Clinical studies on hepatitis B, C, and E virus infection

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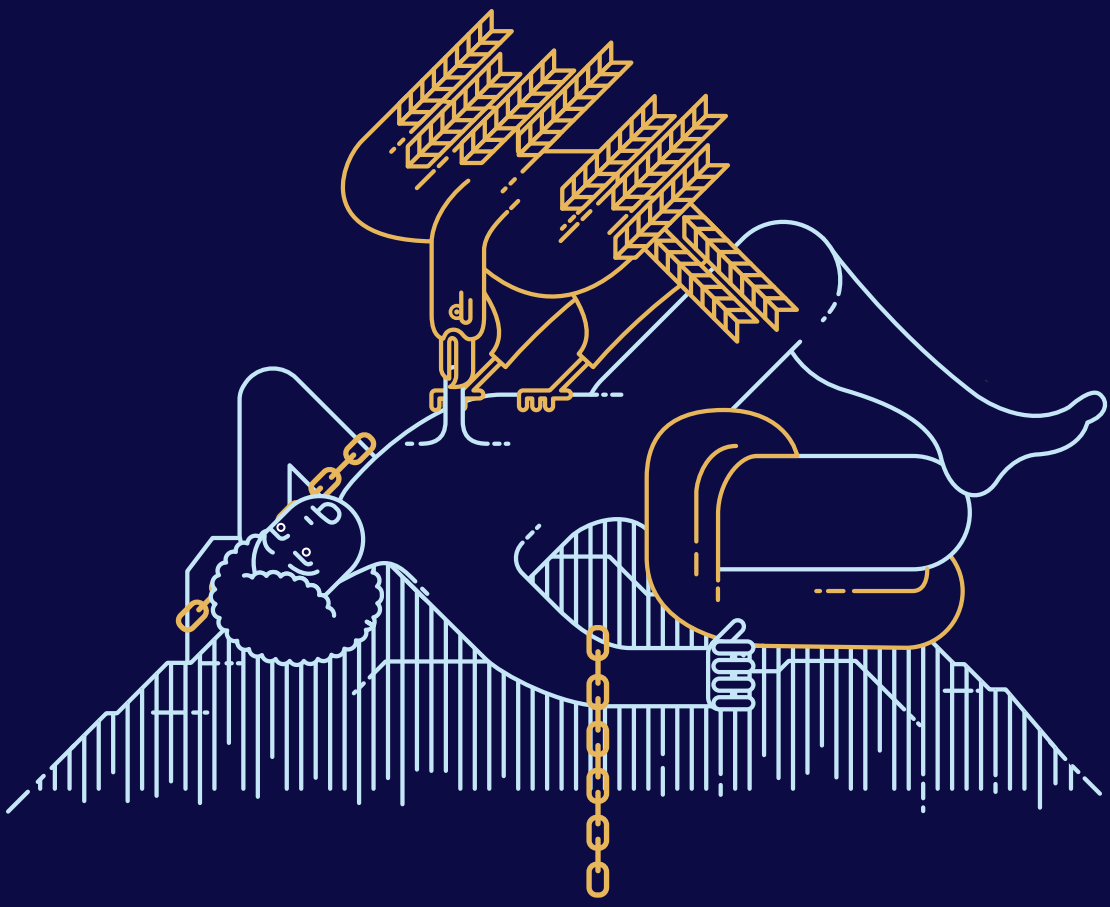
Sophie Willemse



Clinical Studies on Hepatitis B, C, and E Virus Infection

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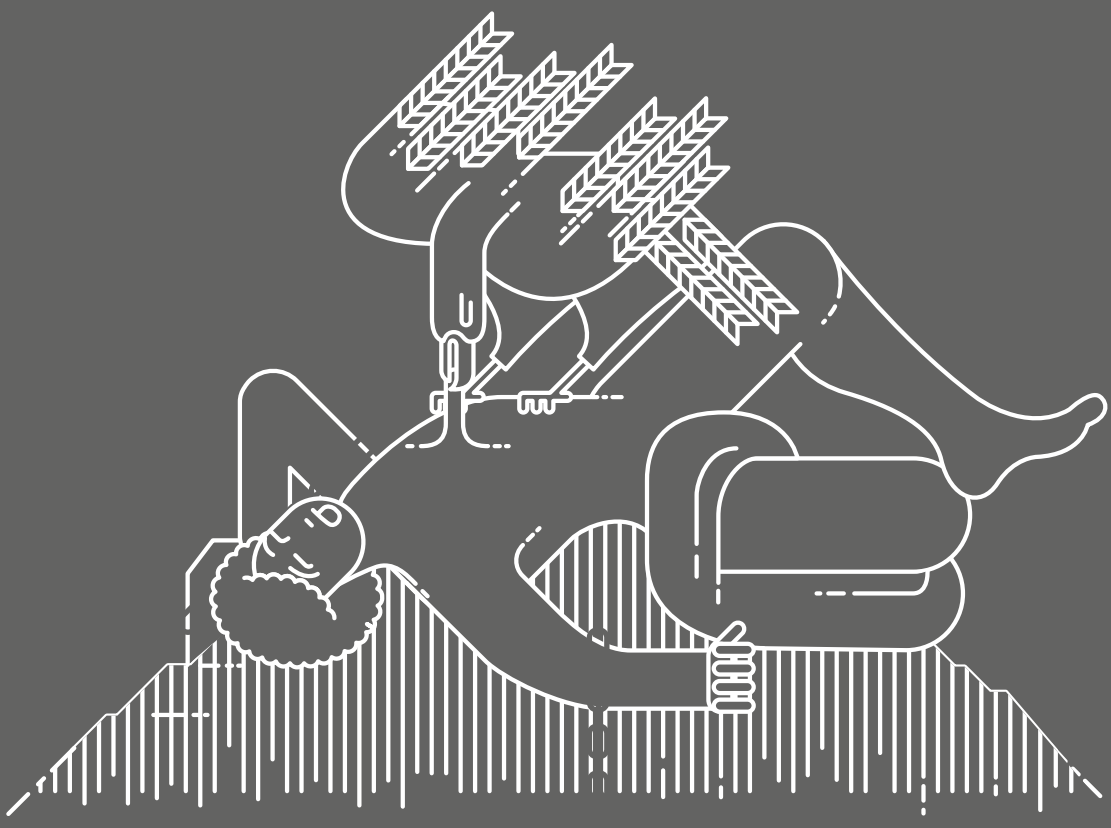

Cover: Prometheus bound after Matthijs Koppen

The titan Prometheus created men from clay and stole fire from Zeus to comfort humanity. He was punished by Zeus for this act by being bound to a rock and have an eagle (Ethon) feed on his liver each day. During the night, Prometheus' liver would grow back, to then have it eaten again next day, making the punishment one of perpetual suffering. Zeus then punished humanity by sending Pandora, and her box, unleashing disasters. In the end, Prometheus was freed by the hero Hercules. Prometheus' persona symbolises audaciousness, inventiveness and perseverance; traits that are essential for every researcher.

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Clinical Studies on Hepatitis B, C, and E Virus Infection

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— voor Piet —

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General Introduction and Outline of the Thesis

GENERAL INTRODUCTION

HEPATITIS B VIRUS INFECTION

Virus and epidemiology

The hepatitis B virus (HBV) is an enveloped double-stranded DNA virus and it belongs to the family of *Hepadnaviridae*. Worldwide, there are over 350 million people chronically infected with HBV, with the highest prevalence in South-East Asia and Africa.[2] As much as one third of the global population has encountered HBV-infection at some point in their life. Every year HBV-infection is responsible for over 780,000 deaths.[2–4] Transmission of HBV may occur via different routes, being sexually, vertically (perinatal), and via blood-blood-contact. Perinatal transmission will lead to chronic HBV-infection in the majority of infected infants (>95%).[3,4] When an acute HBV-infection is encountered later in life, the virus will be spontaneously cleared in most cases, but the chance of chronicity increases again in individuals who are infected with HBV >65 years. Approximately 5–10% of immunocompetent adult patients fail to clear the virus and become chronically infected with HBV.[5] Patients with chronic HBV-infection (CHB) are at risk to develop liver cirrhosis (5-year cumulative incidence of 8–20%)[6,7] and hepatocellular carcinoma (HCC) (annual risk 2–5%).[8]

The prevalence of chronic HBV-infection in The Netherlands is 0.2–0.4% (34,000–68,000 individuals).[9–11] Serological testing in 1995–1996 has shown that 2.1% of the population has ever had contact with the HBV.[12] In 2006, the incidence of acute HBV-infection was highest in the Amsterdam and Rotterdam regions of The Netherlands (3.9 and 3.6 per 100,000 respectively).[13,14] The overall incidence in The Netherlands in 2016 was 0.6 per 100,000.[15] The prevalence and incidence of acute and chronic HBV-infection is higher

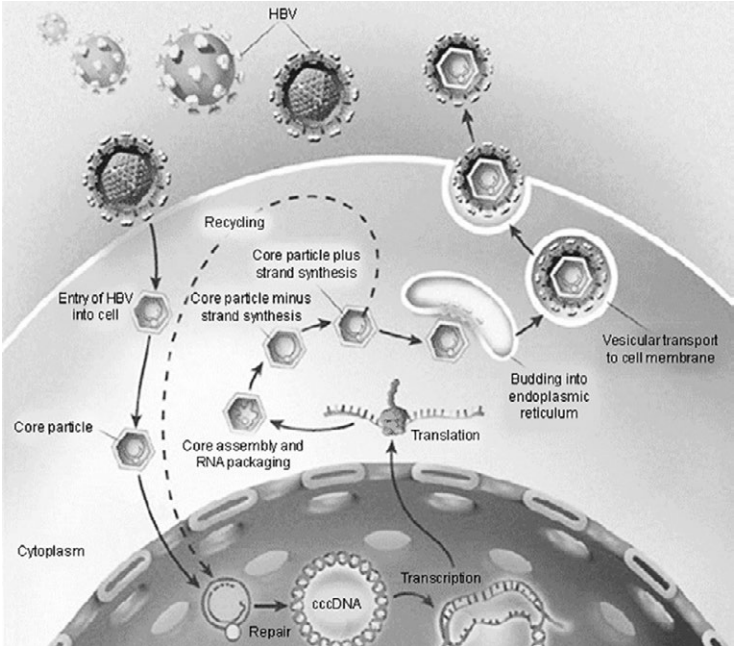

Figure 1. Life cycle of hepatitis B virus. Reproduced with permission from: Ganem D and Prince AM, N Engl J Med 2004.[1] © Massachusetts Medical Society.

among migrants from high-prevalence countries and individuals with risk behaviour (i.e. past injecting drug use, multiple sexual partners, male homosexual activity (MSM)).[3, 4, 10–15]

Phases of HBV-infection

During CHB, multiple phases are distinguishable, which not always occur sequentially (Figure 2.):[7]

– The HBeAg-positive *immune-tolerant phase* is the first phase of CHB. It is characterised by presence of hepatitis B e antigen (HBeAg), high HBV-DNA levels, but normal inflammatory parameters (serum alanine transferase (ALT), necro-inflammation on liver biopsy). This might last for decades, especially in subjects infected during the early years of life. There is no, or slow, progression of liver fibrosis.[16]

– The HBeAg-positive *immune-reactive phase* is the next phase of CHB, which is characterised by high or fluctuating inflammatory activity (reflected by elevated serum ALT) causing liver damage (possibly leading to fibrosis and cirrhosis) and a decrease in HBV-DNA levels. HBeAg-seroconversion might occur, resulting in the next phase.

– The HBeAg-negative inactive HBV *carrier state (or low-replicative phase)* follows the immune-reactive phase, when HBeAg is cleared and anti-hepatitis B e (anti-HBe) antibodies have been formed. HBV-DNA is low (< 2,000 IU/mL) and there is low inflammatory activity (reflected by serum ALT and no necro-inflammation on liver biopsy) resulting in minimal liver damage in most patients. HBsAg-loss and seroconversion to anti-HBs antibody might occur spontaneously in 1–3 % of cases per year, usually after several years with persistently undetectable HBV-DNA.[17]

– The *HBeAg-negative CHB phase* may follow HBeAg-seroconversion during the immune reactive phase or may develop from the *inactive carrier state*.[16] This phase is characterised by fluctuating high HBV-DNA levels (> 2,000 IU/mL) and inflammatory activity (reflected by elevated serum ALT and necro-inflammation in liver biopsy), leading to progressive liver damage (and, again, possibly to liver cirrhosis). Spontaneous clearance of hepatitis B surface antigen (HBsAg) and seroconversion to anti-hepatitis B surface (anti-HBs) antibodies, also named *functional cure,* might occur in a limited percentage of patients (1–3 % per year).[18, 19]

– During the *HBsAg-negative phase*, low-level HBV-replication may persist with detectable HBV-DNA in liver, but not in serum (or in very low levels, < 200 IU/mL), and presence of anti-HBc antibodies with or without anti-HBs.[20] Immunosuppression may lead to reactivation in these individuals.[21] If HBsAg-loss occurs before the development of cirrhosis, the outcome is improved with reduced risk of cirrhosis, decompensation and HCC. However, if cirrhosis has developed before HBsAg-clearance (13 %), patients remain at risk of HCC (3 %) and decompensation (14 %), especially if there are concomitant liver diseases.[22]

Approximately 70% of all CHB patients have HBeAg-negative CHB with minimal inflammatory activity.[23]

Immune reaction in HBV-infection

To eliminate HBV after an acute infection, both the innate and the adaptive immune system are significant.[25–28] The innate immune system is needed for early containment of the virus and initial activation of adaptive immune responses by producing interleukin 6 (IL-6) and type I interferon (IFN-I). IL-6 activates innate effector molecules, and IFN-I has direct antiviral effects, improves antigen presentation and activates natural killer T-cells (NK-cells).[29] NK-cells have a direct antiviral effect by producing interferon-gamma (IFN-y). NK-cells can also modulate T-cell responses, which is important for lysis of virus-infected hepatocytes. When chronic infection is established, a strong adaptive immune response, primed by the innate immune response, is needed for viral clearance.[26, 30, 31]

NK-cells are activated during acute infection.[32, 33] Thereafter, functionally active HBV-specific T-cells can be detected.[26] In most individuals with acute resolving HBV-infection, strong and broad virus specific T-cell responses directed against the HBV-infected hepatocytes can be detected. However, these responses are weak and narrowly focused in patients who develop chronic HBV-infection, resulting in low levels of antiviral cytokines and attenuated cytotoxic T-lymphocyte (CTL) activity.[26, 34–40]

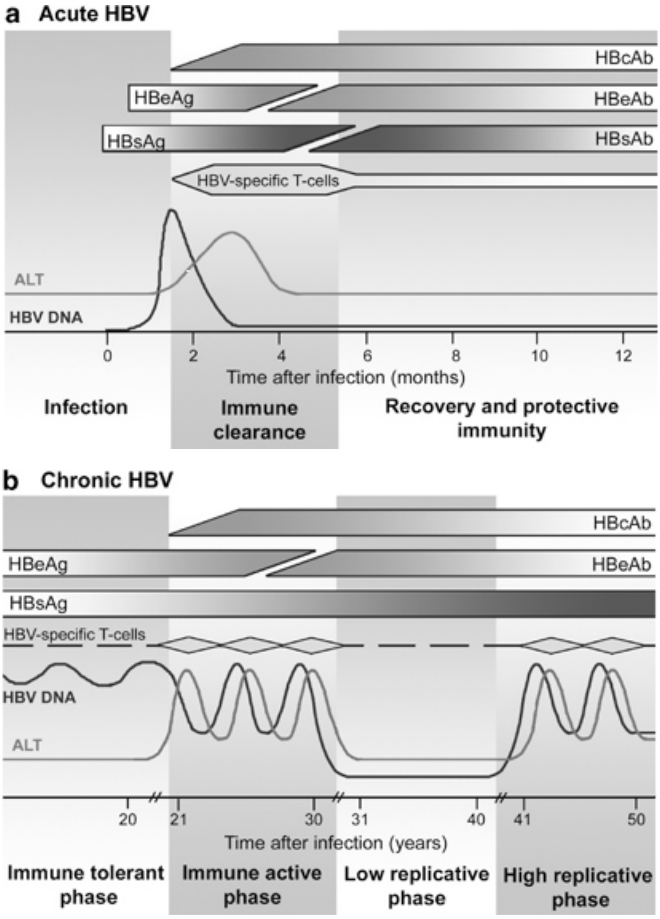

Figure 2. Serology and virology of HBV-infection. Adapted from: Chang JJ and Lewin SR. Immunol Cell Biol 2007.[24] Reproduced with permission, © Nature Publishing Group.

During chronic infection, HBV-specific T-cells are exhausted and their function is impaired.[41] Multiple mechanisms are thought to be responsible for chronicity of HBV-infection. Both the innate and the adaptive immune system may prevent liver damage by inducing a so-called immune-tolerant state. In this phase, HBV-specific CD8 T-cells are deleted by NK-cells[42] and cytotoxic T-cells are deviated to a suppressive or regulatory phenotype by priming through non-professional antigen-presenting cells (APCs). This attenuates the immune reaction which then is needed to clear the virus. However, it is still possible to induce an adequate immune reaction by IFN-I. On the other hand, HBV has the ability to avoid the development of an effective immune response by inhibiting adequate activation of the innate immune system.[43 – 46] This results in an immune tolerant state, hampering viral clearance. A further mechanism by which viral clearance is disturbed, is T-cell exhaustion, which is thought to be caused by repetitive T-cell receptor stimulation by high HBsAg-levels, and aggravated by a lack of signalling from T-helper cells and by unfavourable cytokines such as interleukin-10 or arginase.[47, 48] However, whether HBV-specific T-cells are functionally active during the initial phases of infection is still unknown.

Various markers have been implicated to play a role in viral clearance of HBV and prediction of response to antiviral treatment. Amongst those are pre- or on-treatment HBsAg-levels and different cytokines or chemokines such as arginase, interleukin 10 (IL-10)[47, 48] and interferon-gamma-inducible protein 10 (IP-10). Levels of IP-10 are higher in CHB patients than in healthy controls.[49, 50] In CHB patients, serum IP-10 is positively correlated with serum HBV-DNA, serum ALT-levels, and progressive liver disease.[26,51 – 54] Moreover, high pre-treatment serum IP-10 levels have been associated with HBeAg-loss during or after treatment with peginterferon (pegIFN)[55] or nucleos(t)ide analogues (NAs).[56]

Treatment of chronic HBV-infection

The goal of antiviral treatment in patients with CHB is to prevent progression to cirrhosis, hepatic decompensation and HCC by reducing viral replication and/or loosing HBsAg, (functional cure).[7, 57] Following the current treatment guidelines, the indications for treatment of CHB are generally the same for HBeAg-positive and HBeAg-negative patients, and are mainly based on serum HBV-DNA levels, serum ALT-levels and severity of liver disease. Treatment with antiviral therapy should be considered in CHB patients with the following characteristics:[7]

– HBV-DNA levels above 2,000 IU/mL, ALT above the upper limit of normal (ULN), and severity of liver disease assessment showing at least moderate or severe active necro-inflammation (liver biopsy) or moderate liver fibrosis (liver biopsy/fibroscan) using a standardised scoring system such as HAI,[58] Ishak[59] or METAVIR[60];

– Liver cirrhosis with any detectable HBV-DNA and regardless of ALT-levels;

– HBV-DNA > 20,000 IU/mL and ALT > 2x ULN regardless of degree of fibrosis;

– Family history of HCC or cirrhosis and extrahepatic manifestations;

– Patients > 30 years who are still in the immune-tolerant phase with persistently high HBV-DNA and normal ALT-levels, regardless of degree of liver fibrosis.

For CHB patients with a low viral load, (HBV-DNA < 2,000 IU/mL) and no signs of necro-inflammatory activity or fibrosis, there is currently no indication for treatment, as viral replication is already low, and the progression of liver disease seems slow.[7, 61, 62] However, CHB patients with a low viral load are at risk for the development of cirrhosis and HCC.[61–68] As was shown in earlier cohorts, the risks for developing cirrhosis or HCC are highest in patients with the highest viral load (HBV-DNA > 200,000 IU/mL), however are already increased in those with a low viral load (HBV-DNA < 2,000 IU/mL).[64, 66] Furthermore, CHB patients with a low viral load can progress to HBeAg-negative chronic hepatitis, which could lead to fulminant hepatitis or cirrhosis.[16, 69] The threshold for starting anti-viral therapy based on HBV load has therefore been lowered to > 2,000 IU/mL in the current treatment guidelines[7] compared to 20,000 IU/mL in the older treatment guidelines.[6, 57]

There are three different types of responses to therapy in CHB patients:

– Functional cure, defined as: HBsAg-loss with (HBs-seroconversion) or without formation of anti-HBs antibodies;

– Combined response, defined as: HBeAg-loss, low HBV-DNA (< 2,000 IU/mL) and normal ALT in HBeAg-positive CHB patients; or low HBV-DNA < 2,000 IU/mL) and normal ALT in HBeAg-negative CHB patients;

– No HBeAg-loss, but low HBV-DNA (< 2,000 IU/mL) and normal ALT in HBeAg-positive CHB patients.

Currently available treatment options for CHB are nucleo(s)tide analogues (NAs) and peginterferon (pegIFN). NAs inhibit HBV-DNA synthesis by competitive interaction with the natural substrates of the HBV-polymerase.[70] The compounds registered in The Netherlands are: adefovir, tenofovir disoproxil and tenofovir alafenamide (all nucleotide analogues), lamivudine, telbivudine and entecavir (all nucleoside analogues) and emtricitabine (nucleotide analogue, prescribed in combination with tenofovir for HIV/HBV co-infection). All NAs are to be taken by a single oral dose. Because of their low level of resistance (in contrast to the other NAs), tenofovir and entecavir are the treatment options of first choice amongst the various NAs. The advantages of NAs are their effectiveness in suppressing HBV-replication and their good safety profile. Adequate viral suppression defined as undetectable levels of HBV-DNA in serum is achieved in >90% of patients treated with tenofovir or entecavir for 3 years.[71, 72] Long-term viral suppression improves clinical outcome in CHB patients, in terms of progression to cirrhosis, hepatic decompensation and development of HCC.[71–73] Recent studies have shown that longstanding treatment with NAs can result in a reversion of liver fibrosis or even cirrhosis.[71] NAs only inhibit HBV production, and not the production of viral proteins. Therefore, treatment with NAs rarely leads to loss of HBsAg. Stopping treatment would lead to recurrence of HBV-DNA in most cases,[74] and long-term, usually life-long, treatment is therefore required.

Treatment with pegIFN on the other hand, is of finite duration of 48 weeks with the goal to induce maintained immunological control. PegIFN is administered subcutaneously once per week and has direct antiviral, but mostly immune modulatory effects.[75] The applicability of treatment with pegIFN is generally poor as a result of its many side-effects (such as flu-like symptoms, hematological disorders, and depression), and a relatively low rate of response of 20–30% (HBeAg-seroconversion in HBeAg-positive patients or sustained low HBV-DNA (< 2,000 IU/mL) in HBeAg-negative patients).[76–78] However, in selected HBeAg-positive CHB patients with a combination of certain baseline characteristics, higher response rates (HBeAg-loss) of above 50% may be achieved. These baseline predictors of HBeAg-loss are: genotype A, lower HBV-DNA levels (< 2x108 IU/mL), high ALT levels (> 2 ULN), female sex, older age (> 30 years) and absence of previous pegIFN therapy.[79]

The most favourable treatment outcome of CHB patients is a functional cure.[22, 80–82] This is rarely achieved with a finite course of pegIFN or long-term viral suppression with NAs: only for 0–3% in NA therapy and 4–12% for treatment with pegIFN for 48 weeks.[83]

Theoretically, it would be interesting to combine treatment with pegIFN and NAs because of their effect on modulating the immune response and their additional potent antiviral effect.[75, 84] Earlier attempts using this approach, with different compounds (lamivudine, adefovir, tenofovir and pegIFN) and in different settings (dual finite therapy or pegIFN add-on) showed varying results.[76, 85, 86] Combination of lamivudine and pegIFN showed no benefit compared to either of the two therapies alone in both HBeAg-positive and HBeAg-negative patients.[76–78] Combination therapy with pegIFN and adefovir[86] or tenofovir[85] in CHB patients with high viral load showed higher response rates than monotherapy with either medicament alone (pegIFN or NAs). However, these differences were small and might not weigh up to the increase in side effects caused by the addition of pegIFN. To achieve a better sustained therapeutic effect of treatment, new antiviral agents need to be developed.

HEPATITIS C VIRUS INFECTION

Virus and epidemiology

The hepatitis C virus (HCV) is an enveloped single-stranded RNA virus belonging to the family of *Flaviviridae*. The viral genome contains a long open reading frame of around 3011 codons encoding three structural proteins (core, E1 and E2), an ion channel (p7), and six non-structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B).88 There are seven different genotypes (1–7), differing by 30–35% over the whole genome, which are subdivided into various subtypes (a-k).[89]

HCV-infection is a leading cause of chronic hepatitis affecting over 170 million people worldwide.[90] After being exposed to HCV (via blood-blood exposure), a chronic infection develops in 75–80% of cases.[91] Chronic hepatitis C (CHC) virus infection is characterised by liver inflammation due to pro-inflammatory cytokines and infiltration of specific and non-specific T-lymphocytes.[91] This leads to liver fibrosis and could ultimately cause liver cirrhosis, HCC and death.[92] There is a large geographical variation in prevalence of HCV-infection and in many countries the epidemiology is not well known. At the same time, HCV-related mortality continues to increase as the infected population ages[92] and advances to late-stage liver disease.[93–95] In The Netherlands, estimates on

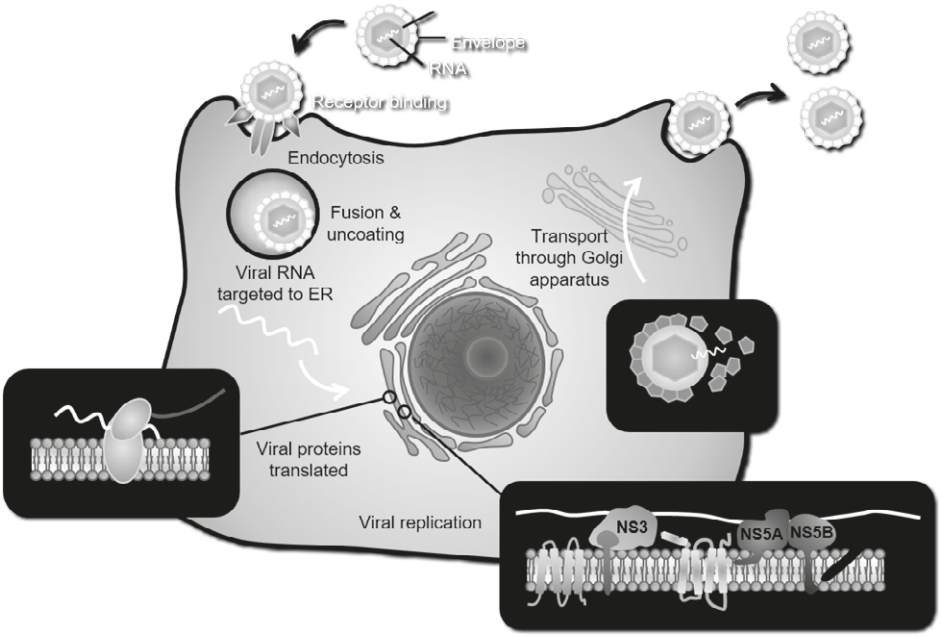

Figure 3. Life cycle of hepatitis C virus. Adapted from: Asselah T and Marcellin P. Liver Int 2011.[87] Reproduced with permission, © John Wiley and Sons.

antibody prevalence of HCV-infection vary from 0.1 to 0.6 %.[11, 96–99] The most recent and reliable nationwide estimate was 0.22 % (0.07 – 0.37 %) in Dutch inhabitants aged 15 – 79 years in 2009, incorporating prevalence data among different subpopulations,[96] corresponding with about 28,000 adult individuals ever infected with HCV. The risk groups of individuals with a chronic HCV-infection are mainly (ex)-drug users or migrants; those at risk of a getting infected with a new (acute) HCV-infection are mainly MSM. This situation is different in The Netherlands compared to many other countries, where HCV transmission among people who inject drugs (PWID) is ongoing, due to the extensive preventive measures among drug users such as awareness and clean needle programs.

Immune reaction in HCV-infection

After infection with HCV, the innate immune system initiates a non-specific immune response through type I IFN, leading to the activation of the intracellular pathway, resulting in the induction of multiple IFN-stimulated genes (ISGs). Type I IFN has also immunomodulatory effects by activating and modulating the function of different kinds of leukocytes, including NK-cells, macrophages, dendritic cells and T-lymphocytes. This results in a strong specific CD4+/CD8+ T-cell response leading ideally to the clearance of the virus.100 In acute HCV-infection, patients with self-limited infection have significant T-cell responses compared to little or no responses in those who evolve to chronicity.[101]

Many cytokines and chemokines have been identified to be involved in immune reactions in response to HCV-infection. Most of these cytokines are modulated by exogenous IFN and play a critical role in viral clearance.[102] In CHC, baseline activation of the immune system tends to be lower prior to treatment with IFN-based therapy

in patients who develop a sustained virological response (SVR) compared to those who do not.[103, 104] This difference of baseline activation of the immune system might be influenced by single nucleotide polymorphisms (SNPs) on chromosome 19 near the interleukin-28B gene (IL28B), encoding IFN-. IL28B gene polymorphisms are highly associated with treatment outcome in CHC patients treated with IFN-based therapy.[105] The gene encoding IP-10 is an ISG[106, 107] that is induced by IFN-y and tumour necrosis factor (TNF) alpha. IP-10 is produced by different kinds of cells such as endothelial cells, fibroblasts, mesangial cells, monocytes, neutrophils and hepatocytes. After binding to its receptor CXCR3, IP-10 functions as a chemotactic cytokine for T-lymphocytes, monocytes and NK-cells, and induces adhesion of activated memory/effector T-cells.[108] Serum levels of IP-10 are higher in CHC patients than in healthy controls.[109]

The relation between IP-10, viral clearance and response to antiviral therapy has extensively been described in acute and chronic HCV-infection.[110–118] Furthermore, it was shown in CHC patients that intrahepatic IP-10 mRNA expression and plasma IP-10 levels are correlated with one another.[113, 117, 118]

Treatment of chronic HCV-infection

Successful treatment of CHC has been challenging for some time. The first antiviral treatment available was recombinant IFN-alpha, which was first described in 1986, before the discovery of the virus in 1989.[119] Success rates of this treatment were 38 % (assessed using ALT-levels as HCV-RNA levels could not yet be measured at the time).[120] Ribavirin (RBV) is a nucleoside analogue with a broad antiviral activity, of which the exact mechanism of action is unknown.[121] The addition of RBV to IFN-alpha ameliorated success rates of treatment, and became the standard of care in 1998.[122] Modification of IFN-alpha by adding a polyethylene glycol (peg) to the molecule increased the half-life, which resulted in a once weekly administration (compared to 3 times a week) and a higher success rate compared to standard IFN-alpha.[123] From the beginning of this century, treatment with pegIFN and RBV for 24 – 48 weeks (depending on genotype) has been the standard of care with SVR rates of 50 % in genotype 1 and 4 after treatment for 48 weeks, and 80 % after treatment for 24 weeks in genotypes 2 and 3.[124] This treatment has multiple side-effects such as flu-like symptoms, fatigue,

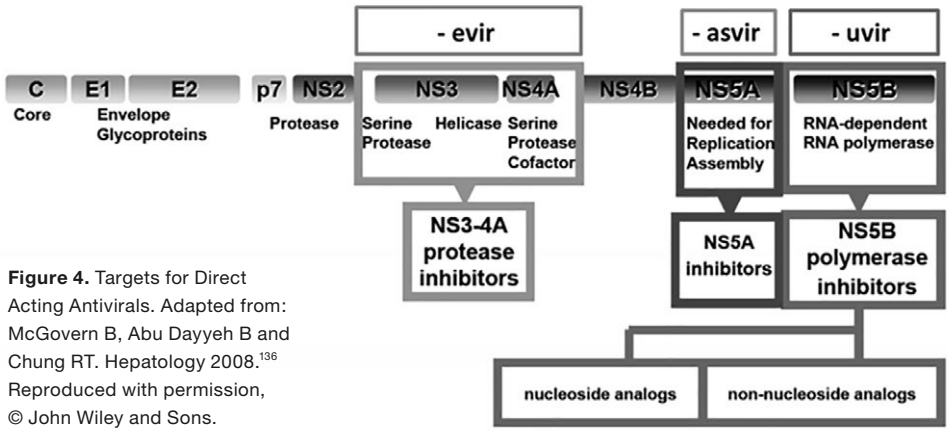

Figure 4. Targets for Direct Acting Antivirals. Adapted from: McGovern B, Abu Dayyeh B and Chung RT. Hepatology 2008.[136] Reproduced with permission, © John Wiley and Sons.

haematological disorders (anaemia, leukopenia, thrombocytopenia), depression, emotional instability, and auto-immune diseases. In 2012, the first direct acting antivirals (DAAs) became available, the protease inhibitors boceprevir and telaprevir, directed against NS3 non-structural protein of the HCV. These medicaments worked exclusively for genotype 1, ameliorating SVR rates to 75 %. However, as they were added to the standard therapy of pegIFN and RBV, the amount of side effects increased substantially, and many patients had difficulties in completing a full course of the therapy, which was still 24 – 48 weeks.[125]
In 2014, more new potent all-oral DAAs were registered, resulting in very high cure rates for patients with all HCV genotypes, and without many side effects.[126–135] There are three classes of DAAs, each targeting different HCV proteins (Figure 4.).

The DAA's that are now European Medicines Agency (EMA) approved:

Protease inhibitors (-evir)
The first group of DAAs targets the NS3/4A protease, inhibiting the cleavage of the precursor HCV protein, from which individual HCV proteins are further derived. The older drugs telaprevir and boceprevir belong to this group (first generation protease inhibitors), but newer drugs in this category are glecaprevir, grazoprevir, paritaprevir, simeprevir and voxilaprevir (second generation protease inhibitors). They all are combined with at least one other DAA from a different group.

NS5A inhibitors (-asvir)
The second group of DAAs targets the NS5A protein, inhibiting viral assembly and replication. There are multiple drugs registered in this category: daclatasvir, elbasvir, ledipasvir, ombitasvir, pibrentasvir and velpatasvir. These drugs also all need to be combined with at least one drug from another group to achieve SVR.

Polymerase inhibitors (-uvir)
The third, and possibly most potent group of DAAs is targeted against the NS5B protein, inhibiting the production of new HCV genomes. The most prescribed NS5B inhibitor is sofosbuvir, which is mostly used in combination with other DAAs, but started off as combination therapy with only RBV for genotype 2, 3 and 4, as other DAAs were not yet registered for these genotypes. Another polymerase inhibitor is dasabuvir, which is only registered in combination with paritaprevir/ritonavir and ombitasvir.

In The Netherlands, DAAs were due to their high price initially only reimbursed for patients with severe liver fibrosis or cirrhosis. Later, in 2015, the Dutch government decided to reimburse treatment with IFN-free DAAs for all CHC patients. The recommendations in the current European HCV treatment guidelines are in line with this decision, stating that all patients with compensated and decompensated chronic liver disease due to HCV-infection should be treated.[137] The Dutch guidelines follow the statements of the EASL.[138] SVR rates with DAAs are 90 – 95 % for all genotypes in non-cirrhotic patients and treatment-naive Child-Pugh A cirrhotic patients. Figure 5. shows the progression of treatment success over the years. Even when there is cirrhosis, treatment success is still high in most genotypes. However, sometimes longer

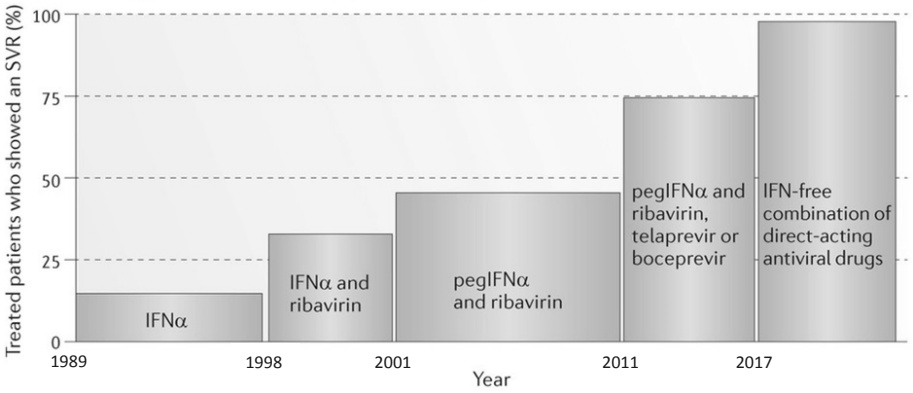

Figure 5. The stepwise increase in sustained virological response rates in the past 28 years. Adapted from: Heim MH. Nature Rev Immunol 2013.[142] Reproduced with permission, © Nature Publishing Group.

treatment or the addition of RBV is advised. Whereas in the past, genotype 3 was one of the "easier" curable genotypes, it is now relatively more difficult to cure, as, especially in treatment-experienced cirrhotic patients, SVR-rates are around 80–86 %.[139–141] Because of the low toxicity profile of many of the DAAs (except from protease inhibitors), even decompensated cirrhotic patients can be safely treated. Moreover, there are multiple regimens possible for patients with renal insufficiency, and treatment may also be given after renal transplantation as pegIFN is no longer required. The remaining questions regarding treatment with DAAs are directed towards DAA failure (mostly genotype 3), and resistance-associated substitutions (RAS), interactions with co-medication, HCC occurrence and recurrence after successful treatment, and the timing of treatment in decompensated cirrhotic patients who are listed for liver transplantation. Some small special groups still require attention, such as patients with short bowel, gastric bypass surgery, patients with low compliance, and patients with acute HCV-infection.

HEPATITIS E VIRUS INFECTION

Virus and epidemiology

Hepatitis E virus (HEV) is a non-enveloped, single-stranded RNA virus, containing three open reading frames (ORF1, ORF2 and ORF3).[144] HEV can be subdivided into at least four genotypes. Genotypes 1 and 2 are human viruses causing acute hepatitis, mainly in young adults in tropical countries. Genotypes 3 and 4 are zoonotic, with pigs as the main reservoir in Europe and parts of Asia.[145, 146] The transmission routes of HEV genotypes 3 and 4 remain unclear, but it has been suggested that the consumption of undercooked meat products, contaminated with HEV, plays an important role.[147, 148] The most prevalent HEV genotype in Europe is genotype 3. After years of decline, the prevalence of HEV-infection in Europe is rising.[149] Studies demonstrated that HEV-infection is widespread among blood donors, with incidences of viraemia varying from 1/762 in The Netherlands[150, 151] to 1 per 2,848 in the United Kingdom,[152] 1 per 3,333 in Spain,[153] and 1 per 8,416 in Austria.[154] HEV-infection among donors is less prevalent in the United States (1 per 9,500).[155]

Immune reaction in HEV-infection

After infection with HEV, a prominent antibody response is observed, directed against immunodominant antigenic epitopes in the ORF1, ORF2 and ORF3 of HEV.[156–158] Early after infection an IgM anti-HEV response appears,[156] followed by anti-HEV IgG a few days later.[159] IgM disappears after 4–5 months,[156] whereas IgG may persist for years to come.[159] HEV-specific antibodies IgM and IgG seem to have a neutralizing function as their titers are shown to be significantly higher in patients with fulminant liver failure (FLF) due to acute HEV-infection than in those without FLF.[160] In immunocompetent individuals, the diagnosis of HEV-infection is based on detection of anti-HEV IgG and IgM antibodies, because in most cases HEV IgM antibodies are detectable as soon as symptoms occur.[161] However, in immunocompromised individuals, the diagnosis of HEV-infection must be based on detection of HEV-RNA, because those patients may remain seronegative, whereas HEV-RNA is detectable during chronic infection. Regarding the cellular immune response to HEV-infection, a robust non-specific IFN-y production directed against the ORF1 and ORF2 region of the viral genome is described.[162–166] Furthermore, in acute resolving HEV-infection, strong and multi-specific HEV-specific T-cell responses are observed,[164–167] whereas in patients with chronic HEV-infection, HEV-specific CD4+ and CD8+ T-cell responses are rather weak.[167]

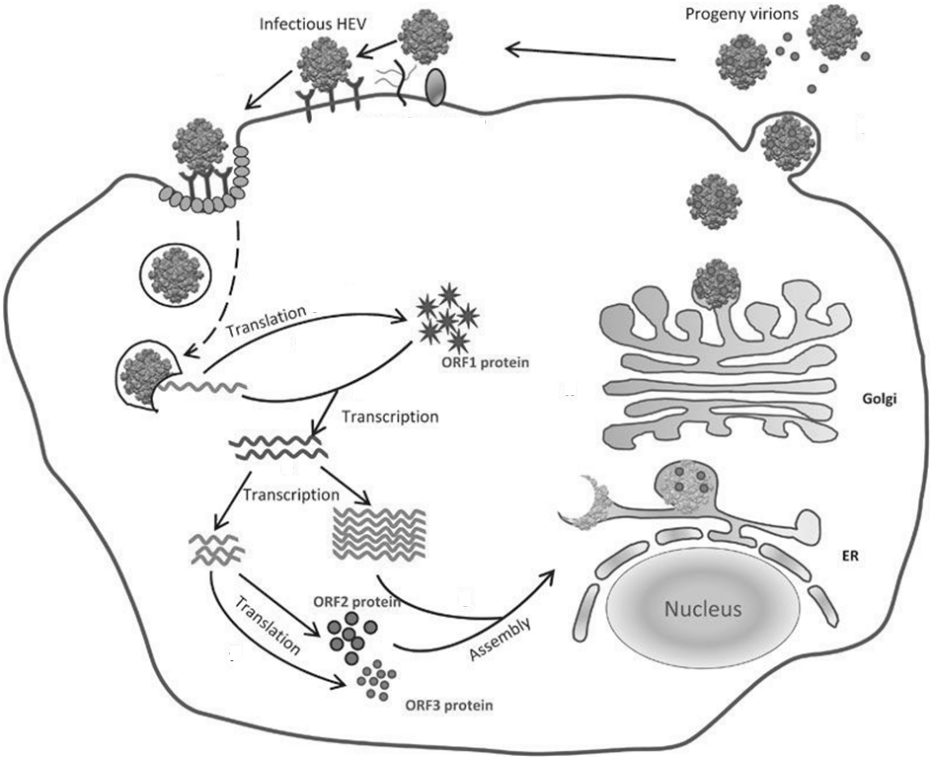

Figure 6. Proposed life cycle of hepatitis E virus. Adapted from: Cao D and Meng XJ. Emerg Microbes Infect 2012.[8] Reproduced with permission, Copyright Nature Publishing Group.

HEV genotype 3 in immunocompromised patients

Typically, HEV genotype 3 infection is asymptomatic and self-limiting. If present, symptoms include anorexia, nausea, fatigue, myalgia and jaundice with elevated bilirubin and liver enzymes. Furthermore, extrahepatic manifestations have been described, such as neurological manifestations (i.e. Guillain Barré syndrome[168]), renal impairment, pancreatitis, cryoglobulinaemia and haematological abnormalities.[169] However, HEV genotype 3 does pose a threat to immunocompromised patients who may develop chronic HEV-infection (58–93%)[170–173] and liver cirrhosis.[171, 174, 175] This was shown in different groups of immunocompromised patients such as solid organ transplanted (SOT) patients[170–173, 175, 176] and HIV-positive patients.[174, 177] In a Dutch cohort of 328 allogeneic hematopoietic stem cell transplantation (alloHSCT) recipients, 8 cases of HEV-infection (2,4 %) were found of which 5 developed chronic hepatitis.[178] This suggests that there is a considerable risk of post-transplant HEV-infection for alloHSCT recipients. Most patients with HEV-infection have increased ALT-levels,[179] and most alloHSCT patients experience one or more episodes of elevated transaminase levels post-transplantation. However, the differential diagnosis of elevated liver enzymes following SOT or alloHSCT is extensive and includes medication toxicity, pre-existing liver conditions such as fatty liver disease, or graft versus host disease (GvHD) of the liver in case of alloHSCT.[180]

Treatment of chronic HEV-infection

Treatment options of chronic HEV-infection are: lowering of immunosuppressant therapy,[162] which is often dangerous and ill advised, or treatment with pegIFN[181] or RBV.[182] Due to the side-effects of pegIFN, RBV is the most frequently chosen treatment. RBV is often dosed as 600 mg per day, based on a case series described in 2014.[182] Most cases and case series describe a 3–6 month duration of treatment. A recent study showed an SVR of 63 % after treatment for 3 months.[183] Therefore, in 2017, a dose of 1000–1200 mg per day rather than 600 mg per day is prescribed if tolerated. Protracted fecal shedding of HEV-RNA may predict treatment failure. Patients with chronic HEV-infection treated for 3 months with RBV who had still HEV-RNA detectable in their stools (excluding in plasma) all had a relapse after stopping therapy.[184]

OUTLINE OF THE THESIS

The following work describes various clinical aspects of viral hepatitis B, C, and E.

Part I of the thesis is focused on HBV-infection. In this part, different aspects of acute and chronic HBV-infection are addressed. **Chapter 1** describes the immune responses of 9 patients with acute HBV-infection, in terms of NK-cell characteristics and HBV-specific T-cell function, with the aim to identify characteristics of patients who develop chronic HBV-infection versus those who spontaneously clear the virus. **Chapter 2** aims to assess plasma- and intrahepatic IP-10 levels as a pre- and on-treatment marker of response to combination therapy with peginterferon (pegIFN) and adefovir in chronic HBV-infection. **Chapter 3** describes a study in patients with chronic HBV-infection with low viral load who were randomised to be treated with a combination of pegIFN and adefovir, pegIFN and tenofovir or no treatment.

Part II of the thesis describes different aspects of chronic HCV-infection, with particular focus on treatment-related topics. In **Chapter 4** the value of plasma IP-10 levels before and during treatment with high-dose induction interferon (IFN) is assessed for the prediction of treatment success. This is investigated in a previously described cohort of 85 chronic hepatitis C (CHC) patients (treatment naïve patients with various HCV genotypes who failed previous interferon-based therapy) who were treated for 6 weeks with high-dose IFN-alpha 2b (18 MU/day for 2 weeks, 9 MU/day for 2 weeks and 6 MU/day for 2 weeks consecutively), combined with ribavirin (RBV) (1000-1200 mg/day), followed by 24 or 48 weeks of pegIFN alpha 2b (1.5 ug/kg once a week) and RBV. **Chapter 5** describes a retrospective multicentre study in the Amsterdam region of The Netherlands, evaluating the efficacy in real-life of combination treatment of sofosbuvir and simeprevir in CHC patients with genotype 4 with advanced liver fibrosis or compensated cirrhosis. In **Chapter 6** a case is described of a CHC patient with HCV genotype 1b, who failed earlier antiviral treatment with a combination of two direct-acting antivirals (DAAs) (a protease- and NS5A-inhibitor), who, after undergoing gastric bypass surgery was successfully treated with a combination of sofosbuvir and simeprevir using therapeutic drug monitoring (TDM). **Chapter 7** describes a prediction model to predict the future HCV disease burden in The Netherlands. In this chapter, a modelling approach is used to predict the effect of different treatment scenarios, in which various CHC patient groups are treated, on the size of the future HCV-viremic population and HCV-related disease burden.

Part III of the thesis is focused on HEV-infection, especially in immunocompromised patients as HEV-infection tends to become chronic in those patient groups. **Chapter 8** assesses the prevalence of chronic HEV-infection in a cohort of 130 patients who underwent allogeneic hematopoietic stem cell transplantation (alloHSCT). The relation between HEV-infection and the occurrence of graft versus host disease (GvHD) in these patients was established.

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