive patients). Concomitant HBsAg and HBV RNA were categorised as both low (HBsAg < 3 log and undetectable HBV RNA), both high (HBsAg > 3 log and detectable HBV RNA), and mixed.
Abbreviations: HBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; HBsAg, Hepatitis B core-related Antigen.

Figure 3. Rates of sustained response (HBV DNA < 2,000 IU/mL six months after treatment withdraw) in the overall cohort, according to HBsAg, HBcrAg and HBV RNA levels at end-of-treatment



Biomarker levels were categorised as low versus high for HBsAg (< 3 log versus > 3 log), HBV RNA (undetectable versus detectable) and for HBcrAg (< 3 log versus > 3 log for HBeAg-negative and < 6 log versus > 6 log for HBeAg-positive patients). Concomitant HBsAg and HBV RNA were categorised as both low (HBsAg < 3 log and undetectable HBV RNA), both high (HBsAg > 3 log and detectable HBV RNA), and mixed. Abbreviations: HBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; HBcrAg, Hepatitis B core-related Antigen.

HBcrAg

Higher HBcrAg levels were associated with off-treatment ALT flares (OR 1.518, 95% CI 1.324 – 1.740, p < 0.001; AUROC 0.720, 95% CI 0.663 – 0.777, p < 0.001). Amongst the 185 patients (57.1%) with high HBcrAg levels (> 3 log for HBeAg-negative and > 6 log for HBeAg-positive), 87 patients (47.0%) experienced an off-treatment flare. On the contrary, only 26/139 patients (18.7%) with low HBcrAg levels (< 3 log for HBeAg-negative and < 6 log for HBeAg-positive patients) experienced a flare (p < 0.001, Figure 2).

Conversely, higher HBcrAg levels at EOT were associated with a lower risk of SR (OR 0.658, 95% CI 0.565 – 0.767, p < 0.001; AUROC 0.240, 95% CI 0.181 – 0.299, p < 0.001). SR was achieved in 42/125 (33.6%) with low HBcrAg levels versus 14/179 (7.8%) in patients with high HBcrAg levels (p < 0.001, Figure 3). HBsAg loss was exclusively observed in patients with low HBcrAg levels (4.4% versus 0.0% in patients with high levels, p = 0.004).

Findings were consistent in multivariate analysis adjusting for other potential predictors (Table 2), in a subgroup of patients with an EOT response (Supplementary Figure 1), and when data was stratified on HBeAg-status and treatment regime (Supplementary Figure 2 – 5).

Table 2. Multivariate analysis

	Flare			**Sustained response**		
	aOR	*95% CI*	*P-value*	*aOR*	*95% CI*	*P-value*
HBsAg*	1.386	1.041 – 1.845	0.025	0.562	0.423 – 0.745	< 0.001
HBV RNA*	1.494	1.129 – 1.976	0.005	0.127	0.017 – 0.919	0.041
HBcrAg*	1.517	1.208 – 1.905	< 0.001	0.476	0.332 – 0.681	< 0.001

** adjusted for age, sex, genotype A, EOT response (HBV DNA < 200 IU/mL), ALT at EOT, and HBeAg-status at baseline.*

Abbreviations: HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; aOR, adjusted odds ratio

Combinations of biomarkers may further stratify ALT flare risk

HBsAg and HBV RNA

Among the 69 patients with both detectable HBV RNA levels and HBsAg > 3 log, 36 patients (52.2%) experienced an off-treatment ALT flare. In contrast, none of the 42 patients (0.0%) with undetectable HBV RNA levels and HBsAg < 3 log experienced a flare ($p < 0.001$, Figure 2). Findings were consistent among patients with an EOT response; 62.5% of the patients with concomitant elevated levels of HBV RNA and HBsAg experienced a flare, compared to 0.0% of whom both biomarkers were low ($p < 0.001$).

Conversely, higher concomitant HBsAg and HBV RNA levels at EOT were associated with a lower risk of SR. SR was achieved in 23/36 (63.9%) with low levels versus 2/65 (3.1%) in patients with high levels ($p < 0.001$, Figure 3). HBsAg loss was exclusively observed in patients with low levels of concomitant HBsAg and HBV RNA (7.3% versus 0.0% in patients with high levels, $p = 0.004$).

HBsAg and HBcrAg: SCALE-B

Higher SCALE-B scores were associated with higher risk of an off-treatment flare. Among the 218 patients with a SCALE-B score of ≥ 320, 98 patients (45.0%) experienced an off-treatment flare. In contrast, a flare was observed in 2/45 patients (4.4%) with a SCALE-B score of < 260 ($p < 0.001$, Figure 2). A similar trend was observed among patients with an EOT response; 20/58 patients (34.5%) with a SCALE-B score of ≥ 320 experienced a flare, compared to 2/39 patients (5.1%) with a SCALE-B score of < 260 ($p = 0.001$).

Conversely, higher SCALE-B score at EOT was associated with a lower risk of SR. SR was achieved in 16/42 (38.1%) with a SCALE-B score of < 260 versus 23/206 (11.2%) in patients with a SCALE-B score of ≥ 320 ($p < 0.001$, Figure 3). HBsAg loss was exclusively observed in patients with SCALE-B score of < 320 (6.0% versus 0.0% in patients with levels of ≥ 320, $p < 0.001$).

DISCUSSION

Off-treatment ALT flares are frequently observed after therapy withdrawal in patients with CHB, with previous studies hinting at a possible beneficial effect.[43,106] This study, a pooled analysis of three randomised controlled trials, demonstrates that off-treatment flares were not associated with increased rates of HBsAg decline or sustained response. Higher EOT levels of HBsAg, HBcrAg and HBV RNA were associated with higher risk of ALT flares and a lower risk of sustained response or HBsAg loss. These biomarkers could therefore be used to stratify relapse risk in patients being evaluated for therapy discontinuation.

Rapid increases in ALT levels, also known as flares, can occur during the natural course of the chronic HBV infection, or in relationship with (discontinuation of) antiviral therapy. It has been debated whether rise in ALT after antiviral therapy withdrawal could be beneficial. This concept is illustrated by a study by Hadziyannis and colleagues, who observed an off-treatment ALT rise in 76% patients of the patients who discontinued NA treatment, with a high subsequent rate of HBsAg loss (20%) within the first year of follow-up, suggesting a beneficial effect of an ALT flare.[106] In addition, on-treatment flares during PEG-IFN therapy have been associated with rapid subsequent HBsAg decline and clearance.[105] However, such a benefit may be restricted to patients with a host-dominant flare (i.e. a flare not preceded by rapid HBV DNA increase),[105,108] and the benefit of off-treatment flares remains uncertain. The findings from the current study provides no support for the hypothesis that off-treatment ALT flares lead to favourable virological outcomes.

Given the absence of sufficient evidence that off-treatment ALT flares increase the chances of treatment response, it is important to consider that ALT flares may also be harmful.[36,108-110] Hepatic decompensation, some with fatal outcome, have been described in patients that discontinued NAs and experienced rise in ALT, particularly in patients with more advanced liver disease.[47,111] Moreover, severe off-treatment ALT flares are not restricted to finite NA therapy, but are also observed in trials with PEG-IFN therapy or novel HBV agents such as nucleic acid polymers (NAPs).[67,110,112] Thus, ALT flares are commonly observed during or after finite treatment with all different antiviral therapies, making risk stratification essential.[36] Identification of patients at high risk of flares may trigger intensive post-treatment follow-up and early re-treatment, which might help prevent hepatic decompensation or other fatalities.

Since HBV DNA levels are often low or undetectable in patients considered eligible for finite treatment, other biomarkers are required for risk stratification. In the last couple of years, several more serum biomarkers have been identified, including quantitative HBsAg, HBV RNA and HBcrAg. These serum biomarkers might reflect intrahepatic viral replication in various degrees during the different phases of HBV infection, through associations with the cccDNA.[21,22,70] Therefore, these biomarkers may serve as marker for intrahepatic transcriptional activity and consequently predict off-treatment sustained response and the risk of flares. In our study, higher levels of these biomarkers were associated with higher risk of flares and lower risk of sustained response or HBsAg loss. These associations were sustained in multivariate analysis and were consistent across subgroups. Albeit the biomarkers are interrelated, a combination of low biomarker levels identified patients with the highest likelihood of favourable outcomes after therapy cessation. Our findings are consistent with previous smaller studies that only studied viral antigens and/or HBV RNA. [113]

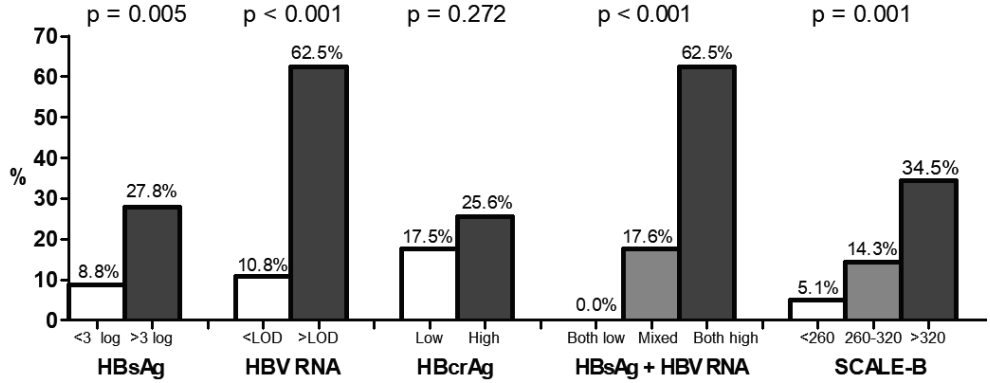
Our findings may have important clinical implications, as they can be used both to select patients most likely to achieve sustained viral suppression (or HBsAg loss) after therapy withdrawal, and to identify patients in need of careful monitoring. Furthermore, the observation that individual biomarkers are also able to predict off-treatment outcomes suggests that they should be evaluated in studies of novel antivirals that target specific parts of the HBV replication cycle and therefore have profound effects on some, but not all biomarkers (e.g. capsid assembly modulators [CAMs], which have a profound effect on HBV DNA and RNA, but less so on HBcrAg and HBsAg).[66,78]

Strengths of this study include the large cohort of both HBeAg-positive and –negative patients who participated in three global randomised controlled trials. In addition, since ALT levels were quantified in the majority of patients every four weeks during a follow-up period of six months, we were able to identify a large number of off-treatment ALT flares. Also, since HBcrAg and HBV RNA levels are frequently low or undetectable in HBeAg negative patients, more sensitive assays may further improve predictive performance. This also applies to HBV RNA, which is frequently below the LOD in virally suppressed patients. Finally, our results are based on patients treated with PEG-IFN (+/-NA) therapy. Validation in NA treated patients and/or patients treated with combination regimens containing novel antivirals is warranted. In addition, despite the large number of patients in the overall cohort, stratification resulted in a limited number of patients per subgroup. Nevertheless, subgroup analysis across HBeAg-status, treatment regime and EOT response categories showed homogeneous results (Supplementary Figures 1-3) with the overall population, supporting the robustness of our findings.

In conclusion, our study demonstrated that off-treatment ALT flares were not associated with favourable virological outcomes and should therefore be considered an undesirable event. Higher levels of HBV RNA, HBcrAg and HBsAg at EOT are associated with higher risk of flares. These findings may be used to select patients most likely to achieve sustained response or HBsAg loss after therapy discontinuation, and to identify patients eligible for intensive post-treatment follow-up.

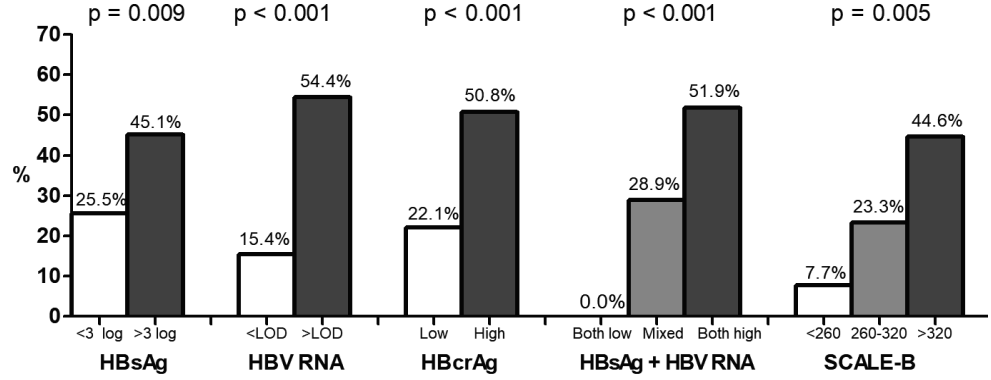
SUPPLEMENTARY FIGURES

Figure S1. Rates of off-treatment ALT flares (ALT ≥ 5x ULN) in patients with an EOT response (HBV DNA < 200 IU/mL at EOT), according to HBsAg, HBcrAg and HBV RNA levels at end-of-treatment



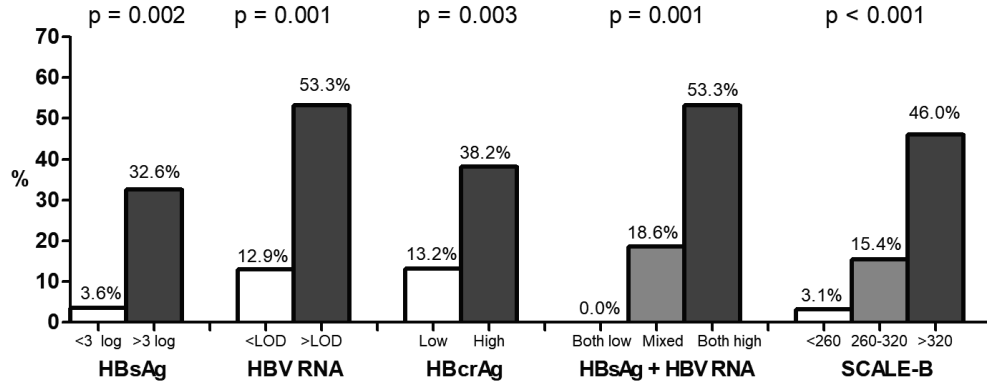
Biomarker levels were categorised as low versus high for HBsAg (< 3 log versus > 3 log), HBV RNA (undetectable versus detectable) and for HBcrAg (< 3 log versus > 3 log for HBeAg-negative and < 6 log versus > 6 log for HBeAg-positive patients). Concomitant HBsAg and HBV RNA were categorised as both low (HBsAg < 3 log and undetectable HBV RNA), both high (HBsAg > 3 log and detectable HBV RNA), and mixed.
Abbreviations: HBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; HBcrAg, Hepatitis B core-related Antigen.

Figure S2. Rates of off-treatment ALT flares (ALT ≥ 5x ULN) in HBeAg-positive patients, according to HBsAg, HBcrAg and HBV RNA levels at end-of-treatment



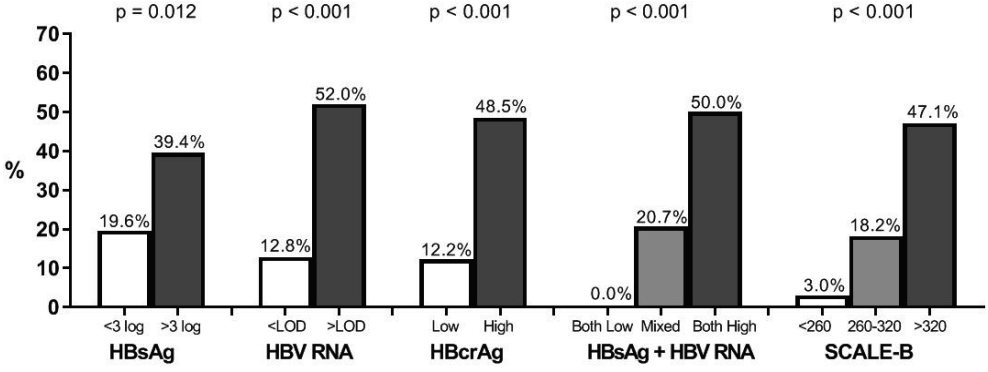
Biomarker levels were categorised as low versus high for HBsAg (< 3 log versus > 3 log), HBV RNA (undetectable versus detectable) and for HBcrAg (< 3 log versus > 3 log for HBeAg-negative and < 6 log versus > 6 log for HBeAg-positive patients). Concomitant HBsAg and HBV RNA were categorised as both low (HBsAg < 3 log and undetectable HBV RNA), both high (HBsAg > 3 log and detectable HBV RNA), and mixed. Abbreviations: HBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; HBcrAg, Hepatitis B core-related Antigen.

Figure S3. Rates of off-treatment ALT flares (ALT ≥ 5x ULN) in HBeAg-negative patients, according to HBsAg, HBcrAg and HBV RNA levels at end-of-treatment



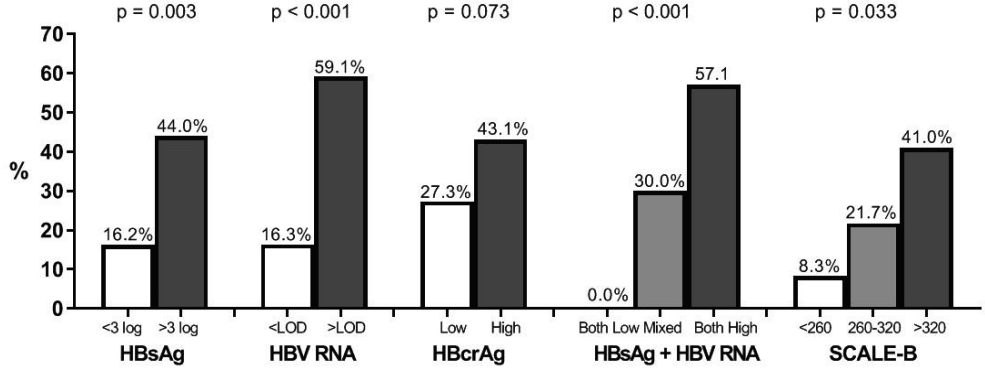
Biomarker levels were categorised as low versus high for HBsAg (< 3 log versus > 3 log), HBV RNA (undetectable versus detectable) and for HBcrAg (< 3 log versus > 3 log for HBeAg-negative and < 6 log versus > 6 log for HBeAg-positive patients). Concomitant HBsAg and HBV RNA were categorised as both low (HBsAg < 3 log and undetectable HBV RNA), both high (HBsAg > 3 log and detectable HBV RNA), and mixed. Abbreviations: HBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; HBcrAg, Hepatitis B core-related Antigen.

Figure S4. Rates of off-treatment ALT flares (ALT ≥ 5x ULN) in patients treated with PEG-IFN mono-therapy, according to HBsAg, HBcrAg and HBV RNA levels at end-of-treatment

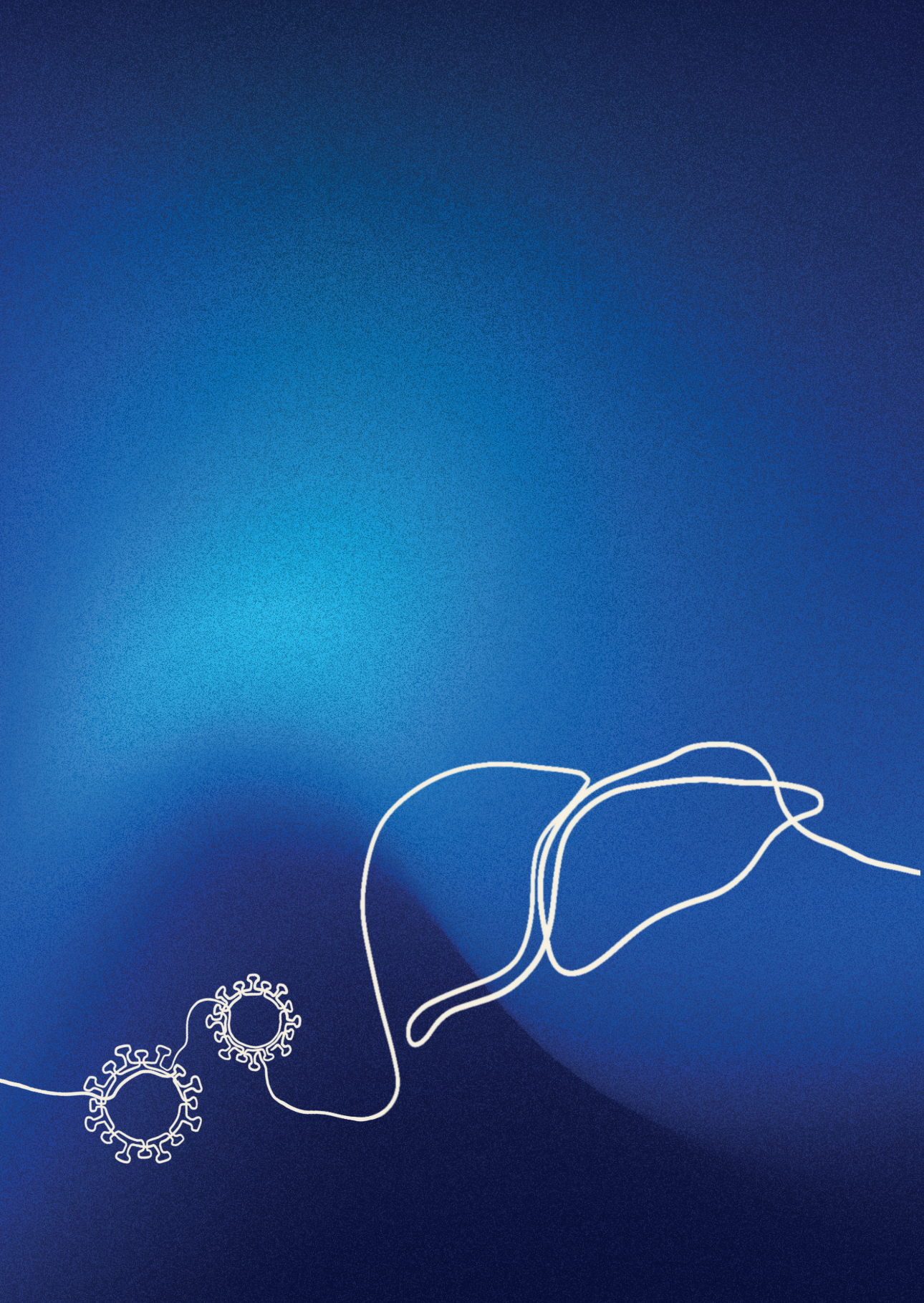


Biomarker levels were categorised as low versus high for HBsAg (< 3 log versus > 3 log), HBV RNA (undetectable versus detectable) and for HBcrAg (< 3 log versus > 3 log for HBeAg-negative and < 6 log versus > 6 log for HBeAg-positive patients). Concomitant HBsAg and HBV RNA were categorised as both low (HBsAg < 3 log and undetectable HBV RNA), both high (HBsAg > 3 log and detectable HBV RNA), and mixed. Abbreviations: HBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; HBcrAg, Hepatitis B core-related Antigen; PEG-IFN, peginterferon.

Figure S5. Rates of off-treatment ALT flares (ALT ≥ 5x ULN) in patients treated with PEG-IFN combination-therapy (PEG-IFN + NA), according to HBsAg, HBcrAg and HBV RNA levels at end-of-treatment

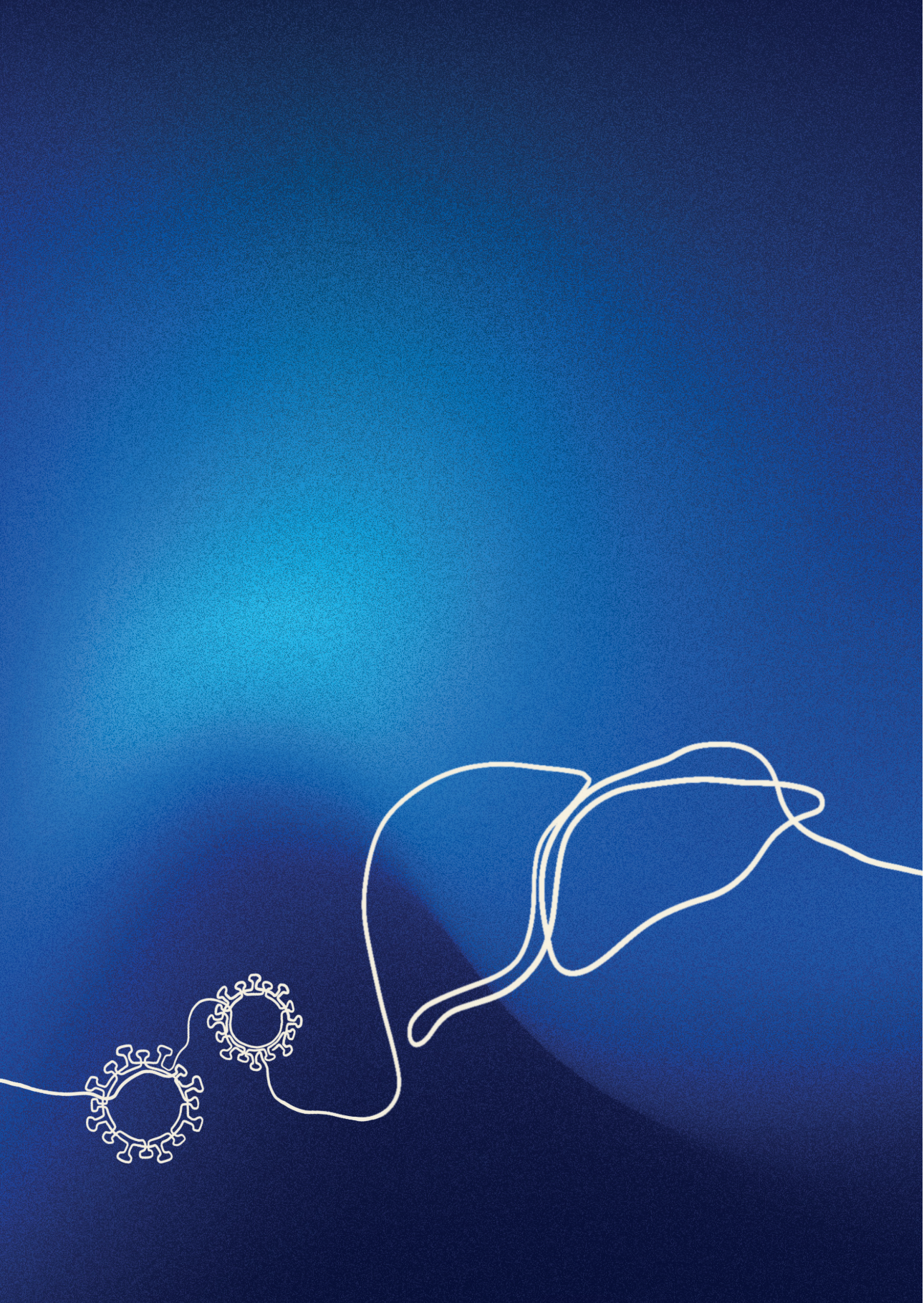


Biomarker levels were categorised as low versus high for HBsAg (< 3 log versus > 3 log), HBV RNA (undetectable versus detectable) and for HBcrAg (< 3 log versus > 3 log for HBeAg-negative and < 6 log versus > 6 log for HBeAg-positive patients). Concomitant HBsAg and HBV RNA were categorised as both low (HBsAg < 3 log and undetectable HBV RNA), both high (HBsAg > 3 log and detectable HBV RNA), and mixed. Abbreviations: HBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; HBcrAg, Hepatitis B core-related Antigen; PEG-IFN, peginterferon; NA, nucleos(t)ide analogues.



PART III

Nationwide elimination of hepatitis C



CHAPTER 7

Hepatitis C Elimination in the Netherlands (CELINE): How nationwide retrieval of lost to follow-up hepatitis C patients contributes to micro-elimination

Sylvia M. Brakenhoff*, Cas J. Isfordink*, Marleen van Dijk*, Patricia A.M. Kracht, Joop E. Arends, Robert J. de Knegt, Marc van der Valk, Joost P.H. Drenth
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ABSTRACT

Background & Aim(s): The number of chronic hepatitis C virus (HCV)-infected patients who have been lost to follow-up (LTFU) is high and threatens HCV elimination. Micro-elimination focusing on the LTFU population is a promising strategy for low-endemic countries like the Netherlands (HCV prevalence 0.16%). We therefore initiated a nationwide retrieval project in the Netherlands targeting LTFU HCV patients.

Methods: LTFU HCV-infected patients were identified using laboratory and patient records. Subsequently, the Municipal Personal Records database was queried to identify individuals eligible for retrieval, defined as being alive and with a known address in the Netherlands. These individuals were invited for re-evaluation. The primary endpoint was the number of patients successfully re-linked to care.

Results: Retrieval was implemented in 45 sites in the Netherlands. Of 20,183 ever-diagnosed patients, 13,198 (65%) were known to be cured or still in care and 1,537 (8%) were LTFU and eligible for retrieval. Contact was established with 888/1,537 (58%) invited individuals; 369 (24%) had received prior successful treatment elsewhere, 131 (9%) refused re-evaluation and 251 (16%) were referred for re-evaluation. Finally, 219 (14%) were re-evaluated, of whom 172 (79%) approved additional data collection. HCV-RNA was positive in 143/172 (83%), of whom 38/143 (27%) had advanced fibrosis or cirrhosis and 123/143 (86%) commenced antiviral treatment.

Conclusion: Our nationwide micro-elimination strategy accurately mapped the ever-diagnosed HCV population in the Netherlands and indicates that 27% of LTFU HCV-infected patients re-linked to care have advanced fibrosis or cirrhosis. This emphasises the potential value of systematic retrieval for HCV elimination.

INTRODUCTION

Achieving hepatitis C virus (HCV) elimination as a global health threat has been a priority of many countries since the World Health Organisation published their elimination targets.[123] In low-endemic countries, like the Netherlands (prevalence 0.16%)[5], micro-elimination may be a favourable approach.[124]

In the Netherlands, HCV is restricted to key populations such as people who inject(ed) drugs, migrants from HCV endemic countries, men who have unsafe sex with men and people with inherited bleeding disorders.[5] These key populations are commonly identified as targets for HCV micro-elimination initiatives. A population worthy of attention are people with HCV who have been lost to follow-up (LTFU). Despite earlier diagnosis they dropped out of the continuum of care before adequate management had been delivered or after antiviral treatment without formal proof of HCV eradication.

Several Dutch regional projects demonstrated that the LTFU rate in people with HCV runs up to 30%.[51-53] These pilot studies drove the development of the current micro-elimination project “Hepatitis C Elimination in the Netherlands (CELINE)”, that aimed to retrieve and re-evaluate LTFU HCV patients in a nationwide manner. Successful implementation would support the concept of micro-elimination in the LTFU HCV population as a tool towards achieving the World Health Organisation (WHO) hepatitis C elimination targets in low endemic countries.[123]

PATIENTS AND METHODS

Study setting and ethics

Care for patients with viral hepatitis in the Netherlands is covered by mandatory health insurance and centred in certified hepatitis treatment centres. Between 2018 and 2020 all 46 certified centres in the Netherlands were invited to participate. If a treatment centre had executed an independent, regional retrieval project, the outcomes were included in this study once a data sharing agreement was reached. Other non-certified centres were invited to participate if there was a close collaboration with a certified hepatitis treatment centre.

Local approval was provided by all participating centres. Retrieval and re-evaluation activities in the CELINE project were part of standard care. Collected clinical data of successfully retrieved patients were analysed for research purposes after patients provided informed consent. Participation in the research was voluntary and did not influence clinical care.

Study population and retrieval strategy

The study protocol has been described in detail previously.[125] An overview can be seen in Supplementary Figure 1. In short, patients with a previously diagnosed HCV infection who had become LTFU were identified based on laboratory results and medical chart review. Patients with severe comorbidity or short life expectancy resulting in an expected lack of benefit from antiviral treatment were excluded. The Municipal Personal Records Database was queried to identify patients eligible for retrieval, defined as being alive and with a registered address in the Netherlands. Subsequently, patients eligible for retrieval were invited by letter for a re-evaluation visit at a hepatitis treatment centre of their choice. Patients younger than 18 were invited for re-evaluation but were not included in data collection.

Study endpoints and statistical analysis

The primary outcome was the number of LTFU patients successfully re-linked to care, defined as at least one visit at the outpatient clinic of a certified hepatitis treatment centre. Secondary outcomes included the total number of diagnosed and number of LTFU individuals, case ascertainment rate (i.e. established contact with invited patients), proportion of HCV-viraemic patients among re-evaluated patients, reasons for becoming LTFU, mode of HCV transmission, proportion of individuals with at least advanced liver fibrosis (liver stiffness measurement value ≥ 9.5 kPa or radiological, histological or clinical signs of cirrhosis[126,127]) among HCV-viraemic patients, and DAA treatment outcome.

Descriptive data are reported as percentage, mean (+/- standard deviation; SD) or median (with interquartile range; IQR). Analyses were performed using IBM SPSS Statistics® version 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

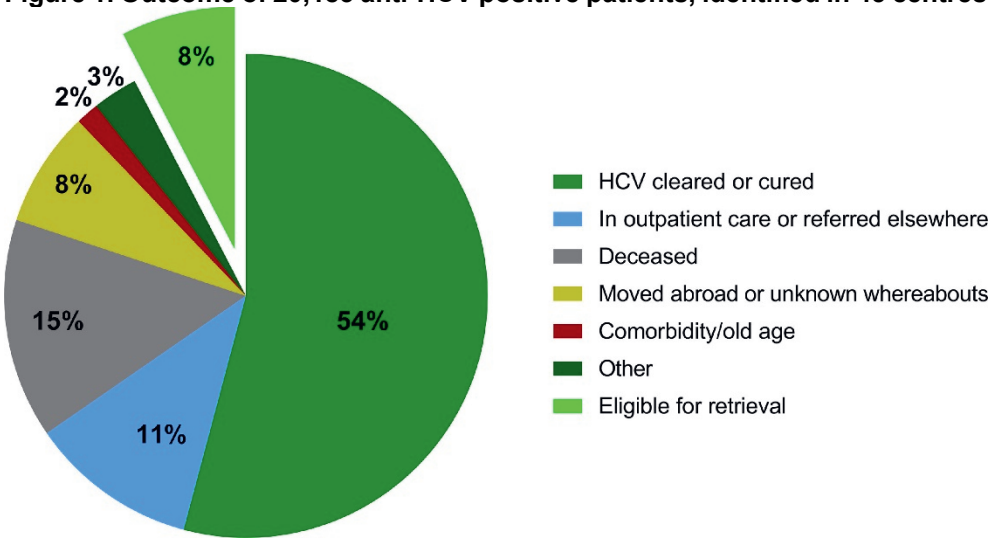
RESULTS

In total, CELINE was implemented in 45 sites, including 39/46 (85%) of certified hepatitis treatment centres in the Netherlands, five non-certified centres and one laboratory mainly serving primary care. Six centres with previously executed regional projects were included.[51-53] Among the remaining seven hepatitis treatment centres not included in the analyses, five centres had initiated own retrieval initiatives prior to CELINE roll-out and were not able to share data while two centres refused participation.

A total of 20,183 previously diagnosed patients were identified using laboratory records spanning median 14 years (IQR 11 – 17 years). The majority (n = 10,929, 54%) had already been successfully treated or spontaneously cleared infection

(Figure 1). In total 1,537 patients (8%) were identified as LTFU and eligible for retrieval.

Figure 1. Outcome of 20,183 anti-HCV positive patients, identified in 45 centres

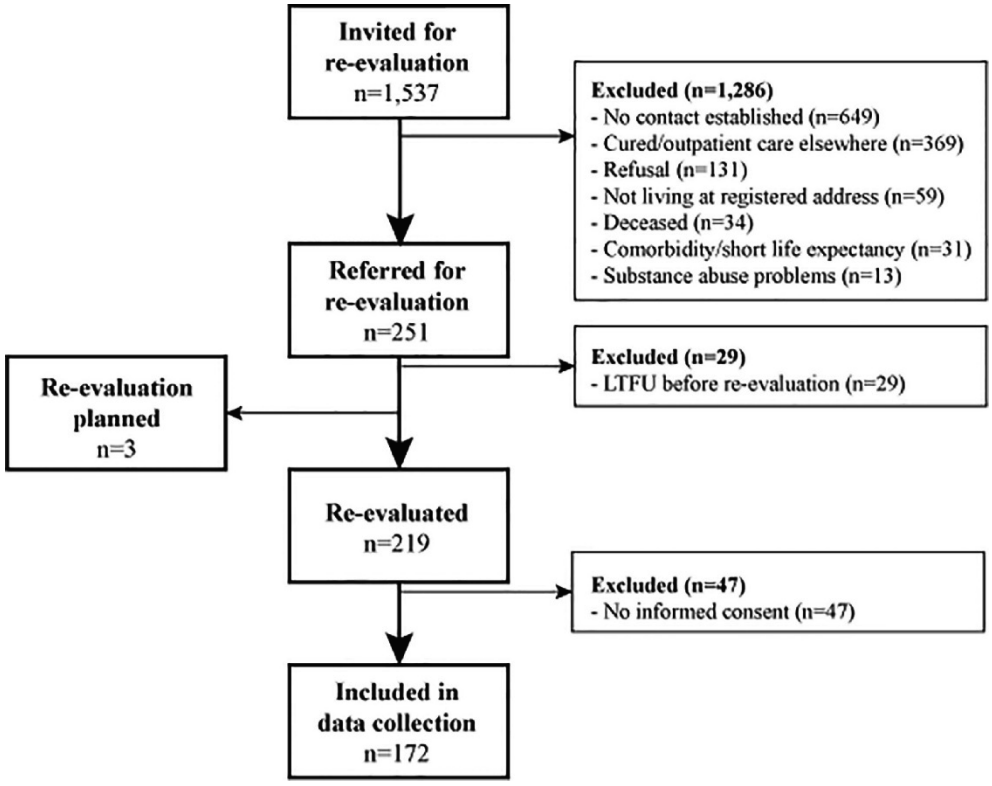


Abbreviations: HCV, hepatitis C virus.

Contact could not be established in 649 cases (Figure 2), resulting in a case ascertainment rate of 58% (888/1,537). Of the 1,537 invited patients, 369 (24%) were already cured or in care elsewhere and 131 (9%) refused to be re-linked to care. In total, 251 (16%) patients were referred, of whom 219 (87%) attended their visit. Three of the remaining 32 patients have their screening visit planned and 29 disregarded their scheduled visit.

Of the 219 screened individuals, 172 (79%) provided informed consent for data collection (Table 1). One hundred and ten patients ever had a liver stiffness measurement (n = 51) and/or abdominal ultrasound (n = 105), of whom 14 patients (13%) had evidence of advanced liver fibrosis or cirrhosis. One LTFU patient had a prior focal hepatocellular carcinoma (HCC). Among the re-evaluated patients, 27 patients (16%) never had a prior HCV-related appointment at an outpatient clinic and 18 patients (11%) reported being unaware of their possible HCV infection. HCV-RNA was positive in 12 of these 18 patients (67%), of whom three (25%) had advanced fibrosis or cirrhosis at the re-evaluation visit.

Figure 2. Flowchart of patients eligible for retrieval, who were invited for re-evaluation



Abbreviations: LTFU: lost to follow-up.

In total, 143/172 patients (83%) tested HCV-RNA positive at re-evaluation (Table 2). HCV-RNA was negative in 24 patients (14%) and not (yet) tested in five (3%). Among the 167 patients with a known HCV-RNA status at re-evaluation, HCV-RNA was positive in 127/145 (88%) of those with a positive HCV-RNA status before becoming LTFU and 16/27 (59%) of those with positive HCV antibodies with unknown HCV-RNA status. At re-evaluation, none of the patients tested HIV-positive, but two patients (1%) had a newly diagnosed hepatitis B virus infection.

Table 1. Characteristics of re-linked patients who provided consent for data collection

	Re-linked patients (n = 172)
Male sex	121 (70%)
Age in years at re-linkage to care (median, IQR)	58 (52 - 63)
Reason for becoming LTFU[1]	
Patient-related	76 (44%)
Therapy-related	44 (26%)
Care-related	41 (24%)
Other/unknown	11 (6%)
Years since last HCV-related hospital visit (median, IQR)	7 (4 - 11)
First-generation migrant	59 (34%)
Route of HCV transmission	
Injecting drug use	119 (69%)
Transfusion	18 (11%)
Other[2]	19 (1%)
Unknown	16 (9%)
(History of) substance abuse	
Injecting drug use	125 (73%)
Alcohol[3]	57 (33%)
Currently on opioid substitution therapy	50 (29%)
HCV treatment experience	44 (26%)[4]
(PEG-)Interferon	40 (23%)
Direct-acting antivirals	7 (4%)
HCV-RNA positive	143 (83%)

[1]Patient-related reasons for LTFU included: multiple no shows, therapy refusal, addiction, or imprisoned. Therapy-related reasons for LTFU included: no indication for therapy, lack of therapy options. Care-related reasons for LTFU included: no consequence given to HCV test, absent SVR check, HCV follow-up postponed due to other comorbidities or pregnancy, absent FU appointment, treatment deferred, waiting for a new appointment. [2]Nosocomial (5), needle prick injury (4), sexual (3), vertical (2), tattoo (1), injecting drug use or transfusion (1), injecting drug use or sexual (2), nosocomial or sexual (1). [3]Defined as > 14 units/week for females and > 21 units/week for males. [4]Several patients received both (PEG-)interferon and direct-acting antivirals. Abbreviations: IQR: interquartile range; LTFU, lost to follow-up; HCV, hepatitis C virus; PEG: pegylated.

Table 2. Characteristics of HCV-RNA positive patients

	HCV-RNA positive (n = 143)
Advanced fibrosis or cirrhosis at re-evaluation[1]	38 (27%)
HCV Genotype	
1a	61 (43%)
1b	29 (20%)
1, other/unknown subtype	4 (3%)
2	9 (6%)
3	27 (19%)
4	10 (7%)
unknown	3 (2%)
Co-infection	
Prior HBV (HBsAg-, anti-HBc+)	50 (35%)
Chronic HBV (HBsAg+)	2 (1%)
HIV	0 (0%)
DAA treatment initiated after retrieval	123 (86%)
SOF/LDV	10 (8%)
SOF/VEL	28 (23%)
GLE/PIB	67 (54%)
ELB/GRZ	13 (11%)
SOF/VEL/VOX	1 (1%)
Unknown	4 (3%)
Treatment outcome	
SVR	91 (75%)
Awaiting SVR-12 measurement	27 (22%)
Discontinued DAA therapy	4 (3%)

[1]*Defined as a liver stiffness value ≥ 9.5 kPa or radiological, histological or clinical signs of cirrhosis.*

Abbreviations: HCV, hepatitis C virus; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBc, antibodies to hepatitis B core antigen; HIV, human immunodeficiency virus; DAA, direct-acting antiviral; SOF, sofosbuvir; LDV, ledipasvir; VEL, velpatasvir; GLE, glecaprevir; PIB, pibrentasvir; ELB, elbasvir; GRZ, grazoprevir; VOX, voxilaprevir; SVR, sustained virological response.

Among HCV-RNA positive patients, 38 (27%) had advanced fibrosis or cirrhosis, of whom two were classified as Child-Pugh B and one as Child Pugh C. Additionally, two patients were diagnosed with an HCC at the time of the re-evaluation visit and another three patients developed an HCC during the period after their re-evaluation visit.

In 86% of HCV-RNA positive patients (123/143) DAA therapy was initiated. Sustained virological response (SVR) was achieved in all of the 91 individuals with a known HCV-RNA result twelve weeks after cessation of treatment. Four patients discontinued DAA, 10 finished the treatment course but became LTFU again without formal proof of SVR, and 27 patients are awaiting their SVR-12 result. Among the

20 patients who did not initiate DAA, six refused treatment, four became LTFU again, five had severe comorbidity or short life expectancy, two died, two had addiction problems, while one will start DAA treatment shortly.

DISCUSSION

CELINE was a nationwide retrieval project aiming to re-engage LTFU HCV patients with care. It was designed as a micro-elimination initiative to advance progress towards the WHO HCV elimination targets in the Netherlands. We demonstrated that the majority of individuals diagnosed in the past with HCV in the Netherlands had been cured prior to rollout of CELINE. We found that 8% was LTFU and eligible for retrieval. Advanced fibrosis or cirrhosis was diagnosed in 27% of HCV-RNA-positive retrieved individuals.

Our retrieval efforts resulted in 219 patients that we could re-link to care, corresponding to 14% of individuals invited for re-evaluation. Thus, the retrieval rate of our nationwide approach was within the bandwidth observed in several previously conducted regional Dutch projects.[51-53] Our study included the vast majority of hepatitis treatment centres in the Netherlands, thereby maximising its impact and providing valuable insight into the epidemiology of patients ever diagnosed with HCV infection in the Netherlands. A higher number of re-linked patients might have been achieved if a national registry had been in place as this would improve adequate coordination of retrieval. Nevertheless, our retrieval was successful as a significant number of patients with advanced fibrosis or cirrhosis were re-linked to care. Furthermore, our study provided valuable insight into the HCV epidemiology of the Netherlands and demonstrated the feasibility of retrieval as a micro-elimination strategy. The robust and extensive framework that was laid out can serve as a blueprint for retrieval of patients with other diseases and in other countries.

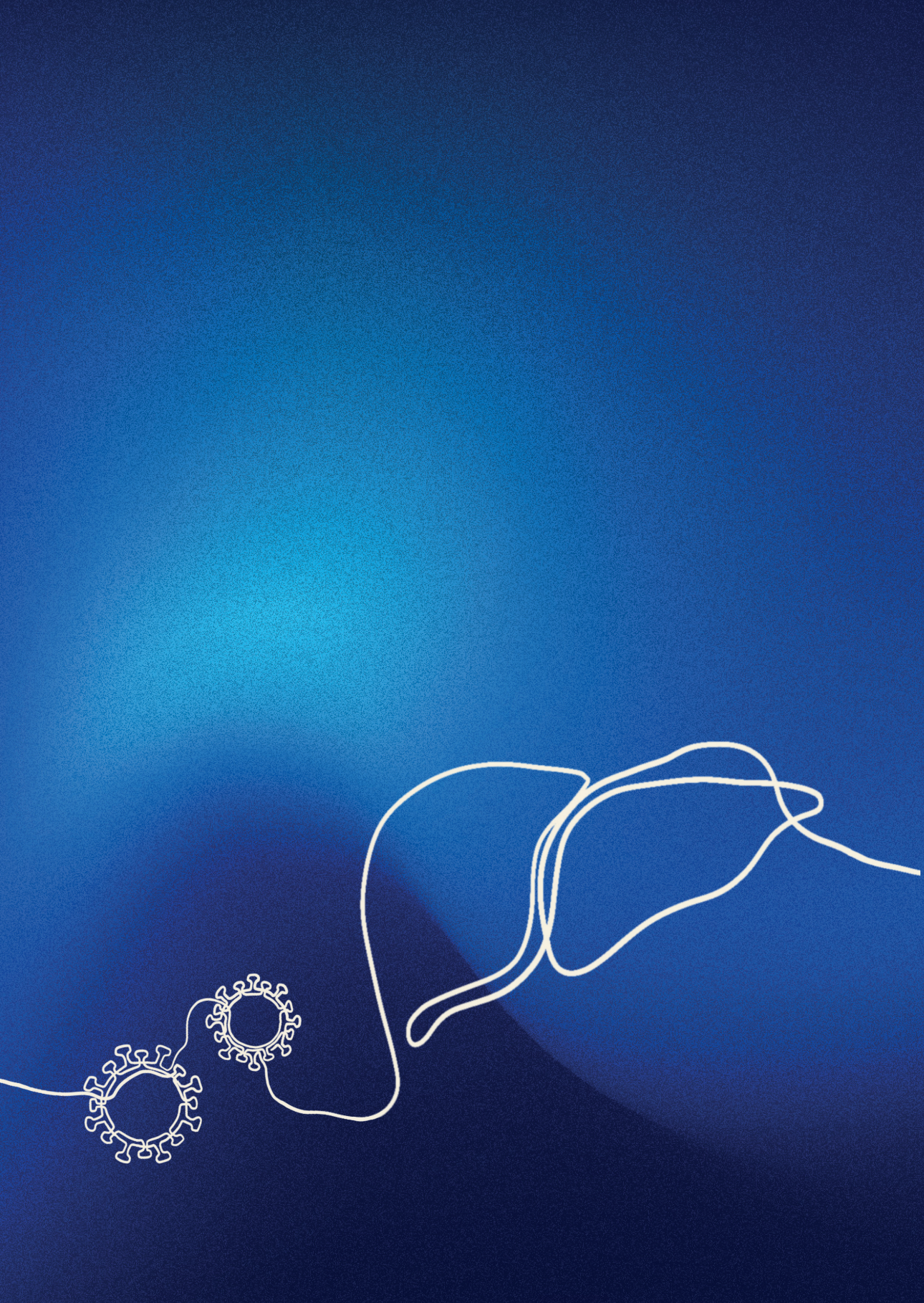
The most common reasons for LTFU in our study were frequent no shows and refusal of HCV therapy. The most common reasons for unsuccessful retrieval were the inability to make contact with the patient, refusal of re-evaluation or substance abuse problems which complicated re-linkage to care. For these individuals it could be beneficial to perform retrieval as a standard annual or bi-annual procedure, instead of a one-time effort. Since current HCV treatment is highly effective, it could be argued that loss to follow-up is an unacceptable outcome and should be prevented or dealt with by all HCV care providers.

An important limitation of retrieval is that retrieval efforts are labour intensive. The current nationwide project was led by three full-time PhD candidates and required a commitment that is most likely impossible to meet by physicians and/or nurse consultants on top of the regular healthcare they provide. There are, however, some measures that can reduce the investments needed for future retrieval projects. First,

make retrieval part of routine care and eliminate the collection of data for research purposes. This will bypass the laborious institutional review board process and will thereby reduce workload. Second, implementing digital innovations such as a case-finding algorithm that successfully identifies diagnosed but untreated HCV patients further reduces workload.[128] Last but not least, the framework now laid out by CELINE will increase efficacy and reduce costs of future retrieval efforts.

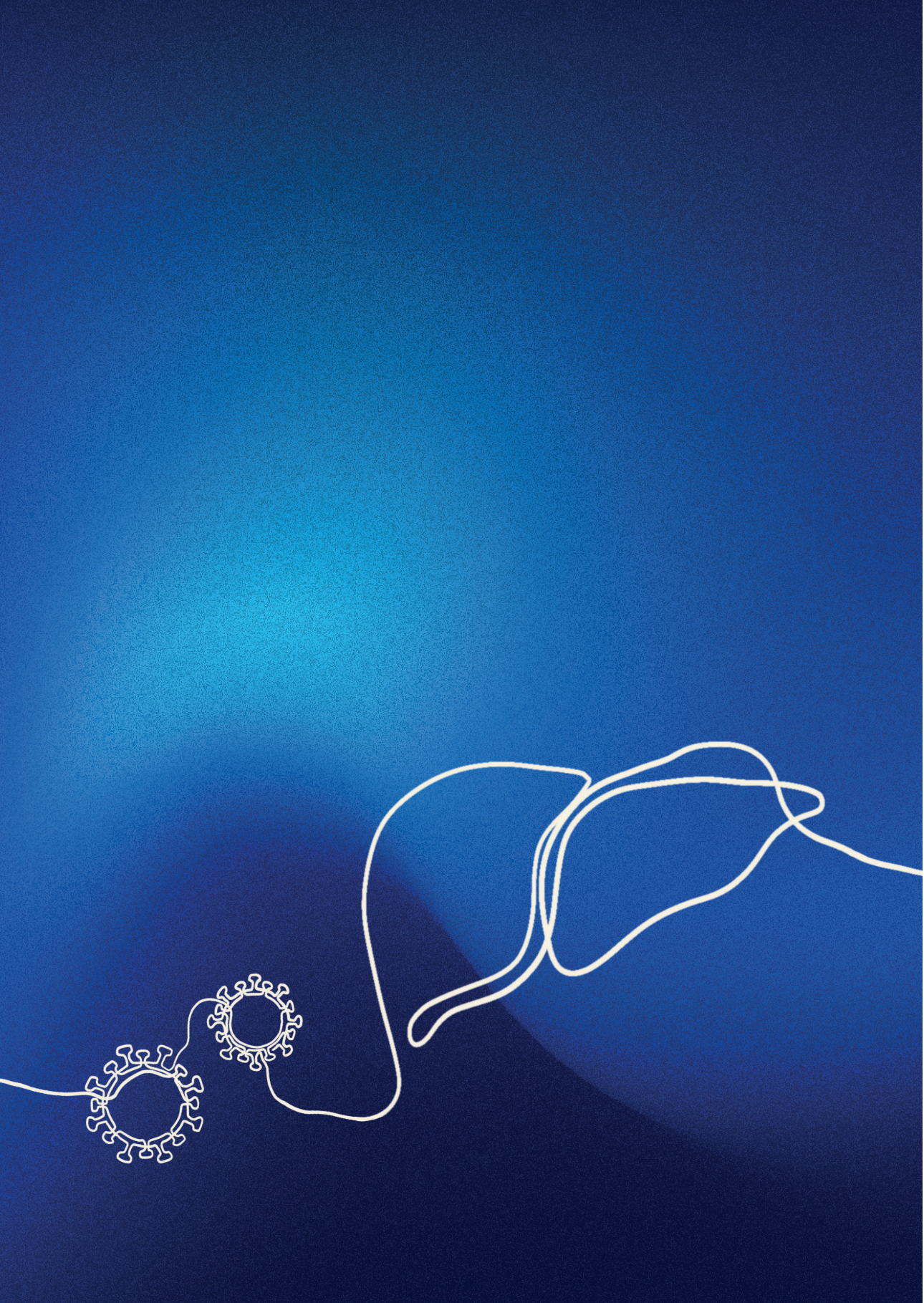
CELINE results must be placed in the greater context of HCV elimination. A recent modelling study predicting the Netherlands' progress towards the WHO HCV elimination targets concluded that the Netherlands is currently on track to meet these targets by 2030.[129] However, this was only met under the assumption that annual HCV diagnosis and treatment rates were maintained at the 2019 levels. HCV micro-elimination in LTFU patients will mainly contribute to maintaining high treatment rates, especially if done repeatedly. In the Netherlands however, this contribution will be minor. Micro-elimination in other subpopulations in the Netherlands has already been highly successful, such as people living with HIV and people with inherited bleeding disorders.[130,131] Increased efforts to find and cure HCV-viraemic individuals in other subpopulations, like migrants from high-endemic countries, PWID and incarcerated individuals, are needed.

To conclude, the majority of patients in the Netherlands who received the diagnosis of chronic HCV infection since the early 2000s has been cured. Our nationwide micro-elimination effort retrieved another 14% of the population who were LTFU and eligible for retrieval. LTFU patients have a high risk of advanced liver disease, illustrated by the 27% of HCV-RNA-positive retrieved individuals with evidence of advanced liver fibrosis or cirrhosis. With CELINE we demonstrated that systematic retrieval provides great value for a better understanding of the HCV epidemiology. Additionally, we established a robust diagnostic pipeline targeting the LTFU population that is worthy of replication in other health care environments. As such, our study supports the view that micro-elimination through retrieval is feasible and contributes to HCV elimination.



PART IV

Adherence to clinical guidelines – evaluating hepatitis care



CHAPTER 8

Patients treated with rituximab are poorly screened for hepatitis B infection: Data from a low-incidence country

Sylvia M. Brakenhoff*, Roos Hoekstra*, Pieter Honkoop, Robert Roomer, Jan G. den Hollander, Geert Bezemer, Robert J. de Knegt, Milan J. Sonneveld, Robert A. de Man
* joint first authorship

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ABSTRACT

Background & Aim(s): Patients with chronic or resolved hepatitis B are at risk of hepatitis B reactivation (HBVr) when treated with high-risk immunosuppressive therapy such as rituximab. Therefore, international guidelines recommend HBV screening prior to rituximab treatment and subsequent antiviral prophylaxis among patients with a (resolved) infection. In this study, we evaluated the adherence to those recommendations.

Methods: This is a retrospective multicentre study including patients treated with rituximab between 2000-2021. Performance of correct screening was assessed, defined as the measurement of hepatitis B surface antigen (HBsAg) and hepatitis B core antibodies (anti-HBc). Next, initiation of antiviral prophylaxis and HBVr rate among patients with a chronic or resolved HBV infection was studied.

Results: We enrolled 3,176 patients of whom 1,448 (46%) were screened correctly. Screening rates differed significantly between academic and non-academic hospitals; respectively 65% vs 32% ($p < 0.001$). In addition, screening rates differed across specialties and improved throughout the years; from 32% before 2012 to 75% after 2020 among academic prescribers, versus 1% to 60% among non-academic prescribers (both $p < 0.001$). Antiviral prophylaxis was initiated in 58% vs 36% of the patients with a chronic or resolved HBV infection. Seven patients experienced HBVr, including one fatal liver decompensation.

Conclusion: Many patients treated with rituximab were not correctly screened for HBV infection and antiviral prophylaxis was often not initiated. Although screening rates improved over time, rates remain suboptimal. With the increasing number of indications for rituximab and other immunosuppressive agents these findings could raise awareness among all medical specialties prescribing these agents.

INTRODUCTION

Hepatitis B virus infection (HBV) is considered a global health threat as it is associated with liver decompensation, liver cirrhosis and primary liver cancer.[1] Worldwide, approximately 269 million individuals have a chronic active hepatitis B infection.[1,132] In the Netherlands, the prevalence of chronic hepatitis B has been estimated at 0.2-0.4%, and approximately 3.5% of the population has a resolved hepatitis B infection.[5,133,134] Since most HBV infections progress asymptomatically, many patients are unaware of their infection. However, both active and quiescent infections can re-activate in the setting of immunosuppressive or cytotoxic treatment.[135]

The anti-CD20 monoclonal agent rituximab is considered a high risk immunosuppressive agent.[135-137] The risk for hepatitis B viral reactivation (HBVr) is 9% among patients with a resolved HBV infection and up to 80% among chronic hepatitis B (CHB) patients when treated with rituximab. [19,20] An HBVr can result in severe and even fatal complications such as symptomatic hepatitis, liver failure, and death. However, HBVr can be prevented using antiviral prophylaxis such as nucleos(t)ide analogues (NAs).[19,138,139]

It is therefore, according current national and international guidelines, recommended to screen patients who start high-risk immunosuppressive therapy.[18,42,121] A correct screening includes testing for both hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core antigen (anti-HBc) to identify both patients with a chronic (HBsAg positive) and resolved (HBsAg negative, anti-HBc positive) infection. In addition, antiviral prophylaxis is recommended for patients with either a chronic or resolved hepatitis infection if treated with rituximab.[18,42]

Nevertheless, findings from several studies suggest that screening might be performed sub optimally (Supplementary Table).[140-145] In the Netherlands, a low endemic country, data on the screening rates among patients treated with high risk immunosuppressive agents are lacking. We therefore aimed to study (1) hepatitis B screening performance in patients treated with rituximab, (2) management of patients with a resolved or chronic hepatitis B infection, and (3) the number of patients that experienced hepatitis B reactivation.

PATIENTS AND METHODS

Study design and patient population

This is a retrospective, observational, multicentre cohort study in the area of Rotterdam, the Netherlands, including one large tertiary academic hospital and four non-academic hospitals. Adult patients who received rituximab between 2000 and 2021 were identified by hospital pharmacy records. Patients were excluded if

rituximab was initiated in a different hospital. Data was retrieved from medical notes and laboratory records of each participating hospital. This study was conducted according to the principles outlined in the 1964 Declaration of Helsinki and its later amendments. The original study protocols have been approved by the medical ethical committees. No patients consent was obtained for the study due to its retrospective design and to prevent selection bias as many patient were deceased.

Outcomes

The main outcome was the proportion of correctly performed HBV screenings in patients who started rituximab treatment. Correct screening was defined as the measurement of both HBsAg and anti-HBc within one year prior to and one month after start of rituximab treatment. Patients were categorised as correctly screened, unscreened, and incorrectly screened. Incorrectly screened patients were divided into subgroups; 1) HBsAg only, 2) anti-HBs or HBV DNA only, and 3) not screened one year prior or one month after start of rituximab treatment.

Secondary outcome included the initiation of antiviral prophylaxis (i.e. NAs) at the start of rituximab treatment in patients with evidence of a chronic hepatitis B [HBsAg(+)] or resolved hepatitis B [HBsAg(-) but anti-HBc(+)] infection. Next, we studied the number of patients that experienced hepatitis B reactivation (HBVr), defined as alanine aminotransferase (ALT) increase in combination with an elevated HBV DNA level (unknown DNA baseline: ≥ 10,000 IU/mL or known HBV DNA baseline: ≥ 2 log increase), and/or HBsAg seroreversion within prior HBsAg negative patients.

Statistical Analysis

Results are presented as mean (± standard deviation [SD]), numbers (in percentages), and medians (with interquartile range [IQR]). Associations between screening performance and prescribers or hospital type (academic vs non-academic) were studied using Chi-square test. To study the screening rates over time, year of rituximab was categorised as screening before 01.01.2012 (< 2012), 01.01.2012 – 31.12.2014 (2012-2015), 01.01.2015 – 31.12.2017 (2015-2018), 01.01.2018 – 31.12.2019 (2018-2020), and after 01.01.2020 (> 2020). Etiological multivariable analysis was used to study risk factors for screening failure,[146,147] including sex, ethnicity, hospital type (academic versus non-academic), setting (outpatient clinic versus hospitalised patients), and year of rituximab treatment. Differences were considered as statistically significant if $p < 0.050$. Statistical analysis was performed using IBM SPSS for Windows, version 25.0 (SPSS Inc., Chicago, Illinois, USA). Graph Pad Prism version 5 for Windows (GraphPad Software, San Diego, California, USA) was used for graphical representation of the results.

RESULTS

Study population

In total 3,176 patients were included; 1,290 academic and 1,886 non-academic. The patient characteristics are displayed in Table 1. High-volume rituximab prescribers included haematologists (61.0%), and low-volume prescribers included neurologists (2.8%), ophthalmologists (2.7%), pulmonologists (0.9%), dermatologists (0.3%), and gastroenterologists (0.3%). Patients received rituximab predominantly in outpatient care setting (94.6%). Haematological malignancies (58.0%) were the most common indications for rituximab.

HBV screening performance was suboptimal and differed significantly between academic and non-academic hospitals, and between rituximab prescribers

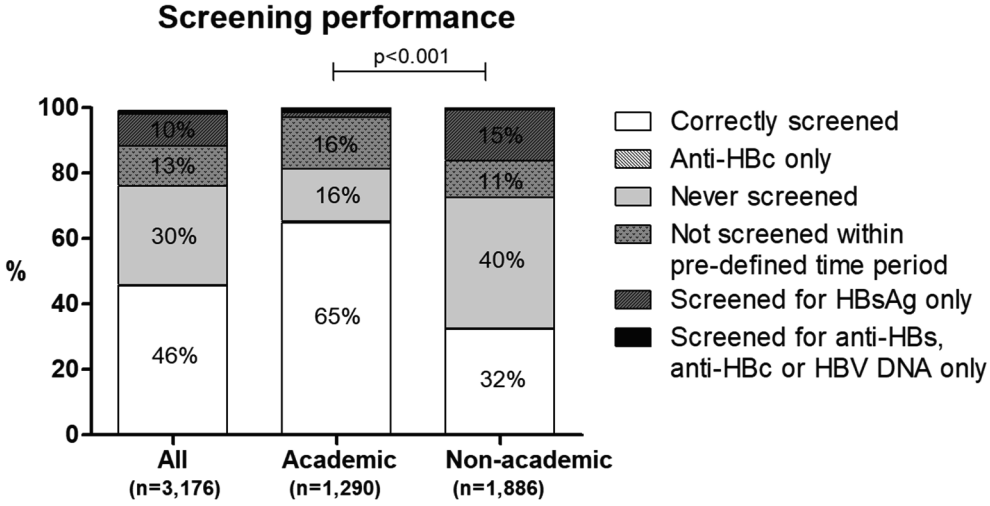
Overall, 1,448 patients (45.6%) were screened correctly and 959 patients (30.2%) were never screened (Figure 1). In addition, 308 patients (9.7%) were screened for HBsAg only, 10 patients for anti-HBc only (0.3%), and 34 patients (1.1%) were screened for other HBV serological markers (i.e. anti-HBs or HBV DNA only). Another 417 patients (13.1%) were screened correctly, but not within the predefined period (Figure 1). The screening rates differed significantly between academic and non-academic hospitals; screenings were performed correctly in 65.0% versus 32.3% of the patients in respectively academic and non-academic hospitals (Figure 1; $p < 0.001$). In addition, screening rates differed between rituximab prescribers (Figure 2), with highest rates among academic rheumatologists but lowest rates among non-academic rheumatologists.

Table 1. Patient characteristics

	Academic n = 1,290	Non-academic n = 1,886	Total n = 3,176
Age at start rituximab (years; median, IQR)	57 (45-66)	66 (56-74)	63 (51-71)
Sex (male; n,%)	708 (54.9)	1,015 (53.8)	1,723 (54.3)
Etnicity (n,%)			
Caucasian, white	1,140 (88.4)	1,714 (90.9)	2,854 (89.9)
Black	73 (5.7)	20 (1.1)	93 (2.9)
Asian	26 (2.0)	21 (1.1)	47 (1.5)
North African/Middle East	35 (2.7)	84 (4.5)	119 (3.7)
Other	16 (1.2)	47 (2.5)	63 (2.0)
Prescriber (n,%)			
Haematologist	610 (47.3)	1,325 (70.3)	1,935 (60.9)
Internist	397 (30.8)	218 (11.6)	615 (19.4)
Rheumatologist	86 (6.7)	314 (16.6)	400 (12.6)
Pulmonologist	26 (2.0)	4 (0.2)	30 (0.9)
Neurologist	73 (5.7)	17 (0.9)	90 (2.8)
Dermatologist	8 (0.6)	0 (0.0)	8 (0.3)
Ophthalmologist	80 (6.2)	7 (0.4)	87 (2.7)
Gastro-enterologist	10 (0.8)	1 (0.1)	11 (0.3)
Clinical setting (n,%)			
Outpatient care	1,140 (88.4)	1,863 (98.8)	3,003 (94.6)
Hospitalised patients (non-ICU)	139 (10.8)	21 (1.1)	160 (5.0)
Hospitalised patients (ICU)	11 (0.9)	2 (0.1)	13 (0.4)
Indication* (n,%)			
Haematological malignancies	574 (44.5)	1,268 (67.2)	1,842 (58.0)
Thrombocytopenia	25 (1.9)	30 (1.6)	55 (1.7)
Anaemia	10 (0.8)	28 (1.5)	38 (1.2)
Vasculitis	150 (11.6)	103 (5.5)	253 (8.0)
Rheumatoide arthritis	62 (4.8)	309 (16.4)	371 (11.7)
Renal disorders	69 (5.3)	63 (3.3)	132 (4.2)
Autoimmune diseases	147 (11.4)	51 (2.7)	198 (6.2)
Encephalopathy	40 (3.1)	1 (0.1)	41 (1.3)
Transplantation-related	66 (5.1)	0 (0.0)	66 (2.1)
Other/unknown	147 (11.4)	33 (1.7)	180 (5.7)
Year of rituximab treatment			
< 2012	47 (3.6)	242 (12.8)	289 (9.1)
2012-2015	174 (13.5)	284 (15.1)	458 (14.4)
2015-2018	476 (36.9)	507 (26.9)	983 (31.0)
2018-2020	404 (31.3)	557 (29.5)	961 (30.3)
> 2020	189 (14.7)	296 (15.7)	485 (15.3)

** Haematological malignancies included lymphoma, leukemia, and monoclonal gammopathy of undetermined significance (MGUS). Trombocytopenia included thrombocytopenia eci, Immune thrombocytopenic purpura (ITP) and Thrombotic thrombocytopenic purpura (TTP). Anaemia included hemoglobinopathy, autoimmune anaemia. Vasculititis included granulomatosis with polyangiitis (Morbus Wegener) and other vasculitis-related diseases. Renal disorders included nephrotic syndrome, glomerulonephritis. Autoimmune disorders included systemic lupus erythematosus (SLE), anti-synthetase syndrome, anti-phospholipid syndrome, and Morbus Graves.*
Abbreviations: ICU, intensive care unit

Figure 1. Screening performance in overall study group, and stratified by academic versus non-academic hospitals



Correct screening was defined as screening on HBsAg and anti-HBc within one year prior or one month after start of rituximab treatment.
Abbreviations: HBsAg, hepatitis B surface antigen; anti-HBc, antibodies to hepatitis B core antigen; anti-HBs, antibodies to hepatitis B surface antigen; HBV, hepatitis B virus.

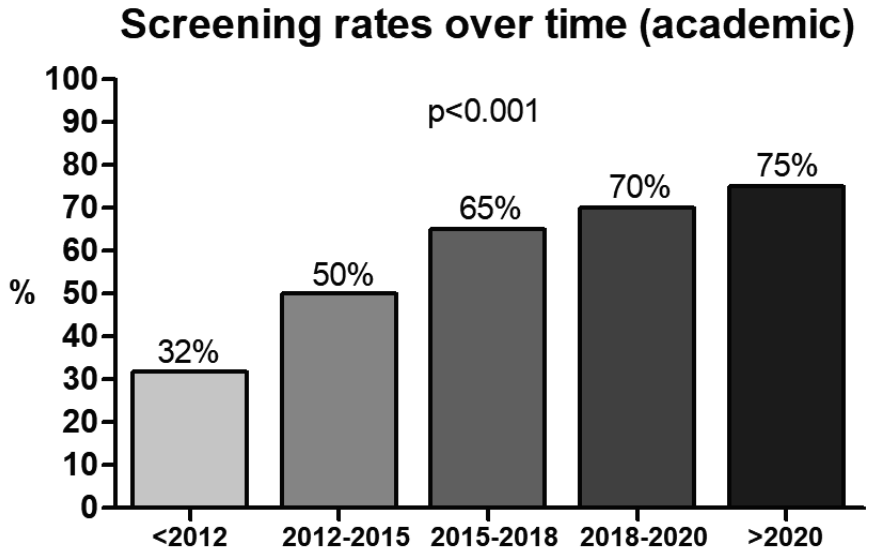
Screening rates improved over time, but remain suboptimal

The screening rates improved over time; from 31.9% before 2012 to 75.1% after 2020 in the academic hospital, and from respectively 1.2% to 59.5% in non-academic hospitals (Figure 2, $p < 0.001$). When data were stratified on prescriber, screening rates also improved over time (< 2018 versus > 2018; Figure 3).

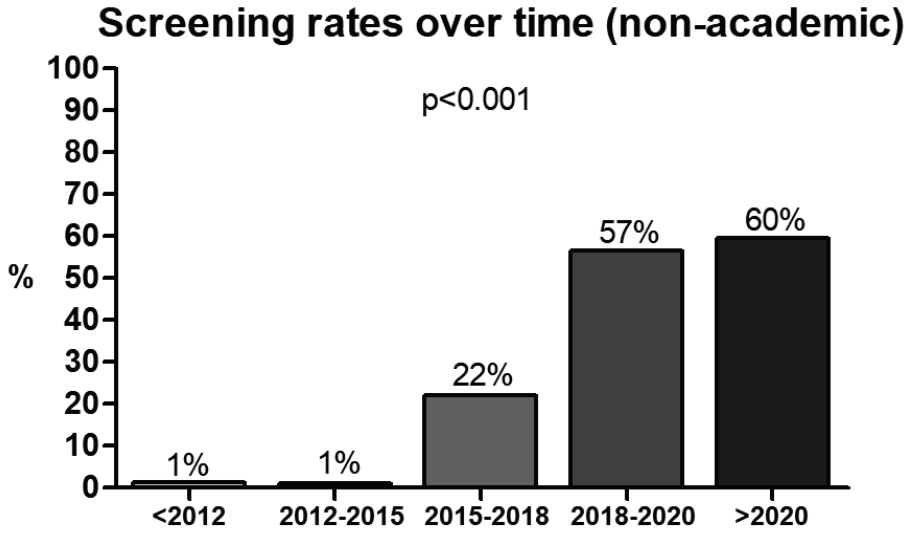
Findings were consistent in multivariable analysis, which demonstrated that hospital type (academic versus non-academic; OR 4.30, 95% CI 3.62 – 5.11, $p < 0.001$) and year of rituximab treatment (OR 1.41, 95% CI 1.37 – 1.46, $p < 0.001$) were significantly associated with screening performance, but not sex, ethnicity, or setting (outpatient clinic versus hospitalised patients). No difference was observed when

screening rates were stratified for race; correct screening among 45/45/40% of the Caucasian/North African or Middle East/Asian patients.

Figure 2. Rates of correct screening over time in academic (A) and non-academic (B) hospitals



A)



B)

Correct screening was defined as screening on HBsAg and anti-HBc within one year prior or one month after start of rituximab treatment.
P-value included the p for trend.Abbreviations: HBsAg, hepatitis B surface antigen; anti-HBc, antibodies to hepatitis B core antigen.

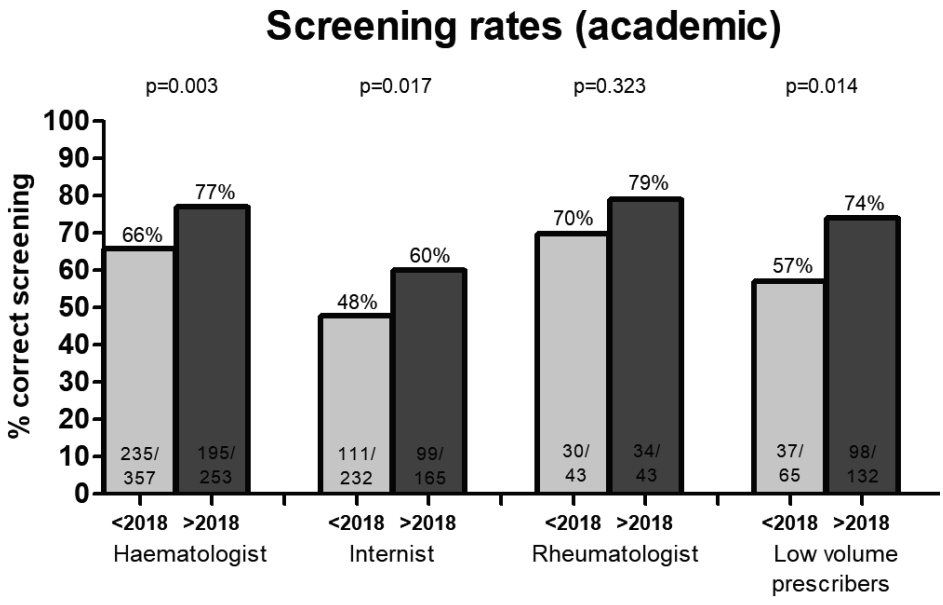
Management of patients with a chronic or resolved hepatitis B infection

Among the 2,183 patients with available HBV serology, 12 patients (0.5%) were HBsAg(+), 111 patients (5.1%) were HBsAg(-) but anti-HBc(+), and two patients were anti-HBc(+) without known HBsAg status (0.1%; Figure 4). Among those 125 patients, 47 received antiviral prophylaxis (37.6%); including 7/12 HBsAg(+) patients (58.3%), 40/111 HBsAg(-) anti-HBc(+) patients (36.0%) and none of the anti-HBc(+) only patients (Figure 4).

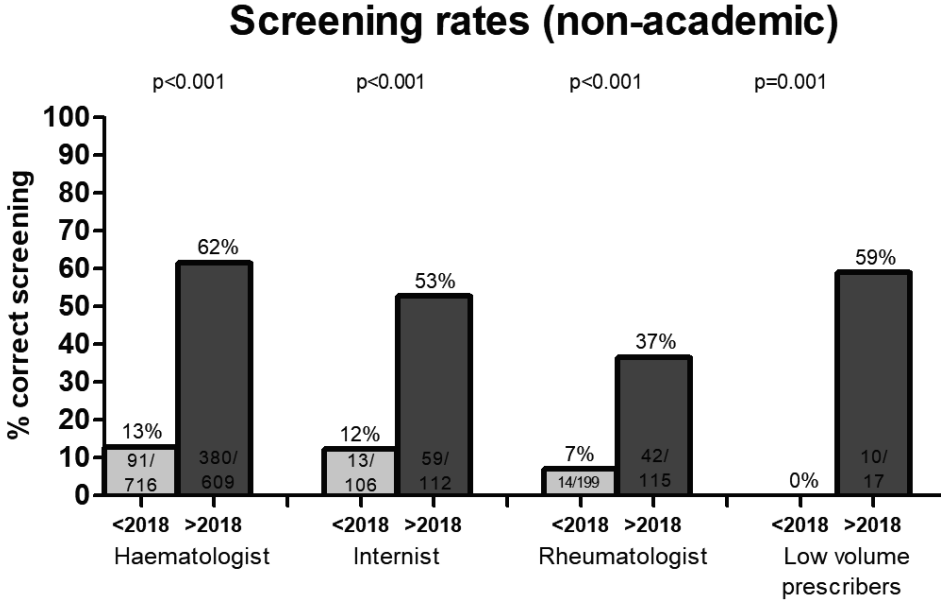
When data were stratified on hospital type, antiviral prophylaxis was started in 31 of the 70 academic patients (44.3%) and in 16 of the 55 non-academic patients (29.1%; $p = 0.082$). In addition, the number of patients receiving antiviral prophylaxis increased over time, from 27.1% before 2018 to 47.0% after 2018 ($p = 0.057$); an increase from 50.0% to 66.7% among HBsAg(+) patients ($p = 0.565$), and 25.0% to 45.8% among HBsAg(-) but anti-HBc(+) patients ($p = 0.035$).

In total, seven patients experienced HBVr, including four chronic hepatitis B patients and three patients with a resolved HBV infection (Figure 4). Of those, two patients experienced hepatic decompensation, of whom one patient died. Among the seven patients with an HBVr, five (71.4%) had an haematological disease. None of the patients who received antiviral prophylaxis experienced HBVr.

Figure 3. Rates of correct screening over time in academic (A) and non-academic hospitals (B), stratified per prescriber



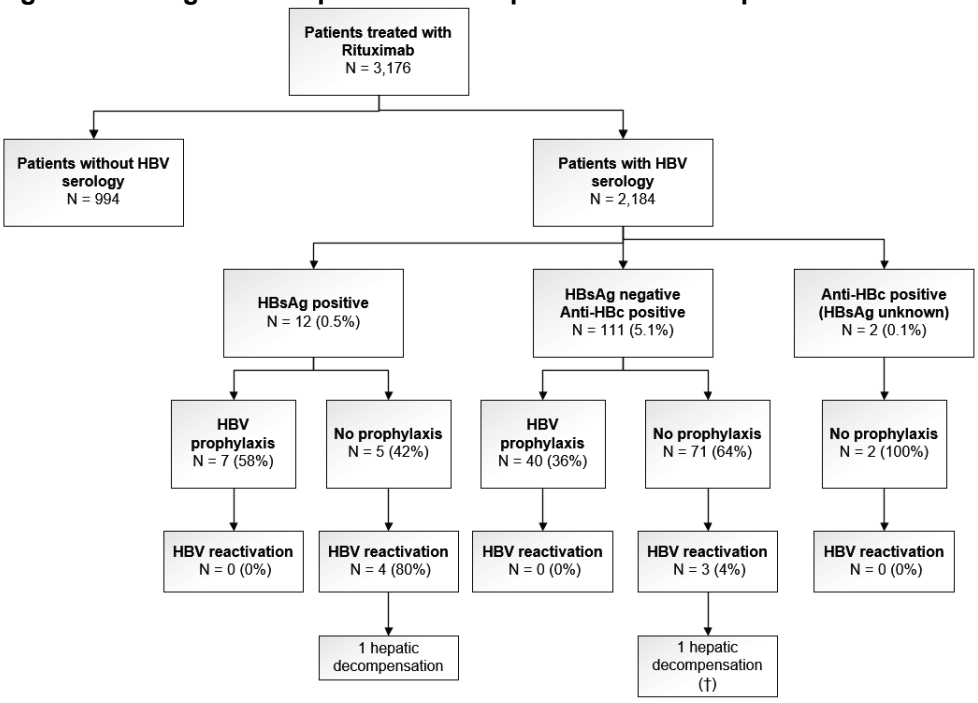
A)



B)

Low volume prescribers included neurologists, ophthalmologists, pulmonologists, dermatologists, and gastroenterologists. Time period was stratified as < 2018 (i.e. before 31.12.2017) and > 2018 (i.e. after 01.01.2018)

Figure 4. Management of patients with a past or chronic hepatitis B infection



HBV prophylaxis included treatment with nucleos(t)ide analogues at start of rituximab. Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBc, antibodies to hepatitis B core antigen.

DISCUSSION

Hepatitis B reactivation (HBVr) is a severe complication among patients with a chronic or resolved hepatitis B infection treated with high risk immunosuppressive agents such as rituximab.[19] In this multi-centre study, including one large academic hospital and four non-academic hospitals in the Netherlands, we demonstrated that screening rates were suboptimal. In seven patients lack of antiviral prophylaxis resulted in HBVr, including one fatal liver decompensation. This stresses the importance of insight in screening performances and HBVr risk to raise more awareness on this issue.

Current international guidelines for hepatitis B are comprehensive regarding screening on HBV markers for patient with high risk immunosuppressive agents.[18,42,121] For instance, the current guideline of the European Association for the Study of the Liver ([EASL] published in 2017)[18] and the previous EASL guideline (published in 2012)[148] raised attention on this topic. In line with the publication of those guideline updates we observed that the screening rates improved significantly after 2012 and 2018. Our findings are in line with a study, performed in the USA,

which demonstrated that testing for anti-HBc and HBsAg increased from 9% to 87% from 2005 to 2017.[149]

Despite the recommendations of those guidelines we observed that hepatitis B screening is not consistently practiced. A possible explanation is that physicians from low endemic countries have limited knowledge about hepatitis B, because they rarely treat patients with an hepatitis B infection. In addition, physicians prescribing high risk immunosuppressive agents, for instance haematologists or rheumatologists, might be unaware of the international hepatitis B guidelines and consult subsequently only guidelines that are published by their medical association(s). A recent survey, conducted among Dutch oncologists showed that only 27% of the respondents indicated to follow a standardised protocol.[150] However, although current (inter)national guidelines (for instance for rheumatoid arthritis and B cell lymphoma) address the risk of HBV reactivation they are unclear on how to screen patients or how to manage patients with resolved or chronic hepatitis B infection.[151-155]

Our results showed a significant difference in screening rates between academic and non-academic hospitals. Several factors could contribute to the discrepancy in screening performance between academic and non-academic hospitals, and between prescribers. First, awareness of HBVr might be higher among medical specialists that prescribe rituximab more frequently and routinely. However, it is remarkable that in this study high-volume prescribers did not necessarily perform screening more adequately compared to low-volume prescribers. Secondly, there is a possibility that within the same institution some specialties have (had) access to computerised screening tools while others did not have this access.

Adequate screening can prevent HBV reactivation and potentially fatal outcomes by administering antiviral prophylaxis.[138,140,156,157] Current guidelines from the European and American associations for liver diseases therefore recommend antiviral prophylaxis over monitoring of HBV DNA, HBsAg and/or ALT levels among patients treated with rituximab.[18,42] However, we observed that many patients with a resolved or chronic hepatitis B did not receive antiviral prophylaxis. Although the administration of antiviral agents increased after 2018 many patients still did not receive adequate management. This resulted in seven HBVr, of whom one patient developed fatal liver decompensation. Therefore, all patients starting on high risk immunosuppressive agents who have evidence of a (resolved) hepatitis B infection should be treated in collaboration with a Hepatologist or infectious disease specialist.

It should be noted that not only rituximab has been listed as high risk immunosuppressive agent, also anthracycline derivates and high dose corticosteroids (prednisone ≥ 20 mg per day for ≥ 4 weeks) are considered high risk agents.[158] Those patients should also been screened for HBV serology and treated

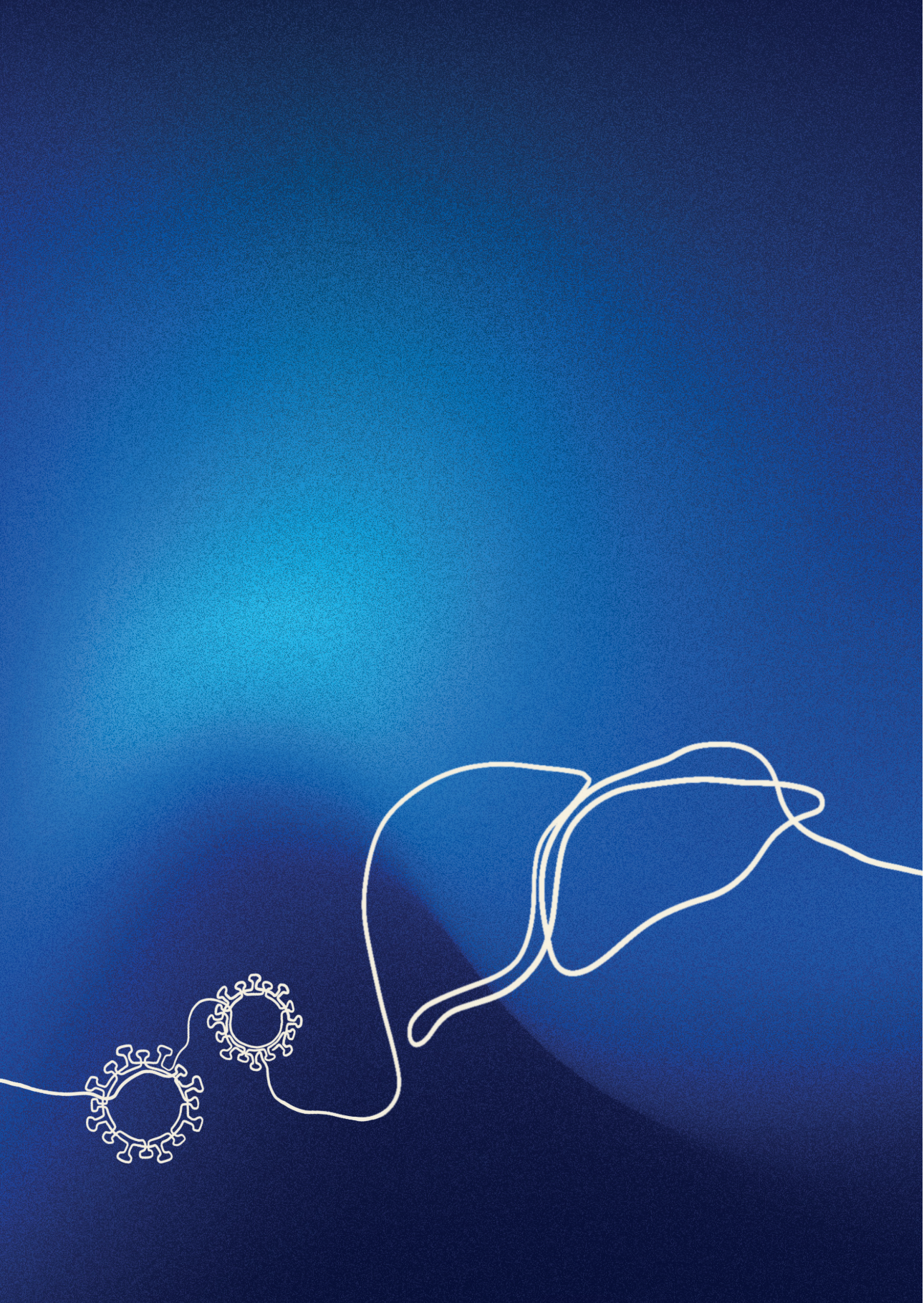
with antiviral prophylaxis if they are HBsAg positive or anti-HBc positive (HBsAg-negative). Pre-emptive therapy (screening for HBsAg and HBV DNA every 1-3 months during and after immunosuppression) is recommended among patients receiving agents with a moderate or low risk of HBV reactivation.[18] Moderate risk agents includes tumour necrosis factor (TNF)-α inhibitors, cytokine inhibitors and integrin inhibitors, tyrosine kinase inhibitors and moderate dose of corticosteroids (prednisone < 20 mg for ≥ 4 weeks). Low risk agents includes low dose corticosteroids and traditional immunosuppression such as azathioprine, 6-mercaptopurine, and methotrexate.[158] Antiviral prophylaxis should be started among HBsAg positive patients. However, each clinician could consider also starting antivirals in anti-HBc positive only patients, as these agents are inexpensive, have limited side effects, and are very effective in preventing HBVr.

Our study included a large cohort of patients that were treated with rituximab in one tertiary and four non-academic hospitals in a time period of 20 years. However, some limitations should be acknowledged. First, the number of HBVr can be an underestimation, since HBVr can occur > 6 months after last rituximab infusion,[159] and some of the included patients started rituximab treatment in 2020 or 2021. Also, we were not able to assess the incidence of HBVr among patients who were not screened. Since rituximab is often combined with cytotoxic agents which can cause toxic hepatitis, it is unknown what could have caused a possible rise in ALT (or even liver failure) among those without any HBV serology/HBV DNA measurements. In addition, there will be patients with unknown toxicity of rituximab probably due to an unknown HBVr. Another issue not addressed in this study is the use of electronical tools for screening which could have affected screening results per medical specialty. Furthermore, we defined correct screening as the measurement of both HBsAg and anti-HBc, in line with the current international guidelines. However, it could be debated that the patients who were screened for anti-HBc only could be considered as correctly screened since both patients with an active and resolved HBV infection (both anti-HBc positive) have an indication for antiviral prophylaxis. Nevertheless, with the limited number of patients in our cohort who were screened for anti-HBc only, screening rates will remain suboptimal when redefining the definition of correct screening. Moreover, additional data that was not part of the first data extraction, such as the treatment duration of antiviral prophylaxis among the patients who received antivirals, could not be collected due to protocol regulation restrictions. Next, we observed a significant difference in screening among academic and non-academic hospitals. However, we included only one large academic hospital. It is therefore unknown whether our findings could be translated to other (Dutch) academic hospitals. Also, the included hospitals are located in a multi-ethnic area of the Netherlands. Therefore, it is unknown whether our findings could be translated to other areas in the Netherlands. However, multivariate analysis demonstrated that ethnicity was not associated with screening performance. Finally,

our findings might only be generalisable to other low-endemic countries, although similar HBV screening rates were reported in both low- and higher-risk areas as well.[140,142,145]

With an increasing number of indications for rituximab treatment, as well as other high risk immunosuppressive and cytotoxic agents such as TNF-α inhibitors and other biologic agents,[160] awareness of risk of HBVr and the importance of HBV screening is necessary among a wide variety of medical specialties. Therefore, both international and national guidelines should be revised and targeted education sessions might improve screening rates, as demonstrated in a study by Dyson *et al*.[161] In addition, screening rates might also be improved using Information Technology (IT) tools. A computer-assisted reminder system alerting physicians of HBV screening might improve screening rates.[162] Another IT system initiated in a Japanese hospital automatically provided information on HBV screening and status of the patient to the attending physician when prescribing rituximab. Implementation of this system was 100% effective, as all patients were treated according to the hospital's hepatitis B guidelines.[163] An Electronic Medical Record (EMR) template in which a result of a recent HBV test is required prior to rituximab prescription could be another solution.

In conclusion, many patients treated with rituximab were not adequately screened for the presence of an HBV infection. Although screening rates improved significantly over the years they remain suboptimal. Lack of adequate screening and antiviral prophylaxis impacted patient outcomes and resulted in HBVr in a couple of patients (including one fatal liver decompensation). Our findings could be used to raise awareness among all medical specialties. Reinforcement of current guidelines, ongoing education, and implementation of electronic systems could play a pivotal role in optimising screening rates and subsequent management of patients with hepatitis B treated with rituximab.



CHAPTER 9

Epidemiology and management of hepatitis B and C in primary care in the Netherlands – data from the Rijnmond Primary Care database

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ABSTRACT

Background & Aim(s): The Dutch guideline for general practitioners (GPs) advises biannual surveillance of hepatitis B (HBV) patients and referral of every hepatitis C (HCV) patient. We aimed to study the prevalence, incidence, and the management of hepatitis B and C in primary care.

Methods: This is a retrospective cohort study using the Rijnmond Primary Care database (RPCD), including health care data of medical records of GPs of approximately 200,000 patients in the area of Rotterdam, the Netherlands. Patient records were selected based on laboratory results, International Classification of Primary Care (ICPC) codes, and free-text words.

Results: In total, 977 patients were included: 717 HBV, 252 HCV, and 8 HBV/HCV coinfected patients. Between 2013 and 2019, the prevalence of HBV and HCV declined from 5.21 to 2.99/1,000 person-years (PYs) and 1.50 to 0.70/1,000 PYs, respectively. We observed that the majority of the patients had been referred to a medical specialist at least once (71% HBV and 89% HCV patients). However, among chronic patients, we observed that 36.2% of the HBV patients did not receive adequate surveillance by their GP (≥ 2 alanine aminotransferase checks within 3 years) or a medical specialist. In addition, 44.4% of the HCV patients had no record about successful antiviral treatment.

Conclusion: This study demonstrated a declining prevalence in viral hepatitis B and C in primary care in the Netherlands. However, a substantial part of the patients did not receive adequate surveillance or antiviral therapy. It is therefore crucial to involve GPs in case finding and in follow-up after treatment.

INTRODUCTION

Chronic infection with viral hepatitis B or C is a global health threat, as it is associated with the development of liver cirrhosis and primary liver cancer (hepatocellular carcinoma). [1,2] Around the world, the prevalence of an infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is estimated at, respectively, 296 million and 58 million.[1,2] In the Netherlands, the prevalence is estimated at 0.3% (40,000 individuals) for HBV and 0.2% (28,000 individuals) for HCV in 2016.[5,164]

During the last years, the treatment of viral hepatitis B and C has improved significantly. Suppression of the hepatitis B virus can be achieved with nucleos(t)ide analoges (NAs)[60,165] and eradication of the hepatitis C virus with direct-acting antiviral agents (DAAs).[166-169] Viral suppression or eradication halts further progression of the liver disease and improves life expectancy.[32,33] Therefore, the World Health Organization (WHO) has adopted a Global Health Sector Strategy on viral hepatitis in 2016, aimed to eliminate viral hepatitis B and C as a public health threat by 2030.[3] In the Netherlands, these targets have been implemented in a National Hepatitis Plan.[48] Despite these effective treatment options and harm reduction strategies in the last decade(s), the annual mortality does not change and approximately 500 individuals die in the Netherland yearly.[164] In Europe, the incidence and mortality rates differs considerably between different countries. The highest prevalence is observed in countries in eastern and southern Europe, especially among high-risk groups such as people who inject drugs (PWID) and men who have sex with men (MSM).[6] In 2015, the annual mortality was 1.3 per 100,000 in Europe. The highest mortality rates were observed in Italy, Germany, and Spain (accounted for two-thirds of all chronic hepatitis related deaths in Europe).[170] Thus, adequate surveillance and treatment of patients with viral hepatitis is important.

General practitioners (GPs) play a key role in case detection and management of viral hepatitis. Indication for HCV and HBV screening include migrants originating from high endemic countries, PWID and MSM, as well as patients with elevated liver enzymes. Patients with an active infection should be referred to a medical specialist to evaluate the presence of liver related complications and initiate antiviral therapy when indicated. However, several studies demonstrated that many patients who are at risk for viral hepatitis infection are not screened accordingly, resulting in many undiagnosed.[171,172]

This could be explained by the fact that most clinical practice guidelines in European countries lack information about the management of patients with viral hepatitis in primary care. In a semi-quantitative study, a few GPs in Germany, Spain, and Italy have indicated to be involved in monitoring of serum liver enzymes, and refer hepatitis B patients based on clinical indicators (such as hepatitis B e antigen [HBeAg], alanine aminotransferase [ALT], HBV DNA, and comorbidities).[173,174]

However, this study also highlighted non-uniform practices in screening and monitoring of patients with viral hepatitis.[174] In 2016, the Dutch guideline for viral hepatitis of the Dutch College of General Practitioners has been updated.[54] Whereas the outdated guideline recommended HBV surveillance for at least 3 years, which could be ceased if no sign of hepatitis (ALT elevation) or HBeAg levels were negative, the updated guideline recommends lifelong surveillance (including ALT measurement every 6 months and hepatitis B surface antigen [HBsAg] measurement every 3 years) and referral of patients with an active viral hepatitis B or C to an hepatitis treatment centre.[54] However, the compliance with the Dutch guideline, as well as the prevalence of viral hepatitis B and C in primary care in the Netherlands, is unknown.

In this study, we therefore aim to provide insight in the prevalence of viral hepatitis B and C in a multi-ethnic area in the Netherlands, as well as in the management of hepatitis B and C patients in primary care.

PATIENTS AND METHODS

Study design

This is a retrospective cohort study using the Rijnmond Primary Care database (RPCD). The RPCD is a region specific product of the Integrated Primary Care Information (IPCI) database, supervised by the department of General Practice of the Erasmus MC, University Medical Center, Rotterdam, the Netherlands. More information about the IPCI database has been prescribed in detail elsewhere.[175] This is a longitudinal observational dynamic database containing medical records of over 200,000 patients from the area of Rotterdam, the Netherlands. These pseudonymised medical records contain demographics, medical notes (free text), diagnoses (including International Classification of Primary Care [ICPC] codes), laboratory results, and drug prescriptions that are routinely collected by GPs. The database included approximately 25% of the population of the area of Rotterdam, equally distributed across the region and including neighbourhoods with different socioeconomic and migration levels. Rotterdam is a dense urban, multi-ethnic area; 52% of the residents have a non-Dutch background and the nearest GP practice has an average distance of 0.6 km (0.37 miles).[176,177] The study period started on 2013 Dec 1 and ended on 2019 Dec 31.

Study population

Patient records were selected based on laboratory results, ICPC codes and/or key words for hepatitis B and C. The ICPC classification is managed by the Dutch College of GPs and adopted by all Dutch GPs.[178] The database covers laboratory results ordered by the GP and is not linked to hospital records. For hepatitis B,

laboratory results included a positive result of HBsAg, ICPC codes D72.02 (acute hepatitis B) or D72.04 (chronic hepatitis B). For hepatitis C, laboratory results included a positive result of HCV antibodies (anti-HCV), ICPC codes D72.03 (acute hepatitis C) or D72.05 (chronic hepatitis C). Patients were excluded if they were identified by an HBV ICPC code, but were also (i) vaccinated for HBV (ATC code J07BC01 or J07BC20), (ii) had a negative HBsAg results within 24 weeks after the ICPC code registration date, or (iii) based on free-text words (including words for prior hepatitis B, vaccination HBV).

After selecting the patients that met these inclusion criteria, the medical charts were reviewed. Cases were labelled as certain cases or uncertain cases (for example if a patient was identified by an ICPC code, but without any additional information in the medical file regarding medical notes and/or laboratory results). All cases were manually categorised as viral hepatitis B (stratified as acute hepatitis B, chronic hepatitis B, and hepatitis B reactivation), hepatitis C (stratified as acute hepatitis C and chronic hepatitis C) and chronic HBV/HCV coinfection. Acute hepatitis B/C was defined as HBsAg positivity for hepatitis B and HCV RNA positivity (or anti-HCV in case of absent HCV RNA testing) for hepatitis C, typically with concomitant jaundice and/or elevation of serum liver enzymes, that occurred within 6 months after viral exposure. Chronic hepatitis B/C was defined as serum HBsAg/HCV RNA positivity (or anti-HCV in case of absent HCV RNA testing) of at least 6 months. HBV reactivation was defined as HBsAg positivity and elevated HBV DNA, in previously HBsAg negative patients but anti-HBc positive patients who underwent high-risk-immunosuppressive treatment. If the medical diagnosis was clearly formulated in a GP note or letter from a hepatitis specialist, this diagnosis was adopted as well. Date of first diagnosis was extracted from the RPCD, but manually altered during chart review if it was evident that the date of diagnosis was different.

End points

First, we studied the prevalence and incidence of viral hepatitis B and C in the study period. Follow-up ended when a patient transferred out of the GP practice, died, or when the end of the study period was reached, whichever occurred first. In addition, follow-up ended as well when a patient cleared the virus spontaneously or after antiviral treatment, i.e. HCV RNA or HBsAg negativity in previous HCV RNA or HBsAg positive patients.

Next, the management of viral hepatitis B and C patients was studied. For chronic hepatitis B patients, management was categorised as surveillance by medical specialist (at least one letter or note of medical specialist about viral hepatitis B), surveillance by GP (at least 2 ALT checks within 3 years), and no surveillance. The management was only determined in a sub-cohort of certain cases with a chronic infection. For hepatitis C, the number of referrals and antiviral treatment was studied.

Prescriptions for antivirals were extracted using the ATC codes: J05AP (antivirals for treatment of HCV infections; direct-acting antiviral agents), J05AF (nucleoside and nucleotide reverse-transcriptase inhibitors), and L03AB (interferons). Subsequent curation rate (HBV suppression with or without antivirals, HBsAg loss among patients with hepatitis B, and sustained virological response [SVR] or spontaneous clearance for hepatitis C patients), with the corresponding date, was recorded.

Statistical analysis

Descriptive data were described as numbers (with percentages), medians (IQR), and means (± SD). Analyses were performed in the overall included study population, as well as a subpopulation of certain cases. Incidence was calculated by dividing the number of incident cases by the midterm population at risk (person-years [PYs] at risk within the study population on July first).[179] Prevalence was calculated as year-prevalence proportion, using the number of patients with diagnosis of hepatitis B or C divided by the total number of PY. Both certain and uncertain patients were included for the prevalence/incidence calculation. IBM SPSS for Windows version 27.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Graph Pad Prism version 5 for Windows (GraphPad Software, San Diego, CA, USA) was used for graphical representation of the results.

RESULTS

Study population

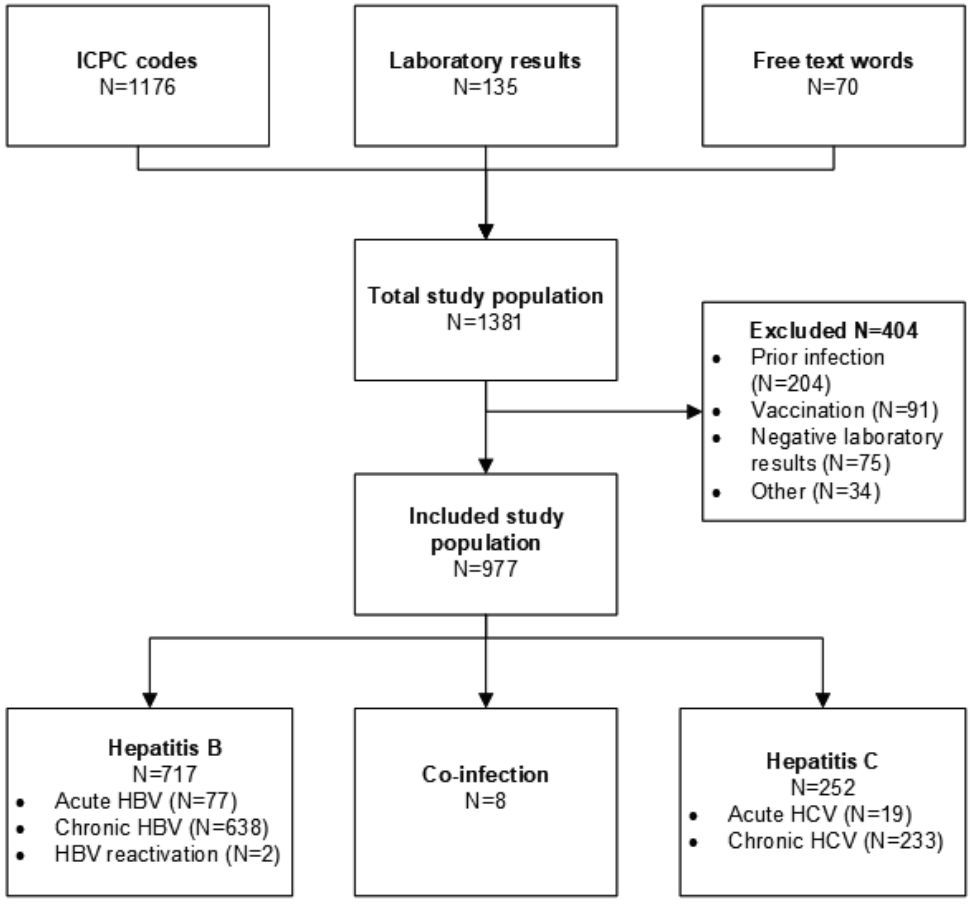
In total, 1,381 patients were identified by the initial search. After reviewing the medical records, 977 patients were included: 717 HBV, 252 HCV, and 8 HBV/HCV coinfection (Figure 1). Baseline patient characteristics are displayed in Table 1. The mean follow-up period was 55 months (IQR 19-98; Supplementary Table 1). After manual validation of these 977 patients, 809 were classified as certain cases: 588 HBV, 214 HCV, and 7 HBV/HCV coinfection.

Table 1. Patient characteristics (all patients, n = 977)

	Hepatitis B n = 717	Hepatitis C n = 252	Co-infection n = 8
Sex (male; n, %)	384 (53.6)	170 (67.5)	7 (87.5)
Age at diagnosis (median, IQR)	37 (27-47)	47 (40-53)	36 (33-45)
Body Mass Index (kg/m^2; mean ±SD)	27.0 (±5.4)	26.6 (±5.4)	24.5 (±5.0)
Diagnosed by (n,%)			
Known infection	251 (35.0)	86 (34.1)	3 (37.5)
Primary care	367 (51.1)	113 (44.8)	4 (50.0)
Hospital	55 (7.7)	40 (15.9)	1 (12.5)
Obstetrics	24 (3.3)	1 (0.4)	0 (0)
Other	20 (2.8)	12 (4.8)	0 (0)
Liver related comorbidities (n,%)			
Compensated cirrhosis	17 (2.4)	27 (10.7)	0 (0)
Decompensated cirrhosis[α]	11 (1.5)	16 (6.3)	0 (0)
Liver transplantation	1 (0.1)	1 (0.4)	1 (12.5)
Hepatocellular carcinoma	14 (2.0)	4 (1.6)	1 (12.5)

[α] Decompensated cirrhosis was defined as ascites, oesophageal or fundus varices, jaundice and/or hepatic encephalopathy.

Figure 1. Flowchart of the included study population



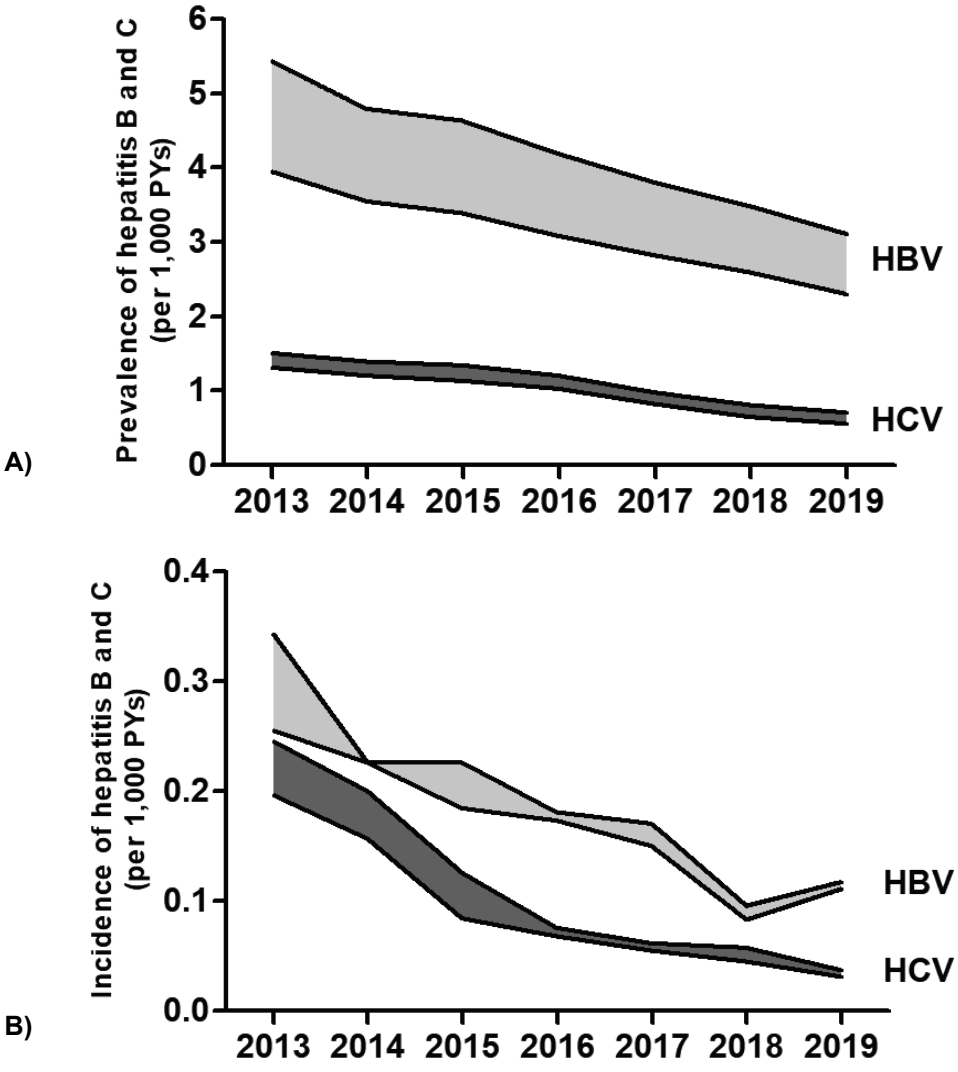
Abbreviations: ICPC, International Classification of Primary Care; HBV, hepatitis B virus; HCV, hepatitis C virus

Incidence and prevalence of hepatitis B and C

The prevalence of viral hepatitis B and C are displayed in Figure 2 and Supplementary Table 2. For HBV, the prevalence declined from 5.21 cases/1,000 PYs (4.16 certain cases/1,000 PYs) in 2013 to 2.99 cases/1,000 PYs (2.42 certain cases/1,000 PYs) in 2019 (–43%). For HCV, the prevalence declined from 1.50 cases/1,000 PYs (1.31 certain cases/1,000 PYs) in 2013 to 0.70 cases/1,000 PYs (0.55 certain cases/1,000 PYs) in 2019 (–53%).

The incidence rates are displayed in Figure 2B and Supplementary Table 3. In 2013, the incidence was 0.34/1,000 PYs for HBV and 0.25/1,000 PY for HCV. In 2019, the incidence was 0.12/1,000 PYs for HBV and 0.03/1,000 PY for HCV.

Fig. 2. Prevalence (A) and incidence (B) of hepatitis B and C (per 1,000 PYs)



The prevalence is presented as both certain (lower line of the shaded area) and certain + uncertain cases (upper line of the shaded area).
Abbreviations: PYs, person years; HBV, hepatitis B virus; HCV, hepatitis C virus

Management of viral hepatitis in primary care

Hepatitis B patients

Among the 588 certain hepatitis B cases, 406 patients chronic hepatitis B patients were studied to assess the management of viral hepatitis in primary care. Among those, 289 patients (71.2%) were referred to a medical specialist at least once (Table 2). However, when studying the actual management, medical specialist performed HBV surveillance in 185 patients (45.6%) and the GP in 59 patients (14.5%). In total, 147 patients (36.2%) received no surveillance of their hepatitis B infection.

Table 2. Management of patients with viral hepatitis in general practice

	Hepatitis B n = 406	**Hepatitis C n = 153**	**Co-infection n = 6**
Referral hepatitis centre (n, %)	289 (71.2)	136 (88.9)	6 (100.0)
Surveillance by GP‡ (n, %)		N/A	
Yes	59 (14.5)		0 (0)
No	147 (36.2)		3 (50.0)
N/A, surveillance specialist	185 (45.6)		3 (50.0)
N/A, cured infection	15 (3.7)		0 (0)
Ultrasound performed in patients without referral	33/112 (29.1)	2/16 (12.5)	-
Medication (n, %)			
Yes	130 (32.0)	113 (73.9)	4 (66.7)
No indication for antivirals	94 (23.2)	1 (0.7)	0 (0)
No, other	174 (42.9)	33 (21.6)	2 (33.3)
Unknown	8 (2.0)	6 (3.9)	0 (0)
Curation (n, %)			
Viral suppressionΣ	126 (31.0)	-	1 (16.7)
HBsAg loss	22 (5.4)	-	0 (0)
SVR	-	82 (53.6)	2 (33.3)
Spontaneous clearance	-	3 (2.0)	0 (0)
No/unknown	258 (63.5)	68 (44.4)	3 (50.0)

Among certain chronic hepatitis B or C patients with at least 2 years of follow-up.

‡ *Surveillance was categorised as surveillance by the GP (at least 2 ALT checks within 3 years), no surveillance by the GP or medical specialist, surveillance by medical specialist (at least 1 letter or note of medical specialist about viral hepatitis B) or absent surveillance due to a cured infection.*

Σ *Viral suppression with or without antivirals*

Abbreviations: GP, general practitioner; HBsAg, hepatitis B surface antigen; SVR, sustained virological response;

To gain insight in the adherence of the updated GP guideline, we extracted a sub-cohort of patients, including certain cases of chronic hepatitis B patients who had at least 2 years of follow-up, including the period after March 2016 (when the GP guideline update was published), and who did not had HBV surveillance by medical specialist at that moment. In total, this sub-cohort included 226 patients of whom 148 patients (65.5%) received ALT surveillance at least once. Among these 148 patients, the mean number of ALT tests was 2.3 per patient (range 1–8) in 4 years. In addition, ALT levels were elevated (> 35/45 U/mL for female/male) at least once in 34/148 patients (23.0%; range 36–275 U/mL). Consequently, after reviewing the medical records of those 34 patients with elevated ALT levels, 20 patients (58.8%) were referred to a medical specialist. Thus, no consequence was given to abnormal liver test in 14/34 patients (41.2%; mean 60 U/mL, range ALT 38–120 U/mL). Among those 14 patients, ALT levels were repeatedly increased in 8 patients (57.1%). HBsAg was only tested among 11 patients (4.9%).

Hepatitis C patients

Among the 153 certain hepatitis C cases who had at least 2 years of follow-up, 136 patients (88.9%) were referred to a specialised hepatitis treatment centre (Table 2). In total, 113 patients (73.9%) received antiviral treatment; of whom 82 patients (53.6%) achieved SVR and 3 patients (2.0%) had a spontaneous clearance of the virus. Thus, 68 patients (44.4%) might still have a chronic hepatitis C infection on 2019 Dec 31, besides the 17 patients without referral to a hepatitis treatment centre.

Tables 3 and 4 display the patient characteristics of, respectively, the 147 chronic hepatitis B patients and 68 chronic hepatitis C patients without adequate surveillance or successful treatment. Notably, among the hepatitis C patients, 54.5% had a registration of (prior) alcohol abuse ($p = 0.005$).

Table 3. Patient characteristics of chronic hepatitis B patients with inadequate management

	Hepatitis B Adequate management N = 259	Hepatitis B Inadequate management N = 147	p-value[§]
Sex (male; n, %)	147 (56.8)	68 (46.3)	0.042
Age at 31 December 2019 (mean ±SD)	50 (±14)	48 (±13)	0.153
Alcohol use[∞] (n, %)	121/136 (89.0)	57/62 (91.9)	0.521
Never/socially active	15/136 (11.0)	5/62 (8.1)	
Alcohol abuses (prior or active)			
Body Mass Index (kg/m^2)[¥] (mean ±SD)	26.6 (±5.4)	27.4 (±4.4)	0.364
Liver related comorbidities (n, %)			
Compensated cirrhosis	13 (5.0)	3 (2.0)	0.138
Decompensated cirrhosis[α]	4 (1.5)	1 (0.7)	0.448
Liver transplantation	0 (0)	0 (0)	0.451
Hepatocellular carcinoma	7 (2.7)	3 (2.0)	0.679

[∞]alcohol use was stratified as none (defined as zero units/day or key words such as "alcohol -") or social use (defined as < 5 units/day, or text words that indicated non-excessive alcohol use), and alcohol abuses (defined as ≥ 5 units/day or ICPC code P15 – Chronic alcohol abuses).

[¥] Body mass index measurements were available among 120 patients with adequate management and 58 patients with inadequate management.

[α] Decompensated cirrhosis was defined as ascites, oesophageal or fundus varices, jaundice, and/or hepatic encephalopathy.

[§] Chi-square test.

Table 4. Patient characteristics of chronic hepatitis C patients with inadequate management

	Hepatitis C Not cured n = 68	Hepatitis C Cured n = 85	p-value[§]
Sex (male; n, %)	51 (75.0)	54 (63.5)	0.129
Age at 31 December 2019 (mean ±SD)	57 (±11)	57 (±9)	0.881
Alcohol use[∞] (n, %)	20/44 (45.5)	45/62 (72.6)	0.005
Never/socially active	24/44 (54.5)	17/62 (27.4)	
Alcohol abuses (prior or active)			
Body Mass Index (kg/m^2)[¥] (mean ±SD)	25.1 (±5.1)	26.6 (±4.8)	0.196
Liver related comorbidities (n, %)			
Compensated cirrhosis	9 (13.2)	12 (14.1)	0.875
Decompensated cirrhosis[α]	3 (4.4)	5 (5.9)	0.685
Liver transplantation	1 (1.5)	0 (0)	0.262
Hepatocellular carcinoma	2 (2.9)	1 (1.2)	0.434

[∞] alcohol use was stratified as none (defined as zero units/day or key words such as "alcohol -") or social use (defined as < 5 units/day, or text words that indicated non-excessive alcohol use), and alcohol abuses (defined as ≥ 5 units/day or ICPC code P15 – Chronic alcohol abuses).

[¥] Body mass index measurements were available among 54 patients with adequate management and 27 patients with inadequate management.

[α] Decompensated cirrhosis was defined as ascites, oesophageal or fundus varices, jaundice, and/or hepatic encephalopathy.

[§] Chi-square test.

DISCUSSION

The Netherlands is a low-endemic country for viral hepatitis B and C.[4,5] However, due to migration, low-endemic countries have local regions with high hepatitis endemicity, such as large cities as Rotterdam. Therefore, more insight in the prevalence and surveillance of viral hepatitis B and C in these areas is important. Using a large longitudinal observational dynamic database containing medical records of GPs in the area of Rotterdam, this study showed a decreasing prevalence of viral hepatitis B and C in primary care between 2013 and 2019. In addition, we demonstrated that the majority of patients with viral hepatitis B and C are referred at least once to a medical specialist. However, we found that a substantial part of the patients did not receive adequate surveillance or curative treatment.

In 2016, the WHO has implemented a global viral hepatitis elimination target.[3] In this report, elimination was defined as a 90% reduction in new infections (95% for HBV and 80% for HCV) and 65% reduction in mortality by 2030, compared with incidence and mortality numbers of 2015. The interim targets for 2020 however, included a 30% reduction in incidence of viral hepatitis B and C in primary care. Our data showed a reduction of HBV incidence of 48% and a reduction of HCV incidence of 77% in 2019. This means that the Netherlands seems on track to reach the incidence target of the hepatitis elimination goal, in contrast to the results of a recent report.[180] A decline in incidence has also been observed among other low-endemic countries such as United Kingdom and Iceland, possibly due to increased antiviral treatments using nationwide retrieval of lost to follow-up patients, and people who are imprisoned or inject(ed) drugs.[181-183] However, the observed decline in incidence might also be caused by a potential decrease in diagnostic test for viral hepatitis in primary care, due to the barriers that GPs experience in case finding such as limited knowledge about viral hepatitis and subsequent risk groups or less attention for follow-up of abnormal liver tests.[171,184] The increase of the number of GP practices in RPCD that originate from low-endemic areas of Rotterdam might also (partly) explain the decline in prevalence.

In the Netherlands, risk groups account for most cases of hepatitis B and C, including (first-generation) migrants, PWID, and men who have sex with men (MSM).[185-189] A possible explanation for the observed decline in prevalence could be the improved treatment options, especially for hepatitis C which can now eradicated with an 8- to 12-week cure with DAAs.[35] However, an absent steep decline in prevalence after introduction of DAAs in 2015 among HCV patients indicates that other factors are also responsible for the decline in HCV prevalence, such as the improved harm reduction strategies for PWID and MSM, HBV surveillance among pregnant women and vaccination among children born from HBV-infected women.

In addition, we demonstrated that the referral rate to a hepatitis specialist was 71–89%. However, our data showed that 36.2% of the hepatitis B patients was not under surveillance by a hepatitis specialist or GP. Furthermore, we observed that many patients received ALT performance at least once, but an annual ALT check was only performed in the minority of patients. This is in line with the study results of Hofman *et al*.[190] In this study, the researchers observed a low performance of annual ALT monitoring among chronic hepatitis B patients in the period 2008–2015. This implies that the updated Dutch GP guideline has not been implemented sufficiently in daily practice. Moreover, we observed that 44.4% of the hepatitis C patients has not been successfully treated (or the GP has not been updated by the hepatitis specialist about viral eradication). In the Netherlands, the nationwide project CELINE has been initiated to retrieve ever diagnosed HCV patients who are not treated because they

have become lost to follow-up (LTFU).[125] Our data suggest that GPs should initiate retrieval of their LTFU patients.

A possible explanation for the suboptimal surveillance of hepatitis B patients by the GP might be the lack of an appointment scheduling system. Consequently, the responsibility for the biannual ALT check lies with the patient instead of the GP. Another explanation could be limited knowledge about the updated guideline or viral hepatitis in general. This could be the consequence of the small number of viral hepatitis patients in every GP practice due to the low prevalence of viral hepatitis in the Netherlands.[5] This has been confirmed in a recent Dutch qualitative study among GPs about case finding of hepatitis B and C patients showed that many GPs indicated that they have limited knowledge about the (updated) GP guideline, lack of time during a consult to address hepatitis screening and an insufficient registration system.[184] Although this study reported barriers for case finding, we believe this barriers (and possible interventions) are also applicable for the suboptimal management for viral hepatitis. However, this warrants confirmation in another (quantitative) study.

Multiple interventions are needed to improve hepatitis B surveillance and retrieval of untreated HCV patients in primary care. First, an appointment scheduling system is warranted to invite hepatitis B patients biannually for their laboratory check. Second, IT changes, such as pop-up messages, could facilitate GPs to perform adequate HBV surveillance, referral of untreated HCV patients or screening among high-risk individuals. Hence, a registration system is crucial, including information about medical diagnosis, laboratory results, and background information such as country of birth. Third, GPs should be encouraged to participate in already available training courses for viral hepatitis. Fourth, a standard set of serum hepatitis markers and liver tests on laboratory forms could facilitate screening, which has been supported by the study of Helsper *et al.*[191] Finally, as mentioned above, retrieval of LTFU HCV and HBV patients can be worthwhile and should therefore be performed in every GP practice.

Despite that this is a large GP-based database, the following limitations need mentioning. Since migrants account for the majority of prevalent HBV and HCV cases, our results cannot be not directly translated to other (low-endemic) areas of the Netherlands, as our results are based on a multi-ethnic area in the Netherlands. Another limitation that should be acknowledged is the retrospective design and the fact that our results depend on the available data within the GP database, which is subjected to the input of individual GPs. Therefore, management and laboratory results of medical specialist are only available if the specialist sends communication to the GP. This could give an underestimation of the real number of patients that receive adequate HBV screening in the hospital or successfully treated hepatitis C patients. Since our data indicated that a few patients with liver cirrhosis or liver

transplantation would not receive surveillance by a hepatitis specialist supports the suggestion that our data is limited by the retrospective design. Finally, due to privacy restrictions, if a patient changes GP within the network of affiliated GP practices of the RPCD, the patient enters the database with a new patient number. This could have resulted in duplicate cases. However, in the Netherlands, very few patients change of GP over time, when they do it is because of moving to a different region. Thus, the impact of duplicate cases to our findings is limited. In addition, for our calculations of the incidence and prevalence, we took the medical history into account. Therefore, a change of GP will not influence the incidence and prevalence rates.

In conclusion, we observe that the prevalence of viral hepatitis B and C is declining in a multi-ethnic area of the Netherlands. This implies that the Netherlands seems on track to achieve the WHO elimination target. However, many patients with hepatitis B and C might not receive adequate surveillance or antiviral therapy. It is therefore crucial to involve primary care in the road to complete elimination.

SUPPLEMENTARY TABLES

Supplementary Table 1. Follow-up

	Overall	Hepatitis B n = 717	Hepatitis C n = 252	Co-infection n = 8
Mean follow-up period (months)	55 (19-98)	57 (19-103)	54 (21-96)	54 (35-101)
No available time	75 (7.7%)	55 (7.7%)	19 (7.5%)	1 (12.5%)
< 1 year	111 (11.4%)	85 (11.9%)	26 (10.3%)	-
1-2 years	96 (9.8%)	70 (9.8%)	26 (10.3%)	-
> 2 years	695 (71.1%)	507 (70.7%)	181 (71.8%)	7 (87.5%)

Supplementary Table 2. Prevalence of viral hepatitis B and C

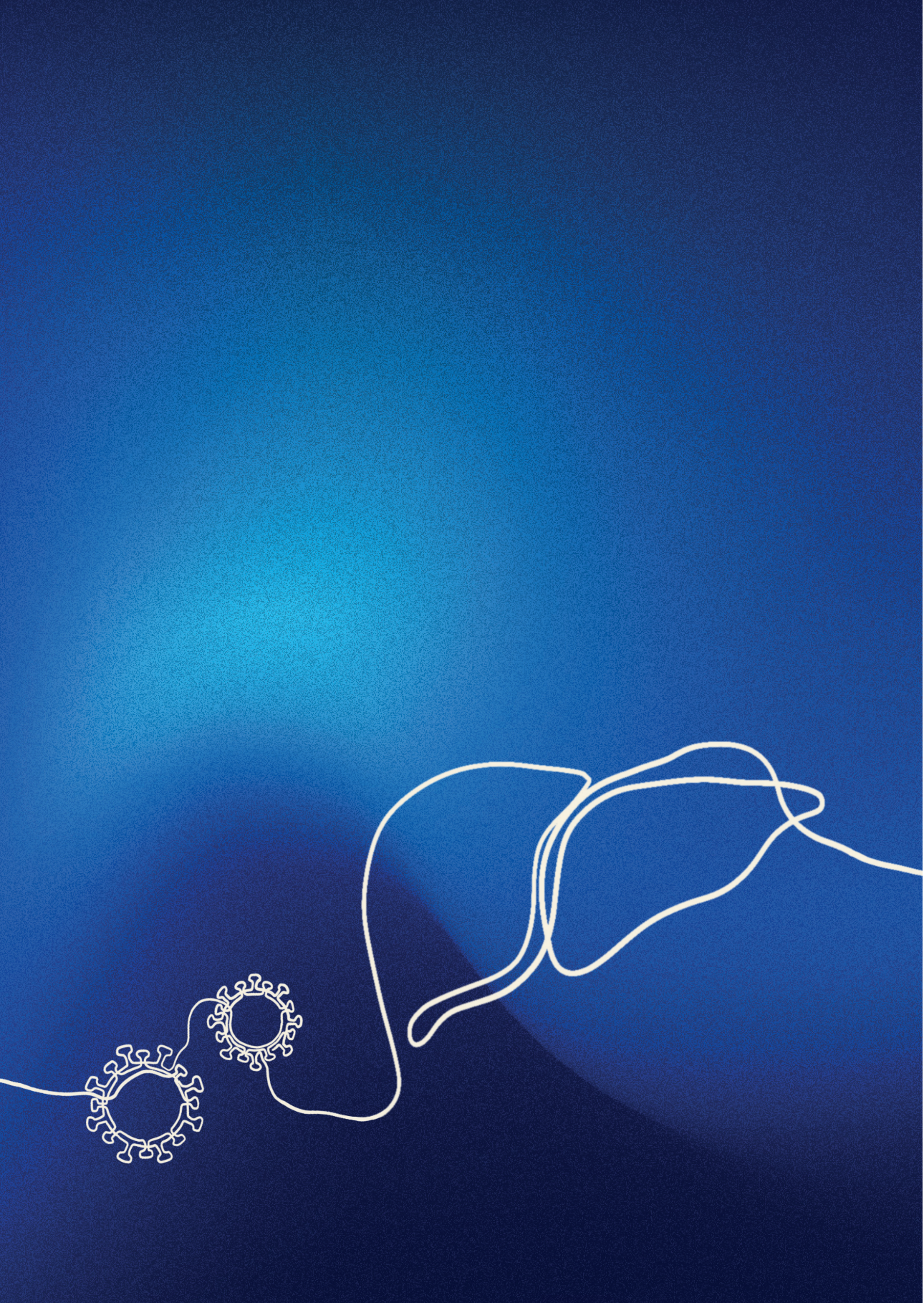
	Total PYs	Number of HBV cases	Number of HCV cases	HBV/1,000 PYs	HCV/1,000 PY
2013	101,860.04	424 - 531	133 - 153	4.16 – 5.21	1.31 – 1.50
2014	115,120.43	429 - 530	138 - 160	3.72 – 4.60	1.20 – 1.39
2015	119,483.61	426 - 531	135 - 160	3.57 – 4.44	1.13 – 1.34
2016	133,048.47	431 - 535	136 - 159	3.24 – 4.02	1.02 – 1.20
2017	146,935.38	435 - 537	120 - 143	2.96 – 3.65	0.82 – 0.97
2018	157,176.59	427 - 526	101 - 126	2.72 – 3.35	0.64 – 0.80
2019	162,214.48	392 - 485	90 - 114	2.42 – 2.99	0.55 – 0.70

Range of number of HBV/HCV cases, with corresponding prevalence per 1,000 PYs, was displayed as total certain cases – certain plus uncertain cases.
Abbreviations: PYs, person years; HBV, hepatitis B virus; HCV, hepatitis C virus

Supplementary Table 3. Incidence of viral hepatitis B and C

	Total PYs	Number of HBV cases	Number of HCV cases	HBV/1,000 PYs	HCV/1,000 PY
2013	101,860.04	26 – 35	20 – 25	0.26 – 0.34	0.20 – 0.25
2014	115,120.43	26 – 26	18 – 23	0.23 – 0.23	0.16 – 0.20
2015	119,483.61	22 – 27	10 – 15	0.18 – 0.23	0.08 – 0.13
2016	133,048.47	23 – 24	9 – 10	0.17 – 0.18	0.07 – 0.08
2017	146,935.38	22 – 25	8 – 9	0.15 – 0.17	0.05 – 0.06
2018	157,176.59	13 – 15	7 – 9	0.08 – 0.10	0.04 – 0.06
2019	162,214.48	18 – 19	5 – 6	0.11 – 0.12	0.04 – 0.03

Range of number of HBV/HCV cases, with corresponding prevalence per 1,000 PYs, displayed as total certain cases – certain plus uncertain cases.
Abbreviations: PYs, person years; HBV, hepatitis B virus; HCV, hepatitis C virus



CHAPTER 10

Assessment of adherence to clinical guidelines in patients with chronic hepatitis B

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ABSTRACT

Background & Aim(s): Adherence to guidelines is associated with improved long-term outcomes in patients with chronic hepatitis B (CHB). We aimed to study the degree of adherence and determinants of non-adherence to management guidelines in a low endemic country.

Methods: We reviewed the medical records of all CHB patients who visited our outpatient clinic in 2020. Adherence to guidelines was assessed based on predefined criteria based on the EASL guidance, and included initiation of antiviral therapy when indicated, optimal choice of antiviral therapy based on comorbidities, assessment of HAV/HCV/HDV/HIV serostatus, renal function monitoring and enrolment in HCC surveillance program if indicated. Adherence rates were compared across types of outpatient clinic (dedicated viral hepatitis clinic versus general hepatology clinic).

Results: We enrolled 482 patients. Among the 276 patients with an indication for antiviral therapy, 268 (97.1%) received treatment. Among patients with renal and/or bone disease, 26/29 patients (89.7%) received the optimal choice of antiviral agent. Assessment of HAV/HCV/HDV/HIV serostatus was performed in 86.1/91.7/94.4/78.4%. Among the 91 patients treated with tenofovir disoproxil, 57 (62.6%) underwent monitoring of renal function. Of 241 patients with an indication for HCC surveillance, 212 (88.3%) were enrolled in a surveillance program. Clinics dedicated to viral hepatitis had superior adherence rates compared to general hepatology clinics (complete adherence rates 63.6% versus 37.2%, $p < 0.001$).

Conclusion: Follow-up at a dedicated viral hepatitis clinic was associated with superior adherence to management guidelines.

INTRODUCTION

Chronic hepatitis B (CHB) is a global healthcare problem which currently affects approximately 248 million persons worldwide.[1,18] The prevalence of CHB varies widely across countries. In Western nations the prevalence of CHB is generally low (< 2%) whereas the prevalence may be up to 5% in North-Africa, 5-8% in parts of Asia and is estimated to be over 8% in sub-Saharan Africa.[192] In the Netherlands, the prevalence has been estimated at 0.34%.[5] Various studies have hinted that the uptake of recent recommendations with regard to optimal choice of antiviral therapy and hepatocellular carcinoma (HCC) surveillance is suboptimal, leading to worse outcomes for patients.[193-196] This may be particularly relevant in countries with a low hepatitis B virus (HBV) prevalence, as expertise may vary across physicians. We therefore sought to investigate the degree of adherence to management guidelines in a low endemic country.

PATIENTS AND METHODS

Study design and study population

This study is part of an Initiative to Improve CARe for cUrrent and future patientS with chronic hepatitis B in the Netherlands (ICARUS). For this study, all consecutive patients with HBV mono-infection (defined as HBsAg positivity for at least six months) who visited the outpatient clinic of the department of Gastroenterology and Hepatology of the Erasmus MC in 2020 were enrolled. The Erasmus MC, University Medical Center, is a large tertiary referral hospital located in the centre of Rotterdam, the second largest city in the Netherlands.

Patients with CHB are seen at either one of the dedicated viral hepatitis outpatient clinics or at one of several general hepatology outpatient clinics. Both the viral hepatitis and general hepatology clinics were overseen by experienced Hepatologists. General hepatitis clinics care for patients with a variety of liver diseases, with viral hepatitis accounting for a minority of the patient population, whereas the viral hepatitis clinics cater exclusively to patients with viral hepatitis. Patients with viral hepatitis are preferably allocated to one of the viral hepatitis clinics. However, due to lack of capacity and/or patient preference some patients cannot attend one of the viral hepatitis clinics and are therefore managed at general hepatology clinics. Allocation is therefore unrelated to severity of liver disease or phase of HBV infection.

Data collection

For all eligible patients, patient charts were reviewed and data was obtained regarding patient demographics, virology, stage of liver disease and relevant comorbidities. Diagnosis of cirrhosis was based on histology, ultrasound findings

compatible with cirrhosis in the presence of signs of portal hypertension or a liver stiffness of ≥ 12.5 kPa (based on Fibroscan®, Paris France).

Definition of standard of care

Standard of care was based upon the recommendations set forth in the European Association for the Study of the Liver (EASL) guideline.[18] Adherence to the guideline was assessed through ascertainment of compliance with several predefined criteria covering the whole spectrum of CHB care, including (1) treatment indications, (2) optimal choice of therapy in the presence of renal and/or bone disease, (3) assessment HAV, HCV, HDV and HIV serostatus, (4) monitoring of renal function during high-risk treatment and (5) enrolment in HCC surveillance programs if indicated.

Patients meeting any of the following criteria were considered to be in need of antiviral therapy with nucleo(s)tide analogues: presence of cirrhosis with detectable HBV DNA levels, ≥ F2 liver fibrosis with HBV DNA > 2,000 IU/mL and ALT > the upper limit of normal (ULN), HBV DNA > 20,000 IU/ml and ALT > 2x ULN, or serum HBV DNA > 10^7 IU/ml (irrespective of fibrosis stage), positive family history for cirrhosis or HCC, presence of extra-hepatic symptoms, patients starting on high-risk immunosuppressive agents, and HBV DNA > 200,000 UI/mL in pregnant women.

Optimal choice of antiviral therapy in patients with renal and/or bone disease was defined as use of tenofovir alafenamide (TAF) or entecavir (ETV) among patients with an indication for antiviral therapy.

Presence of (protection against) co-infections were studied by assessment of the presence of IgG anti-HCV, IgG anti-HIV, IgG anti-HAV and IgG anti-HDV at least once prior to patient's visit to the outpatient clinic in 2020.

Adequate monitoring of renal function was defined as regular (at least yearly) assessment of serum creatinine levels and serum phosphate levels in patients treated with tenofovir disoproxil (TDF).

Indications for HCC surveillance comprised either a positive family history of HCC, presence of liver cirrhosis, ethnicity (Sub-Saharan patients aged ≥ 20 years, Asian male patients aged ≥ 40 years and Asian female patients aged ≥ 50 years). Adequate HCC surveillance was defined conservatively as the presence of at least three imaging studies in the previous two years.

Outcomes and statistical analysis

The primary outcome of this study was the proportion of patients not managed according to guidelines as assessed using the individual indicators described above. We also calculated composite measure of complete adherence, which was based

on adherence to all individual components. Adherence rates were assessed in the overall population, and stratified according to type of outpatient clinic (dedicated viral hepatitis clinic versus general hepatology clinic).

Descriptive data were described as numbers (with percentages), medians (with interquartile range; IQR) and means (± standard deviation; SD). The association between type of outpatient clinic and adherence was explored using chi square test. Differences were considered statistically significant when $p < 0.05$. For statistical data analysis, IBM SPSS for Windows version 25.0 (SPSS Inc., Chicago, Illinois, USA), was used.

Ethical considerations

The study was conducted in accordance with the guidelines of the declaration of Helsinki and the principles of Good Clinical Practice. The study was also reviewed by the Institutional Review Board of the Erasmus MC (MEC-2020-0823).

RESULTS

Patient characteristics

We enrolled 482 patients. The patient characteristics are shown in Table 1. The mean age was 49 years (± 14) and 54.4% were male. The most common ethnicities were Asian (38.2%) and North-African/Middle Eastern (26.1%; Table 1). Liver cirrhosis was present in 12.0% of the patients.

Table 1. Patient characteristics

	n = 482
Age (years; mean, ±SD)	49 (±14)
Sex (male; n, %)	262 (54.4)
Ethnicity (n, %)	
Caucasian, white	78 (16.2)
Asian	184 (38.2)
Black, Sub-Saharan	66 (13.7)
Black, non-Sub-Saharan	24 (5.0)
North-African or Middle Eastern countries	126 (26.1)
Hispanic	4 (0.8)
Comorbidities (n, %)	
Osteoporosis	5 (1.0)
Renal dysfunction[α]	32/395 (8.1)
Liver stiffness (kPa; median, IQR)	6.1 (4.7-8.3)
Liver cirrhosis[β] (n, %)	58 (12.0)

[α] Renal dysfunction was defined as an eGFR under 60 ml/min/1.73 m2.
[β] Diagnosis of cirrhosis was based on histology, ultrasound findings compatible with cirrhosis in the presence of signs of portal hypertension or a liver stiffness measurement of ≥ 12.5 kPa.

Adherence rates in the overall population

Overall, complete adherence to all assessed components was observed in 254 (52.7%).

In total, 276 (57.3%) patients had an indication for antiviral therapy. The most common indication was HBV DNA > 20,000 IU/ml and ALT > 2x ULN (116/276, 42.0%). Among patients with indication for antiviral therapy, 268 (97.1%) patients received antiviral therapy (Table 2).

Among the 29 patients with renal and/or bone disease, 26 (89.7%) were treated with TAF or ETV (Table 2).

IgG anti-HAV, anti-HCV, anti-HDV were assessed in the majority of patients (> 86%), whereas anti-HIV was assessed in 378 (78.4%; Table 2).

In total, 91 patients were treated with TDF. None of the patients were treated with ADV in 2020. Creatinine and phosphate level measurements were regularly performed in 57/91 (62.6%) patients (Table 2).

In total, 241 (50.0%) had at least one HCC risk factor and had therefore an indication for HCC surveillance. Among these, 212 (88.3%) received adequate surveillance (Table 2). Among the 58 patients with cirrhosis, 52 patients (89.7%) underwent adequate surveillance.

Table 2. Adherence of clinical guideline[18]

	Adequate adherence guideline	Inadequate adherence guideline	Total
Indication to start therapy (n, %)	**268 (97.1)**	**8 (2.9)**	**276**
- Cirrhosis with detectable HBV DNA levels (> 20 IU/mL)	56 (100)	0 (0)	56[β]
- HBV DNA > 2,000 IU/mL with ALT > ULN and at least F2 fibrosis	35 (97.2)	1 (2.8)	36
- HBV DNA >20,000 IU/ml with ALT > 2 x ULN	111 (95.7)	5 (4.3)	116
- HBV DNA > 10^7 IU/ml	24 (96.0)	1 (4.0)	25
- Positive family history for cirrhosis or HCC	15 (100)	0 (0)	15
- Presence of extra-hepatic symptoms	0 (0)	0 (0)	0
- Starting immunosuppressive agents	25 (96.2)	1 (3.8)	26
- HBV DNA > 200,000 UI/mL in pregnant women	2 (100.0)	0 (0)	2
Optimal choice of antiviral (n, %) Use of TAF or ETV in patients with renal or bone disease	26 (89.7)	3 (10.3)	29
Assessment of serostatus[Σ] (n, %)			482
- anti HAV (IgG)	415 (86.1)	67 (13.9)	
- anti-HCV (IgG)	442 (91.7)	40 (8.5)	
- anti HDV (IgG)	455 (94.4)	27 (5.6)	
- anti-HIV (IgG)	378 (78.4)	104 (21.6)	
Monitoring creatinine and phosphate levels among patients treated with TDF[¥] (n, %)	57 (62.6%)	34 (37.4)	91
HCC surveillance among patients with an indication[π] (n, %)	212 (88.3)	28 (11.7)	241

[β] Total cohort included 58 patients with liver cirrhosis. However, two patients were excluded in the analysis as the viral load was undetectable.

[α] One patient ceased antiviral therapy in 2020 in study context.

[Σ] Assessment of the presence of IgG anti HCV, IgG anti HIV, IgG anti HAV and IgG anti HDV at least once during follow-up

[¥] Adequate monitoring was defined as the quantification of serum creatinine and phosphate levels at least once a year among patients treated with ADV, TDF or TAF.

[π] Enrolment in an HCC surveillance program if indicated based on family history of HCC, presence of liver cirrhosis, ethnicity (Sub-Saharan patients aged ≥ 20 years, Asian male patients aged ≥ 40 years and Asian female patients aged ≥ 50 years). Adequate HCC surveillance was defined conservatively as at least three ultrasounds (or one MRI) performances in the previous two years.

Abbreviations: ALT, alanine aminotransferase; ETV, entecavir; HAV, hepatitis A virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis delta virus; HIV, human immunodeficiency virus; IU/mL, international units/millilitre; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil; ULN, upper limit of normal.

Dedicated viral hepatitis clinics have superior adherence rates

Among the 283 patients treated at a dedicated viral hepatitis clinic, complete adherence to all indicators was observed in 180 patients (63.6%), compared to 74/199 patients (37.2%) treated at a general hepatology clinic ($p < 0.001$). Findings were consistent across the individual indicators, such as renal function monitoring (68.9 vs 34.6%, $p < 0.001$), assessment of HAV (94.0 vs 74.9%, $p < 0.001$), HCV (94.0 vs 88.4%, $p = 0.030$), HDV (96.8 vs 91.0%, $p = 0.006$) and HIV (84.5 vs 69.8%, $p < 0.001$) serostatus. Adequate HCC surveillance rates were higher among dedicated viral hepatitis clinics (92.4 vs 82.3%, $p = 0.016$), particularly among patients without liver cirrhosis (94.1 vs 76.9%, $p < 0.001$). Adherence to guidelines for initiation of antiviral therapy was high regardless of clinic type ($p = 0.159$). Findings were consistent after adjustment for patient age, sex and ethnicity.

DISCUSSION

Adherence to guidelines is of paramount importance for improving patient care. In the current study conducted in a large tertiary care hospital in a low endemic country, the majority of patients received optimal care based on EASL guideline recommendations. However, a significant number of patients remained untested for viral co-infections, did not receive the optimal antiviral agent based on comorbidities and, perhaps most importantly, did not receive adequate HCC surveillance. A major risk factor for non-adherence to guideline recommendations was management at a non-viral hepatitis specialised liver clinic, suggesting that centralising care for CHB patients may be critical in optimising clinical management.

CHB is a complex disease with a heterogeneous natural history. Treatment indications are established on multiple factors based on biochemistry, virology and stage of liver disease, as well as patient factors including family history of liver-related complications. When deciding on initiating antiviral therapy, the optimal choice of antiviral agent is not just based on virological factors, but should also take into consideration the presence of comorbidities such as renal or bone diseases. During treatment, follow-up for treatment related complications is mandatory in some, but not all, agents. We observed that the majority of patients with a treatment indication received antiviral therapy, although ~3% of patients with obvious treatment indications remained untreated. Even though the specific reason for under-treatment are difficult to ascertain from our retrospective study (patient-related or physician-related), this is a missed opportunity as antiviral therapy may improve liver histology and reduce the risk of HCC and development of cirrhosis.[197]

Recent studies indicate that treatment with TDF may be a risk factor for impaired proximal tubular reabsorption known as the Fanconi syndrome.[198] Monitoring of renal function and serum phosphate levels is therefore advised in both guidelines

and Summary of Product Characteristics (SMPCs). However, we observed that 63% of the patients treated with TDF were not monitored for creatinine and phosphate levels, suggesting that this potentially devastating complication requires more attention.

In addition, we observed a suboptimal screening rate for co-infections, especially the measurement of anti-HIV. HIV-HBV co-infection accelerates the progression of liver related complications such as liver cirrhosis or HCC compared to patients with an HBV mono-infection. Moreover, among patients with an undiagnosed HIV co-infection, treatment with a single antiviral agent could induce drug resistance.[199] Therefore, the identification of patients with a co-infection is of clinical importance.

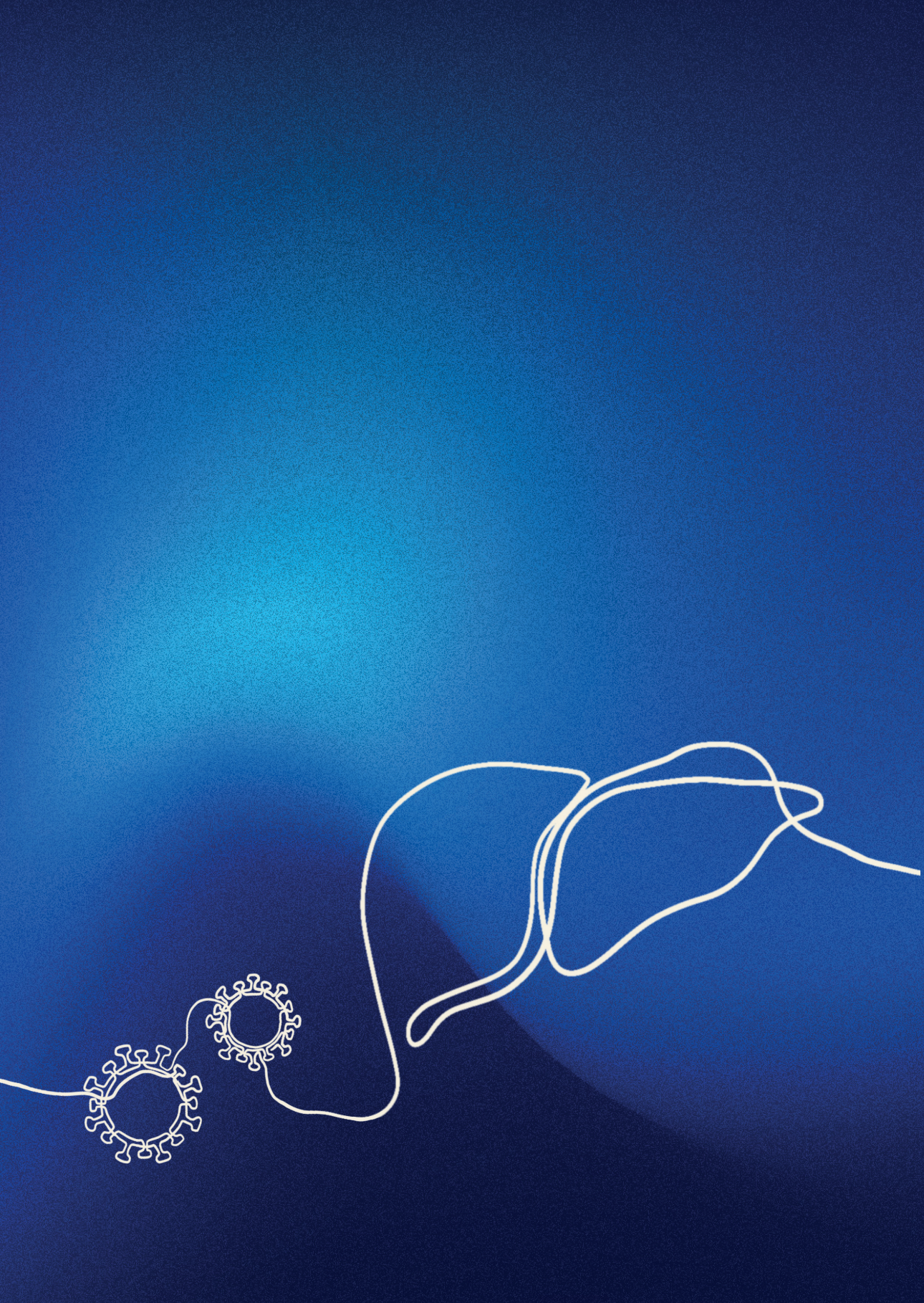
A final important aspect of CHB management is identifying patients at high risk of developing HCC. Besides the presence of cirrhosis as an established risk factor, various other subgroups have also been identified as high risk and considered eligible for enrolment in HCC surveillance programs, such as combinations of ethnicity, age and family history. It is therefore not surprising that management of CHB is challenging for most physicians, especially in low-endemic countries where physicians may care for limited numbers of CHB patients and therefore build limited experience. In the current study, 50% of patients were potentially eligible for HCC surveillance. Only 88% of these patients underwent adequate HCC surveillance during the study timeframe. Notably, HCC surveillance was adequately performed among 82% of patients managed at general hepatology clinics, compared to 92% in patients seen at a dedicated viral hepatitis clinic ($p = 0.016$). The suboptimal HCC surveillance rates are in line with previous data,[200] and are unfortunate as HCC surveillance has been associated with improved outcomes.[193]

The findings reported here corroborate those from a previous study,[201] and are in line with reports from other fields.[202-204] Furthermore, we have recently published a study showing that general practitioners generally do not provide adequate follow-up to patients with viral hepatitis despite the availability of a specific guideline,[205] further underscoring the importance of centralising care in CHB. Additional interventions that could potentially improve adherence are focused training sessions and/or implementation of tools in the electronic medical records that support standardised care, for example through reflex testing for co-infections in patients with viral hepatitis.

Strengths of this study include a large cohort of CHB patients that visited a large academic hospital in 2020. Several limitations of this study should be considered. First, the retrospective design of the study utilised data from patient charts, which may not always contain complete information on potential reasons for deviating from the guidelines. Secondly, this study has been conducted in a tertiary centre with high expertise for liver-related care. Whether our findings could be extrapolated to other

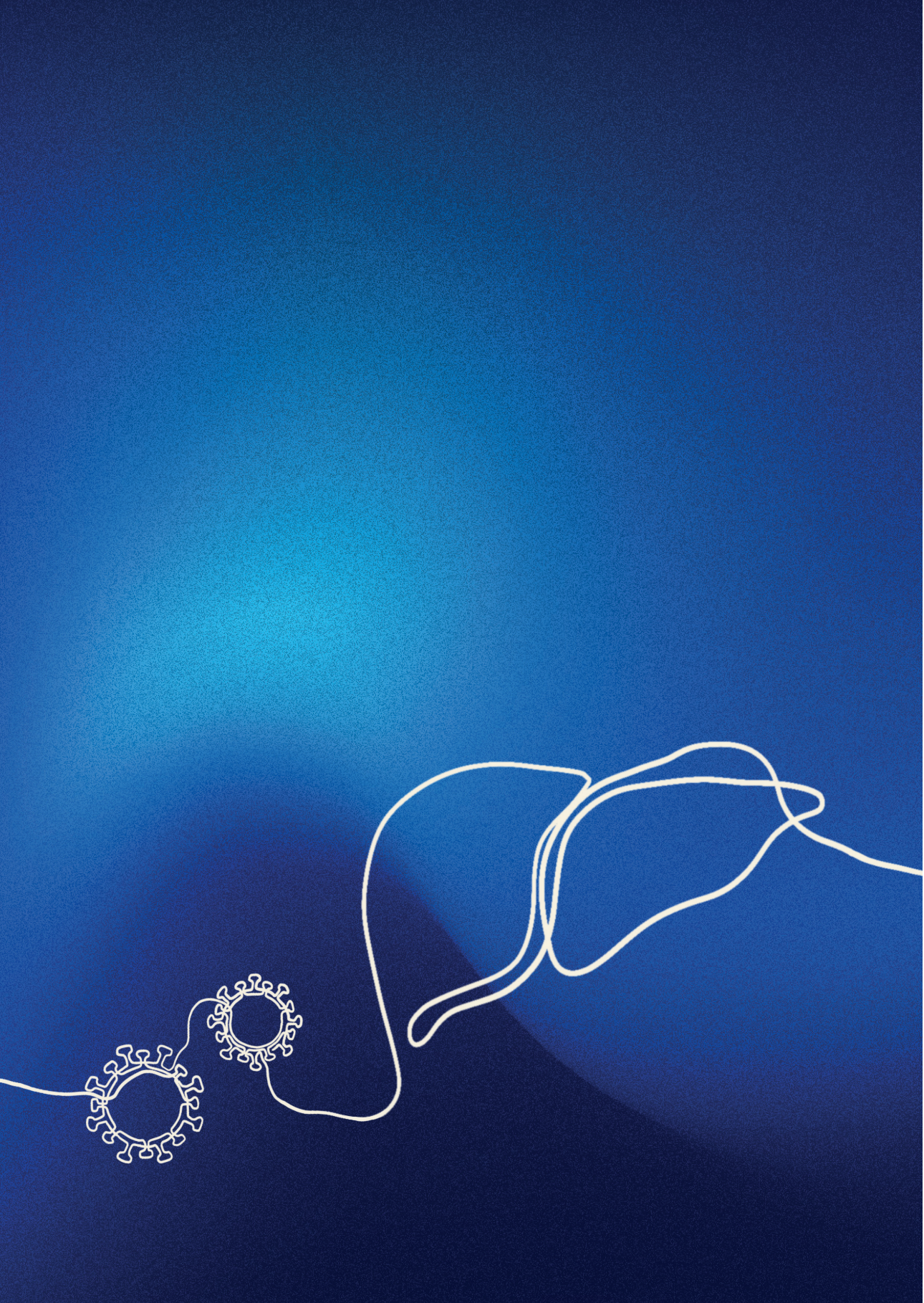
(non-)academic hospitals, or other low endemic countries, warrants further exploration.

In conclusion, the majority of CHB patients in our hospital were monitored and treated according to the management guidelines. Follow-up at a dedicated viral hepatitis outpatient clinic was associated with superior adherence rates.



PART VI

General Discussion



CHAPTER 12.1

Summary

Part II of this thesis investigated how serum HBV biomarkers could be used to predict off-treatment outcomes and the prospects of nucleos(t)ide analogues cessation in chronic hepatitis B patients.

In **chapter 2**, we investigated the on-treatment viral kinetics of HBV biomarkers and treatment response among patients that received one year of peginterferon-based therapy. We demonstrated that at on-treatment week 24, a decline in HBV RNA levels was associated with a higher probability of sustained response (HBV DNA < 2,000 IU/mL six months post-treatment); 27% versus 13% among patients without HBV RNA decline. However, 56% of the patients with an HBV RNA decline did not experience a concomitant decline in HBsAg levels. Among those patients, the chance of a sustained response was significantly lower compared to patients who did experience a concomitant decline in HBsAg; sustained response was achieved in 48% versus 16% among patients with > 1 log HBsAg decline versus those with < 0.5 log HBsAg decline. Findings were consistent for HBcrAg and if treatment response was defined as HBsAg loss. These findings suggest that combinations of viral biomarkers should be used to accurately assess response to antiviral therapy.

Chapter 3 studied the correlation between serum anti-HBc and other HBV biomarkers, intra-hepatic inflammatory activity, and treatment response. We found that anti-HBc levels correlated with age, IP-10, HBV DNA, HBcrAg, HBsAg, and HBV RNA levels among untreated HBeAg-positive patients, but not among HBeAg-negative patients. In addition, anti-HBc levels varied across HBV genotypes, with the highest levels among patients with HBV genotype A and the lowest among patients with HBV genotype D. Next, we showed that anti-HBc levels correlated with the severity of intrahepatic inflammatory activity, with a higher risk of severe inflammation among patients with high anti-HBc levels. Finally, we assessed the association between anti-HBc levels and treatment response. We showed that anti-HBc levels were higher among patients that did not receive antiviral treatment compared to patients treated with peginterferon. In line with this finding, anti-HBc levels declined during antiviral therapy. Higher anti-HBc levels were associated with favourable treatment outcomes such as HBeAg loss, sustained response, HBsAg decline, and HBsAg clearance. These findings stress the importance of B cells in the control of HBV infection and suggest that quantitative anti-HBc levels may be useful in clinical practice.

In **chapter 4**, we assessed the relationship with off-treatment ALT flares and virological outcomes and if these flares could be predicted using HBV biomarkers. We demonstrated in a pooled analysis of peginterferon-treated patients that off-treatment ALT flares (ALT levels > 5x ULN within six months after end-of-treatment) were associated with lower rates of sustained response compared to patients without an off-treatment ALT flare (3.5% vs 26.8%). Therefore, off-treatment ALT flares must be seen as an undesired event. Next, we observed that higher end-of-treatment

HBsAg, HBV RNA, and/or HBcrAg levels were associated with a higher risk of off-treatment ALT flares, but with a lower chance of sustained response or HBsAg loss. Especially, when these biomarkers were combined (HBsAg + HBV RNA or HBsAg + HBcrAg), the off-treatment outcome could be predicted more accurately. These findings could be used in clinical practice, as they can select patients eligible for antiviral therapy discontinuation and guide off-treatment follow-up.

Chapter 5 includes a prospective study in which 33 HBeAg-negative patients ceased nucleos(t)ide analogue (NA) therapy. We demonstrated that after 96 weeks, 39% of the patients achieved a sustained response (HBV DNA < 2,000 IU/mL) and 12% achieved HBsAg loss. However, a severe hepatic flare (ALT > 10x ULN) was observed in 21% of the patients. None of the patients developed hepatic decompensation or died. **Chapter 6** describes a case of an HBeAg-positive patient that ceased NA treatment and was scheduled for a follow-up appointment after several months. In the meantime, this patient developed a viral relapse, that processed into acute liver failure. Although the patient had undergone liver transplantation, he died because of post-operative complications. These two chapters showed that NA cessation is possible, but only in a strict selection of HBeAg-negative patients and if close follow-up can be guaranteed. The higher chance of HBsAg loss must be balanced against the risk of severe hepatic flares.

Part III of this thesis investigated how retrieval of lost to follow-up (LTFU) patients could contribute to hepatitis C elimination in the Netherlands. In **chapter 7**, we performed a nationwide retrieval project. In this study, we included 45 sites in the Netherlands. LTFU patients were identified based on laboratory results and medical chart review. Every patient eligible for retrieval (defined as being alive and with a registered address in the Netherlands) was invited for re-evaluation at the outpatient clinic and antiviral therapy. We showed that the majority (65%) of ever-diagnosed patients were already treated or still in outpatient care. In total, 8% of the patients were eligible for retrieval. Of them, 14% were relinked to care. This project supports that micro-elimination through retrieval of LTFU patients is feasible and might contribute to HCV elimination, but also time-consuming.

Part IV of this thesis investigated adherence to clinical guidelines.

HBV reactivation (HBVr) is a severe complication in patients treated with high-risk immunosuppressive agents, particularly the anti-CD20 monoclonal agent rituximab. As this risk is present in both patients with an active and quiescent hepatitis B infection, the guidelines recommend that every patient starting with rituximab must be screened for hepatitis B. Next, it is recommended to initiate antiviral prophylaxis in every patient with a (resolved) hepatitis B infection. In **chapter 8**, we performed a multi-centre cohort study and found that only 46% of the patients were screened correctly (measurement of both HBsAg and anti-HBc). Interestingly, screening rates

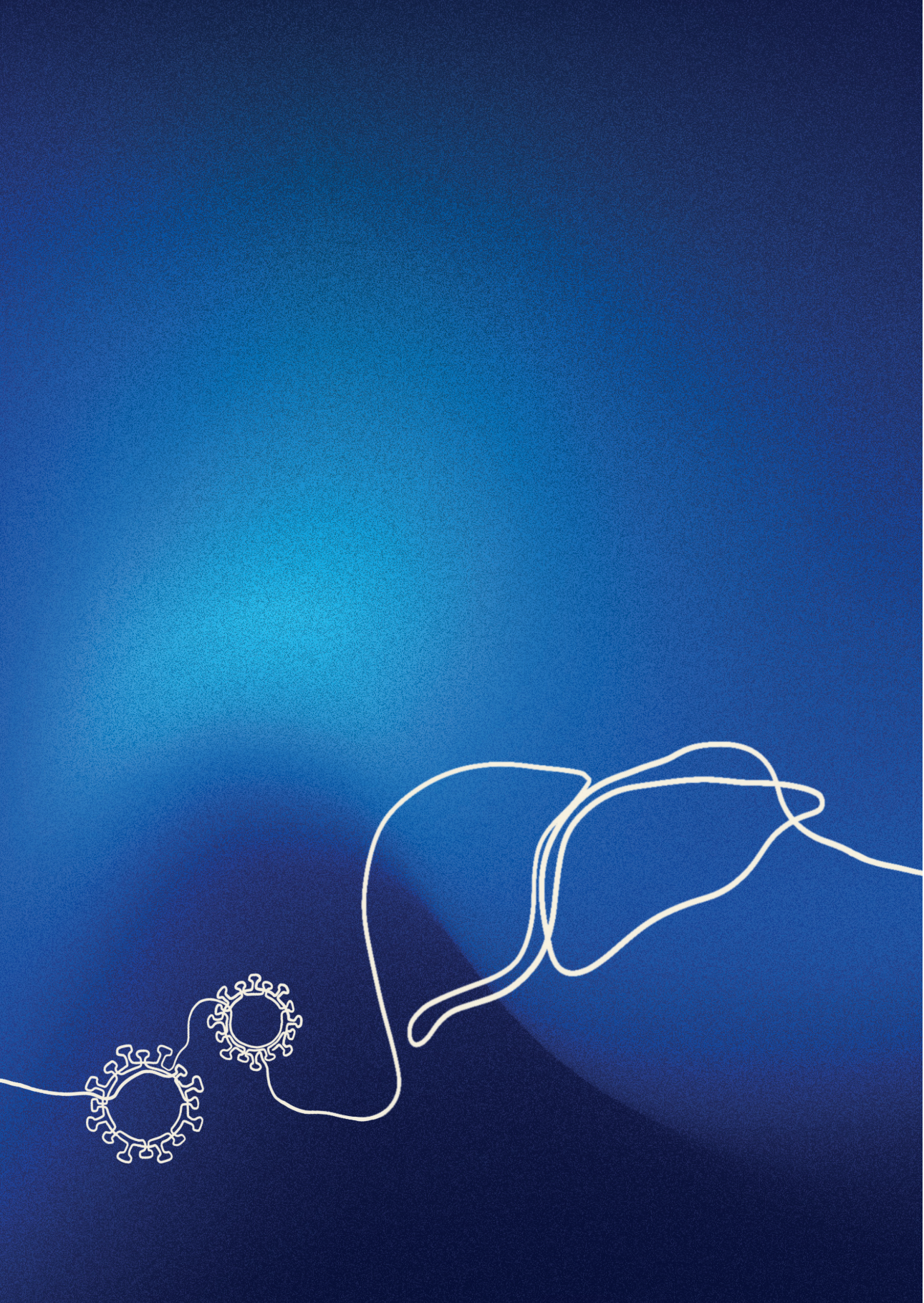
differed significantly between academic and non-academic hospitals (65% versus 32% respectively), as well as across medical specialties. Screening rates were not necessarily higher among high-volume prescribers (such as haematologists) compared to low-volume prescribers. In addition, screening rates improved throughout the years; 32% before 2012 to 75% after 2020 in the academic hospital and respectively 1% to 60% in non-academic hospitals. Among patients with a chronic or resolved HBV infection, antiviral prophylaxis was initiated in 58% versus 36% respectively. In total, seven patients experienced HBVr of whom one patient died due to liver decompensation. This addresses the importance of screening patients that start treatment with high-risk immunosuppressive agents, and initiation of antiviral prophylaxis among patients with a (resolved) hepatitis B infection.

In the Netherlands, general practitioners (GPs) play an important role in the identification of patients with hepatitis B and C, and the surveillance of chronic hepatitis B patients. The current guideline for GPs recommends biannual surveillance (ALT levels) and referral of every patient with an active hepatitis B or C infection to a specialised hepatitis treatment centre. In **chapter 9,** we assessed the performance of this GP guideline using a large healthcare database of medical records of GPs in a multi-ethnic area in the Netherlands. We observed that 71% and 89% of respectively chronic hepatitis B and C patients were referred to a hepatitis treatment centre at least once in their life. However, a substantial part of the patients did not receive adequate surveillance or curative treatment during follow-up. Among chronic hepatitis B patients, only 15% received regular ALT measurements ordered by the GP and 46% were still in care with a medical specialist. In total, 36% of the patients did not receive surveillance of their HBV infection. Among the chronic hepatitis C patients, 74% received antiviral therapy, but only 54% had a registration of successful viral eradication. These findings are important to create awareness among GPs and medical specialists, and suggest that GPs should initiate retrieval of their LTFU patients.

In **chapter 10,** we studied the adherence and determinants of non-adherence to the clinical hepatitis B guideline in a high-expert academic hospital in the Netherlands. Every chronic hepatitis B patient that visited the outpatient clinic in 2020 was included. We observed that 97% of the patients with an indication for antiviral therapy received antiviral treatment, and 90% of the patients with renal/bone disease were treated with entecavir or tenofovir alafenamide. Screening for co-infections hepatitis A, C, or D was performed in > 86% of the patients, but the presence of a co-infection with HIV was assessed in only 78% of the patients. The majority of patients with an indication for HCC surveillance did receive adequate surveillance (88%). Interestingly, we observed that adherence to medical guidelines was significantly better among patients treated at a dedicated viral hepatitis clinic compared to

patients that were treated at a general hepatology clinic. These findings suggest that hepatitis B care should be centralised in expert clinics.

Part V of this thesis investigated if hepatitis care can be individualised beyond the general clinical guidelines. In **chapter 11,** we assessed the added value of an add-on guideline for the treatment of chronic hepatitis C patients. This add-on guideline, the HCV TherapySelector, displays patient-profiled data from high-quality studies on the treatment of hepatitis C patients. Using a mobile or web-based application, the efficacy of several treatment regimens is shown for an individual patient based on his/her specific patient profile. This application is updated monthly and offers herewith up-to-date information. In our study, we showed that the treatment of patients would have been optimised if the TherapySelector was used. The use of such an add-on might be of great interest in diseases with suboptimal curation rates or clinically relevant adverse effects for which treatment options rely on specific patient characteristics. This article should therefore be seen as a proof of concept for other diseases.



CHAPTER 12.2

Discussion and further perspectives

General

Viral hepatitis B and C are major global health problems, as a chronic infection can progress to liver cirrhosis and hepatocellular carcinoma (HCC). Nowadays, very effective antiviral therapies are available that can suppress the hepatitis B virus (HBV) or eradicate the hepatitis C virus (HCV).[18,35]

However, many patients die every year due to the consequences of their infection. In the Netherlands, the annual mortality has been estimated at 500 individuals.[164] Therefore, the world health organization (WHO) has called for action to eliminate viral hepatitis as a global health threat.[3]

In this thesis, we investigated how to optimise the care of patients infected with HBV or HCV. First, we studied off-treatment follow-up after discontinuation of antiviral therapy in chronic hepatitis B patients and how serum biomarkers can contribute to patient selection and off-treatment follow-up guidance. Secondly, we investigated how nationwide retrieval of chronic hepatitis C patients who have become lost to follow-up can contribute to micro-elimination. Thirdly, hepatitis care was evaluated in primary care and in specialised hepatitis treatment centres. Fourthly, we tested the possibilities to improve medical decision-making using a guideline add-on which is based on individual patient characteristics.

Novel biomarkers for treatment response prediction

The main barrier to HBV cure is covalently closed circular DNA (cccDNA). Intrahepatic cccDNA acts as a template for the transcription of all HBV RNAs responsible for HBV DNA replication and viral antigen production. The elimination of cccDNA has therefore been seen as the true (sterilizing) cure.[224] However, as this is difficult to achieve, functional cure, i.e. loss of hepatitis B surface antigen (HBsAg), is (currently) the goal of antiviral therapy.[18]

Nowadays, available biomarkers in clinical practice are HBV DNA, HBeAg, anti-HBe, HBcAg, anti-HBc, HBsAg, and anti-HBs. Besides HBV DNA, the majority of laboratories can only measure the qualitative results of these biomarkers (i.e. positive or negative). These are useful in the diagnosis of hepatitis B and/or to gain insight into the disease stage, but have imperfect sensitivity to predict treatment response.

Current antiviral options include one year of pegylated interferon (PEG-IFN) or long-term nucleos(t)ide analogues (NA).[148] NAs block reverse transcriptase and herewith suppress viral replication. However, NAs do not impact cccDNA. In contrast, PEG-IFN acts as an immune modulator and stimulates a cell-mediated immune response. This immune response targets HBV-infected hepatocytes which act as a reservoir for cccDNA, causing degradation of cccDNA in HBV-infected hepatocytes.[225]

The aim of PEG-IFN is the induction of long-term immunological control. However, the response to PEG-IFN differs greatly between patients. Predictors of treatment response include HBV genotype, ALT levels, HBV DNA levels, sex, and age.[226] Nevertheless, sustained response or HBsAg loss is only achieved in a fraction of patients with the most favourable characteristics.[226] In both clinical and scientific worlds, there is a growing interest in the use cccDNA transcriptional activity or the hosts' immune response to estimate the probability of treatment response more accurately. [70,227,228] As cccDNA can only be invasively measured using liver biopsy, the use of serum biomarkers reflecting cccDNA activity is more attractive. Growing evidence suggests that hepatitis B core-related antigen (HBcrAg) and HBV RNA can be good candidates. In addition, antibodies to HBcAg (anti-HBc) might be useful reflecting the host's immune activity.

HBcrAg

HBcrAg is a marker that incorporates several viral antigens, including HBcAg, HBeAg, and p22 core-related antigen. A liver biopsy-proven study demonstrated that HBcrAg correlated with cccDNA levels and cccDNA transcriptional activity. In addition, HBcrAg levels were higher among HBeAg-positive compared to HBeAg-negative patients, and correlated with serum HBV DNA, intrahepatic HBV DNA, and pre-genome HBV RNA (pgRNA). They found lower levels of cccDNA and lower cccDNA activity among patients with undetectable HBcrAg levels.[229] Chuaypen *et al.* demonstrated that HBcrAg decline during PEG-IFN treatment was associated with durable off-treatment HBV DNA suppression (sustained response).[228] In addition, studies with finite NA therapy discovered that low HBcrAg levels were associated with sustained response and HBsAg loss.[230,231] A prediction algorithm has been developed including HBcrAg, HBsAg, age, and tenofovir use (SCALE-B score), showing that HBcrAg and HBsAg levels were predictors for clinical relapse after NA withdrawal.[107] In this thesis, we also demonstrated that low levels of HBcrAg were associated with a higher probability of sustained response and a lower risk for ALT flares among PEG-IFN treated patients (**chapter 4**). In addition, a decline in both HBV RNA and HBcrAg during PEG-IFN therapy was associated with a favourable off-treatment response (**chapter 2**).

However, most clinical studies exploring the clinical use of HBcrAg are limited by small sample sizes and included predominantly Asian patients.[232,233] Also, as the level of HBcrAg is influenced by the HBeAg status and HBV DNA levels, these patient characteristics need to be taken into account for the interpretation of HBcrAg levels.[234] Subsequently, no defined cut-off values are available yet to guide medical decision-making. Finally, HBcrAg is currently only available for experimental purposes, but not in clinical practice.

HBV RNA

The next biomarker includes HBV RNA. pgRNA is the product of cccDNA transcription, and serum HBV RNA serves as a surrogate marker of pgRNA and herewith cccDNA activity.[235] HBV RNA levels also correlate with HBeAg-status, with higher levels of HBV RNA among HBeAg-positive patients.[234] Different studies have been conducted exploring the predictive value of HBV RNA for treatment response. Van Bömmel *et al.* demonstrated that HBV RNA levels before PEG-IFN initiation and during therapy were associated with treatment response (HBeAg seroconversion at week 24 post-treatment).[70] In line with these findings, we observed that HBV RNA kinetics during PEG-IFN therapy and at end-of-treatment could predict off-treatment outcomes (**chapter 2** and **chapter 4**). Just like HBcrAg, HBV RNA use is also limited in clinical practice due to unavailable cut-off values, and because it is currently only available for experimental purposes.

Anti-HBc

Another barrier to HBV cure is an impaired immune response of the host against the virus. Evidence suggests that B cells play an important role in the natural course of a chronic hepatitis B infection.[83,86,95] In **chapter 3,** we explored the role of B cells, using anti-HBc, and treatment response. We showed that higher anti-HBc levels were associated with a higher probability of treatment response. This suggests that patients with low levels of anti-HBc might have an impaired immune response, resulting in a lower chance of a favourable outcome after immunomodulatory therapy. In addition, we found that higher levels of anti-HBc were associated with hepatic inflammation. This supports the hypothesis that the enhanced immune activity of the host, in order to attack HBV, causes intrahepatic damage. Finally, anti-HBc levels were correlated with HBV RNA, HBcrAg, and HBsAg. Thus, anti-HBc levels might also reflect cccDNA activity.

Our findings are in line with the study by Fan *et al.*[89] and Chi *et al.* [236], showing that higher anti-HBc levels are associated with favourable off-treatment outcomes. Conversely, Hu *et al.,* using a large cohort of Asian HBeAg-negative patients without antiviral treatment, found that low levels of anti-HBc were associated with undetectable HBV DNA and HBsAg loss.[237] It should be noted that this study mainly included patients with low baseline HBV DNA and ALT levels. These patients might have suppressed immune activity (and thus low anti-HBc levels), which has resulted in these conflicting findings. Currently, the use of anti-HBc in clinical/experimental settings is limited, as more evidence is needed to explore the role of anti-HBc and treatment response prediction, and also because quantitative levels can only be measured using commercial kits.

Recommendations and future perspectives

The findings of this thesis support the literature that HBcrAg, HBV RNA, and/or anti-HBc could be used to assess treatment response.

Although we studied patients treated with PEG-IFN, which is a treatment regime that is currently not often used in clinical practice, the findings of this thesis might be translated into current practice. First, our studies are in line with studies exploring off-treatment outcomes after finite NA therapy. These showed that the combination of viral antigens and/or HBV RNA did improve the prediction of off-treatment outcomes.[230,231] In addition, the quest for novel antiviral agents is going on. PEG-IFN is currently experiencing a revival, as it is often used in combination with novel agents.[66,67] Therefore, the findings of this thesis might be used in the studies of novel (immunomodulatory) agents.

In addition, we observed in **chapter 2** and **chapter 4** that the kinetics of sole HBV biomarkers can be used for response prediction, but that the combination of biomarkers was able to predict treatment outcomes more accurately. A combination of biomarkers should therefore be used for patient selection, off-treatment guidance, and to assess antiviral efficacy. However, we also found that quantitative HBsAg levels, a biomarker that has been studied for many years and is available in clinical practice, remains essential for response prediction.

Furthermore, a promising new marker includes particular components of HBsAg. HBsAg consists of three co-carboxyterminal proteins: small, medium, and large surface proteins (respectively SHBs, MHBs, and LHBs).[238] Recent studies found an association between LHBs and MHBs levels, and HBsAg loss. These researchers found a more profound decline of LHBs and (in particular) MHBs levels among patients with HBsAg loss compared to patients without. Among the patients that achieved HBsAg loss, LHBs and MHBs levels became undetectable early on-treatment (i.e. several months before HBsAg clearance) and showed superior predictive value compared to total HBsAg levels.[239,240]

Thus, although the search for the implementation of novel biomarkers is going on, HBsAg levels should be included in patient selection, follow-up during and after therapy, and research exploring novel agents. However, more translational and clinical research is needed to explore the relationship between serum biomarkers HBsAg, HBcrAg, HBV RNA, and anti-HBc, and treatment response, including studying viral kinetics, the host immune response, and to establish clinically relevant cut-off values.

ALT flares

ALT flares occur during the natural course of a chronic hepatitis B (CHB) infection, but also in relation to antiviral therapy.[36] It has been debated that ALT flares could be beneficial.[106] However, flares can also cause hepatic decompensation (**chapter 6)**.[36] For a long time, physicians and researchers aim to differentiate ALT flares into "good" (or "host-dominating") and "bad" (or "virus-dominating") flares.[108] A good flare might reflect an effective immune response of the host against the HBV, whereas a bad flare reflects an ineffective immune response. Usually, every flare is preceded by a rise in HBV DNA. Among patients with bad flares, HBV DNA remains high or ascends during the ALT flare, followed by a little decline in viral antigens and ongoing hepatitis. These patients require immediate re-treatment to prevent liver damage or even decompensation. In contrast, patients with a good flare experience a concomitant decline in viral antigens, which are followed by a decline in HBV DNA.[108,110] However, the kinetics of serum antigens and HBV DNA could only differentiate between good and bad flares during or after the flare. Therefore, viral- and/or host-related predictors that can be quantified before the rise in ALT (or even HBV DNA) might be preferred.

In **chapter 4,** we observed that higher levels of serum HBsAg, HBV RNA, and HBcrAg were associated with a higher risk of ALT flares compared to patients with low levels of these biomarkers. In our cohort, we did not observe that patients with off-treatment ALT flares had a higher probability of sustained response or HBsAg loss.

Recommendations and future perspectives

We did not observe an association between ALT flares and favourable off-treatment outcomes such as sustained response or HBsAg loss. Therefore, ALT flares should be seen as an undesirable event, as they can potentially lead to hepatic decompensation and death. As PEG-IFN therapy and many novel antiviral agents require a finite treatment duration, and the clinical world is exploring the possibilities of stopping NA therapy after long-term viral suppression, it is important to have access to predictors for off-treatment ALT flares. Serum biomarkers such as HBsAg, HBcrAg, and HBV RNA at end-of-treatment can be used for patient selection off-treatment follow-up guidance. However, pre-defined cut-off values of these biomarkers are not yet available.

Finally, instead of aiming for or defining characteristics of a "good" flare, severe ALT flares should not be pursued or seen as a (potential) favourable outcome.

Selecting and monitoring chronic hepatitis B patients eligible for finite NA therapy

NA therapy is the backbone of current antiviral treatment in CHB patients, as it is very effective in suppressing viral replication. This halts further progression of the liver disease and leads to liver fibrosis regression in the majority of patients. But although the HCC risk decreases among patients with on-treatment viral suppression, they remain at risk to develop HCC.[18] Since HBsAg loss during NA treatment is very rare, several studies explored the possibility of ceasing NA therapy before the loss of HBsAg.[18,114] Combining the results of the studies exploring the possibilities of ceasing NA therapy, it can be concluded that NA withdrawal could be associated with favourable outcomes but also with substantial risks. These are displayed in Table 1.

Table 1. Pro's en con's of NA cessation

Pro's	Con's
• Higher rates of HBsAg loss • Durable viral suppression in a substantial proportion of patients	• Viral rebound in the majority of patients • Risk of severe hepatic flares, and herewith risk of hepatic decompensation and death • Close off-treatment monitoring • Careful patient selection

Pro's

One of the advantages of NA discontinuation includes the higher rates of HBsAg loss compared to patients that remain on-NA therapy. Although the probability of HBsAg loss differed greatly between the studies (from 2% to 18% after two years of follow-up),[106,116] the majority of studies showed higher HBsAg rates among patients that stopped NA therapy compared to patients that continue NA therapy.[43,44,114] In our prospective stop study, we observed an HBsAg clearance rate of 12% after two years of follow-up (**chapter 5**). This is higher than the expected HBsAg rate of < 1% per year among patients that remain on-treatment.[115,241] Besides HBsAg loss, many patients achieved durable viral suppression (HBV DNA levels < 2,000 IU/mL). The higher probability of HBsAg loss could be explained by the restoration of T-cell response and induction of immune activation.[242] This concept was first introduced by Hadziyannis *et al*. In this study, re-treatment was withheld in patients with a virological rebound or even (mild) hepatic flare but only initiated among patients with a persistent ALT rise or severe hepatitis. Many patients in this study achieved HBsAg loss.[106]

Another advantage is that we can now discuss this option with patients that desire to stop daily medication intake and inform them about the advantages but also about

the potential risks. Finally, although some researchers pointed out the rationale of NA cessation to prevent antiviral resistance to NA agents,[39,117] this is not applicable in many Western countries that have access to entecavir and tenofovir (both agents with no or limited risk to resistance).[18]

Con's

Besides these favourable outcomes, there are several risks that need to be taken into account when considering NA cessation. First, almost every patient experiences a viral rebound after NA discontinuation.[45] This HBV reactivation leads to a rise in ALT levels in the majority of patients and severe ALT flares (ALT > 5x or > 10x ULN) in a substantial proportion of these patients.[43,45,62] In our prospective stop study, we observed that 21% of the patients experienced a rise of ALT levels > 10x ULN (**chapter 5**). Although some researchers claim that these flares might be beneficial,[106,243] cases of hepatic decompensation have been described, some with fatal outcomes.[38,47] These occurred predominantly in cirrhotic patients, but also in non-cirrhotic patients (**chapter 6**).[46,47,111,119] Patients with severe hepatitis (ALT > 10x ULN) should be re-treated immediately to prevent hepatic decompensation. Since the ALT flares can occur anytime during follow-up, close off-treatment monitoring is essential. This results in additional visits, which require a time investment for the physician and patient, and more costs due to the extra laboratory tests. Finally, it can be hypothesized that severe ALT flares or persistent HBV load can cause liver damage and increase the HCC risk. Recently, a large multicentre study observed no differences in HCC risk among non-cirrhotic patients who ceased NA therapy compared to a matched control group of patients. However, the median follow-up duration of this study was 44 months.[244] As HCC development could take many years,[245] this follow-up period might be too short to rule out an increased HCC risk. Long-term follow-up data is currently still lacking.

Furthermore, it should be emphasised that results from previously published NA stop papers are heterogeneous. This could be the consequence of the differences in study design, patient selection, and re-treatment criteria. Many Asian studies had a retrospective design, as the NA cessation approach is mainly driven by reimbursement policies. In Asia, NAs are also ceased in cirrhotic patients, leading to a higher risk of severe safety outcomes. Additionally, the differences in outcome could also be related to ethnicity. It has been suggested that Asian individuals have a lower risk of favourable outcomes compared to Caucasians. If this is the result of genetic differences, differences in HBV genotype, duration of HBV infection, or type of NA agent (older regimens are still approved in most Asia Pacific countries[121]) is still unknown. Finally, as re-treatment criteria have not been yet defined, every NA stop study (especially those with a retrospective design) applied different re-treatment definitions. This might also have led to the heterogeneous off-treatment results.

Recommendations and future perspectives

Emerging evidence suggests that NA therapy can be ceased before HBsAg loss. However, due to the potential off-treatment risks, patient selection and strict off-treatment follow-up are crucial.

Patients eligible for NA discontinuation include HBeAg-negative patients with long-term on-treatment viral suppression (i.e. undetectable HBV DNA levels). The duration of on-treatment viral suppression has not been explored in detail, but current guidelines suggest at least one year of viral suppression after durable on-treatment HBeAg seroconversion among patients who were HBeAg-positive at the start of NA treatment, and at least two/three years among patients who were HBeAg-negative at start of NA treatment.[18,117] Next, to limit the risk of hepatic decompensation, only patients without (history of) cirrhosis, hepatic decompensation or HCC should be included. Moreover, although current guidelines do not include HBsAg levels at the end-of-treatment as inclusion criteria to select patients, evidence suggests that HBsAg levels are the best reliable predictor for off-treatment outcomes. These should therefore also be included in patient selection, excluding patients with high HBsAg levels (for instance > 1,000 IU/mL, and possibly > 100 IU/mL among Asian patients) as these patients have a very limited chance of favourable outcomes but an increased risk of ALT flares.[114] Therefore, a complete evaluation of every patient before NA cessation is crucial. This includes at least a full laboratory assessment, comprising HBeAg, HBV DNA levels, quantitative HBsAg levels, liver enzymes, and (if not performed in the past) serology to exclude co-infections (HCV, HDV, HIV). Liver elastography could be performed to assess the risk for the presence of advanced fibrosis or cirrhosis. Finally, NA cessation should only be considered among patients who are willing to be objected to close follow-up and who are informed about the potential risks.

Current EASL guideline states that *"NAs may be discontinued only in patients who can be followed closely with ALT and HBV DNA determinations at least during the first year following NAs cessation"*.[18] However, they are not specific about how this off-treatment follow-up should be managed. As HBV reactivation and ALT flares can occur any time during off-treatment follow-up but especially during the first 6 months,[45] it could be advocated that off-treatment follow-up might include monthly evaluation in the first 3-6 months, thereafter every 3 months and every 6 months after 1 year (i.e. the follow-up schedule described in **chapter 5**). Every visit should include the assessment of liver enzymes, coagulation tests, HBeAg, HBV DNA, and if possible HBsAg levels. In case of a profound rise in HBV DNA levels or any elevation in ALT levels, additional visits should be planned and re-treatment should be initiated in case of imminent severe hepatitis or hepatic decompensation. Also, re-treatment should be considered in case of persistent flares or HBeAg seroreversion. In addition, treatment might also be re-started after one or two years

if the patients then again meet the indication criteria for antiviral therapy according to the medical guidelines.[18]

The remaining challenges include reliable predictors to assist patient selection and off-treatment follow-up. Promising predictors might include biomarkers reflecting cccDNA transcriptional activity such as HBV RNA and HBcrAg, or the host's immunological state using anti-HBc. But although some studies demonstrated that these biomarkers could be useful,[230,231,236] more research is needed to determine the cut-off values of these biomarkers. Also, various studies hinted that ethnicity might be another important predictor.[114,246] More research is needed to confirm these differences and to search for the underlying mechanisms. Next, long-term outcomes of untreated patients without HBsAg loss should be evaluated.

Nationwide retrieval of lost to follow-up patients contributes to micro-elimination

The Netherlands is a low-endemic country for viral hepatitis C, making nationwide screening not (cost-) effective. Instead, we should target key populations, including patients with a high risk of viral hepatitis infection. This is also described as micro-elimination. One of these key populations includes patients who have become lost to follow-up (LTFU) before they were successfully treated.[48] **Chapter 7**, includes a nationwide retrieval project (CELINE) in which the majority of hepatitis treatment centres in the Netherlands were included. Using laboratory results and medical records
of 20,183 patients with a positive HCV test result, we were able to re-link 219 LTFU patients to care. Although invitation letters were sent and patients were contacted by phone, contact could have been established in only 58% of the patients. In addition, among the patients in which contact could be established, 9% refused evaluation and some retrieved patients became LTFU again before treatment could have been initiated, indicating that this remains a hard-to-reach population. Among the patients that were successfully re-linked to care, 83% tested HCV RNA positive. The majority of those patients were successfully treated with DAA treatment. In addition, 27% of the HCV RNA positive patients had liver stiffness measurements indicating advanced fibrosis or cirrhosis, stressing the importance of identifying these infected patients.

Recommendations and future perspectives

Chapter 7 demonstrated that systematic evaluation of laboratory records and medical files is able to identify patients eligible for retrieval. However, it is also a time-consuming project. Nevertheless, CELINE can be used as a blueprint for other low-endemic countries and for future retrieval efforts. It remains important to continue

retrieval of patients who have become LTFU, for instance annually. This could be less time-consuming compared to this nationwide project, which included laboratory records over the last 15 years, and could be more effective as contact information is more likely to be still up-to-date.

Another option to increase the efficacy of retrieval could be the decentralising of care. Among the retrieved patients, the main transmission route of HCV infection was injecting drug abuse. A proportion of LTFU patients were still struggling with drug abuse and consequently might have declined evaluation and became LTFU again after re-linkage to care. If evaluation and treatment could be offered in addiction centres, the re-linkage rate might increase. Soon, the first results of the chain of addiction care (CAC) project will be expected, in which antiviral treatment is offered in addiction care centres.[247]

Hence, the retrieval of LTFU patients can be seen as low-hanging fruit and is able to re-link patients in care for antiviral therapy. This contributes to micro-elimination. The remaining challenges to achieving the elimination of viral hepatitis in the Netherlands include the identification of infected individuals in other key populations. These include men who have unsafe sex with men (MSM), people who (have) inject(ed) drugs (PWID), migrants, and incarcerated people.

For MSM, multiple projects have been initiated. The NoMoreC project offers information, HCV self-tests (a service that was offered in the past but unfortunately cannot be offered anymore), and toolboxes including protective materials such as gloves, condoms, and sterile needles.[248] In addition, high-risk individuals using pre-exposure prophylaxis (PrEP) for HIV prevention (which is offered to people with high-risk behaviour) are screened regularly for HCV infection.[249] Finally, current harm reduction strategies for PWID include opioid substitution therapy and needle and syringe exchange programs have led to a reduction of infection rate of HBV and HCV.[250]

Next, one of the key populations in the Netherlands includes migrants.[5] Therefore, screening (first-generation) migrants would be an important strategy.[251,252] Currently, migrants are only screened for tuberculosis. In the past, a pilot study added voluntary HBV and HCV testing to tuberculosis screening for immigrants. Among the individuals that were tested (only 54% of the approached accepted screening), up to 4.5% tested positive for HBsAg and up to 1.2% for anti-HCV.[253] Although it has been suggested that screening of migrants from countries with an HBV and HCV prevalence of ≥ 0.41% and ≥ 0.22% are cost-effective,[254] no screening programs have been initiated (yet). Only GPs are encouraged to perform hepatitis C and B screening among migrants.[178] The uptake of this recommendation is unknown. A qualitative study showed that many GPs are unaware of their responsibility to screen patients that originate from high-risk countries.[184] In addition, according to the law

for the protection of privacy (AVG) it is not allowed to register the country of origin in the patient file, making it challenging to select patients eligible for screening.[184]

Recently, the Dutch Health Council (Gezondheidsraad) declared that screening of first-generation migrants of non-Western origin is in line with the Population Screening Act (in Dutch: Wet op het Bevolkingsonderzoek).[255] Therefore, screening does not require permission from the Ministry of Health. The remaining barriers that must be overcome include funding and a care pathway comprising the identification of migrants eligible for retrieval, testing, and linkage to care. Migrants with residence permits could be identified using the Municipal Personal Records Database, as this database contains information about their place of birth. Next, these individuals must be informed, preferably in their native language, and invited for screening. The screening could be performed in health facilities using blood tests but also using self-tests. These self-tests, using dry blood spots or saliva, might be attractive as people can experience a low threshold to participate. In addition, people are more familiar with self-tests since the covid-19 crisis. Finally, individuals with positive test results should be referred to a hepatitis treatment centre for complete evaluation and (if necessary) treatment. However, asylum seekers and refugees without residence permits are not registered, making this care pathway more complicated. In addition, as these patients do not have health insurance, funding for the screening and antiviral treatment is another barrier to screen this population.

Finally, prisons should also be included in our strategy to achieve elimination. Incarcerated people can be considered a high-risk group, as many of them have a history of intravenous drug use and have tattoos/piercings.[256] Although exact numbers are missing, the prevalence of viral hepatitis C is therefore expected to be high. In 2010, a report was published in which the prevalence of hepatitis C was studied using the medical files of inmates in 11 prisons in the Netherlands, showing an HCV prevalence of 2-8%.[257] Multiple (foreign) reports demonstrated that screening and treatment of incarcerated individuals with hepatitis C is essential to reach elimination while it is feasible and cost-effective. These reports not only show a high prevalence of hepatitis C in prisons, but also a high uptake of DAA therapy when treatment is offered in prison setting.[256,258] However, testing and treatment of hepatitis C (and B) in Dutch prisons are currently low. Systematic screening is not (yet) performed due to restrictions by the Custodial Institutions Agency (in Dutch: ‘Dienst Justitiële Inrichtingen’). The main reason might be the fact that the treatment of hepatitis C must be financed by the Ministry of Justice, as incarcerated individuals do not have health insurance.[259] Thus, funding and/or policy changes might be required before screening in prisons could be performed.

Nevertheless, in **chapter 9** we observed a decline in the prevalence and incidence of viral hepatitis B and C in a multi-ethnic area in the Netherlands. This implies that

the Netherlands seems to be on track to reach the WHO elimination target by 2030. However, exact numbers in the remaining area of the Netherlands are lacking.

Adherence to medical guidelines – how to improve hepatitis care

Current guidelines used in the Netherlands include the international guidelines prepared by the EASL,[18,35] the national richtsnoer for hepatitis specialists,[55,56] and the NHG guideline for general practitioners (GPs)[178]. For HCC, various studies showed that limited adherence to the guidelines leads to worse patient outcomes.[193,194] This highlights the importance of adherence to medical guidelines. In this thesis, we investigated the adherence to current guidelines including HBV screening among patients treated with rituximab (**chapter 8**), the adherence to surveillance of viral hepatitis B and referral of hepatitis C patients in the GP practice (**chapter 9**), and the adherence to the international guideline among hepatitis B patients who visited the outpatient clinic in a large tertiary hospital (**chapter 10**).

In **chapter 8**, we found that many patients that were treated with rituximab did not receive the correct screening for HBV. Screening is recommended by the guidelines, as patients with a (prior) hepatitis B infection require antiviral prophylaxis to prevent hepatitis B reactivation. Several potential explanations for inadequate adherence can be imagined. First, a lack of knowledge of the risk of HBV reactivation may lead to unawareness about the need for screening. Secondly, the lack of knowledge about HBV serology results might result in ordering the wrong serology markers (for instance HBsAg only) as well as the misinterpretation of the test results. Consequently, patients with prior HBV infections remain undiagnosed and receive inadequate management. Thirdly, local guidelines, generated by hospitals, might not be available or lack information about HBV screening in patients treated with rituximab. Consequently, many physicians are unaware of the need for screening.

Our findings are, unfortunately, not unique. Several studies have highlighted the poor screening rates and antiviral prophylaxis initiation among patients with a (prior) HBV infection.[140,141,143-145,149] Although these studies used different definitions for correct screening (anti-HBc testing, HBsAg + anti-HBc, and HBsAg + anti-HBs + anti-HBc), screening rates were 27-71% and initiation of antiviral prophylaxis 17-74%.[140,141,143-145,149] These findings are in line with our study, highlighting the need for improvement. Our findings could raise awareness among physicians in the Netherlands and in other countries.

Next, in **chapter 9**, we studied the management of hepatitis B and C patients in GP practices and adherence of the Dutch guideline for GPs, which advises biannual surveillance of hepatitis B patients and referral of every hepatitis C patient. Although we observed that the majority of patients have been referred to a hepatitis specialist at least once in their life, many hepatitis B patients did not receive adequate

surveillance by either the GP or hepatitis specialist accordingly. In addition, many hepatitis C patients did not have a confirmed HCV eradication following antiviral therapy.

Due to the low prevalence of viral hepatitis in the Netherlands, each GP only has a few patients in care. It can therefore be imagined that the knowledge about viral hepatitis and/or the guideline is limited, leading to inadequate management of these patients in the GP practice. Another reason could be the fact that Dutch GPs do not have an automated appointment scheduling system, hampering the organisation of regular screening visits.

Finally, we observed that the majority of patients with a chronic hepatitis B infection received care in line with the international hepatitis B guideline (**chapter 10**).[18] Interestingly, if follow-up was performed by one of the viral hepatitis specialists, adherence rates were superior compared to patients that were in care with a general Hepatologist.

Recommendations and future perspectives

To increase adherence to current guidelines, first (ongoing) education is essential for all healthcare providers prescribing rituximab or other high-risk immunosuppressive agents, GPs, and Hepatologists. However, although annual training is offered to GPs, it seems that the number of GPs attending this course is decreasing. GPs are offered a large number of trainings for various diseases, making it impossible to attend all, especially due to the increasing workload in GP practises.[260] For Hepatologists, biannual education is offered during the national Digestive Disease Days Meetings. How the education for other healthcare providers prescribing rituximab has been organised is unknown.

Next, Information Technology (IT) tools might enhance adherence rates. These might include an alert (if possible also showing recent laboratory results) linked to the prescription of certain agents. For example, a warning to screen for hepatitis B in case of high-risk immunosuppressive agents and to check the renal function among patients treated with tenofovir disoproxil fumarate. In addition, a standard set of laboratory tests might facilitate screening and the management of hepatitis patients. Finally, a standard note in the patient file including patient characteristics, treatment, and HCC screening information might be useful as “checklist’. More research is warranted about which IT tools are able to optimise hepatitis care.

Another IT tool that might facilitate the management of viral hepatitis patients is the guideline add-on the TherapySelector. At one glance, up-to-date information about treatment options is offered, based on high-quality studies, adjusted for specific patient characteristics. Currently, the TherapySelector is only validated for hepatitis

C. In **chapter 11**, we showed that the management of patients treated for chronic hepatitis C could have been improved when the TherapySelector would have been used. However, due to the development of pangenotypic direct-acting antiviral agents (DAAs), the need for such a guideline add-on might be limited for HCV. When novel HBV agents are licensed, the TherapySelector could be extended for this patient group. As the application is updated monthly, maintenance could be time-consuming and expensive. In spite of this, these limitations are marginal when applying a very specific search string to select relevant high-quality studies (**chapter 11, Supplementary Table 1**). Next, to enhance its use in clinical practice, publicity among physicians will be required.

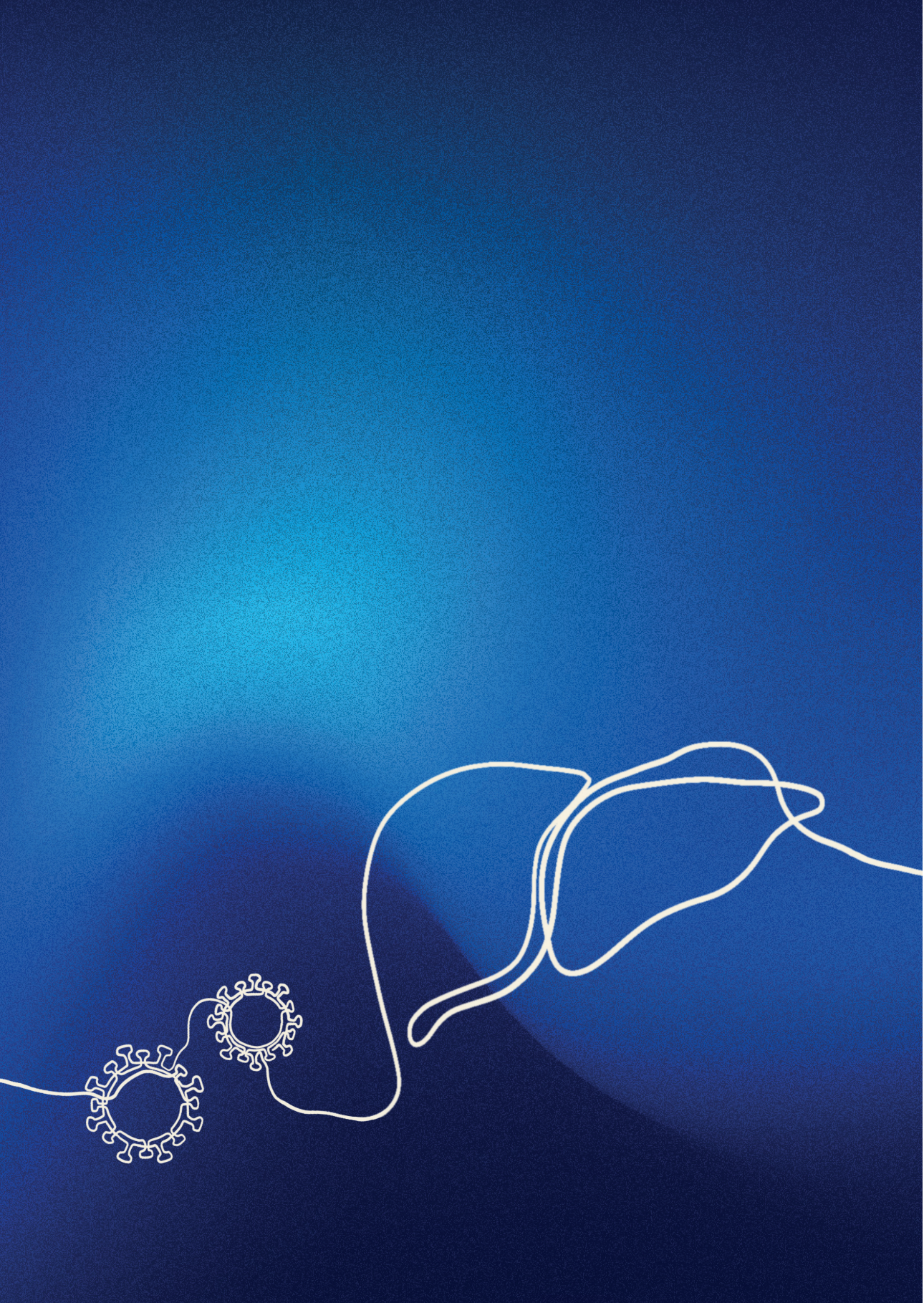
Finally, based on the findings of **chapter 10**, it might be debated whether viral hepatitis care should be centralised in high-expert hepatitis treatment centres. But first, it should be determined whether this is supported by Hepatologists and whether they have sufficient capacity to reschedule the care of hepatitis patients from the GPs to Hepatology departments.

Conclusion

Hepatitis B is a dynamic disease that is associated with liver-related morbidity and mortality. Currently, antiviral therapy is only able to suppress viral replication, but novel agents are emerging to eradicate HBV. To accurately evaluate the efficacy of these agents, a combination of serum biomarkers associated with cccDNA transcriptional activity must be used. Ceasing NA therapy must be handled with caution and only in a very selected group of patients and preferably executed in high-expert (academic) hospitals. In the years to come, trials exploring the efficacy of novel agents and how serum biomarkers can be used to evaluate the treatment outcome are needed.

For hepatitis C, very effective antivirals make it possible to cure every patient. However, before we can reach global elimination, LTFU patients must be retrieved, and undiagnosed individuals need to be identified and linked to care. Until high-risk groups such as migrants and incarcerated individuals are neglected, the elimination target may be jeopardized.

In conclusion, the care of patients with hepatitis B and C could be optimised when using serum biomarkers correlated with cccDNA activity. In addition, the use and adherence of clinical guidelines are essential, but also new innovations such as the add-on guideline TherapySelector could optimise the care for hepatitis patients further.



CHAPTER 12.3

Nederlandse samenvatting

Deel II van dit proefschrift beschrijft het onderzoek hoe HBV-biomarkers kunnen worden ingezet om de response op antivirale therapie te voorspellen en wat de uitkomsten zijn van het staken van nucleos(t)ide analogen bij chronisch hepatitis B patiënten.

Hoofdstuk 2 beschrijft hoe we de kinetiek van HBV-biomarkers tijdens behandeling onderzochten bij patiënten die gedurende één jaar met peginterferon alfa-2a (PEG-IFN) werden behandeld. Vervolgens bestudeerden we of deze biomarkers de response op behandeling konden voorspellen. We toonden aan dat een daling in HBV RNA 24 weken na het starten van de behandeling, geassocieerd was met een hogere kans op een duurzame response (HBV DNA < 2,000 IU/mL zes maanden na einde van behandeling); 27% versus 13% bij patiënten zonder daling in HBV RNA. Echter, werd er bij 56% van de patiënten met een daling in HBV RNA geen gelijktijdige daling in HBsAg-spiegels waargenomen. Bij deze patiënten was de kans op een duurzame response significant lager ten opzichte van patiënten die wel een gelijktijdige daling in HBsAg hadden; duurzame response werd bereikt in 48% versus 16% van de patiënten met > 1 log HBsAg-daling versus de patiënten met < 0.5 log HBsAg-daling. De bevindingen waren consistent voor de biomarker HBcrAg en wanneer response werd gedefinieerd als HBsAg-verlies. Deze bevindingen suggereren dat een combinatie van virale biomarkers moet worden gebruikt om accuraat de response op antivirale therapie te voorspellen.

Hoofdstuk 3 beschrijft de studie van de correlatie tussen anti-HBc en andere HBV-biomarkers, intra-hepatisch inflammatoire activiteit, en response op behandeling. We observeerden dat anti-HBc-spiegels correleren met leeftijd, IP-10, HBV DNA, HBcrAg, HBsAg en HBV RNA-spiegels bij onbehandelde HBeAg-positieve patiënten, maar niet bij HBeAg-negatieve patiënten. Daarnaast varieerden anti-HBc-spiegels tussen de verschillende HBV-genotypen. De hoogste spiegels werden waargenomen bij patiënten met HBV-genotype A, en de laagste spiegels bij patiënten met HBV-genotype D. Daarnaast toonden we aan dat anti-HBc-spiegels correleren met de ernst van intra-hepatische inflammatie. Patiënten met hoge anti-HBc-spiegels hadden een verhoogd risico op ernstige inflammatie. Tot slot onderzochten we de associatie tussen anti-HBc en response op behandeling. We observeerden dat anti-HBc-spiegels hoger waren bij patiënten die niet werden behandeld, dan patiënten die wel werden behandeld met PEG-IFN. Overeenkomstig met deze bevindingen daalden de anti-HBc-spiegels tijdens de behandeling met antivirale therapie. Hogere anti-HBc-spiegels waren geassocieerd met gunstige uitkomsten zoals HBeAg-verlies, duurzame response, HBsAg-daling en HBsAg-verlies. Deze bevindingen benadrukken het belang van B-cellen in de beheersing van de HBV-infectie en suggereren dat de toepassing van kwantitatieve anti-HBc-spiegels nuttig zou kunnen zijn in de klinische praktijk.

Hoofdstuk 4 beschrijft het onderzoek naar de relatie tussen ALAT-opvlammingen en virologische uitkomsten plus de vraag of serum HBV-biomarkers deze ALAT-opvlammingen konden voorspellen. Gebruikmakend van een gepoold cohort van PEG-IFN-behandelde patiënten, toonden we aan dat ALAT-opvlammingen (ALAT-spiegels > 5x ULN), die optraden binnen zes maanden na het staken van de behandeling, geassocieerd waren met een lagere kans op een duurzame response ten opzichte van patiënten zonder een ALAT-opvlamming (3.5% versus 26.8%). Derhalve zullen ALAT-opvlammingen die ontstaan na staken van antivirale therapie, moeten worden gezien als een ongewenste gebeurtenis. Daarnaast observeerden we dat hogere HBsAg-, HBV RNA- of HBcrAg-spiegels op het einde van behandeling geassocieerd waren met een hoger risico op ALAT-opvlammingen, maar met een lagere kans op een duurzame response of HBsAg-verlies. In het bijzonder kon de uitkomst accuraat worden voorspeld indien deze biomarkers werden gecombineerd (HBsAg + HBV RNA of HBsAg + HBcrAg). Deze bevindingen kunnen worden toegepast in de klinische praktijk voor het selecteren van patiënten die mogelijk geschikt zijn om hun antivirale therapie te staken en om handvatten te bieden ten aanzien van de begeleiding van deze patiënten.

Hoofdstuk 5 beschrijft een prospectieve studie waarin de nucleos(t)ide analogen (NA) werden gestaakt bij 33 HBeAg-negatieve patiënten. We toonden aan dat na 96 weken, 39% van de patiënten een duurzame response hadden bereikt (HBV DNA < 2,000 IU/mL) en 12% HBsAg-verlies. Echter, werd er een ernstige hepatische opvlamming (ALAT > 10x ULN) in 21% van de patiënten geobserveerd. Geen van de patiënten ontwikkelde leverdecompensatie of is overleden.

Hoofdstuk 6 beschrijft een casus van een HBeAg-positieve patiënt die zijn nucleotide analoog staakte en waarbij vervolgens een vervolgafspraak na een aantal maanden werd gepland. Hij ontwikkelde in de tussentijd een virale relapse, wat uiteindelijk acuut leverfalen veroorzaakte. Ondanks dat deze patiënt een levertransplantatie onderging, overleed hij aan de gevolgen van postoperatieve complicaties. De onderzoeken die beschreven zijn in deze twee hoofdstukken, tonen aan dat het staken van NAs mogelijk is, maar alleen in een zeer selecte groep HBeAg-negatieve patiënten en indien nauwgezette follow-up kan worden gegarandeerd. De hogere kans op HBsAg-verlies moet dus worden afgewogen tegen het risico op ernstige ALAT-opvlammingen.

Deel III van dit proefschrift beschrijft het onderzoek hoe heropsporing van uit zorg verdwenen patiënten kan bijdragen aan hepatitis C eliminatie in Nederland.

Hoofdstuk 7 beschrijft een nationaal heropsporingsproject, waarbij in deze studie 45 centra in Nederland zijn geïncludeerd. Patiënten die uit zorg waren verdwenen, werden geïdentificeerd op basis van laboratoriumuitslagen en de beoordeling van patiëntendossiers. Patiënten die geschikt waren voor heropsporing (gedefinieerd als

in leven zijn en met een adres geregistreerd in Nederland), werden uitgenodigd voor herevaluatie en behandeling op de polikliniek. We toonden aan dat de meerderheid (65%) van de ooit-gediagnosticeerde al reeds behandeld, of nog onder poliklinische controle waren. In totaal was 8% van de patiënten geschikt voor heropsporing. Van deze patiënten werd uiteindelijk 14% succesvol naar de poli verwezen. Dit project ondersteunt dat micro-eliminatie middels heropsporing van deze groep patiënten haalbaar is en zou kunnen bijdragen aan de eliminatie van hepatitis C, maar dat het ook tijdrovend is.

Deel IV van dit proefschrift beschrijft het onderzoek van de navolging van klinische richtlijnen.

Hepatitis B reactivatie (HBVr) is een ernstige complicatie bij patiënten die worden behandeld met hoog-risico immunosuppressiva, met name met het anti-CD20 monoklonaal geneesmiddel rituximab. Aangezien dit HBVr-risico aanwezig is bij zowel patiënten met een actieve als doorgemaakte hepatitis B infectie, moet elke patiënt voorafgaand aan de start met rituximab worden gescreend. Vervolgens wordt aangeraden om te starten met antivirale profylaxe bij elke patiënt met een (doorgemaakte) hepatitis B infectie. **Hoofdstuk 8** beschrijft de uitvoering en resultaten van een multicenter cohortstudie, waarbij de bevinding was dat slechts 46% van de patiënten correct werd gescreend (bepaling van zowel HBsAg en anti-HBc). Opvallend was dat de screeningspercentages significant verschilden tussen academische en niet-academische ziekenhuizen (respectievelijk 65% versus 32%), en tussen medische specialismen. Screeningspercentages waren opvallend genoeg niet per se hoger onder hoog-volume voorschrijvers (zoals hematologen) ten opzichte van laag-volume voorschrijvers. Wel verbeterden de screenings-percentages door de jaren heen; 32% voor 2012 tot 75% na 2020 in het academisch ziekenhuis en respectievelijk 1% tot 60% in de niet-academische ziekenhuizen. Bij patiënten met een chronische of doorgemaakte hepatitis B infectie werd antivirale profylaxe gestart bij respectievelijk 58% versus 36%. Zeven patiënten ontwikkelde een HBVr. Van deze patiënten overleed een patiënt aan de gevolgen van leverdecompensatie. Dit benadrukt het belang van het screenen van patiënten die worden behandeld met hoog-risico immunosuppressiva, alsmede het starten van antivirale profylaxe bij patiënten met een (doorgemaakte) hepatitis B infectie.

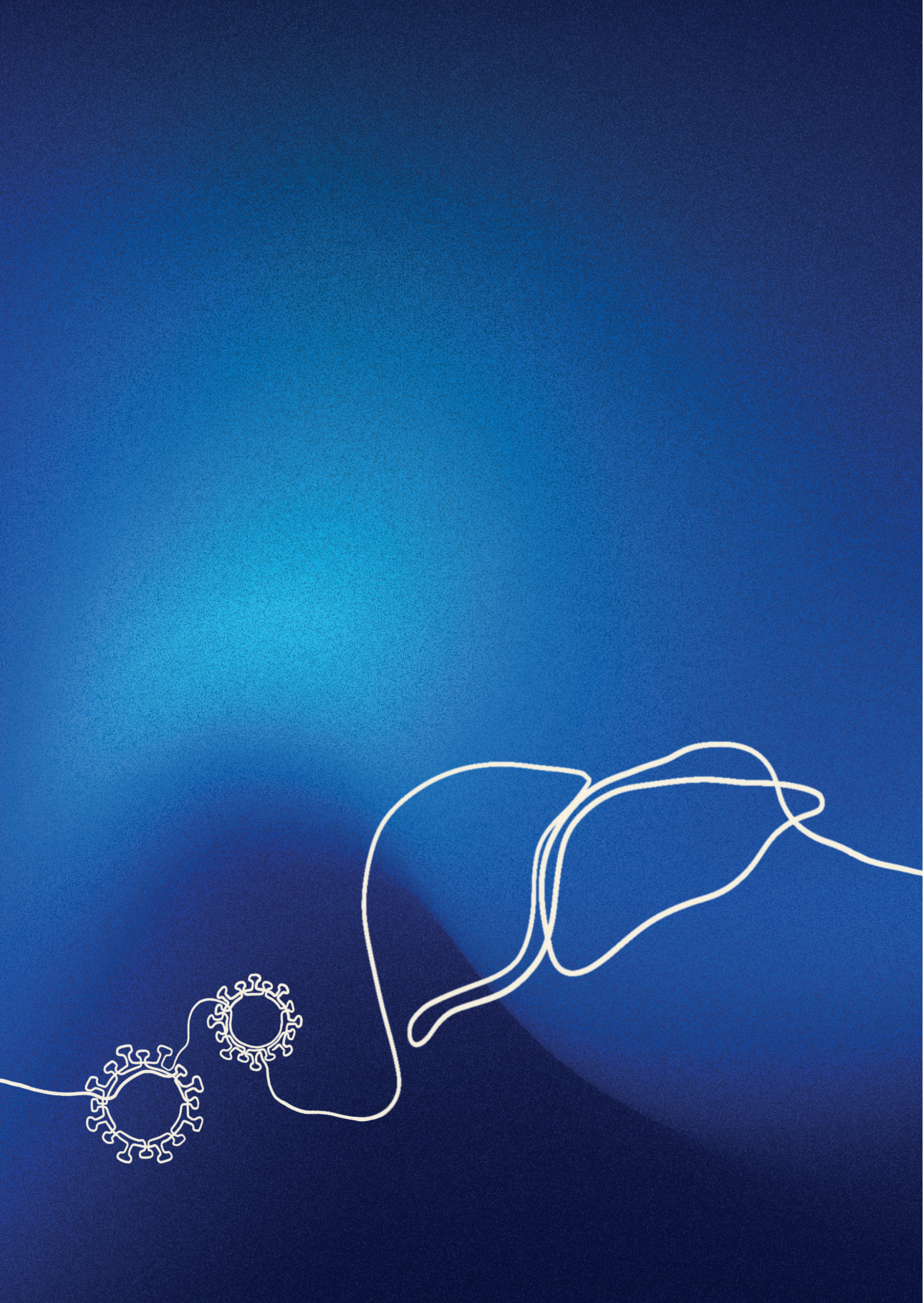
De huisarts speelt in Nederland een belangrijke rol bij de identificatie van patiënten met hepatitis B en C. De huidige richtlijn voor huisartsen adviseert halfjaarlijkse ALAT-controle en verwijzing van elke patiënt met een actieve hepatitis B of C infectie naar een gespecialiseerd hepatitisbehandelcentrum. **Hoofdstuk 9** beschrijft het onderzoek naar de navolging van deze richtlijn middels een grote database van huisartsendossiers afkomstig uit een multi-etnisch gebied in Nederland. We observeerden dat respectievelijk 71% en 89% van de chronische hepatitis B en C patiënten ooit in hun leven zijn verwezen naar een hepatitisbehandelcentrum.

Echter, werd een substantieel deel van de patiënten niet adequaat gesurveilleerd of curatief behandeld gedurende follow-up. Bij slechts 15% van de chronische hepatitis B patiënten werden regelmatig ALAT-controles uitgevoerd door de huisarts en 46% was reeds onder behandeling van een medisch specialist. Dit betekent dat 36% van de patiënten niet adequaat werd gesurveilleerd. Daarnaast werd 74% van de hepatitis C patiënten ooit behandeld met antivirale middelen. Echter, had slechts 54% een bewezen virale eradicatie. Deze bevindingen zijn belangrijk om het bewustzijn te vergroten onder huisartsen en medisch specialisten, en het impliceert dat heropsporing van uit zorg verdwenen patiënten in de huisartspraktijk waardevol kan zijn.

Hoofdstuk 10 beschrijft het onderzoek naar de naleving van de klinische hepatitis B richtlijn, met eventuele voorspellers van inadequate naleving, in een high-expert academisch ziekenhuis in Nederland. Chronische hepatitis B patiënten die in 2020 de polikliniek hadden bezocht, werden geïncludeerd. We observeerden dat 97% van de patiënten met een indicatie voor antivirale behandeling ook daadwerkelijk werd behandeld, en dat 90% van de patiënten met een verminderde nierfunctie of botziekte werd behandeld met entecavir of tenofovir alafenamide. Screening op co-infecties met hepatitis A, C of D was uitgevoerd in > 86% van de patiënten, maar screening op HIV bij slechts 78% van de patiënten. De meerderheid van de patiënten met een indicatie voor HCC-surveillance werd ook adequaat gesurveilleerd (88%). Opvallend was de bevinding dat de navolging van de richtlijn beter was bij patiënten die onder controle waren op de gespecialiseerde hepatitiskliniek ten opzichte van de algemene hepatologiekliniek. Deze bevindingen suggereren dat hepatitis B behandeling zou moeten worden gecentraliseerd naar high-expert klinieken.

Deel IV van dit proefschrift beschrijft het onderzoek naar het individualiseren van de zorg van hepatitispatiënten buiten de algemene klinische richtlijnen om.

Hoofdstuk 11 beschrijft het onderzoek naar de toegevoegde waarde van een add-on richtlijn voor de behandeling van chronische hepatitis C patiënten. Deze add-on richtlijn, de HCV TherapySelector, toont middels een mobiele of web-gebaseerde applicatie de effectiviteit van verschillende behandelopties voor de individuele patiënt op basis van zijn/haar specifieke patiënten profiel, gebaseerd op hoogwaardige studies. Doordat deze applicatie maandelijks wordt bijgewerkt, toont het up-to-date informatie. In ons onderzoek toonden we dat de behandeling van patiënten geoptimaliseerd kon worden als de TherapySelector zou zijn gebruikt. Het gebruik van een dergelijke add-on richtlijn kan nuttig zijn bij ziekten met suboptimale genezingspercentages of klinisch relevante bijwerkingen, waarvoor behandelings-opties afhangen van specifieke patiëntkenmerken. Dit artikel moet daarom worden gezien als een proof of concept.



APPENDICES

References
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Abbreviations

AASLD	American Association for the Study of Liver Diseases
ALT	alanine aminotransferase
anti-HBc	antibodies to hepatitis B core antigen
anti-HBs	antibodies to hepatitis B surface antigen
anti-HCV	antibodies to hepatitis C virus
aOR	adjusted odds ratio
APASL	The Asian Pacific Association for the Study of the Liver
BL	baseline
c/mL	copies/millilitre
CAMs	capsid assembly modulators
cccDNA	covalently closed circular DNA
cDNA	complementary DNA
CELINE	Hepatitis C Elimination in the Netherlands
CHB	chronic hepatitis B
CHC	chronic hepatitis C
CI	confidence interval
DAA	direct-acting antivirals
DCV	daclatasvir
DSV	dasabuvir
EASL	European Association for the Study of the Liver
ELB	elbasvir
EMA	European Medicine Agency
EOF	end of follow-up
EOT	end-of-treatment
ETV	entacavir
FDA	Food and Drug Administration
GLE	glecaprevir
GP	general practitioner
GRZ	grazoprevir
HAI	histological activity index
HAV	hepatitis A virus

HBcAg	hepatitis B core antigen
HBcrAg	hepatitis B core-related antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HBVr	hepatitis B reactivation
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDV	hepatitis delta virus
HIV	human immunodeficiency virus
ICARUS	Improve CARe for cUrrent and future patientS with chronic hepatitis B in the Netherlands
ICPC	International Classification of Primary Care
ICU	intensive care unit
int	intermediate
IP-10	interferon-y inducible protein 10
IQR	interquartile range
IU	international units
LAM	lamivudine
LDV	ledipasvir
LLOQ	with a lower limit of quantification
LOD	lower limit of detection
LTFU	people who inject(ed) drugs
MEB	Medicine Evaluation Board
MSM	men who have sex with men
n	number
NA	nucleos(t)ide analogues
NA	not applicable
NAPs	nucleic acid polymers
OMV	ombitasvir
OR	odds ratios
ORF	open reading frames
PCR	real-time polymerase chain reaction
PEG-IFN	pegylated interferon
PIB	pibrentasvir

PPDRTD	patient profile therapy regimen data
PTV	paritaprevir
PWID	people who inject(ed) drugs
PY	person-year
RACE	rapid amplification of complimentary DNA (cDNA)-ends
RBV	ribavirin
RCT	radomised controlled trial
RTV	ritonavir
SD	standard deviation
SHM	Stichting HIV monitoring
SIM	simeprevir
SMPCs	Summary of Product Characteristics
SOF	sofosbuvir
SR	sustained response
SVR	sustained virological response
TAF	tenofovir alafenamide
TDF	tenofovir disoproxil
tiab	title and abstract
TNF	tumour necrosis factor
TS	TherapySelector
U	units
ULN	upper limit of normal
VEL	velpatasvir
VOX	voxilaprevir
WHO	World Health Organisation
wk	week

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20. **A new area of medical decision-making: The TherapySelector as an add-on to clinical guidelines.** Brakenhoff SM, Theijse T, van Wijngaarden P, Trautwein C, Brozat JF, Tacke F, Honkoop P, Vanwolleghem T, Posthouwer D, Zeuzem S, Mihm U, Wedemeyer H, Berg T, Schalm S, de Knegt RJ. *Submitted*.

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About the Author

Sylvia Merel Brakenhoff was born on June 18th 1992, in Hilversum, the Netherlands. In 2010, she graduated secondary school at the Comenius College, Hilversum. From 2010 until 2012 she studied Gezondheid & Leven at de Vrije Universiteit, Amsterdam, before being admitted to medical school at the University of Amsterdam in 2012. During the bachelor, she attended the Honours Programme.

During the study Medicine, her interest in hepatology, especially viral hepatitis, emerged. She investigated the immuno-logical response during an acute hepatitis B infection (bachelorthesis) and liver stiffness improvement among successfully treated hepatitis C patients (masterthesis). She went to the Diakonesse hospital in Paramaribo, Suriname, for an additional internship. Her semi-arts internship was at the department of Gastroenterology and Hepatology of the Rode Kruis hospital, Beverwijk.

After medical school she started her PhD program under supervision of Prof. dr. R.A. de Man and dr. R.J. de Knegt, at the department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam. During her PhD program, she was member of HepNed and HCV richtsnoer.

In December 2021, she started as resident not in training (ANIOS) at the department of internal medicine at the Reinier de Graaf Gasthuis, Delft. From March 2023 onwards, she started with her Internal Medicine residency at the Reinier de Graaf Gasthuis as part of her specialist training in Gastroenterology and Hepatology at the Erasmus MC, Rotterdam.