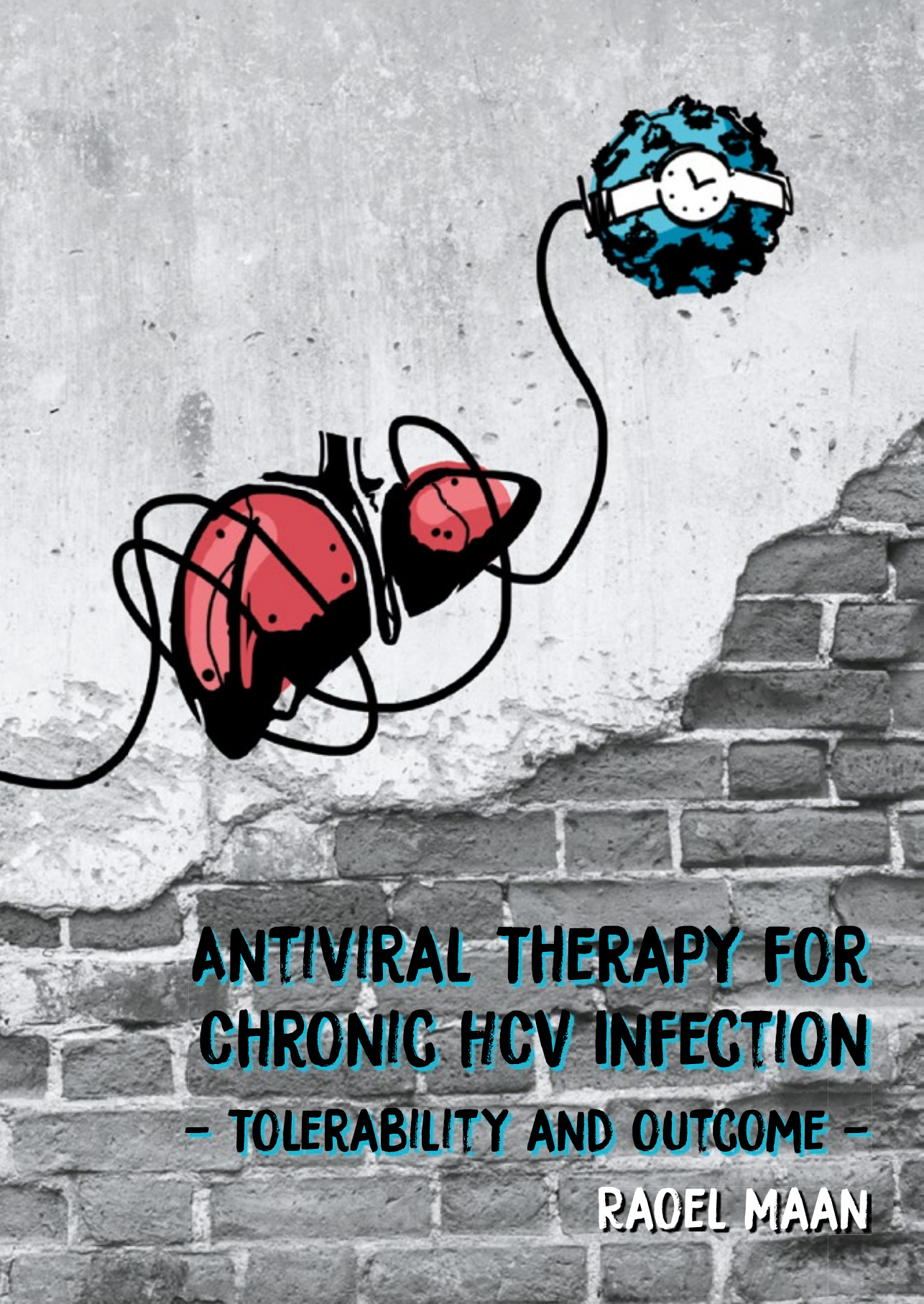

# ANTIVIRAL THERAPY FOR CHRONIC HCV INFECTION

## – TOLERABILITY AND OUTCOME –

RAOEL MAAN

# Antiviral Therapy for Chronic HCV Infection

## - Tolerability and Outcome -

*Raoel Maan*

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## EPIDEMIOLOGY

Chronic hepatitis C virus (HCV) infection is a major global public health problem, with recent estimates suggesting that 64–103 million people are infected worldwide.[1] Moreover, it is thought to be responsible for over 350,000 deaths each year and is the predominant reason for liver transplantation in the Western world.[2] The prevalence of HCV infection, however, shows a substantial geographical variation. Worldwide, Egypt, Mongolia and Cameroon have the highest prevalence of more than 20%.[2] In Europe the prevalence of HCV infection varies from 0.1 to 6.0%, with the highest occurrence in Southern and Eastern Europe.[3] The Netherlands represents a country in which HCV infection is not frequently observed. A recently performed epidemiological study indicated that the seroprevalence of anti-HCV antibodies was 0.2%, around the lowest in the world.[4] Nevertheless, this would mean that almost 40,000 inhabitants of the Netherlands have been in contact with the HCV, leading to approximately 30,000 Dutch patients with a chronic HCV infection. First-generation migrants from HCV-endemic countries and injecting drug users (IDU) form important risk groups in the Netherlands.[5] [6] Furthermore, there was an increasing prevalence among HIV-positive men who have sex with men before 2010, but this appeared to level off in the years after.[7]

## THE HEPATITIS C VIRUS

From the mid 1970's it was known that transfusion-associated hepatitis did often not concur with serological markers against the hepatitis A or B viruses.[8] [9] This so called non-A, non-B hepatitis was sporadically found in absence of blood transfusion or receipt of other blood products as well.[10] In 1989, after many years of research, dr. Michael Houghton and colleagues isolated and characterized the viral agent thought to be the predominant cause of hepatitis following blood transfusion.[11] This pathogen was designated to be the HCV. Later, nearly all cases with non-A non-B post-transfusion hepatitis were shown to be attributable to HCV infection.[12]

Hepatitis C virus is a small enveloped virus of approximately 55–65 nanometers in size and is a member of the genus Hepacivirus, belonging to the Flaviviridae family. It contains a single-stranded RNA genome of positive polarity. This genome is approximately 9600 nucleotides in length and consists of a highly conserved 59 untranslated region, followed by a single open reading frame that encodes a polyprotein of 3010 to 3033 amino acids. Cellular and viral proteases cleave this large protein into ten smaller viral gene products: three structural proteins (core, E1, and E2); an ion channel (p7); and six nonstructural proteins (NS2, NS3A, NS4A, NS4B, NS5A, and NS5B) (Figure i.1). Structural proteins are required for assembly and are used for the determination of the seven main HCV genotypes (and sub genotypes).[13] The p7 and NS2 protease are required for the release of infectious particles. The other nonstructural proteins (NS3A, NS4A, NS4B, NS5A, and NS5B) are closely involved in HCV replication.[14] NS3 and its cofactor NS4A form a stable heterodimeric complex, which cleaves the HCV polyprotein at four sites. NS4B is the presumed central organizer of the HCV replicase complex and a main inducer of intracellular membrane rearrangements. The NS5A protein is essential for RNA replication and

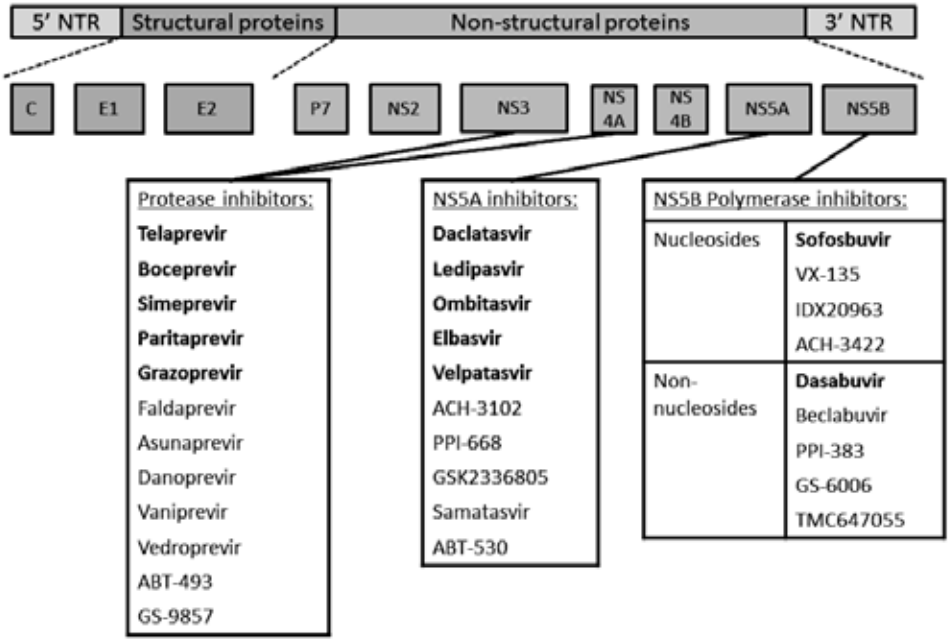


**Figure i.1 | The hepatitis C virus (HCV) genome**

The hepatitis C virus (HCV) genome encoding three structural proteins and seven non-structural proteins. The direct-acting antivirals are listed below the proteins and include the NS3/4A (or protease) inhibitors, the NS5A inhibitors, and the NS5B polymerase inhibitors (both nucleosides and non-nucleosides). The direct-acting antivirals approved by the US Food and Drug Administration and the European Medicines Agency are highlighted in bold.

assembly of infectious virus particles. The RNA-dependent NS5B protein is the RNA polymerase catalyzing the amplification of the viral RNA genome.[14 15] Figure i.2 shows the entry of HCV into the hepatocytes, as well as its life cycle and replication process.[15] In addition, several host factors have been involved in the HCV life cycle. These include epidermal growth factor receptor (EGFR) and ephrin receptor A2 (EphA2), which are two receptor tyrosine kinases that have recently been identified as HCV entry factors.[16] Cyclophilin A (CypA), another host factor, is a protein that is involved in the replication of HCV by binding to the NS5A protein of all HCV genotypes.[17] Apolipoprotein E (apoE) is a component of lipoviral particles, which is involved in the HCV infection of hepatocytes.[18]

Although HCV might be present in extrahepatic tissues such as bone marrow and lymphocytes, hepatocytes form the primary host cells in which the virus replicates extremely fast.[19] A possible explanation for the hepatotropicity of HCV might be the distinct set of microRNAs, which are small non-coding regulatory RNAs, which are expressed by hepatocytes. Possibly the most important host factor, microRNA-122 (miR-122), is the most abundant hepatocyte-derived microRNA, and actually constitutes about 70% of the total microRNA population in the liver.[20-23] As other tissues showed no or

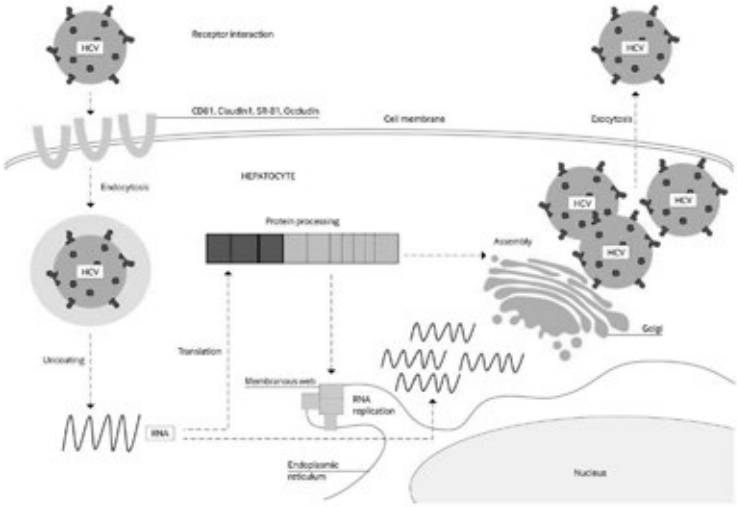


**Figure i.2 | Life cycle of the hepatitis C virus**

Adapted from Feeney et al.[26] Schematic overview of the life cycle of the hepatitis C virus (HCV). In order to enter the hepatocyte, HCV interacts with co-receptors, resulting in its endocytosis. Then the virus fuses with the endosome and uncoats its RNA. Host ribosomes translate the RNA into a polyprotein, which is cleaved by host and virally encoded proteases into the three structural and seven non-structural proteins. The non-structural proteins form a complex on a "membranous web" that replicates HCV RNA. The Golgi assembles the HCV RNA with viral structural proteins, leading to the formation of infectious viral particles, which are exocytosed from the cell. 

only minor expression of miR-122, this microRNA can be considered liver-specific.[20 22 23] Importantly, miR-122 seems to be essential for HCV RNA stability and propagation.[24 25] By binding to two closely spaced target sites in the highly conserved 5'UTR of the HCV genome, miR-122 is thought to protect the HCV genome from nucleolytic degradation and/or from host innate immune responses.[25-27]

Each infected hepatocyte is estimated to produce around fifty viral particles a day, leading to a total daily production of $10^{12}$ new viruses in an HCV-infected liver.[28] As the virus mutates quickly due to the high error rate of the viral RNA-dependent RNA polymerase, patients are considered to be infected with a HCV quasispecies of genetic variants.[29] Seven HCV genotypes have been identified, which differ by 30-35% of the nucleotide sites over the complete genome and are further subdivided into various subtypes.[30 31] The HCV genotypes are not equally distributed around the globe. In the Western world HCV genotype 1 is most prevalent, while HCV genotype 2 and 3 are the predominant variants in Asia. In Egypt HCV genotype 4 exists almost exclusively. Other HCV genotypes are less frequently found and originate from South Africa (HCV genotype 5), South East Asia (HCV genotype 6) and Central Africa (HCV genotype 7). Because the HCV genotypes may have different clinical consequences and treatment outcomes, it is relevant to know with which HCV genotype a patient is infected.

## NATURAL COURSE OF HCV INFECTION

**I**n the Western world, the transmission of HCV has declined substantially over the last two decades and the currently reported incidence rates of acute HCV infection are below 1 per 100,000 individuals.[32-34] This incidence might be underestimated, however, because diagnosing HCV infection in the acute phase, i.e. the first 6 months of infection, remains a clinical challenge as the majority of patients are asymptomatic.[35 36] Already 1 to 3 weeks after exposure, HCV RNA can be detected in the circulation and this represents the first manifestation of the disease.[37 38] Anti-HCV antibody seroconversion usually takes several weeks longer. Spontaneous resolution of HCV infection may occur within 3-4 months after acquisition of the virus. Based on a systematic review of longitudinal studies 1 in every 4 patients with acute HCV infection is expected to have self-limiting disease, but reported rates of spontaneous clearance vary from 10% to more than 50%.[39 40]

If serum HCV RNA is still detectable six months after exposure, spontaneous clearance is unlikely to follow and patients are considered to have chronic HCV infection.[41] Around 75% of patients who acquired HCV are thought to develop a chronic infection. Most patients who are referred for active HCV infection are diagnosed in this chronic phase. Patients may present with non-specific symptoms, although also chronic HCV infection often remains asymptomatic. As a consequence, the majority of patients are currently unaware that they are chronically infected with HCV.[42] Nevertheless, various degrees of hepatic fibrosis may be present among these patients. Development of hepatic fibrosis or even cirrhosis represents the predominant consequences of chronic HCV infection and negatively impacts clinical outcomes among patients with this disease.

Chronic HCV-infected patients may experience various non-specific symptoms such as fatigue, nausea, abdominal or musculoskeletal pain, loss of weight and pruritus. More specific liver-related manifestations are mostly restricted to patients with end-stage cirrhosis, and include variceal bleeding, ascites, jaundice and hepatic encephalopathy. Often, symptoms can be attributed to the presence of extrahepatic manifestations, which are reported in up to 74% of patients.[43] Extrahepatic manifestations include mixed cryoglobulinemia vasculitis, lymphoproliferative disorders such as B-cell non-Hodgkin lymphoma, renal disease, type II diabetes mellitus, porphyria cutanea tarda and lichen planus. Depression, irritability, malaise and other neuropsychiatric symptoms have been observed among these patients as well, with depression being most frequently reported.[44] Importantly, the extrahepatic symptoms and manifestations may impact morbidity, treatment opportunities as well as mortality.[45]

Of all symptoms, fatigue is most frequently reported. Although fatigue can have a variety of physical and psychological causes, it has indeed been associated with chronic HCV infection. A recent study among 401 patients with chronic HCV genotype 1 infection indicated that approximately 50% experienced fatigue.[46] Patients with cirrhosis had fatigue more frequently and of more severe intensity, as was reported by a previous study as well.[47] Fatigue should not be underestimated as it has a major impact on the health-related quality of life. The health-related quality of life is often impaired patients with chronic HCV infection, even in case of asymptomatic disease or normal aminotransferase levels.[47 48]

Patients with chronic HCV infection often have low grade hepatic inflammation, which may be evidenced through liver biopsy or suggested by elevations of the serum aminotransferases. Although the ALT level is often raised to approximately one to three times the upper limit of normal, many patients with chronic hepatitis C infection have normal ALT levels. Together with the absence of (liver-specific) symptoms this certainly challenges us to diagnose HCV infection. Frequently, patients are diagnosed with chronic HCV infection because routine blood tests showed signs of hepatitis. The chronic inflammatory activities in the liver, as a response to the continuous presence of HCV, stimulate fibrogenesis by activation of hepatic stellate cells. These activated cells transdifferentiate to myofibroblasts and produce many extracellular matrix components as well as mediators leading to accumulation of these proteins.[49] Therefore, chronic HCV infection is often accompanied by the development of hepatic fibrosis. The degree of fibrosis, i.e. the stage of liver disease, is determined according to semi-quantitative histopathological scoring systems, of which the METAVIR and Ishak classification are most frequently used.[50 51] In both classifications cirrhosis is the most advanced stage, which can be considered as the general end-stage of many chronic liver diseases. In case of cirrhosis the normal architecture of the liver parenchyma is completely compromised. Cirrhosis is characterized by nodules of regenerating hepatocytes which are surrounded by fibrotic septa. These septa may be vascularized and can stretch between portal areas or between the portal areas and the central veins. Especially the porto-caval septa are relevant in the pathophysiology of cirrhosis as these can form anastomoses between efferent and afferent vessels.[52 53] Next to the clinical implications of shunting of blood, by which the functionally active hepatocytes are bypassed, this also leads to relative hypoxemia of the liver parenchyma resulting in further liver injury and neovascularization.[54] The vascular abnormalities within a cirrhotic liver are indeed thought to have an important role in the development of the clinical sequelae of chronic HCV infection. Presence of cirrhosis surely impedes the prognosis of patients with this disease.[55 56] Next to the manifestations of liver failure (also called decompensated cirrhosis), patients with HCV-induced cirrhosis are in danger of hepatocellular carcinoma (HCC). Both liver failure and HCC, which are rarely seen in absence of cirrhosis, may result in the need for liver transplantation or liver-related mortality. A recent meta-analysis indicated patients with chronic HCV infection and advanced hepatic fibrosis have an overall annual risk of 2.9% to develop liver failure, 3.2% to progress to HCC and 2.7% to die of liver-related causes.[57] Although these rates were based on nonresponders to interferon-based therapy, they are likely to be representative of the natural history of untreated chronic hepatitis C. Indeed, the earlier interferon-based treatments which did not lead to viral clearance had no impact on the natural course of the disease.[55 58-60]

It should be considered, though, that not all patients with chronic HCV infection show progression of hepatic fibrosis. Accurate data regarding the natural history of HCV-induced fibrosis progression and the occurrence of its clinical complications are difficult to obtain. Studies are subject to selection bias as diagnosing chronic HCV infection favors symptomatic patients who utilize medical resources. Other relevant issues include the high prevalence of co-factors which may already cause liver damage by themselves, such as alcohol abuse or co-infection with the hepatitis B virus (HBV) or HIV, and the uncertainty regarding the moment of HCV acquisition. Nevertheless, it has been estimated that approximately 16% of the patients will establish cirrhosis within 20 years of HCV infection.[61] Because fibrosis progression may accelerate over time, however, the number of patients which will progress to

cirrhosis might be higher as estimated in the earlier natural history studies. Importantly, because the majority of Western patients with chronic HCV infection are thought to have acquired their infection in the 1960s and ´70s, the proportion of patients with HCV-induced cirrhosis is rapidly rising.[62 63]

## ANTIVIRAL THERAPY FOR HCV INFECTION

Unmatched progress in the treatment of chronic HCV infection has been made over the last two decades. Interferon monotherapy was the first approved antiviral treatment for non-A, non-B hepatitis. Now, for the last two years, direct-acting antivirals (DAAs) have replaced interferon-based therapy completely.

**Mechanism of action of antiviral therapy**

Although still not fully elucidated, interferon alfa is thought to induce a large number of genes (called interferon-stimulated genes) with antiviral properties, leading to a multi-faceted attack on the virus. In addition, it also has some direct antiviral actions as well as important interactions with the adaptive and innate immune responses.[64] Standard interferon was administered subcutaneously three times a week. Since the beginning of the 21st century, however, polyethylene glycol-modified (pegylated) interferon is used. Pegylated interferon is more stable as compared to standard interferon and has a longer half-life which allows once weekly dosing.[65]

Ribavirin is a guanosine analogue with activity against several RNA and DNA viruses. Different hypotheses regarding its mechanism of action have been proposed, of which the theory of lethal mutagenesis seems most reasonable.[64] It has been part of the standard-of-care in the treatment of chronic HCV infection since 1998.

Over the last decades, major advances in the understanding of the HCV life cycle resulted in the development of DAAs for the treatment of HCV infection.[66] Multiple classes of DAAs have been developed which interact with various nonstructural viral proteins and thereby target different steps in the replication of HCV RNA. The three main DAA groups concern the NS3/4A protease inhibitors, the NS5B polymerase inhibitors and the NS5A inhibitors. In general, treatment with DAAs increases the virological efficacy of antiviral regimens and allows shortening pegylated interferon treatment duration. In fact, more recent DAA treatment regimens with a high barrier to resistance showed to be able to eradicate the chronic HCV infection without the need for pegylated interferon. The development of highly effective interferon-free therapy for chronic HCV infection should be regarded as one of the most important milestones in hepatology, and perhaps even in overall medicine, of the 21st century.

Protease inhibitors target the NS3/4A serine protease and thereby inhibit the cleavage of this protein and thus HCV replication.[15] Current approved "-previrs" include telaprevir, boceprevir, simeprevir, paritaprevir and grazoprevir (Figure i.1). The NS5A inhibitors, also known as "-asvirs", target another nonstructural protein and block the replication of HCV RNA at the stage of membranous web biogenesis.[67] So far, daclatasvir, ledipasvir, ombitasvir, velpatasvir and elbasvir have been approved (Figure i.1). Both NS3/4A protease inhibitors and NS5A inhibitors have very potent antiviral activity

but exhibit a low barrier to viral resistance. The NS5B inhibitors, or "-buvirs", can be divided into two main classes: nucleos(t)ide inhibitors and non-nucleotide inhibitors (Figure i.1). By binding to the active site of the NS5B RNA-dependent RNA polymerase, nucleos(t)ide inhibitors (e.g., sofosbuvir) cause premature chain termination. The non-nucleotide inhibitors (e.g., dasabuvir) bind outside the active site, causing a conformational change, and thereby decrease the polymerase activity of the enzyme.[15]

**Rationale for antiviral therapy for chronic HCV infection**

The development of hepatic fibrosis is probably the most common indication to initiate antiviral treatment for chronic HCV infection. Antiviral therapy might indeed be postponed among patients with chronic HCV infection who do not seem to developed hepatic fibrosis. In fact, some patients may never need to be treated as they will not develop clinical complications of their chronic HCV infection. However, extrahepatic symptoms and manifestations of chronic HCV infection, which can reduce the health-related quality of life, may represent another relevant reason to initiate antiviral therapy. Furthermore, some patients might choose to be treated out of fear for further HCV transmission.

Nowadays, antiviral therapy for chronic HCV infection is considered successful in case sustained virological response (SVR) is attained. This virological endpoint is defined as HCV RNA negativity in the circulation 24 weeks following cessation of therapy. Importantly, SVR showed to have a long-term durability.[68] Because virological relapse is already extremely rare in case a patient is HCV RNA negative at 12 weeks following the end of therapy, the post-treatment follow-up duration was shortened to this time point in order to determine sustained response rates in more recent clinical trials.[69]

While we rely on SVR to determine treatment success, achievement of SVR should not be considered as the primary goal of antiviral therapy. Rather, we treat patients to improve their health-related quality of life and to prevent HCV-related morbidity and mortality. Therefore, it is important that there is an increasing body of evidence which suggests that antiviral therapy resulting in SVR also benefits the patients from a clinical point of view. Eradication of HCV was shown to decrease the frequency and severity of fatigue, and increase the quality of life.[46 70-72] Histological studies showed that patients who attained SVR had regression of hepatic fibrosis, even in case cirrhosis was established prior to treatment initiation.[73] The regression of fibrosis following antiviral therapy among patients with HCV-induced cirrhosis has been related to improved clinical outcome.[74] Following SVR, the hepatic venous pressure gradient was found to decline as well.[75-78] Multiple large cohort studies with extensive follow-up duration among patients with chronic HCV infection and advanced liver disease indicated a strong and independent association between SVR and reduced incidence of liver failure, hepatocellular carcinoma and liver-related death.[75 79-81] More important, recent data showed that there was an association between SVR and all-cause mortality, the most definite and robust clinical endpoint].[81-84] Additional analyses among patients with compensated advanced liver disease who attained SVR, showed that they even had a similar life expectancy compared to a matched general population.[85 86]

**Antiviral therapy for acute HCV infection**

Early monotherapy with 24 weeks of pegylated interferon in patients with acute HCV infection showed to prevent chronicity in 71 to 98%, depending on treatment adherence.[87] The efficacy was found to decline when antiviral therapy was initiated longer after HCV exposure, so that early treatment may be advised.[88] However, immediate therapy may result in overtreatment of those patients who might also spontaneously resolve their HCV infection.[87 89] A 12-week delay from the moment of exposure, to evaluate natural HCV RNA kinetics among those with symptomatic acute HCV infection, has therefore been suggested.[90-92] The recent results of the Hep-Net Acute HCV III study from Germany, in which 107 patients with symptomatic acute HCV infection were randomized to receive 24 weeks of pegylated interferon immediately or after 12 weeks with the addition of ribavirin, confirmed the effectiveness of such a delayed treatment strategy as long as proper adherence during follow-up and treatment can be assured (90% and 93% with undetectable HCV RNA after 60 weeks, respectively).[93] However, as was found in this study, compliance might be difficult in the specific population with acute HCV infection in the Western world. The timing of antiviral therapy thus remains subject to debate and the preferred strategy should probably be determined on an individual basis.

There is insufficient data to generally recommend the use of ribavirin for acute HCV infection.

Santantonio et al. performed a randomized trial in 130 patients with acute HCV infection.[94] All patients started therapy by week 12 after onset and were randomized to receive 12 or 24 weeks of pegylated interferon monotherapy, or 12 weeks of pegylated interferon and ribavirin. There was no difference in SVR rate between any of these three regimens, demonstrating that 12 weeks of pegylated interferon might be sufficient to treat acute HCV infection. A non-randomized trial in which HIV-infected patients with newly acquired HCV infection were treated with pegylated interferon and ribavirin did suggest, however, that ribavirin improved the early HCV RNA decline.[95] This was particularly seen among those patients who were expected to have a longer duration of infection. In some cases, when treatment is initiated after more than 12 weeks of onset, adding ribavirin to pegylated interferon to treat acute HCV infection might therefore be considered.

A relevant issue which does not necessarily promote the treatment of acute HCV infection concerns the possibility of re-infection due to continuous risk behavior, for instance among IDU and HIV-infected men who have sex with men.[96] Even more important is the current availability of highly effective interferon-free regimens. Recently, data from the Hep-Net Acute HCV IV study from Germany showed that six weeks of antiviral therapy with ledispasvir and sofosbuvir resulted in a virological response in all 20 patients with HCV genotype 1.[97] Although these results are promising, it could be questioned whether treatment in these patients should be deferred and physicians should wait until chronic HCV infection has been established. As the price of DAAs is an important issue, overtreatment should be prevented. Especially when these patients could be easily treated with highly effective and safe treatment regimens once they have established a chronic infection. Cost-effectiveness studies are needed in order to conclude on the timing of antiviral therapy for acute HCV infection.

**Interferon-based antiviral therapy for chronic HCV infection**

Jay H. Hoofnagle was the first who described the treatment of so called chronic non-A, non-B hepatitis.[98] Ten patients were treated with standard interferon in different doses, frequencies and

duration. As HCV was still not identified, this study assessed the response to therapy by measuring ALT activity rather than HCV RNA levels. Next to ALT normalization, standard interferon therapy indeed showed potential to eradicate the viral infection. However, the virological efficacy of this early treatment strategy was poor with only about 10% of treated patients attaining SVR.[99] The SVR rate even declined to 2% among patients with HCV genotype 1 infection and advanced hepatic fibrosis, which are probably the most well-known factors associated with a poor virological response to interferon-based antiviral therapy.[100]

In 1994 Brillanti et al. showed that adding ribavirin to interferon could be beneficial for patients with chronic HCV infection.[101] While monotherapy with ribavirin did not result in substantial HCV RNA declines, this drug did seem to prevent virological relapse when added to interferon or pegylated interferon therapy.[99 102 103] Pegylated interferon instead of standard interferon further increased the virological efficacy of antiviral therapy. A 48-week regimen of pegylated interferon and ribavirin resulted in SVR in about 60% of patients.[104-106] This combination therapy was optimized during the first decade of this century with different treatment durations and response-guided therapy, depending on host and viral factors which were associated with virological responses. Still, among those with HCV genotype 1 infection and advanced hepatic fibrosis, cure rates with pegylated interferon and ribavirin remained limited. Only 35% of this difficult-to-treat population was able to attain SVR with this combination regimen.[107] Pegylated interferon and ribavirin treatment duration depended on HCV genotype, the stage of liver disease and the on-treatment HCV RNA kinetics, but largely varied between 24 or 48 weeks.

Unfortunately, interferon-based therapy is accompanied by numerous adverse effects, including fatigue, flu-like symptoms, hair loss, gastro-intestinal symptoms, headaches, dermatological abnormalities and neuropsychological symptoms such as concentration difficulties, depression and irritability.[108] These symptoms further deteriorate the health-related quality of life of HCV-infected patients and interfere with patients' ability to perform daily activities.[109] Furthermore, interferon-based therapy causes bone marrow suppression so that neutropenia, thrombocytopenia and anemia may develop. It should be realized, of course, that also ribavirin therapy is associated with adverse events, of which the induction of (hemolytic) anemia is probably the most well-known. Treatment-induced anemia might even necessitate ribavirin dose reductions, erythropoietin therapy or recurrent blood transfusions.

Especially the hematological abnormalities represent important limitations of pegylated interferon therapy, as they are common causes for dose reductions due to fear of severe infections or bleedings. [110] Interferon dose reductions, however, negatively impact the virological efficacy of antiviral therapy.[111 112] Moreover, patients with advanced liver disease are often excluded from interferon-based therapy in case they already present with thrombocytopenia at baseline as a result of portal hypertension and/or reduced thrombopoietin production.[113] Based on the limited association between treatment-induced cytopenia and clinically relevant infections or bleedings, it has been suggested that current guidelines with respect to pegylated interferon dose reductions may be too strict.[114 115] However, limited data is available among patients with advanced liver disease, while these are the patients with the highest risk of infections and bleedings. Taken together, the limited virological

efficacy and substantial side effects associated with (pegylated) interferon-based treatment urged physicians to carefully consider initiation of antiviral treatment in each individual patient.

**Interferon-based triple therapy for chronic HCV infection**

As a result of the growing understanding of the HCV life cycle, ciluprevir (BILN-2061) was the first HCV protease inhibitor which advanced into clinical trials.[116] Although development of the drug was stopped due to toxicity in animals, studies showed the antiviral potential of protease inhibitors. This eventually led to the approval of two NS3/4A protease inhibitors in 2011, telaprevir and boceprevir, representing the first class of DAAs in clinical practice.[117 118] Although these drugs were a major advance for the treatment of chronic HCV infection and their efficacy was higher than dual therapy with pegylated interferon and ribavirin, they had several limitations.[119] These included the following: not very effective against HCV genotypes other than HCV genotype 1; treatment became more complex with various dosing schedules, durations, and stopping rules; the pill burden was large; the rates of resistance-associated variants (RAVs) were high; and there were many potential drug-drug interactions. Moreover, pegylated interferon and ribavirin remained a necessity, and these first triple therapy regimens only increased the side effects of antiviral treatment.[120 121] Overall, SVR rates with the addition of telaprevir or boceprevir were around 70%, which was markedly higher as compared to the 50% with pegylated interferon and ribavirin among patients with HCV genotype 1 infection.[122-126] Still, these response rates were largely dependent on the degree of hepatic fibrosis, as SVR rates decreased to approximately 50% among those patients with cirrhosis. In this subgroup, caution with telaprevir or boceprevir triple therapy was advised as the first real-world experiences in France indicated many severe side effects, especially in case of low platelets counts and/or low albumin levels.[121] Due to the further improvement and development of DAAs, the use of these drugs is not recommended anymore in the Western world.

From a second wave of first-generation NS3/4A protease inhibitors, only simeprevir was approved by the Food and Drug Administration (FDA) and the European Medical Agency (EMA) for the treatment of chronic HCV infection.[127] Although still in combination with pegylated interferon and ribavirin, simeprevir improved tolerability considerably as it could be dosed once daily. Clinical trials which assessed the combination of simeprevir, pegylated interferon and ribavirin showed that around 80% of treatment-naïve patients with HCV genotype 1 infection attained SVR.[128 129] Importantly, the majority (around 90%) of these patients could be treated with only 24 weeks of pegylated interferon and ribavirin. As with the first-generation protease inhibitors, the SVR rate among patients who had relapsed after a prior pegylated interferon-based treatment course was not inferior to that among treatment-naïve patients, while treatment was markedly less successful among those with a prior null-response (reduction of less than 2 log10 in HCV RNA after 12 weeks of therapy).[130 131] Among patients chronically infected with HCV genotype 4, against which simeprevir is active as well, similar SVR results have been obtained.[132] However, a new dilemma arose, which is still subject to an ongoing discussion: should baseline RAVs be assessed? It was found that among ~50% of the patients with HCV genotype 1a the highly fit Q80K variant was present. Patients with this variant who received a triple therapy regimen including simeprevir, pegylated interferon and ribavirin, achieved SVR rates

that were similar to those patients who received dual therapy with pegylated interferon and ribavirin. It was therefore recommended to test for this variant when treatment was considered for patients with HCV genotype 1a.

**Interferon-free treatment regimens for chronic HCV infection**

Lok et al. were the first to describe an interferon-free regimen including a protease inhibitor, (asunaprevir) and an NS5A inhibitor (daclatasvir).[133] Four of the 11 patients with HCV genotype 1 (subtype 1a: 9 patients; subtype 1b: 2 patients) treated with this regimen for 24 weeks achieved SVR. Later on it was found that this regimen was much more effective in Japan, with SVR rates up to 90%, due to the fact that all patients in the Japanese study were infected with HCV genotype 1b. Currently, this regimen is approved in Japan.

Even greater strides in the treatment of chronic HCV infection have been made by the development of sofosbuvir, which is a nucleotide NS5B inhibitor with activity against all HCV genotypes and a high barrier to resistance.[134] Addition of sofosbuvir to only 12 weeks of pegylated interferon and ribavirin therapy showed SVR rates around 90% among both treatment-naïve patients with HCV genotype 1, 4, 5 or 6 infection as well as treatment-naïve and treatment-experienced patients with HCV genotype 2 or 3 infection.[135-137] Even in case of compensated cirrhosis, 80% of patients were able to eradicate their chronic HCV infection with this short triple regimen. The first robust data on interferon-free treatment with sofosbuvir were derived from patients with HCV genotype 2 and 3 infection. A 12 week regimen of sofosbuvir and ribavirin resulted in SVR rates above 90% among those with HCV genotype 2 infection.[137-139] At first, patients with HCV genotype 3 infection showed less optimal results with this combination. By extending the treatment duration to 24 weeks, however, the SVR rate increased to 85% in this group of patients as well.[138] For interferon-experienced HCV genotype 3-infected patients with cirrhosis this sofosbuvir and ribavirin combination therapy was not very effective. Small studies suggested that inclusion of pegylated interferon might be useful, leading to the Boson trial. Patients with HCV genotype 3, including ~30% cirrhotic patients, were randomized to receive 16 or 24 weeks of sofosbuvir and ribavirin, or 12 weeks of sofosbuvir, pegylated interferon and ribavirin. Although 24 weeks of dual therapy was superior to 16 weeks, it was less effective than 12 weeks of triple therapy. Among cirrhotic patients the benefit of adding pegylated interferon was most evident as SVR rates increased from 82% to 91% for naïve patients and from 76% to 86% for patients who were treatment-experienced.[140] At the cost of side effects, addition of pegylated interferon certainly increases the virological efficacy among all patients with HCV genotype 3. Although sofosbuvir was first approved in combination with pegylated interferon and/ or ribavirin, it has proven to be highly effective in combination with one or two DAAs from other classes, and is now mainly used as part of interferon-free regimens.

Most recent studies indicated that combining sofosbuvir with other DAAs, either as dual or triple therapy, is likely to represent a much more attractive alternative to optimize the virological efficacy as well as the safety profile of antiviral therapy. Daclatasvir was the first NS5A inhibitor that was evaluated in combination with sofosbuvir for the treatment of HCV genotype 1. In this phase II trial, patients received 12 or 24 weeks of therapy with or without the addition of ribavirin. From the

126 patients included, only three patients were counted as non-SVR since they were lost to follow-up.[141] In the COSMOS trial, simeprevir was the first protease inhibitor combined with sofosbuvir for 12 or 24 weeks with or without ribavirin. Despite having not more than 30 patients per treatment arm, this phase II trial led to the use of this regimen after both agents were approved for its use with pegylated interferon and ribavirin.[142] In phase III trials this regimen was evaluated for 8 to 12 weeks in patients without cirrhosis and 12 weeks for those with cirrhosis.[143 144] Again, the baseline Q80K variant was an important predictor of response for patients with HCV genotype 1a. The OPTIMIST-1 showed that among non-cirrhotic patients who were treated for 8 weeks the Q80K variant was associated with a lower SVR rate of 73% while 84% of the patients without this variant achieved SVR. However, extending the treatment duration to 12 weeks could overcome this difference with SVR rates of 96-9% for all patients.[144] The OPTIMIST-2, including solely patients with cirrhosis, also showed the importance of this variant. Presence of the Q80K variant led to an SVR rate of 74%, while 92% of the patients without this variant achieved SVR.[143] These studies show that once a regimen is shortened or initiated in a population that is difficult to cure, baseline RAVs become more important. Prolongation of treatment or the addition of ribavirin can possibly be a solution.

These promising results led to the studies of sofosbuvir combined with ledipasvir, another NS5A inhibitor. This first single-tablet regimen is very potent, but less pangenotypic than the combination of sofosbuvir and daclatasvir. The combination has been studied for the duration of 8-24 weeks with and without the addition of ribavirin.[145-147] For both treatment-naïve and treatment-experienced patients with HCV genotype 1, there was no difference in treatment duration. Furthermore, there was no additional benefit when ribavirin was added. However, since only a few patients with cirrhosis were included, these trials were not powered to conclude on an optimal regimen for treatment-experienced cirrhotic patients. In the ION-2 trial, 82-86% of the treatment-experienced patients with cirrhosis achieved SVR after 12 weeks of therapy with or without ribavirin, whereas this rate was 100% for both 24-week arms.[145 148] A subsequent trial, including cirrhotic patients who were treatment-experienced, randomized patients to receive either 12 weeks of sofosbuvir-ledipasvir and ribavirin or 24 weeks of sofosbuvir-ledipasvir without ribavirin. With 77 and 78 patients in the two arms, this study suggested that a 12-week regimen with the addition of ribavirin is similar to extending sofosbuvir-ledipasvir therapy to 24 weeks, since the SVR rates were 96% and 97%, respectively.[149] For the first time in the history of chronic HCV infection, patients with decompensated cirrhosis could be included in clinical trials. The SOLAR-1 and SOLAR-2 trials evaluated 12 and 24 weeks of sofosbuvir-ledipasvir and ribavirin. Even in this population with advanced cirrhosis, SVR rates were close to 90%, with no difference between 12 or 24 weeks of therapy.[150 151]

The first regimen that combined drugs from all three classes included paritaprevir (protease inhibitor that is ritonavir-boosted), ombitasvir (NS5A inhibitor) and dasabuvir (non-nucleotide NS5B inhibitor).[152-157] This 12-week regimen is highly effective against HCV genotype 1b, with SVR rates of 95-100% irrespective of the patients' profile. For patients with HCV genotype 1a, SVR rates were 90-97% and depended on the addition of ribavirin. Moreover, the duration of 24 weeks has been recommended for patients with cirrhosis and HCV genotype 1a. Again, regimens with a low barrier to resistance benefit from extending duration and/ or the addition of ribavirin. Pockros et al. assessed

the efficacy of this regimen in patients with HCV genotype 1 and end-stage renal disease. Although the number of included patients was limited, 12 weeks of triple therapy with paritaprevir/ritonavir, ombitasvir and dasabuvir with or without ribavirin resulted in an SVR rate of 90%.[158]

The development of second-generation protease inhibitors has increased the physicians' choice for the treatment of HCV genotype 1 even more. Recently, grazoprevir (protease inhibitor) and elbasvir (NS5A inhibitor) have been approved for the treatment of HCV genotype 1 and 4.[159] The advantage of this second-generation protease inhibitor is that it has a higher barrier to resistance compared to the first-generation protease inhibitors. A recent trial showed that this co-formulation plus ribavirin for 12 weeks resulted in an SVR rate of 96% in patients who had previously failed therapy with pegylated interferon ribavirin in addition to telaprevir, boceprevir or simeprevir.[160] The regimen is also highly effective for treatment-naïve and treatment-experienced patients, with different regimens recommended for HCV genotype 1a and 1b.[161] Moreover, it was shown to have an excellent efficacy in patients with renal failure.[162] This study is the largest study in this specific population so far and resulted in an SVR rate of 99%.

Due to the fact that HCV genotype 1 is most prevalent in the Western world, clinical trials mainly focused on the development of treatment regimens for this genotype. In the era of pegylated interferon and ribavirin, HCV genotype 1 and 4 were placed in the same group when treatment decisions, like dosage and duration were concerned. Most DAAs that are active against HCV genotype 1 are also active against HCV genotype 4. Although not extensive assessed in clinical trials, the combination of simeprevir and sofosbuvir, sofosbuvir and ledipasvir, paritaprevir and ombitasvir without the addition of dasabuvir and grazoprevir-elbasvir have shown to be effective against HCV genotype 4. Subsequently, clinical guidelines recommend the use of these regimens for patients with HCV genotype 4.[35 36]

For patients with HCV genotype 2, the combination of sofosbuvir and ribavirin is very effective. However, if there is a contra-indication for ribavirin, these patients can be treated with sofosbuvir and daclatasvir for 12 weeks. For HCV genotype 3, the combination of sofosbuvir and daclatasvir was also evaluated. In non-cirrhotic patients, 12 weeks of this combination resulted in an SVR rate of 96%. When this regimen was initiated in patients with cirrhosis, only 63% of patients achieved SVR.[163] These disappointing results led to the small ALLY 3+ study, including patients with HCV genotype 3 and advanced hepatic fibrosis.[164] Patients were randomized to receive sofosbuvir and daclatasvir plus ribavirin for 12 or 16 weeks and resulted in SVR rates of 83 to 89%, respectively. However, the small sample size makes it difficult to draw strong conclusions or to distinguish the preferred duration. Additional data from the French compassionate use program, including mostly cirrhotic patients, showed that 24 weeks of sofosbuvir and daclatasvir with or without ribavirin led to SVR rates above 80%.[165] When the effectiveness was assessed according to the Child Pugh score, patients with Child Pugh A achieved SVR rates of 85% or higher. Again, these data should be interpreted with some caution, as patients were not randomized and treatment regimen as well as duration were decided at the discretion of the treating physician.

In the near future, pangenotypic regimens will be approved, which will simplify treatment decisions considerably. The combination of sofosbuvir and velpatasvir will probably be the first pangenotypic regimen and has shown excellent efficacy in phase III trials.[166-168] With this combination,

the use of ribavirin is redundant and even patients with decompensated cirrhosis can be effectively treated. However, the treatment of HCV genotype 3 remains a challenge.

Since the first approval of interferon-free regimens, there is an increasing amount of data on real-world experiences. Both small retrospective cohort studies as well as large registries report on the outcome of antiviral treatment for chronic HCV infection. In contrast to the first real-world data on telaprevir and boceprevir, no major concerns were raised from these studies. As patients in these real-world registries did not have to fulfill strict inclusion criteria, effectiveness was not as high as in phase III trials, but were still proven to be very effective.[169] Although these DAAs were very well tolerated and generally had a good safety profile, reports on possible hepatotoxicity have emerged in patients with decompensated cirrhosis.[170 171] Moreover, drug-drug interactions always need to be assessed since it can cause important side effects as was seen for sofosbuvir and amiodarone.[172 173] In general, treatment with DAAs is very safe in non-cirrhotic patients and patients with compensated cirrhosis. Most problems occur in patients with decompensated cirrhosis, who were often withheld from antiviral treatment in the era of pegylated interferon and ribavirin. Whether these adverse events could be attributed to the toxic effect of drugs or whether it is part of the patient's natural history is not always clear and remains matter of debate. Likewise, the occurrence of hepatic decompensation during antiviral treatment has been reported for several treatment regimens, leading the FDA to discourage the use of dasabuvir, ombitasvir, and paritaprevir/ritonavir for patients with decompensated liver disease.[174] Also, because of the real-world safety issues which were encountered with the first-generation protease inhibitors telaprevir and boceprevir among patients with cirrhosis and low platelets or low albumin levels, one could argue that protease inhibitors may not represent an ideal class of DAAs for those with the most severe cirrhosis.[121]

The near future will tell us whether the use of DAAs is always justified and whether no important safety issues will change our perspective on timing of antiviral therapy. As longer follow-up will be available for patients with decompensated cirrhosis, there may be a subgroup identified that does not really benefit from antiviral therapy before liver transplantation. Furthermore, there were some early observations in small cohorts of patients that reported early tumor recurrence in patients with HCV-related HCC who underwent interferon-free antiviral therapy.[175 176] Pol, however, did not observe an increased risk of HCC recurrence after DAA treatment in three distinct prospective cohorts.[177] These unexpected findings will probably dominate HCV research during the upcoming years.

## SCOPE AND AIMS OF THIS THESIS

Clearly, the development of antiviral therapy has moved at an incredible pace during the 3 years following the first proof-of-concept that chronic HCV infection could be eradicated without pegylated interferon.[133] The implementation of interferon-free treatment regimens has broadened the horizon for patients with chronic HCV infection tremendously. Within a timeframe of 5 years, important treatment developments resulted in near-perfect SVR rates, even among patients with the most advanced liver disease. As successful antiviral therapy may be lifesaving, these developments were long awaited. Some hurdles have to be taken, however, before the health burden of this chronic disease can truly

be reduced. For instance, the access to DAAs needs to be broadened so that patients can be treated regardless of the severity of hepatic fibrosis. Reducing the costs of these drugs probably remains a key factor before this goal can be achieved. Pegylated interferon and ribavirin therapy is therefore likely to remain an important treatment option for many countries. As developments are still ongoing, prices will hopefully fall as a result of mutual competition. Also, it is important to increase the number of patients who are diagnosed, as the majority of patients are currently unaware of their chronic viral hepatitis. Nevertheless, we have seen a true paradigm shift regarding the treatment of chronic HCV infection. For patients affected by this virus the nearby future has never looked more promising.

This thesis focuses on the tolerability and outcome of antiviral treatment for patients with chronic HCV infection. First, in order to optimize interferon-based treatment regimens for patients with advanced hepatic fibrosis, the need for dose reductions because of interferon-induced thrombocytopenia or neutropenia was assessed in relation to the occurrence of on-treatment bleeding episodes and infections. Next, the association of functional variants in the inosine triphosphatase (*ITPA*) gene and hematological side effects during interferon-based regimens were evaluated. In order to see which patients would be considered for interferon-free treatment regimens, the epidemiological changes in patient and disease characteristics of patients referred to a tertiary center were assessed. As these interferon-free regimens rouse a broad discussion on the costs of antiviral therapy, the costs of interferon-based treatment were assessed among patients with advanced hepatic fibrosis. Next, the safety and effectiveness of interferon-free regimens were assessed in patients with compensated and decompensated cirrhosis as well as in patients with HCV genotype 3 specifically. Finally, the occurrence of renal impairment during sofosbuvir-based antiviral therapy was reported.

**Aims**

The aims of this thesis were to assess:

1. the association between interferon-induced thrombocytopenia and bleeding episodes as well as the association between interferon-induced neutropenia and infections among patients with chronic HCV infection and advanced hepatic fibrosis.
2. the association between the functional *ITPA* variants and hematological side effects during antiviral therapy with pegylated interferon and ribavirin
3. the epidemiological changes in patient and disease characteristics among individuals with chronic HCV infection
4. the direct medical costs during interferon-based antiviral treatment and the costs per SVR among patients with advanced hepatic fibrosis
5. safety and effectiveness of DAA-therapy in patients with cirrhosis and HCV genotype 3
6. the occurrence of renal impairment during sofosbuvir-based antiviral therapy

# CHAPTER 1.1

## EFFECT OF THROMBOCYTOPENIA ON TREATMENT TOLERABILITY AND OUTCOME IN PATIENTS WITH CHRONIC HCV INFECTION AND ADVANCED HEPATIC FIBROSIS

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## ABSTRACT

**Background & Aims**

Pegylated interferon is still the backbone of hepatitis C treatment and may cause thrombocytopenia, leading to dose reductions, early discontinuation, and eventually worse clinical outcome. We assessed associations between interferon-induced thrombocytopenia and bleeding complications, interferon dose reductions, early treatment discontinuation, as well as SVR and long-term clinical outcome.

**Methods**

All consecutive patients with chronic HCV infection and biopsy-proven advanced hepatic fibrosis (Ishak 4–6) who initiated interferon-based therapy between 1990 and 2003 in 5 large hepatology units in Europe and Canada were included.

**Results**

Overall, 859 treatments were administered to 546 patients. Baseline platelets (in $10^9$/L) were normal (≥150) in 394 (46%) treatments; thrombocytopenia was moderate (75–149) in 324 (38%) and severe (<75) in 53 (6%) treatments. Thrombocytopenia-induced interferon dose reductions occurred in 3 (1%); 46 (16%), and 15 (30%) treatments respectively ($p$ <0.001); interferon was discontinued due to thrombocytopenia in 1 (<1%), 8 (3%), and in 8 (16%) treatments respectively ($p$ <0.001). In total, 104 bleeding events were reported during 53 treatments. Only two severe bleeding complications occurred. Multivariate analysis showed that cirrhosis and a platelet count below 50 were associated with on-treatment bleeding. Within thrombocytopenic patients, patients attaining SVR had a lower occurrence of liver failure ($p$ <0.001), hepatocellular carcinoma ($p$ <0.001), liver related death or liver transplantation ($p$ <0.001), and all-cause mortality ($p$ = 0.001) compared to patients without SVR.

**Conclusions**

Even in thrombocytopenic patients with chronic HCV infection and advanced hepatic fibrosis, on-treatment bleedings are generally mild. SVR was associated with a marked reduction in cirrhosis-related morbidity and mortality, especially in patients with baseline thrombocytopenia.

# INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major cause of cirrhosis, hepatocellular carcinoma (HCC), and end-stage liver disease.[178] Cirrhosis and portal hypertension lead to splenomegaly and subsequent thrombocytopenia (platelet count <150x10$^9$/L) by the sequestration of platelets. Thrombopoietin is produced by the liver and production decreases with impaired synthetic function, which contributes to a reduced platelet count as well. In addition, virus-induced thrombocytopathy and bone marrow suppression are described as potential mechanisms.[113 179-181] Therefore, thrombocytopenia is a frequent manifestation in chronic liver disease and serves as an indicator of disease severity.[182]

Sustained virological response (SVR), i.e., HCV RNA negativity in blood six months after cessation of antiviral therapy, is the goal of antiviral therapy, with important clinical implications. Patients with advanced hepatic fibrosis who achieve SVR have lower liver-related and all-cause mortality as well as a reduced risk of hepatocellular carcinoma.[79-81] Current therapy involves pegylated interferon alfa (PegIFN) and ribavirin (RBV). This combination treatment leads to SVR in 10–44% of patients with compensated cirrhosis and HCV genotype 1 or 4 infection, compared to 33–72% among those with HCV genotype 2 or 3.[107] Unfortunately, treatment with PegIFN and RBV is associated with many side effects. One of the major side effects is the induction or aggravation of thrombocytopenia. Especially among patients with cirrhosis this frequently necessitates dose reductions, which lead to reduced treatment efficacy.[111 112]

Recently, telaprevir or boceprevir is added to the treatment regimen for HCV genotype 1 infection. Although the addition of a protease inhibitor increased the SVR rate for those patients with HCV genotype 1 and advanced liver disease, cure rates remain poor.[122-126] Furthermore, real-world data have shown that triple therapy was associated with a high risk of severe on-treatment complications including infection, liver failure, and even death among patients with cirrhosis, especially for those with low platelets (≤100x10$^9$/L) or serum albumin <35 g/L.[183]

Current guidelines advise to reduce the dose of PegIFN when platelet counts fall below 50x10$^9$/L and to stop treatment when platelet counts fall below 25x10$^9$/L.[35 36] As a result patients with platelet counts below 75x10$^9$/L are often excluded from antiviral therapy or have to stop treatment prematurely.[35 36] However, patients with thrombocytopenia are those with most advanced liver disease, who are most likely to have a clinical benefit from successful antiviral treatment.[79-81 184] Under the assumption that the risk of disease progression outweighs the risk of interferon-induced adverse events, some clinicians do treat patients with chronic HCV infection and severe thrombocytopenia. Currently, little is known about the safety, antiviral efficacy or long-term clinical outcome of antiviral therapy among these patients.

The primary aim of this study was to assess the association between thrombocytopenia and interferon dose reductions, early treatment discontinuation and bleeding complications among patients with chronic HCV infection and bridging fibrosis or cirrhosis. The secondary aim was to assess the SVR rates and long-term clinical outcome among patients having bridging fibrosis or cirrhosis, with or without thrombocytopenia.

## PATIENTS AND METHODS

### Patients

The study is based on all patients included in our previously described international, multicenter cohort from 5 large hepatology units in Europe and Canada.[81 185] This cohort included all consecutive patients with chronic HCV infection who started an interferon-based treatment between 1990 and 2003 and had histological proof of bridging fibrosis or cirrhosis (Ishak fibrosis score 4–6). Patients co-infected with human immunodeficiency virus (HIV) or hepatitis B virus (HBV) and patients with a history of decompensated liver disease were excluded. All charts were re-reviewed by a single investigator (RM) in order to collect detailed data on platelet counts, PegIFN and/or RBV dose reductions or treatment cessation, and bleeding episodes during antiviral treatment.

Thrombocytopenia was defined as a platelet count below $150x10^9$/L; moderate thrombocytopenia was defined as a platelet count of 75–$149x10^9$/L and severe thrombocytopenia as a platelet count below $75x10^9$/L.[36 186] The platelet count closest to the start of therapy was considered as baseline platelet count, not exceeding six months before treatment. A bleeding episode was defined as severe if it resulted in hospital admission, requirement of blood transfusion, permanent disability, or death. All other bleedings were defined as mild. Episodes of bleeding were registered if bleeding was reported by the patient or if the patient was referred for further analysis because of bleeding.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. The study protocol was reviewed and approved by the ethics committee in the center of the primary investigators, which was the Erasmus MC University Medical Center in Rotterdam, the Netherlands. Ethical approval in the participating centers was obtained according to the local regulations. According to the standards of the local ethics committees, written informed consent was obtained from patients in an earlier phase.[81 185]

### Outcome

The primary outcome measures were thrombocytopenia-induced interferon (IFN) dose reductions, early treatment discontinuation, and bleeding complications during antiviral therapy. Secondary outcome measures were SVR and clinical events, such as liver failure, HCC, and (liver-related) mortality or liver transplantation. Follow-up started 24 weeks after the end of treatment, so that SVR status could be assessed at the start of follow-up.

Liver failure was defined as an episode of either ascites confirmed by ultrasonography, variceal bleeding, jaundice or overt hepatic encephalopathy. The diagnosis of HCC was based on histological confirmation or two coincident imaging techniques (computed tomography, magnetic resonance imaging, or contrast-enhanced ultrasonography), showing a focal lesion of more than two cm with arterial-phase enhancement or one imaging technique showing a focal lesion of more than two cm with arterial-phase enhancement in combination with the presence of an α-fetoprotein level of more than 400 ng/ml.[187]

Liver transplantation and liver-related death were analyzed as a combined endpoint. Death caused by liver failure, primary liver malignancy, or variceal bleeding was considered liver related.

Death due to extrahepatic malignancy, cardiovascular or cerebrovascular events, or other causes was considered as not liver related. The cause of death was determined by the treating physician.

**Statistical analyses**

Continuous variables were summarized as median (interquartile range [IQR]) and categorical variables as frequencies (percentages). Comparisons between groups were performed using the v2 test for categorical variables or the Mann-Whitney U test for comparing medians. Data were adjusted for multiple testing.

Treatments were classified according to the baseline platelet count (normal platelets, moderate thrombocytopenia, and severe thrombocytopenia) as well as according to the fibrosis state (bridging fibrosis and cirrhosis). To take into account multiple measurements of platelet counts per patient during treatment, a repeated measurement model with a random intercept and slope was applied. In order not to force linearity, restricted cubic splines were used to model the dynamics of platelet counts during treatment for different groups.

Kaplan-Meier methods and log-rank test were used to analyze the time to reach a platelet count of $<50x10^9$/L during treatment.

Logistic regression techniques, adjusting for multiple measurement within a patient, were used to analyze the association of platelet counts measured during therapy with a succeeding on-treatment bleeding event.

Dose reductions and early treatment discontinuation were assessed in treatments for which detailed data regarding the treatment period were available.

Logistic regression was used to analyze which of the baseline characteristics were associated with attaining SVR after the last registered treatment. Platelet count at baseline was analyzed as a dichotomous variable, using the cut-off value of $<150x10^9$/L. Age, sex, treatment naïve, and variables with a *p* value of ≤0.2 in univariate analyses were included in multivariate analyses.

The cumulative incidence of liver failure, HCC and (liver-related) mortality after the last registered treatment were assessed using the Kaplan-Meier method. The log-rank test was applied to compare different groups of patients based on SVR-status and baseline platelet count at the last treatment.

Since PegIFN is still the backbone of hepatitis C treatment, sensitivity analyses were done among patients treated with PegIFN for the association of platelet counts with on-treatment bleeding and SVR.

A *p* value <0.05 was considered statistically significant and all statistical tests were two-tailed. IBM SPSS 20.0.0.1 statistical package (SPSS, Inc., Chicago, IL) and SAS 9.3 were used.

# RESULTS

### Patients

Overall, 546 patients with chronic HCV infection and bridging fibrosis or cirrhosis who started interferon-based therapy between 1990 and 2003 were included. Of the 421 (77%) patients without SVR, 215 patients received at least one subsequent antiviral treatment regimen. Overall, 859 treatment courses were registered.

In 377 of 859 (44%) treatments thrombocytopenia was present at baseline. Thrombocytopenia was moderate in 324 of 377 (86%) treatments and severe in 53 of 377 (14%) treatments (Table 1.1.1). For 88 (10%) treatments there was no platelet count available within 6 months prior to treatment initiation (Supplementary Fig. 1.1.1).

### Dynamics of platelet counts during treatment

The dynamics of the platelet counts during treatment are illustrated in Fig. 1.1.1. The group with severe thrombocytopenia reached a platelet count <50x$10^9$/L more often as well as in an earlier phase of treatment compared to the group with moderate thrombocytopenia and the group with normal platelets (median time until the first visit with a platelet count <50x$10^9$/L was respectively 1, 24, and 46 weeks, all $p$ <0.001) (Supplementary Fig. 1.1.2). The platelet count dropped below 50x$10^9$/L during 3 (<1%) of 357 treatments among patients with normal platelet count prior to treatment, during 78 (25%) of the 310 treatments in patients with moderate baseline thrombocytopenia and in 37 (73%) of 51 treatments among patients with severe thrombocytopenia ($p$ <0.001, Fig. 1.1.2).

### Effect of thrombocytopenia on interferon dose

Dose reductions and early treatment discontinuation could be assessed in 684 (89%) and 720 (93%) treatments, respectively. In 3 (1%) of 338 treatments among those with normal platelet count, 46 (16%) of 296 treatments among patients with moderate thrombocytopenia and in 15 (30%) of 50 treatments among patients with severe thrombocytopenia prior to therapy, thrombocytopenia was the main reason for at least one (peg)interferon dose reduction ($p$ <0.001, Fig. 1.1.2). In univariate analysis, the use of PegIFN was associated with the occurrence of dose reductions due to thrombocytopenia (OR 2.16, 95%CI 1.25–3.72, $p$ = 0.006).

Treatment was prematurely discontinued due to thrombocytopenia in 1 (<1%) of 354 treatments among patients with normal platelet count, 8 (3%) of 314 treatments among those with moderate baseline thrombocytopenia, and 8 (15%) of 52 treatments among patients with severe baseline thrombocytopenia ($p$ <0.001, Fig. 1.1.2).

### Effect of thrombocytopenia on safety and bleeding complications

In total, 109 (13%) of the 859 treatment courses had at least one visit with anemia. The occurrence of anemia was significantly different between treatment courses among patients with a normal platelet count and patients with baseline thrombocytopenia (33/394 (8.4%) vs. 76/377 (20.2%), $p$ <0.001). Further safety details are shown in Table 1.1.2.

**Table 1.1.1 | Baseline characteristics according to baseline platelet count**

| Variable | Treatments with normal platelets (n=294) | Treatments with moderate TCP (n=324) | Treatments with severe TCP (n = 53) |
|---|---|---|---|
| Male | 281 (71%) | 222 (69%) | 37 (70%) |
| Age, in years[†] | 48 (42-55) | 51 (45-58) | 51 (44-59) |
| BMI, in kg/m$^2$ | 26.1 (23.5-29.0) | 26.6 (23.7-29.4) | 26.8 (23.7-31.3) |
| HCV genotype | | | |
| 1 | 267 (68%) | 212 (65%) | 38 (72%) |
| 2 | 33 (8%) | 22 (7%) | 5 (9%) |
| 3 | 64 (16%) | 55 (17%) | 4 (8%) |
| 4 | 16 (4%) | 16 (5%) | 3 (6%) |
| Other/unknown | 14 (4%) | 19 (6%) | 3 (6%) |
| Treatment naïve | 212 (54%) | 192 (59%) | 26 (49%) |
| Cirrhosis[#†*] | 259 (66%) | 271 (84%) | 51 (96%) |
| Fibrosis score | | | |
| Ishak 4 | 135 (34%) | 53 (16%) | 2 (4%) |
| Ishak 5 | 81 (21%) | 57 (18%) | 5 (9%) |
| Ishak 6 | 178 (45%) | 214 (66%) | 46 (87%) |
| Platelet count, in 10$^9$/L[#†*] | 197 (174-231) | 115 (95-135) | 63 (55-69) |
| Albumin, in g/L[#†*] | 43 (40-45) | 41 (37-43) | 37 (34-41) |
| Bilirubin, in µmol/L[#†*] | 12 (9-15) | 14 (11-20) | 22 (14-33) |
| AST/ALT ratio[†*] | 0.68 (0.55-0.84) | 0.78 (0.60-1.0) | 0.89 (0.68-1.1) |
| Treatment with PegIFN/RBV | 184 (47%) | 151 (47%) | 23 (43%) |
| Treatment duration, in weeks | 29 (21-48) | 25 (17-48) | 24 (14-47) |
| Alcohol abuse ever | 94 (24%) | 74 (23%) | 8 (15%) |
| Diabetes mellitus[†] | 39 (10%) | 60 (19%) | 8 (15%) |

Abbreviations: TCP, thrombocytopenia; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PegIFN, pegylated interferon; RBV, ribavirin.

a. Medians are presented as number, (IQR, interquartile range).
b. Numbers are presented as n, (percentage of the whole group).
c. Variables that were significantly different among two groups were marked with # for moderate vs. severe thrombocytopenia, [†] for moderate thrombocytopenia vs. normal platelets and/or for severe thrombocytopenia vs. normal platelets.

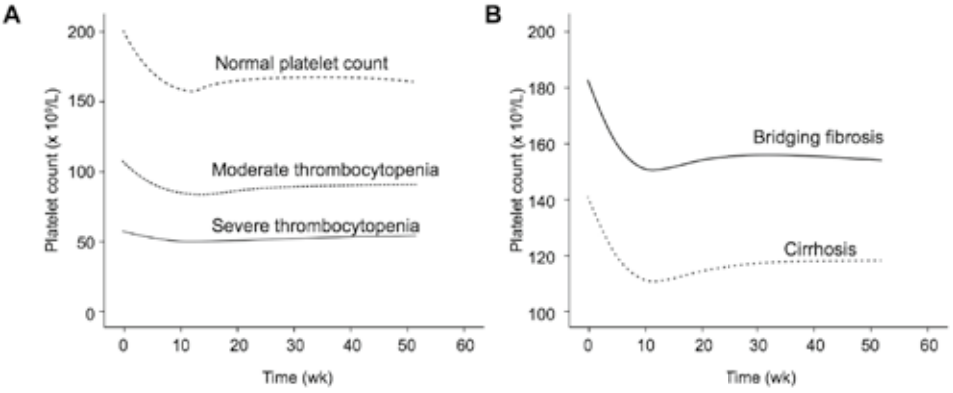



**Figure 1.1.1 | Dynamics of the platelet counts during treatment**

(A) Shows the dynamics of platelet counts during treatment among patients with normal platelet count, moderate thrombocytopenia and severe thrombocytopenia at baseline. (B) Shows the dynamics of platelet counts during treatment among patients with bridging fibrosis and cirrhosis.

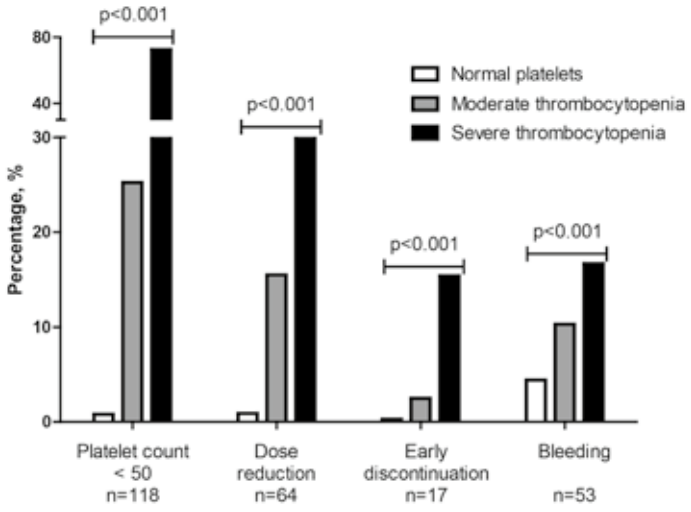



**Figure 1.1.2 | Primary endpoints**

Platelet count <50x109/L, dose reductions due to thrombocytopenia, early treatment discontinuation due to thrombocytopenia, and on-treatment bleeding, according to baseline platelet count.

Bleeding complications could be assessed in 678 (88%) treatments for which detailed data regarding the treatment period were available. In total, 104 bleeding events were registered during 53 treatments in 48 patients. Details on bleedings are shown in Table 1.1.3. Eleven (11%) of 104 bleedings required treatment or further diagnostic procedures; sigmoidoscopy, gastroscopy, or consultation of another specialist. At least one bleeding episode occurred in 15 (4%) of 338 treatments in patients with a normal platelet count, in 30 (10%) of the 292 treatments in patients with moderate thrombocytopenia, and in 8 (17%) of 48 treatments in patients with severe thrombocytopenia ($p$ <0.001, Fig. 1.1.2).

Two bleeding complications were severe. Both were esophageal variceal hemorrhage requiring hospital admission and transfusion. Nevertheless, treatment was not discontinued in these two cases. Fatal bleedings did not occur. All other bleeding episodes were mild and included gingival bleeding (n = 19), hematuria (n = 14), and epistaxis (n = 45).

In multivariate analysis adjusted for age and gender, the presence of cirrhosis (OR 4.6, 95%CI 1.6–13.9, $p$ = 0.006), and a platelet count <50x10$^9$/L at the previous visit (OR 5.4, 95%CI 2.8–10.5, $p$ <0.001) were independently associated with the occurrence of on-treatment bleeding (Table 1.1.4). As a sensitivity analysis, only those patients treated with PegIFN were considered, showing that a platelet count <50x10$^9$/L remained independently associated with the occurrence of on-treatment bleeding (OR 4.5, 95%CI 2.3–9.0 $p$ <0.001, Table 1.1.4).

**Table 1.1.2 | Adverse events during treatment**

| Reason | Treatments with normal/ unknown platelets (n=392) | Treatments with TCP (n=361) |
|---|---|---|
| Discontinuation | | |
| Hematological side effects | 9 (2.5%) | 2 (0.5%) |
| Severe infection | 6 (1.7%) | 1 (0.3%) |
| Decompensation | 5 (1.4%) | none |
| Other side effects/ events (psychiatric, cardiac or not specified) | 14 (3.9%) | 13 (3.3%) |
| Admission | | |
| Bleeding (decompensation) | 2 (0.6%) | None |
| Severe infection | 14 (3.9%) | 10 (2.6%) |
| Transfusion | None | 4 (1.0%) |
| Adverse events | 7 (1.9%) | 6 (1.5%) |

Abbreviations: TCP, thrombocytopenia

a. Numbers are presented as n, (percentage of the total amount of treatment courses)
b. Reasons for admission defined as adverse events, included a ruptured ovary cyst, stroke, renal impairment, atriumfibrillation, chest pain (no diagnosis), abdominal pain (no diagnosis), pleural effusion, decompensation cordis and hematological side effects

**Table 1.1.3 | Details on bleedings**

| Type of bleeding | Treatments with normal platelets (n=338) | Treatments with moderate TCP (n=292) | Treatments with severe TCP (n=48) |
|---|---|---|---|
| Major | | | |
| Variceal bleeding | None | 2 (3.3%) | None |
| Minor | | | |
| Epistaxis | 12 (48%) | 31 (51%) | 7 (39%) |
| Gingival | 3 (12%) | 13 (21%) | 2 (11%) |
| Hematuria | 4 (16%) | 4 (6.6%) | 6 (33%) |
| Vaginal | 5 (20%) | 4 (6.6%) | None |
| Rectal | None | 4 (6.6%) | 2 (11%) |
| Hemoptoe | 1 (4%) | 2 (3.3%) | None |
| Other | None | Skin (1.6%) | Subconjunctival (5.6%) |
| Total | 25 | 61 | 18 |

Abbreviations: TCP, thrombocytopenia

a. Numbers are presented as n, (percentage of the total amount of bleedings)

**Effect of thrombocytopenia on SVR**

In total, 193 (35%) of 546 patients attained SVR. Details on SVR rates are shown in Table 1.1.5. To assess the association between platelet counts and virological response, only the last treatment that a patient received was considered.

Of 394 treatments that were started in patients with a normal baseline platelet count, 113 (29%) resulted in SVR. This differed significantly from the group with baseline thrombocytopenia, in which 70 (19%) of the 377 treatments resulted in SVR ($p = 0.001$). Within the group of patients with baseline thrombocytopenia, there was no difference in SVR rates between those with moderate and those with severe thrombocytopenia: 61 (19%) of 324 treatments in patients with moderate baseline thrombocytopenia and 9 (17%) of 53 treatments in patients with severe baseline thrombocytopenia ($p = 0.749$).

Thrombocytopenia prior to treatment was inversely associated with SVR (OR 0.41 95%CI 0.28–0.60 $p$ <0.001). In multivariate analysis, adjusting for age, gender, cirrhosis, baseline diabetes mellitus, HCV genotype, AST/ALT ratio, treatment year, type of therapy, and treatment naivety, baseline thrombocytopenia was an independent negative predictor of SVR (OR 0.49, 95%CI 0.31–0.76 $p$ = 0.002, Table 1.1.6). As a sensitivity analysis, only those patients treated with PegIFN were considered, showing that baseline thrombocytopenia remained an independent negative predictor of SVR (OR 0.45, 95%CI 0.27–0.77 $p = 0.004$, Table 1.1.6).

**Effect of thrombocytopenia on long-term clinical outcome**

Liver failure, HCC, liver-related death, and all-cause mortality occurred less often among patients who attained SVR compared to patients who did not attain SVR within the group of patients with normal platelets and the group of patients with baseline thrombocytopenia (Fig. 1.1.3). Among the

**Table 1.1.4 | Univariate and multivariate logistic regression analysis for on-treatment bleeding**

| Variable | Univariate | | Multivariate | | Multivariate (Peg-IFN only) | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | p-value | OR (95% CI) | p-value | OR (95% CI) | p-value |
| Age | 1.0 (0.97-1.03) | 0.867 | 0.98 (0.95-1.02) | 0.31 | 0.97 (0.94-1.00) | 0.09 |
| Female gender | 1.61 (0.82-3.19) | 0.169 | 1.61 (0.88-2.97) | 0.12 | 3.02 (1.46-6.25) | 0.003 |
| BMI | 1.03 (0.96 – 1.11) | 0.42 | | | | |
| Cirrhosis | 6.0 (2.0-17.7) | 0.001 | 4.64 (1.55-13.9) | 0.006 | 2.59 (0.75-8.98) | 0.135 |
| Baseline albumin | 0.93 (0.88-1.00) | 0.042 | | | | |
| Baseline bilirubin | 1.04 (1.00-1.08) | 0.035 | | | | |
| Platelets (categorical) | | | | | | |
| Normal | 1.00 | | | | | |
| Moderate | 2.72 (1.31-5.64) | 0.007 | | | | |
| Severe | 4.32 (1.62-11.5) | 0.003 | | | | |
| Weeks on treatment | 0.98 (0.97-1.00) | 0.057 | | | | |
| Type of IFN (Peg-IFN vs IFN) | 0.75 (0.38-1.48) | 0.41 | | | | |
| Platelet count < 50 at previous visit | 6.90 (3.62-13.1) | <0.001 | 5.38 (2.76-10.5) | <0.001 | 4.51 (2.25-9.03) | <0.001 |

Abbreviations: BMI, body mass index; IFN, interferon; Peg-IFN, pegylated interferon

**Table 1.1.5 | SVR rates according to genotype, previous response (naïve or not) and type of therapy**

| | Interferon | | Interferon + RBV | | PegIFN + RBV * | |
|---|---|---|---|---|---|---|
| | TCP | Normal platelets | TCP | Normal platelets | TCP | Normal platelets |
| Naïve (n=430) | | | | | | |
| HCV genotype 1 + 4 | 1/45 (2.2%) | 0/51 (0.0%) | 4/43 (9.3%) | 9/41 (22.0%) | 14/61 (23.0%) | 20/51 (39.2%) |
| HCV genotype 2 + 3 | 1/16 (6.2%) | 1/10 (10.0%) | 5/12 (41.7%) | 7/14 (50.0%) | 11/23 (47.8%) | 27/34 (79.4%) |
| HCV genotype 5, 6 or unknown | 3/14 (21.4%) | 2/8 (25.0%) | 1/3 (33.3%) | 1/2 (50.0%) | 0/1 (0.0%) | 1/1 (100%) |
| Previously treated (n=341) | | | | | | |
| HCV genotype 1 + 4 | 0/14 (0.0%) | 0/19 (0.0%) | 5/34 (14.7%) | 6/34 (17.6%) | 15/72 (20.8%) | 22/87 (25.3%) |
| HCV genotype 2 + 3 | 0/1 (0.0%) | 0/5 (0.0%) | 4/9 (44.4%) | 5/10 (50.0%) | 5/25 (20.0%) | 11/24 (45.8%) |
| HCV genotype 5, 6 or unknown | - | 0/1 (0.0%) | 0/3 (0.0%) | 1/1 (100%) | 1/1 (100%) | 0/1 (0.0%) |

Abbreviations: SVR, sustained virological response; HCV, hepatitis C virus; TCP, thrombocytopenia; RBV, ribavirin

a. * Including 27 treatments with peginterferon monotherapy

patients who did not attain SVR, liver failure, HCC, liver-related death, and all-cause mortality occurred significantly more often in patients with thrombocytopenia compared to the patients with normal platelets at baseline. In contrast, the difference in cumulative occurrence of liver failure, HCC, liver-related death, and all-cause mortality among the patients with SVR was not statistically significant between those with and without thrombocytopenia.

## DISCUSSION

This large cohort study describes the course of antiviral treatment and post-treatment clinical outcome among patients with chronic HCV infection and advanced liver disease, including those with moderate and severe thrombocytopenia. These data are unique since severe thrombocytopenia normally is a contraindication for interferon-based therapy in randomized controlled trials in patients with chronic HCV infection. Severe thrombocytopenia was found to be the reason to refrain from interferon-based antiviral therapy in approximately 4% of patients with chronic HCV infection, mainly patients with advanced liver disease.[188] Importantly, the proportion of patients with chronic HCV infection and cirrhosis is rapidly rising.[63]

In the present study, almost one third of patients with severe thrombocytopenia needed at least one dose reduction due to thrombocytopenia and treatment was discontinued prematurely in 16%. Dose reductions and early treatment discontinuation were less frequent among patients with

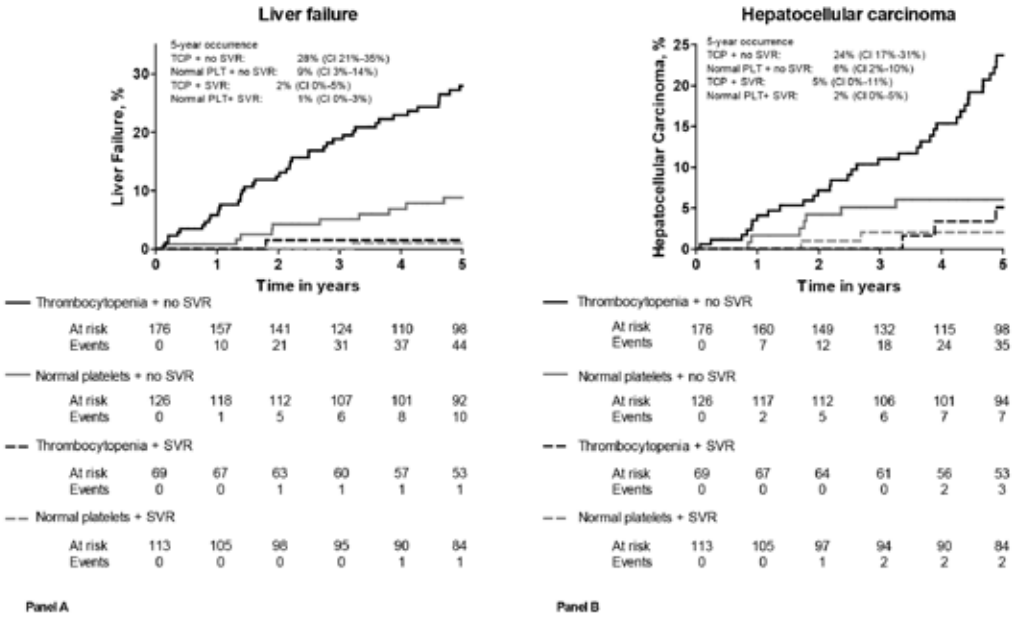



**Figure 1.1.3 | Kaplan-Meier curves showing the occurrence of long-term outcome after the last treatment based on SVR-status and baseline platelet count**

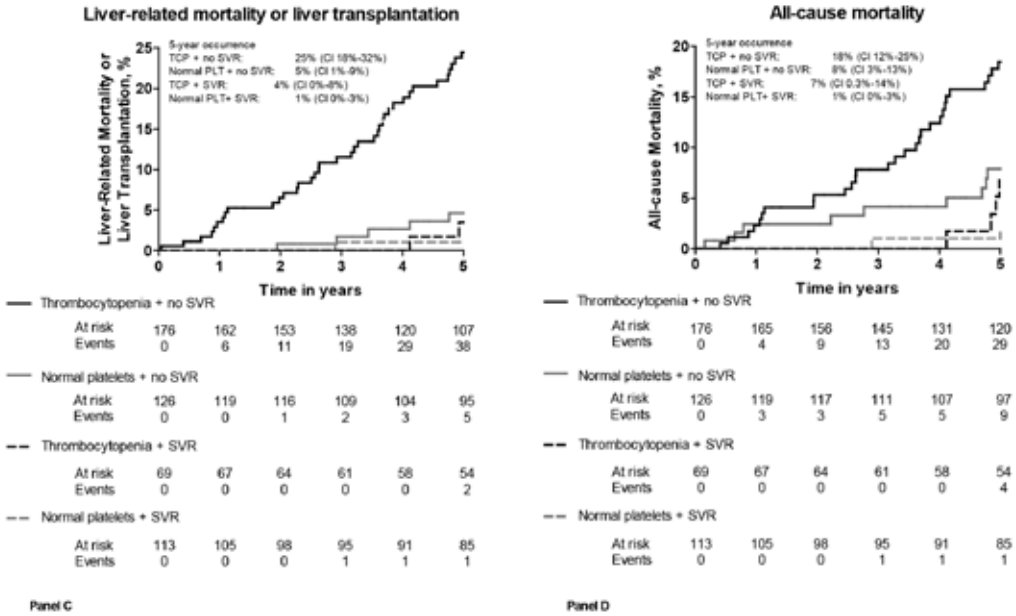



**Figure 1.1.3 | Continued**

(A) Occurrence of liver failure over time. p=0.001 for SVR vs. no SVR within patients with normal PLT; p<0.001 for SVR vs. no SVR within patients with TCP; p<0.001 for TCP vs. normal PLT within non-responding patients; and p=0.606 for TCP vs. normal PLT within patients attaining SVR. (B) Occurrence of HCC over time. p=0.008 for SVR vs. no SVR within patients with normal PLT; p<0.001 for SVR vs. no SVR within patients with TCP; p<0.001 for TCP vs. normal PLT within non-responding patients; and p=0.079 for TCP vs. normal PLT within patients attaining SVR. (C) Occurrence of liver-related death or liver transplantation over time. p=0.008 for SVR vs. no SVR within patients with normal PLT; p<0.001 for SVR vs. no SVR within patients with TCP; p<0.001 for TCP vs. normal PLT within non-responding patients; and p=0.332 for TCP vs. normal PLT within patients attaining SVR. (D) Occurrence of all-cause mortality over time. p=0.032 for SVR vs. no SVR within patients with normal PLT; p=0.001 for SVR vs. no SVR within patients with TCP; p<0.001 for TCP vs. normal PLT within non-responding patients; and p=0.260 for TCP vs. normal PLT within patients attaining SVR. TCP, thrombocytopenia; PLT, platelets; SVR, sustained virological response.

**Table 1.1.6 | Univariate and multivariate logistic regression analysis for SVR at the last treatment**

| Baseline variable | Univariate OR (95% CI) | p-value | Multivariate OR (95% CI) | p-value | Multivariate (Peg-IFN only) OR (95% CI) | p-value |
|---|---|---|---|---|---|---|
| Age | 0.98 (0.96-1.00) | 0.013 | | | | |
| Male gender | 1.31 (0.89-1.93) | 0.131 | | | | |
| Genotype 2/3 | 3.58 (2.39-5.36) | <0.001 | 3.04 (1.89-4.89) | <0.001 | 2.40 (1.37-4.23) | 0.002 |
| Cirrhosis | 0.70 (0.46-1.06) | 0.091 | | | | |
| BMI | 0.96 (0.92-1.00) | 0.069 | | | | |
| DM | 0.45 (0.26-0.80) | 0.006 | 0.50 (0.26-0.97) | 0.041 | 0.40 (0.18-0.90) | 0.026 |
| AST/ALT ratio | 0.27 (0.14-0.53) | <0.001 | 0.34 (0.15-0.77) | 0.009 | 0.39 (0.15-1.02) | 0.056 |
| Albumin | 1.08 (1.03-1.12) | <0.001 | | | | |
| Bilirubin | 0.95 (0.93-0.98) | <0.001 | | | | |
| Thrombocytopenia | 0.41 (0.28-0.60) | <0.001 | 0.49 (0.31-0.76) | 0.002 | 0.45 (0.27-0.77) | 0.004 |
| Treatment year | 1.11 (1.05-1.16) | <0.001 | | | | |
| Treatment naïve | 1.21 (0.85-1.73) | 0.284 | 1.80 (1.16-2.79) | 0.009 | 3.02 (1.71-5.33) | <0.001 |
| Treatment: | | | | | | |
| - Peg-IFN vs IFN | 7.03 (3.42-14.4) | <0.001 | 18.7 (5.57-63.0) | <0.001 | | |
| - Peg-IFN vs IFN +RBV | 0.96 (0.63-1.46) | 0.856 | 0.95 (0.57-1.58) | 0.831 | | |
| - IFN + RBV vs IFN | 7.30 (3.37-15.8) | <0.001 | 19.8 (5.64-69.6) | <0.001 | | |

Abbreviations: BMI, body mass index; DM, diabetes mellitus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; IFN, interferon; Peg-IFN, pegylated interferon; RBV, ribavirin

moderate thrombocytopenia, in line with findings from earlier studies that excluded patients with platelets below 75x10$^9$/L.[100 189]

The registration trials of PegIFN showed lower rates of dose modifications (<10%) due to thrombocytopenia, probably because they included few patients with bridging fibrosis or cirrhosis and no patients with a platelet count below 90x10$^9$/L.[104-106] In a previous single-center study among 321 patients with chronic HCV infection, including 68 patients with cirrhosis, doses of 12 patients (4%) were reduced and antiviral treatment was discontinued in two patients because of thrombocytopenia. Twenty-four patients in that study were also included in the present study, due to its center and an overlapping inclusion period.[115]

Although cirrhosis and low platelet count were associated with on-treatment bleeding, our data confirm that treatment of patients with on-treatment platelet counts below 50x10$^9$/L is generally safe.[115] Even in this cohort, including only patients with advanced liver disease, bleedings were relatively rare and mostly mild. Moreover, the two severe (variceal) bleedings observed might also have occurred in the natural course of advanced liver disease, independent of antiviral therapy.[55]

Although several interferon-free regimens are currently under development, pegylated interferon is still the backbone of antiviral treatment that is used in patients today. Awaiting further treatment developments is often not an option for patients with cirrhosis as they have a 1–5% annual risk of developing HCC; 3–6% of hepatic decompensation; and 2–4% of death.[190] Despite established cirrhosis, attaining SVR may reduce these risks and improve prognosis in terms of liver-related death and all-cause mortality.[79-81] We showed that clinical disease progression or death was more common in non-responders with thrombocytopenia than in non-responders with normal platelet counts, which suggests that thrombocytopenia predicts worse clinical outcome. Moreover, clinical disease progression or death was less common among thrombocytopenic patients who had attained SVR, in line with previous results from this cohort.[81 185] Thus, successful therapy may outweigh the side-effects of treatment in patients with advanced liver disease.

It is hard to detangle whether thrombocytopenia is responsible for lower SVR rates rather than serving as a marker for more severe disease and portal hypertension, thus defining a more difficult to treat patient population.[191 192] In general, dose reductions will compromise treatment efficacy as seen in patients with HCV genotype 1 infection.[111 112] If dose reductions due to thrombocytopenia could be avoided, either by using platelet growth factors or by applying less strict stopping rules, SVR rates might be expected to increase. Importantly, the two severe bleedings that occurred in our cohort were variceal bleedings, which are caused by portal hypertension rather than thrombocytopenia per se, and the relevance of dose reductions because of thrombocytopenia in this setting may therefore be questionable. Still, only two studies, the ENABLE-1 and ENABLE-2 study, showed improvement in SVR rates after interventions to improve the degree of thrombocytopenia, probably due to low sample sizes in other studies.[193-195] Eltrombopag enabled treatment initiation and improved treatment efficacy when compared with placebo. On the other hand, eltrombopag was associated with decompensation and thromboembolic complications, which warrants monitoring of safety of this treatment on the long term.[193]

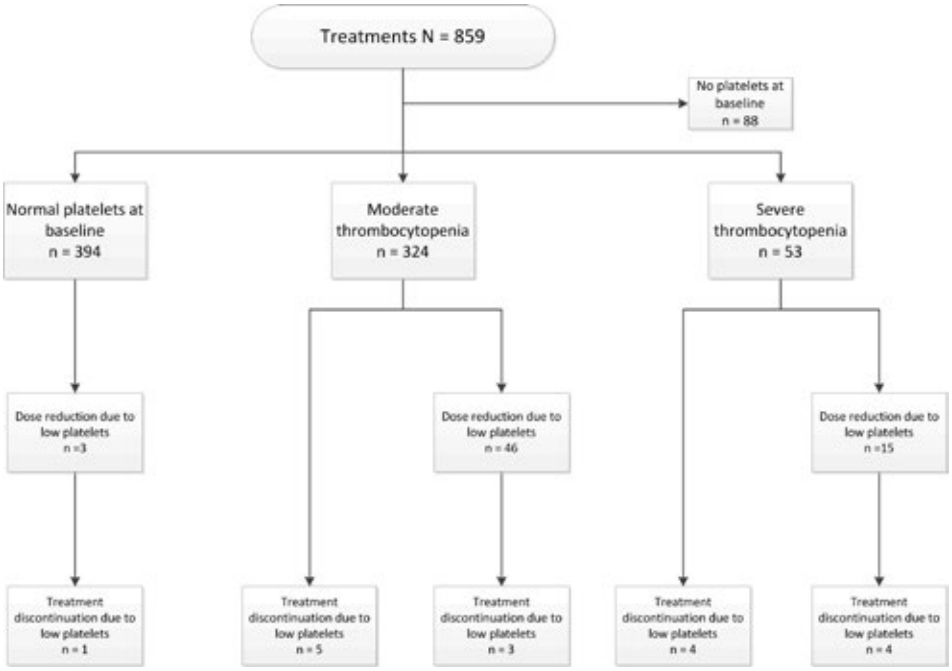
One limitation of the study is selection bias, since the patients were all treated in tertiary centers. However, the strength of our study is that all consecutive patients have been included, even those

with severe thrombocytopenia who would not be eligible for treatment in randomized trials. A limitation of this approach is that there is heterogeneity in the treatments that have been administered, varying from interferon monotherapy in the past to combination therapy with PegIFN and RBV in more recent years. Due to the retrospective nature of the study, we depended on the patient charts for reporting of bleeding episodes. In this way, clinically insignificant bleedings such as minor hematomas or skin bleedings that may be expected to occur during severe thrombocytopenia are possibly underreported.

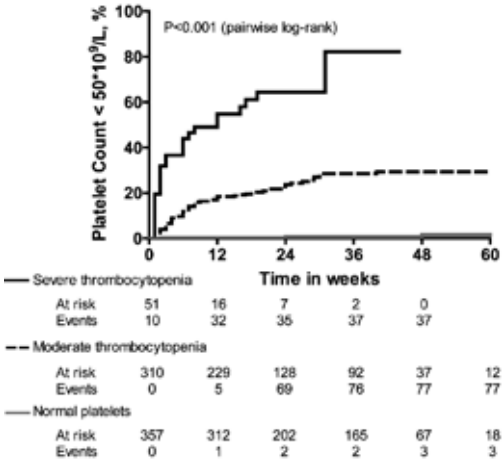
Currently, the standard triple therapy for chronic HCV genotype 1 is PegIFN and RBV, with telaprevir or boceprevir. This therapy was registered in 2012 and consequently long term follow-up data were not available at the time of this study. In general, triple therapy leads to higher SVR rates. In the registration trials, telaprevir or boceprevir did not seem associated with higher occurrence of on-treatment thrombocytopenia.[122-126] A recent report from the French early access program for use of protease inhibitors in patients with HCV-induced cirrhosis showed platelet counts below $50x10^9$/L in 13% of the patients treated with telaprevir, PegIFN, and RBV and in 6% of the patients treated with boceprevir, PegIFN, and RBV.[183] These results indicate that in the era of triple therapy we should still be aware that treatment-induced declines in platelet counts are likely to occur.

In conclusion, thrombocytopenia was an important cause for dose reductions and for treatment discontinuation in patients with chronic HCV infection and bridging fibrosis or cirrhosis treated with interferon-based regimens. Cirrhosis and a platelet count below $50x10^9$/L were associated with on-treatment bleedings, which were mostly mild. SVR was associated with a marked reduction in cirrhosis-related morbidity and mortality, especially in patients with baseline thrombocytopenia.

## SUPPLEMENTARY FIGURES




**Supplementary Figure 1.1.1 |** Overview of baseline platelet counts, dose reductions, and early treatment discontinuation during the treatments.




**Supplementary Figure 1.1.2 |** Kaplan-Meier curve showing the occurrence of a platelet count below 50x109/L during treatments started in patients with normal platelet count, moderate thrombocytopenia and severe thrombocytopenia at baseline. Ten patients had a platelet count below 50x109/L at baseline.

# CHAPTER 1.2

## ELTROMBOPAG FOR THROMBOCYTOPENIC PATIENTS WITH CHRONIC HCV INFECTION

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*Gastroenterology 2014, Jul; 147(1): 254-5*

## DEAR EDITOR:

Afdhal et al found that eltrombopag increased platelet count in thrombocytopenic (<75x109/L) patients with chronic HCV infection and advanced fibrosis and cirrhosis.[193] By using eltrombopag they achieved increased rates of sustained virological response (SVR) in patients that would otherwise be ineligible to begin or maintain antiviral therapy. Both ENABLE-1 and ENABLE-2 are well designed trials and the results seem encouraging; yet, several aspects do require some attention.

Both trials had an initiation phase using eltrombopag to increase platelet count above the threshold for treatment initiation (ENABLE-1: 90x109/L, using pegylated interferon α [PegIFN]-2a and ENABLE-2: 100x109/L, using PegIFN-2b). Afterwards patients were randomized to eltrombopag or placebo during the whole course of antiviral treatment. As the authors acknowledge in the discussion, the placebo group is not representative for the real-life setting since the investigators were instructed to follow the current local product labels for dose reductions and discontinuations. In many centers, treating physicians are applying less strict rules when dose reductions or discontinuations are considered. Data on the efficacy in such centers is needed, in order to be able to assess the incremental benefit of eltrombopag on SVR rates. Moreover, no discrimination is made between the reasons for the PegIFN induced dose reductions. In the light of eltrombopag, only the avoidance of dose reductions due to thrombocytopenia seems essential.

The risk of bleeding during PegIFN induced thrombocytopenia has never been fully elucidated. We previously showed that the risk of bleeding during PegIFN and ribavirin treatment is associated with cirrhosis and a platelet count below 50x109/L. However, these bleedings were mild and mostly not clinically relevant.[115] One could therefore question whether, with eltrombopag, we are not merely treating the physician's fear for bleeding than a real clinical problem. Even in a large cohort of 546 patients with advanced hepatic fibrosis, we found that the risk of bleeding is generally mild during treatment with interferon-based regimens, also among patients with interferon-induced thrombocytopenia.[196]

Regarding side-effects, the safety of eltrombopag remains a concern, as the rates of hepatic decompensation and thromboembolic events were significantly higher in the group receiving eltrombopag. This is in line with a previous study of eltrombopag.[197] Moreover, by allowing patients with severe thrombocytopenia to undergo antiviral treatment, the risk of hepatic decompensation is considerably increased, as Hezode et al showed in a cohort of cirrhotic patients treated with triple therapy.[183] Especially when the full dose of PegIFN is maintained, this risk is substantial. This also raises the question whether we will be able to safely use eltrombopag in patients undergoing triple therapy.

In our opinion, eltrombopag is a potent agent for treating thrombocytopenia, thereby allowing antiviral treatment. However, more data are needed to determine whether the side effects outweigh the benefit on SVR rates and bleeding risk of thrombocytopenic patients during triple therapy.

# CHAPTER 2

## RISK OF INFECTIONS DURING INTERFERON-BASED TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION AND ADVANCED HEPATIC FIBROSIS

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*Journal of Gastroenterology and Hepatology. 2015, Jun; 30(6): 1057-64*

## ABSTRACT

**Background & Aims**

Pegylated interferon-based treatment is still the backbone of current hepatitis C therapy and is associated with bone marrow suppression and an increased risk of infections. The aim of this retrospective cohort study was to assess the risk of infections during interferon-based treatment among patients with chronic hepatitis C virus (HCV) infection and advanced hepatic fibrosis and its relation to treatment-induced neutropenia.

**Methods**

This cohort study included all consecutive patients with chronic HCV infection and biopsy-proven bridging fibrosis or cirrhosis (Ishak 4–6) who started treatment between 1990 and 2003 in five large hepatology units in Europe and Canada. Neutrophil counts between 500–749/μL and below 500/μL were considered as moderate and severe neutropenia, respectively.

**Results**

This study included 723 interferon-based treatments, administered to 490 patients. In total, 113 infections were reported during 88 (12%) treatments, of which 24 (21%) were considered severe. Only one patient was found to have moderate neutropenia and three patients were found to have severe neutropenia at the visit before the infection. Three hundred and twelve (99.7%) visits with moderate neutropenia and 44 (93.6%) visits with severe neutropenia were not followed by an infection. Multivariable analysis showed that cirrhosis (odds ratio [OR] 2.85, 95% confidence interval [CI] 1.38–5.90, $p = 0.005$) and severe neutropenia at the previous visit (OR 5.42, 95% CI 1.34–22.0, $p = 0.018$) were associated with the occurrence of infection, while moderate neutropenia was not. Among a subgroup of patients treated with pegylated interferon, severe neutropenia was not significantly associated (OR 1.63, 95% CI 0.19–14.2, $p = 0.660$).

**Conclusions**

In this large cohort of patients with bridging fibrosis and cirrhosis, infections during interferon-based therapy were generally mild. Severe interferon-induced neutropenia rarely occurred, but was associated with on-treatment infection. Moderate neutropenia was not associated with infection, suggesting that current dose reduction guidelines might be too strict.

## INTRODUCTION

Hepatitis C virus (HCV) infection is a major cause of cirrhosis, hepatocellular carcinoma, and end-stage liver disease.[178] It has been estimated that by 2030, 45% of the US patients chronically infected with HCV will have cirrhosis.[63] Today, antiviral treatment efficacy among patients with cirrhosis is unsatisfactory.[107 122 126]

Although it is expected that even in this difficult-to-treat subgroup of patients, the sustained virological response (SVR) rates will improve substantially by using interferon-free regimes,[142 145 156] pegylated interferon (PegIFN) and ribavirin (RBV) are still the backbone of antiviral therapy in most countries. In fact, due to the high costs of the new direct-acting antivirals (DAAs), PegIFN and RBV might remain the primary treatment option for many patients with chronic HCV infection around the globe. Treatment with PegIFN is, however, associated with major side effects, of which bone marrow suppression and subsequent neutropenia is one of the most frequently reported. Out of concern for infections, product labels and guidelines currently advise physicians to reduce the dose of PegIFN when neutrophil counts drop below 750/μL and to stop PegIFN when neutrophil counts drop below 500/μL.[35 36] This is largely based on prior experiences in oncology, where patients receiving chemotherapy showed an increased risk of infection when neutrophil counts dropped below 500/μL, with the greatest risk below 100/μL.[198] While patients undergoing interferon-based therapy are indeed more susceptible to bacterial and fungal infections, prior studies did not find these to be related to the treatment-induced neutropenia.[114 199-201] Especially because interferon dose adjustments compromise antiviral treatment efficacy for patients with chronic HCV infection, it was suggested that the current guidelines regarding dose reductions might be too strict.[111 112]

However, whereas patients with cirrhosis are considered to be immunocompromised and thus at the highest risk of infections, data regarding the relation between neutropenia and infections during interferon-based antiviral therapy within this population are scarce.

## PATIENTS AND METHODS

### Patients

The current study is based on all patients included in our previously described international, multi-center cohort.[81] This cohort included all consecutive patients from five large hepatology units in Europe and Canada, who had chronic HCV infection and started an interferon-based treatment between 1990 and 2003. They all had histological proof of bridging fibrosis (Ishak fibrosis score 4) or cirrhosis (Ishak fibrosis score 5 or 6).[51] Patients co-infected with the human immunodeficiency virus or the hepatitis B virus were excluded, as well as patients with a history of decompensated liver disease. For the current study, all consecutive treatment courses with available on-treatment data, including assessment of infection and laboratory results, were included.

All charts were re-reviewed by a single investigator (RM) in order to collect detailed baseline and on-treatment data. Data were obtained on patient characteristics (age, gender, body mass index), severity of liver disease (Ishak fibrosis score), presence of diabetes mellitus (DM), severe alcohol

use, and antiviral treatment (type of medication, treatment period, previous and current virological response). Furthermore, laboratory data (neutrophil count, platelet count, hemoglobin, albumin, bilirubin, aspartate aminotransferase, alanine aminotransferase, and glucose) and virology data (HCV genotype, HCV-RNA, and anti-hepatitis B core antigen) were collected. The period within 6 months before treatment was considered as baseline. During antiviral treatment, all (Peg)IFN and/or RBV dose reductions or treatment cessations were collected as well as all infectious episodes and all other adverse events.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. The study protocol was reviewed and approved by the ethics committee in the center of the primary investigators, which was the Erasmus Medical Center in Rotterdam, the Netherlands. Ethical approval in the participating centers was obtained according to the local regulations.

**Outcome measures**

The primary outcome measure was the occurrence of infections during antiviral therapy or within 2 weeks after treatment cessation. Secondary outcome measures were on-treatment neutropenia and the severity of infections.

Neutrophil counts from 500/μL to 749/μL were defined as moderate neutropenia, since interferon dose reductions are advised at these levels. Neutrophil counts below 500/μL were considered as severe neutropenia. DM at baseline was defined by an elevated fasting glucose (>6.1 mmol/L), a positive glucose tolerance test, or if patients were using anti-diabetic medication.

Infections were considered as all the episodes of clinically suspected infection according to the treating physician, when antibiotic therapy was prescribed or when there was evidence of an infection (radiology, positive culture). Severe infections were defined as infections resulting in death, hospital admission, or treatment discontinuation. All other infections were defined as mild.

**Statistical analyses**

Continuous variables were summarized as median (interquartile range [IQR]) and categorical variables as frequencies (percentages). Comparisons between groups were performed using $\chi^2$ test for categorical variables or the Mann–Whitney U-test for continuous variables.

The dynamics of neutrophil counts during treatment for different groups were studied with a repeated measurement model. A restricted cubic spline was fitted per group, and to take into account multiple measurements per patient during treatment, a model with random intercept and slope was applied.

Logistic regression was used to assess which baseline factors were associated with the occurrence of infections during antiviral therapy. Age, sex, cirrhosis, DM, and variables with a $p$ value of ≤0.2 in univariable analyses were included in multivariable analyses. Adjusting for multiple measurements within a patient, the association between on-treatment neutrophil counts and succeeding infectious events was assessed. By using a backward stepwise method, we selected the variables significantly associated with infections and included these in the final model. Akaike's Information Criteria were

used to select the best model. A sensitivity analysis was performed among only those patients treated with PegIFN-based regimens, since standard interferon is not used nowadays.

The cumulative incidence of infections was assessed using the Kaplan–Meier method. The log-rank test was applied to compare patients with bridging fibrosis and cirrhosis.

A $p$ value <0.05 was considered statistically significant and all statistical tests were two tailed. The significance level of interactions was set at 0.01 in order to correct for multiple testing. [81] PASW statistics 21.0 for Windows (SPSS, IBM, Armonk, NY, USA) and SAS 9.3 (SAS Institute, Cary, NC, USA) were used.



## RESULTS

### Patients

Overall, 546 patients with chronic HCV infection and bridging fibrosis or cirrhosis started interferon-based antiviral therapy between 1990 and 2003. In 490 (90%) patients, detailed on-treatment data were available to assess the occurrence of infections during at least one interferon-based regimen. Of the 361 (74%) patients without SVR, 174 received at least one subsequent antiviral treatment regimen. Overall, 723 treatment courses could be included in the analyses. Table 2.1 summarizes the baseline characteristics of the patients at their first included treatment. Median age was 49 years (IQR 43–56), 336 (69%) patients were male, and 372 (76%) presented with cirrhosis.

### Neutropenia and dose of interferon

The median neutrophil count prior to the start of all treatment courses was 3000/μL (IQR 2400–3900). Median time between visits was 2 weeks (IQR 1–4 weeks). During therapy, the median neutrophil count decreased to a nadir of 1200/μL (IQR 800–1700) in a median time of 11 weeks (IQR 4–22). Patients receiving PegIFN had a lower median nadir neutrophil count as compared with patients receiving standard interferon (1000/μL [IQR 700–1400] vs 1400/μL [IQR 1000–2000], $p$ <0.001), whereas baseline neutrophil counts did not differ between these groups ($p$ = 0.729). Figure 2.1 shows the course of neutrophil counts during treatment. During 88 (12%) treatment courses moderate neutropenia occurred at least once, and during 23 (3%) treatment courses, severe neutropenia occurred at least once. Because of neutropenia, the (Peg)IFN dose was reduced during 58 (8%) treatment courses and 3 (<1%) treatments were discontinued.

### Infections

In total, 113 infections were reported during 88 (12%) treatments among 81 (17%) patients. Table 2.2 summarizes the type of infections. Dermatological infections were most common (24%), followed by upper respiratory tract infections (20%) and urinary tract infections (18%). None of the patients were diagnosed with spontaneous bacterial peritonitis during treatment. Median time until the first infection was 12 weeks (IQR 6–24). Time until infection was not significantly different for mild or severe infections ($p$ = 0.648). Twenty-four infections among 23 (3.2%) patients were defined as severe. Treatment was discontinued due to infection in seven patients, and 23 hospital admissions due to

**Table 2.1 | Baseline characteristics at first registered treatment**

| Variable (missing cases [%]) | Patients (n=490) |
|---|---|
| Male | 336 (69%) |
| Age, in years | 49 (43–56) |
| BMI, in kg/m$^2$ * | 26.5 (23.7–29.4) |
| HCV genotype | |
| 1 | 316 (65%) |
| 2 | 46 (9%) |
| 3 | 79 (16%) |
| 4 | 23 (5%) |
| Other/ unknown | 26 (5%) |
| Treatment naïve | 397 (81%) |
| Cirrhosis | 372 (76%) |
| Fibrosis score | |
| Ishak 4 | 118 (24%) |
| Ishak 5 | 98 (20%) |
| Ishak 6 | 274 (56%) |
| Neutrophil count per µL * | 3040 (2500–3900) |
| Platelet count, in $10^9$/L | 146 (108–197) |
| Albumin, in g/L * | 42 (39–44) |
| Bilirubin, in µmol/L * | 13 (10–19) |
| Treatment with PegIFN | 201 (41%) |
| Treatment duration, in weeks | 26 (21–48) |
| Diabetes mellitus | 64 (13%) |
| Alcohol abuse ever * | 109 (22%) |

Abbreviations: PegIFN ; pegylated interferon

a. Medians are presented as number, (IQR, interquartile range). Numbers are presented as n, (percentage of the whole group)
b. Variables with an asterisk (*) had ≥10% missing values

**Table 2.2 | Type of infections**

| Type of infection | n (%) | Severe infection (%)[c] |
|---|---|---|
| Urinary tract | 20 (18%) | 3 (3%) |
| Upper respiratory tract | 22 (20%) | 2 (2%) |
| Pulmonary | 15 (13%) | 6 (5%) |
| Dermatological | 27 (24%) | 8 (7%) |
| Gastrointestinal | 3 (3%) | 2 (2%) |
| Oral | 12 (11%) | 1 (1%) |
| Other[b] | 14 (12%) | 2 (2%) |
| Total | 113 (100%) | 24 (21%) |

[a] Data presented as number, (percentage of the total number of infections)

[b] Other infections included a case of endocarditis (severe), dental and eye infections

[c] Severe infection was defined as infection requiring hospital admission or treatment discontinuation



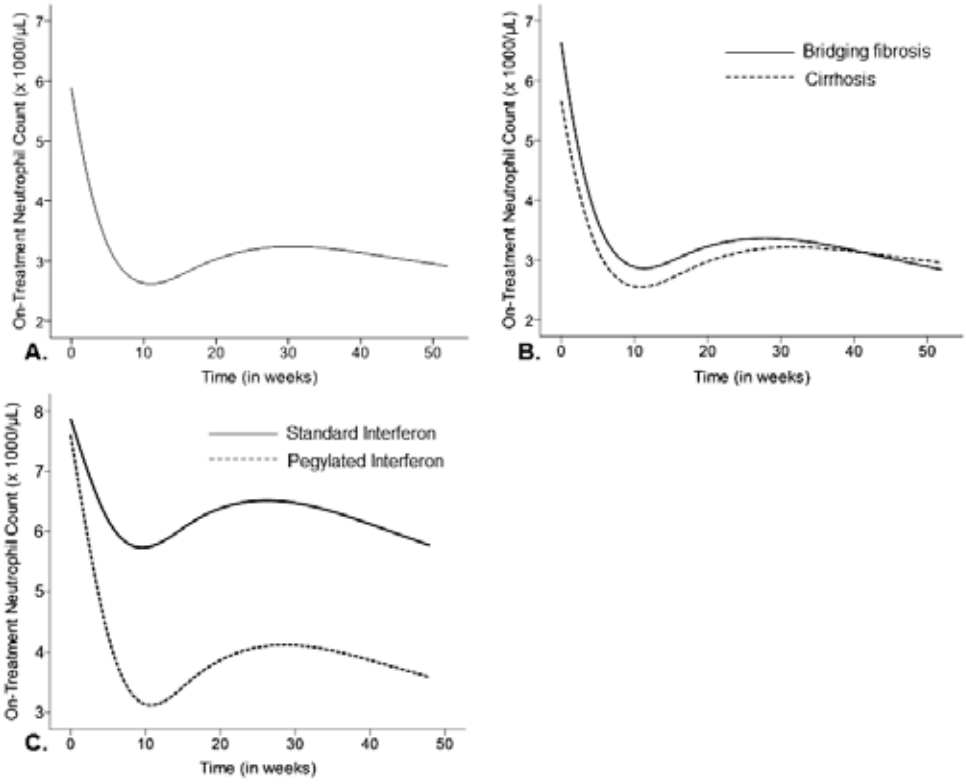



**Figure 2.1 | Dynamics of the neutrophil counts during treatment**

Panel A shows the dynamics of neutrophil counts among all patients. Panel B shows the dynamics of neutrophil counts among patients with bridging fibrosis and cirrhosis. Panel C shows the dynamics of neutrophil counts among patients treated with standard interferon and pegylated interferon (p<.001).

**Table 2.3 | Logistic regression analysis for infection**

| Variable | Univariable OR (95% CI) | *p* value | Multivariable OR (95% CI) | *p* value | Multivariable (PegIFN only[c]) OR (95% CI) | *p* value |
|---|---|---|---|---|---|---|
| Age | 1.02 (1.00-1.04) | 0.075 | 1.02 (0.99-1.05) | 0.296 | 1.02 (0.99-1.06) | 0.218 |
| Female gender | 1.58 (1.01-2.47) | 0.044 | 1.67 (0.95-2.94) | 0.077 | 1.03 (0.52-2.10) | 0.913 |
| BMI | 1.03 (0.98-1.07) | 0.263 | | | | |
| Cirrhosis at baseline | 2.14 (1.19-3.85) | 0.011 | 2.85 (1.38-5.90) | 0.005 | 3.22 (1.16-8.95) | 0.025 |
| DM at baseline | 1.58 (0.96-2.59) | 0.073 | 1.48 (0.78-2.81) | 0.224 | 1.17 (0.53-2.56) | 0.694 |
| Weeks on treatment | 1.01 (1.00-1.02) | 0.032 | 1.01 (0.99-1.02) | 0.322 | | |
| Type of interferon (PegIFN vs standard interferon) | 1.38 (0.88-2.16) | 0.155 | 0.80 (0.45-1.41) | 0.435 | | |
| Moderate neutropenia at previous visit (500-749/μL) | 0.19 (0.03-1.40) | 0.103 | | | | |
| Severe neutropenia at previous visit (<500/μL) | 4.25 (1.21-14.9) | 0.024 | 5.42 (1.34-22.0) | 0.018 | 1.63 (0.19-14.2) | 0.660 |
| Platelet count ≤ 100 * $10^9$/L | 0.86 (0.49-1.50) | 0.589 | | | | |
| Albumin < 35 g/L | 1.36 (0.59-3.15) | 0.471 | | | | |

Abbreviations: BMI, body mass index; DM, Diabetes mellitus; PegIFN, pegylated interferon

a. All analyses were corrected for multiple measurements within a patient

b. PegIFN based therapy, including 25 treatments with PegIFN monotherapy

an infection were reported. The median duration of admission was 9 days (IQR 3–12). None of the patients died as a result of an on-treatment infection. During 92 (81%) of the infections, antibiotic therapy was prescribed.

**Neutropenia and infections**

Eleven patients (1.5%) had a baseline neutrophil count below 1500/μL, of which 10 patients had a grade 2 neutropenia (i.e. neutrophil count <1500/μL) and only one patient had a grade 3 neutropenia (i.e. neutrophil count <1000/μL). Three of these patients needed a dose reduction due to neutropenia, of which one patient discontinued due to side effects, and one of these patients needed dose reductions due to thrombocytopenia. In only one patient, an infection was reported, which was severe. This patient required hospital admission for 23 days due to a Staphylococcus aureus bacteremia following cellulitis. The infection resolved with intravenous antibiotics, while interferon and RBV were continued.

For 81 infections (72%), the neutrophil count at the visit prior to the infection was available. Median neutrophil count at the visit prior to an infection was 1740/μL (IQR 1300–2650). The median

**Table 2.4 | Logistic regression analysis for severe infection[c]**

| Variable# | Univariable | | Multivariable | |
|---|---|---|---|---|
| | OR (95% CI) | *p* value | OR (95% CI) | *p* value |
| Age | 1.01 (0.97-1.06) | 0.572 | 1.00 (0.95-1.05) | 0.942 |
| Female gender | 3.63 (1.41-9.34) | 0.008 | 3.94 (1.54-10.1) | 0.004 |
| BMI | 0.98 (0.87-1.11) | 0.759 | | |
| Cirrhosis at baseline | 1.47 (0.50-4.36) | 0.484 | 1.23 (0.40-3.77) | 0.721 |
| DM at baseline | 3.04 (1.29-7.20) | 0.011 | 3.84 (1.52-9.70) | 0.004 |
| Weeks on treatment | 0.89 (0.72-1.10) | 0.280 | | |
| Type of interferon (PegIFN vs standard interferon) | 0.80 (0.34-1.87) | 0.600 | | |
| Platelet count ≤ 100 * $10^9$/L | 1.52 (0.58-4.00) | 0.396 | | |
| Albumin < 35 g/L | 2.45 (0.69-8.65) | 0.164 | | |

Abbreviations: BMI, body mass index; PegIFN, pegylated interferon

a. All analyses were corrected for multiple measurements within a patient
b. Severe infections were defined as infections were treatment was discontinued or when admission was needed
c. There were too few cases with moderate (n=1) or severe (n=1) neutropenia and severe infection to include these variables in the analysis.

neutrophil count at the previous visit was not different for mild and severe infections ($p$ = 0.399). Only one patient was found to have moderate neutropenia (neutrophil count of 690/μL) and three patients were found to have severe neutropenia (neutrophil counts of 300/μL, 330/μL, and 390/μL) at the visit before the infection. Three hundred and twelve (99.7%) visits with moderate neutropenia and 44 (93.6%) visits with severe neutropenia were not followed by an infection. Patients who underwent a dose reduction of (Peg)IFN did not experience less infections, compared with patients who did not undergo a dose reduction (10.8% vs 10.6%, $p$ = 1.00). During nine treatment courses, dose reductions were performed after an infection was reported.

Table 2.3 summarizes the results of univariable logistic analysis, which showed that female gender (odds ratio [OR] 1.58, 95% confidence interval [CI] 1.01–2.47, $p$ = 0.044), the presence of cirrhosis (OR 2.14, 95% CI 1.19–3.85, $p$ = 0.011), and number of weeks on treatment (OR 1.01, 95% CI 1.00–1.02, $p$ = 0.032) were significantly associated with infection. While moderate neutropenia at the visit prior to infection was not significantly associated (OR 0.19, 95% CI 0.03–1.40, $p$ = 0.103) with infection, severe neutropenia at the previous visit was (OR 4.25, 95% CI 1.21–14.9, $p$ = 0.024).

Multivariable logistic analyses, adjusted for age, gender, DM, number of weeks on treatment, and type of interferon showed that cirrhosis (OR 2.85, 95% CI 1.38–5.90, $p$ = 0.005) and severe neutropenia at the visit prior to the infection (OR 5.42, 95% CI 1.34–22.0, $p$ = 0.018) were independently associated with infections. The interaction terms between the variables in the final model were not statistically significant. In a sensitivity analysis including only the 356 (49%) PegIFN-based treatment courses among 292 patients, during which 71 infections were reported, severe neutropenia at the visit prior

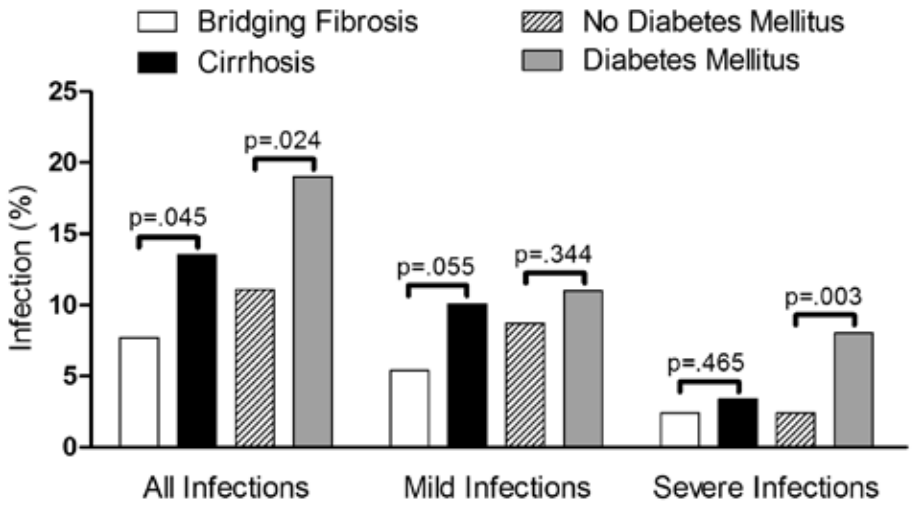



**Figure 2.2 |** The bars represent the percentage of treatments with at least one mild or severe infection among specific subgroups of patients based on the presence of cirrhosis and diabetes mellitus. Fifteen infections were reported among patients that had both cirrhosis and diabetes mellitus, of which 10 were mild infections and 5 were severe infections.

to infection was not significantly associated (OR 1.63, 95% CI 0.19–14.2, $p = 0.660$). In this analysis, cirrhosis (OR 3.22, 95% CI 1.16–8.95, $p = 0.025$) was the only factor associated with infection. Table 2.4 shows the variables associated with severe infection during antiviral treatment. After adjustment for age and cirrhosis, female gender (OR 3.94, 95% CI 1.54–10.1, $p = 0.004$) and DM (OR 3.84, 95% CI 1.52–9.70, $p = 0.004$) were associated with the occurrence of severe infections. Figure 2.2 illustrated the infection rates according to the presence of cirrhosis or DM.

## DISCUSSION

In this large cohort study, infections which occurred during interferon-based antiviral therapy were generally mild. The percentage of 12% of treatment courses complicated by infections is in line with previous studies reporting an infection rate of 4–23%.[114 200 202 203] Moderate (Peg)IFN-induced neutropenia was not associated with infection, whereas severe neutropenia was. However, a sensitivity analysis among patients treated with PegIFN could not confirm this. Furthermore, approximately 94% of the visits of patients with severe neutropenia were not followed by an infection. Cirrhosis and diabetes were important risk factors for the occurrence and severity of infection.

This real-life study was not limited by strict interferon dose modification rules which are used in clinical trials and thereby allows for assessment of lower neutrophil counts in relation to infections. Severe neutropenia occurred during only 3% of antiviral treatment regimens, and only four (17%) of these treatment courses with severe neutropenia were complicated by an infection. When only

PegIFN-based regimens were considered, the association between severe neutropenia and occurrence of infections was not statistically significant. This is in line with the findings of Antonini et al.[202] who showed that neither the presence nor duration of neutropenia was associated with the occurrence of infections among 319 chronic HCV-infected patients treated with PegIFN and RBV. This was confirmed by another retrospective study among 321 patients treated with PegIFN and RBV, as also in this study, on-treatment neutropenia and its duration were not associated with infectious episodes.[114] The Individualized Dosing Efficacy versus Flat Dosing to Assess Optimal Pegylated Interferon Therapy (IDEAL) study is the largest clinical study to date, which prospectively assessed the role of PegIFN-induced cytopenias on the occurrence of infections.[203] Again, no association between nadir neutrophil count and occurrence of infection was found. The authors did report, however, that the nadir lymphocyte count was associated with infectious episodes. Because the nadir lymphocyte count was analyzed rather than the lymphocyte count at the most recent visit prior to infection, it remains difficult to determine causality and to translate this finding into clinical recommendations with respect to the dose of PegIFN. In our cohort, lymphocyte counts were not available as these are not collected during antiviral therapy in daily practice.



In contrast to our cohort, the above described studies included a relatively low number of patients with advanced fibrosis (11–30%) and did not show results for this subgroup specifically. We found that patients with cirrhosis more frequently experienced infections during interferon-based therapy as compared with those with bridging fibrosis. Roomer et al.[114] also described a higher rate of infectious episodes among the 68 (21%) patients with cirrhosis included in their study, and from other cohorts, similar results were obtained with odds ratios ranging from 2.7 to 4.9.[114 199-201] Triple therapy regimens with DAAs were not included in our study and often reduce treatment duration to 12 or 24 weeks.[130 135 137] Shortened PegIFN therapy could prevent infections, as we found a linear infection rate during treatment with a median of 12 weeks from initiation of therapy to the occurrence of an infection (Supplementary Fig. 2.1). However, first real-world data regarding the addition of telaprevir or boceprevir to PegIFN and RBV therapy indicate a substantially elevated risk of severe infections among patients with cirrhosis, likely because patients with advanced cirrhosis who would have met stopping rules with PegIFN and RBV alone were able to achieve viral suppression and stay on therapy longer, thus increasing their risk of infection.[121] Cirrhosis is considered an immunocompromised state that may lead to a variety of infections, also outside of the scope of interferon-based therapy.[204] Translational studies have suggested that patients with cirrhosis have impaired neutrophil function, limiting the first immunological defense against bacterial pathogens.[205 206]

As this cohort study included solely patients treated with an interferon-based regimen, it lacks a control group of untreated cirrhotic patients. However, a previous study among cirrhotic patients awaiting liver transplantation found that there was a higher incidence of infections in the group that was treated with PegIFN and RBV compared with the matched control group that did not receive treatment, respectively 13 events and 2 events ($p = 0.012$).[207] This study indicated that PegIFN-based antiviral treatment increases the risk of bacterial infections in a cirrhotic population. Although apparently not through a reduction in neutrophil counts, infectious episodes are more frequently encountered during the use of interferon-based therapy. The mechanisms for this association are, however, not well understood. It has been hypothesized that PegIFN alters neutrophil function,

but limited data are available.[208] Others have suggested that PegIFN boosts other factors among cirrhotic patients which facilitate infections, such as bacterial translocation, dysfunction of the reticuloendothelial system, and reduction of serum and ascitic fluid complement levels.[209] Thus, an impaired function of the immune system might be responsible for the increased infection rate during interferon-based therapy.

Current recommendations regarding PegIFN dose reductions are based on experience among cancer patients receiving chemotherapy.[198] In this situation, however, other mechanisms such as mucosal damage and hampered organ function caused by the underlying disease play important roles in the susceptibility for infections as well. Dose reductions due to neutropenia may need to be carefully considered as long as the neutrophil counts remain stable between 500 and 750/μL. Dose reductions reduce the chance to attain SVR, which is likely to affect clinical outcome.[111 112 210] A study among African American and Caucasian American patients, in whom dose reductions were not undertaken until the neutrophil count dropped below 500/μL, did not report a higher rate of infections than expected.[211] Our study further suggests that dose reductions could not prevent infectious episodes during antiviral therapy as the number of patients who underwent a dose reduction of (Peg)IFN did not experience less infections (10.8% vs 10.6%). This should be interpreted with caution as dose reductions were at the discretion of the treating physicians and the number of infections could be higher if these dose reductions were not applied. The treating physicians could have applied dose reductions only to those patients that had a higher initial risk for infectious events. Due to its retrospective character, one could not really conclude that reductions of IFN could not affect the occurrence of infections. Ideally, one would design a randomized controlled trial with one group of patients undergoing dose reductions and the other group continuing the dose despite low neutrophil counts (or other hematological adverse events). Such studies are however hardly feasible. One could also debate whether dose reductions were performed too late. Unfortunately we were unable to assess this issue. Dose reductions were not performed according to the guidelines, making it impossible to conclude on timing of dose reductions. Furthermore, dose reductions were also performed for other reasons than neutropenia.

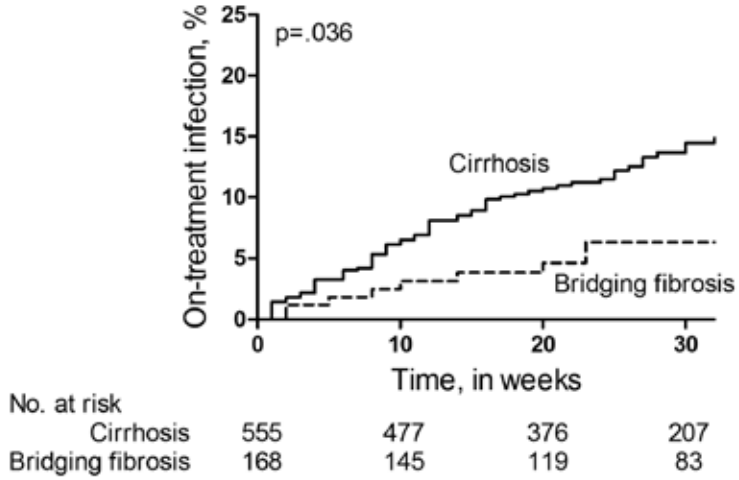
Our finding of an association between DM and more severe infection was not unexpected. Higher infection rates among diabetic patients could be explained by vascular insufficiency, depressed leucocyte, and natural killer cell function, as well as impaired antioxidant systems involved in antibacterial activity.[212 213] Others have previously shown that the risk of infections during PegIFN-based therapy was around twofold to threefold higher among patients with chronic HCV infection and DM.[114 201]

The association of female gender and the occurrence of severe infection was also seen in the largest prospective study that assessed the risk for infections during antiviral treatment.[203] In this study, they found an increased incidence of urinary tract infections and Candida infections, partly explaining the finding. Furthermore, they also found an increased incidence of respiratory tract infections among women. This was also the case in our study, where more women required hospital admission due to respiratory tract infections. There was no higher incidence of infections of the urinary tract. Although the underlying mechanism of our findings is not clear, clinicians should be aware of a possible increased risk for severe infection among women treated with PegIFN and RBV.

A limitation of our study is that, due to its retrospective character, not all infections may have been reported. However, it is unlikely that severe infections, which are clinically most relevant, were missed. Also, there was heterogeneity in the treatment regimens administered, varying from interferon monotherapy to combination therapy with PegIFN and RBV. Because neutrophil counts showed deeper declines with PegIFN, a sensitivity analysis was performed including only PegIFN-containing regimens.

In conclusion, patients with chronic HCV infection and bridging fibrosis and cirrhosis undergoing interferon-based therapy experience generally mild infections. Patients with cirrhosis are at elevated risk of interferon-associated infections, and among patients with DM, the course of infection seems to be more severe, indicating these groups should be carefully monitored. Although on-treatment severe neutropenia (<500/μL) might be associated with an elevated risk of infection, such deep declines in neutrophils do not frequently occur during PegIFN-based therapy. Furthermore this finding could not be confirmed among a subgroup of patients treated with PegIFN, which is used in current daily practice. Moderate neutropenia was not associated with an increased infection rate, suggesting that dose reductions due to neutropenia may need to be carefully considered as long as the neutrophil counts remain stable between 500 and 750/μL. Current guidelines regarding PegIFN dose reductions might be too strict and may unnecessarily compromise the virological efficacy of interferon-based therapy.

## SUPPLEMENTARY FIGURE




**Supplementary Figure 2.1 |** Kaplan-Meier curve showing the occurrence of infection within the first 30 weeks of treatment within two groups based on the presence of cirrhosis.

# CHAPTER 3

## ITPA POLYMORPHISMS ARE ASSOCIATED WITH HEMATOLOGICAL SIDE EFFECTS DURING ANTIVIRAL THERAPY FOR CHRONIC HCV INFECTION

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## ABSTRACT

**Background & Aims**

Genetic polymorphisms in the inosine triphosphatase (*ITPA*) gene have been associated with the protection from early ribavirin (RBV)-induced hemolytic anemia among patients with chronic hepatitis C virus (HCV) infection. The aim of the present study was to investigate the association between the functional *ITPA* variants and hematological side effects during antiviral therapy with pegylated interferon (PegIFN) and RBV.

**Methods**

This cohort study included all consecutive Caucasian patients treated for chronic HCV infection with PegIFN and RBV between 2000 and 2009 for whom a serum sample was available for genetic testing. The predicted inosine triphosphate pyrophosphatase (ITPase) activity was based on the genotypes of the SNPs rs1127354 and rs7270101. Decline in hemoglobin (Hb) during antiviral therapy, as well as dose reductions, blood transfusions and use of erythropoietin were assessed.

**Results**

In total, 213 patients were included. The predicted ITPase activity was normal among 152 (71%) patients; 61 (29%) patients had ITPase deficiency. By multivariable linear regression, RBV dose in mg per kilogram (Beta 0.09, 95%CI 0.04–0.13, $p$ <0.001) and normal ITPase activity (Beta 0.89, 95%CI 0.64–1.14, $p$ <0.001) were associated with more Hb decline at week 4 of treatment. Patients with normal ITPase activity underwent more dose adjustments of RBV than patients with ITPase deficiency (19 (13%) vs 1 (2%), $p$ = 0.014) and received erythropoietin more frequently (12 (8%) vs 0 (0%), $p$ = 0.024).

**Conclusions**

Genetic variants in the *ITPA* gene protected against RBV treatment-induced anemia among Caucasian patients with chronic HCV infection. Patients with normal ITPase activity underwent more dose reductions of RBV and received erythropoietin more frequently.

## INTRODUCTION

Currently, there is a changing paradigm in the treatment of chronic hepatitis C virus (HCV) infection. Although it is expected that the efficacy and safety of antiviral therapy improves considerably with the introduction of direct acting antivirals (DAAs), the high costs may limit the availability of these new drugs. Therefore, pegylated interferon (PegIFN) and ribavirin (RBV) containing regimens are likely to remain important treatment options in many countries around the world, also in high-income countries. Furthermore, some studies showed that the addition of RBV to DAAs could be beneficial in selected cases.[146 155] Unfortunately, PegIFN and RBV are associated with many side effects, including cytopenias. These cytopenias occur frequently and are the most important reasons for dose reductions.[110 214] As these dose reductions compromise treatment efficacy,[111 112] it is of great importance to select patients who are at greatest risk for these hematological side effects. These patients may benefit from strategies to optimize treatment adherence, such as early administration of supportive hematopoietic growth factors.

Recently, two genetic polymorphisms in the inosine triphosphatase (*ITPA*) gene on chromosome 20 were shown to be associated with protection against early RBV-induced hemolytic anemia during therapy with PegIFN and RBV.[215] The first polymorphism concerns a missense variant in exon 2 (rs1127354), the second concerns a splicing-altering single nucleotide polymorphism (SNP) in intron 2 (rs7270101). These two functional variants cause ITPase deficiency, subsequently preventing the depletion of erythrocyte adenosine triphosphate (ATP) and oxidative damage on the erythrocyte membrane.[216] Although ITPase deficiency is protective against anemia, it has been associated with a greater decline in platelet count during PegIFN and RBV therapy.[217 218] It has been suggested that patients with normal ITPase activity have a higher chance to develop thrombocytosis in reaction to the decline in hemoglobin (Hb). Although the exact mechanisms for this reactive thrombocytosis have not been completely elucidated, the increased stimulation of megakaryocyte-erythroid progenitor cells by erythropoietin (EPO) production is thought to be of major importance.[219 220]

The association of the *ITPA* variants with the occurrence of RBV-induced hemolytic anemia has been previously assessed.[217 218 221 222] However, these studies were based on patients included in randomized controlled trials with strict inclusion criteria and dosing rules. It can be questioned whether these findings also apply for the general patient population treated with PegIFN and RBV in field practice, where dose reductions are less strictly applied. Therefore, the aim of this study was to investigate the relationships between functional *ITPA* variants and hematological side effects of PegIFN and RBV therapy in routine daily practice. Secondly, the relations between these genetic polymorphisms and the occurrence of PegIFN and RBV dose reductions, administration of EPO and blood transfusions and virological response to antiviral therapy was analyzed.

## PATIENTS AND METHODS

### Patients

All Caucasian patients of whom a blood sample was available for genetic testing were included from our previously described cohort, which includes all consecutive patients with chronic HCV infection who were treated with PegIFN alfa-2a or -2b and RBV between 2000 and 2009 in our center.[114 115] The inclusion and exclusion criteria for this study are described elsewhere.[114 115] Briefly, patients were included if they were treated with PegIFN and RBV between 2000 and 2009. Patients treated with conventional interferon and patients co-infected with human immunodeficiency virus or the hepatitis B virus were excluded. In order to prevent confounding by ethnic origin, only Caucasian patients were included in the present study.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. The ethical review board of the Erasmus Medical Center, Rotterdam, The Netherlands approved this study as it was considered to be a low-risk study using retrospective and anonymized patient data. Written informed consent was obtained from each patient for storage of serum samples.

### Data acquisition

We obtained baseline data on gender, age, race, body mass index, METAVIR score, HCV genotype, previous interferon-based treatment, platelet count, absolute neutrophil counts, Hb, bilirubin and albumin concentration, glucose levels, presence of hemophilia and use of anticoagulants and anti-platelet therapy, diabetes mellitus (DM), history of heroin use and/or smoking.

During therapy all patients visited the outpatient clinic in one to six weeks intervals. At every visit blood tests were performed and patients were assessed for dose reductions and discontinuation of antiviral therapy. Among patients who were treated within a standard of care protocol, PegIFN and RBV dose reductions were made at the discretion of the treating physician. Patient characteristics such as age, physical condition, virological response, comorbidities and side effects of antiviral therapy were taken into account when considering a dose reduction. All study protocols of the clinical trials stated that dose reductions should be made according to product labels. However, these guidelines were not applied in some patients, due to the expected decrease of antiviral efficacy. Therefore these patients were treated at the discretion of the treating physician as well. The use of blood transfusion and EPO were also registered.

### Endpoints

The primary endpoint was the decline in Hb (mmol/L) and platelet count ($x10^9$/L) which was assessed at week 4 (+/-7 days). This time point was chosen in order to limit the influence of dose reductions on these hematological outcomes.[215] A clinically significant decline in Hb was defined as a decrease of at least 3.0 g/dL (1.86 mmol/L) or an absolute value lower than 10 g/dL (6.21 mmol/L). These thresholds were also used in other studies on ITPase deficiency.[221-223] Anemia was defined according to the thresholds used by the World Health Organization; for women a Hb concentration below 7.45 mmol/L and for men below 8.1 mmol/L were used as cut-off. Thrombocytopenia was defined as a

platelet count below 150x10$^9$/L. A clinical relevant thrombocytopenia was defined as a platelet count below 50x10$^9$/L, since current guidelines advise to reduce the dose of PegIFN when platelet counts fall below 50x10$^9$/L.[35]

As a secondary endpoint, the decline in Hb and platelet counts were also assessed at week 8 and 12 (+/-7 days) of antiviral therapy, as well as the nadir values of these parameters.

Sustained virological response (SVR) was defined as HCV RNA negativity in blood six months after cessation of antiviral therapy. Dose reductions of RBV and PegIFN, as well as the administration of EPO and blood transfusion, were considered as clinical endpoints.

**Genotyping methods**

Serum samples stored at -20° or -80° Celsius were used for DNA extraction and genotyping procedures, which were carried out centrally at LGC genomics. Purified genomic DNA of ≥5 ng was used for genotyping. Genotypes were assigned using all of the data from the study simultaneously. Genotype sequences were derived from NCBI. Genetic analyses were performed at the polymorphic sites rs12979860 (19:39248147, near *IL28B*, also known as interferon-λ3), rs1127354 (20:3213196, *ITPA*–1) and rs7270101 (20:3213247, *ITPA*–2). The IL28B SNP rs12979860 was chosen, since it best describes the association with sustained SVR for all genotypes.[224-226] Linkage disequilibrium and Hardy-Weinberg equilibrium (HWE) were tested for these SNPs using SNAP and OEGE.[227]

**Predicted ITPase activity**

As freshly acquired erythrocytes were lacking in order to directly measure ITPase activity, the predicted ITPase activity was based on genotypes of both *ITPA*–1 and *ITPA*–2 as is determined by previous analyses.[228 229] Patients with normal ITPase activity (i.e. 100%) were defined as patients with the combined presence of CC-genotype and AA-genotype for rs1127354 and rs7270101 respectively (Supplementary Table 3.1). Patients with less than 100% ITPase activity were defined as patients with ITPase deficiency (non-CC-genotype and non-AA-genotype for rs1127354 and rs7270101 respectively, Supplementary Table 3.1).

**Statistical Analysis**

Continuous variables were summarized as median (interquartile range [IQR]) and categorical variables as frequencies (percentages). Comparisons between groups were performed using $X^2$ test for categorical variables or the Mann-Whitney U test for comparing medians. The genetic association analyses for *ITPA* and *IL28B* polymorphisms consisted of a dominant genetic model (CC- and AA-genotype vs non-CC- and non-AA-genotype for ITPA and CC-genotype vs non-CC genotype for IL28B).[222 230] Linear regression analysis determined which variables were associated with the absolute decline in Hb and platelet count at week 4. Logistic regression was performed to determine which variables were associated with a clinically significant Hb decline at week 4, SVR and virological relapse. For decline in Hb and platelet count, a sensitivity analysis was performed which excluded patients whom were treated with a high PegIFN dose induction regimen. Age, sex and variables with a *p* value of ≤0.2 in univariable analyses were included in multivariable analyses. All final models were created by using a backward stepwise method, in order to select the variables that were significantly and

independently associated. Potential confounding was checked. All statistical tests were two-tailed, and $p$ <0.05 was considered to be statistically significant. The significance level of interactions was set at 0.01 in order to correct for multiple testing. SPSS version 21.0 (SPSS, Chicago, IL) was used.

## RESULTS

### Patients

In total, 321 consecutive patients with chronic HCV infection were treated with PegIFN and RBV between 2000 and 2009, of which 256 were Caucasian. Two hundred thirteen (83%) of these patients, who had a sample available for genetic testing and could be genotyped for both *ITPA* polymorphisms, were included in the current analyses (Supplementary Figure 3.1). Median age was 45 years (IQR 39–50), 145 (68%) were male, 105 (49%) had HCV genotype 1, and 39 (18%) had cirrhosis. Of the included patients, 140 were treated within a standard of care protocol. The remaining 73 patients were treated within clinical studies: 61 patients participated in three clinical trials and received a standard of care with PegIFN alfa-2a (180µg/week) or -2b (1.5µg/kg/week) plus weight based RBV. The remaining 12 patients received a PegIFN induction regimen with either PegIFN alfa-2a (270–360 µg/week) for 24 weeks or PegIFN alfa-2b (2.0–3.0 µg/kg/week) for 24 weeks followed by 48 weeks of PegIFN and daily weight-based ribavirin.

### Genotyping

The majority of patients were homozygous carriers of the major allele for *ITPA*–1 (rs1127354 C) and *ITPA*–2 (rs7270101 A), respectively 200 (89%) and 170 (76%) patients. The minor allele frequency (MAF) was 0.04 for *ITPA*–1 (rs1127354 A) and 0.13 for *ITPA*–2 (rs7270101 C). Eighty-two of the patients (36%) had the favorable *IL28B* genotype (rs12979860 CC); MAF was 0.39 (rs12979860 T). All SNPs were in HWE and not in linkage disequilibrium ($r^2 \leq 0.012$). The call rates were 96% (217/226), 98% (221/226) and 96% (217/226), for *ITPA*–1, *ITPA*–2 and *IL28B* respectively.

Supplementary Table 1 shows the distribution of the predicted ITPase activity according to the genotype of *ITPA*–1 and *ITPA*–2. In total, 152 (71%) patients had normal ITPase activity and 61 (29%) patients had ITPase deficiency. Baseline characteristics were compared between patients with normal ITPase activity and patients with ITPase deficiency (Table 3.1).

### ITPase deficiency and on-treatment hemoglobin concentration

In total, 182 (85%) patients experienced at least one episode of anemia during antiviral treatment and in 157 (74%) patients a clinically significant decline in Hb was reported. At baseline, median Hb concentration was 9.2 mmol/L (IQR 8.7–9.9) for patients with normal ITPase activity and 9.3 mmol/L (IQR 8.7–9.9) for patients with ITPase deficiency ($p = 0.793$). At treatment weeks 4, 8 and 12, median Hb concentration was significantly lower among patients with normal ITPase activity ($p$ <0.001 for all time points, Figure 3.1A). The nadir median Hb concentration was lower for patients with normal ITPase activity compared to patients with ITPase deficiency, respectively 6.6 mmol/L (IQR 5.8–7.2) and 7.2 mmol/L (IQR 6.6–8.1, $p$ <0.001). Furthermore, patients with ITPase deficiency had a lower occurrence

**Table 3.1 | Baseline characteristics**

| Baseline variables | Total N=213 | Normal ITPase activity n=152 | ITPase deficiency n=61 | p-value |
|---|---|---|---|---|
| Age | 45 (39-50) | 44 (38–49) | 46 (40-54) | 0.25 |
| Male | 145 (68%) | 100 (66%) | 45 (74%) | 0.26 |
| BMI in kg/m$^2$ [c] | 26.0 (23.7-28.1) | 25.8 (23.3–28.0) | 26.4 (24.1-30.3) | 0.13 |
| HCV Genotype | | | | 0.20 |
| 1 | 105 (49%) | 76 (50%) | 29 (48%) | |
| 2 | 19 (9%) | 11 (7%) | 8 (13%) | |
| 3 | 76 (36%) | 53 (35%) | 23 (38%) | |
| 4 | 13 (6%) | 12 (8%) | 1 (2%) | |
| Histology/ elastography | | | | 0.47 |
| METAVIR score [d] | | | | |
| F0-1 | 69 (32%) | 54 (38%) | 15 (28%) | |
| F2 | 67 (32%) | 46 (33%) | 21 (39%) | |
| F3 | 20 (9%) | 16 (11%) | 4 (7%) | |
| F4 | 39 (18%) | 25 (18%) | 14 (26%) | |
| Hemoglobin, in mmol/L | 9.3 (8.7-9.9) | 9.2 (8.7–9.9) | 9.3 (8.7-9.9) | 0.79 |
| Anemia [e] | 8 (4%) | 5 (3%) | 3 (5%) | 0.57 |
| Platelet count, in platelet x10$^9$/L | 197 (152-234) | 198 (154–234) | 191 (145-241) | 0.38 |
| Thrombocytopenia [e] | 48 (23%) | 31 (20%) | 17 (28%) | 0.24 |
| Absolute neutrophil count, in cells/μL [c] | 3200 (2500-4200) | 3400 (2700–4400) | 2800 (2300-3500) | 0.022 |
| Albumin, in g/L | 44 (42-46) | 44 (42–46) | 44 (42-45) | 0.22 |
| Bilirubin, in μmol/L | 10 (7-13) | 10 (7–13) | 10 (7-14) | 0.75 |
| Prothrombin time, in seconds | 12.4 (11.7-13.2) | 12.4 (11.7–13.2) | 12.5 (11.8-13.5) | 0.76 |
| AST/ALT ratio [c] | 0.73 (0.54-1.0) | 0.74 (0.55-0.98) | 0.72 (0.52-1.0) | 0.91 |
| Gamma-gt, in IU/L [c] | 62 (33-118) | 62 (33-111) | 63 (39-150) | 0.29 |
| Creatinin, in mmol/L [c] | 71 (63-80) | 71 (63-80) | 70 (67-76) | 0.57 |
| HCV RNA load < 800,000 IU/mL [c] | 60 (28%) | 35 (26%) | 25 (46%) | 0.007 |
| Use of anticoagulants | 7 (3%) | 4 (3%) | 3 (5%) | 0.40 |
| Presence of haemophilia | 11 (5%) | 8 (5%) | 3 (5%) | 0.92 |
| Presence of DM | 12 (6%) | 11 (7%) | 1 (2%) | 0.11 |
| Smoking | 129 (61%) | 92 (66%) | 37 (69%) | 0.71 |
| History of IV drug use | 121 (57%) | 82 (55%) | 39 (66%) | 0.15 |
| Peginterferon alfa-2a | 151 (71%) | 112 (74%) | 39 (64%) | 0.16 |
| Peginterferon induction regimen | 12 (6%) | 10 (7%) | 2 (3%) | 0.36 |
| Dose of RBV, in mg/kg | 13.2 (11.8-14.5) | 13.5 (12.0–14.6) | 12.8 (10.3–14.1) | 0.036 |

Abbreviations: ITPase, inosine triphosphaye pyrophosphatase; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; DM, diabetes mellitus; IV, intravenous; RBV, ribavirin

a. Medians are presented as number (IQR). Numbers are presented as n, (percentage of whole group)
b. Variables with a 'c' were missing in ≥ 10%
c. Liver biopsy or elastography was available in 195 patients
d. Anemia was defined as a Hb concentration below 8.1 mmol/L for men and below 7.45 mmol/L for women, thrombocytopenia was defined as a platelet count below 150x10$^9$/L

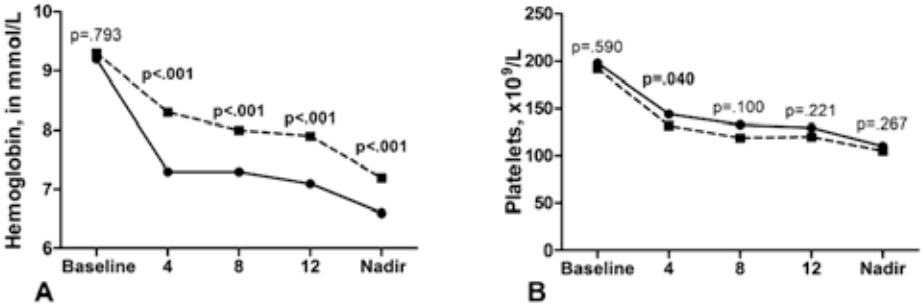



**Figure 3.1 | Median hemoglobin and platelet count**

Median hemoglobin **(A)** and platelet count **(B)** at baseline, at week 4, 8 and 12 and the nadir hemoglobin and platelet count during treatment. Dashed line represents the patients with ITPase deficiency and the black line represents patients with normal ITPase activity. Abbreviations: ITPase, inosine triphosphaye pyrophosphatase.

**Table 3.2 | Univariable and multivariable linear regression analysis for absolute Hb decline at week 4**

| | Univariable | | Multivariable | |
|---|---|---|---|---|
| **Baseline variables** | **Beta (95% CI)** | **p-value** | **Beta (95% CI)** | **p-value** |
| **Age, per year** | 0.05 (-0.09-0.02) | 0.473 | 0.04 (-0.01-0.02) | 0.535 |
| Female gender | -0.09 (-0.38-0.20) | 0.545 | 0.24 (-0.03-0.50) | 0.086 |
| Cirrhosis | 0.09 (-0.26-0.44) | 0.628 | | |
| DM | -0.01 (-0.60-0.59) | 0.984 | | |
| BMI | -0.01 (-0.05-0.02) | 0.490 | | |
| Platelet count, per 10x10$^9$/L | -0.02 (-0.04-0.00) | 0.115 | -0.02 (-0.04- -0.00) | 0.022 |
| Hemoglobin, per mmol/L | 0.36 (0.21-0.52) | <0.001 | 0.44 (0.29-0.59) | <0.001 |
| Peg 2b vs Peg 2a | -0.08 (-0.38-0.22) | 0.583 | | |
| Induction therapy | 0.37 (-0.20-0.94) | 0.203 | | |
| RBV dose, per mg/kg | 0.11 (0.05-0.16) | <0.001 | 0.09 (0.04-0.13) | <0.001 |
| Treatment naïve | 0.05 (-0.31-0.42) | 0.776 | | |
| Presence of hemophilia | 0.44 (-0.21-1.09) | 0.184 | | |
| Use of anticoagulants | -0.26 (-1.00-0.48) | 0.490 | | |
| HCV Genotype (2/3 vs 1/4) | -0.38 (-0.65- -0.11) | 0.006 | | |
| *IL28B* (CC vs CT/TT) | -0.12 (-0.40-0.17) | 0.422 | | |
| *ITPA-1* (CC vs CA/AA) | 1.02 (0.52-1.51) | <0.001 | | |
| *ITPA-2* (AA vs AC/CC) | 0.81 (0.50-1.12) | <0.001 | | |
| Normal ITPase activity | 0.93 (0.66-1.21) | <0.001 | 0.89 (0.64-1.14) | <0.001 |

Abbreviations: DM, diabetes mellitus; BMI, body mass index; RBV, ribavirin; *IL28B*, interleukin-28B; *ITPA*, inosine triphosphatase; ITPase, inosine triphosphaye pyrophosphatase

a. The final model was created by using a backward stepwise method. Confounding was checked.

of a clinically significant decline in Hb as compared to patients without ITPase deficiency at week 4, 8 and 12 ($p$ <0.001 for all timepoints, Supplementary Figure 3.2).

Multivariable linear regression analysis showed that baseline platelet count (Beta -0.02, 95% CI -0.04–-0.00, $p$ = 0.022), baseline Hb concentration (Beta 0.44, 95% CI 0.29–0.59, $p$ <0.001), RBV dose per kilogram body weight (Beta 0.09, 95% CI 0.04–0.13, $p$ <0.001) and normal ITPase activity (Beta 0.89, 95% CI 0.64–1.14, $p$ <0.001) were associated with the absolute decline in Hb concentration at week 4 of treatment (Table 3.2). The interaction terms between the variables in the final model were not statistically significant. Also in a sensitivity analysis for which patients with a PegIFN induction regimen were excluded the presence of normal ITPase activity was associated with the occurrence of a significant decline in Hb concentration at week 4 (Beta 0.91, 95% CI 0.65–1.18, $p$ <0.001).

In multivariable logistic regression analysis, the occurrence of a significant decline, as a dichotomous variable, was associated with baseline Hb concentration (OR 2.31, 95% CI 1.47– 3.64, $p$ <0.001), RBV dose per kilogram body weight (OR 1.41, 95% CI 1.19–1.67, $p$ <0.001) and normal ITPase activity (OR 11.5, 95% CI 4.24–31.1, $p$ <0.001) (Supplementary Table 3.2). Again, in a sensitivity analysis among patients without a PegIFN induction regimen, the presence of normal ITPase activity was associated with the occurrence of a significant decline in Hb concentration at week 4 (OR 13.4, 95% CI 4.61–39.0, $p$ <0.001).



**ITPase deficiency and on-treatment platelet counts**

Median platelet count was only significantly higher at week 4 of treatment among patients with normal ITPase activity compared to patients with ITPase deficiency (144x$10^9$/L, (IQR 103– 196) vs 132x$10^9$/L, (IQR 99–160); $p$ = 0.040, Figure 3.1B). At least one episode of thrombocytopenia was present among 166 (78%) patients, of which 22 (10%) had a platelet count below 50x$10^9$/L. Only ten (5%) patients experienced a platelet count below 50x$10^9$/L at week 4 of treatment. The occurrence of a platelet count below 50x$10^9$/L among patients with normal ITPase activity (4%) and patients with ITPase deficiency (7%) was comparable ($p$ = 0.418). When the whole treatment period was taken into account, the occurrence of a platelet count below 50x$10^9$/L was still similar between patients with normal ITPase activity and ITPase deficiency (11% vs 10%, respectively, $p$ = 0.881).

In multivariable linear regression analysis, adjusted for Hb decline, baseline platelet count (per 10x$10^9$/L, Beta 2.55, 95%CI 1.73–3.38, $p$ <0.001) and cumulative dose of PegIFN (per 100 mcg, Beta 4.86, 95%CI 2.75–6.98, $p$ <0.001) were associated with more decline in platelet count at week 4, whereas the presence of normal ITPase activity (Beta -18.5, 95%CI -29.7–-7.31, $p$ = 0.001) was significantly associated with less decline in platelet count at week 4 (Table 3.3). When patients with a PegIFN induction regimen were excluded, presence of normal ITPase activity was still associated with less decline in platelet count (Beta -16.5, 95%CI -27.6–-5.33, $p$ = 0.004).

**ITPase deficiency, dose reductions, EPO and blood transfusions**

In total, 20 (9%) patients underwent at least one dose reduction of RBV and 44 (21%) patients at least one dose reduction of PegIFN. At least one blood transfusion was given to 27 (13%) patients and 12 (6%) patients received at least one dose of EPO. Nineteen (13%) patients with normal ITPase activity underwent at least one dose reduction of RBV, whereas one (2%) patient with ITPase deficiency

**Table 3.3 | Univariable and multivariable linear regression analysis for absolute decline in platelet count at week 4**

| | Univariable | | Multivariable | |
|---|---|---|---|---|
| **Baseline variables** | **Beta (95% CI)** | **p-value** | **Beta (95% CI)** | **p-value** |
| Age, per year | -0.75 (-1.35- -0.15) | 0.015 | -0.44 (-9.46-2.32) | 0.233 |
| Female gender | 7.13 (-5.25-19.5) | 0.257 | 6.15 (-4.69-17.0) | 0.265 |
| Cirrhosis | -5.10 (-19.8-9.62) | 0.495 | | |
| DM | -3.23 (-28.2-21.8) | 0.799 | | |
| BMI | -0.22 (-1.71-1.28) | 0.776 | | |
| Platelet count, per $10x10^9$/L | 2.49 (1.60-3.37) | <0.001 | 2.55 (1.73-3.38) | <0.001 |
| Hb, per mmol/L | -1.06 (-8.04-5.92) | 0.765 | | |
| Hb decline, per mmol/L | -7.89 (-13.8- -1.93) | 0.010 | -3.57 (-9.46-2.32) | 0.233 |
| Peg 2b vs Peg 2a | -15.0 (-27.5- -2.43) | 0.020 | | |
| Induction therapy | 13.9 (-10.0-37.9) | 0.253 | | |
| Cumulative dose of PegIFN, per 100 mcg | 4.45 (2.12-6.78) | <0.001 | 4.86 (2.75-6.98) | <0.001 |
| RBV dose, per mg/kg | -1.21 (-3.61-1.19) | 0.320 | | |
| HCV Genotype (2/3 vs 1/4) | 3.69 (-8.00-15.4) | 0.534 | | |
| *IL28B* (CC vs CT/TT) | -5.76 (-17.9-6.40) | 0.351 | | |
| *ITPA-1* (CC vs CA/AA) | -18.2 (-39.8-3.26) | 0.096 | | |
| *ITPA-2* (AA vs AC/CC) | -9.54 (-23.4-4.33) | 0.177 | | |
| Normal ITPase activity | -14.9 (-27.5- -2.19) | 0.022 | -18.5 (-29.7- -7.31) | 0.001 |

Abbreviations: DM, diabetes mellitus; BMI, body mass index; Hb, hemoglobin; RBV, ribavirin; *IL28B*, interleukin-28B; *ITPA*, inosine triphosphatase; ITPase, inosine triphosphaye pyrophosphatase

a. The final model was created by using a backward stepwise method. Confounding was checked.

underwent at least one dose reduction ($p = 0.014$, Figure 3.2). The dose of PegIFN was reduced among 36 (24%) patients with normal ITPase activity and among eight (13%) patients with ITPase deficiency ($p = 0.085$). Blood transfusion and EPO were administered to 23 (15%) and twelve (8%) patients with normal ITPase; and to 4 (7%) and none (0%) of the patients with ITPase deficiency ($p = 0.089$ and $p = 0.024$, respectively).

**ITPase deficiency and virological response**

In total, 123 (58%) patients attained SVR, 45 (21%) patients had a virological relapse and 43 (20%) were non-responder. Two patients, who were both HCV RNA negative at the end of treatment, were lost to follow-up before being able to assess the sustainability of their virological response. Neither polymorphisms in the *ITPA* gene (*ITPA*–1 CC vs. CA/AA, OR 1.84 95% CI 0.66–5.15, $p = 0.24$; *ITPA*–2 AA vs AC/CC, OR 1.03 95% CI 0.54–1.97, $p = 0.92$) nor the presence of normal ITPase activity (OR 1.23, 95% CI 0.68–2.24, $p = 0.50$) were associated with SVR. In multivariable logistic regression analyses, age (per year, OR 0.94, 95% CI 0.90–0.98, $p = 0.004$), baseline gamma-glutamyltransferase (per U/L, OR 0.99,

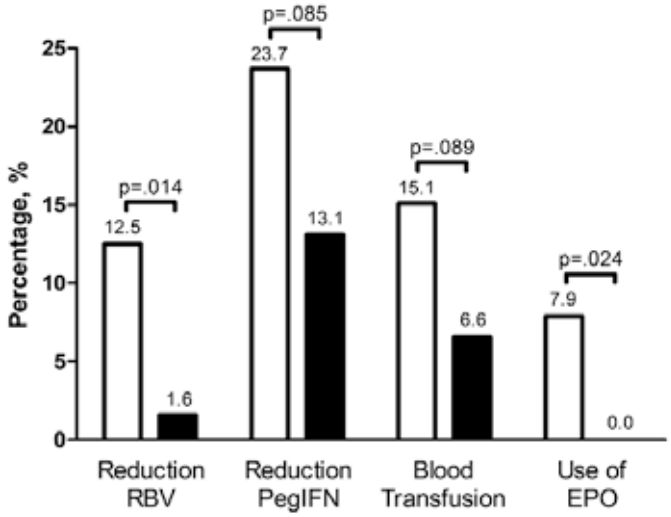



**Figure 3.2 | ITPase deficiency, dose reductions, EPO and blood transfusions**

Percentage of patients with at least one dose reduction of RBV or PegIFN, at least one blood transfusion or one dose of EPO during treatment. White bars represent the patients with normal ITPase activity and the black bars represent patients with ITPase deficiency. Abbreviations: ITPase, inosine triphosphate pyrophosphatase; RBV, ribavirin; PegIFN, pegylated interferon; EPO, erythropoietin.

95% CI 0.99–1.00, $p = 0.016$), HCV genotype (2/3 vs 1/4, OR 4.26, 95% CI 1.89–9.63, $p < 0.001$) and *IL28B* genotype (CC vs CT/TT, OR 5.06, 95% CI 2.16–11.9, p<0.001) were associated with SVR (Supplementary Table 3.3).

Univariable logistic regression analysis showed that the presence of normal ITPase activity was not significantly associated with virological relapse (OR 0.67, 95% CI 0.34–1.36, $p = 0.27$). Multivariable logistic regression analysis showed that only age (OR 1.05, 95% CI 1.01–1.10, $p = 0.02$) was significantly associated with virological relapse.

## DISCUSSION

This large cohort study in a real world setting found that genetic polymorphisms in the *ITPA* gene, resulting in ITPase deficiency, were associated with less Hb decline during PegIFN and RBV therapy among Caucasian patients with chronic HCV infection. Patients with normal ITPase activity were at higher risk to undergo dose reductions of both PegIFN and RBV, receive blood transfusions and be administered EPO. These patients may benefit from early administration of supportive hematopoietic growth factors in order to improve treatment adherence. Furthermore it could be a helpful tool in the decision of adding RBV, which has shown to be of additional value for some IFN-free regimens.[146 155]

Patients with ITPase deficiency (27% of our cohort) experienced lower declines in Hb concentration compared to patients with normal ITPase activity during the first four weeks of antiviral treatment. As expected, besides the presence of normal ITPase activity, RBV dose was also associated with a significant decline in Hb at week 4. The results of the present study confirm observations described in previous studies.[217 221 222] More important, our study revealed that patients with normal ITPase activity underwent more dose adjustments for RBV (13% vs 2%), and more often EPO was administered (8% vs 0%). Two previous studies among patients with HCV genotype 2 and 3 found no association between the ITPase activity and the need for RBV dose reductions.[217 222] In the first study, including 238 patients with HCV genotype 2 and 3, RBV dose was reduced in 35 patients (15%).[222] The presence of ITPase deficiency was not statistically associated with a lower hazard ratio (HR) for RBV dose reductions (HR 0.80, 95% CI 0.35–1.71, $p = 0.57$). This study used weight-based RBV and the dose was not reduced until Hb concentration fell below 5.9 mmol/L (i.e. 9.5 g/dL). The second study included 349 patients with HCV genotype 2 and 3, which showed that 6% of the patients did not receive the full planned dose of RBV. This low rate can be attributed to the use of low-dose RBV (i.e. 800 mg/ day) and the shorter treatment duration among this patients. In contrast, and in line with our data, a study among solely HCV genotype 1 infected patients did describe that the presence of ITPase deficiency resulted in fewer RBV dose reductions.[221] A high rate of RBV dose reductions was found in this study (47%), attributed to the fact that they included missed doses in this rate. Only four patients in this trial received EPO. In general, these data were based on clinical trial cohorts, solely including selected patients with specific HCV genotypes, different doses of RBV as well as limited use of blood transfusion and EPO. Furthermore these studies used strict dosing rules, instead of dose adjustments according to the treating physician, which is more representative for the clinical setting. Recently, another real-world study, also including all HCV genotypes, found that patients with ITPase deficiency required less RBV dose reductions and less EPO.[223] In 18% of the patients with ITPase deficiency a RBV dose reductions was required, whereas 41% of the patients with normal ITPase activity underwent at least one dose reductions of RBV. However, this study used strict dosing rules as well, which is not an optimal reflection of daily practice. Moreover, they did not report on the ethnic background of patients, which is essential in genetic studies.[230]

The presence of ITPase deficiency was also associated with a deeper decline in platelet count at week 4 of treatment, which could be explained by the absence of thrombocytosis in reaction to the hemolytic anemia. This is in line with previous studies which showed that reduced ITPase activity, which protects against RBV-induced anemia, is associated with the occurrence of treatment-induced thrombocytopenia.[217 218 231] ITPase deficiency was not related to the occurrence of a platelet count below $50x10^9$/L, but this rarely happens among patients with chronic HCV infection who are treated with PegIFN and RBV. Indeed, also in the current study, only 10% of patients had a platelet count below $50x10^9$/L during their treatment course. Nevertheless, it could be a predictive tool among patients with cirrhosis, who are more prone to develop severe thrombocytopenia.[196]

We did not find ITPase deficiency to be associated with virological response to PegIFN and RBV, perhaps because of limited power in our current study. Data on the influence of ITPase activity on the virological response is inconclusive, probably due to the inclusion of various HCV genotypes, heterogeneity in treatment regimens and the various ways in which the association was analyzed. Our

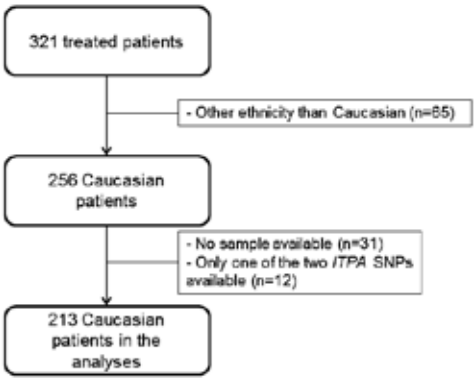
results are in line with the largest study to date, among patients with HCV genotype 1, which did not show a relation between ITPase deficiency and SVR either.[221] Nevertheless, it could be hypothesized that the higher frequency of dose reductions among patients with normal ITPase activity would compromise treatment efficacy, as was reported previously.[111 112] However, the mechanism by which anemia and ITPase deficiency influence virological response is still not fully unraveled.

The clinical importance of *ITPA* polymorphisms in the era of regimes with DAAs could be debated. Although limited data is available, previous studies among patients treated with triple therapy including telaprevir also showed that a *ITPA* polymorphism (rs1127354) was associated with the development of on-treatment anemia.[232-234] Unfortunately, these studies are solely among patients of Asian ancestry, and these patients are monoallellic for *ITPA* polymorphism rs7270101. In contrast to these results, the limited data available for Caucasian patients showed contrasting results.[235] Among patients with advanced hepatic fibrosis, *ITPA* polymorphisms were associated with the severity of Hb decline at week 4, but not at week 12 of therapy. This effect was attributed to the increased plasma levels of RBV after the first 4 weeks of triple therapy.[236] Recently, it was shown that ITPA polymorphisms were also associated with anemia during IFN-free therapy.[237] Studies on the impact of RBV dose reductions among patients treated with triple therapy, including PegIFN, RBV and boceprevir, demonstrated that a lower dose of RBV did not affect SVR rates.[238 239] Moreover, RBV dose reductions among patients with HCV genotype 2 and 3, treated with IFN-free regimens, also suggested no effect on SVR rates.[138 139] Nevertheless, as RBV will still be a component of IFN-free treatment regimens, *ITPA* polymorphisms can select patients who are prone to develop RBV-induced hemolytic anemia in order to apply more conservative and/ or earlier dose reductions or early administration of supportive agents. Secondly, it may be used as an additive tool to select a specific IFN-free regimen for the individual patient as not all regimens require addition of RBV for optimal virological efficacy. However, more data is needed in order to conclude on the clinical utility of these SNPs.

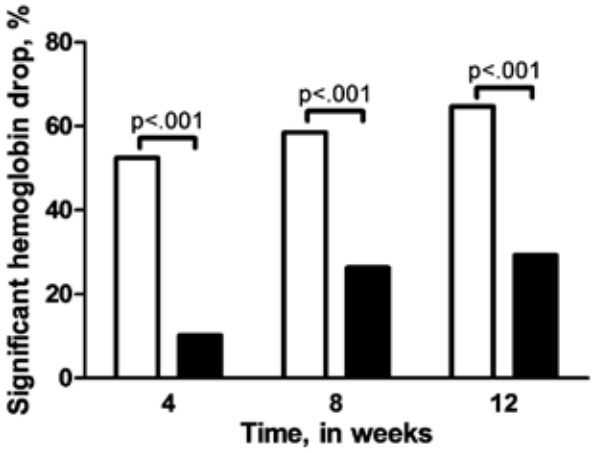
A limitation of this study is that RBV concentrations were not available. A previous study showed higher RBV concentrations at week 4 among patients with normal ITPase activity compared to patients with ITPase deficiency.[217] This could be explained by the reduced hemolytic anemia that was present in the patients with ITPase deficiency, generating a larger distribution volume for the intracellular forms of RBV, leading to lower extracellular concentrations of RBV. On the other hand, lower plasma concentrations of RBV could have led to the protection against anemia. In contrast, another study among 546 patients showed no association between ITPase deficiency and RBV levels ($p$ = 0.11).[221] Finally, due to the retrospective character of the study, we were not able to measure ITPase activity directly, as this requires freshly acquired erythrocytes. Nevertheless, classification of the predicted ITPase activity by combining the two genotypes has been suggested to be reliable.[228 229]

In conclusion, this real-world study showed that ITPase deficiency is associated with the protection against hemolytic anemia among Caucasian patients with chronic HCV infection who are treated with PegIFN and RBV. This led to less dose reductions of RBV and PegIFN as well as less administration of blood transfusions and EPO. Since treatment efficacy is hampered by dose reductions, patients with normal ITPase activity may benefit from early strategies in order to improve treatment adherence.

## SUPPLEMENTARY FIGURES AND TABLES




**Supplementary Figure 3.1 |** Abbreviations: ITPA, inosine triphosphatase; SNP, single nucleotide polymorphism.




**Supplementary Figure 3.2 |** Percentage of patients with a clinically significant decline in Hb within the first twelve weeks. A significant decline was defined as a decrease of at least 1.86 mmol/L (3.0 g/dL) or an absolute value lower than 6.21 mmol/L (10 g/dL). White bars represent the patients with normal ITPase activity and the black bars represent patients with ITPase deficiency. Abbreviations: Hb, hemoglobin; ITPase, inosine triphosphate pyrophosphatase.

**Supplementary Table 3.1 |** Predicted ITPase activity according to genotype of *ITPA*-1 and *ITPA*-2

| ***ITPA*-1 genotype** [a] | ***ITPA*-2 genotype** [a] | **Predicted ITPase activity (%)** [a] | **ITPase deficient** [a] | **Distribution within cohort n (%)** |
|---|---|---|---|---|
| Wild type (CC) | Wild type (AA) | 100 | No | 152 (67%) |
| Wild type (CC) | Heterozygote (AC) | 60 | Yes | 40 (18%) |
| Wild type (CC) | Homozygote (CC) | 30 | Yes | 5 (2.2%) |
| Heterozygote (CA) | Wild type (AA) | 25 | Yes | 12 (5.3%) |
| Heterozygote (CA) | Heterozygote (AC) | 10 | Yes | 4 (1.8%) |
| Homozygote (AA) | Wild type (AA) | <5 | Yes | (0%) |

Abbreviations: ITPase, inosine triphosphate pyrophosphatase; *ITPA*, inosine triphosphatase

**Supplementary Table 3.2 |** Univariable and multivariable logistic regression analysis for significant hemoglobin decline at week 4

| | **Univariable** | | **Multivariable** [c] | |
|---|---|---|---|---|
| **Baseline variable** | **OR (95% CI)** [a] | **p-value** | **OR (95% CI)** [a] | **p-value** |
| Age, per year | 1.01 (0.98-1.04) | 0.49 | 1.00 (0.96-1.04) | 0.95 |
| Female gender | 0.74 (0.40-1.36) | 0.33 | 1.07 (0.47-2.44) | 0.87 |
| Cirrhosis | 1.43 (0.70-2.90) | 0.33 | | |
| DM [a] | 1.06 (0.33-3.47) | 0.92 | | |
| BMI [a] | 0.98 (0.91-1.05) | 0.55 | | |
| Platelet count, per $10x10^9$/L | 0.98 (0.93-1.02) | 0.31 | | |
| Hb, per mmol/L [a] | 1.69 (1.18-2.42) | 0.004 | 2.32 (1.48-3.65) | <0.001 |
| PegIFN 2a vs PegIFN 2b [a] | 1.29 (0.69-2.39) | 0.42 | | |
| PegIFN induction regimen [a] | 2.17 (0.67-7.10) | 0.198 | | |
| RBV dose, per mg/kg [a] | 1.31 (1.14-1.50) | <0.001 | 1.41 (1.19-1.67) | <0.001 |
| Treatment naïve | 0.58 (0.28-1.20) | 0.143 | | |
| Presence of hemophilia | 2.32 (0.63-8.48) | 0.204 | | |
| Use of anticoagulants | 0.58 (0.11-3.07) | 0.52 | | |
| HCV Genotype (2/3 vs 1/4) [a] | 0.36 (0.20-0.65) | 0.001 | 0.58 (0.27-1.22) | 0.15 |
| *IL28B* (CC vs CT/TT) [a] | 0.99 (0.55-1.76) | 0.96 | | |
| *ITPA-1* (CC vs CA/AA) [a, b] | | | | |
| *ITPA-2* (AA vs AC/CC) [a] | 6.42 (2.58-16.0) | <0.001 | | |
| Normal ITPase activity [a] | 9.72 (3.93-24.0) | <0.001 | 12.1 (4.48-32.8) | <0.001 |

Abbreviations: CI, confidence interval; DM, diabetes mellitus; BMI, body mass index; Hb, hemoglobin; PegIFN, pegylated interferon; RBV, ribavirin; HCV, hepatitis C virus; *IL28B*, interleukin-28B; *ITPA*, inosine triphosphatase; ITPase, inosine triphosphaye pyrophosphatase

a. Within the CA/AA genotype no significant decline in Hb occurred
b. The final model was created by using a backward stepwise method. Confounding was checked.

**Supplementary Table 3.3 |** Univariable and multivariable logistic regression analysis for SVR

| | Univariable | | Multivariable [b] | |
|---|---|---|---|---|
| **Baseline variable** | **OR (95% CI) [a]** | **p-value** | **OR (95% CI) [a]** | **p-value** |
| Age, per year | 0.93 (0.90-0.96) | <0.001 | 0.93 (0.89-0.98) | 0.002 |
| Female gender | 1.85 (1.01-3.38) | 0.046 | | |
| Cirrhosis | 0.29 (0.14-0.61) | 0.001 | 0.38 (0.13-1.1) | 0.07 |
| DM [a] | 0.35 (0.10-1.18) | 0.090 | 0.54 (0.08-3.5) | 0.51 |
| BMI [a] | 0.95 (0.88-1.01) | 0.12 | | |
| Gamma-glutamyltransferase, per U/L | 0.99 (0.98-0.99) | <0.001 | 0.99 (0.99-1.00) | 0.004 |
| Baseline platelet count (per $10x10^9$/L) | 1.08 (1.03-1.14) | 0.002 | | |
| HCV Genotype 2/3 vs 1/4 [a] | 7.44 (3.91-14.2) | <0.001 | 4.55 (2.02-10.2) | <0.001 |
| PegIFN 2a vs PegIFN 2b [a] | 0.98 (0.54-1.79) | 0.95 | | |
| Treatment naïve | 1.78 (0.87-3.64) | 0.11 | | |
| HCV RNA < 800,000 IU/mL [a] | 1.55 (0.83-2.91) | 0.17 | | |
| *IL28B* (CC vs CT/TT) [a] | 4.04 (2.15-7.59) | <0.001 | 4.20 (1.85-9.55) | 0.001 |
| Significant Hb decline at week 4 [a] | 0.50 (0.28-0.88) | 0.017 | | |
| *ITPA*-1 CC vs CA/AA [a] | 1.84 (0.66-5.15) | 0.24 | | |
| *ITPA*-2 AA vs AC/CC [a] | 1.03 (0.54-1.97) | 0.92 | | |
| Normal ITPase activity [a] | 1.23 (0.68-2.24) | 0.50 | | |

Abbreviations: SVR, sustained virological response; CI, confidence interval; DM, diabetes mellitus; BMI, body mass index; HCV, hepatitis C virus; PegIFN, pegylated interferon; *IL28B*, interleukin-28B; Hb, hemoglobin; *ITPA*, inosine triphosphatase; ITPase, inosine triphosphaye pyrophosphatase

c. The final model was created by using a backward stepwise method. Confounding was checked.

# CHAPTER 4

## IMPROVEMENT OF PLATELETS AFTER SVR AMONG PATIENTS WITH CHRONIC HCV INFECTION AND ADVANCED HEPATIC FIBROSIS

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## ABSTRACT

**Background & Aims**

Patients with chronic hepatitis C virus (HCV) infection may develop cirrhosis with portal hypertension, reflected by decreased platelet count and splenomegaly. This retrospective cohort study aimed to assess changes in platelet counts after antiviral therapy among chronic HCV-infected patients with advanced fibrosis.

**Methods**

Platelet counts and spleen sizes were recorded in an international cohort of patients with Ishak 4–6 fibrosis who started antiviral therapy between 1990 and 2003. Last measured platelet counts and spleen sizes were compared with their pre-treatment values (within 6 months prior to the start of therapy). All registered platelet count measurements from 24-week following cessation of antiviral therapy were included in repeated measurement analyses.

**Results**

This study included 464 patients; 353 (76%) had cirrhosis and 187 (40%) attained sustained virological response (SVR). Among patients with SVR, median platelet count, increased by $35\times10^9$/L (IQR 7–62, $p$ <0.001). In comparison, patients without SVR showed a median decline of $17\times10^9$/L (IQR 5–47, $p$ <0.001). In a subgroup of 209 patients, median decrease in spleen size was 1.0 cm (IQR 0.3–2.0) for patients with SVR, while median spleen size increased with 0.6 cm (IQR 0.1–2.0, $p$ <0.001) among those without SVR. The changes in spleen size and platelet count were significantly correlated (R=0.41, $p$ <0.001).

**Conclusions**

Among chronic HCV-infected patients with advanced hepatic fibrosis, the platelet counts improved following SVR and the change in platelets correlated with the change in spleen size following antiviral therapy. These results suggest that HCV eradication leads to reduced portal pressure.

## INTRODUCTION

The continuous inflammation in livers of patients with chronic hepatitis C virus (HCV) infection may cause hepatic fibrogenesis. Progression of this process may eventually lead to cirrhosis, at which stage patients have an unfavorable prognosis due to the elevated risk of hepatocellular carcinoma (HCC) and liver failure.[55] In one of the largest studies on the fibrosis progression rate, dating back to 1997, it was estimated that 33% of patients with chronic HCV infection develop cirrhosis within 20 years.[178] However, the number of patients who develop cirrhosis could be higher over a longer period of time as fibrosis development may not be linear. In fact, as the population with chronic hepatitis C is aging, it is expected that the incidence of HCV-related cirrhosis will increase during the upcoming years.[63]
The treatment of chronic HCV infection improved enormously during the last two decades. Even in case of advanced hepatic fibrosis, sustained virological response (SVR) rates over 90% can be achieved with combination regimens of direct-acting antiviral agents.[142 145 146 156 240] Several studies showed that hepatic fibrosis can regress once HCV is eradicated as causative agent of liver injury, also among patients with advanced hepatic fibrosis.[74 241-247] However, these studies are limited by a short follow-up duration or low number of patients with cirrhosis. Also, there is significant sampling error with respect to percutaneous liver biopsy, which remains an invasive procedure with potentially severe complications so that repeated assessments of liver histology are often not feasible.[247-249] The longitudinal pattern of hepatic fibrosis regression is thus difficult to study.

The platelet count is strongly related to the degree of hepatic histopathological abnormalities and portal pressure, especially among those patients with bridging fibrosis or cirrhosis.[250-257] Indeed, lower platelets have been repeatedly associated with a higher risk for cirrhosis-related morbidity and mortality, which supports that the platelet count is representative of the stage of liver disease.[55 79-81] Importantly, the change in platelets correlated with the change in hepatic fibrosis following antiviral therapy among patients with chronic HCV infection, including those who attained SVR.[258 259] Changes in platelets thus represent a noninvasive alternative to assess the evolution of the stage of liver disease and portal pressure. Because splenic sequestration of blood cells as a direct result of elevated portal pressure causes the spleen to increase in size, splenomegaly is also considered a non-invasive marker of the degree of portal hypertension or presence of esophageal varices.[250-252]

The aim of our study was to assess the change in platelet counts following SVR in a large cohort of consecutively interferon-treated patients with chronic HCV infection and biopsy-proven bridging fibrosis or cirrhosis.

## PATIENTS AND METHODS

### Patients

All consecutive patients with chronic HCV infection and bridging fibrosis or cirrhosis (Ishak fibrosis score 4–6) who initiated interferon-based antiviral therapy between 1990 and 2003 were included from five large hepatogy units in Europe and Canada. The design of this retrospective cohort study has been described in detail previously.[81] For the current study, the patients were assessed from the

last received interferon-based treatment course onwards. Hereby, it was prevented that interferon-induced bone marrow suppression influenced the platelet counts during follow-up as a result of retreatment. Excluded were patients with a human immunodeficiency virus or hepatitis B virus co-infection, patients who had developed HCC or liver failure prior to start of follow-up or prior to the first available platelet count measurement during follow-up, and patients who received long-term low-dose pegylated interferon maintenance therapy. Patients without follow-up beyond January 1st 2010 were invited for a single visit to the outpatient clinic.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. According to the standards of the local ethics committees, written informed consent was obtained from patients visiting the outpatient clinics.

**Outcome measures**

Pre-treatment markers of liver disease severity closest to the start of therapy were included, as long as these were available within 6 months before the start of antiviral therapy. All available platelet count measurements were registered from 24 weeks after cessation of interferon-based antiviral therapy. A platelet count $<150\times10^9$/L was defined as thrombocytopenia. Measurements obtained after the diagnosis of HCC or liver failure were not considered. The diagnosis of HCC was based on histopathological confirmation or two coincident imaging techniques (computed tomography, magnetic resonance imaging, or contrast-enhanced ultrasonography) showing a focal lesion larger than 2 cm with arterial-phase hyper-enhancement or one imaging technique showing a focal lesion larger than 2 cm with arterial-phase hyper-enhancement in the presence of an α-fetoprotein level greater than 400 ng/mL.[187] Liver failure was defined as an episode of ascites, bleeding varices, jaundice, or overt hepatic encephalopathy. Only the first episode of liver failure was considered in case patients experienced multiple liver failure events. The last available spleen size measurement (in centimeters), as determined by radiological examination (ultrasound, computed tomography or magnetic resonance imaging), was registered. Spleen sizes that were measured after initiation of treatment for HCC or portal hypertension were not considered, and in such cases, the last spleen size prior to these events was used.

**Statistical analyses**

Baseline characteristics were compared between patients with SVR and patients without SVR using the Mann-Whitney test for continuous and the chi-square test for categorical variables. To assess the difference in median platelet count per Ishak fibrosis scores (4, 5, or 6), the Kruskal–Wallis was used. Correlations were analyzed with Spearman's rank correlation coefficient. The relation between baseline laboratory markers and the degree of hepatic fibrosis (Ishak 4/5 versus 6 and Ishak 4 versus 5/6) was assessed with logistic regression and receiver operating characteristic curve analysis.

Per virological response group, the statistical significance of the change in platelets from baseline to the last available measurement during follow-up was assessed with the Wilcoxon signed-rank test. The difference in the change of platelets between patients with SVR and patients without SVR was assessed with the Mann- Whitney test. Per virological response group, McNemar's test was used to

assess the difference in the percentage of patients with thrombocytopenia at baseline and last follow-up. The change in spleen size was assessed accordingly.

Linear regression analysis was used to determine which baseline variables were associated with the change in platelets from baseline to last follow-up among the patients who attained SVR. Repeated measurement analyses with a random intercept and slope per patient and an unstructured covariance matrix were performed to analyze the evolution of platelets over time, correcting for potential non-linearity by including the squared time to the platelet count measurement into the model. As chronic HCV infection and interferon therapy can influence the platelets as well, 24 weeks after cessation of antiviral therapy was considered as time zero in the repeated measurement analyses.
All statistical tests were two sided, and a $p$ value <0.05 was considered to be statistically significant. SPSS version 17.0.2 (SPSS Inc., Chicago, IL, USA) and SAS 9.2 PROC GENMOD (SAS institute, Cary, NC, USA) were used for all statistical analyses.

## RESULTS

**Study population**

Between 1990 and 2003, 546 patients with chronic HCV infection and histological proof of advanced hepatic fibrosis received interferon-based antiviral therapy. Eight patients who were lost-to-follow-up and eight patients who developed HCC or liver failure before 24 weeks after their initial treatment course were excluded. Of the remaining 530 patients, 125 (24%) attained SVR, and 405 (76%) did not. During follow-up, 204 patients without SVR were retreated at least once, which resulted in SVR for another 67 patients. Among retreated patients, seven experienced a cirrhosis-related complication before 24 weeks following their last treatment course, 14 received long-term low-dose pegylated interferon maintenance therapy, and 45 had no available platelet count measurement during follow-up. These patients were excluded as well, so that the total study cohort consisted of 464 patients: 187 with SVR and 277 without SVR (Figure 4.1). Table 4.1 summarizes the baseline characteristics according to the virological response to the last antiviral treatment course.



**Platelet count in relation to hepatic fibrosis**

At baseline, platelet counts were associated with histological stage of fibrosis: the median platelet count was $186\times10^9$/L (interquartile range [IQR] 143–226) among patients with Ishak fibrosis score 4, $160\times10^9$/L (IQR 134–209) among patients with Ishak fibrosis score 5 and $133\times10^9$/L (IQR 92–176) among patients with Ishak fibrosis score 6 ($p$ <0.001). Accordingly, the percentage of patients with thrombocytopenia increased with higher Ishak fibrosis score (31%, 40%, and 60% for Ishak fibrosis score 4, 5, and 6, respectively, $p$ <0.001). The platelet count could largely discriminate patients with Ishak fibrosis score 4/5 from those with Ishak 6 (area under the curve [AUC] 0.70, 95% confidence interval [CI] 0.65–0.75, $p$ <0.001). The AUC of the platelet count was similar to differentiate between Ishak 4 and 5/6 (AUC = 0.69, 95% CI 0.63–0.74, $p$ <0.001). The discriminating abilities of bilirubin, albumin, or the ratio between the aspartate and alanine aminotransferase were lower (AUCs ranging

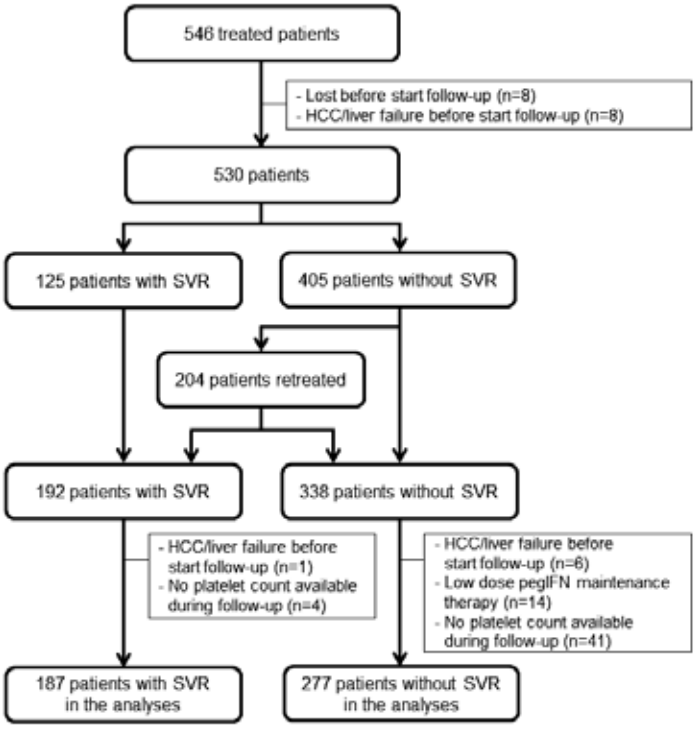


**Figure 4.1 | Study flow chart**

Abbreviations: HCC; hepatocellular carcinoma, SVR; sustained virological response, PegIFN; pegylated interferon

**Table 4.1 | Baseline characteristics according to virological response**

| | Overall (n = 464) | With SVR (n = 187) | Without SVR (n = 277) | *p* value |
|---|---|---|---|---|
| Age, years | 51 (44-57) | 49 (44-57) | 51 (44-58) | 0.071 |
| Male, *n/total* (%) | 321/464 (69) | 137/187 (73) | 184/277 (66) | 0.118 |
| BMI, kg/m$^2$ [a] | 25.9 (23.4-28.7) | 25.6 (23.1-28.6) | 26.1 (23.8-29.0) | 0.135 |
| Fibrosis score, *n/total* (%) | | | | 0.429 |
| - Ishak 4 | 111/464 (24) | 50/187 (27) | 61/277 (22) | |
| - Ishak 5 | 91/464 (20) | 33/187 (18) | 58/277 (21) | |
| - Ishak 6 | 262/464 (56) | 104/187 (55) | 158/277 (57) | |
| HCV genotype, *n/total* (%) | | | | <0.001 |
| - 1 | 300/440 (68) | 93/179 (52) | 207/261 (79) | |
| - 2 | 42/440 (10) | 32/179 (18) | 10/261 (4) | |
| - 3 | 79/440 (18) | 46/179 (26) | 33/261 (13) | |

**Table 4.1 | Continued**

| | Overall (n = 464) | With SVR (n = 187) | Without SVR (n = 277) | *p* value |
|---|---|---|---|---|
| - 4 | 15/440 (3) | 6/179 (3) | 9/261 (3) | |
| - Other | 4/440 (1) | 2/179 (1) | 2/261 (1) | |
| Type of treatment, *n/total* (%) | | | | <0.001 |
| - IFN mono | 70/464 (15) | 9/187 (5) | 61/277 (22) | |
| - IFN and RBV | 110/464 (24) | 49/187 (26) | 61/277 (22) | |
| - PegIFN mono | 12/464 (3) | 4/187 (2) | 8/277 (3) | |
| - PegIFN and RBV | 253/464 (54) | 118/187 (63) | 135/277 (49) | |
| - ConsensusIFN (+/- RBV) | 11/464 (2) | 2/187 (1) | 9/277 (3) | |
| - PegIFN and RBV and PI | 8/464 (2) | 5/187 (3) | 3/277 (1) | |
| Laboratory markers of liver disease severity [b] | | | | |
| - Platelet count, x$10^9$/L | 150 (112-199) | 162 (132-205) | 142 (100-191) | <0.001 |
| - Albumin, g/L | 42 (39-44) | 42 (40-44) | 41 (38-44) | 0.016 |
| - Bilirubin, µmol/L | 13 (10-18) | 12 (9-15) | 14 (10-19) | <0.001 |
| - AST/ALT ratio | 0.73 (0.59-0.92) | 0.68 (0.55-0.82) | 0.76 (0.62-0.97) | <0.001 |
| Spleen size, cm | 12.5 (11.0-14.3) | 12.0 (10.6-13.8) | 12.9 (11.2-14.8) | 0.012 |
| Treatment naïve, *n/total* (%) | 267/464 (58) | 109/187 (58) | 158/277 (57) | 0.789 |
| Year treatment started | 2001 (1998-2003) | 2002 (2000-2003) | 2001 (1998-2003) | 0.003 |
| Treatment duration, weeks | 31 (22-48) | 48 (25-49) | 24 (16-48) | <0.001 |
| Diabetes mellitus, *n/total* (%) | 71/464 (15) | 20/187 (11) | 51/277 (18) | 0.024 |
| History of severe alcohol use, *n/total* (%) [c] | 100/437 (23) | 38/180 (21) | 62/257 (24) | 0.460 |
| AntiHBc positivity, *n/total* (%) | 168/364 (46) | 63/141 (45) | 105/223 (47) | 0.654 |

Data are presented as median (interquartile range), unless otherwise noted. Abbreviations: BMI; Body Mass Index, HCV; Hepatitis C Virus, IFN; interferon, PegIFN; pegylated interferon, AST; Aspartate aminotransferase, ALT; Alanine aminotransferase, antiHBc; anti-hepatitis B core antigen, PI; protease inhibitor.

a. BMI was missing in 24 (13%) patients with SVR, and 61 (22%) patients without SVR.
b. Platelet count was missing in 9 (5%) patients with SVR and 29 (10%) patients without SVR. Albumin was missing in 13 (7%) patients with SVR and 48 (17%) patients without SVR. Total bilirubin was missing in 14 (7%) patients with SVR and 36 (13%) patients without SVR. The AST/ALT ratio was missing in 15 (8%) patients with SVR and 37 (15%) patients without SVR. The spleen size was missing in 88 (47%) patients with SVR and 130 (47%) patients without SVR.
c. Severe alcohol use was defined as the use of more than 50 gram of alcohol per day.

from 0.56 to 0.62). Logistic regression analyses indicated that the platelet count was the only objective laboratory marker of liver disease severity that had an independent statistically significant association with the baseline stage of hepatic fibrosis (Table 4.2).

**Changes in platelets following antiviral therapy**

During the follow-up, which started 24 weeks post-treatment, 3387 platelet count measurements were registered. The median interval between platelet count measurements was 0.45 years (IQR 0.13–0.79), which differed between patients with SVR (0.54 years, IQR 0.28–1.02) and patients without SVR (0.31 years, IQR 0.12– 0.61, $p$ <0.001). The last available platelet counts were measured after a median of 5.7 (IQR 2.1–7.6) years among patients with SVR and after 4.4 (IQR 1.9–7.1) years among patients without SVR ($p$ = 0.111). The median last platelet count was $198\times10^9$/L (IQR 166–248) in the group with SVR and $113\times10^9$/L (IQR 73–167) in the group without SVR ($p$ <0.001). In 426 patients, a platelet count measurement was available both at baseline as well as during follow-up. Among those with SVR, 44 (62%) of the patients with thrombocytopenia at baseline showed a normal platelet count at the time of the last measurement ($p$ <0.001), while only 2 (2%) patients with SVR and normal platelets at baseline had thrombocytopenia at the final measurement (Table 4.3). The platelet counts in these patients were $122\times10^9$/L and $134\times10^9$/L at the end of follow-up, respectively. Among patients without SVR, 47 (43%) patients changed from a normal platelet count at baseline to thrombocytopenia at the end of follow-up ($p$ <0.001), while 14 (10%) patients with thrombocytopenia at baseline had normal platelet counts at the time of the last measurement. From baseline to the last available measurement, the platelet count showed a median increase of $35\times10^9$/L (IQR 7–62) among the patients with SVR

**Table 4.2 | Logistic regression analyses for the stage of hepatic fibrosis**

| | Univariate analyses | | | Multivariate analyses | | |
|---|---|---|---|---|---|---|
| **Ishak score 4 versus 5/6** | **OR** | **95%CI** | ***p* value** | **OR** | **95%CI** | ***p* value** |
| Age, per year | 1.05 | 1.02-1.07 | <0.001 | 1.05 | 1.02-1.08 | 0.003 |
| Males | 1.17 | 0.74-1.84 | 0.511 | - | - | - |
| BMI, per kg/m$^2$ | 1.07 | 1.00-1.13 | 0.036 | 1.05 | 0.98-1.11 | 0.150 |
| HCV genotype 3 | 1.53 | 0.89-2.63 | 0.121 | - | - | - |
| Laboratory data | | | | | | |
| - Platelet count, per 10x10$^9$/L | 0.92 | 0.89-0.96 | <0.001 | 0.93 | 0.89-0.97 | 0.001 |
| - Albumin, per g/L | 0.94 | 0.89-0.99 | 0.012 | 0.98 | 0.91-1.05 | 0.505 |
| - Bilirubin, per µmol/L | 1.04 | 1.00-1.07 | 0.037 | 1.00 | 0.97-1.04 | 0.817 |
| - AST/ALT ratio, per 0.1 | 1.01 | 0.96-1.06 | 0.713 | - | - | - |
| Diabetes mellitus | 1.66 | 0.86-3.21 | 0.135 | - | - | - |
| History of severe alcohol use | 1.17 | 0.70-1.94 | 0.546 | - | - | - |
| AntiHBc positivity | 0.93 | 0.57-1.51 | 0.760 | - | - | - |

Abbreviations: ALT; Alanine aminotransferase, AST; Aspartate aminotransferase, BMI; Body mass index, HCV; hepatitis C virus, CI; Confidence interval, OR; Odds Ratio.

and a median decrease of 17×10$^9$/L (IQR 5–47) among patients without SVR ($p$ <0.001; for the paired analyses within each response group as well as for the comparison between both response groups). Linear regression analyses were performed to assess which factors were associated with improvement of platelets following SVR (Table 4.4). Higher body mass index (BMI) was negatively associated with the change in the platelet count (β =1.59, standard error 0.78, $p$ = 0.043). These analyses were corrected for the baseline platelet count and the time from SVR to the last platelet count measurement.

Repeated measurement analyses showed a gradual and almost linear increase in platelets beyond the moment of SVR, while the platelet counts further declined among those patients who did not attain SVR ($p$ <0.001; for the change within each response group as well as for the comparison between both response groups) (Figure 4.2).

**Spleen size**

Spleen sizes were available within 6 months prior to initiation of antiviral therapy in 99 (53%) patients with SVR and 147 (53%) patients without SVR. The median baseline spleen size was 12.0 cm (IQR 10.6–13.8) among those patients who would later achieve SVR and 12.9 (IQR 11.2–14.8) among those without subsequent SVR ($p$ = 0.012). The median spleen sizes were 11 cm (IQR 9.8–12.6), 12.5 cm (IQR 11.4–14.0) and 13.0 cm (IQR 11.4–15.0) among patients with Ishak 4, 5, and 6 fibrosis, respectively ($p$ <0.001). The AUC to discriminate Ishak 4/5 from Ishak 6 fibrosis using the spleen size was 0.65 (95%CI 0.58–0.72, $p$ <0.001) and 0.74 (95%CI 0.66–0.81, $p$ <0.001) to discriminate Ishak 4 from Ishak 5/6. At baseline, the spleen size was significantly correlated to the platelet count (R =0.44, $p$ <0.001).



**Table 4.3 | Thrombocytopenia at baseline versus follow-up**

| **Patients with SVR** | **Last platelets <150** | **Last platelets ≥150** | **total** |
|---|---|---|---|
| Baseline platelets <150 | 27 (38%) | **44 (62%)** | 71 |
| Baseline platelets ≥150 | **2 (2%)** | 105 (98%) | 107 |
| total | 29 (16%) | 149 (84%) | 178 |
| | | **McNemar's test:** $p$<0.001 | |

| Patients without SVR | Last platelets <150 | Last platelets ≥150 | total |
|---|---|---|---|
| Baseline platelets <150 | 124 (90%) | **14 (10%)** | 138 |
| Baseline platelets ≥150 | **47 (43%)** | 63 (57%) | 110 |
| total | 171 (69%) | 77 (31%) | 248 |
| | | **McNemar's test:** $p$<0.001 | |

Included in these analyses were all 426 patients (178 with SVR and 248 without SVR) who had both a platelet count measurement at baseline and a platelet count measurement during follow-up available. The grey-shaded cells indicate the groups of patients who showed a change in platelet count category from baseline to the last measurement during follow-up. Platelets are measured in x10$^9$/L.

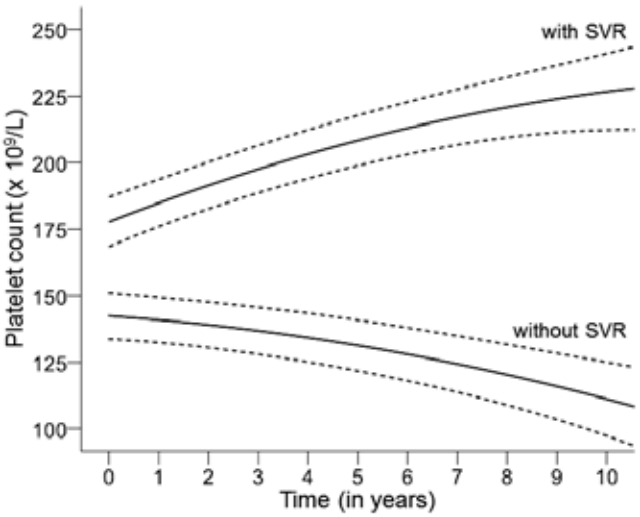


**Figure 4.2 | Evolution of platelet counts according to virological response**

Repeated measurement analyses with a random intercept and slope per patient and an unstructured covariance matrix were performed to analyze the evolution of platelets over time, correcting for potential non-linearity by including the squared time to the platelet count measurement into the model. The mean and 95% confidence interval (dotted line) are presented for patients with sustained virological response (SVR) and patients without SVR. Twenty-four weeks after cessation of antiviral therapy was considered as time 0. The statistical significance refers to both the change within the group with SVR, the change within the group without SVR as well as the difference between both virological response groups.

In 145 (78%) patients with SVR and 235 (85%) of patients without SVR, a spleen size was registered during follow-up. The last median spleen size was 10.4 cm (IQR 9.5–12.0) among patients with SVR versus 13.6 cm (IQR 11.6–16.0) among patients without SVR ($p$ <0.001). Paired data on pre-treatment and end-of-follow-up spleen sizes were available in 209 (45%) patients. Among patients with SVR the spleen size showed a median decrease of 1.0 cm (IQR 0.3–2.0) and among patients without SVR the spleen size showed a median increase of 0.6 cm (IQR 0.1–2.0) from baseline to the last measurement ($p$ <0.001; for the paired analyses within each response group as well as for the comparison between both response groups). The change in spleen size was statistically significantly correlated with the change in platelet count (R =0.41, $p$ <0.001).

## DISCUSSION

With this study, we showed that the platelet counts improved following eradication of chronic HCV infection among patients with bridging fibrosis or cirrhosis. With a repeated measurement analysis

**Table 4.4 | Linear regression analyses for the change in platelets among patients with SVR [a]**

| | Univariate analyses | | | Multivariate analyses | | |
|---|---|---|---|---|---|---|
| **SVR patients** | **β [b]** | **SE** | ***p* value** | **β [b]** | **SE** | ***p* value** |
| Age, per year | 0.36 | 0.35 | 0.307 | - | - | - |
| Males | -3.88 | 7.46 | 0.603 | - | - | - |
| BMI, per kg/m$^2$ | -1.75 | 0.83 | 0.037 | -1.59 | 0.78 | 0.043 |
| Ishak score 4 vs. 5/6 | -1.90 | 7.37 | 0.797 | - | - | - |
| HCV genotype 3 | 0.81 | 7.80 | 0.917 | - | - | - |
| Laboratory markers at baseline | | | | | | |
| - Platelet count, per 10x10$^9$/L | -1.43 | 0.53 | 0.007 | -1.84 | 0.05 | 0.001 |
| - Albumin, per g/L | -0.44 | 0.83 | 0.596 | - | - | - |
| - Bilirubin, per µmol/L | 0.10 | 0.46 | 0.835 | - | - | - |
| - AST/ALT ratio, per 0.1 | -1.51 | 1.13 | 0.183 | - | - | - |
| Diabetes mellitus | 16.78 | 10.65 | 0.117 | - | - | - |
| History of severe alcohol use | -9.62 | 8.27 | 0.246 | - | - | - |
| Time to last platelet count, per year | 3.46 | 0.92 | <0.001 | 3.37 | 0.99 | 0.001 |
| AntiHBc positivity | 13.11 | 7.87 | 0.098 | - | - | - |

Abbreviations: ALT; Alanine aminotransferase, AST; Aspartate aminotransferase, BMI; Body mass index, HCV; hepatitis C virus, SE; Standard error.

a. The unstandardized coefficients are reported.
b. The β indicates that for each unit increase of the predictor variable, the change in platelets will increase (in case of a positive β) or decrease (in case of a negative β) by β units. So, when specified to our multivariate analysis above, for every 1 kg/m2 increase in BMI at baseline, the change in platelets will be 1.59 x109/L lower.

including over 3000 platelet count evaluations, a rather linear increase in platelets was observed from the moment of SVR onwards. In contrast, patients who did not attain SVR showed a further decline. The increase in platelets continued for many years after SVR, suggesting that the histopathological abnormalities and portal pressure gradually improve among chronic HCV-infected patients with advanced liver disease who were successfully treated. This was further substantiated by the reduction in spleen size among the patients who had attained SVR.

Four previous studies have assessed the change in platelets after SVR among Western patients with chronic HCV infection.[80 242 260 261] George et al. followed 150 patients with interferon-induced clearance of their chronic HCV infection for 5 years.[242] In this study, the mean pre-treatment platelet count (232×10$^9$/L) did not significantly differ from the mean last measured platelet count during follow-up (235×10$^9$/L). However, only 16 (11%) patients with cirrhosis were included in this study, at which stage platelet counts are most affected. Although limited by the inclusion of only 10 (10%) patients with cirrhosis as well, another follow-up study did show that the mean platelets significantly increased from 209×10$^9$/L at baseline to 239×10$^9$/L at the final follow-up (which ranged from 1 to 22 years after successful therapy) among 100 patients with SVR.[261] The change in platelets following

antiviral therapy was also assessed in the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) trial, which only included patients with at least Ishak F3 fibrosis.[80] In contrast to both earlier described studies, a control group without SVR was included. As compared with pre-treatment levels, the mean platelet count was already significantly increased at the time SVR was attained (24 weeks after cessation of therapy). An additional post-hoc assessment among the patients with SVR after approximately 5 years of follow-up indicated that the platelet counts further increased with time. In line with our findings, the platelet counts declined among patients without SVR.

With repeated measurement analyses including all platelet count assessments during follow-up, we have showed the evolution of platelets according to the virological response following antiviral therapy among patients with bridging fibrosis or cirrhosis in more detail. Pre-treatment platelet counts were not included in these analyses as we started the follow-up at the time of SVR. This is important, because other mechanisms than splenic sequestration as a result of elevated portal pressure may reduce the platelet counts among patients with chronic HCV infection as well, such as reduced thrombopoietin (TPO) production, HCV-mediated bone marrow suppression and the presence of autoantibodies, causing chronic immune thrombocytopenic purpura.[113] At the time of SVR, when HCV has been suppressed for more than 6 months, bone marrow suppression due to HCV or interferon-based therapy should no longer be of influence.[115 196] Increasing TPO levels could continue to be relevant for the rise of platelets after SVR, because the production of TPO has been negatively correlated with the degree of hepatic fibrosis.[262 263] In this case, regression of hepatic fibrosis and improvement of liver function would thus remain the underlying cause for the improved platelet counts. Nevertheless, lowering of portal pressure and reversal of splenomegaly is likely to remain a predominant reason for the increase in platelets once HCV is eradicated as cause of liver injury. Indeed, the change in platelets and change in spleen size were correlated in our study, which has not been shown by any of the previous studies. A limitation of the current study, however, is that data on baseline spleen size were available in only a limited number of the included patients, also because we restricted the baseline period to 6 months prior to the start of antiviral therapy.

Another interesting result of our study was that higher BMI was negatively associated with the increase in platelet counts among the patients with SVR. Currently, risk factors for disease progression following viral eradication remain largely unknown. Our finding might be explained by the presence of hepatic steatosis and inflammation among patients with high BMI.[264 265] Although data on the presence of steatosis is lacking in our cohort, its association with higher BMI is well known.[266] To what extend these or other aspects of the metabolic syndrome may impact the clinical course of cirrhosis after patients have cleared their chronic HCV infection requires further study. For now, life-style modifications to reduce obesity may be advocated. [267]

Despite the limitations of percutaneous liver biopsy (ethical concerns regarding multiple measurements and the subjective character of hepatic fibrosis assessment), the impact of antiviral therapy on liver histology among patients with chronic HCV infection has been studied. [73 241 242 246 247] The largest study to date included almost 700 patients with at least METAVIR F2 fibrosis at baseline from 4 randomized controlled trials.[246] Even though all patients were biopsied already 24 weeks after cessation of antiviral therapy, patients with SVR showed regression of hepatic fibrosis while patients without SVR had rather stable liver disease, with median estimated annual METAVIR fibrosis progression rates

of -0.591 and 0, respectively. However, 24 weeks after cessation of antiviral therapy might be too early to assess the true impact of successful antiviral therapy on liver histology. Indeed, a prior study including 183 patients with various degrees of HCV-induced hepatic fibrosis and SVR indicated that regression of hepatic fibrosis takes time, as the regression of fibrosis was more pronounced in case of longer post-SVR follow-up.[247] Our data, showing the continued increase in platelets for many years after SVR, is in line with this finding. A recent histological study including patients with HCV-related cirrhosis and SVR showed that 23 (61%) patients had a reduction of the METAVIR F4 fibrosis score in their liver biopsy obtained after a median of approximately 5 years following treatment cessation.[241] Interestingly, even though the METAVIR F4 score was not reduced in the remaining 15 (39%) patients, morphometric analyses indicated that their total liver collagen content was still significantly reduced. Because the semi-quantitative hepatic fibrosis scores, by which regression of hepatic fibrosis has been largely assessed so far, may thus be somewhat too crude, objective and continuous variables for the assessment of changes in hepatic fibrosis are relevant to fully appreciate the impact of SVR on liver disease severity. As the change in platelets has been linked to the change in hepatic fibrosis, the platelet count represents an easily accessible biomarker to assess histological improvement.[258 259] We remained with the platelet count as outcome measure in our study because many other laboratory-based non-invasive fibrosis markers showed a poor accuracy for hepatic fibrosis assessment among patients with SVR.[268] Perhaps, this is explained by the fact that most include parameters of hepatic inflammation, which is also affected by viral eradication. Regression of hepatic fibrosis, especially in case of cirrhosis, was recently shown to be clinically relevant. In a pivotal study by Mallet et al., clinical outcome was superior among chronic HCV-infected patients with cirrhosis who showed a substantial reduction of their post-treatment METAVIR score as compared with patients who did not regress.[74] In addition, other large cohort studies have indicated that SVR was also associated with a reduced occurrence of liver failure, HCC and mortality among patients with advanced liver disease.[75 79-83] Only a few studies have directly measured the hepatic venous pressure gradient (HVPG) before and after antiviral therapy.[77 78 269] All included only a small number of patients, but showed that SVR was significantly associated with a reduction in HVPG, which remains one of the best validated surrogate markers in hepatology. Our finding of a decrease in spleen size following SVR in a large group of patients with bridging fibrosis or cirrhosis further substantiates the reduction of portal pressure once the chronic HCV infection is eradicated.

In conclusion, the platelet counts gradually increased, and the spleen size decreased following achievement of SVR among chronic HCV-infected patients with bridging fibrosis or cirrhosis. This suggests that successful antiviral therapy leads to a reduction in portal pressure, probably because of regression of the histopathological abnormalities, which have resulted from long-term liver injury due to chronic HCV infection.

# CHAPTER 5

## EPIDEMIOLOGICAL TRENDS AMONG THE POPULATION WITH CHRONIC HCV INFECTION IN THE NETHERLANDS

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## ABSTRACT

**Background & Aims**

As the field of antiviral therapy for chronic HCV infection is rapidly evolving, this study aimed to assess the epidemiological changes in patient and disease characteristics among individuals with chronic HCV infection.

**Methods**

This study included all consecutive patients with chronic HCV monoinfection who were referred between 1990 and 2013 to the Erasmus MC University Medical Center Rotterdam, a large tertiary center in the Netherlands. To identify trends over time, the study population was divided into six equal eras based on date of first visit to the outpatient clinic.

**Results**

A total of 1,779 patients were diagnosed with chronic HCV infection. Mean age increased over time from 43.6 (SD 13.8) years to 51.7 (SD 11.2) years ($p < 0.001$). The number of patients who were referred with cirrhosis increased over time, from 31 (25%) patients in Era 1 to 118 (42%) patients in Era 6 ($p < 0.001$), respectively. More patients were referred with HCV genotype 1a and 3 in the last era, with 27 (48.2%) and 15 (14.0%) patients in Era 1 and 58 (54.2%) and 60 (21.8%) patients in Era 6 ($p < 0.001$ both), respectively. The vast majority of patients (69.5%) were born between 1950 to 1975, with 62.5% of the patients being born between 1945 and 1965.

**Conclusions**

The HCV-infected population is ageing and is more often referred with severe liver disease. This study stresses the importance of urgently implementing national HCV screening programs in order to be able to decrease the future burden of chronic HCV infection in the Netherlands.

## INTRODUCTION

Chronic infection with HCV is a major public health problem. The estimated prevalence of chronic HCV infection globally is 2–3%, with a high geographical variation.[270-272] Recently, it has been estimated that in the Netherlands around 22,000 individuals have a chronic HCV infection, of whom 12,000 have a known diagnosis.[4] In the Western world HCV genotype 1 is most common, while HCV genotype 6 is the predominant variant in Southeast Asia. In the Netherlands, HCV genotype 1 is the most prevalent (50%), followed by HCV genotype 3 (30%) and subsequently HCV genotype 2 and 4 (both approximately 10%).[5 13] The result of the continuous presence of HCV is a chronic inflammatory activity in the liver, causing progressive fibrosis and ultimately cirrhosis. Fortunately, the disease is characterized by a slow progression and not all patients with chronic HCV infection show progression of hepatic fibrosis. However, once cirrhosis is established, patients have an annual risk of approximately 3% of developing hepatic decompensation as well as hepatocellular carcinoma (HCC).[61 273-277] Early detection and successful treatment could reduce this risk and slow down the development of cirrhosis and HCC.

Since the discovery of HCV in 1989 and the implementation of blood screening tests shortly thereafter, there has been a significant reduction in the transmission of the virus.[11 278-281] In the US, it has been identified that the majority of HCV patients were infected between the 1960s and 1970s. As cirrhosis is slowly developed, many infected people remain undetected so far. Consequently, due to ageing of this HCV-infected population, approximately 25% of the US patients with chronic HCV infection are expected to have cirrhosis. This proportion is likely to increase to 45% by the year 2030.[63] Birth cohort screening could identify these patients in an earlier stage of disease. Early detection could prevent morbidity and mortality due to chronic HCV infection. Therefore, in the US, birth cohort screening has recently been recommended for people born between 1945–1965, since more than 75% of HCV patients in the US are born during these years.[282-284] So far, a screening program has not been implemented in the Netherlands. This can be attributed to the low prevalence, which ranges from 0.13% to 0.22%.[4 6 285] From a public health perspective it might be important to implement screening programs when there is an increase in newly referred patients with a more advanced stage of the disease. These patients do benefit from successful antiviral treatment as it may avoid or reduce cirrhosis-related complications and improve the overall survival.[81] However, as Koh et al.[261] suggested, the greatest benefit from successful antiviral treatment may be in patients without cirrhosis.

In 2011, it was estimated that in the Netherlands 900 patients were treated annually.[4] With the introduction of very safe and effective interferon (IFN)- and ribavirin (RBV)-free antiviral treatment, it is expected that more patients can be treated. Currently, sofosbuvir, simeprevir and daclatasvir are being reimbursed by Dutch insurance companies for the treatment of patients with chronic HCV infection and bridging fibrosis or cirrhosis. As a pan-genotypic antiviral regimen has not yet been introduced in daily care, current antiviral treatment is still genotype-specific.

Recently, data was published on the present and future burden of chronic HCV infection in the Netherlands based on historical data.[4 286 287] In 2013, the prevalence of cirrhosis among the patients with chronic HCV infection was 11%, of whom 1.3% had decompensated cirrhosis. The rate of both HCC and liver-related mortality was 0.5%. Additional data on the trends in patient and disease

characteristics from the last 23 years are important for Dutch health policy makers to be informed about the shifting trends in the patient population with chronic HCV infection. This also provides information on the patients that will be considered for IFN-free therapy. Moreover, it could help deciding on the implementation of efficient screening programs in the Netherlands. Therefore, the aim of this study was to assess the epidemiological trends in patient and disease characteristics over time among individuals with chronic HCV infection that were newly referred to a tertiary center in the Netherlands from 1990 to 2013.

## PATIENTS AND METHODS

**Study population and data collection**

This study included all consecutive patients with chronic HCV infection who were referred between 1990 and 2013 to the Erasmus MC University Medical Center Rotterdam (EMC), a large tertiary center in the Netherlands. The EMC is considered to have the largest viral hepatitis unit in the country and is an important referral center for the surrounding hospitals. In addition, it has the largest unit for liver transplantation in the country. All charts were reviewed by two investigators in order to collect detailed data on patient and disease characteristics and treatment history at their first visit to our clinic. Patients were included if they had detectable HCV RNA by polymerase chain reaction (PCR). Patients who were coinfected with HIV or HBV were excluded, as well as patients who underwent a liver transplantation before referral.[288-290] Baseline characteristics included the date of birth, gender, ethnicity, body mass index (kg/m$^2$), HCV genotype and subtype, treatment history, probable mode of transmission, history of/or current alcohol abuse (≥50 g/day), diabetes mellitus and other comorbidities. Ethnicity was categorized as Caucasian, Black, Asian or Mediterranean. The probable mode of transmission was determined by the presence of risk factors such as intravenous drug use (IDU), blood transfusion or tattoos. The laboratory data that were obtained included alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin and platelet count. The AST/ALT ratio, AST to platelet ratio index (APRI) and FIB-4 index were calculated from AST, ALT, platelet count and age at time of referral. To assess the severity of liver disease, detailed information was obtained on liver biopsy, transient elastography, ultrasound and/or clinical (liver) history. Liver biopsy was taken as a golden standard and a METAVIR score of F4 was considered as cirrhosis.[50] When there was no liver biopsy performed, results of transient elastography were assessed, in which a liver stiffness of 12.5 kilopascals or higher was considered as cirrhosis.[291] If data on liver biopsy or transient elastography was not available, ultrasound reports and clinical history were assessed in order to determine whether cirrhosis was likely to be present. Clinical cirrhosis was defined as an episode of decompensated cirrhosis (that is, ascites, spontaneous bacterial peritonitis, icterus, overt hepatic encephalopathy or variceal bleeding) or the presence of esophagus varices during gastroscopy. The cutoff values that were used to define cirrhosis were AST/ALT ratio ≥1.00, APRI>2.00 and FIB-4 index >3.25.[292-294] The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. The study was considered to be a low-risk study using retrospective and anonymized patient data. Ethical approval was obtained from the local ethics committee.

**Virology**

Information on amplification of RNA was obtained from results of polymerase chain reaction (PCR), to determine if RNA genome was detectable. The following tests were used: Roche Cobas AmpliPrep/ Cobas Amplicor HCV (Roche, Pleasanton, CA, USA) test version 2.0 with limit of detection (LOD)=100 IU/ml (from 01-01-2000 to 01-10-2008), Roche Cobas AmpliPrep/Cobas Taqman HCV test version 1.0 with LOD=15 IU/ml and limit of quantification (LOQ)=43 IU/ml (from 01-10-2008 to 15-03-2012), Roche Cobas AmpliPrep/Cobas TaqMan HCV test version 2.0 with LOD=LOQ=15 IU/ ml (from 15-03-2012 to present). Before 2000, multiple assays were used for testing HCV RNA, including outsourced assays. The HCV genotype was determined by sequential analyses. Supplementary Table 5.1 shows information on all the tests.

**Statistical analyses**

Descriptive analyses were conducted to determine means, medians and proportions of baseline patient characteristics. Pearson $X^2$ test was used for the comparison of dichotomous or categorical variables and Mann–Whitney U-test, Student's t-test or ANOVA was used for the comparison of continuous variables. To identify trends over calendar time in patient characteristics, the study population was divided in six eras based on date of first visit at the Hepatology Unit of the EMC. In order to obtain equal eras, a 4-year range was applied: Era 1 (1990–1993), Era 2 (1994–1997), Era 3 (1998–2001), Era 4 (2002–2005), Era 5 (2006–2009) and Era 6 (2010–2013). Linear trend test (ANOVA or Mantel–Haenszel $X^2$ for trend) was performed to assess differences in proportions or means across the six eras. To assess whether there was a birth year cohort in our study, we applied two time frames from earlier studies that described a birth year cohort for the Netherlands (1950–1975) and a US birth year cohort (1945–1965).[261 284] All statistical tests were two-tailed and $p < 0.05$ was considered statistically significant. Statistical analyses were performed in SPSS for windows, version 21.0 (IBM, Armonk, NY, USA).



## RESULTS

**Baseline characteristics**

Between 1990 and 2013 a total of 1,971 patients with chronic HCV infection were referred to the Department of Hepatology at the EMC. Patients coinfected with HIV or HBV and patients who underwent liver transplantation before referral (n=192) were excluded. In total, 1,779 patients were included in the current analyses. Table 5.1 presents an overview of baseline characteristics of the study population. In this population, 1,210 (68.0%) patients were male and mean age was 46.5 years (SD 11.7). Cirrhosis was present among 459 patients (26.2%). The presence of cirrhosis was biopsy-proven in 174 (37.9%) patients and diagnosed by transient elastography in 97 (21.1%) patients. The remaining patients with cirrhosis were diagnosed by ultrasound (38.6%) or by the presence of a clinical cirrhotic event (2.4%). Figure 5.1 presents the number of patients born per birth year. The vast majority of patients (69.5%) were born between 1950 to 1975, with 62.5% of the patients being born between 1945 and 1965.

**Table 5.1 | Baseline characteristics at time of referral**

| Baseline variables | Total (N=1,779) |
|---|---|
| Age, *in years* | 46.5 (11.7) |
| Male | 1,210 (68.0%) |
| HCV genotype | |
| 1 | 915 (51.4%) |
| *Subtype 1a* | *239 (26.1%)* |
| *Subtype 1b* | *300 (32.8%)* |
| *Unknown subtype* | *376 (41.1%)* |
| 2 | 192 (10.8%) |
| 3 | 414 (23.3%) |
| 4 | 132 (7.4%) |
| Other[b] | 12 (0.7%) |
| Unknown | 114 (6.4%) |
| Cirrhosis | 459 (22.8%) |
| Diabetes mellitus | 170 (9.6%) |
| Ethnicity | |
| Caucasian | 1,238 (69.6%) |
| Black | 219 (12.3%) |
| Mediterranean | 229 (12.9%) |
| Asian | 72 (4.0%) |
| Mode of infection | |
| Blood transfusion | 280 (15.7%) |
| IDU | 727 (40.9%) |
| Tattoo | 43 (2.4%) |
| medical procedures | 76 (4.3%) |
| Positive partner | 24 (1.3%) |
| Multiple risk factors | 44 (2.5%) |
| Unknown | 585 (32.9%) |
| Alcohol abuses | |
| Yes | 525 (29.5%) |
| *Active* | *299 (57.0%)* |
| *In past* | *226 (43.0%)* |
| No | 1,041 (58.5%) |
| Unknown | 213 (12.0%) |
| Treatment experienced | 294 (16.5%) |
| BMI, $kg/m^2$ | 24.9 (22.2-28.2) |
| Albumin, *in g/L* | 44 (40-46) |
| Platelet count, $x10^9/L$ | 186 (127-237) |
| AST/ALT ratio | 0.77 (0.58-1.06) |
| APRI | 0.99 (0.50-2.27) |
| FIB-4 Index | 1.62 (0.93-3.81) |

Abbreviations: HCV, hepatitis C virus; IDU, intravenous drug use; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, AST to platelet ratio index

a. Continuous variables are presented as mean (standard deviation) or median (interquartile range); dichotomous variables are presented as n, (percentage of whole group)

b. Other genotypes included HCV genotype 5 and 6 or double genotypes

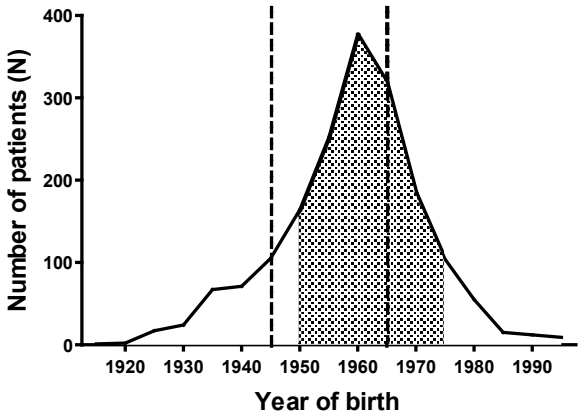


**Figure 5.1 | Birth year of the patients that were included in the study**

The shaded area shows that 69.5% of the referred patients were born between 1950 and 1975. The dashed lines mark the period between 1945 and 1965 in which 62.5% of the patients that were referred were born.

**Demographics**

Mean age at first visit increased over time (Table 5.2), from 43.6 (SD 13.8) years in Era 1 to 51.7 (SD 11.2) years in Era 6 ($p < 0.001$). When the percentage of patients with an age of 50 or higher was considered, it increased from 32.5% in the first era to 66.2% in the last era ($p < 0.001$). Most patients were male, but this percentage did not change over time ($p = 0.780$), ranging from 66.6% to 69.5%. There was no statistically significant trend for ethnicity among the patients ($p = 0.654$); 1,238 (70.4%) patients were Caucasian.

**Mode of transmission**

There were significant changes in mode of infection during the six different eras ($p = 0.003$). Among patients that were infected via blood transfusion, a significant decrease in percentage was observed, from 39 (31.2%) patients in Era 1 to 47 (16.4%) patients in Era 6 ($p < 0.001$). In contrast, the number of patients infected through IDU significantly increased over time, from 34 (27.2%) patients in Era 1 to 100 (35.0%) patients in Era 6 ($p = 0.036$), with even higher percentages in Era 3, 4 and 5, respectively 46.6, 44.0 and 48.2%. Figure 5.2 shows that IDU was most frequent mode of infection (61.4%) among patients with HCV genotype 3, and to a lesser extent among patients with HCV genotype 1, 2 and 4 (respectively 39.8%, 17.0% and 29.5%).

**HCV genotype**

HCV genotype 1 was predominant in all time periods, and the distribution of HCV genotypes did not differ significantly among the various eras ($p = 0.474$). Nevertheless, there was a decrease observed among patients with genotype 2 (from 20.6% to 6.9%) and an increase among patients with genotype 3 (from 14.0% to 21.8%).

**Table 5.2 | Comparison over the six eras**

| Variable | Era 1 (n=126) 1990-1993 | Era 2 (n=259) 1994-1997 | Era 3 (n=331) 1998-2001 | Era 4 (n=365) 2002-2005 | Era 5 (n=411) 2006-2009 | Era 6 (n=287) 2010-2013 | *p* value |
|---|---|---|---|---|---|---|---|
| Gender | | | | | | | 0.797 |
| Male | 85 (67.5%) | 174 (67.2%) | 230 (69.5%) | 243 (66.6%) | 280 (68.1%) | 198 (69.0%) | |
| Age | | | | | | | <0.001 |
| In years | 43.6 (13.8) | 45.2 (11.9) | 44.4 (11.7) | 45.4 (11.4) | 47.5 (10.6) | 51.7 (11.2) | |
| HCV genotype | | | | | | | 0.474 |
| 1 | 62 (57.9%) | 119 (53.4%) | 137 (45.5%) | 196 (57.0%) | 232 (57.6%) | 169 (61.5%) | |
| 1a | 27 (43.5%) | 33 ( 27.7%) | 39 (28.5%) | 39 (19.9%) | 43 (18.5%) | 58 (34.3%) | 0.001[c] |
| 1b | 29 (46.8%) | 77 (64.7%) | 67 (48.9%) | 49 (25.0%) | 29 (12.5%) | 49 (29.0%) | |
| Unknown subtype | 6 (9.7%) | 9 (7.6%) | 31 (22.6%) | 108 (55.1%) | 160 (69.0%) | 62 (63.3%) | |
| 2 | 22 (20.6%) | 31 (13.9%) | 43 (14.3%) | 41 (11.9%) | 36 (8.9%) | 19 (6.9%) | |
| 3 | 15 (14.0%) | 53 (23.8%) | 100 (33.2%) | 89 (25.9%) | 97 (24.1%) | 60 (21.8%) | |
| 4 | 8 (7.5%) | 20 (9.0%) | 21 (7.0%) | 18 (5.2%) | 38 (9.4%) | 27 (9.8%) | |
| Ethnicity | | | | | | | 0.654 |
| Caucasian | 88 (70.4%) | 179 (69.1%) | 224 (68.1%) | 257 (71.2%) | 299 (74.6%) | 191 (67.5%) | |
| Black | 15 (12.0%) | 29 (11.2%) | 40 (12.2%) | 49 (13.6%) | 50 (12.5%) | 36 (12.7%) | |
| Mediterranean | 16 (12.8%) | 32 (12.4%) | 50 (15.2%) | 41 (11.4%) | 44 (11.0%) | 47 (16.6%) | |
| Asian | 6 (4.8%) | 19 (7.3%) | 15 (4.6%) | 14 (3.9%) | 8 (2.0%) | 9 (3.2%) | |
| Mode of infection | | | | | | | 0.003 |
| Blood transfusion | 39 (31.2%) | 55 (21.8%) | 49 (15.1%) | 44 (12.1%) | 46 (11.2%) | 47 (16.4%) | |
| IDU | 34 (27.2%) | 85 (33.7%) | 151 (46.6%) | 160 (44.0%) | 197 (48.2%) | 100 (35.0%) | |
| Other[d] | 52 (41.6%) | 112 (44.4%) | 124 (38.3%) | 160 (44.0%) | 166 (40.6%) | 139 (48.6%) | |
| Treatment experienced | | | | | | | 0.001 |
| Yes | 7 (5.6%) | 46 (18.9%) | 50 (15.5%) | 51 (14.1%) | 77 (19.0%) | 63 (22.0%) | |

**Table 5.2 | Continued**

| Variable | Era 1 (n=126) 1990-1993 | Era 2 (n=259) 1994-1997 | Era 3 (n=331) 1998-2001 | Era 4 (n=365) 2002-2005 | Era 5 (n=411) 2006-2009 | Era 6 (n=287) 2010-2013 | *p* value |
|---|---|---|---|---|---|---|---|
| Cirrhosis | | | | | | | <0.001 |
| Yes | 31 (24.6%) | 44 (17.2%) | 66 (20.2%) | 84 (23.3%) | 116 (28.9%) | 118 (41.7%) | |

Abbreviations: HCV, hepatitis C virus; IDU, intravenous drug use

a. Patients characteristics were compared between 6 eras with linear trend test, using ANOVA test for continuous variables and the Mantel-Haenszel $\chi^2$ statistics
b. Numbers are presented as N (percentage of the whole group), means are presented as number (Standard Deviation)
c. Linear trend test performed with known subtypes
d. Other mode of transmission were defined as: tattoo, other injections or medical procedure, positive partner, multiple mode of infection or unknown

**Table 5.3 | AST/ALT ratio, APRI and FIB 4 index over the eras**

| | | Era 1 (n=101) 1990-1993 | Era 2 (n=248) 1994-1997 | Era 3 (n=303) 1998-2001 | Era 4 (n=341) 2002-2005 | Era 5 (n=385) 2006-2009 | Era 6 (n=282) 2010-2013 | *p* value |
|---|---|---|---|---|---|---|---|---|
| Total | AST/ALT | 0.61 (0.48-0.77) | 0.62 (0.50-0.80) | 0.69 (0.53-0.97) | 0.80 (0.59-1.11) | 0.88 (0.67-1.18) | 0.93 (0.72-1.27) | <0.001[c] |
| | APRI | 0.70 (0.40-1.80) | 0.61 (0.36-1.33) | 1.14 (0.55-2.62) | 1.11 (0.52-2.34) | 1.06 (0.52-2.47) | 1.06 (0.57-2.68) | 0.028[c] |
| | FIB 4 index | 1.07 (0.69-2.63) | 1.12 (0.64-2.58) | 1.54 (0.87-3.96) | 1.66 (0.94-3.64) | 1.78 (1.08-4.20) | 2.22 (1.25-5.01) | <0.001[c] |
| Cut-off for cirrhosis[d] | AST/ALT (≥1.00) | 10 (9.9%) | 37 (14.9%) | 70 (23.1%) | 113 (33.1%) | 148 (38.4%) | 118 (41.8%) | <0.001 |
| | APRI (>2.00) | 20 (19.8%) | 40 (16.1%) | 103 (34.0%) | 100 (29.3%) | 115 (29.9%) | 94 (33.3%) | <0.001 |
| | FIB 4 index (>3.25) | 19 (18.8%) | 50 (20.2%) | 84 (27.7%) | 96 (28.2%) | 118 (30.6%) | 108 (38.3%) | <0.001 |

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, AST to platelet ratio index

a. Results are based on patients that could be assessed on all three markers
b. Continuous variables (total score) are presented as median (interquartile range); dichotomous variables (cirrhosis based on cut-off values) are presented as n, (percentage of whole group)
c. *P* values are based on the comparison of the median value in Era 1 and Era 6 by Mann-Whitney *U*-test.
d. The cut-off values were compared between 6 eras with linear trend test, using the Mantel-Haenszel $\chi^2$ statistics

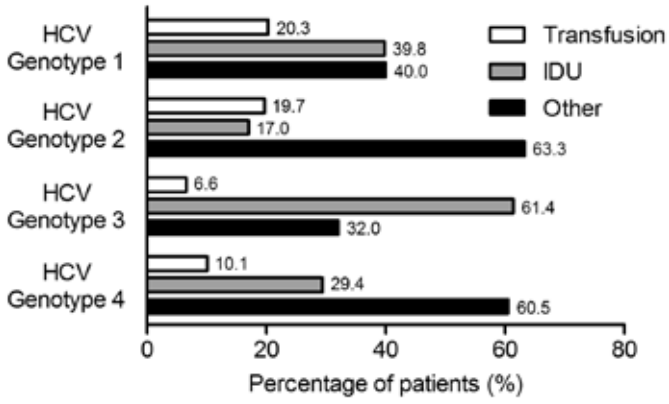


**Figure 5.2 | The mode of infection among patients with HCV genotype 1 to 4**

White bars represent the patients that were infected due to a blood transfusion. Grey bars represent the patients that were infected due to IDU. The black bars represent all other modes of infection, including tattoo, medical procedures, positive partner, multiple risk factors or unknown mode of infection. Abbreviations: IDU, intravenous drug use.

Among patients with an available subtype for HCV genotype 1, a significant linear trend was observed, with a shift in predominant subtype. In Era 1, 27 (48.2%) patients had subtype 1a and 29 (51.8%) patients had subtype 1b. This was significantly different from Era 6, in which 58 (54.2%) patients were diagnosed with subtype 1a and 49 (45.8%) patients were diagnosed with subtype 1b ($p = 0.001$).

**Treatment experience**

In Era 1, only 7 (5.6%) patients that were referred were treatment-experienced. The percentage of patients that were treatment experienced gradually increased to 22.0% in the last era ($p = 0.001$). A major increase of treatment-experienced patients was seen among patients with cirrhosis at baseline (10.0% in Era 1 to 32.2% in Era 6; $p = 0.012$). This increase was less pronounced, and not statistically significant, among patients without cirrhosis (from 4.2% to 14.6%, respectively; $p = 0.873$).

**Severity of liver disease**

There was an increase seen in number of patients with cirrhosis at first visit, from 31 patients (24.6%) in Era 1 to 118 patients (41.7%) in Era 6 ($p < 0.001$). When considering the group of patients with an age below 50, there was no increase observed among patients diagnosed with cirrhosis (18.8% in Era 1 and 19.8% in Era 6; $p = 0.223$). In contrast, there was a significant increase among patients aged 50 or higher (from 50.0% to 67.3%; $p < 0.001$). Table 5.3 presents the median AST/ALT ratio, APRI and FIB-4 index that were calculated among the patients over the six eras. These markers increased from respectively 0.59 (0.48–0.76), 0.69 (0.38–1.79) and 1.07 (0.69–2.63) in the first era to 0.92 (0.72–1.26), 1.06 (0.57–2.71) and 2.22 (1.25–5.01) in the last era ($p < 0.001$ for all). When using the cutoff values for the presence of cirrhosis, the percentage of patients that were defined as cirrhotic patients by the non-invasive markers gradually increased ($p < 0.001$ for all).

## DISCUSSION

This study showed the epidemiological trends among 1,779 patients with chronic HCV infection that were newly referred to a large hepatology unit in a tertiary center in the Netherlands. There was a significant increase in mean age over the succeeding time periods, showing that the population with chronic HCV infection is ageing. The proportion of newly referred patients who were diagnosed with cirrhosis increased over time. The majority of patients were infected with HCV genotype 1, and there was an increasing number of patients with HCV genotype 1a and 3.

In our study population there was a significant increase in the age of patients at time of referral, from 43.6 years to 51.7 years. This finding is in line with a recent study that was performed in the US, also showing an increase in mean age (from 45 to 56 years) among patients who were referred to a tertiary liver center.[295] In fact, it is a cohort of patients that were probably infected a few decades ago, in the peak incidence years. As ageing of the population will continue, treatment of elderly patients will increase. Innes et al.[296] showed that non-cirrhotic patients aged 60 or older have the lowest benefit of achieving sustained virological response (SVR) to prevent liver failure and liver-related death. In the past, IFN-based antiviral treatment was being withheld among older patients, due to increased risk for side effects and reduced efficacy. As the new direct-acting antivirals (DAAs) were shown to have no safety issues, physicians will not be hampered in applying these regimens to the elderly. However, as the costs of these IFN-free regimens are high, it could be questioned whether the application of these strategies will be cost-effective in this growing subgroup. Therefore, as our study underlines, we need to assess the clinical relevance of antiviral therapy for chronic HCV infection among the elderly. There might be a threshold in age, when patients with advanced hepatic disease will have less clinical benefit from successful antiviral therapy as there will be a higher burden of non-liver-related diseases.

In all six eras, HCV genotype 1 was the predominant genotype. Historically, HCV genotype 1 had the lowest response rates to antiviral therapy with pegylated IFN and ribavirin.[106] With the introduction of the first generation protease inhibitors these response rates increased but were still suboptimal.[122 126] Among those patients with HCV genotype 1, the incidence of subtype 1a increased compared to subtype 1b. This increase of subtype 1a was also observed in Belgium and the US.[295 297] A possible explanation of this finding could be that the response rates of triple therapy with telaprevir and boceprevir were higher among patients with subtype 1b, due to the lower rate of drug-resistant variants.[298] Even with the newer IFN-free DAA-regimens, differences in SVR rates between genotype 1a and 1b remain relevant.[128 129 145 156 157] Another, maybe more important finding, is the increase of HCV genotype 3. Although it was not statistically significant, probably due to the lack of power, it has major clinical implications. First, it was found that HCV genotype 3 was associated with disease progression for both severity of liver disease as well as clinical outcome.[299] Second, treatment of HCV genotype 3 seems to be the most challenging in the era of IFN-free regimens, when treatment efficacy is concerned.[138 163]

In the last era (2010–2013), the number of patients with cirrhosis at their first visit was high (41.7%). First, this finding may be explained by the reduced transmission rate after the discovery of HCV, leading to the ageing of a cohort of patients that has been infected with the virus in the peak incidence years and consequently an increase of patients presenting with cirrhosis.[300] Second, late referral to

our center could have contributed to the large number of patients with cirrhosis, who were more difficult to treat in the era of IFN-based treatment. Third, the slow course of the disease is responsible for the late diagnosis of the infection. However, the high rate of patients with cirrhosis should be interpreted with care, since it might be subject to selection bias as patients with significant disease are those who are seeking medical care and will be referred to a tertiary center as they are considered difficult to treat. In a recent study among more than 14,000 patients, late diagnosis of chronic HCV infection was associated with more hospitalization (59% versus 35%) and death (33% versus 9%), with a median time of 4.8 years from initial HCV diagnosis until death.[301] In France, an observational multicenter study showed an increase in number of newly referred patients with cirrhosis with 7.4% of the patients in 1995, 10.4% in 2001 and 16.1% in 2010. Even though an increase was observed, this percentage in France was lower than in the current study.[302] This is probably due to the fact that, in contrast to the Netherlands as well as many other countries in Europe, France has been actively screening and treating patients over the last two decades. This might have led to more patients being cured in an earlier phase of disease.[303] With the combination of multiple DAAs, it is expected that more patients, regardless of the presence of cirrhosis, will achieve SVR in the near future. Although, there is a reduced risk for developing clinical complications of cirrhosis among patients who attained SVR, the risk cannot be fully eliminated.[81 82] Therefore, these patients will continue to be included in intensive surveillance programs.

It is interesting to note that 70% of all patients were born between 1950 to 1975. This is in line with recent published data showing that 72% of the HCV population in the Netherlands was born between 1950–1975.[286] Another recent study that was performed in the US found a birth year cohort of 1945 to 1965 in which 75% of the patients were born, which is higher than the 63% in the present study.[283] The wider range in the Netherlands might be due to variability in risk factors between the countries and different time of introduction of the virus. In the US, the prevalence in the 1945 to 1965 birth year cohort was 3× higher (3.5%) compared to the prevalence in the general population.[304] Since it was shown that US birth cohort screening is cost-effective from a minimum prevalence of 0.84%, screening of patients born between 1945 and 1965 is recommended.[285] When screening is concerned, it has to be noted that regulation of health-care systems differs per country and therefore these findings concerning cost-effectiveness of HCV screening cannot directly be extrapolated to the Dutch health-care system. Since we found an increasing number of patients that were referred with more advanced disease, probably the point is reached at which urgent screening is warranted. Due to the low prevalence in the Netherlands, it is a country where chronic HCV infection could be fully eradicated. As this study showed that 70% of the patients were born between 1950 and 1975, those people could be included in screening programs. In addition, first-generation migrants could be screened, since it was found that these patients account for many infections.[6] In order to prevent the development of cirrhosis among patients with chronic HCV infection and identify the patients in an early stage of disease, it is of utmost importance to implement effective screening programs. Especially, since we now have very efficient treatment regimens.

A salient strength of the current study was the large sample size of 1,779 chronic HCV patients, which ensured an adequate power. Furthermore, by including consecutive patients from 1990 to 2013, epidemiological trends from the discovery of the virus to the present time could be accurately

assessed. Nevertheless, the study also has some limitations. First, retrospective data were used, which resulted in missing variables as data was not gathered for the purpose of research. Using data of a tertiary center might have caused selection bias resulting in the overrepresentation of difficult to treat patients, like patients with advanced liver disease. Finally, cirrhosis was diagnosed in heterogeneous ways. Nevertheless, we performed sensitivity analyses, including solely patients that were diagnosed by biopsy or transient elastography, showing comparable results (data not shown). Non-invasive fibrosis markers showed the same trends, assuring us that these results were legitimate.

In conclusion, the HCV-infected population in the Netherlands is ageing and more often referred with severe liver disease. This stresses the importance of urgently implementing national HCV screening programs in order to be able to decrease the future burden of chronic HCV infection in the Netherlands.

# SUPPLEMENTARY TABLE

**Supplementary Table 5.1 |** HCV RNA tests that have been used

| Date | Test |
|---|---|
| **Quantitative/qualitative HCV RNA test** | |
| Before 2000 | Multiple assays, including outsourced assays |
| 01-01-2000 to 01-10-2008 | Roche Cobas AmpliPrep/cobas Amplicator HCV test version 2.0<br>LOD: 100 IU/mL |
| 01-10-2008 to 15-03-2012 | Roche Cobas AmpliPrep/cobas Taqman HCV test version 1.0<br>LOD: 15 IU/mL; LOQ: 43 IU/mL |
| 15-03-2012 to present | Roche Cobas Ampli/Prep/cobas Taqman HCV test version 2.0<br>LOD=LOQ= 15 IU/mL |
| **Sequential tests** | |
| ~ to 08-08-2005 | Sanger sequencing on 5'UTR |
| 08-08-2005 to 10-12-2003 | Sanger sequencing on NS5-gen |
| 10-12-2003 to 16-07-2007 | Versant HCV genotype version 1.0 assay (LiPA) |
| 16-07-2007 to present | Versant HCV genotype version 2.0 assay (LiPA) |

Abbreviations: LOD, limit of detection; LOQ, limit of quantification

# CHAPTER 6

## REAL-WORLD MEDICAL COSTS OF ANTIVIRAL THERAPY AMONG PATIENTS WITH CHRONIC HCV INFECTION AND ADVANCED HEPATIC FIBROSIS

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## ABSTRACT

**Background & Aims**

Very potent direct acting antivirals for the treatment of chronic hepatitis C virus (HCV) infection were recently introduced into daily clinical practice. Currently, treatment uptake is hampered by their high costs, eliciting prioritization of treatment. We aimed to evaluate the direct medical costs during interferon (IFN)-based antiviral treatment and the costs per sustained virological response (SVR) among patients with advanced hepatic fibrosis.

**Methods**

This retrospective cohort study included all consecutive patients with chronic HCV infection and biopsy-proven bridging fibrosis or cirrhosis (Ishak 4-6) treated with IFN-based regimens in five hepatology units of tertiary care centers in Europe and Canada. Direct medical costs, expressed in 2013 Euros, during therapy were assessed. The components of care were quantified by three distinct categories: treatment, safety/ monitoring and complications. Cost per SVR was calculated by dividing the mean cost by the SVR rate.

**Results**

In total, 672 interferon-based treatments administered to 455 patients were included. Total medical costs per patient were averaged to €14,559 (95%CI, €13,323-€15,836). The mean cost per SVR was €38,514 (95%CI, €35,244-€41,892). The costs per SVR were €26,105 (95%CI, €23,068-€29,296) for patients with a normal platelet count and €50,907 (95%CI, €44,151-€59,612) for patients with thrombocytopenia, with the costs per SVR of €74,961 (95%CI, €55,463-€103,541) among those patients with a platelet count below $100x10^9$/L.

**Conclusions**

Due to the lower SVR rates, the cost per SVR of IFN-based treatment increased when patients with more advanced liver disease were treated. Additional costs of IFN-free therapy could be limited among these patients.

## INTRODUCTION

The burden of chronic hepatitis C virus (HCV) infection is expected to increase over the next years.[305] Today, it is the leading cause of chronic liver disease, hepatocellular carcinoma (HCC) and the most common indication for liver transplantation in the Western world.[41 178] It has been estimated in the United States that by 2030, 45% of the HCV population will have cirrhosis, which may be reflected by the development of thrombocytopenia.[63 113] Indeed, even though the pathophysiology of thrombocytopenia is thought to be multifactorial, it appears to be largely related to the severity of liver disease.[180 181] Since the number of patients with cirrhosis will increase, it can be expected that the prevalence of those with thrombocytopenia will increase as well.[306] It could be questioned to what extent the presence of thrombocytopenia reflects a functional problem which causes on-treatment safety issues such as bleeding complications, or whether it is merely serving as a marker for more severe liver disease with portal hypertension. Nevertheless, thrombocytopenia defines a more difficult to cure patient population, who are in urgent need of successful antiviral therapy.[191 192]

Until now, treatment for chronic HCV infection consisted of pegylated interferon (PegIFN)-based regimens leading to suboptimal sustained virological response (SVR) rates and important safety issues, especially among patients with advanced liver disease.[107] These side effects frequently necessitate dose reductions or treatment discontinuation, leading to reduced treatment efficacy.[111 112] The recent discovery of direct-acting antivirals (DAAs) has revolutionized treatment for patients with chronic HCV infection. With interferon-free therapy even cirrhotic patients have very high chances of attaining SVR.[145 156]

Unfortunately, the costs of DAAs are very high and this currently limits the availability of these drugs for the majority of patients worldwide. Also in wealthy countries it is deemed necessary to prioritize DAA treatment, in order to reduce the budget impact. Because patients with cirrhosis, especially in case of thrombocytopenia, are at highest risk of cirrhosis-related complications, these patients are treated first. Indeed, they have most direct clinical benefits of the highly effective IFN-free treatment regimens.[196 307] In light of the discussion on costs and availability of IFN-free therapy it is noteworthy that there have only been a limited number of real-world economic studies published. The true cost of IFN-based therapy applied to a population with advanced hepatic disease is thus largely unknown. For this reason we aimed to investigate the real-world healthcare costs per SVR for patients with advanced hepatic fibrosis in Europe and Canada that were treated with IFN-based regimens.



## PATIENTS AND METHODS

### Study population

In this cohort all consecutive patients from five large hepatology units in Europe (the Netherlands, Germany and Switzerland) and Canada, who had chronic HCV infection, histological proof of bridging fibrosis or cirrhosis (Ishak fibrosis score 4-6) and started an interferon-based treatment between 1990 and 2003 were included.[81] For the current study, all consecutive treatment courses with available on-

treatment clinical data were assessed. Cases for whom data were available for less than three visits during the course of therapy, the respective treatment course was not considered.
The charts of all included patients were re-reviewed by a single investigator (RM) in order to collect detailed information at baseline and during treatment. Details on the data that were collected were described previously.[196 308] The period within six months before treatment was considered as baseline.

Components of care, from initiation of therapy until 24 weeks after stopping treatment (i.e. the moment when SVR could be assessed), were quantified by three distinct categories: treatment, safety-monitoring and complications. Total medical costs attributable to each cost component were calculated. Laboratory costs were based on a detailed inventory of the resource use of patient charts. Costs of hospitalization (inpatient care episode) were calculated as the per diem prices multiplied by the length of stay. Outpatient costs were assigned based on visits to the hospital. Specialist fees are (all-)inclusive in the unit costs of outpatient or inpatient visits.

A list of major unit prices and their sources, as well as the profile of the health care systems for each country, are presented in Supplementary Tables 6.1 and 6.2.[309-315] All medical costs were expressed in 2013 Euros, using publicly available currency exchange rates. Where necessary, costs were adjusted to 2013 prices using consumer price index.[309] Costs were presented with mean, median, standard deviation, standard error and 95% confidence intervals.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. The study protocol was reviewed and approved by the ethics committee in the center of the primary investigators, which was the Erasmus Medical Center in Rotterdam, the Netherlands. Ethical approval in the participating centers was obtained according to the local regulations.[81 185]

**Objectives**

The primary objective was to assess direct medical costs during antiviral therapy among patients with bridging fibrosis or cirrhosis and to assess the cost per SVR. Secondary objectives were the assessment of medical costs in the five different centers as well as in the four different countries and for different components during treatment. To assess the role of the severity of liver disease, the direct medical costs and cost per SVR were also assessed according to the presence of bridging fibrosis or cirrhosis (Ishak 4 vs Ishak 5-6) and for three different categories based on the baseline platelet count (a valid marker of the severity of liver disease). A platelet count ≥150x$10^9$/L was considered as a normal platelet count; a platelet count <150x$10^9$/L as thrombocytopenia and a platelet count < 100x$10^9$/L as a severe thrombocytopenia.

**Definitions**

Three major components were defined to display the total costs. Treatment costs were based on the total dose of (Peg)IFN and RBV, taking into account all dose adjustments for one of these compounds. The costs of safety monitoring included the costs for all laboratory tests, visits to day clinic, admissions to the hospital, referrals to others physicians and additional diagnostic procedures for reasons other than an infection, bleeding episode or another adverse event. The costs of complications contained costs for hospital admission, diagnostic procedures, referrals to others physicians and treatment for

an infection, bleeding episode or another adverse event. On-treatment resource utilization was presented for the whole treatment period of each individual patient.

**Statistical analyses**

Continuous variables were summarized as median (interquartile range [IQR]) and categorical variables as frequencies (percentages). Mean costs were estimated separately for disease-related components. Total costs were calculated per patient. Standard error and 95% confidence interval of the mean were calculated by means of bootstrapping the cost results with 1000 bootstrap samples. Comparisons between groups were performed using $X^2$ test for categorical variables or the Mann-Whitney U test for continuous variables. The costs per SVR were calculated by using the following formula: costs per SVR = mean cost per patient * (1/SVR rate). A *p* value <0.05 was considered statistically significant and all statistical tests were two-tailed. PASW statistics 22.0 for Windows (SPSS, IBM, Armonk, New York, USA) was used.

**Sensitivity Analysis**

One-way deterministic sensitivity analysis was performed to determine the influence of components of care on medical costs. In addition, the SVR rate of the study population was varied over a range of ± 50% when the costs per SVR were calculated.

## RESULTS

**Patients**

In total, 546 patients with chronic HCV infection and bridging fibrosis or cirrhosis started IFN-based antiviral therapy between 1990 and 2003. In 672 (78%) of the 859 treatment courses among these patients detailed on-treatment data were available to assess the occurrence of bleeding, infection and laboratory tests. These treatment courses were registered among 455 patients. Of the 334 (73%) patients without SVR after their treatment of inclusion, 169 patients received at least one subsequent antiviral treatment regimen. Table 6.1 summarizes the baseline characteristics of the patients at their first included treatment. In order to compare the baseline characteristics according to the presence of thrombocytopenia, these data were also shown in Table 6.1. Median age was 48 years (IQR 43-56), 317 (70%) patients were male and 346 (76%) presented with cirrhosis (Ishak 5 or 6). At the first registered treatment, platelet count was available for 432 (95%) patients. Baseline thrombocytopenia was present among 226 (52%) patients, of whom 87 (38%) had a platelet count below $100x10^9$/L.

**On-treatment resource utilization**

Fourteen (7%) patients with a normal platelet count experienced 28 bleeding episodes in total, for which none received treatment for the bleeding. Among patients with thrombocytopenia, 34 (15%) patients experienced 76 bleeding episodes, for which 9 (26%) required treatment. Seventeen (20%) patients and 37 bleeding episodes were reported among patients with a platelet count below $100x10^9$/L, of which 4 (24%) needed treatment. The number of infections that were experienced by

**Table 6.1 | Baseline characteristics according to the presence of thrombocytopenia at the first registered treatment course**

| Variable | Total (N=455) | Treatments with normal platelets (n=206) | Treatments with TCP (n=226) | *p* value |
|---|---|---|---|---|
| Male | 317 (70%) | 146 (71%) | 154 (68%) | 0.538 |
| Age, in years | 48 (43–56) | 47 (42–55) | 50 (44–57) | 0.009 |
| BMI, in kg/m$^2$ | 26.6 (23.8–29.4) | 26.2 (23.4–29.0) | 26.8 (24.1–29.8) | 0.149 |
| Center | | | | 0.002 |
| Rotterdam | 63 (14%) | 21 (10%) | 41 (18%) | |
| Toronto | 210 (46%) | 118 (57%) | 88 (39%) | |
| Hannover | 93 (20%) | 32 (16%) | 52 (23%) | |
| Homburg | 15 (3%) | 7 (3%) | 5 (2%) | |
| Bern | 74 (16%) | 28 (14%) | 40 (18%) | |
| HCV genotype | | | | 0.282 |
| 1 | 310 (68%) | 139 (68%) | 154 (68%) | |
| 2 | 45 (10%) | 26 (13%) | 18 (8%) | |
| 3 | 75 (17%) | 32 (16%) | 38 (17%) | |
| 4 | 21 (5%) | 9 (4%) | 12 (5%) | |
| 5 or 6 | 4 (1%) | - | 4 (2%) | |
| Treatment naïve | 367 (81%) | 168 (82%) | 180 (80%) | 0.617 |
| Cirrhosis | 346 (76%) | 134 (65%) | 196 (87%) | <0.001 |
| Fibrosis score | | | | <0.001 |
| Ishak 4 | 109 (24%) | 72 (35%) | 30 (13%) | |
| Ishak 5 | 93 (20%) | 45 (22%) | 42 (19%) | |
| Ishak 6 | 253 (56%) | 89 (43%) | 154 (68%) | |
| Platelet count, in $10^9$/L | 147 (109–197) | 198 (174–225) | 111 (89-132) | <0.001 |
| Albumin, in g/L | 42 (39–44) | 43 (41–45) | 40 (37–43) | <0.001 |
| Bilirubin, in **µmol**/L | 13 (10–19) | 12 (9–15) | 15 (11–23) | <0.001 |
| AST/ALT ratio | 0.70 (0.57–0.91) | 0.67 (0.52–0.80) | 0.78 (0.62–1.02) | <0.001 |
| Treatment with PegIFN* | 204 (45%) | 97 (47%) | 102 (45%) | 0.684 |
| Alcohol abuse ever[d] | 103 (24%) | 49 (25%) | 52 (25%) | 0.929 |
| Diabetes mellitus | 58 (13%) | 24 (12%) | 34 (15%) | 0.301 |

Abbreviations: TCP, thrombocytopenia; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PegIFN, pegylated interferon;

a. Medians are presented as number, (IQR, interquartile range)
b. Numbers are presented as n, (percentage of the whole group)
c. * Including respectively 10 and 4 treatment courses with PegIFN monotherapy for patients with normal platelets and TCP
d. Missing in 37 patients

the patients with a normal platelet count, thrombocytopenia or platelet count below 100x10$^9$/L, were 46 (among 34 (17%) patients), 58 (among 42 (19%) patients) and 11 (among 9 (10%) patients), respectively. Antibiotics were prescribed for 43 (93%), 45 (78%) and 9 (82%) of the infections, and 8 (17%), 12 (21%) and 6 (55%) of the infections were considered severe. Six patients (3%) with a normal platelet count underwent 9 radiological procedures and 13 (6%) patients needed a hospital admission during therapy. Twenty (9%) patients with thrombocytopenia underwent 31 radiological procedures and 26 (12%) patients were admitted to the hospital during therapy. For the patients with a platelet count below 100x10$^9$/L, 11 (13%) patients underwent 20 radiological procedures and 13 (15%) patients need a hospital admission. When laboratory assessments were considered, patients with a normal platelet count had a mean of 3.5 lab values that were determined per week, whereas patients with thrombocytopenia and with a platelet count below 100x10$^9$/L had a mean of 4.9 and 6.7 lab values determined per week, respectively.

**Treatment duration and dose reductions**

Median treatment duration was 34 weeks (IQR 19-48) for patients with bridging fibrosis and 26 weeks (IQR 21-48) for patients with cirrhosis ($p$ = 0.878). At least one dose reduction of IFN was applied in 24.7% of the treatments among patients with bridging fibrosis and in 33.7% of the treatments among patients with cirrhosis ($p$ = 0.028). Treatment was discontinued in 32.4% of the treatments given to patients with bridging fibrosis and in 38.1% of the treatments among cirrhotic patients ($p$ = 0.177). Side effects were the reason for treatment discontinuation in 18.2% and 29.3% of the discontinuations among patients with bridging fibrosis and cirrhosis, respectively ($p$ = 0.100).

The median duration per treatment was 31 weeks (IQR 21-48) for patients with a normal platelet count and 26 weeks (IQR 20-48) for patients with thrombocytopenia ($p$ = 0.507), also including patients with a platelet count below 100x10$^9$/L whom received treatment for a median of 24 weeks (IQR 16-48). The dose of IFN was reduced in 24.1% of treatments among patients with a normal platelet count and in 40.6% of treatments among patients with a thrombocytopenia ($p$ <0.001). Treatment was discontinued in 33.4% of the treatments among patients with a normal platelet count, including 22.1% of these discontinuations due to side effects. Among patients with thrombocytopenia, 40.0% of the treatments were discontinued, of which 32.8% were due to side effects ($p$ = 0.088 and $p$ = 0.071, respectively). For patients with a platelet count below 100x10$^9$/L, in 51.4% of the treatments at least one dose reduction was applied as well as early treatment discontinuation. Almost half of the discontinuations were due to side effects (48%).

**Costs during treatment**

Total medical costs per patient were averaged to €14,559 (95% CI €13,323-€15,836). Table 6.1 depicts country-specific mean costs per patient with their respected sums. The highest average costs per patient were in Switzerland (€18,777 (95%CI €14,608-€25,226)). The lowest average cost per patient was in Germany (€11,388 (95%CI €9,377-€13,757)). The mean per patient costs in the Netherlands and Canada were comparable, €15,520 (95%CI €13,122-€17,983) and €14,414 (95%CI €12,887-€16,113), respectively (Table 6.2). Figure 6.1 shows country-specific costs (per patient) for all medical components (treatment, safety-monitoring, complications). The most influential cost driver was the anti-

**Table 6.2 | Medical costs per country**

| | | | | Bootstap[a] | |
|---|---|---|---|---|---|
| | | | | 95% Confidence Interval | |
| **Country** | | **Medical costs** | **Standard error** | **Lower** | **Upper** |
| The Netherlands | Number of patients | 63 | | | |
| | Sum of costs | €977.779,00 | | | |
| | Mean of costs | €15.520,30 | €1.249,25 | €13.121,74 | €17.983,75 |
| | Standard deviation | €9.604,45 | €971,33 | €7.601,23 | €11.382,00 |
| Canada | Number of patients | 210 | | | |
| | Sum of costs | €3.026.964,00 | | | |
| | Mean of costs | €14.414,11 | €828,00 | €12.887,77 | €16.113,13 |
| | Standard deviation | €12.075,46 | €1.078,24 | €10.074,07 | €14.248,22 |
| Germany | Number of patients | 108 | | | |
| | Sum of costs | €1.229.995,00 | | | |
| | Mean of costs | €11.388,84 | €1.117,93 | €9.377,48 | €13.757,87 |
| | Standard deviation | €11.231,37 | €957,72 | €9.285,58 | €12.969,94 |
| Switzerland | Number of patients | 74 | | | |
| | Sum of costs | €1.389.559,00 | | | |
| | Mean of costs | €18.777,82 | €2.676,13 | €14.608,86 | €25.226,37 |
| | Standard deviation | €23.470,06 | €8.491,23 | €10.293,84 | €36.689,81 |

a. Bootstrap results are based on 1000 samples

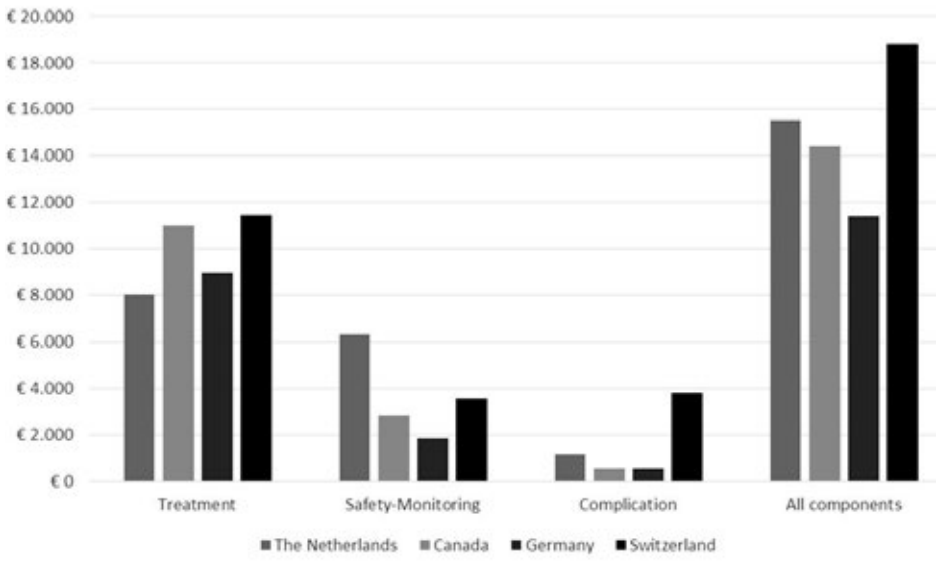


**Figure 6.1 | Medical costs of key components of care per country per patient**

Mean medical costs in 2013 Euro of the key components of care per country per patient. These key components included treatment, safety-monitoring and complications. The last four bars represent the total mean medical costs.

viral therapy component (in particular (Peg)IFN), with a mean cost of €9,579 (95%CI €8,722-€10,408). Costs of safety-monitoring and complications during antiviral treatment were € 3,198 (95%CI €2,973-€3,454) and €1,176 (95%CI €505-€2,206), respectively. Figure 6.2 shows results by means of a tornado diagram when key medical components were varied in one-way sensitivity analysis.

**Costs by severity of liver disease**

In total, 346 (76%) patients presented with cirrhosis at the first registered treatment. The remaining 109 (24%) patients had histological proof of bridging fibrosis. The mean costs per patient according to Ishak score 4 and Ishak score 5-6 were €15,346 (95%CI €13,327-€17,626) and €14,311 (95% CI €12,930-€15,894), respectively (Table 6.3).

The mean costs among patients with and without thrombocytopenia were €14,416 (95%CI €12,503-€16,598) and €12,419 (95% CI €10,974-€13,937), respectively (Table 6.4). The analyses among patients with platelet count below 100x10$^9$/L showed comparable results (€13,786 (95%CI €10,200-€19,042).

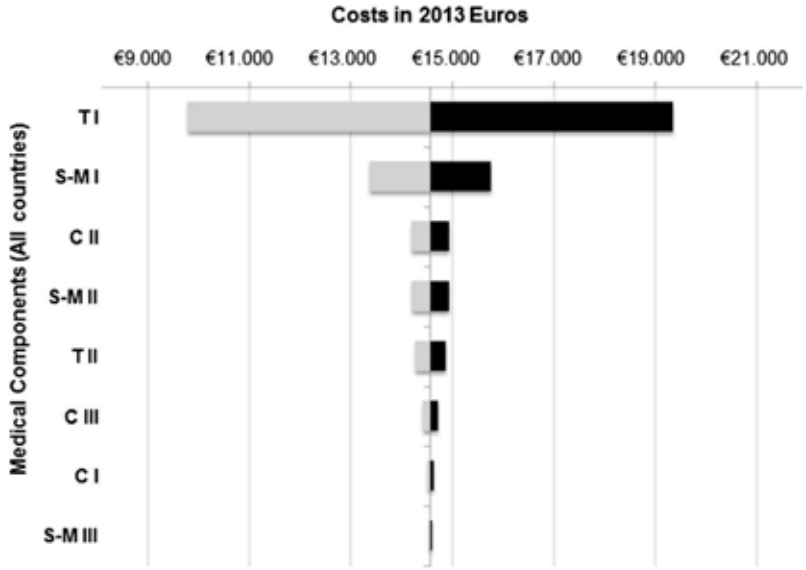


**Figure 6.2 | Tornado diagram showing one-way sensitivity analysis**

Tornado diagram showing one-way sensitivity analysis for the medical costs for all countries. The components of these costs are defined below the figure.

| | |
|---|---|
| T I | Costs of (peg)interferon |
| S-M I | Costs of visits |
| C II | Costs due to an infection |
| S-M II | Costs of laboratory assessments during treatment |
| T II | Costs of ribavirin |
| C III | Costs due to admissions for adverse events, other than an infection or bleeding |
| C I | Costs due to a bleeding |
| S-M III | Costs due to admissions per protocol |

**Table 6.3 | Medical costs for patients according to the platelet count**

| | | | | Bootstap[a] | |
|---|---|---|---|---|---|
| | | | | 95% Confidence Interval | |
| **Country** | | **Medical costs** | **Standard error** | **Lower** | **Upper** |
| Normal platelet | Number of patients | 206 | | | |
| count (≥150x10$^9$/L) | Sum of costs | €2.558.314,00 | | | |
| | Mean of costs | €12.418,89 | €763,31 | €10.902,75 | €13.986,88 |
| | Standard deviation | €10.677,34 | €814,22 | €9.096,20 | €12.310,23 |
| Thrombocytopenia | Number of patients | 226 | | | |
| (<150x10$^9$/L) | Sum of costs | €3.258.156,00 | | | |
| | Mean of costs | €14.416,62 | €1.060,18 | €12.503,21 | €16.598,63 |
| | Standard deviation | €16.223,66 | €3.945,55 | €10.100,82 | €23.385,11 |
| Severe | Number of patients | 88 | | | |
| Thrombocytopenia | Sum of costs | €1.213.166,00 | | | |
| (<100x10$^9$/L) | Mean of costs | €13.785,98 | €2.325,09 | €10.200,16 | €19.042,14 |
| | Standard deviation | €21.770,30 | €8.156,54 | €8.964,83 | €34.385,86 |
| Thrombocytopenia | Number of patients | 64 | | | |
| with SVR | Sum of costs | €1.211.576,00 | | | |
| (<150x10$^9$/L) | Mean of costs | €18.930,88 | €2.868,68 | €14.354,62 | €24.919,52 |
| | Standard deviation | €23.998,97 | €9.715,90 | €7.320,03 | €38.487,99 |

Abbreviations: SVR, sustained virological response

a. Bootstrap results are based on 1000 samples

**Table 6.4 | Medical costs according to the presence of bridging fibrosis and cirrhosis**

| | | | | Bootstrap[a] | |
|---|---|---|---|---|---|
| | | | | 95% Confidence Interval | |
| **Country** | | **Medical costs** | **Standard error** | **Lower** | **Upper** |
| Bridging fibrosis | Number of patients | 109 | | | |
| (Ishak score 4) | Sum of costs | €1.672.683,00 | | | |
| | Mean of costs | €15.345,72 | €1.074,51 | €13.327,90 | €17.625,57 |
| | Standard deviation | €11.655,84 | €934,06 | €9.590,22 | €13.411,27 |
| Cirrhosis | Number of patients | 346 | | | |
| (Ishak score 5/6) | Sum of costs | €4.951.614,00 | | | |
| | Mean of costs | €14.311,02 | €722,20 | €12.930,57 | €15.894,47 |
| | Standard deviation | €14.998,31 | €2.829,15 | €10.631,60 | €20.491,02 |

a. Bootstrap results are based on 1000 samples

**Costs per SVR**

The SVR rate of the overall population was 38% (172/455). The mean cost per SVR was €38,514 (95%CI, €35,244-€41,892). One-way sensitivity analysis revealed that when the SVR rate varied (±50%), mean costs ranged from €25,746 (95%CI, €23,496-€27,928) to €77,027 (95%CI, €70,488-€83,783) (Table 6.5). The SVR rate was 46% (50/109) among patients with bridging fibrosis and 35% (122/346) among patients with cirrhosis ($p = 0.046$). The mean costs per SVR were €33,454 (95%CI €29,053-€38,425) for patients with bridging fibrosis, whereas the costs per SVR were €40,587 (95%CI, €36,670-€45,076) for patients with cirrhosis.

Again, when one-way sensitivity analysis was performed with the SVR rate varied (±50%), mean costs ranged from €22,303 (95%CI, €19,369-€25,616) to €66,909 (95%CI, €58,106-€76,849) among patients with bridging fibrosis. Similarly, when SVR rate among patients with cirrhosis varied (±50%), mean costs ranged from €27,058 (95%CI, €24,447- €30,051) to €81,174 (95% CI, €73,341-€90,153), respectively (Table 6.5).

For patients with an available platelet count at baseline, the SVR rates were 28% (64/226) among patients with thrombocytopenia and 48% (98/206) among patients with a normal platelet count ($p < 0.001$). The mean cost per SVR was €50,907 (95%CI, €44,151-€58,612) for patients with thrombocytopenia, whereas the mean cost per SVR was €26,105 (95%CI, €23,068-€29,296) for patients with a normal platelet count. For patients with a platelet count below $100x10^9$/L, the SVR rate was 18% (16/87). Within this specific group, the mean cost per SVR was €74,961 (95%CI, €55,463-€103,541).

One-way sensitivity analysis revealed that when SVR rate among patients with thrombocytopenia varied (±50%), mean costs ranged from €33,938 (95%CI, €29,434-€39,074) to €101,813 (95%CI, €88,302-€117,223). Similarly, when SVR rate among patients without thrombocytopenia varied (±50%), mean costs ranged from €17,403 (95%CI €15,379-€19,530) to €52,210 (95% CI, €46,136-€58,592), respectively. For patients with a platelet count below 100*109/L, costs per SVR ranged from €49,974 (95%CI €36,975-€69,027) to €149,923 (95%CI €110,925-€207,082) (Table 6.5).

Table 6.5 also shows these data according to the following baseline characteristics: the use of conventional IFN and PegIFN; having HCV genotype 1/4 and HCV genotype 2/3; being treatment naïve and treatment experienced.



## DISCUSSION

Our study quantified real-world healthcare costs of IFN-based treatment among patients with advanced hepatic fibrosis who were treated in the Netherlands, Germany, Switzerland and Canada. Although the direct medical costs did not differ among the patients with more severe thrombocytopenia, the cost per SVR was higher among patients with lower platelet count. This could be attributed to the lower SVR rate of IFN-based treatment among these patients.

As antiviral therapy, rather than the costs for management of adverse events, was the most influential cost driver in this study, it is not unexpected that the costs during treatment were not higher among patients with more severe liver disease. First, patients with more severe liver disease were treated for a shorter period as antiviral treatment is prematurely discontinued in case of an

**Table 6.5 | Costs per SVR according to baseline variables**

| Subgroup | Mean costs (95% confidence interval) | SVR rate | Costs per SVR (95% confidence interval) | Mean costs per SVR when SVR rate varied (± 50%) |
|---|---|---|---|---|
| Total population | €14,559 (€13,323-€15,836) | 172/455 (38%) | €38,514 (€35,244-€41,892) | €25,746 - €77,027 |
| Severity of liver disease | | | | |
| Bridging fibrosis | €15,346 (€13,327-€17,626) | 50/109 (46%) | €33,454 (€29,053-€38,425) | €22,303 - €66,909 |
| Cirrhosis | €14,311 (€12,930-€15,894) | 122/346 (35%) | €40,587 (€36,670-€45,076) | €27,058 - €81,174 |
| Baseline platelet count | | | | |
| Normal platelet count | €12,419 (€10,974-€13,937) | 98/206 (48%) | €26,105 (€23,068-€29,296) | €17,403 - €52,210 |
| Thrombocytopenia | €14,416 (€12,503-€16,598) | 64/226 (28%) | €50,907 (€44,151-€58,612) | €33,938 - €101,813 |
| Platelet count <100x10$^9$/L | €13,786 (€10,200-€19,042 | 16/87 (18%) | €74,961 (€55,463-€103,541) | €49,974 - €149,923 |
| Type of interferon used | | | | |
| Conventional interferon | €12,754 (€11,265-€14,242) | 74/251 (30%) | €43,260 (€38,210-€48,307) | €28,840 - €86,520 |
| Pegylated interferon | €16,780 (€14,534-€19,026) | 98/204 (48%) | €34,930 (€30,254-€39,605) | €23,287 - €69,860 |
| HCV genotype | | | | |
| HCV genotype 1/4 | €14,824 (€13,488-€16,159) | 100/335 (30%) | €49,660 (€45,185-€54,133) | €33,107 - €99,321 |
| HCV genotype 2/3 | €14,222 (€10,806-€17,638) | 72/120 (60%) | €23,703 (€24,013-€29,397) | €15,802 - €47,407 |
| Previous treatment response | | | | |
| Naive | €14,092 (€12,600-€15,585) | 143/367 (39%) | €36,166 (€32,337-€39,998) | €24,111 - €72,332 |
| Experienced | €16,505 (€13,850-€19,159) | 29/88 (33%) | €50,084 (€42,028-€58,138) | €33,389 - €100,168 |

Abbreviations: SVR, sustained virological response; HCV, hepatitis C virus

insufficient virological response. Second, out of fear of bleeding and infection, dose reductions are often applied when platelet and neutrophil counts drop substantially. Although previous studies showed that on-treatment platelet counts below 50x10$^9$/L were associated with the risk of bleeding among patients undergoing IFN-based treatment, these bleeding episodes were generally without major clinical complications, and thereby do not generate more healthcare costs.[115 196] On-treatment infections were generally mild as well.[308]

In a previous study, the diagnosis of thrombocytopenia among patients with chronic HCV infection was associated with increased medical resource utilization ($37,924 vs $12,174, $p$ <0.001).[316] However, this study cannot be truly compared to our study as they included patients with various stages of disease and assessed resource utilization in general, instead of on-treatment costs. The increased medical resource utilization among these patients could be attributed to the stage of liver disease rather than the presence of thrombocytopenia as thrombocytopenia is associated with the severity of liver disease.[182] Patients with thrombocytopenia and cirrhosis, which represent a more diseased population, are indeed at higher risk to develop hepatic decompensation, HCC and liver transplantation, which increases their medical resource utilization.[75 79-81 191 317]

More important was our finding that the costs per SVR were higher among patients with thrombocytopenia compared to patients with a normal platelet count, especially among those who had a platelet count below 100x10$^9$/L. This can be predominantly attributed to the lower SVR rates among these patients. In line with this finding were the mean costs per SVR for other subgroups that are associated with a hampered treatment response, i.e. patients treated with conventional IFN, HCV genotype 1/4 and those whom are treatment experienced. As the costs per SVR for patients with a platelet count below 100x10$^9$/L were high, it would be interesting to compare these to the real-world costs per SVR with IFN-free therapy. One could argue, that the additional costs of IFN-free antiviral therapy will be less than expected for patients with more severe liver disease. Especially, when one would take into account the important safety issues that are associated with IFN-based therapy among patients with low platelet counts. Phase 3 studies showed high SVR rates around 95% and negligible safety issues with IFN-free regimens, which could result in lower costs per SVR. Moreover, these high SVR rates prevent patients from necessitating re-treatment, which would generate even higher costs.

A recent study assessing the costs per SVR in a cohort of patients that were treated with triple therapy (telaprevir-based) showed that the median cost per SVR was $189,338.[318] These costs were much higher than what we found. First, this study included only 52 (35%) patients with advanced hepatic fibrosis, leading to more patients completing the full therapy of 48 weeks. Second, triple therapy with telaprevir is more expensive than dual therapy. Third, only including patients with HCV genotype 1, result again in a longer treatment duration and a reduced antiviral efficacy, when compared to patients with HCV genotypes 2 and 3. Moreover first real-world data on triple therapy, showed much more safety concerns than dual therapy, which would increase the costs for the management of adverse events even further.[121] In our study, treatment efficacy was limited among patients with a platelet count below 100x10$^9$/L, and as a result these patients had the highest costs per SVR (€74,961 (95%CI €55,463-€103,541). Jonk et al. performed a comparable analysis among patients with HCV genotype 1 and 2 that were treated with PegIFN and RBV. They found that the costs

per SVR were $47,324, with higher costs per SVR among HCV genotype 1 patients ($63,448) compared to patients with HCV genotype 2 ($25,152).[319]

Our study has some limitations. The time span of the patient accrual does not cover important treatment advances such as the introduction of the first generation DAAs. Due to its retrospective character, another limitation is missing data. When comparing baseline variables between patients that were included and excluded in the study (Supplementary Table 6.3), more data was missing for treatment courses applied earlier during the period of inclusion. However, this may in fact make the included patients more representative of the current HCV population. Lastly, costs for over-the-counter medications, reduced productivity at work and quality of life were not included in this study and could have an influence on the economic burden of antiviral treatment. However, the study was not conducted for this purpose. When considering these costs for interferon-free regimens, recent data showed that both work productivity, as well as health-related quality of life improved during and after successful antiviral treatment.[320 321] This is in contrast with the results during IFN-based regimens, for both dual and triple therapy.[322 323]

A salient strength is that the study was conducted as a multi-country, multi-center study in which healthcare utilization and costs were quantified in four countries, located in two continents. Moreover the study included a large cohort of patients, who had histological evidence of advanced liver disease. For the presence of thrombocytopenia, readily available lab results were used rather than the ICD-9 codes that are commonly used, which makes the diagnosis very reliable.

The cost per SVR was high among patients with advanced liver disease. Taking into account the possible risks of IFN-based treatment, as well as the high chance of necessitating re-treatment, the additional costs per SVR of the highly-effective and well-tolerated IFN-free regimens could be limited, especially in the specific subset of patients with most advanced liver disease who are in urgent need of successful antiviral therapy.

# SUPPLEMENTARY TABLES

**Supplementary Table 6.1 |** Unit costs and their sources for each country

| Unit costs/ country (2013) | The Netherlands | Canada | Germany | | Switzerland |
|---|---|---|---|---|---|
| | | | Hannover | Homburg | |
| **Antiviral treatment** | | | | | |
| Interferon | €20.22/6 MU | $218.76/18 MU | €20.22/6 MU | €20.22/6 MU | 415.90/25 MU |
| Pegylated interferon | | | (Dutch price) | (Dutch price) | |
| 2a | €184.97/180 mcg | $478.70/180 mcg | €295.87/180 mcg | €295.87/180 mcg | 333.65/180 mcg |
| 2b | €184.98/100 mcg | $467.46/120 mcg | €288.23/100 mcg | €288.23/100 mcg | 338.31/100 mcg |
| Ribavirin | €2.94/200 mg | $1.48/200 mg | €5.98/200 mg | €5.98/200 mg | 7.16/200 mg |
| **Laboratory tests** | | | | | |
| Albumin | € 1.81 | $2.59 | € 3.30 | € 1.75 | CHF 2.50 |
| Bilirubin | € 1.81 | $2.59 | € 0.88 | € 4.08 | CHF 3.20 |
| yGT | € 2.10 | $2.59 | € 0.88 | € 2.33 | CHF 2.50 |
| AST | € 2.10 | $2.59 | € 0.88 | € 2.33 | CHF 2.50 |
| ALT | € 2.25 | $2.59 | € 0.88 | € 2.33 | CHF 2.50 |
| Creatinine | € 1.96 | $2.59 | € 0.88 | € 2.33 | CHF 2.50 |
| Glucose | € 1.00 | $2.59 | € 0.88 | € 2.33 | CHF 2.50 |
| Hemoglobin | € 1.81 | $8.27 (hematology) | € 2.10 (hematology) | € 3.50 (Hematology) | CHF 10.00 (hematology) |
| Platelets | € 1.81 | | | | |
| Leucocytes | € 1.81 | | | | |
| Neutrophils | € 1.81 | | | € 1.17 | |
| PT/ Quick | € 4.73 | $6.20 Together with PT | € 1.75 | € 2.91 | CHF 6.00 Together with PT |
| INR | € 11.32 | | € 1.75 | € 2.91 | |
| HCV RNA | € 120.13 | $105.64 | € 66.30 | € 145.72 | CHF180 |
| **Radiology** | | | | | |
| X-ray | € 51.64 | $32.65 | € 53.77 | € 27.26 | CHF 100.26 |
| CT Thorax | €248,16 | $64.95 | € 370.00 | € 187.27 | CHF 284.62 |
| Ultrasound abdomen | € 73.92 | $81.95 | € 100.00 | € 14.30 | CHF 150.00 |
| CT abdomen | € 211.64 | $86.60 | € 470.00 | € 187.27 | CHF 284.12 |
| Ultrasound extremity | € 73.92 | $44.20 | € 80.00 | € 44.20 | CHF 120.00 |
| Endoscopy | | | | | |
| Gastroscopy | € 91.38 | $125.10 | € 600.00 | € 199.76 | CHF 345.49 |
| Colonoscopy | € 407.91 | $197.20 | € 800.00 | € 201.94 | CHF 453.80 |

**Supplementary Table 6.1 |** Continued

| Unit costs/ country (2013) | The Netherlands | Canada | Germany | | Switzerland |
|---|---|---|---|---|---|
| | | | Hannover | Homburg | |
| **Visits** | | | | | |
| Outpatient clinic | € 273.00 | $157.00 | € 60.00 | € 30.60 | CHF 200.00 |
| Consultation | € 140.00 | $157.00 | € 60.00 | € 30.60 | CHF 200.00 |
| Admission (1 day) | € 626.16 | $2,657 | € 600.00 | € 600.00 | CHF 3840.00 |
| **Antibiotics** | | | | | |
| Mean costs/ week | € 31.03 | $22.88 | € 58.09 | € 8.91 | CHF 218.88 |
| Sources / References | - Dutch Healthcare Authority[314]<br>- The National Healthcare Institute[313] | - Ontario schedule of Benefits – Physician and laboratory services<br>- Ontario Drug Benefit Formulary/ Comparative Drug Index<br>- Management of chronic hepatitis B: Consensus guidelines[315] | - MHH list for hospital-based costs and medication | - Internal list from Saarland University Hospital | - The Swiss Compendium[312] |

**Supplementary Table 6.2 |** Profile of health care system per country[310]

| | |
|---|---|
| The Netherlands | **The Netherlands** has statutory health insurance system, with universally-mandated private insurance (of national exchange). The Dutch government regulates and subsidizes insurance. Public financing consists earmarked payroll tax, community-rated insurance premiums and general tax revenue. Private plans provide statutory benefits while 85% of the population buy complementary coverage for benefits excluded from statutory package. Children are exempt from cost-sharing and there are premium subsidies for low-income. |
| Switzerland | **Switzerland** has statutory health insurance system, with universally mandated private insurance (of regional exchange). There is federal legislation on health insurance, with cantonal (state) government responsible for provider supervision, capacity planning, and financing through subsidies. Switzerland has community-rated insurance premiums and general tax revenue. Private plans provide universal core benefits; some people buy complementary (services not covered by statutory insurance) and supplementary (improved amenities and access). The copayment exemptions are for <19-year-olds. Income-related premium assistance is 30%. |
| Germany | **Germany** has statutory health insurance system, with 131 competing SHI insurers ("sickness funds" in national exchange). People with high income can opt out for private coverage. Public financing consists employer/employee earmarked payroll tax and general tax revenue. 11% of the population opt out from statutory insurance and buy private substitutive coverage. Some complementary (minor benefit exclusions from statutory scheme, copayments) and supplementary coverage (for improved amenities). The caps on cost-sharing include 2% of household income and 1% of income for chronically ill. Children and adolescents <18 years of age are exempt. |
| Canada | **Canada** has regionally administered universal public insurance program that plans and funds (mainly private) provision. Public financing consists provincial/federal general tax revenue. 67% of the population buy private complementary coverage for non-covered benefits (e.g., private rooms in hospitals, drugs, dental care, optometry). There is no cost-sharing for publicly covered services. The protection for low-income people from cost of prescription drugs varies by region. |

**Supplementary Table 6.3 | Baseline characteristics at the first registered treatment of patients included and excluded in the analyses**

| Variable | Patients included in analyses (n=455) | Patients excluded from analyses (n=91) | *p* value |
|---|---|---|---|
| Male | 317 (70%) | 59 (65%) | 0.363 |
| Age, in years | 48 (43–56) | 48 (42–57) | 0.746 |
| BMI, in kg/m$^2$ | 26.6 (23.8–29.4) | 25.1 (23.6–29.5) | 0.288 |
| Center | | | <0.001 |
| Rotterdam | 63 (14%) | 1 (1%) | |
| Toronto | 210 (46%) | 34 (37%) | |
| Hannover | 93 (20%) | 25 (28%) | |
| Homburg | 15 (3%) | 13 (14%) | |
| Bern | 74 (16%) | 18 (20%) | |
| HCV genotype | | | 0.547 |
| 1 | 310 (68%) | 39 (65%) | |
| 2 | 45 (10%) | 5 (8%) | |
| 3 | 75 (17%) | 15 (25%) | |
| 4 | 21 (5%) | 1 (2%) | |
| 5 or 6 | 4 (1%) | - | |
| Treatment naïve | 367 (81%) | 85 (93%) | 0.003 |
| Cirrhosis | 346 (76%) | 60 (66%) | 0.044 |
| Fibrosis score | | | 0.054 |
| Ishak 4 | 109 (24%) | 31 (34%) | |
| Ishak 5 | 93 (20%) | 11 (12%) | |
| Ishak 6 | 253 (56%) | 49 (54%) | |
| Platelet count, in 10$^9$/L | 147 (109–197) | 159 (101-203) | 0.755 |
| Albumin, in g/L | 42 (39–44) | 40 (36–44) | 0.166 |
| Bilirubin, in µmol/L | 13 (10–19) | 13 (9–17) | 0.369 |
| AST/ALT ratio | 0.70 (0.57–0.91) | 0.74 (0.57–0.92) | 0.508 |
| Treatment with PegIFN* | 204 (45%) | 20 (22%) | <0.001 |
| Alcohol abuse ever[d] | 103 (24%) | 18 (23%) | 0.823 |
| Diabetes mellitus | 58 (13%) | 11 (12%) | 0.863 |

Abbreviations: BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PegIFN, pegylated interferon;

a. Medians are presented as number, (IQR, interquartile range)
b. Numbers are presented as n, (percentage of the whole group)
c. * Including respectively 10 and 4 treatment courses with PegIFN monotherapy for patients with normal platelets and TCP
d. Missing in 37 patients

# CHAPTER 7

## SAFETY AND EFFECTIVENESS OF DAA-BASED THERAPY FOR THE TREATMENT OF PATIENTS WITH CHRONIC HCV INFECTION AND CIRRHOSIS

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## ABSTRACT

**Background & Aims**

Direct-acting antivirals (DAAs) have revolutionized treatment for patients with chronic hepatitis C virus (HCV) infection, leading to a high rates of sustained virological response. This study assessed the real-world safety and effectiveness of DAA-based antiviral therapy for the treatment of cirrhotic patients with chronic HCV infection.

**Methods**

This international, multicenter cohort study included all consecutive patients with chronic HCV infection and cirrhosis who underwent antiviral therapy with second-generation DAAs. Data on all patients were analyzed to assess treatment response. Predictors of hepatic decompensation during antiviral therapy were assessed using Cox proportional hazards regression analyses.

**Results**

Until June 2015, 433 cirrhotic patients with chronic HCV infection started DAA-based treatment. Their mean age was 57.8 (±8.7) years, 277 (64.0%) patients were male, and 114 (26.3%) had a Child–Pugh (CP) score of B/C cirrhosis. The sustained virological response rate at 12 weeks was similar among patients with a CP score of A (261 of 304 [85.9%]) and a CP score of B/C (83 of 101 [82.2%]; $p = 0.37$). A baseline albumin level less than 35 g/L (hazard ratio [HR], 3.11; 95% confidence interval [CI], 1.23–7.84; $p = 0.005$), baseline MELD score of 14 or higher (HR, 1.63; 95% CI, 1.03–2.61; $p = 0.037$), and HCV genotype 3 (HR, 2.05; 95% CI, 1.09–3.88; $p = 0.033$) were associated independently with hepatic decompensation during antiviral treatment among patients with a CP score of B/C.

**Conclusions**

This large cohort study showed that therapy is safe and effective in patients with compensated (CP score of A) cirrhosis. For patients with decompensated (CP score of B/C) cirrhosis, albumin level less than 35 g/L, MELD score of 14 or greater, and HCV genotype 3 are important risk factors for hepatic decompensation during DAA-based treatment. Therefore, these patients require close monitoring during antiviral therapy or treatment should be deferred until after transplantation.

## INTRODUCTION

Chronic hepatitis C virus (HCV) infection continues to be a major global public health problem, with recent estimates suggesting that 64 to 103 million people are infected worldwide.[1] Continuous inflammatory activity in the liver may lead to hepatic fibrosis, which eventually may progress to cirrhosis, putting patients at risk for hepatic decompensation and hepatocellular carcinoma (HCC).[274] Consequently, chronic HCV infection is still the leading indication for liver transplantation in the Western world.[13] Although the prevalence of HCV infection is decreasing, it is estimated that the disease burden of chronic HCV will increase as the infected population ages, leading to an increasing incidence of cirrhosis and its complications.[63] Successful viral eradication (sustained virological response [SVR]) is associated with a reduced incidence in mortality and morbidity among patients with cirrhosis.[81]

The discovery of oral direct-acting antivirals (DAAs) has revolutionized treatment for chronic HCV infection. Within multiple phase 3 studies, shorter and better tolerated therapies without pegylated interferon alfa (PegIFN) have been reported to lead to SVR rates as high as 80% to 100% among patients with compensated cirrhosis.[143 145 156] Moreover, given the excellent safety profile of the new regimens, physicians have increasing confidence that patients with hepatic decompensation can be treated safely. However, case reports and small case series have reported patients with cirrhosis who experienced worsening of hepatic decompensation during therapy.[170 171 324] Understanding who is at risk for liver-related complications will be critical to help clinicians identify which patients require closer monitoring and which patients potentially should be left untreated until after liver transplantation.

This study aimed to assess the safety and effectiveness of DAA-based regimens for the treatment of cirrhotic patients with chronic HCV infection. Patients with compensated cirrhosis were compared with patients with decompensated cirrhosis.

## PATIENTS AND METHODS

### Study Population and Design

This international cohort study included all consecutive patients with chronic HCV infection and cirrhosis from 4 academic, tertiary care, referral centers including 2 in Canada (Toronto Centre for Liver Disease and Gastrointestinal Research Institute Vancouver), 1 in Germany (Hannover Medical School), and 1 in The Netherlands (Erasmus MC, University Medical Center Rotterdam). All patients received DAA-based antiviral therapy. The specific regimen was at the discretion of the treating physician. Patients enrolled in phase II/III clinical trials were excluded, as well as patients who underwent liver transplantation before antiviral therapy was initiated. All charts were reviewed by a single investigator to obtain details on demographics, virological, and clinical data.

The protocol was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice and was reviewed and approved by each local ethics review board.

**Definitions**

***Cirrhosis.*** The presence of cirrhosis was based on the results of liver biopsy or transient elastography. Pretreatment liver biopsy showing cirrhosis, defined as a METAVIR score of F4, was considered the gold standard.[50] When no liver biopsy was performed, a liver stiffness value of 13.0 kPa or greater was considered as cirrhosis.[325] If liver biopsy or transient elastography resulted in a METAVIR score of less than F4, patients were considered cirrhotic if they had at least 2 of the following: esophageal varices; platelet count less than $140x10^9$/L; a nodular liver, signs of portal hypertension, or ascites on imaging; noninvasive serum panels compatible with stage 4 fibrosis (FIB-4 >3.25 or FibroTest ≥0.75).[293 326]

***Hepatic decompensation.*** The criteria for hepatic decompensation were as follows: (1) new-onset variceal bleeding, ascites, jaundice leading to hospital admission, or hepatic encephalopathy in patients who had never experienced hepatic decompensation; (2) if patients experienced worsening of existing hepatic decompensation (ie, required increased dose of diuretics, addition of rifaximin for pre-existing hepatic encephalopathy, or hospital admission for a decompensation event); or (3) if patients experienced a new decompensation event other than that already present at the start of antiviral therapy.

***Serious adverse events.*** Adverse events were considered as follows: (1) comprised hepatic decompensation as described earlier, (2) required hospitalization, (3) resulted in discontinuation of HCV therapy, or (4) resulted in death. Virological response was measured 12 or 24 weeks after cessation of antiviral therapy. HCV RNA was measured using the COBAS AmpliPrep/COBAS TaqMan, version 2 (lower limit of detection, 15 IU/mL) (Roche, Pleasanton, CA) or the RealTime HCV assay (lower limit of quantification, 12 IU/mL) (Abbott Molecular, Des Plaines, IL) according to the manufacturer's instructions.

**Statistical Analysis**

Data are presented as means with ±SD, medians with interquartile range, or as proportions. For comparisons of patients with Child–Pugh (CP) score of A vs a CP score of B/C, the Student *t* test, chi-square, Pearson correlation, or their nonparametric equivalents were used.

For analysis of adverse events, all patients who started therapy were included. Cox proportional hazards regression analyses were used to assess factors associated with the occurrence of hepatic decompensation from the start of therapy until 12 weeks after cessation. Patients were censored at the 12-week SVR (SVR12) assessment or at death/liver transplantation. Because patients could experience more than 1 event, all events that occurred in a given patient were included in the analyses and time to subsequent decompensation as well as factors associated with more than 1 episode of decompensation were assessed. Analyses were stratified by the number of events that the patient experienced (ie, at risk for first event, at risk for second event, third event, and so forth). Interaction terms between patient characteristics and event strata (first event, second event, and so forth) were studied to determine whether different factors were associated with the first or second event. All factors with a P value of .2 or less in univariable analyses as well as clinical factors known to be important, such as model for end-stage liver disease (MELD) score, age, and sex, were included in multivariable Cox models. By using a backward stepwise method, after exclusion of collinear variables, variables significantly associated with outcome were included in the final model. Results are presented as a

hazard ratio (HR) and the 95% confidence interval (95% CI). For any laboratory parameter associated with outcome as a continuous variable, dichotomous thresholds also were evaluated to potentially improve clinical utility.

For the effectiveness analysis, a per-protocol analysis was performed, including all patients who completed therapy and had complete follow-up data. A second analysis was performed including all patients, regardless of treatment duration, and including patients who were lost to follow-up evaluation or died as non-SVR.

A *p* value less than .05 was considered statistically significant and all statistical tests were 2-tailed. The significance level of interactions was set at 0.01 to correct for multiple testing. PASW statistics 21.0 for Windows (SPSS, IBM, Armonk, NY) was used.

## RESULTS

### Patient Characteristics

Between January 1, 2014, and July 1, 2015, there were 433 cirrhotic patients who started treatment with a DAA-based regimen. Demographic features are summarized in Table 7.1. Ascites was the most common prior decompensation event (105 [84%] patients). Supplementary Table 7.1 shows the treatment regimens used. In total, 238 (55.0%) of the treatments included ribavirin (RBV) and 130 (30.0%) included a protease inhibitor (PI). Patients with a CP score of A were treated more often with a regimen including a PI (107 of 319; 33.5%) compared with those with a CP score of B/C (23 of 114, 20.2%; $p = .008$). There was no difference in use of RBV among these subgroups (172 of 319 [53.9%] vs 66 of 114 [57.9%]; $p = .464$).

### Adverse Events

In total, 104 (24.0%) patients reported 164 serious adverse events (SAEs) (Supplementary Table 7.2). Among patients with a CP score of A, 50 (13.2%) reported at least 1 SAE compared with 53 (46.5%) patients with a CP score of B/C ($p < .001$). There were 2 cases of suspected treatment-induced hepatotoxicity. Both patients (1 receiving sofosbuvir [SOF]/simeprevir [SMV] and 1 receiving SOF/RBV) had an increase in direct bilirubin level with no other precipitating cause. Five (1.2%) patients underwent liver transplantation between initiation of antiviral therapy and assessment of SVR12; 2 patients for HCC (5 and 10 weeks after stopping therapy); 2 patients for hepatic decompensation, including 1 patient with suspected drug-induced liver injury at week 10 of treatment and 1 patient received a transplant for both HCC and hepatic decompensation (8 weeks after stopping therapy). Four patients had undetectable HCV RNA at the time of transplantation and achieved SVR. Only 1 patient, who had a detectable viral load of less than 15 IU/mL at the time of transplantation, relapsed after transplant.

Six (1.4%) patients (1 CP score of A, 3 CP scores of B, and 2 CP scores of C) died between starting therapy and SVR12. Five deaths were liver-related (2 from acute-on chronic hepatic decompensation as a result of pneumonia, 1 from deteriorating liver function caused by suspected drug-induced liver injury, 1 from multi-organ failure after esophageal variceal bleeding, and 1 from cryoglobulinemia and end-stage liver failure in the absence of a clear precipitating event). The non–liver-related death occurred as a result of a motor vehicle accident 2 weeks after cessation of therapy.

**Table 7.1 | Baseline characteristics according to Child Pugh score (A vs. B/C)**

| Baseline variable | Child Pugh A (n=319) | Child Pugh B/C (n=114) | Total (N=433) | *p* value |
|---|---|---|---|---|
| Male sex | 206 (64.6%) | 71 (62.3%) | 277 (64.0%) | 0.661 |
| Age, in years | 58.0 (± 8.6) | 57.1 (± 9.3) | 57.8 (± 8.7) | 0.334 |
| ≥65 years | 57 (17.9%) | 22 (19.3%) | 79 (18.2%) | 0.734 |
| BMI, kg/m$^2$ [b] | 27.3 (± 4.9) | 27.5 (± 5.5) | 27.4 (± 5.1) | 0.672 |
| Caucasian | 268 (84.0%) | 93 (81.6%) | 361 (83.4%) | 0.583 |
| Black | 2 (0.6%) | 2 (1.8%) | 4 (0.9%) | |
| Asian | 15 (4.7%) | 4 (3.5%) | 19 (4.4%) | |
| Other/unknown | 34 (10.7%) | 15 (13.2%) | 49 (11.3%) | |
| Treatment history | | | | 0.097 |
| Naïve | 104 (32.6%) | 47 (41.2%) | 151 (34.9%) | |
| Experienced | 67 (58.8%) | 67 (58.8%) | 282 (65.1%) | |
| IFN-based failures | 157 (73.0%) | 59 (88.1%) | 216 (76.6%) | |
| DAA failures | 58 (27.0%) | 8 (11.9%) | 66 (23.4%) | |
| Prior decompensation | 36 (11.3%) | 89 (78.1%) | 125 (28.9%) | <0.001 |
| Child Pugh score | | | | |
| A | 319 (100.0%) | | 319 (73.7%) | |
| B | | 105 (92.1%) | 105 (24.2%) | |
| C | | 9 (7.8%) | 9 (2.1%) | |
| MELD score | 8 (6-20) | 13 (6-20) | 9 (6-20) | <0.001 |
| 0-9 | 244 (76.5%) | 19 (16.7%) | 264 (61.0%) | |
| 10-15 | 67 (21.0%) | 79 (69.3%) | 146 (33.7%) | |
| 16-21 | 3 (0.9%) | 16 (14.0%) | 19 (4.4%) | |
| Unknown | 5 (1.6%) | - | 4 (0.9%) | |
| HCV Genotype | | | | 0.105 |
| 1a | 143 (44.8%) | 33 (28.9%) | 176 (40.6%) | |
| 1b | 90 (28.2%) | 38 (33.3%) | 128 (29.6%) | |
| 1 (unspecified) | 7 (2.2%) | 3 (2.6%) | 10 (2.3%) | |
| 2 | 11 (3.4%) | 6 (5.3%) | 17 (3.9%) | |
| 3 | 50 (15.7%) | 23 (20.2%) | 73 (16.9%) | |
| 4 | 10 (3.1%) | 6 (5.3%) | 16 (3.7%) | |
| 5 | 0 (0.0%) | 1 (0.9%) | 1 (0.2%) | |
| Mixed genotype | 8 (2.5%) | 4 (2.6%) | 12 (2.8%) | |
| Platelet count, x10$^9$/L | 108 (16-599) | 75 (14-734) | 98 (14-734) | <0.001 |
| Albumin, in g/L | 37.9 (± 4.7) | 31.1 (± 5.2) | 36.1 (± 5.7) | <0.001 |
| Bilirubin, in mmol/L | 14 (3-76) | 33 (3-100) | 17 (3-100) | <0.001 |
| Creatinine, in umol/L | 70 (36-500) | 71 (43-193) | 70 (36-500) | 0.296 |
| Comorbidities | | | | |
| Diabetes mellitus | 74 (23.2%) | 24 (21.1%) | 98 (22.6%) | 0.639 |
| Alcohol abuse[b] | 57 (31.8%) | 32 (38.1%) | 89 (33.8%) | 0.318 |
| HIV coinfection | 4 (2.2%) | 1 (1.2%) | 5 (1.9%) | 0.620 |

Abbreviations: BMI, body mass index; HCV, hepatitis C virus; IFN, interferon; DAA, direct-acting antivirals; HIV, human immunodeficiency virus

a. Continuous variables are presented as mean (±SD) or median (range); categorical variables are presented as n (%). *P* values correspond to the comparison of patients with Child-Pugh A vs Child-Pugh B/C.

b. BMI was missing in 15% of the patients; alcohol abuse was missing in 40% of the patients; alcohol abuse was defined as either a history of alcohol abuse or a current use of more than 21 units per week.

An SAE was reported during 63 (22.3%) treatments that included RBV compared with 32 (16.4%) treatments without RBV ($p = .012$). Overall, there was no difference in the occurrence of SAEs between regimens that included a PI compared with those that did not (PI, 25 of 130 [19.2%] vs no PI, 70 of 303 [23.1%]; $p = .37$). In patients with a CP score of B/C cirrhosis, 14 of 23 (60.9%) patients receiving a PI reported at least 1 SAE compared with 39 of 91 (42.9%) of patients not receiving a PI ($p = .12$).

Of the 32 patients treated with an IFN-containing regimen, 30 (93.8%) patients had a CP score of A and 2 had CP scores of B/C cirrhosis. Only 1 patient discontinued treatment early owing to side effects. Six (18.8%) patients experienced at least 1 SAE, including hepatic decompensation (ascites, n = 1), liver transplantation for HCC (n = 1), anemia (n = 1), recurrent gingival bleeding (n = 2), and hypokalemia (n = 1).

**Hepatic Decompensation**

Fifty (11.5%) of the 433 patients experienced at least 1 hepatic decompensation event during or within 12 weeks after therapy with a median time to the first decompensation event of 8 weeks (range, 0–36 wk). Nineteen patients experienced more than 1 decompensation event, leading to a total of 90 events in 50 patients. Of these, 50 (55.6%) events comprised worsening of pre-existing hepatic decompensation, 26 (28.9%) new events in the presence of existing hepatic decompensation, and 14 (15.6%) de novo events in patients without a history of decompensation (Supplementary Figure 7.1). The most common decompensation event was ascites, either new-onset (10.0%) or worsening ascites (38.9%). Five (5.6%) patients experienced recurrent variceal bleeding. Of the 50 patients with an event, 33 (66%) required at least 1 hospital admission for decompensation. In 8 (14%) patients, treatment was discontinued, however, 2 patients still achieved SVR. In total, 8 (14%) of these 50 patients died (5 before SVR12 was assessed and 3 after having achieved SVR12).

Univariable Cox regression showed that baseline characteristics associated with an increased risk of first hepatic decompensation included the following: treatment-naive status (vs treatment-experienced: HR, 1.84; 95% CI, 1.06–3.21; $p = .034$), HCV genotype 3 (vs non–genotype 3: HR, 2.42; 95% CI, 1.35–4.36; $p = .005$), a CP score of B/C (vs a CP score of A: HR, 18.1; 95% CI, 8.51 to infinity; $p < .001$), albumin level less than 35 g/L (HR, 5.89; 95% CI, 3.19–10.9; $p < .001$), bilirubin level of 40 mmol/L or greater (HR, 7.35; 95% CI, 4.16–13.0; $p < .001$), MELD score of 14 or greater (HR, 8.28; 95% CI, 4.74–14.5; $p < .001$), and platelet count less than $100x10^9$/L (HR, 2.72; 95% CI, 1.49–5.13; $p = .001$). In a multivariable Cox model, HCV genotype 3 (vs non–genotype 3: HR, 2.08; 95% CI, 1.16–3.74; $p = .020$), a CP score of B/C (vs a CP score of A: HR, 12.63; 95% CI, 5.57 to infinity; $p < .001$), and albumin level less than 35 g/L (HR, 2.03; 95% CI, 1.05–3.93; $p = .026$) were associated independently with the occurrence of the first decompensation event during treatment (Table 7.2). Because MELD score and CP score were highly collinear, CP score could be replaced by a MELD score of 14 or higher (HR, 4.68; 95% CI, 2.60–8.42; $p < .001$). A sensitivity analysis, excluding patients treated with an IFN-based regimen, did not affect the model (data not shown). Because CP score was a very strong factor, the analyses were repeated for a CP score of A and a CP score of B/C as separate groups. Univariable Cox regression among patients with a CP score of A showed that only a history of hepatic decompensation was associated with on-treatment hepatic decompensation (HR, 13.42; 95% CI, 3.21 to infinity; $p < .001$). Among a CP score of B/C patients, HCV genotype 3, albumin level less than 35 g/L, and MELD score of 14 or higher

**Table 7.2 | Cox Proportional Hazards Regression Analyses for Hepatic Decompensation among all patients**

| | Univariable | | Multivariable | |
|---|---|---|---|---|
| **Baseline variable** | **HR (95% CI)** | ***p* value likelihood ratio** | **HR (95% CI)** | ***p* value likelihood ratio** |
| Age | 1.002 (0.977-1.028) | 0.867 | | |
| Age ≥ 65 | 1.001 (0.568-1.765) | 0.997 | | |
| Male gender | 1.043 (0.663-1.639) | 0.856 | | |
| Treatment naïve | | | | |
| For 1st event | 1.839 (1.055-3.205) | 0.034 | | |
| For subsequent event | 0.994 (0.524-1.888) | 0.986 | | |
| RBV-based | 0.911 (0.597-1.392) | 0.667 | | |
| PI-based | 0.998 (0.599-1.665) | 0.995 | | |
| HCV genotype 3 | | | | |
| For 1st event | 2.424 (1.347-4.364) | 0.005 | 2.080 (1.156-3.744) | 0.020 |
| For subsequent event | 0.678 (0.331-1.389) | 0.277 | 0.584 (0.283-1.202) | 0.134 |
| Child Pugh B/C vs A | | | | |
| For 1st event | 18.139 (8.509-∞) | <0.001 | 12.628 (5.570-∞) | <0.001 |
| For subsequent event[a] | - | - | - | - |
| Albumin | 0.879 (0.848-0.911) | <0.001 | | |
| Albumin <35 g/L | | | | |
| For 1st event | 5.894 (3.187-10.900) | <0.001 | 2.030 (1.047-3.934) | 0.026 |
| For subsequent event | 7.345 (0.993-∞) | 0.007 | 4.581 (0.594-∞) | 0.070 |
| Bilirubin | 1.031 (1.021-1.042) | <0.001 | | |
| Bilirubin ≥40 µmol/L | | | | |
| For 1st event | 7.345 (4.162-12.963) | <0.001 | | |
| For subsequent event | 0.937 (0.499-1.759) | 0.839 | | |
| Creatinine | 1.004 (0.999-1.009) | 0.184 | | |
| MELD score | 1.241 (1.169-1.317) | <0.001 | | |
| MELD score ≥14 | | | | |
| For 1st event | 8.279 (4.741-14.458) | <0.001 | 4.675 (2.596-8.422)[b] | <0.001 |
| For subsequent event | 1.634 (0.823-3.241) | 0.152 | 1.266 (0.632-2.538) | 0.502 |
| Platelet count | 0.996 (0.992-1.000) | 0.061 | | |
| Platelet count <100x$10^9$/L | | | | |
| For 1st event | 2.724 (1.448-5.126) | 0.001 | | |
| For subsequent event | 1.052 (0.530-2.090) | 0.884 | | |

Abbreviations: HR, hazards ratio; CI, confidence interval; RBV, ribavirin; PI, protease inhibitor; HCV, hepatitis C virus

a. There were no subsequent events among patients with CP A.

b. Model if Child Pugh was replaced by MELD score ≥14; HR presented for HCV genotype 3 and albumin <35 g/L were adapted from the model including Child Pugh

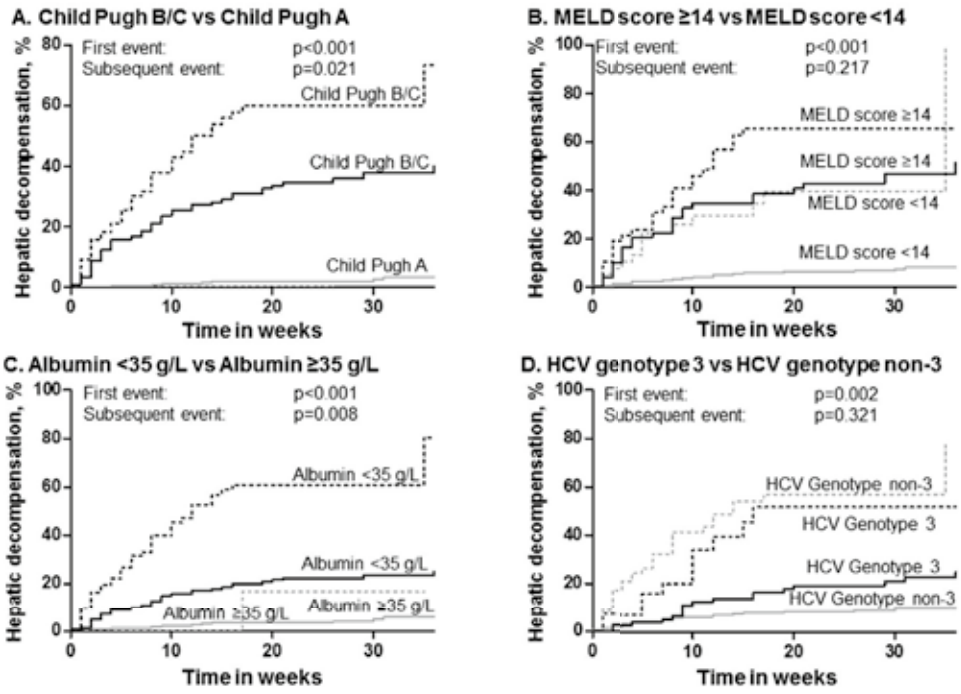



**Figure 7.1 | Kaplan Meier curves showing the occurrence of hepatic decompensation from start of therapy to SVR12 for 4 important baseline factors**

Straight lines represent the first event; dotted lines represent subsequent events. Among patients with Child Pugh A, there were no subsequent events. Abbreviations: HCV, hepatitis C virus

| | | 24-week cumulative incidence for first event |
|---|---|---|
| Panel A | Child Pugh A | 1.9% (95%CI 0.3-3.5) |
| | Child Pugh B/C | 34.5% (95%CI 25.7-43.3) |
| Panel B | MELD score <14 | 5.3% (95%CI 2.9-7.7) |
| | MELD score ≥14 | 39.4% (95%CI 27.6-51.2) |
| Panel C | Albumin ≥35 g/L | 3.4% (95%CI 1.2-5.6) |
| | Albumin <35 g/L | 22.2% (95%CI 15.7-28.7) |
| Panel D | HCV genotype non-3 | 8.7% (95%CI 5.8-11.6) |
| | HCV genotype 3 | 19.2% (95%CI 10.2-28.2) |

were associated independently with the occurrence of decompensation (Table 7.3). There was a trend toward increasing risk of decompensation with PI use in CP score B/C patients (HR, 1.60; 95% CI, 0.90–2.85; $p = .12$). Figure 7.1 shows the occurrence of first and subsequent decompensation by CP score, MELD score, albumin level, and the presence of HCV genotype 3.

**Treatment Response**

Per-protocol analysis showed that 344 (84.9%) of 405 patients achieved SVR12. Of patients who did not attain SVR12, 60 (98.4%) relapsed and 1 (1.6%) had a partial response. When including all patients, 350 (80.8%) patients achieved SVR12, 63 (14.5%) relapsed, 3 had a partial response, 6 patients died, and 11 patients were lost to follow-up evaluation, including 3 patients with undetectable HCV RNA levels 4 weeks after cessation of therapy and 6 patients with undetectable HCV RNA levels at the end of treatment. The SVR12 rate was similar among patients with a CP score of A (261 of 304 [85.9%]) and a CP score of B/C (83 of 101 [82.2%]; $p = .37$) in per-protocol analysis, however, there was a trend toward better response rates among patients with a CP score of A compared with a CP score of B/C cirrhosis when all patients were considered (CP score A, 264 of 319 [82.8%] vs CP score B/C, 86 of 114 [75.4%]; $p = .088$).

Excluding regimens in which RBV is considered standard of care (ie, DAA/PegIFN/RBV and SOF/RBV), the addition of RBV did not influence SVR12. The SVR12 rate was 94.8% (92 of 97) for regimens with RBV compared with 93.0% (172 of 185) for regimens without RBV ($p = .54$). Only HCV genotype was associated significantly with treatment response because SVR12 was 70.6% (48 of 68) among patients with HCV genotype 3 compared with 87.8% (296 of 337) among patients with other genotypes ($p < .001$). Among patients with HCV genotype 1, SVR was 89.0% (202 of 227) for a CP score of A cirrhosis and 83.1% (54 of 65) for a CP score of B/C cirrhosis ($p = .20$). Thirty-six (72.0%) of 50 patients who experienced hepatic decompensation achieved SVR12, compared with 314 of 383 (82.0%) patients without decompensation during therapy ($p = .092$). Supplementary Tables 7.3 and 7.4 show SVR12 rates (full-case analysis) stratified by treatment regimen/duration, HCV genotype, treatment experience, and CP score at baseline. Figure 7.2 shows the changes in MELD score according to CP score and treatment response.

Table 7.4 shows the cumulative incidence of hepatic decompensation and SVR according to the presence of any combination of the 3 factors at baseline (HCV genotype 3, albumin level <35, and MELD score ≥14). As noted, in patients with all 3 baseline predictors of decompensation, the probability of decompensation was higher than that of achieving SVR, whereas for patients with 0 or even 1 factor, SVR was much more likely than decompensation.

**Table 7.3 | Cox Proportional Hazards Regression Analyses for Hepatic Decompensation among patients with Child Pugh B/C**

| | Univariable | | Multivariable | |
|---|---|---|---|---|
| **Baseline variable** | **HR (95% CI)** | ***p* value likelihood ratio** | **HR (95% CI)** | ***p* value likelihood ratio** |
| Age | 1.005 (0.980-1.032) | 0.680 | | |
| Age ≥ 65 | 0.897 (0.489-1.646) | 0.722 | | |
| Male gender | 1.089 (0.678-1.750) | 0.723 | | |
| Treatment naïve | | | | |
| For 1st event | 1.704 (0.929-3.127) | 0.086 | | |
| For subsequent event | 0.921 (0.486-1.748) | 0.801 | | |
| RBV-based | 0.835 (0.535-1.304) | 0.431 | | |
| PI-based | 1.583 (0.931-2.691) | 0.102 | 1.598 (0.897-2.848) | 0.120 |
| HCV genotype 3 | | | | |
| For 1st event | 2.210 (1.173-4.161) | 0.019 | 2.054 (1.088-3.878) | 0.033 |
| For subsequent event | 0.651 (0.318-1.331) | 0.228 | 0.685 (0.321-1.459) | 0.320 |
| Albumin | 0.939 (0.900-0.980) | 0.004 | | |
| Albumin <35 g/L | | | | |
| For 1st event | 3.338 (1.189-9.372) | 0.007 | 3.107 (1.232-7.837) | 0.005 |
| For subsequent event | 3.182 (0.428-∞) | 0.174 | 3.257 (0.420-∞) | 0.182 |
| Bilirubin | 1.010 (0.998-1.022) | 0.113 | | |
| Bilirubin ≥40 µmol/L | | | | |
| For 1st event | 1.868 (1.016-3.432) | 0.047 | | |
| For subsequent event | 0.792 (0.422-1.483) | 0.464 | | |
| Creatinine | 1.007 (0.999-1.016) | 0.122 | | |
| MELD score | 1.097 (1.021-1.178) | 0.012 | | |
| MELD score ≥14 | | | | |
| For 1st event | 1.843 (1.159-2.930) | 0.009 | 1.634 (1.025-2.606) | 0.037 |
| For subsequent event | 1.333 (0.678-2.621) | 0.398 | 1.289 (0.647-2.570) | 0.466 |
| Platelet count | 1.001 (0.998-1.004) | 0.696 | | |
| Platelet count <100x10$^9$/L | | | | |
| For 1st event | 0.966 (0.485-1.921) | 0.921 | | |
| For subsequent event | 1.074 (0.541-2.134) | 0.837 | | |

Abbreviations: HR, hazards ratio; CI, confidence interval; RBV, ribavirin; PI, protease inhibitor; HCV, hepatitis C virus

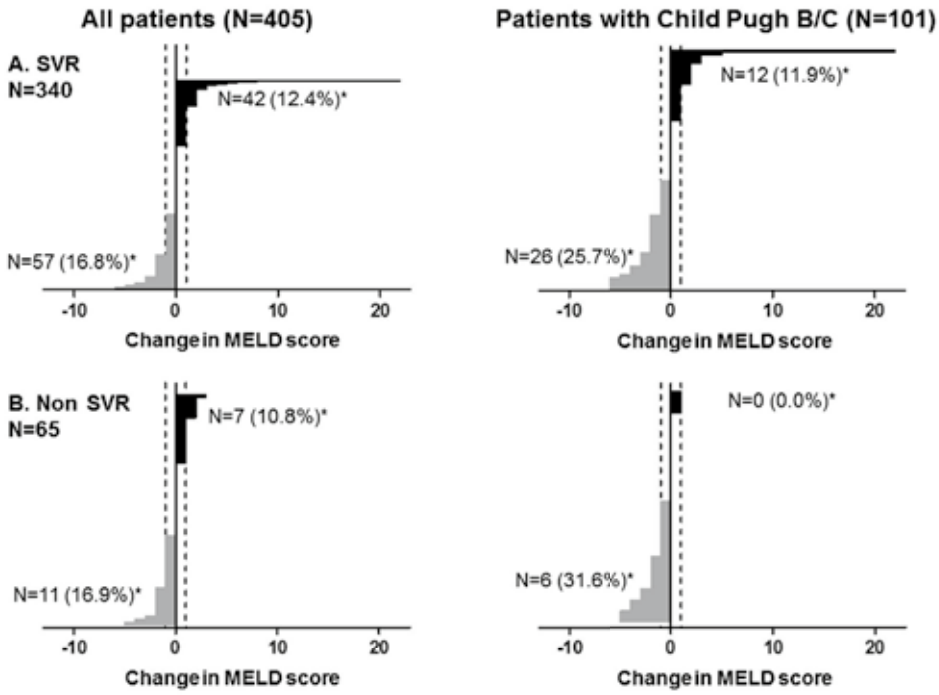



**Figure 7.2 | Change in MELD score at SVR12 visit for all patients and patients with Child Pugh B/C**

Patients who underwent liver transplantation, died or were lost to follow-up were not included. Panel A shows changes in MELD score for patients who achieved SVR and panel B shows changes in MELD score for patients who did not attain SVR. Grey bars represent improvement of MELD score and black bars represent worsening of MELD score. The asterisk (*) represents the number of patients (%) with a significant change in MELD score (i.e. ≥ 2 points).

**Table 7.4 | Cumulative incidence of hepatic decompensation and SVR12 rate according to three risk factors**

| | | 24 week cumulative incidence (95% CI) hepatic decompensation (first event) | SVR12 rate[b] (%) |
|---|---|---|---|
| **0 factor** | N | | |
| No factor | 212 | 3.3% (0.9-5.7) | 182/212 (85.6%) |
| **1 factor** | | | |
| HCV genotype 3 | | | |
| Yes | 73 | 19.2% (10.2-28.2) | 48/73 (65.8%) |
| No | 360 | 8.7% (5.8-11.6) | 302/360 (83.9%) |
| Albumin <35 g/L[a] | | | |
| Yes | 163 | 22.2% (15.7-28.7) | 124/163 (76.1%) |
| No | 269 | 3.4% (1.2-5.6) | 225/269 (83.6%) |
| MELD score ≥14[a] | | | |
| Yes | 66 | 39.4% (27.6-51.2) | 54/66 (81.8%) |
| No | 362 | 5.3% (2.9-7.7) | 292/362 (80.7%) |
| Any factor | 217 | 17.6% (12.5-22.7) | 165/217 (76.0%) |
| **2 factors** | | | |
| HCV genotype 3 + | | | |
| Albumin <35 g/L | 28 | 46.4% (28.0-64.8) | 16/28 (57.1%) |
| MELD score ≥14 | 15 | 60.0% (35.3-84.7) | 11/15 (73.3%) |
| Albumin <35 g/L + | | | |
| MELD score ≥14 | 54 | 46.3% (33.0-59.6) | 42/54 (77.8%) |
| Any two factors | 73 | 39.7% (28.5-50.9) | 53/73 (72.6%) |
| **3 factors** | | | |
| HCV genotype 3 + Albumin <35 g/L + MELD score ≥14 | 12 | 75.0% (50.5-99.5) | 8/12 (66.7%) |

Abbreviations: CI, confidence interval; SVR, sustained virological response; HCV, hepatitis C virus

a. MELD score at baseline was missing for 5 patients; albumin at baseline was missing for 1 patient

b. SVR12 rates were presented as full case analysis

## DISCUSSION

This large multicenter cohort study assessed real-world safety and the effectiveness of DAA regimens in HCV patients with cirrhosis. Patients with a CP score of A cirrhosis tolerated therapy well, with only 8 patients (2.5%) experiencing an episode of decompensation. In contrast, decompensating events occurred in 36.8% of patients with a CP score of B/C and were associated with 3 specific risk factors: HCV genotype 3, serum albumin level less than 35 g/L, and a MELD score of 14 or higher. The overall SVR12 rates were lower than those reported in clinical trials, especially for patients with decompensated cirrhosis and patients with HCV genotype 3.

Hepatic decompensation during DAA therapy occurred in 11.5% of patients. More than 50% of these events comprised worsening of pre-existing (stable) hepatic decompensation and the remainder were new events, mostly in patients who already had experienced another decompensation event. De novo decompensation in a patient with previously well-compensated cirrhosis was rare, accounting for 14 episodes. Among patients with a CP score of B/C at baseline, factors predictive of decompensation were baseline albumin level less than 35 g/L, a MELD score of 14 or higher, and infection with HCV genotype 3. The importance of albumin also was highlighted in a recent study by Foster et al.[327] They found that albumin level less than 35 g/L was associated with an increased risk of "harm" during therapy, defined as an increase in MELD score by 2 or more points, and/or the occurrence of any serious adverse event. Albumin level was particularly important in patients older than age 65, a trend that we did not observe.[327] Although serum albumin is a well-known parameter of liver synthetic function, it is not clear why it is specifically more useful in predicting adverse outcomes in patients treated with DAAs. Beyond reflecting hepatocyte protein synthesis, a low albumin level may worsen complications of portal hypertension such as ascites, and albumin may have a specific role in reducing the risk of infection, a major precipitant for hepatic decompensation.[328] In the era of PegIFN and RBV, Dultz et al[329] found that every 1-point increase in MELD score increased the risk for decompensation 1.56-fold. Our data, as well as those from other studies, emphasize that MELD is a useful predictor of safety and possibly response to IFN-free DAA-based therapy.

Our finding that HCV genotype 3 was associated with the occurrence of hepatic decompensation was somewhat surprising. Genotype 3 HCV infection was associated with an accelerated course of fibrosis, but this is unlikely to be of major significance during the short duration of DAA therapy. Although more patients with HCV genotype 3 had a MELD score of 14 or higher, genotype remained a significant factor in multivariable analysis and an interaction with MELD was not observed. A simple explanation may be that the lower success of therapy for HCV genotype 3 meant that fewer patients with this genotype achieved SVR and more patients relapsed after therapy. However, the rate of decompensation was similar between patients who did and did not achieve SVR, suggesting that this is not the full explanation. Alternatively, the issue may be the use of SOF/RBV combination therapy, which was the most commonly used regimen, in part because daclatasvir was not approved in Canada during the study period. However, SOF and RBV also frequently were components of the treatment regimens administered to patients with HCV genotype non-3 (102 of 291; 35.1%) and the use of this combination was not associated independently with decompensation. Univariable analysis did not show that the use of RBV or the cumulative exposure to RBV were associated with the risk of

decompensation. One possible hypothesis is that the metabolism of DAAs may be different among patients with different genotypes. Recently, Younossi et al[330] assessed the cholesterol pathway in patients with HCV genotypes 2 and 3 and found that the presence of HCV genotype 3 was associated with reduced circulation of lipids, resulting in relative hypocholesterolemia, a possible basis for the hampered virological response with this genotype. Whether this same pathway also could be involved in increasing the risk of adverse events requires further study. In addition to the safety issue, lower SVR rates in genotype 3 patients highlight the need for better therapy for this group of patients. Fortunately, multiple regimens in development, including SOF/velpatasvir, are highly active against genotype 3 and should be available in the near future.[168] Hopefully more effective therapy for HCV genotype 3 also will prove to be safer than current regimens.

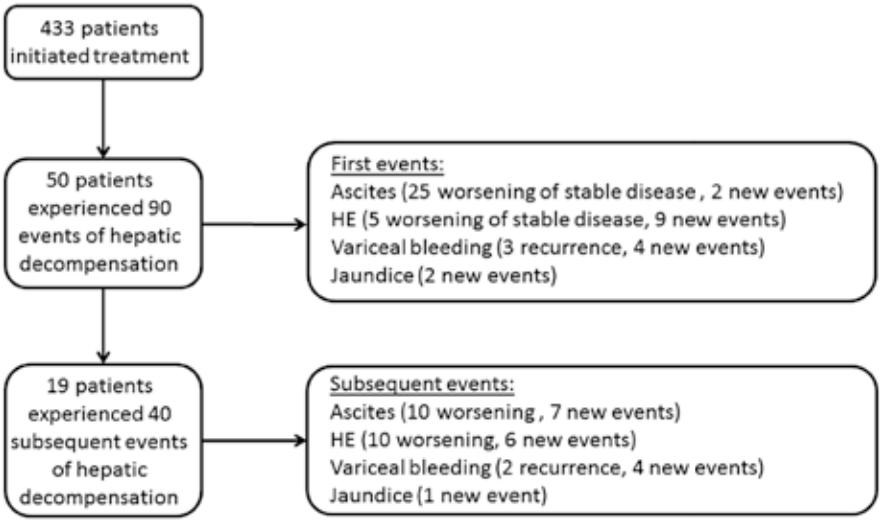
Cox regression analyses among patients with a CP score of B/C showed a trend toward increasing the risk of decompensation with the use of a protease inhibitor (HR, 1.60; 95% CI, 0.90–2.85). Upon initial approval of SOF/SMV, many of the most advanced patients were treated with this regimen to salvage them from decompensated cirrhosis. However, numerous cases of hepatotoxicity with SOF/SMV were reported, reinforcing that SMV is not recommended in patients with decompensated cirrhosis.[127 324 331] The issue is likely to be a class effect related to hepatic metabolism and the potential for accumulation of PIs in the liver of cirrhotic patients. More recently, the US Food and Drug Administration indicated that the use of dasabuvir, ombitasvir, and paritaprevir/ritonavir is contraindicated in patients with decompensated liver disease.[174] Similarly, the most recently approved PI, grazoprevir, is not recommended in people with a CP score of B/C because of the risk of PI-induced hepatotoxicity.

Before starting therapy, it is important to weigh the risks and benefits of antiviral treatment. For patients with a CP score of A cirrhosis, the risk of decompensation appears low in this study and in multiple clinical trials and real-world reports, suggesting that these patients can be treated safely and are likely to bene t from therapy. However, in patients with a CP score of B/C at baseline, there is clearly a risk of causing harm. By identifying 3 useful factors, physicians potentially can assess the risk for hepatic decompensation and weigh this risk against the chance of attaining SVR. One could argue that when the risk of decompensation is equal to or higher than the chance of attaining SVR, delaying therapy would be a better option. Recent data from patients treated after liver transplantation have shown excellent safety and high rates of SVR, particularly in patients treated early after transplantation.[150 332 333] Moreover, the clinical bene t of attaining SVR in patients with a CP score of B/C still is unknown. Some studies have shown improvement in MELD score at 12 or 24 weeks after the cessation of therapy,[327 334] but whether small decreases in MELD score truly indicate clinical bene t and whether these benefits will translate into improved clinical outcomes remains to be seen. More data are needed urgently on long-term outcomes of decompensated cirrhosis to elucidate which patients benefit from successful antiviral therapy and, more importantly, which patients do not. Our analysis showed that therapy is safe and effective in patients with compensated CP score of A cirrhosis. For patients with a CP score of B/C, an albumin level less than 35 g/L, a MELD score of 14 or greater, and genotype 3 infection were associated with decompensation during therapy. If only 1 of these factors was present, the SVR rate still was approximately 75%, compared with an approximately 20% risk of decompensation, thus favoring treatment with careful monitoring. However, when 2 or all 3 risk factors were present, the rate of decompensation was almost equivalent or even higher than

the chance of SVR, arguing that these patients would likely be better off deferring therapy until after transplantation. Although some of the decompensation events may be owing to the natural history of advanced liver disease rather than treatment itself, the high event rate and marginal benefit of pre-transplant treatment in these patients still would argue for delaying therapy.

This study had important limitations, including its retrospective nature and the potential for underreporting of decompensation events and missing data. However, it is unlikely that severe adverse events, which are clinically most relevant, were missed. Second, because of the heterogeneous regimens used, it was not possible to conclude that a specific regimen would be preferred in patients with decompensated cirrhosis. Finally, the limited follow-up period of 12 weeks after treatment allowed for assessment of only short-term benefits of therapy. In conclusion, in a large cohort of patients with HCV cirrhosis, SVR rates with second-generation DAAs were lower than reported in clinical trials. Treatment was safe and effective in patients with compensated cirrhosis. However, in patients with a CP score of B/C at baseline, there was a significant risk of worsening decompensation during therapy. An albumin level less than 35 g/L, a MELD score of 14 or higher, and HCV genotype 3 infection are useful tools to assess the risk and benefits when antiviral treatment with DAAs is under consideration for patients with advanced cirrhosis.

## SUPPLEMENTARY FIGURE AND TABLES




**Supplementary Figure 7.1 |** Flow chart for hepatic decompensation from start of therapy to the SVR12 visit. In total, 50 patients experienced at least one decompensating event. In 19 (38%) patients a subsequent decompensating event was reported. HE, hepatic encephalopathy

**Supplementary Table 7.1 |** Treatment allocation

| | DAA/PEG/ RBV[a] | SOF/RBV | SOF/SMV ± RBV | SOF/LDV ± RBV | OBV/PTV/r/ DSV ± RBV | SOF/DCV ± RBV |
|---|---|---|---|---|---|---|
| Patients, n (%) | 32 (7.4%) | 99 (22.9%) | 106 (24.5%) | 115 (26.6%) | 24 (5.5%) | 57 (13.2%) |
| RBV used | 32 (100.0%) | 99 (100.0%) | 27 (25.5%) | 36 (31.3%) | 20 (83.3%) | 24 (42.1%) |
| Completed treatment | 31 | 97 | 101 | 102 | 21 | 47 |
| Early treatment discontinuation | 1 | 4 | 5 | 0 | 1 | 6 |
| Reason for stopping: | | | | | | |
| Adverse event | 0 | 2 | 1 | 0 | 0 | 0 |
| Decompensation | 0 | 2 | 2 | 0 | 0 | 2 |
| Only RBV/PegIFN Stopped | 1 | 0 | 2 | 0 | 1 | 4 |
| Lost to follow-up/ died | 0 | 4 | 2 | 6 | 2 | 3 |
| SVR 12[b] | 23/32 (71.9%) | 57/99 (57.6%) | 95/106 (89.6%) | 101/115 (87.8%) | 22/24 (91.7%) | 52/57 (91.2%) |

Abbreviations: DAA, direct-acting antiviral; PEG, pegylated interferon α; RBV, ribavirin; SOF, sofosbuvir; SMV, simeprevir; LDV, ledipasvir; OBV, ombitasvir; PTV/r, paritaprevir/ritonavir; DSV, dasabuvir ; DCV, daclatasvir; SVR, sustained virological response

a. Including 30 treatments with SOF/PEG/RBV, 1 treatment with SIM/PEG/RBV and 1 treatment with SOF/PEG/RBV/boceprevir

b. b. Patients who died or were lost to follow-up were included as non-SVR

**Supplementary Table 7.2 |** Safety issues reported

| Event | Child-Pugh A 319 patients | Child-Pugh B/C 114 patients | Total 433 patients |
|---|---|---|---|
| **SAEs** | | | |
| Total | 62 | 116 | 178 |
| At least one | 49 (15.4%) | 54 (47.4%) | 103 (23.8%) |
| 2 events | 10 (3.1%) | 7 (6.1%) | 17 (3.9%) |
| > 2 events | 1 (0.3%) | 18 (15.8%) | 19 (4.4%) |
| **Hepatic decompensation** | 8 (12.9%) | 82 (71.9%) | 90 (50.6%) |
| Variceal bleeding | 5 (62.5%) | 8 (9.8%) | 13 (14.4%) |
| Hepatic encephalopathy | 0 (0.0%) | 30 (36.6%) | 30 (33.3%) |
| Ascites | 3 (37.5%) | 41 (50.0%) | 44 (48.9%) |
| Jaundice[a] | 0 (0.0%) | 3 (3.7%) | 3 (3.3%) |
| **Other SAEs** | | | |
| Cardiac | 3 (4.8%) | 0 (0.0%) | 3 (1.7%) |
| Gastrointestinal | 1 (1.6%) | 0 (0.0%) | 1 (0.6%) |
| Infection | 8 (12.9%) | 3 (2.6%) | 11 (6.2%) |
| Symptomatic anemia[b] | 14 (22.6%) | 9 (7.9%) | 23 (12.9%) |
| Liver transplantation | 1 (1.6%) | 4 (3.5%) | 5 (2.8%) |
| Death[c] | 1 (1.6%) | 5 (4.4%) | 6 (3.4%) |
| Other | 17 (27.4%) | 16 (12.3%) | 31 (17.4%) |
| **Laboratory adverse events** | | | |
| Anemia (Hb <8.0 g/dL) | 5 (1.6%) | 6 (5.3%) | 11 (2.5%) |
| Creatinine increase (>26.5 µmol/L) | 10 (3.1%) | 11 (9.6%) | 21 (4.8%) |

Abbreviations: SAEs, serious adverse events; HCC, hepatocellular carcinoma; Hb, hemoglobin

a. Jaundice was defined as an episode of a total serum bilirubin ≥ 40 µmol/L, and requiring hospital admission
b. Symptomatic anemia was defined as the presence of anemia (all grades) requiring dose reduction of RBV or blood transfusion
c. Causes of death included two deaths due to acute on chronic hepatic decompensation caused by pneumonia; two deaths due to end-stage liver disease (including one case of suspected drug induced liver injury); one death due to multi-organ failure after variceal bleeding; and one death due to a motor vehicle accident.

**Supplementary Table 7.3 |** SVR12 rates for a treatment duration of 12 weeks (full case analysis)

| | HCV genotype | No addition of RBV | | RBV-based regimen | | |
|---|---|---|---|---|---|---|
| | **Treatment experience** | **Child-Pugh A** | **Child-Pugh B/C** | **Child-Pugh A** | **Child-Pugh B/C** | **Total** |
| **DAA/PEG/ RBV** | HCV genotype 1a | | | | | |
| | Naïve | - | - | 3/4 | 0/1 | 3/5 |
| | TE | - | - | 4/4 | - | 4/4 |
| | HCV genotype 1b | | | | | |
| | Naïve | - | - | 2/3 | - | 2/3 |
| | TE | - | - | 2/4 | - | 2/4 |
| | HCV genotype 1 no subtype | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | - | - | 2/2[a] | - | 2/2 |
| | HCV genotype 3 | | | | | |
| | Naïve | - | - | 1/1 | - | 1/1 |
| | TE | - | - | 5/6 | - | 5/6 |
| | HCV genotype 4 | | | | | |
| | Naïve | - | - | 0/1 | - | 0/1 |
| | TE | - | - | - | - | - |
| | HCV genotype 5 | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | - | - | - | 1/1 | 1/1 |
| | | | | | | **20/27** |
| **SOF/RBV** | HCV genotype 2 | | | | | |
| | Naïve | - | - | 3/4 | 2/2 | 5/6 |
| | TE | - | - | 2/3 | 1/2 | 3/5 |
| | | | | | | **8/11** |
| **SOF/SMV** | HCV genotype 1a | | | | | |
| | Naïve | 3/4 | 1/1 | 7/7 | 2/2 | 13/14 |
| | TE | 17/20 | - | 12/13 | 1/1 | 30/34 |
| | HCV genotype 1b | | | | | |
| | Naïve | 9/11 | 4/5 | - | - | 13/16 |
| | TE | 19/20[b] | 5/6 | - | - | 24/26 |
| | HCV genotype 1 no subtype | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | 1/1 | 2/3 | - | - | 3/4 |
| | HCV genotype 4 | | | | | |

**Supplementary Table 7.3 |** Continued

| | HCV genotype | No addition of RBV | | RBV-based regimen | | |
|---|---|---|---|---|---|---|
| | **Treatment experience** | **Child-Pugh A** | **Child-Pugh B/C** | **Child-Pugh A** | **Child-Pugh B/C** | **Total** |
| | Naïve | 2/2 | 1/1 | - | - | 3/3 |
| | TE | 1/1 | 1/1 | - | - | 2/2 |
| | | | | | | **88/99** |
| **SOF/LDV** | HCV genotype 1a | | | | | |
| | Naïve | 10/10 | 3/3[c] | 2/2 | 2/3[d] | 17/18 |
| | TE | 1/2 | 1/1 | 8/9[e] | 5/5[f] | 15/17 |
| | HCV genotype 1b | | | | | |
| | Naïve | 2/4 | 2/3 | - | 1/2 | 5/9 |
| | TE | 1/1 | - | 3/3 | 2/2 | 6/6 |
| | HCV genotype 1 no subtype | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | - | - | 2/2 | - | 2/2 |
| | HCV genotype 4 | | | | | |
| | Naïve | 1/1 | - | - | - | 1/1 |
| | TE | - | - | - | 1/1 | 1/1 |
| | | | | | | **47/54** |
| **OBV/PTV/r/ DSV** | HCV genotype 1a | | | | | |
| | Naïve | - | - | 6/6 | 1/1 | 7/7 |
| | TE | - | - | - | - | - |
| | HCV genotype 1b | | | | | |
| | Naïve | 1/1 | - | 4/4 | - | 5/5 |
| | TE | - | - | 4/4 | 0/1 | 4/5 |
| | HCV genotype 1 no subtype | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | 1/1 | - | - | - | 1/1 |
| | | | | | | **17/18** |
| **SOF/DCV** | HCV genotype 1a | | | | | |
| | Naïve | 0/1 | - | 1/1 | - | 1/2 |
| | TE | - | - | 3/3 | 1/1 | 4/4 |
| | HCV genotype 1b | | | | | |
| | Naïve | - | - | 1/1 | - | 1/1 |
| | TE | 1/1 | - | 2/2[g] | - | 3/3 |
| | HCV genotype 3 | | | | | |

**Supplementary Table 7.3 |** Continued

| HCV genotype | No addition of RBV | | RBV-based regimen | | |
|---|---|---|---|---|---|
| **Treatment experience** | **Child-Pugh A** | **Child-Pugh B/C** | **Child-Pugh A** | **Child-Pugh B/C** | **Total** |
| Naïve | - | - | - | - | - |
| TE | 1/1 | - | - | - | 1/1 |
| HCV genotype 4 | | | | | |
| Naïve | - | - | - | - | - |
| TE | - | - | 1/1 | 1/1 | 2/2 |
| | | | | | **12/13** |

Abbreviations: SVR, sustained virological response; HCV, hepatitis C virus; RBV, ribavirin; DAA, direct-acting antiviral; PEG, pegylated interferon α; TE, treatment experienced; SOF, sofosbuvir; SMV, simeprevir; LDV, ledipasvir; OBV, ombitasvir; PTV/r, paritaprevir/ritonavir; DSV, dasabuvir ; DCV, daclatasvir

a. Including one patient with HCV genotype 1 receiving 12 weeks of SOF in addition to BOC/PEG/RBV, which was used for 24 weeks already
b. One patient had a mixed infection with HCV genotype 1b/4, this patient achieved SVR12
c. One patient had a mixed infection with HCV genotype 1a/1b, this patient achieved SVR12
d. One patient had a mixed infection with HCV genotype 1a/2, this patient achieved SVR12
e. One patient had a mixed infection with HCV genotype 1a/1b, this patient achieved SVR12
f. One patient had a mixed infection with HCV genotype 1a/2, this patient achieved SVR12
g. This patient had a mixed infection with HCV genotype 1b/3

**Supplementary Table 7.4 |** SVR12 rates for a treatment duration of 16 or 24 weeks (full case analysis)

| | **HCV genotype** | **No addition of RBV** | | **RBV-based regimen** | | **Total** |
|---|---|---|---|---|---|---|
| | **Treatment experience** | **Child-Pugh A** | **Child-Pugh B/C** | **Child-Pugh A** | **Child-Pugh B/C** | |
| **DAA/PEG/RBV[c]** | HCV genotype 1a | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | - | - | 1/1 | - | 1/1 |
| | HCV genotype 1b | | | | | |
| | Naïve | - | - | 1/1[a] | - | 1/1 |
| | TE | - | - | 0/1 | - | 0/1 |
| | HCV genotype 3 | | | | | |
| | Naïve | - | - | 0/1 | - | 0/1 |
| | TE | - | - | 1/1 | - | 1/1 |
| | | | | | | **3/5** |
| **SOF/RBV** | HCV genotype 1a | | | | | |
| | Naïve | - | - | 0/2 | - | 0/2 |
| | TE | - | - | 1/2 | - | 1/2 |
| | HCV genotype 1b | | | | | |
| | Naïve | - | - | 2/4 | 2/2 | 4/6 |
| | TE | - | - | 4/10 | 2/6 | 6/16 |
| | HCV genotype 2 | | | | | |
| | Naïve | - | - | 2/2[b] | 1/1[b] | 3/3 |
| | TE | - | - | 2/2[b] | 0/1[b] | 2/3 |
| | HCV genotype 3 | | | | | |
| | Naïve | - | - | 11/17 | 7/12 | 18/29 |
| | TE | - | - | 9/17 | 5/8[b] | 14/25 |
| | HCV genotype 4 | | | | | |
| | Naïve | - | - | 1/1 | - | 1/1 |
| | TE | - | - | - | 0/1 | 0/1 |
| | | | | | | **49/88** |
| **SOF/SMV** | HCV genotype 1a | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | - | - | 3/3 | - | 3/3 |
| | HCV genotype 1b | | | | | |
| | Naïve | - | 1/1 | - | - | 1/1 |
| | TE | 2/2 | - | - | - | 2/2 |
| | HCV genotype 4 | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | - | - | 1/1 | - | 1/1 |
| | | | | | | **7/7** |

**Supplementary Table 7.4 |** Continued

| | HCV genotype | No addition of RBV | | RBV-based regimen | | Total |
|---|---|---|---|---|---|---|
| | Treatment experience | Child-Pugh A | Child-Pugh B/C | Child-Pugh A | Child-Pugh B/C | |
| **SOF/LDV** | HCV genotype 1a | | | | | |
| | Naïve | 1/1 | 2/3 | 1/1[c] | - | 2/3 |
| | TE | 25/28[d] | 4/5[e] | 3/3[f] | 1/2 | 33/38 |
| | HCV genotype 1b | | | | | |
| | Naïve | 1/1 | 2/2 | - | - | 3/3 |
| | TE | 7/8 | 6/6 | - | - | 13/14 |
| | HCV genotype 3 | | | | | |
| | Naïve | - | - | - | - | |
| | TE | - | - | 1/1 | - | 1/1 |
| | | | | | | **54/61** |
| **OBV/PTV/r/ DSV** | HCV genotype 1a | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | 2/2 | - | 2/3 | - | 4/5 |
| | HCV genotype 1 no subtype | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | - | - | 1/1 | - | 1/1 |
| | | | | | | **5/6** |
| **SOF/DCV** | HCV genotype 1a | | | | | |
| | Naïve | 2/2 | - | - | 1/1 | 3/3 |
| | TE | 13/13[g] | 5/6 | 1/2 | 0/1 | 19/22 |
| | HCV genotype 1b | | | | | |
| | Naïve | - | - | - | 1/1 | 1/1 |
| | TE | 5/5 | - | 1/1 | 1/1 | 7/7 |
| | HCV genotype 3 | | | | | |
| | Naïve | 1/1 | - | 1/2[b] | - | 2/3 |
| | TE | - | - | 2/2 | 3/3 | 5/5 |
| | HCV genotype 4 | | | | | |
| | Naïve | - | - | - | - | - |
| | TE | 2/2 | 1/1 | - | - | 3/3 |
| | | | | | | **40/44** |

Abbreviations: SVR, sustained virological response; HCV, hepatitis C virus; RBV, ribavirin; DAA, direct-acting antiviral; PEG, pegylated interferon α; TE, treatment experienced; SOF, sofosbuvir; SMV, simeprevir; LDV, ledipasvir; OBV, ombitasvir; PTV/r, paritaprevir/ritonavir; DSV, dasabuvir ; DCV, daclatasvir

a. This patient received 24 weeks of SMV/PEG/RBV
b. 16 weeks for 6 patients with HCV genotype 2 (SOF/RBV [SVR 5/6]) and two patients with HCV genotype 3 (SOF/RBV [SVR] and SOF/DCV/RBV [SVR])

c. This patient had a missing HCV genotype
d. Including two patients who had a double infection with HCV genotype 1a/2, both patients achieved SVR12
e. One patient had a mixed infection with HCV genotype 1a/1b
f. One patient had a mixed infection with HCV genotype 1a/1b
g. One patient had a mixed infection with HCV genotype 1a/1b

# CHAPTER 8

## EFFECTIVENESS AND SAFETY OF SOFOSBUVIR-BASED REGIMENS FOR CHRONIC HCV GENOTYPE 3 INFECTION: RESULTS OF THE HCV-TARGET STUDY

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## ABSTRACT

**Background & Aims**

Sofosbuvir (SOF) is active against all hepatitis C virus (HCV) genotypes, and SOF-based therapies lead to high rates of sustained virological response (SVR). However, genotype 3 (GT3) HCV remains a challenge with lower SVR rates reported, particularly in patients with cirrhosis. This study reports the effectiveness and safety of SOF-based therapy in patients with GT3 HCV treated in clinical practice.

**Methods**

Hepatitis C Virus Therapeutic Registry and Research Network is an international, prospective observational study evaluating patients treated in usual clinical practice. Patients with GT3 HCV were analyzed to assess predictors of treatment response and adverse events using descriptive statistics and multivariable logistic regression.

**Results**

Treatment outcomes were available for 197 patients treated with SOF and ribavirin (RBV), with or without peginterferon, including 54% with cirrhosis and 49% who failed prior therapy. Of 178 patients treated with SOF/RBV, 60% achieved SVR at 12 weeks (SVR12), compared with 84% of 19 patients treated with SOF/peginterferon/RBV. For patients treated with SOF/RBV, the SVR12 rate was 58% in treatment-naive patients with cirrhosis, and 42% in those with cirrhosis who failed prior therapy. In noncirrhotic patients, SVR12 rates were 89% in treatment-naive and 88% in treatment-experienced patients. After controlling for age and sex, absence of cirrhosis (odds ratio [OR], 6.4; 95% confidence interval [CI], 2.78–14.74), albumin levels ≥3.2 g/dL (OR, 12.48; 95% CI, 3.86–40.33), and platelet count $\geq 10^5$ cells/μL (OR, 7.44; 95% CI, 3.51–15.78) were associated with greater odds of SVR12.

**Conclusions**

SVR rates were acceptable in patients with GT3 HCV without cirrhosis; however, in those with cirrhosis, treatment with SOF/RBV was suboptimal, highlighting the need for new therapies for this population.

## INTRODUCTION

Chronic hepatitis C virus (HCV) infection continues to be a major global public health problem, with recent estimates suggesting that >103 million people are infected worldwide.[1] There is wide geographical variation in HCV genotype distribution; genotype 1 is most common (46%), followed by genotype 3 (GT3) (22%).[1] Chronic infection leads to progressive liver fibrosis that may eventually lead to cirrhosis, putting patients at risk of liver failure and/or hepatocellular carcinoma (HCC).[274-276] Several studies have shown that both the risk for fibrosis progression and the risk of developing HCC are increased among patients with HCV GT3.[299 335]

Therapy for chronic HCV was originally based on interferon, and resulted in different response rates by genotype.[107] Genotypes 2 (GT2) and 3 are relatively interferon sensitive and required shorter duration of therapy with lower doses of ribavirin (RBV) to achieve high rates of sustained virological response (SVR). Even in the interferon era, data suggested that GT3 was more difficult to cure than GT2, particularly in patients with established cirrhosis.[336] The discovery of direct-acting antivirals (DAAs) has revolutionized treatment of chronic HCV infection. Sofosbuvir (SOF) is a well-tolerated nucleotide polymerase inhibitor with activity against all HCV genotypes. However, despite initially promising results, large clinical trials reported lower rates of SVR in patients with GT3 infection receiving SOF-based therapy than with other HCV genotypes.[337]

The FUSION, FISSION, and POSITRON trials showed that the combination of SOF and RBV was more effective and much better tolerated than pegylated interferon (peg-IFN)/ RBV in patients with GT2 and GT3 HCV.[137 139] However, a breakdown of the results by genotype and cirrhosis status revealed that patients with GT3, particularly those with cirrhosis, had high rates of relapse. With extension of therapy to 24 weeks, the SVR rates rose to 93% in treatment-naive patients; however, in patients who had failed prior peg-IFN/RBV, the SVR rates were 85% without cirrhosis, but only 60% in those with cirrhosis.[138]

Studies then evaluated adding peg-IFN to SOF/RBV for 12 weeks and showed that SVR rates increased to 85%, even in treatment-experienced patients with cirrhosis.[140 338] Based on these results, guidelines recommend either a 12-week course of SOF/peg-IFN/RBV or a 24-week course of SOF/RBV for patients with GT3 infection.[35 36] Daclatasvir (DCV), a relatively pan-genotypic NS5A inhibitor, has also been combined with SOF, and the combination has been recommended as an alternative therapy for GT3 infection.[35 36] The efficacy and safety of these regimens are based on treatments administered to patients who fulfilled the eligibility criteria for clinical trials. Whether these results apply in daily clinical practice is unknown. This study reports the safety and effectiveness of 2 SOF-based regimens for the treatment of chronic GT3 HCV in an international, prospective observational study.

## PATIENTS AND METHODS

### Study Population and Design

Hepatitis C Virus Therapeutic Registry and Research Network (HCV-TARGET) is an international, prospective observational study enrolling patients from academic (n = 44) and community (n = 17) centers in the United States, Canada, Germany, and Israel who receive antiviral treatment for chronic HCV infection. Sequentially enrolled patients with no history of liver transplant or prior DAA treatment, ≥18 years old with GT3 HCV who received treatment with SOF and RBV, with or without peg-IFN, were included. The treatment regimen was chosen by the treating physician. Redacted medical records were collected by a central team of trained coders and reviewed, and baseline and on-treatment demographic, clinical, and virological data were entered into a common, standardized database. Data were managed using REDCap electronic data capture tools hosted at the University of North Carolina at Chapel Hill and reviewed for completeness and accuracy by study monitors. Extreme or unlikely values were verified or resolved with additional queries.

The protocol was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The protocol was approved by each local institutional review board (IRB). All patients provided written informed consent or an informed consent waiver was granted by the IRB overseeing the site.

### Definitions

The following clinical definitions were applied to the study population.

***Cirrhosis.*** Cirrhosis was defined by an algorithm implemented for HCV-TARGET analyses.[169] The primary indicator of cirrhosis was a liver biopsy with Metavir score F4. Patients with a liver biopsy reported as Metavir score F3 with at least 1 secondary indicator were also defined as having cirrhosis. Secondary indicators included serum fibrosis scores above thresholds for cirrhosis (FibroSure/FibroTest, FibroSpect, Hepascore); transient elastography (Fibroscan) ≥12.5 kPa; or signs of portal hypertension (esophageal/gastric varices, portal gastropathy, or platelet count $<140x10^9$/L). In the absence of a biopsy, patients with ≥2 secondary indicators were defined as having cirrhosis.[120]

Decompensated cirrhosis was defined as documented presence of current or past ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatic hydrothorax or variceal hemorrhage, or use of medications specifically prescribed for one of these indications.

***Adverse Event.*** An adverse event (AE) was defined as any reported AE regardless of the need for dose reduction or discontinuation of HCV therapy.

***Serious Adverse Event.*** A serious adverse event (SAE) was defined as an AE that either required hospitalization or met criteria for expedited reporting per US Food and Drug Administration form MEDWATCH 3500.

***Anemia.*** Anemia was reported as an AE, or documented RBV dose reduction, treatment with erythropoietin, or blood transfusion.

**Statistical Analyses**

Demographics, baseline laboratory values, and frequencies of AEs were collected and analyzed by treatment regimen for the evaluable population (N = 197), which comprised patients who ended treatment with a known virological outcome or were confirmed to be lost to posttreatment follow-up (counted as non– virological failures). Those lost to follow-up during treatment or who withdrew consent were excluded.

The per-protocol population (n = 174) comprised patients who completed treatment or ended treatment early due to virological failure only, and have a known virological outcome. The unadjusted rates of SVR were calculated for the evaluable and per-protocol populations, and multivariable analyses of factors associated with response and results in subgroups of interest are reported for the per-protocol population, including treatment history, presence of cirrhosis, and features of cirrhosis. Confidence intervals (CIs) of unadjusted rates were calculated using exact binomial methods.

The association between baseline covariates and SVR was estimated by logistic regression adjusted for age and sex for the per-protocol population. Covariates significant after adjustment for age and sex without significant collinearity were combined into multivariable models. Predictor variables were selected a priori based on consensus of clinical expertise and included well-established covariates associated with SVR: treatment regimen, age, sex, race, albumin level (<3.2 g/dL, ≥3.2 g/dL), platelet count (<$10^5$ cells/μL, ≥$10^5$ cells/μL), creatinine clearance, total bilirubin (mg/dL), hemoglobin (g/dL), cirrhosis status, and history of prior antiviral treatment as well as Model for End-Stage Liver Disease score and history of decompensating event in patients with cirrhosis. Results are presented as an odds ratio (OR) with 95% CIs. Analyses were performed using SAS version 9.4 software (SAS Institute, Cary, North Carolina).

## RESULTS

**Patient Disposition**

Between December 2013 and January 2015, 210 patients with GT3 HCV infection with no history of liver transplant or prior DAA therapy started treatment with SOF/RBV or SOF/ peg-IFN/RBV. A total of 185 patients completed the prescribed regimen. Eighteen patients discontinued treatment early due to AEs [4], lack of efficacy [4], noncompliance [3], or other reasons [3] (eg, loss of insurance or planned surgery). Twelve patients were excluded due to loss to follow-up during treatment [4], withdrawal of consent [1], or ongoing posttreatment follow-up at the time of reporting [5].

The majority of patients (178/197 [90%]) were treated with SOF/RBV, with 87% receiving 24±2 weeks of therapy, while 19 (10%) patients received 12 (n = 12), 16 (n = 1), or 24 (n = 5) weeks of SOF/peg-IFN/RBV, including 1 patient who discontinued treatment at week 4. In total, 112 (57%) were male, 81% were white, and the mean age was 56 years (range, 27–77 years). Just under half (49%) were treatment experienced and 3 patients (1.5%) were coinfected with human immunodeficiency virus (HIV). Of 107 (54%) patients with cirrhosis, 49 (46%) had a history of prior hepatic decompensation. Of cirrhotic patients with complete MELD data (70%), the median baseline MELD score was 10 (range, 6–24). MELD score was <10 in 49% (n = 37), 10–15 in 44% (n = 33), and >15 in 7% (n = 5) of patients with cirrhosis (Table 8.1; Supplementary Table 8.1).

**Table 8.1 | Baseline demographic and clinical characteristics of patients with G3 HCV who completed therapy with sofosbuvir and ribavirin +/- peginterferon**

| Characteristic | SOF/PEG/RBV (n=19) | SOF/RBV (n=178) | Total (n=197) |
|---|---|---|---|
| Median age (range), yr | 56.0 (33-67) | 56.0 (27-77) | 56.0 (27-77) |
| 18-39 | 3 (15.8%) | 19 (10.7%) | 22 (11.2%) |
| 40-64 | 15 (78.9%) | 140 (78.7%) | 155 (78.7%) |
| 65+ | 1 (5.3%) | 19 (10.7%) | 20 (10.2%) |
| Male sex, n (%) | 13 (68.4%) | 99 (55.6%) | 112 (56.9%) |
| Race, n (%) | | | |
| White | 14 (73.7%) | 146 (82.0%) | 160 (81.2%) |
| Black | 0 (0.0%) | 4 (2.2%) | 4 (2.0%) |
| Other/pending | 5 (26.3%) | 28 (15.7%) | 33 (16.8%) |
| Hispanic ethnicity, n (%) | 2 (10.5%) | 15 (8.4%) | 17 (8.6%) |
| Prior HCV treatment, n (%) | | | |
| Naive | 7 (36.8%) | 94 (52.8%) | 101 (51.3%) |
| Experienced | 12 (63.2%) | 84 (47.2%) | 96 (48.7%) |
| Cirrhosis, n (%) | 11 (57.9%) | 96 (53.9%) | 107 (54.3%) |
| History of hepatic decompensation, n (%) | 3 (15.8%) | 46 (25.8%) | 49 (24.9%) |
| Baseline MELD ≥ 10 (n=75) | 2 (25.0%) | 36 (53.7%) | 38 (50.7%) |
| HCV RNA (mean), $\log_{10}$ IU/mL | 6.3 (4-7) | 5.9 (0-8) | 5.9 (0-8) |
| Median total bilirubin (range), mg/dl | 0.9 (0.2-2.8) | 0.7 (0.1-6.6) | 0.8 (0.1-6.6) |
| Median albumin (range), g/dl | 4.0 (2.4-5.0) | 3.9 (2.3-5.0) | 3.9 (2.3-5.0) |
| Median ALT (range), mean IU/l | 74.0 (28.0-375.0) | 73.0 (12.0-362.0) | 73.0 (12.0-375.0) |
| Median platelet count (range) ($x10^3$) per µl | 132 (59.0-302.0) | 134 (22.0-418.0) | 133 (22.0-418.0) |

Abbreviations: ALT, alanine aminotransferase; HCV, hepatitis C virus; MELD, Model for End-Stage Liver Disease; peg-IFN, pegylated interferon; RBV, ribavirin; SOF, sofosbuvir

a. Data are presented as No. (%) unless otherwise indicated

**Treatment Response**

SVR12 was achieved by 107 of 178 (60.1%) patients treated with SOF/RBV. The reasons for virological failure included relapse in 50 (28%) patients, viral breakthrough in 6 patients (3.4%), and lack of efficacy in 1 patient (<0.5%). Fourteen patients (8%) were lost to posttreatment follow-up and were counted as failing treatment. Of those who received SOF/peg-IFN/RBV, 16 of 19 (84%) achieved SVR12; 1 was a nonresponder and 2 relapsed (Table 8.2).

In the per-protocol population, the SVR12 rate was similar in patients without cirrhosis who received SOF/RBV, whether treatment naive (41/46 [89%]) or experienced (22/25 [88%]). In patients with cirrhosis treated with SOF/RBV, the SVR12 rate was 58% (19 of 33) in treatment-naive patients and fell to 42% (22 of 52) in those who had failed prior therapy (Table 8.2). In contrast, of 8 treatment-experienced patients with cirrhosis who received SOF/peg-IFN/RBV, 7 (88%) achieved SVR12 (Table 8.2). Overall, treatment with SOF/RBV was associated with a lower rate of SVR than treatment with

**Table 8.2 | Virological response in patients with G3 HCV who completed therapy with sofosbuvir and ribavirin +/- peginterferon**

| Treatment Regimen | SOF/PEG/RBV (n=19) | | | | SOF/RBV (n=178) | | | | Total (n=197) |
|---|---|---|---|---|---|---|---|---|---|
| Overall $SVR_{12}$ (Evaluable) | 16/19 (84.2%) | | | | 107/178 (60.1%) | | | | 123/197 (62.4%) |
| Per Protocol $SVR_{12}$ | 16/18 (88.9%) | | | | 104/156 (66.7%) | | | | 120/174 (69.0%) |
| | **Non-Cirrhotic** | | **Cirrhotic** | | **Non-cirrhotic** | | **Cirrhotic** | | |
| Overall $SVR_{12}$ (Evaluable) | 87.5% (7/8) | | 81.8% (9/11) | | 78.1% (64/82) | | 44.8% (43/96) | | |
| Per Protocol $SVR_{12}$ | 87.5% (7/8) | | 90% (9/10) | | 88.7% (63/71) | | 48.2% (41/85) | | |
| | **TN** | **TE** | **TN** | **TE** | **TN** | **TE** | **TN** | **TE** | |
| SVR12 (Evaluable) | 100% (4/4) | 75% (3/4) | 66.7% (2/3) | 87.5% (7/8) | 75.0% (42/56) | 84.6% (22/26) | 55.3% (21/38) | 37.9% (22/58) | |
| SVR12 (PP) | 100% (4/4) | 75% (3/4) | 100% (2/2) | 87.5% (7/8) | 89.1% (41/46) | 88.0% (22/25) | 57.6% (19/33) | 42.3% (22/52) | |
| Virological failure (EP) | | | | | | | | | |
| Breakthrough | - | - | - | - | 1 | 1 | 2 | 2 | 6 |
| Relapse | - | 1 | - | 1 | 7 | 3 | 12 | 28 | 52 |
| Non-response | - | - | 1 | - | - | - | - | 1 | 2 |
| Non-virological failure | - | - | - | - | 6 | - | 3 | 5 | 14 |

Abbreviations: EP, evaluable population; peg-IFN, pegylated interferon; PP, per protocol; RBV, ribavirin; SOF, sofosbuvir; $SVR_{12}$, sustained virological response at 12 weeks; TE, treatment experienced; TN, treatment naïve

SOF/peg-IFN/RBV (OR, 0.25; 95% CI, .06–1.13), but the difference did not reach statistical significance given the few patients treated with triple therapy. After controlling for the presence of cirrhosis, neither treatment history nor treatment duration was associated with SVR.

After controlling for age and sex, the absence of cirrhosis (OR, 6.4; 95% CI, 2.78–14.74), albumin levels of ≥3.2 g/dL (OR, 12.48; 95% CI, 3.86–40.33), and platelet count $\geq 10^5$ cells/μL (OR, 7.44; 95% CI, 3.51–15.78) were associated with greater odds of achieving SVR12, whereas male sex (OR, 0.44; 95% CI, .22–.86) and increasing baseline bilirubin levels (OR, 0.37; 95% CI, .22–.63) were negative predictors of response (Figure 8.1).

Among patients with cirrhosis, after controlling for age and sex, patients with baseline albumin ≥3.2 g/dL (OR, 7.13; 95% CI, 2.09–24.32) and those with platelet count $\geq 10^5$ cells/μL (OR, 5.27; 95% CI, 1.84–15.08) were more likely to achieve SVR. Increasing baseline total bilirubin (OR, 0.43; 95% CI, .23–.79), baseline MELD score (OR, 0.75; 95% CI, .61–.92), and history of decompensating events (OR,

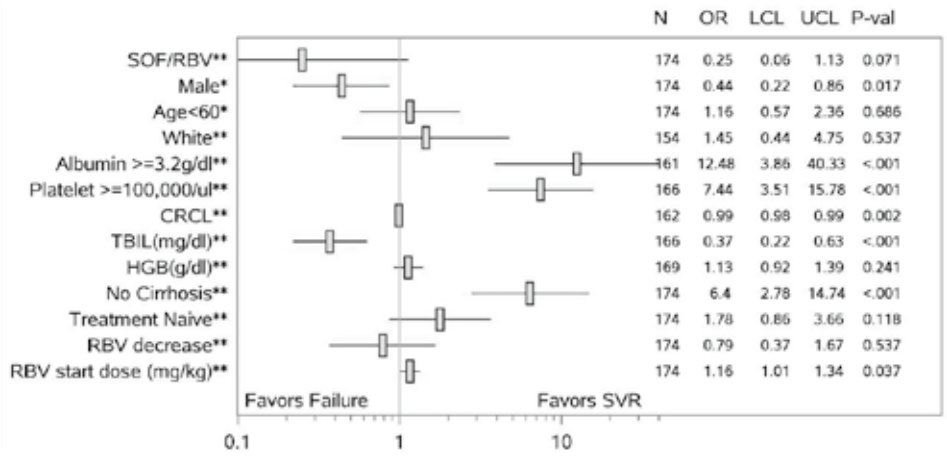


**Figure 8.1 |** Baseline predictors of sustained virologic response (SVR) among patients with genotype 3 hepatitis C virus treated with sofosbuvir (SOF) and ribavirin (RBV) with or without peginterferon with available virological outcomes. Factors associated with SVR are shown with an estimate of the odds ratios (ORs) with 95% confidence interval determined by multivariable logistic regression for each variable controlled for either age and sex**, or age or sex*. Abbreviations: CrCl, creatinine clearance; HGB, hemoglobin; LCL, lower confidence limit; TBIL, total bilirubin; UCL, upper confidence limit.

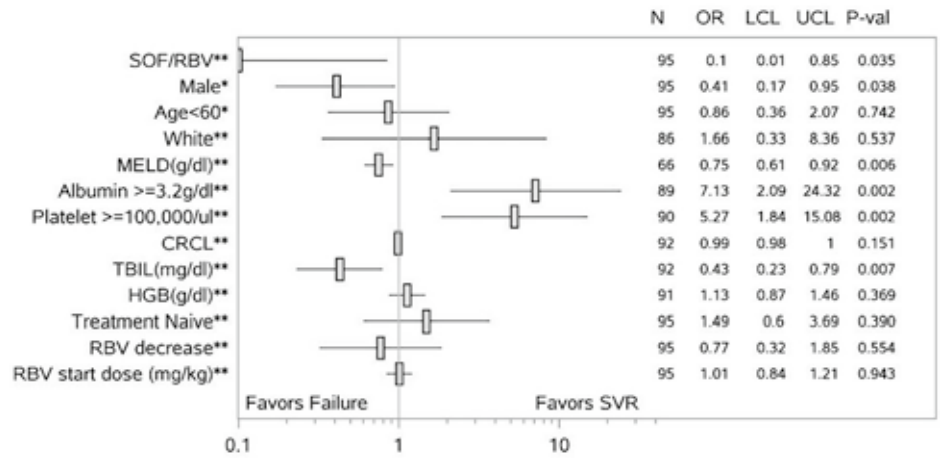


**Figure 8.2 |** Baseline predictors of sustained virological response (SVR) among patients with genotype 3 hepatitis C virus and cirrhosis treated with sofosbuvir (SOF) and ribavirin (RBV) with or without peginterferon with available virological outcomes. Factors associated with SVR among patients with cirrhosis are shown with an estimate of the odds ratios (ORs) with 95% confidence interval determined by multivariable logistic regression for each variable controlled for either age and sex**, or age or sex*. Abbreviations: CrCl, creatinine clearance; HGB, hemoglobin; LCL, lower confidence limit; MELD, Model for End-Stage Liver Disease; TBIL, total bilirubin; UCL, upper confidence limit.

0.29; 95% CI, .12–.70) were each associated with a decreased likelihood of SVR (Figure 8.2). The SVR rate in patients treated with SOF/RBV with compensated cirrhosis was 64.8% (35/54) compared with 36% (4/11) in those with a history of decompensated disease ($p$ = .08). Baseline HCV RNA was not predictive of SVR. Week 4 HCV RNA values were available in 153 patients (81%) and although patients with undetectable HCV RNA by week 4 were more likely to achieve SVR, this did not reach statistical significance ($p$ = .09).

**Adverse Events**

At least 1 AE was reported by 86% (169/197), whether treated with SOF/RBV or SOF/peg-IFN/RBV (Table 8.3). The most commonly reported AEs were fatigue (40%), headache (21%), and anemia (20%) (Table 8.3). Rash was reported in 5 (26%) patients treated with SOF/peg-IFN/RBV and 23 (13%) of those treated with SOF/RBV. Depression was documented in 25 (14%) patients on SOF/RBV compared to none on SOF/peg-IFN/RBV. In total, 11 patients experienced hepatic decompensation during therapy, all of whom had a prior history of decompensation (Table 8.4). Hepatic encephalopathy (n = 10) was the most frequently reported decompensating event, followed by ascites (n = 2) and variceal hemorrhage (n = 2).

Anemia was reported in 37 (21%) patients on SOF/RBV and 3 (16%) patients on SOF/peg-IFN/RBV. The median hemoglobin decline was 2.1 g/dL in SOF/RBV-treated patients, and 2.7 g/dL in patients treated with SOF/peg-IFN/RBV. Anemia led to RBV dose reduction in 33 (18.5%) and discontinuation in 2 (10.5%) patients. The initial RBV dose ranged from 4.6 mg/kg to 19.2 mg/kg (mean, 13.1 mg/kg). As shown in the forest plots (Figures 8.1 and 8.2), initial RBV dose was not associated with SVR in those with cirrhosis but was associated with SVR in the overall population, suggesting the effect was greatest in those without cirrhosis. The correlation between RBV dose and SVR is shown in Figure 8.3. RBV dose reduction was not associated with SVR (Figure 8.1 and 8.2). Addition of erythropoietin was needed by 7 patients (4%) and a blood transfusion by 4 (2%) individuals, all in patients treated with SOF/RBV. A total of 18 SAEs were reported in 13 patients (all treated with SOF/RBV), of which 8 SAEs in 7 patients were likely related to HCV therapy per the treating physician.

## DISCUSSION

This large observational study evaluated the effectiveness and safety of 2 SOF-based therapies in patients with GT3 HCV infection. Although treatment was generally safe and fairly well tolerated, the SVR12 results were suboptimal (69%), particularly in patients with cirrhosis treated with SOF/RBV alone, highlighting the need for improved therapies for this population.

As reported in clinical trials, the main determinant of outcome in GT3 infection was the presence of cirrhosis.[137-139] Less than half (48%) of those with cirrhosis treated with SOF/ RBV achieved SVR12. Notably, cirrhosis was a negative predictor of response even in patients who were previously untreated. Although the VALENCE trial reported a 92% SVR rate in treatment- naive patients with cirrhosis, only 13 such patients were included in the trial.[138] In this real-world study, only 19 of the 33 (58%) treatment-naive patients with cirrhosis achieved SVR12. Whether this reflects differences

**Table 8.3 | Adverse events, and management of anemia**

| | SOF/PEG/RBV (n=19) | SOF/RBV (n=178) | Total (n=197) |
|---|---|---|---|
| **Event** | **N Patients (%)** | **N Patients (%)** | **N Patients (%)** |
| Any adverse event (AE) | 15 (79.0) | 154 (86.5) | 169 (85.8) |
| Fatigue | 8 (42.1) | 70 (39.3) | 78 (39.6) |
| Headache | 4 (21.1) | 38 (21.4) | 42 (21.3) |
| Anemia | 3 (15.8) | 37 (20.8) | 40 (20.3) |
| Insomnia | 2 (10.5) | 35 (19.7) | 37 (18.8) |
| Nausea | 1 (5.3) | 33 (18.5) | 34 (17.3) |
| Rash | 5 (26.3) | 23 (12.9) | 28 (14.2) |
| Dyspnea | 2 (10.5) | 25 (14.0) | 27 (13.7) |
| Influenza-like illness | 5 (26.3) | 21 (11.8) | 26 (13.2) |
| Depression | 0 (0.0) | 25 (14.0) | 25 (12.7) |
| Dizziness | 2 (10.5) | 21 (11.8) | 23 (11.7) |
| Irritability | 3 (15.8) | 19 (10.7) | 22 (11.2) |
| Pruritus | 2 (10.5) | 20 (11.2) | 22 (11.2) |
| Decreased appetite | 3 (15.8) | 15 (8.4) | 18 (9.1) |
| Abdominal pain | 3 (15.8) | 13 (7.3) | 16 (8.1) |
| Asthenia | 2 (10.5) | 13 (7.3) | 15 (7.6) |
| Diarrhea | 2 (10.5) | 11 (6.2) | 13 (6.6) |
| Muscle spasms | 2 (10.5) | 11 (6.2) | 13 (6.6) |
| Cough | 2 (10.5) | 11 (6.2) | 13 (6.6) |
| Serious adverse events (18 events)[b] | **0 (0.0)** | **13 (7.3)** | **13 (6.6)** |
| Anemia management | | | |
| RBV dose reduction | 2 (10.5) | 33 (18.5) | 35 (17.8) |
| Erythropoietin use | 0 (0.0) | 7 (3.9) | 7 (3.6) |
| Blood transfusion | 0 (0.0) | 4 (2.2) | 4 (2.0) |
| RBV discontinuation | 0 (0.0) | 1 (0.6) | 1 (0.5) |

Abbreviations: peg-IFN, pegylated interferon; RBV, ribavirin; SOF, sofosbuvir.

a. Data are presented as No. (%) of patients.

b. Anemia, colitis, gastrointestinal hemorrhage, esophageal varices hemorrhage, chest pain, hepatocellular carcinoma (2), hepatic encephalopathy (4), dizziness (2), depression, psychotic disorder, renal failure acute (2), epistaxis.

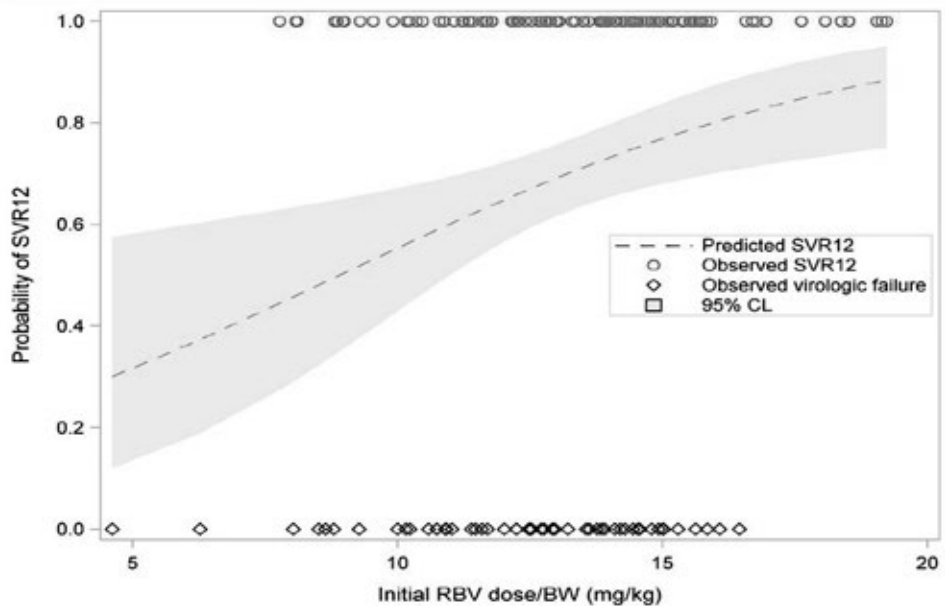


**Figure 8.3 |** Correlation of probability of sustained virological response at 12 weeks (SVR12) and initial ribavirin (RBV) dose (by body weight [BW]; mg/kg). The impact of the initial dose of RBV is shown by estimating the probability of SVR according to initial RBV dose using observed SVR (circle) and observed virological failure (diamond), The shaded area shows the 95% confidence interval (CI) for the estimated correlation.

between clinical trials and clinical practice, or inclusion of patients with more advanced disease, or highlights the uncertainty of drawing conclusions from the small numbers included in the trial is difficult to determine.

How cirrhosis affects response to therapy is not clear but is likely multifactorial.[339] Data from patients with genotype 1 infection suggest that even in a cirrhotic liver, SOF is effectively taken up by hepatocytes and phosphorylated to the active compound.[339] Shunting due to portal hypertension may affect local drug concentrations and cirrhosis has important effects on innate and adaptive immune function, which are likely still important for viral clearance even with potent DAA therapy.[339] Younossi and colleagues recently reported that patients with GT3 infection had lower levels of lipids and, specifically, lower levels of metabolites from late in the cholesterol biosynthesis pathway.[330] Although the specific mechanisms remain unclear, these findings support the hypothesis that the unique effects of GT3 HCV on lipid metabolism may affect responses to antiviral therapy, an effect that appears to be more pronounced in the presence of cirrhosis. Unfortunately, cirrhosis is important even when SOF is combined with other approved DAAs for GT3 infection. Only 63% of patients with cirrhosis achieved SVR when SOF was combined with DCV for 12 weeks.[163] In the small ALLY 3+ study, patients with GT3 HCV and cirrhosis who received SOF/DCV plus RBV for 12 or 16 weeks achieved SVR rates of 83%–89%; however, the small sample size makes it difficult to draw strong conclusions or to distinguish the preferred duration.[164] Additional data from the French compassionate use program showed that 24 weeks of SOF/DCV with or without RBV led to SVR rates >80% despite inclusion of

**Table 8.4 | Details on 14 decompensation events in 11 patients during antiviral therapy**

| Patient ID | Decompensation Event | Regimen | Releated to Treatment | Timing of Event, wk | Past History of Decompensation | Treatment Outcome |
|---|---|---|---|---|---|---|
| 1 | Hepatic Encephalopathy | SOF/RBV | N | 8.3 | Y | SVR |
| 2 | Hepatic Encephalopathy | SOF/RBV | Y | 16.1 | Y | Relapse |
| 3 | Variceal Hemorrhage | SOF/RBV | N | 11.7 | Y | Relapse |
| 4 | Hepatic Encephalopathy | SOF/RBV | Y | 4.9 | Y | Relapse |
| 4 | Hepatic Encephalopathy | SOF/RBV | Y | 24.4 | Y | Relapse |
| 5 | Variceal Hemorrhage | SOF/RBV | Y | 0.4 | Y | SVR |
| 6 | Hepatic Encephalopathy | SOF/RBV | Y | 0.1 | Y | Relapse |
| 7 | Hepatic Encephalopathy | SOF/RBV | Y | 19.4 | Y | SVR |
| 7 | Hepatic Encephalopathy | SOF/RBV | Y | 16.6 | Y | SVR |
| 8 | Hepatic Encephalopathy | SOF/RBV | Y | 0.1 | Y | LTFU |
| 8 | Ascites | SOF/RBV | Y | 9 | Y | LTFU |
| 9 | Hepatic Encephalopathy | SOF/RBV | N | 4.3 | Y | BT |
| 10 | Hepatic Encephalopathy | SOF/RBV | Y | 4.9 | Y | BT |
| 11 | Ascites | SOF/RBV | Y | 13.3 | Y | SVR |

Abbreviations: BT, breakthrough (on treatment); LTFU, lost to posttreatment follow-up; SOF/RBV, sofosbuvir/ribavirin; SVR, sustained virological response

some patients with decompensated disease.[340] SOF/RBV plus LDV has also been evaluated in GT3 HCV. With the very limited activity of LDV against GT3 in vitro, it was perhaps surprising to see SVR rates as high as 73% in patients with cirrhosis.[341] These results clearly leave room for improvement for patients with GT3 infection and cirrhosis.

The disappointing outcomes with SOF/RBV therapy led to evaluation of the reintroduction of peg-IFN. Similar to the initial small studies evaluating this approach, the SOF/peg-IFN/ RBV regimen proved quite effective in this cohort, most notably in the 8 patients with cirrhosis who had failed prior therapy, 7 of whom achieved SVR. A randomized controlled trial comparing SOF/RBV for 16 or 24 weeks to SOF/peg-IFN/RBV for 12 weeks in patients with GT3 HCV[140] showed that the inclusion of peg-IFN increased response rates in all patient subgroups but most notably in those with cirrhosis, whether treatment naïve or experienced. Peg-IFN was well tolerated, with only 1 patient discontinuing treatment prematurely. Similarly, in this real-world experience, peg-IFN was well tolerated, with similar

rates of AEs and higher rates of SVR reported compared with SOF/ RBV alone. However, despite these encouraging results, SOF/ peg-IFN/RBV was prescribed to only 19 of the 197 (9.6%) patients with GT3 who have started therapy in HCV-TARGET to date. Clearly there is a great reluctance by both clinicians and patients to accept interferon-based therapies. Fortunately, recent studies have shown that SOF combined with velpatasvir, a pangenotypic NS5A inhibitor, is highly effective for GT3 HCV infection, leading to SVR12 rates of 90% even in patients with cirrhosis, without the need for RBV.[168] Other promising DAA combinations hold promise for GT3 HCV as well.[342] With this in mind, although SOF/ peg-IFN/RBV may be considered for patients, particularly those with cirrhosis, waiting for approval of new therapies may be prudent, as it is unclear if an unsuccessful course of SOF/RBV or SOF/DCV will affect responses and/or access to future therapies.

Among patients with cirrhosis, low baseline albumin and low platelet count were associated with much lower odds of achieving SVR12. Only 20% of patients with albumin levels <3.2 g/dL achieved SVR, whereas 39% with baseline platelet count $<10^5$ cells/µL achieved SVR12 with SOF/RBV. Of the 18 patients with albumin <3.2 g/dL and platelets $<10^5$ cells/µL, only 4 (22%) achieved SVR, suggesting that for such individuals, waiting for availability of new multi-DAA regimens would be advisable. This study has some important limitations. The lack of randomization limits the ability to directly compare treatment groups, which is further compounded by the small number of patients who received SOF/ peg-IFN/RBV therapy. However, the results clearly demonstrate the limitations of SOF/RBV in patients with GT3 HCV infection and cirrhosis. In addition, by including a broader population of patients, many of whom may not have qualified for clinical trials, the safety and effectiveness profile provide very useful data for clinicians and patients.

## CONCLUSIONS

This large observational, real-world study demonstrated that GT3 remains a clinical challenge. SOF/ RBV for 24 weeks resulted in low SVR rates in patients with cirrhosis, particularly those with low baseline albumin and platelet levels. Although high efficacy and acceptable tolerability were seen in patients treated with SOF/peg-IFN/RBV, the extremely low uptake of this regimen highlights the poor acceptance of peg-IFN among patients and clinicians. Therapies in development will hopefully improve outcomes for this difficult-to-cure population.

# SUPPLEMENTARY TABLE

**Supplementary Table 8.1 |** Disposition of patients with G3 HCV who underwent therapy with sofosbuvir and ribavirin +/- peginterferon

| | SOF PEG RBV | SOF RBV | Total |
|---|---|---|---|
| | (N=19) | (N=191) | (N=210) |
| Started treatment | 19 (100.0%) | 191 (100.0%) | 210 (100.0%) |
| Treatment ongoing | 0 (0.0%) | 1 (0.5%) | 1 (0.5%) |
| Discontinued prematurely | 1 (5.3%) | 23 (12.0%) | 24 (11.4%) |
| Adverse event | 0 (0.0%) | 5 (2.6%) | 5 (2.4%) |
| Lack of efficacy | 0 (0.0%) | 5 (2.6%) | 5 (2.4%) |
| Withdrawal by subject | 0 (0.0%) | 1 (0.5%) | 1 (0.5%) |
| Lost to follow up | 0 (0.0%) | 5 (2.6%) | 5 (2.4%) |
| Non compliance | 1 (5.3%) | 3 (1.6%) | 4 (1.9%) |
| Other | 0 (0.0%) | 4 (2.1%) | 4 (1.9%) |
| Completed treatment | 18 (94.7%) | 167 (87.4%) | 185 (88.1%) |
| Lost to post-treatment follow-up | 0 | 14 | 14 |
| In post treatment follow up | 0 | 6 | 6 |

Abbreviations: PEG, pegylated infterferon; RBV, ribavirin; SOF, sofosbuvir

# CHAPTER 9

## FREQUENCY OF RENAL IMPAIRMENT IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION TREATED WITH SOFOSBUVIR-BASED REGIMENS

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## ABSTRACT

**Background & Aims**

Guidelines recommend withholding sofosbuvir (SOF) in patients with an estimated glomerular filtration rate (eGFR) of less than 30 mL/min. However, little is known about the risk of renal impairment (RI) in patients with no renal contraindications for SOF-based treatment.

**Methods**

This multicenter retrospective observational study included all consecutive patients that were treated with SOF-based or telaprevir (TVR)/boceprevir (BOC)-based regimens at two tertiary university centers in North America. RI was defined as an increase of ≥0.3 mg/dL in serum creatinine level. Multivariable logistic regression analysis was used to identify risk factors for the occurrence of RI.

**Results**

In total, 426 patients were included and treated with a SOF-based regimen (n=233, 54.7%) or TVR/BOC-based regimen (n=193, 45.3%). Among patients treated with a TVR/BOC-based regimen 34 (18%) of 193 patients experienced RI compared to 26 (11%) of 233 patients treated with SOF-based regimens ($p$ = 0.056). Multivariable logistic regression analysis showed that the presence of ascites (OR 4.44, 95%CI 1.46-13.54, $p$ = 0.009) and the use of NSAIDs (OR 4.47, 95%CI 1.32-15.19, $p$ = 0.016) were associated with a risk of RI during SOF-based antiviral therapy. Creatinine levels returned to normal at end of follow-up in 23 (88%) of the 26 patients that experienced RI and had a creatinine level available during follow-up.

**Conclusions**

Although the risk for RI was lower than for patients treated TVR/BOC-based regimens, RI was seen during 11% of SOF-based regimens and was mostly reversible. Patients with ascites and patients using NSAIDs have an increased risk for RI during SOF-based antiviral therapy.

## INTRODUCTION

It has been estimated that 64-103 million people worldwide are chronically infected with the hepatitis C virus (HCV).[1] The continuous inflammatory activity in the liver may lead to hepatic fibrosis and subsequently to cirrhosis. Although not linear, approximately 15-20% of patients with chronic HCV infection will develop cirrhosis within 20 years after their encounter with the virus.[61] These patients have an increased risk of developing liver failure and hepatocellular carcinoma (HCC), associated with an increased mortality rate.[55] However, chronic HCV infection is a multifaceted disease, which is not restricted to the liver. Extrahepatic manifestations, including mixed cryoglobulinemic vasculitis and renal disease, may occur long before cirrhosis has been established.[45] Notably, the non-hepatic complications of HCV are clinically relevant, with a recent study documenting increased non-liver-related mortality among patients with HCV.[343]

Until 2011, physicians depended on the administration of pegylated interferon alpha (PegIFN) and ribavirin (RBV) for the treatment of chronic HCV infection. These regimens were suboptimal in most patients and were associated with important safety issues.[104-106] The introduction of the first protease inhibitors, telaprevir (TVR) and boceprevir (BOC), for the treatment of HCV genotype 1 led to improved sustained virological response (SVR) rates. Although the registration trials reported a relatively benign renal safety profile, shortly after approval, data emerged on significant renal adverse events[344 345] along with a more significant overall adverse event profile than that documented in the registration trials.[120 121] The approval of sofosbuvir (SOF), a very potent nucleotide analogue inhibitor of the HCV RNA-dependent RNA polymerase, has revolutionized the treatment of chronic HCV infection. SOF is active against all HCV genotypes and when combined with other direct-acting antivirals (DAAs) leads to very high rates of SVR in most patient populations.[141 147] The main active metabolite of SOF, GS331007, is excreted by the kidneys, and higher drug and metabolite concentrations are found in those patients with an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m2 or end-stage renal disease (ESRD) on hemodialysis.[346] Therefore, guidelines do not recommend the use of SOF in patients with an eGFR <30mL/min or patients with ESRD, and recommend regular monitoring of renal function in patients receiving SOF.[35 36] The major trials examining the safety and efficacy of SOF demonstrated an excellent renal safety profile[139 141 147], however whether this also applies in real-world clinical practice is currently unknown. Therefore, we aimed to examine the renal safety profile in patients treated with SOF-based therapies, and compared it to the profile of TVR/BOC-based antiviral therapy.

## PATIENTS AND METHODS

**Study population**

This multicenter retrospective observational study included all consecutive patients who initiated treatment with a SOF-based or TVR/BOC-based regimen prior to January 31st, 2015. Patients were treated in the Toronto Centre for Liver Disease or the University of Arkansas for Medical Sciences. All included patients had available creatinine results before, during and after therapy and all creatinine levels were evaluated. Patients on hemodialysis or treated post-liver transplantation were excluded.

All charts were reviewed by five investigators (RM, SA, JSK, SMH and KDR) in order to collect detailed data on baseline demographics, on-treatment laboratory results and clinical events and outcome of therapy.

**Definitions**

On-treatment RI was defined as an increase of ≥0.3 mg/dL or ≥50% in serum creatinine level when compared to baseline value.[347] Normalization of creatinine was considered whenever follow up determinations were no more than 0.2 mg/dL from baseline. The eGFR was calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation: eGFR (mL/min/1.73m2) = 186 x (creatinine / 88.4) -1.154 x (age) -0.203 x (0.742 if female) x (1.210 if black). The presence of cirrhosis was based on liver biopsy, transient elastography (kPa>13), or clinical suspicion (i.e. the presence of varices, ascites, hepatic encephalopathy). The Child-Turcotte-Pugh (CTP) score was used to make a distinction between compensated and decompensated cirrhosis (decompensated if CTP ≥7 points). An undetectable viral load 12 weeks after cessation of SOF-based therapy and 24 weeks after TVR/BOC-based therapy was considered an SVR. The Abbott RealTime HCV assay (Abbott Molecular, Des Plaines, IL, USA) with a lower limit of quantification of 12 IU/mL, and Roche COBAS AmpliPrep/TaqMan 48 HCV Test (Roche Diagnostics, Indianapolis, IN, USA) with a lower limit of quantification of 15 IU/mL, were used in Canada and USA, respectively.

**Outcomes**

The primary outcome of this study was the occurrence of RI during antiviral therapy with SOF-based and TVR/BOC-based regimens. Secondary outcomes included factors associated with the occurrence of RI, the influence of RI on attaining SVR, and the on-treatment dynamics of creatinine among patients who experienced RI.

**Statistical analyses**

Data were presented as means with standard deviation (SD), medians with interquartile range (IQR) or as frequency (percentage), as appropriate. For comparisons of patients treated with SOF-based regimens versus TVR/BOC-based regimens, Student's t-test, Chi-square, Pearson correlation or their non-parametric equivalents were used.

Univariable logistic regression analyses were used to assess factors associated with RI. All clinical factors of importance, including age, the presence of cirrhosis, CTP score, Model for End-stage Liver Disease (MELD) score, the presence of ascites, hypertension, the use of nonsteroidal anti-inflammatory drugs (NSAIDs), and antiviral treatment regimens were included in multivariable analyses. The original MELD score was used for calculations in order to avoid the flooring effect from the United Network for Organ Sharing (UNOS)-modified version. Results are presented with an odds ratio and 95% confidence intervals. The dynamics of on-treatment creatinine levels were plotted for all patients, with a special focus on patients who experienced RI. A $p$ value <0.05 was considered statistically significant and all statistical tests were two-tailed. The significance level of interactions was set at 0.01 in order to correct for multiple testing. Analyses were performed using PASW statistics 21.0 for Windows (SPSS, IBM, Armonk, New York, USA) and R statistical software version 3.2.2 (online available).

## RESULTS

### Patients

In total, 426 patients with chronic HCV infection who underwent antiviral treatment with SOF-based regimens (n=233, 54.7%) or TVR/BOC-based regimens (n=193, 45.3%) were included. Table 9.1 shows baseline characteristics according to the treatment regimen applied. Mean age was 54.7 (±9.0) years, 253 (59.4%) patients were male and 221 (52.0%) had evidence of cirrhosis. Of those patients treated with a SOF-based regimen, 68 (29.2%) received SOF/simeprevir (SMV) ± RBV, 77 (33.0%) received SOF/PegIFN/RBV, 77 (33.0%) received SOF/RBV, and 11 (4.7%) received SOF/ledipasvir (LDV) ± RBV. A total of 44 patients (22.8%) received BOC/PegIFN/RBV and 149 (77.2%) patients received TVR/PegIFN/RBV. At baseline, a higher proportion of patients treated with SOF-based regimens had evidence of cirrhosis, including decompensated cirrhosis, and they had higher MELD scores compared to patients treated with TVR/BOC-based regimens. Supplementary Table 9.1 shows baseline characteristics of the 32 (7.5%) patients with a baseline eGFR ≤60 mL/min of whom none had a baseline eGFR <30 mL/min.

### Renal impairment

From the total cohort of 426 patients, 60 (14.1%) patients experienced a rise in creatinine ≥0.3 mg/dL during antiviral treatment. Of these, 34 (17.6%) patients treated with TVR/BOC-based regimens experienced RI compared to 26 (11.2%) patients who received SOF-based regimens ($p = 0.056$). Table 9.2 shows the characteristics of the patients who experienced RI and were treated with SOF-based antiviral therapy. Among the 13 patients with human immunodeficiency virus (HIV)/HCV coinfection, one (7.7%) experienced RI. Six (18.8%) of 32 patients with a baseline eGFR ≤60 mL/min experienced RI. When analyzing patients with an increase in creatinine ≥50%, 14 (7.3%) patients treated with TVR/BOC-based regimens experienced RI, compared to 8 (3.4%) treated with SOF-based regimens ($p = 0.076$). In order to better identify the risk factors potentially associated with RI we used the ≥0.3 mg/dL definition for subsequent analyses.

Univariable logistic regression analyses showed that higher age (OR 1.06, 95%CI 1.02-1.10, $p = 0.004$), lower baseline eGFR (OR 0.99, 95%CI 0.97-1.00, $p = 0.035$), lower baseline albumin (OR 0.44, 95%CI 0.27-0.40, $p = 0.001$), CTP class B/C (OR 2.26, 95%CI 1.19-4.32, $p = 0.013$), the presence of ascites (OR 3.29, 95%CI 1.73-6.22, $p <0.001$), hypertension (OR 2.66, 95%CI 1.52-4.65, $p = 0.001$), the use of NSAIDs (OR 2.41, 95%CI 1.02-5.69, $p = 0.045$) and the use of angiotensin-converting-enzyme inhibitors (ACE) or angiotensin II receptor antagonists (ARA) (OR 1.96, 95%CI 1.07-3.58, $p = 0.029$) were significantly associated with the occurrence of RI (Table 9.3). As the presence of ascites and the use of diuretics were highly collinear, only the presence of ascites was used in the model in order to avoid redundancy. There was a trend toward a lower risk of RI with SOF-based regimens compared to TVR/BOC-based regimens (OR 0.59, 95%CI 0.34-1.02, $p = 0.058$). When these parameters were tested in a multivariable logistic regression model adjusted for age and MELD score, only the presence of ascites (OR 4.69, 95%CI 2.10-10.5, $p <0.001$), hypertension (OR 2.96, 95%CI 1.58-5.55, $p = 0.001$), and the use of NSAIDs (OR 2.69, 95%CI 1.08-6.72, $p = 0.034$) remained independently associated with an increased risk for RI; whereas the use of a SOF-based regimen (OR 0.40, 95%CI 0.22-0.74, $p = 0.004$) was

**Table 9.1 | Baseline characteristics according to the treatment regimen that was applied**

| Variable | Total N=426 | TVR/BOC-based therapy n=193 | SOF-based therapy n=233 | *p* value |
|---|---|---|---|---|
| Age | 54.7 (±8.98) | 54.0 (±8.85) | 54.9 (±9.20) | 0.62 |
| Male sex | 253 (59.4%) | 120 (62.2%) | 133 (57.1%) | 0.29 |
| BMI | 28.7 (±6.04) | 29.0 (±5.74) | 28.4 (±6.26) | 0.27 |
| | | | | |
| HCV genotype | | | | Not |
| *1* | 355 (83.3%) | 192 (99.5%) | 163 (70.0%) | performed |
| *2* | 42 (9.9%) | - | 42 (18.0%) | |
| *3* | 20 (4.7%) | - | 20 (8.6%) | |
| *4* | 8 (1.9%) | 1 (0.5%) | 7 (3.0%) | |
| *6* | 1 (0.2%) | - | 1 (0.4%) | |
| Cirrhosis | 221 (51.9%) | 88 (45.6%) | 133 (57.3%) | 0.016 |
| CTP B/C | 68 (16.0%) | 23 (11.9%) | 45 (19.3%) | 0.040 |
| MELD score | 5.2 (±3.8) | 4.7 (±3.4) | 5.7 (±4.0) | <0.001 |
| | | | | |
| Cryoglobulins (+)[b] | 94 (25.7%) | 36 (22.9%) | 58 (27.8%) | 0.30 |
| Proteinuria[c] | 60 (21.1%) | 29 (22.7%) | 31 (19.7%) | 0.65 |
| Diabetes mellitus | 79 (18.5%) | 35 (18.1%) | 44 (18.9%) | 0.84 |
| Hypertension | 168 (39.4%) | 72 (37.3%) | 96 (41.2%) | 0.41 |
| NSAIDs | 30 (7.0%) | 12 (6.2%) | 18 (7.7%) | 0.55 |
| ACE/ARA | 89 (20.9%) | 36 (18.7%) | 53 (22.7%) | 0.30 |
| Diuretics | 101 (23.7%) | 41 (21.2%) | 60 (25.8%) | 0.32 |
| | | | | |
| Creatinine | 0.86 (±0.20) | 0.85 (±0.21) | 0.86 (±0.19) | 0.42 |
| eGFR | 87.5 (±20.8) | 90.0 (±22.5) | 85.4 (±19.2) | 0.022 |
| Platelet count | 167 (±89) | 177 (±90) | 159 (±87) | 0.038 |
| ALT | 63 (39-100) | 61 (37-103) | 63 (40-100) | 0.50 |

Abbreviations: TVR, telaprevir; BOC, boceprevir; SOF, sofosbuvir; BMI, body mass index; HCV, hepatitis c virus; CTP, Child-Turcotte-Pugh; MELD, Model for End-stage Liver Disease; NSAIDs, nonsteroidal anti-inflammatory drugs; ACE, angiotensin-converting-enzyme inhibitors; ARA, angiotensin II receptor antagonists; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase

a. Data are presented as n (%), mean (±SD) or median (interquartile range), p-values are the result of Student's t-test, Chi-square, Pearson correlation or their non-parametric equivalents.
b. Missing for 60 (14.1%) patients
c. Spot or quantitative proteinuria not tested in 141 (33.1%) patients

**Table 9.2 | Characteristics of patients experiencing renal impairment during SOF-based therapy**

| Patient | Age | Sex | Therapy | Cirrhosis | Baseline eGFR | Baseline creatinine | Maximum creatinine | Hypertension | NSAIDs | Reversible RI |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 65 | Female | SOF/SMV±RBV | CTP B/C | 84.0 | 0.7 | 1.0 | Yes | No | Yes |
| 2 | 58 | Male | SOF/SMV±RBV | CTP B/C | 86.7 | 0.9 | 1.3 | Yes | No | Yes |
| 3 | 55 | Female | SOF/SMV±RBV | No | 86.9 | 0.7 | 1.0 | Yes | No | Yes |
| 4 | 59 | Male | SOF/SMV±RBV | CTP B/C | 68.5 | 1.1 | 1.4 | Yes | No | Yes |
| 5 | 59 | Female | SOF/SMV±RBV | CTP A | 85.6 | 0.7 | 1.0 | Yes | Yes | Yes |
| 6 | 57 | Female | SOF/RBV | CTP B/C | 86.2 | 0.7 | 1.5 | No | No | Yes |
| 7 | 50 | Female | SOF/RBV | CTP B/C | 66.3 | 0.9 | 1.2 | No | No | Yes |
| 8 | 53 | Male | SOF/RBV | CTP B/C | 78.2 | 1.0 | 1.3 | No | Yes | Yes |
| 9 | 59 | Female | SOF/RBV | CTP A | 73.4 | 0.8 | 1.1 | Yes | No | Yes |
| 10 | 48 | Male | SOF/RBV | CTP B/C | 90.1 | 0.9 | 1.4 | No | No | Yes |
| 11 | 60 | Female | SOF/RBV | No | 73.2 | 0.8 | 1.1 | No | No | Yes |
| 12 | 61 | Female | SOF/PEG/RBV | No | 72.9 | 0.8 | 1.2 | Yes | Yes | Yes |
| 13 | 57 | Male | SOF/PEG/RBV | CTP A | 62.4 | 1.2 | 1.6 | Yes | No | Yes |
| 14 | 57 | Male | SOF/PEG/RBV | No | 56.9 | 1.3 | 1.6 | Yes | No | Yes |
| 15 | 63 | Male | SOF/PEG/RBV | No | 85.2 | 0.9 | 2.0 | Yes | No | Yes |
| 16 | 49 | Female | SOF/PEG/RBV | CTP A | 88.9 | 0.7 | 1.2 | No | Yes | Yes |
| 17 | 49 | Female | SOF/PEG/RBV | No | 76.2 | 0.8 | 2.0 | Yes | No | Yes |
| 18 | 52 | Female | SOF/RBV | CTP B/C | 65.8 | 0.9 | 1.3 | Yes | No | Yes |
| 19 | 60 | Male | SOF/SMV±RBV | CTP B/C | 70.0 | 1.1 | 2.0 | No | No | Yes |
| 20 | 69 | Male | SOF/SMV±RBV | CTP A | 64.0 | 1.1 | 2.9 | No | No | Yes |
| 21 | 31 | Male | SOF/RBV | No | 93.0 | 1.0 | 1.3 | No | Yes | Yes |
| 22 | 57 | Male | SOF/SMV±RBV | CTP B/C | 87.0 | 0.9 | 1.4 | No | No | Yes |
| 23 | 44 | Female | SOF/SMV±RBV | CTP B/C | 52.0 | 1.1 | 1.7 | Yes | No | Yes |
| **24[a]** | **66** | **Male** | **SOF/RBV** | **No** | **37.9** | **1.8** | **2.1** | **Yes** | **No** | **No** |
| **25[a]** | **68** | **Female** | **SOF/RBV** | **CTP A** | **55.1** | **1.0** | **3.5** | **No** | **No** | **No** |
| **26[a]** | **61** | **Male** | **SOF/PEG/RBV** | **No** | **85.8** | **0.9** | **1.2** | **Yes** | **No** | **No** |

Abbreviations: SOF, sofosbuvir; eGFR, estimated glomerular filtration rate; NSAIDs, nonsteroidal anti-inflammatory drugs; RI, renal impairment SMV, simeprevir; RBV, ribavirin; CTP, Child-Turcotte-Pugh; PEG, pegylated interferon alpha

a. The three highlighted patients did not have reversible renal impairment.

**Table 9.3 | Logistic regression analyses for renal impairment**

| Variable | Univariable | *p* value | Multivariable | *p* value |
|---|---|---|---|---|
| Age | 1.06 (1.02-1.10) | 0.004 | 1.03 (0.99-1.08) | 0.17 |
| Sex | 0.64 (0.37-1.11) | 0.11 | | |
| BMI | 1.04 (0.99-1.08) | 0.10 | | |
| | | | | |
| Cirrhosis | 1.15 (0.66-1.99) | 0.62 | | |
| MELD score | 1.04 (0.97-1.12) | 0.23 | 1.00 (0.92-1.09) | 0.99 |
| CTP B/C | 2.26 (1.19-4.32) | 0.013 | | |
| Ascites | 3.29 (1.73-6.22) | <0.001 | 4.69 (2.10-10.45) | <0.001 |
| | | | | |
| Hypertension | 2.66 (1.52-4.65) | 0.001 | 2.96 (1.58-5.55) | 0.001 |
| Diabetes mellitus | 1.26 (0.64-2.46) | 0.50 | | |
| NSAIDs | 2.41 (1.02-5.69) | 0.045 | 2.69 (1.08-6.72) | 0.034 |
| Diuretics | 3.22 (1.82-5.68) | <0.001 | | |
| ACE/ARA | 1.96 (1.07-3.58) | 0.029 | | |
| | | | | |
| Creatinine | 1.01 (0.55-1.85) | 0.98 | | |
| eGFR | 0.99 (0.97-1.00) | 0.035 | | |
| ALT | 1.00 (0.99-1.00) | 0.085 | | |
| Albumin | 0.44 (0.27-0.40) | 0.001 | | |
| | | | | |
| SOF-based vs TVR/ BOC-based | 0.59 (0.34-1.02) | 0.058 | 0.40 (0.22-0.74) | 0.004 |

Abbreviations: BMI, body mass index; MELD, Model for End-stage Liver Disease; CTP, Child-Turcotte-Pugh; NSAIDs, nonsteroidal anti-inflammatory drugs; ACE, angiotensin-converting-enzyme inhibitors; ARA, angiotensin II receptor antagonists; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; SOF, sofosbuvir; TVR, telaprevir; BOC, boceprevir

associated with a decreased risk (Table 9.3). There was no significant difference in incidental RI across the different SOF-based regimens. A sensitivity analysis, excluding patients with HIV/HCV coinfection, did not change the results (data not shown).

When only the patients treated with SOF-based regimens were considered, univariable analysis showed that baseline MELD score (OR 1.14, 95%CI 1.03-1.25, $p = 0.009$), the presence of ascites (OR 5.61, 95%CI 2.38-13.24, $p < 0.001$), the use of NSAIDs (OR 3.55, 95%CI 1.15-10.95, $p = 0.027$), lower baseline eGFR (OR 0.97, 95%CI 0.94-0.99, $p = 0.003$), and lower baseline albumin (OR 0.31, 95%CI 0.16-0.60, $p = 0.001$) were associated with the occurrence of RI (Table 9.4). Although multivariable analysis was limited by the number of events, in a model adjusted for MELD score, the presence of ascites (OR 4.44, 95%CI 1.46-13.54, $p = 0.009$), and the use of NSAIDs (OR 4.47, 95%CI 1.32-15.19, $p = 0.016$) were associated with increased risk of RI in patients treated with SOF-based regimens.

**Table 9.4 | Logistic regression analyses for renal impairment among patients treated with SOF-based regimens**

| Variable | Univariable | *p* value | Multivariable[a] | *p* value |
|---|---|---|---|---|
| Age | 1.02 (0.97-1.07) | 0.36 | | |
| Sex | 0.73 (0.32-1.64) | 0.44 | | |
| BMI | 1.03 (0.97-1.10) | 0.29 | | |
| | | | | |
| Cirrhosis | 1.47 (0.62-3.44) | 0.38 | | |
| MELD score | 1.14 (1.03-1.25) | 0.009 | 1.04 (0.92-1.18) | 0.501 |
| CTP B/C | 4.18 (1.73-10.12) | 0.002 | | |
| Ascites | 5.61 (2.38-13.24) | <0.001 | 4.44 (1.46-13.54) | 0.009 |
| | | | | |
| Hypertension | 2.12 (0.93-4.85) | 0.075 | | |
| Diabetes mellitus | 1.33 (0.50-3.55) | 0.56 | | |
| NSAIDs | 3.55 (1.15-10.95) | 0.027 | 4.47 (1.32-15.19) | 0.016 |
| Diuretics | 4.06 (1.76-9.38) | 0.001 | | |
| ACE/ARA | 1.96 (0.82-4.70) | 0.13 | | |
| | | | | |
| Creatinine | 1.14 (0.67-1.94) | 0.64 | | |
| eGFR | 0.97 (0.94-0.99) | 0.003 | | |
| ALT | 1.00 (0.99-1.01) | 0.58 | | |
| Albumin | 0.31 (0.16-0.60) | 0.001 | | |

Abbreviations: SOF, sofosbuvir; BMI, body mass index; MELD, Model for End-stage Liver Disease; CTP, Child-Turcotte-Pugh; NSAIDs, nonsteroidal anti-inflammatory drugs; ACE, angiotensin-converting-enzyme inhibitors; ARA, angiotensin II receptor antagonists; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase

a. Three variables were forced into the model, because extensive multivariable analyses could not be performed due to the number of events (n=26)

**Outcome of antiviral treatment**

Among the patients treated with TVR/BOC-based regimens, 138 of the 193 (71.5%) patients achieved SVR. A higher SVR rate was achieved among the 233 patients treated with SOF-based regimens (193/233 [82.8%], $p = 0.005$) when compared to TVR/BOC-based regimens. There was no difference in SVR rate between patients that were treated with TVR-based regimens (107/150, 71.3%) and BOC-based regimens (31/43, 72.1%, $p = 0.922$). Among the 60 patients who experienced RI, 45 (75.0%) achieved SVR, compared to 286 (78.1%) of the 366 patients that did not experience RI ($p = 0.59$). The rate of SVR among patients who experienced RI during SOF-based therapy was 69.2% (18/26) compared to 84.5% (175/207) among those treated with SOF who did not experience RI, $p = 0.051$, Figure 9.1). Multivariable regression analysis among patients treated with SOF-based regimens, only

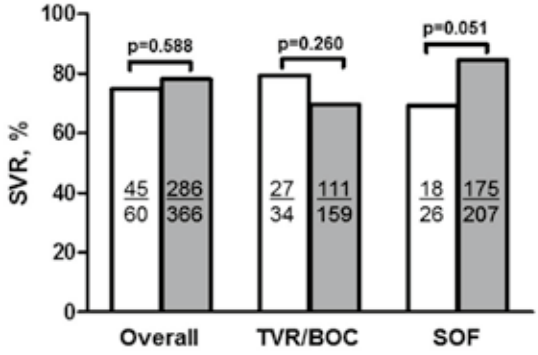


**Figure 9.1 | SVR rates**

The SVR rates according to the treatment regimen applied and for patients with (white bars) and without renal impairment (grey bars).

including the presence of cirrhosis and RI as factors, showed that both variables were associated with a lower chance of attaining SVR (presence of cirrhosis: OR 0.53, 95%CI 0.25-1.11, $p = 0.094$; RI: OR 0.43, 95%CI 0.17-1.09, $p = 0.074$).

**Dynamics of creatinine changes**

The mean creatinine levels during and after antiviral treatment with TVR/BOC-based regimens (a) and SOF-based regimens (b) are shown in Figure 9.2 for all subjects, and in Figure 9.3 for those who experienced RI. The mean rise in creatinine was 0.16 (range -0.4-2.5), and differed between patients treated with TVR/BOC-based regimens (0.20 [range -0.1-1.3]) and those treated with SOF-based regimens (0.12 [range -0.4-2.5], $p < 0.001$). Median time to RI was 9 weeks (IQR 4-16), and the mean maximum rise in creatinine was 0.55 mg/dL (range 0.30-2.5) for patients who experienced RI. There was no statistically significant difference between patients treated with TVR/BOC-based regimens (0.50 [range -0.3-1.3]) and those treated with SOF-based regimens (0.60 [range 0.3-2.5], $p = 0.52$).

Creatinine levels returned to normal at end of follow-up in 51 (86.4%) of the 59 patients who experienced RI and had a creatinine level available at the end of follow-up. Among those patients without normalization of creatinine, 3 (11.5%) were treated with a SOF-based regimen and 5 (15.2%) with a TVR/BOC-based regimen (see Table 9.2 for patient characteristics). In patients who experienced RI and had available creatinine level during follow-up, the mean difference in creatinine between baseline and follow-up was 0.06 mg/dL (range -0.30 – 0.90).

## DISCUSSION

This multicenter study showed that RI was observed in 11% of patients treated with a SOF-based regimen, which was lower than the 18% observed in patients treated with TVR/BOC-based regimens. However, as RI was reversible in all but 3 cases, SOF-based treatment can be considered to have a good

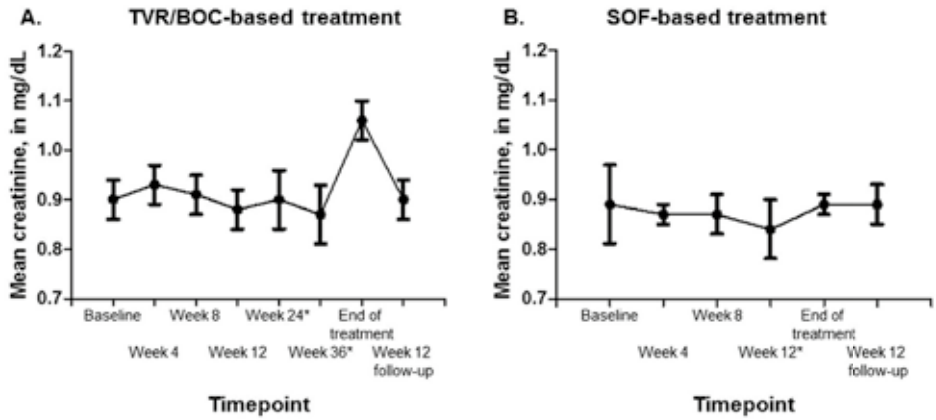


**Figure 9.2 | Dynamics of creatinine for all patients**

Panel A shows the mean creatinine level (and standard error) at baseline, during and after TVR/BOC-based antiviral therapy. The asterisks (*) indicate the mean creatinine level for patients at week 24 and 36 who were treated for 48 weeks. Panel B shows the mean creatinine level (and standard error) at baseline, during and after SOF-based antiviral therapy. The asterisks (*) indicate the mean creatinine level for patients at week 12 who were treated for 24 weeks.

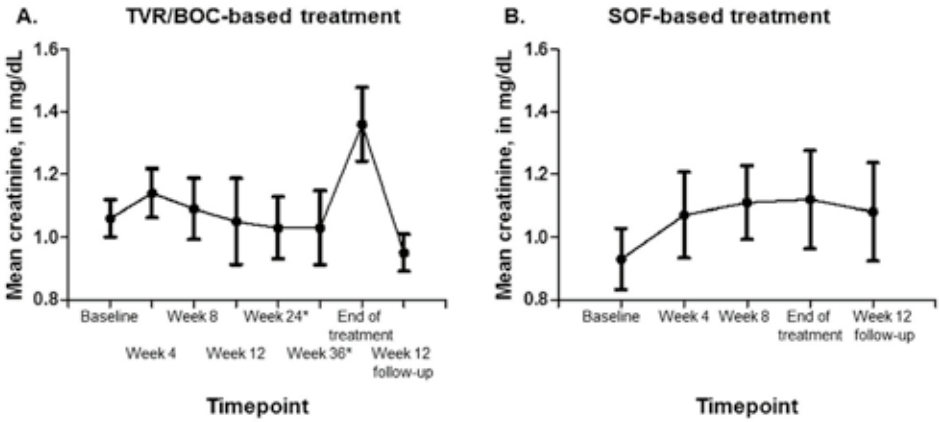


**Figure 9.3 | Dynamics of creatinine for patients experiencing renal impairment**

Panel A shows the mean creatinine level (and standard error) at baseline, during and after TVR/BOC-based antiviral therapy. The asterisks (*) indicate the mean creatinine level for patients at week 24 and 36 who were treated for 48 weeks. Panel B shows the mean creatinine level (and standard error) at baseline, during and after SOF-based antiviral therapy. The asterisks (*) indicate the mean creatinine level at week 12 for patients who were treated for 24 weeks.

renal safety profile. Patients with ascites, hypertension and those who use NSAIDs had an increased risk of RI during antiviral therapy. Among patients treated with SOF-based regimens, those with advanced liver disease, particularly those with decompensated cirrhosis (ascites), had an increased risk for RI.

The large phase 3 trials with SOF-based antiviral therapy did not report on the occurrence of RI as an important safety issue.[148 346] Large clinical trials often include a very selected patient population, which is not always representative of the broad population of patients with chronic HCV infection. Recently, some studies have reported on the safety and effectiveness of SOF-based therapy among patients with impaired renal function (eGFR <30mL/min), with notably heterogeneous results. Bhamidimarri et al. reported on 15 patients with ESRD (both pre-dialysis and on dialysis) who underwent treatment with half-dose SOF and SMV. During the treatment period no major adverse events were reported and 87% of the patients achieved SVR.[348] A report from the US included seventeen patients with advanced renal disease who received full-dose SOF in addition to SMV. Fifteen of these patients were on dialysis and two were pre-dialysis. All patients achieved SVR, there were no specific severe adverse events and none of the patients needed a dose reduction during therapy.[349] Recently, Desnoyer et al. reported on careful pharmacokinetics in 12 patients with renal disease who underwent treatment with different SOF-based regimens. All patients were on hemodialysis and received either daily full-dose SOF or full-dose SOF given three times a week after dialysis. Ten (83.3%) of the 12 patients achieved SVR, including all 7 who received daily full-dose SOF and no major adverse events were reported.[350] Notably, the GS331007 metabolite did not accumulate beyond the first days of treatment and 53% was removed by dialysis. Although these three small studies did not report major safety issues, detailed information on creatinine levels during treatment was not discussed. Recently, data on the safety and efficacy of SOF-based regimens among 73 patients with an eGFR ≤45 ml/min/1.73m2 were reported from the HCV-TARGET registry.[351] Although they used only chart review rather than objective evidence of RI, they found that the presence of impaired renal function (eGFR ≤45 ml/min/1.73m2) at baseline and a lower baseline hemoglobin level were associated with a higher risk for RI during SOF-based therapy. Renal outcomes after discontinuation of SOF were not reported. Worsening renal function occurred in 2% of all patients, but was higher among patients with an eGFR ≤45 ml/min/1.73m2 (15%), a high proportion of whom were post-transplant (49%). This study even reported one death among the group of patients with impaired renal function; a liver transplant recipient died due to worsening of renal failure and hepatic decompensation. There was no statistically significant difference in SVR rates based on the baseline eGFR. In contrast, we used a robust definition of RI based on recommendations of the International Club of Ascites (ICA) for patients with cirrhosis[347], and eliminated post-transplant patients and those with a baseline eGFR <30 ml/min/1.73m2 (or on hemodialysis). This strategy allowed us to create a homogeneous cohort with no increased risk for RI at baseline, and one that resembles the majority of patients treated in clinical practice throughout the world. We identified an incidence of on-treatment RI of 11%, which was associated with a trend toward a lower rate of SVR. It is likely that this finding can be explained by the fact that the patients who experienced RI and did not attain SVR were patients with more advanced liver disease. Indeed, 7 (87.5%) of the 8 patients who did not attain SVR in this group had cirrhosis, of whom 6 had decompensated cirrhosis (data not shown). It was not unexpected that the use of a TVR/BOC-based regimen was associated with a higher occur-

rence of RI, as previous studies also showed that renal adverse events were not uncommon.[344 345] Although they used another definition for renal impairment, Mauss et al. showed that up to 5% of patients on triple therapy experienced renal insufficiency stage 3 (eGFR <60 mL/min). However, some authors have questioned whether the decrease in eGFR noted is clinically significant, as TVR was shown to inhibit drug transporter OCT2 (an organic cation transporter on the basolateral membrane of the tubular cell), which interacts with creatinine tubular transport.[352] Thus, the rise in creatinine may not represent true deterioration of glomerular filtration, but a transient change in tubular excretion of creatinine with no associated kidney damage. Although the true nephrotoxicity of protease inhibitors (PIs) remains unclear, studies (including the current one) have found that the early rise in creatinine normalizes after the cessation of antiviral therapy in almost all individuals suggesting that renal issues are likely not a major issue with first generation PIs. Studies using more specific markers of glomerular filtration (i.e. iothalamate) before and during antiviral treatment have not been performed but would help clarify the true degree of renal toxicity of these agents.

New generation PIs like SMV, paritaprevir and grazoprevir are all entirely hepatically metabolized and thus expected to have excellent renal safety profiles.[353-355] We did not find an increased frequency of RI in patients using SOF/SMV when compared to patients taking SOF without SMV (data not shown). Renal elimination of SMV and grazoprevir is negligible at <1%, and similarly, the regimen containing paritaprevir/ritonavir, ombitasvir and dasabuvir is minimally renally excreted (<11.3%).[127 159 356] Based on their limited renal excretion, grazoprevir and elbasvir as well as the combination of paritaprevir/ritonavir, ombitasvir and dasabuvir have been evaluated in patients with advanced chronic kidney disease or on hemodialysis showing that these agents are extremely safe and highly effective.[158 162]

The finding that ascites was associated with the occurrence of RI during antiviral therapy stresses the importance of close monitoring of patients with decompensated cirrhosis. Renal function gradually deteriorates due to hemodynamic changes associated with decompensated liver disease.[357] Whether our observation of impaired renal function in these patients is induced by antiviral therapy or whether it is related to progression of liver disease and/or its treatment, is not clear. Patients taking diuretics were at higher risk of RI during treatment as were patients taking NSAIDs, a class of drugs that are contraindicated in patients with cirrhosis due to their impairment of renal blood flow, particularly in the setting of portal hypertension.[358]

There are some limitations of our study. Due to its retrospective character, the choice of antiviral regimens was made by the treating physician, however patients were treated in academic centers and all consecutive patients were included, limiting selection bias. Although missing data always present challenges, we had very low rates of missing data and do not believe that they significantly affected the results of our study. Given the reduced number of RI events noted in the subgroup of patients with SOF-based regimens, we had limited ability to do a comprehensive multivariable analysis to identify more factors associated with RI (i.e. hypertension). Finally, the HCV TARGET registry and others have suggested that patients with baseline impairment of renal function are at higher risk of RI during SOF-based therapy, however, we had few patients with eGFR below 60 ml/min at baseline and a subanalysis of this population could thus not be performed. Nevertheless, approximately 19% of these patients experienced RI.

Despite inclusion of patients with more advanced liver disease, the risk for RI was lower for patients treated with SOF-based regimens compared to TVR/BOC-based regimens. Although RI was seen in 11% of the patients treated with SOF-based therapy, it was reversible in almost all cases. Patients with ascites and patients using NSAIDs have an increased risk for RI during SOF-based antiviral therapy. Monitoring of renal function and standard nephroprotective measures are suggested when using SOF-based regimens, particularly in patients with advanced cirrhosis and portal hypertension, or in those with comorbidities potentially affecting the kidneys.

# SUPPLEMENTARY TABLE

**Supplementary Table 9.1 |** Characteristics of the 32 patients with a baseline eGFR ≤60 mL/min

| Variable | N | |
|---|---|---|
| Age | 32 | 58.1 (±6.54) |
| Male sex | 32 | 14 (43.8%) |
| BMI | 32 | 27.6 (±5.75) |
| | | |
| HCV genotype | 32 | |
| *1* | | 28 (87.5%) |
| *2* | | 1 (3.1%) |
| *3* | | 2 (6.3%) |
| *4* | | 1 (3.1%) |
| Cirrhosis | 32 | 15 (46.9%) |
| CTP B/C | 31 | 7 (21.9%) |
| MELD score | 31 | 10 (7-13) |
| | | |
| Cryoglobulins (+) | 29 | 6 (18.8%) |
| Diabetes mellitus | 32 | 9 (28.1%) |
| Hypertension | 32 | 16 (50.0%) |
| NSAIDs | 32 | 3 (9.4%) |
| ACE/ARA | 32 | 5 (15.6%) |
| Diuretics | 32 | 9 (28.1%) |
| | | |
| Creatinine | 32 | 1.23 (±0.26) |
| eGFR | 32 | 52.2 (±6.8) |
| Platelet count | 32 | 206 (±127) |
| ALT | 32 | 49 (33-82) |
| | | |
| Treatment regimen | 32 | |
| *BOC/P/R* | | 1 (3.1%) |
| *TVR/P/R* | | 11 (34.4%) |
| *SOF/P/R* | | 8 (25.0%) |
| *SOF/R* | | 6 (18.8%) |
| *SOF/SMV/±R* | | 5 (15.6%) |
| *SOF/LDV/±R* | | 1 (3.1%) |

Abbreviations: eGFR, estimated glomerular filtration rate; BMI, body mass index; HCV, hepatitis c virus; CTP, Child-Turcotte-Pugh; MELD, Model for End-stage Liver Disease; NSAIDs, nonsteroidal anti-inflammatory drugs; ACE, angiotensin-converting-enzyme inhibitors; ARA, angiotensin II receptor antagonists; ALT, alanine aminotransferase; BOC, boceprevir; P, pegylated interferon alpha; R, ribavirin; TVR, telaprevir; SOF, sofosbuvir; SMV, simeprevir; LDV, ledipasvir

a. Data are presented as n (%), mean (±SD) or median (interquartile range).

# GENERAL DISCUSSION AND CONCLUSIONS

## BACKGROUND

Chronic hepatitis C virus (HCV) infection continues to be a major global public health problem. Although the incidence of HCV infection is declining in the West, it has been estimated that the incidence of patients with HCV-induced cirrhosis will not peak until 2030.[63]

With the approval of multiple direct-acting antivirals (DAAs), the therapeutic horizon has been broadened for patients with chronic HCV infection. After two decades with merely minor improvements of interferon-based antiviral treatment regimens, current DAAs allow patients to be treated with high effectiveness and excellent tolerability. Until 2011, treatment of chronic HCV infection consisted solely of pegylated interferon and ribavirin. Almost 50% of the patients with chronic HCV infection were treated successfully and were considered having a sustained virological response (SVR) when serum HCV RNA was absent at 24 weeks after the cessation of therapy. The SVR rates greatly varied between patients and several viral, host and treatment factors could be used as predictors of successful treatment outcome.[107 339 359] Once SVR is attained, patients have an improved long-term outcome compared to patients without SVR.[75 79-82 84] This beneficial effect of SVR was best assessed in patients with advanced hepatic fibrosis, who are at highest risk for developing liver failure, hepatocellular carcinoma (HCC) and all-cause mortality. Due to one of the most important milestones in hepatology, even patients with severe hepatic disease can be offered antiviral therapy. Interferon-based therapy was namely contra-indicated in patients with decompensated cirrhosis, due to the occurrence of severe adverse events, including worsening of hepatic decompensation, severe bacterial infections and even death.[121 207] Interferon-free regimens are likely to change their future, but this will remain a matter of debate during the upcoming years.

## THE OLD LANDSCAPE: OPTIMIZING INTERFERON-BASED THERAPY

Until 2014, pegylated interferon remained the backbone of the treatment for chronic HCV infection. The first DAAs were combined with pegylated interferon and ribavirin.[128 129 135] Although these regimens could be shortened to 12 or 24 weeks and were associated with improved SVR rates, interferon-induced side effects remained and some patients could not receive treatment.

Hereafter, combinations of multiple classes of DAAs showed overwhelming SVR rates and excellent safety profiles. The high costs of these new drugs, however, have limited their availability. Indeed, also in high-income countries the access to DAAs is still subject to debate. In the Netherlands, partly due to the low prevalence of this disease, all patients with chronic HCV infection can be treated with the approved DAAs. Yet, pegylated interferon is still expected to remain a necessary treatment option for many patients with chronic HCV infection around the globe. Consequently, optimizing the use of pegylated interferon in order to maximize the chance of SVR is thus still relevant. Especially among patients with compensated advanced liver disease it is not an option to await the availability of interferon-free treatment options.

Interferon-associated side effects limit treatment adherence and may lead to dose reduction or treatment discontinuation, by which the virological efficacy of antiviral therapy is compromised.[110-112 214] Interferon-based regimens, which can be shortened to 12-24 weeks with the addition of a next-generation DAA, remained to have the pegylated interferon-associated side effects. The most commonly reported side effects are cytopenias, such as thrombocytopenia, neutropenia and anemia.[110 214] These interferon-induced cytopenias were actually the most frequent causes of pegylated interferon dose reductions.[104] Out of fear for bleeding episodes and infections, current guidelines and product labels recommend to reduce the dose of pegylated interferon when the platelet count drops below 50x109/L or when the absolute neutrophil count (ANC) drops below 750/µL. Treatment is even advised to be discontinued in case of a platelet count below 25x109/L or a ANC below 500/µL.[35 36] It has been suggested, however, that these recommendations are overly cautious and unnecessarily impede treatment success. [114 115] The only study which assessed the risk of on-treatment bleeding episodes did show a relation with interferon-induced thrombocytopenia, but all registered bleeding episodes were deemed minor expect for one, which actually occurred in a patient without thrombocytopenia.[115] Although chronic HCV-infected patients are at an increased risk of bacterial infections during interferon-based therapy, these infections were generally mild.[114 200 203 360-362] Moreover, a relation between the infection rate and interferon-induced neutropenia has never been described.

Yet, the above-cited studies included only a limited number of patients with advanced hepatic fibrosis. Because of an increase in portal pressure and a reduced thrombopoietin production those with cirrhosis are also most prone for thrombocytopenia. In fact, thrombocytopenia may already be present in absence of interferon-based therapy.[113] As a result, cirrhosis was probably found to be a risk factor for bleeding episodes during pegylated interferon therapy. [115] Patients with cirrhosis, however, can be considered as immunocompromised and are at highest risk of infections. [204] In **chapters 1a and 2**, we have investigated the risk of bleeding episodes and infections during interferon-based antiviral therapy among the patients with chronic HCV infection and bridging fibrosis or cirrhosis (Ishak fibrosis score 4–6). This cohort study included all consecutive patients with chronic HCV infection who started an interferon-based treatment between 1990 and 2003. In total, 104 bleeding episodes were registered during 53 (8%) of the 678 interferon-based treatments which could be included for this endpoint. In line with the results as reported by Roomer et al., cirrhosis and a platelet count <50x$10^9$/L at the previous visit were significantly associated with bleeding episodes.[115] However, nearly all registered bleeding episodes were considered to be minor and remained without serious clinical consequences. The two (2%) bleeding episodes which were considered to be severe both concerned variceal bleeding episodes for which the patients were hospitalized. Antiviral therapy was not discontinued in these patients. Considering the natural history of HCV-related cirrhosis, two variceal bleeding episodes in such a large cohort of patients with advanced liver disease might also be expected outside of the scope of interferon-based therapy. [55] In fact, both patients had a previous variceal bleeding episode and both had pretreatment thrombocytopenia as a measure of portal hypertension (89x$10^9$/L and 90x$10^9$/L respectively). In order to overcome thrombocytopenia-induced dose reductions of pegylated interferon as well as the fear for bleeding episodes, efforts have been

made in order to increase the platelet count before and during antiviral therapy by treating patients with eltrombopag, an oral thrombopoietin receptor agonist.[193] Although, eltrombopag was shown to be a potent agent for treating thrombocytopenia, some important safety issues on its use during interferon-based antiviral therapy were addressed in **chapter 1b**. The rates of hepatic decompensation and thromboembolic events were significantly higher in the group receiving eltrombopag. By allowing patients with severe thrombocytopenia to undergo interferon-based therapy, the risk for hepatic decompensation is considerably increased. It could be questioned whether these risks outweigh the little benefit on SVR rates which were seen in the groups that received eltrombopag. Besides, patients with severe portal hypertension may not benefit from successful antiviral therapy as they are beyond the 'point of no return' and their natural history is unlikely to be reversed.

In this same cohort, 113 infections were registered during 88 (12%) of the 723 interferon-based treatments. In 23 of these treatments the ANC dropped below 500/μL, but an infection was observed during only three of these treatments. Nevertheless, on-treatment ANC <500/μL was significantly associated with the occurrence of infection. However, a sensitivity analysis including only those regimens containing pegylated interferon could confirm this finding, which is in line with other studies including patients treated with pegylated interferon and ribavirin only.[114 200 202 203] Importantly, the majority of infections which occurred during interferon-based therapy were mild, both in our study as well in the studies of others. As expected, we found that cirrhosis and diabetes mellitus were independent risk factors for on-treatment infections.

With respect to infections and bleeding episodes, our study among patients with advanced liver disease confirmed that pegylated interferon-based therapy is generally safe. In case of risk factors such as cirrhosis or diabetes mellitus, infections and/or bleeding episodes may be expected more frequently. These patients should therefore to be monitored more closely. Because patients with advanced liver disease seem to have much to gain by attaining SVR, it may be considered to maintain the dose of pegylated interferon as long as the ANC and platelet counts remain above 500/μL and 25x109/L, respectively. Although we rarely observed cytopenias below these values (the ANC dropped below 500/μL in 3% of treatments and the platelet counts dropped below 25x109/L in 2% of treatments), it should be noted that interferon dose reductions were at the discretion of the treating physicians. Prospective studies would obviously be warranted to validate these alternative recommendations, especially when regimens of 12 to 24 weeks can be used. However, considering the field is moving toward interferon-free therapy, these studies are unlikely to be executed.

Although not in general, ribavirin is still being used next to the DAAs to optimize the virological efficacy in some specific subgroups of patients. The use of ribavirin has been associated with hemolytic anemia and is responsible for hemoglobin declines during antiviral therapy. Two genetic polymorphisms in the inosine triphosphatase (*ITPA*) gene on chromosome 20 were shown to be associated with protection against early ribavirin-induced hemolytic anemia during treatment with pegylated interferon and ribavirin.[215] As the findings on these two *ITPA* polymorphisms were mostly derived from patients who were included in randomized controlled trials, we assessed this association in **chapter 3** for patients who were treated in daily clinical practice.[217 218 221 222] In total, 213 Caucasian patients could be included who had a blood sample available for genetic testing. In 74% patients a clinically significant decline in hemoglobin was reported. Patients who were homozygous carriers

of the major allele of the two *ITPA* genes (representing normal ITPase activity) experienced deeper declines in hemoglobin and had a higher occurrence of clinically significant anemias. More important, these patients often underwent dose reductions of both ribavirin and pegylated interferon, and more often were administrated blood transfusions and/or erythropoietin.

Since treatment efficacy is hampered by dose reductions, patients with normal ITPase activity may benefit from early strategies to maintain adequate hemoglobin levels in order to improve treatment adherence. These polymorphisms may also be used as an additive tool to select a specific interferon-free regimen for the individual patient as not all regimens require addition of ribavirin for optimal virological efficacy. However, whether the association between ITPase activity and anemia remains in case of ribavirin containing interferon-free therapy needs to be assessed.

The importance of platelets was also highlighted in **chapter 4**. Besides an improved prognosis, SVR has been repeatedly associated with reductions in hepatic fibrosis and portal pressure, which are linked to a favorable clinical outcome.[74 78 241-247 269 363-365] Evaluating liver histology or the hepatic venous pressure gradient requires invasive procedures, so that multiple assessments are difficult to accomplish. In **chapter 4** we have therefore investigated the evolution of platelet counts over time following antiviral therapy in patients with chronic HCV infection and advanced hepatic fibrosis. Especially among patients with more advanced liver disease, the platelet count represents a non-invasive and objective parameter of the degree of portal hypertension and hepatic fibrosis.[253 255 256] Previously, changes in platelet count following antiviral therapy have been linked to changes in hepatic fibrosis.[258 259] With a repeated measurement analysis we observed a significant increase in platelet counts for many years following achievement of SVR, which showed to be rather linear. Importantly, the change in platelet count correlated with the change in spleen size, which represents another marker of portal pressure. These findings suggest a gradual improvement in portal pressure and liver histology after HCV eradication. In contrast, the platelet counts further declined during follow-up among patients who were not successfully treated.

## THE CHANGING LANDSCAPE

Since the discovery of HCV in 1989 and the implementation of blood screening tests shortly thereafter, there has been a significant reduction in the transmission of the virus.[11 278-281] As fibrosis slowly develops, and patients may be asymptomatic or with non-specific symptoms only, many infected people remain undetected so far. Due to ageing of the HCV-infected population, approximately 25% of the US patients with chronic HCV infection are expected to have cirrhosis. This proportion is likely to increase to 45% by the year 2030.[63] With the introduction of safe and effective interferon-free antiviral regimens, it is expected that more patients can be effectively treated, including those with advanced liver disease.

Based on historic data, the present and future burden of chronic HCV infection in the Netherlands were recently modeled as well.[4 286 287] Additional details on the trends in patient and disease characteristics from the last 2 decades are important for Dutch health policy makers to be informed about the shifting trends in the patient population with chronic HCV infection. This provides

information on the burden of patients who will be considered for interferon-free therapy during the upcoming years. **Chapter 5** describes the epidemiological trends in patient and disease characteristics over time among 1779 individuals with chronic HCV infection that were newly referred to a tertiary care center in the Netherlands from 1990 to 2013. As expected, with a reduced incidence of chronic HCV infection, more recently referred patients were older and more often had advanced hepatic fibrosis as compared to the patients who were referred in the early years. Also, more patients were infected with HCV genotype 1a. This could possibly explained by the lower response rates of patients with chronic HCV genotype 1a compared to patients with chronic HCV genotype 1b, especially when treated with first-generation protease inhibitors telaprevir and boceprevir.[298] The reason for this difference between HCV genotype 1a and 1b was most likely the resistance-associated variants that were present at baseline or emerge during antiviral therapy.[366-369] Although it was not statistically significant, there was also an increase in the number of patients referred with chronic HCV genotype 3. As this genotype is associated with a more accelerated disease progression, this finding is important.[299] Moreover, treatment with interferon-free regimens had suboptimal efficacy among patients with HCV genotype 3.

Another interesting finding was that 70% of all patients were born between 1950 and 1975. Although screening for chronic HCV infection has not been considered cost-effective in the Netherlands due to the low prevalence of the disease in light of suboptimal treatment options, this should be re-evaluated for specific subgroups with highest prevalence now that effective treatment regimens are available. An effective screening strategy could really decrease the disease burden with respect to both prevalence and clinical impact are concerned. At the moment, the fact that patients are unaware of their disease seems to represent the greatest limitation to make a real impact on HCV-related mortality.[370]

The approval of various DAAs induced an enormous debate on the price of these drugs. Currently, the high costs of these DAAs hamper global access. Policy makers, therefore, are forced to prioritize treatment to those patients who are considered to have an urgent need for successful antiviral therapy. This situation, however, is far from ideal, especially since patients without cirrhosis have the greatest benefit from successful antiviral treatment as was suggested by Koh et al.[261] Since the introduction of DAAs there was an increasing number of studies on the cost-effectiveness of these drugs with different conclusions on who to treat and when to treat.[371-374] In order to place the costs of DAA into broader perspective, **chapter 6** describes the costs per SVR for patients with advanced hepatic fibrosis who were treated with interferon-based antiviral therapy. Patients that were included in chapter 1 and 2, were also included in this study if detailed information was available. For the total cohort, the mean costs per SVR was €38,514. These costs were higher for patients with cirrhosis (€40,587) as compared to patients with bridging fibrosis (€33,454). When the baseline platelet count was used as indicator for the severity of liver disease, we found that for patients with a platelet count $<100x10^9$/L the mean costs per SVR were €74,961, which was substantially higher than for patients with a normal platelet count at baseline (€26,105). These differences can probably be attributed to the lower chance of attaining SVR in these subgroups, which is important since the currently approved interferon-free regimens seem overcome this with universal high SVR rates. Taking into account the possible risks of interferon-based treatment as well as the high chance of necessitating re-treatment, the additional

costs per SVR of the DAAs could be limited, especially among those with most severe liver disease. Of course, these data should be interpreted with caution and not be used to justify the high costs of the new drugs. In order to be able to treat more patients, these costs should be substantially reduced.

## THE NEW LANDSCAPE: ANTIVIRAL THERAPY WITH DIRECT-ACTING ANTIVIRALS

After the approval of telaprevir and boceprevir, the first DAAs, real-world studies warned the treating physicians for important safety issues, especially in patients with advanced hepatic fibrosis.[120 121] Real-world data is of utmost importance after the approval of new drugs. The efficacy and safety of these regimens are based on treatments administered to patients who fulfilled the eligibility criteria for clinical trials. In **chapter 7** we assessed the safety and effectiveness of DAA-based antiviral therapy in patients with cirrhosis. Data from 4 large university hospitals in Canada, Germany and the Netherlands were combined. In total, 433 cirrhotic patients were included of whom 114 had evidence of decompensated cirrhosis (defined as a Child Pugh B or C). The overall SVR rate was 81% (350/433) and there was a trend towards a better response rate among patients with CP A compared to CP B/C patients (264/ 319 [82.8%] vs. 86/ 114 [75.4%], $p = 0.088$). More importantly, 50 (11.5%) patients experienced worsening or new onset of hepatic decompensation during antiviral therapy. Factors that were associated with the occurrence of hepatic decompensation were HCV genotype 3 (vs non-genotype 3), Child Pugh B/C (vs Child Pugh A) and albumin <35 g/L. When Child Pugh score was replaced by MELD score, we found that a MELD score ≥14 was associated with hepatic decompensation. When three risk factors (HCV genotype 3, albumin <35 g/L and MELD score ≥14) were present, the 24-week cumulative incidence for hepatic decompensation was 75.0% (50.5-99.5) and the chance of attaining SVR was 67%. By identifying these three parameters, physicians could potentially assess the risk for hepatic decompensation, which should be weighed against the chance of SVR. One could argue that when the risk of decompensation is more or less equal or higher than the chance of attaining SVR, delaying therapy would be a better option. This study also highlighted the importance of HCV genotype 3. Previous data has shown that HCV genotype 3 is associated with faster progression to cirrhosis, the development of HCC and overall survival.[299 335] Our data shows that HCV genotype 3 is still relevant among patients with decompensated cirrhosis as we found that it has also been associated with the development/worsening of hepatic decompensation. Fortunately, more effective therapy for HCV genotype 3 were recently presented and reported on multiple combinations of further developed DAAs (sofosbuvir and velpatasvir, especially with the addition of GS-9857, and ABT-493 and ABT-530).[168 375-377] Approval of these agents should be considered a high priority.

The need for more effective therapy for HCV genotype 3 was also highlighted in **chapter 8**. This study, part of the large registry HCV-TARGET, included 197 patients with HCV genotype 3, who were treated with sofosbuvir and ribavirin with or without the addition of pegylated interferon. Of 178 patients treated with sofosbuvir and ribavirin, 60% achieved SVR, compared to 84% of 19 patients treated with sofosbuvir, pegylated interferon and ribavirin. After controlling for age and sex, absence

of cirrhosis, albumin levels ≥3.2 g/dl and platelet count >100x$10^9$/L were associated with greater odds of SVR. Although this study was not powered for this purpose, it showed that adding pegylated interferon to the treatment of HCV genotype 3 could increase the chance of SVR. This was also highlighted in a randomized controlled trial comparing sofosbuvir and ribavirin for 16 or 24 weeks to sofosbuvir, pegylated interferon and ribavirin for 12 weeks in patients with HCV genotype 3.[378] This study showed that the inclusion of pegylated interferon increased response rates in all patient subgroups, but most notably in those with cirrhosis. Among these patients the SVR rates increased from 82% with sofosbuvir and ribavirin for 24 weeks to 91% with sofosbuvir, pegylated interferon and ribavirin in treatment-naïves and from 77% to 86% in case of prior treatment-experience. Pegylated interferon was tolerated well with only 1 patient discontinuing treatment prematurely. Similarly, pegylated interferon was well tolerated with similar rates of adverse events and higher rates of SVR reported than with sofosbuvir and ribavirin alone. However, despite these encouraging results, sofosbuvir, pegylated interferon and ribavirin was prescribed to only 19 of the 197 (9.6%) patients with HCV genotype 3 infection who have started therapy within the HCV-TARGET to date. Clearly both clinicians and patients are reluctant to accept interferon-based therapies. Fortunately recent studies have shown that sofosbuvir combined with velpatasvir, a pan-genotypic NS5A inhibitor, is highly effective for chronic HCV genotype 3 infection, leading to SVR rates of 90% even in patients with cirrhosis. Ribavirin no longer seems to be required with this new DAA combination.[168]

Despite the fact that sofosbuvir is considered very safe, its use is not recommended in patients with an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73$m^2$ or patients with end-stage renal disease. This is because the main active metabolite of sofosbuvir, GS331007, is excreted by the kidneys, and higher drug and metabolite concentrations are found in those patients with an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73$m^2$ or end-stage renal disease on hemodialysis.[346] Although the major trials examining the safety and efficacy of sofosbuvir demonstrated an excellent renal safety profile, it currently remains unknown whether this also applies in daily clinical practice. Previously, the registration trials of telaprevir and boceprevir also reported a relatively benign renal safety profile, while shortly after its approval data on significant renal safety toxicity emerged.[344 345] In **Chapter 9** the occurrence of renal impairment was assessed during sofosbuvir-based antiviral therapy. This large study included 426 patients with chronic HCV infection who were treated with sofosbuvir-based (n=233) or telaprevir/ boceprevir-based (n=193) regimens at two tertiary university centers in North America. The risk for renal impairment was lower for patients treated with sofosbuvir-based regimens compared to telaprevir/ boceprevir-based regimens. Although renal impairment was seen in 11% of the patients treated with sofsobuvir-based therapy, it has a good renal safety profile as renal impairment was reversible in almost all cases. Patients with ascites and patients using NSAIDs have an increased risk for renal impairment during sofosbuvir-based antiviral therapy. Monitoring of renal function and standard nephroprotective measures are suggested when using sofosbuvir-based regimens, particularly in patients with advanced cirrhosis and portal hypertension, or in those with comorbidities potentially affecting the kidneys. Fortunately, two regimens have shown promising results in patients with renal failure. Based on their limited renal excretion, grazoprevir and elbasvir as well as the combination of paritaprevir/ritonavir, ombitasvir and dasabuvir have been evaluated in patients with advanced chronic kidney disease or on hemodialysis showing that these agents are extremely safe and highly effective.[158 162]

## CONCLUSIONS

With the approval of multiple oral DAAs, physicians now have the ability to initiate highly effective and very safe antiviral therapy in patients with chronic HCV infection, even in those with most advanced hepatic disease. Although the costs limit global access to these drugs, a recent study on the use of generic DAAs showed promising results.[379] Until that time, pegylated interferon may be necessary, yet, for a shorter duration than before 2014. With the right monitoring, even patients with compensated cirrhosis could be safely treated with pegylated interferon. In absence of DAA combinations, physicians should not be too reluctant to initiate interferon-based antiviral therapy, especially not in patients with HCV genotype 3 who may actually benefit from this addition. Chronic HCV infection is par excellence a disease that needs a patient tailored approach when treatment is concerned. While there is almost no limitation for the use of DAAs, some patients may have no clinical benefit from successful antiviral therapy as the natural course of their disease cannot be reversed anymore. With the upcoming pangenotypic regimens, treatment decisions will be simplified, although this will not be the case for every patient. Close collaboration between physicians, health authorities and pharmaceutical companies is urgently needed to reduce the enormous burden of chronic HCV infection. Patients need to be diagnosed, linked to care and treated in order to eradicate the virus, especially now we have a cure.

# SAMENVATTING

## ACHTERGROND

Chronische hepatitis C virus (HCV) infectie blijft wereldwijd een belangrijk probleem voor de volksgezondheid. Hoewel de incidentie van chronische HCV infectie afneemt in het Westen, denkt men dat de incidentie van patiënten met HCV-geïnduceerde cirrose, verlittekening van de lever, pas in 2030 zijn hoogtepunt zal bereiken.[63]

Door de goedkeuring van meerdere medicijnen die direct op het virus inwerken (DAAs, *direct-acting antivirals*), heeft de behandelaar van patiënten met chronische HCV infectie momenteel een zeer ruime keuze. Na twee decennia van slechts geringe verbeteringen van 'de oude' interferon-gebaseerde antivirale behandelingen, maken de huidige DAAs het mogelijk om patiënten te behandelen met een hoge effectiviteit en uitstekende verdraagbaarheid. Tot 2011 bestond de behandeling van chronische HCV infectie uitsluitend uit gepegyleerd interferon en ribavirine, medicijnen die het immuun systeem van de patiënt stimuleren het virus op te ruimen (interferon) en ervoor te zorgen dat het virus muteert (ribavirine). Bijna 50% van de patiënten met chronische HCV infectie werden hiermee succesvol behandeld. We beschouwen patiënten als genezen indien er 24 weken na het staken van de behandeling geen virus meer aantoonbaar is in het bloed. Dit wordt een blijvende virologische respons genoemd (SVR, *sustained virological response*). De SVR percentages varieerden aanzienlijk tussen patiënten en verschillende virale-, patiënt- en behandel factoren konden worden gebruikt als voorspellers van een succesvol behandelingsresultaat.[107 339 359] Zodra SVR is bereikt, hebben patiënten een betere uitkomst op de lange termijn in vergelijking met patiënten zonder SVR.[75 79-82 84] Dit gunstige effect van SVR is het beste onderzocht bij patiënten met gevorderde leverfibrose, zij hebben namelijk het grootste risico op het ontwikkelen van leverfalen, hepatocellulair carcinoom (HCC) en sterfte. Door een van de belangrijkste mijlpalen in de hepatologie, kunnen zelfs patiënten met een ernstige leveraandoening antivirale therapie ontvangen. Interferon-gebaseerde therapie was namelijk gecontra-indiceerd bij patiënten met gedecompenseerde levercirrose, vanwege het risico op ernstige bijwerkingen, waaronder verergering van leverdecompensatie, ernstige bacteriële infecties en zelfs overlijden.[121 207] Interferon-vrije behandelingen zullen de toekomst van patiënten met chronische HCV infectie waarschijnlijk veranderen, maar of dit voor iedere patiënt zo is zal de komende jaren een punt van discussie blijven.

## HET OUDE LANDSCHAP: OPTIMALISEREN VAN INTERFERON-GEBASEERDE THERAPIE

Tot 2014 was gepegyleerd interferon de basis van de behandeling van chronische HCV infectie. De eerste DAAs werden namelijk gecombineerd met gepegyleerd interferon en ribavirine.[128 129 135] Hoewel deze regimes konden worden ingekort tot 12 of 24 weken en een hogere kans op genezing hadden, bleven de interferon-geïnduceerde bijwerkingen bestaan, waardoor sommige patiënten niet konden worden behandeld.

Hierna volgden combinaties van meerdere soorten DAAs met een hoge kans op slagen van de behandeling en een uitstekend veiligheidsprofiel. De hoge kosten van deze nieuwe geneesmiddelen

zorgen echter voor een beperkte beschikbaarheid. Sterker nog, ook in landen met hoge inkomens is de beschikbaarheid van DAAs nog steeds onderwerp van discussie. In Nederland, mede door de lage prevalentie van deze ziekte, kunnen alle patiënten met chronische HCV infectie worden behandeld met de geregistreerde DAAs. Verwacht wordt dat wereldwijd, voor veel patiënten met chronische HCV infectie, gepegyleerd interferon een belangrijk onderdeel van de behandeling blijft. Daarom is het belangrijk om optimaal gebruik te maken van gepegyleerd interferon en daardoor de kans op SVR te vergroten. Vooral bij patiënten met gecompenseerde levercirrose is het geen optie om de beschikbaarheid van interferon-vrije behandelingsopties af te wachten.

Interferon-geassocieerde bijwerkingen beperken de therapietrouw en kunnen leiden tot dosisverlaging of staken van de behandeling, waarbij de kans op succesvolle behandeling in gevaar komt.[110-112 214] Interferon-gebaseerde behandelopties kunnen worden ingekort tot 12 of 24 weken met de toevoeging van de nieuwe generatie DAAs. Helaas gaat dit wel gepaard met de interferon-geassocieerde bijwerkingen. De meest voorkomende bijwerkingen zijn afwijkingen in het bloed, zoals trombocytopenie (lage bloedplaatjes), neutropenie (lage neutrofielen) en anemie (laag hemoglobine).[110 214] Deze interferon-geïnduceerde afwijkingen zijn de meest voorkomende oorzaken van dosisverlagingen van gepegyleerd interferon.[104] Uit angst voor bloedingen en infecties wordt in de huidige richtlijnen aanbevolen om de dosering van gepegyleerd interferon te verlagen wanneer het aantal bloedplaatjes daalt onder $50x10^9$/l of als het absolute aantal neutrofielen (ANC) daalt onder 750/µl. Indien het aantal bloedplaatjes daalt beneden $25x10^9$/l of bij een ANC lager dan 500/µl wordt zelfs geadviseerd de behandeling te staken.[35 36] Gesuggereerd wordt dat deze adviezen te voorzichtig zijn en onnodig de kans op een succesvolle behandeling verlagen.[114 115] De enige studie die het risico op bloedingen tijdens behandeling heeft geëvalueerd toonde een relatie met interferon-geïnduceerde trombocytopenie. Gelukkig waren alle geregistreerde bloedingen waren mild, behalve één bloeding, die optrad bij een patiënt zonder trombocytopenie.[115] Hoewel patiënten met een chronische HCV infectie een verhoogd risico op bacteriële infecties hebben tijdens interferon-gebaseerde therapie, waren deze infecties over het algemeen mild.[114 200 203 360-362] Bovendien is een relatie tussen een infectie en de ernst van een interferon-geïnduceerde neutropenie nooit beschreven.

Helaas includeerden de bovengenoemde studies slechts een beperkt aantal patiënten met vergevorderde leverfibrose. Door een toename van portale druk en een verminderde productie trombopoietine hebben patiënten met cirrose een grote kans op het ontwikkelen van trombocytopenie. Vaak hebben deze patiënten al een trombocytopenie zonder de aanwezigheid van interferon-gebaseerde therapie.[113] Als gevolg hiervan, werd cirrose als een risicofactor voor bloedingen tijdens behandeling met gepegyleerd interferon beschouwd.[115] Patiënten met cirrose kunnen ook worden beschouwd als immuun gecompromitteerd, waardoor zij een verhoogd risico hebben op infecties.[204] In **hoofdstuk 1.1 en 2**, hebben we het risico op bloedingen en infecties tijdens interferon-gebaseerde antivirale therapie onderzocht bij patiënten met chronische HCV infectie en ernstige fibrose of cirrose (Ishak fibrose score 4-6). In deze studie werden uit vijf verschillende centra alle patiënten met een chronische HCV infectie geïncludeerd, die hun eerste interferon-gebaseerde behandeling hadden ondergaan tussen 1990 en 2003. In totaal werden 678 interferon-gebaseerde behandelingen bekeken, waarbij in 53 (8%) behandelingen ten minste één bloeding werd geregistreerd. In lijn met de resultaten zoals gerapporteerd door Roomer et al., was het hebben van

cirrose en het hebben van bloedplaatjes onder de $50x10^9$/l bij het vorige bezoek aan de polikliniek significant geassocieerd met bloedingen.[115] Echter, bijna alle geregistreerde bloedingen waren mild en bleven zonder ernstige klinische consequenties. De twee (2%) bloedingen die als ernstig werden beschouwd, waren beide varices bloedingen waarvoor de patiënten werden opgenomen in het ziekenhuis. De antivirale therapie werd bij deze patiënten overigens niet gestopt. Aangezien onze studie alleen patiënten includeerde met gevorderde leverziekte, kunnen de twee varices bloedingen ook passen bij het natuurlijk beloop van de ziekte en hoeft het geen relatie te hebben met de interferon-gebaseerde therapie.[55] Bovendien hadden beide patiënten een eerdere varices bloeding gehad en beiden hadden voorafgaand aan de behandeling trombocytopenie als maat voor portale hypertensie (respectievelijk $89x10^9$/l en $90x10^9$/l). Om ervoor te zorgen dat het aantal bloedplaatjes voorafgaand aan de behandeling hoog genoeg was, met als doel om dosisverlagingen en bloedingen te voorkomen, werd onderzocht of patiënten konden worden behandeld met eltrombopag, een orale trombopoietine receptoragonist.[193] Hoewel werd aangetoond dat eltrombopag een krachtig middel is voor de behandeling van thrombocytopenie, werden een aantal belangrijke bijkomende problemen bij het gebruik tijdens interferon-gebaseerde antivirale therapie besproken in **hoofdstuk 1b**. De kans op het ontwikkelen van leverdecompensatie en trombo-embolische bijwerkingen was significant hoger in de groep die eltrombopag kreeg. Doordat patiënten met ernstige trombocytopenie langer behandeld werden met interferon, was het risico op hepatische decompensatie aanzienlijk toegenomen. Men zou zich kunnen afvragen of deze risico's opwegen tegen het minimale voordeel met betrekking tot de kans op SVR bij patiënten die werden behandeld met eltrombopag. Bovendien is het de vraag of patiënten met ernstige portale hypertensie baat hebben bij een succesvolle antivirale therapie. Het is goed mogelijk dat deze patiënten voorbij een 'point of no return' zijn, waardoor het natuurlijke beloop van de ziekte niet meer kan worden omgedraaid.

**Hoofdstuk 2** laat zien dat binnen dezelfde groep patiënten als hierboven beschreven, 113 infecties gerapporteerd werden tijdens 88 (12%) van de 723 interferon-gebaseerde behandelingen. In 23 van deze behandelingen daalden de neutrofielen onder 500/µl, maar een infectie werd slechts bij drie van deze behandelingen waargenomen. Een ANC <500/µl bij het voorafgaande bezoek was wel significant geassocieerd met het optreden van een infectie. Echter, bij een analyse waarbij alleen patiënten werden geïncludeerd die waren behandeld met gepegyleerd interferon, kon deze associatie niet worden aangetoond. Dit komt overeen met eerdere studies, waarbij hetzelfde is onderzocht.[114 200 202 203] Belangrijk is dat de meeste infecties, die tijdens de behandeling met interferon optraden, mild waren, zowel in onze studie als in andere studies. Zoals verwacht bleek dat ook cirrose en diabetes mellitus onafhankelijke risicofactoren zijn voor infecties tijdens interferon-gebaseerde behandeling.

Met betrekking tot infecties en bloedingen laten onze studies zien dat bij patiënten met gevorderde leverziekte, gepegyleerd interferon-gebaseerde therapie over het algemeen veilig is. Bij risicofactoren zoals cirrose of diabetes mellitus kunnen infecties en/of bloedingen vaker voorkomen. Deze patiënten moeten daarom nauwlettend worden gecontroleerd tijdens de behandeling. Omdat patiënten met gevorderde leverziekte veel baat hebben bij het behalen van SVR, kan worden overwogen om de dosis van interferon te handhaven zolang ANC en bloedplaatjes respectievelijk boven de 500/µl en $25x10^9$/l zijn. Hoewel we zelden deze waarden hebben waargenomen (ANC <500/µl in 3% van de behandelingen en de bloedplaatjes $<25x10^9$/l in 2% van de behandelingen), moet

worden opgemerkt dat de dosisverlagingen van interferon niet volgens een bepaald protocol werden toegepast, maar werden gekozen door de behandelende artsen. Prospectieve studies zouden moeten worden uitgevoerd om te kijken of minder strenge richtlijnen rondom de dosisverlagingen niet tot een groter risico op infecties en bloedingen leidt, vooral wanneer de behandelduur verkort kan worden tot 12 of 24 weken. Echter, gezien de snelle veranderingen waarbij interferon-vrije therapie de eerste keuze is, zullen deze studies waarschijnlijk nooit worden uitgevoerd.

Waarschijnlijk zal ribavirine nog steeds worden gebruikt in combinatie met DAAs om in bepaalde subgroepen van patiënten de virologische effectiviteit te optimaliseren. Het gebruik van ribavirine is geassocieerd met hemolytische anemie en is verantwoordelijk voor hemoglobine dalingen tijdens antivirale therapie. Genetische polymorfismen in het inosine triphosphatase (*ITPA*) gen op chromosoom 20 bleken tijdens de behandeling met gepegyleerd interferon en ribavirine geassocieerd te zijn met de bescherming tegen vroegtijdige ribavirine-geïnduceerde hemolytische anemie.[215] Aangezien de bevindingen over deze twee *ITPA* polymorfismen meestal afkomstig waren van patiënten die waren geïncludeerd in gerandomiseerde gecontroleerde studies, onderzochten we deze associatie in **hoofdstuk 3** bij patiënten die in de dagelijkse klinische praktijk werden behandeld.[217 218 221 222] In totaal konden 213 Kaukasische patiënten worden geïncludeerd, omdat zij een bloedmonster beschikbaar hadden voor de genetische testen. In 74% van de patiënten werd een klinisch significante daling van de hemoglobine gemeld. Patiënten die homozygote dragers waren van de meest voorkomende allel van beide *ITPA* genen (wat overeenkomt met een normale ITPase activiteit), ondervonden een diepere daling van hemoglobine en daardoor trad een klinisch significante anemie vaker op. Deze patiënten ondergingen ook meer dosisverlagingen van zowel ribavirine en gepegyleerd interferon en kregen vaker bloedtransfusies en/of erytropoëtine toegediend.

Omdat de effectiviteit van de behandeling wordt verminderd door dosisreducties kunnen patiënten met een normale ITPase activiteit profiteren van de vroege strategieën ter bevordering van het hemoglobinegehalte. Deze polymorfismen kunnen ook worden gebruikt als criterium om de keuze voor een bepaald interferon-vrij regime voor de individuele patiënt te selecteren, aangezien niet alle regimes de toevoeging van ribavirine nodig hebben voor een optimaal virologisch effect. De vraag blijft echter wel of de associatie tussen ITPase activiteit en anemie ook wordt gevonden indien patiënten worden behandeld met interferon-vrij therapie in combinatie met ribavirine.

Het belang van bloedplaatjes werd opnieuw benadrukt in **hoofdstuk 4.** Naast een verbeterde prognose, is SVR herhaaldelijk geassocieerd met vermindering van leverfibrose en portale druk, welke verband houden met een gunstige klinische uitkomst.[74 78 241-247 269 363-365] Het evalueren van verandering in de architectuur van de lever of de hepatische veneuze drukgradiënt vereist invasieve procedures, zodat het moeilijk is om meerdere keren deze uitkomsten te evalueren. In **hoofdstuk 4** hebben we daarom het beloop van bloedplaatjes na antivirale therapie onderzocht bij patiënten met chronische HCV infectie en gevorderde leverfibrose. Vooral bij patiënten met gevorderde leverziekte is de hoogte van bloedplaatjes een niet-invasieve en objectieve parameter van de mate van portale hypertensie en leverfibrose.[253 255 256] Eerder zijn de veranderingen in het aantal bloedplaatjes na antivirale therapie in verband gebracht met veranderingen in leverfibrose.[258 259] Met herhaaldelijke metingen zagen we na enkele jaren een aanzienlijke toename van bloedplaatjes, welke lineair bleek te zijn, indien patiënten SVR hadden behaald,. Belangrijk is dat de verandering in het aantal thrombocyten gecorreleerd is

aan de verandering in miltgrootte, welke een maat is van portale druk. Deze bevindingen wijzen op een geleidelijke verbetering van de portal druk en leverhistologie nadat een chronische HCV infectie succesvol is behandeld. In tegenstelling tot bij patiënten met SVR, namen de bloedplaatjes verder af tijdens de follow-up bij patiënten die zonder succes werden behandeld.

## HET VERANDERENDE LANDSCHAP

Sinds de ontdekking van HCV in 1989 en de implementatie van het screenen van bloedproducten kort daarna, werd een significante daling van de overdracht van het virus geconstateerd.[11 278-281] Aangezien leverfibrose zich langzaam ontwikkelt en patiënten vaak asymptomatisch zijn of niet-specifieke symptomen hebben, blijven veel besmette mensen tot nu onopgemerkt. Als gevolg van veroudering van de HCV-geïnfecteerde populatie, wordt verwacht dat ongeveer 25% van de Amerikaanse patiënten met chronische HCV infectie levercirrose heeft. Dit percentage zal waarschijnlijk in de komende jaren verder toenemen en in 2030 tot 45% zijn gestegen.[63] Met de introductie van veilige en effectieve interferon-vrij antivirale behandelingen, wordt verwacht dat meer patiënten effectief kunnen worden behandeld, zelfs patiënten met gevorderde leverziekte.

Op basis van historische gegevens werd een model gemaakt voor de huidige en toekomstige ziektelast van chronische HCV infectie in Nederland.[4 286 287] Aanvullende informatie over de trends in de patiënt- en ziektekenmerken van de laatste twee decennia is belangrijk voor de Nederlandse gezondheidszorg en voor beleidsmakers om te worden geïnformeerd over de veranderende trends in de patiëntenpopulatie met een chronische HCV infectie. Dit geeft informatie over de kenmerken van de patiënten die tijdens de komende jaren voor interferon-vrije therapie in aanmerking komen. **Hoofdstuk 5** beschrijft de epidemiologische veranderingen van de patiënt- en ziektekenmerken van 1.779 nieuwe patiënten met een chronische HCV infectie die tussen 1990 tot 2013 werden verwezen naar een tertiair centrum in Nederland. Zoals verwacht met een verminderde incidentie van chronische HCV infectie werden in de laatste jaren patiënten verwezen die ouder waren en vaker gevorderde leverfibrose hadden in vergelijking met de patiënten die in de eerste jaren werden verwezen. Ook waren de patiënten vaker besmet met HCV genotype 1a. Dit kan mogelijk verklaard worden door de lagere kans op genezing bij patiënten met chronische HCV genotype 1a in vergelijking met patiënten met chronische HCV genotype 1b, vooral bij behandeling met de eerste generatie proteaseremmers telaprevir en boceprevir.[298] De oorzaak van dit verschil tussen HCV genotype 1a en 1b is waarschijnlijk de resistentie-geassocieerde varianten die aanwezig waren voorafgaand aan de behandeling of welke zijn ontstaan tijdens antivirale therapie.[366-369] Hoewel het niet statistisch significant was, werd tevens een toename van het aantal patiënten met chronische HCV genotype 3 gevonden. Aangezien dit genotype geassocieerd is met een snellere progressie van de ziekte, is deze bevinding erg belangrijk.[299] Bovendien is de behandeling met interferon-vrije therapie minder effectief bij patiënten met HCV genotype 3.

Een andere interessante bevinding was dat 70% van alle patiënten werd geboren tussen 1950 en 1975. Hoewel screening voor chronische HCV infectie in Nederland door de lage prevalentie van de ziekte en bij suboptimale behandelingen niet als kosteneffectief werd beschouwd, moet dit nu er

effectieve behandelingen beschikbaar zijn opnieuw worden beoordeeld voor specifieke subgroepen met de hoogste prevalentie. Met het oog op de prevalentie en de klinische impact van de ziekte kan een effectieve screening strategie de ziektelast van chronische HCV infectie echt verminderen. Om de HCV-gerelateerde sterfte te verminderen moeten patiënten, die nog niet op de hoogte zijn van hun chronische HCV infectie, worden gediagnosticeerd.[370]

De goedkeuring van verschillende DAAs leidde tot een discussie over de prijs van deze geneesmiddelen. Op dit moment belemmeren de hoge kosten van deze DAAs wereldwijde beschikbaarheid. Beleidsmakers worden daarom gedwongen om prioriteit te geven aan het behandelen van patiënten die een dringende behoefte hebben aan succesvolle antivirale therapie. Deze situatie is echter verre van ideaal, vooral omdat gedacht wordt dat patiënten zonder cirrose het grootste voordeel van succesvolle antivirale behandeling hebben.[261] Sinds de introductie van DAAs werden steeds meer studies verricht die de kosteneffectiviteit van deze geneesmiddelen onderzocht, waarbij verschillende conclusies worden gevormd over wie te behandelen en op welke moment.[371-374] Om de kosten van DAAs in een breder perspectief te plaatsen, hebben we in **hoofdstuk 6** de kosten per SVR bekeken bij patiënten met gevorderde hepatische fibrose die werden behandeld met interferon-gebaseerde antivirale therapie. De patiënten die waren geïncludeerd in de studies uit hoofdstuk 1 en 2, werden ook geïncludeerd in dit onderzoek indien gedetailleerde informatie beschikbaar was. Voor de totale studiegroep waren de gemiddelde kosten per SVR €38.514. Deze kosten waren hoger voor patiënten met cirrose in vergelijking met patiënten met ernstige fibrose. Indien de hoeveelheid bloedplaatjes werd gebruikt als indicator voor de ernst van de leverziekte, bleek dat voor patiënten met bloedplaatjes <100x10$^9$/l de gemiddelde kosten per SVR €74,961 waren, wat aanzienlijk hoger was dan voor patiënten met normale bloedplaatjes bij aanvang van behandeling (€26,105). Deze verschillen kunnen waarschijnlijk worden toegeschreven aan de lagere kans op het bereiken van SVR in deze subgroepen, wat belangrijk is, omdat dit bij de huidige goedgekeurde interferon-vrije behandelingen niet het geval is vanwege hoge SVR percentages binnen alle subgroepen van patiënten. Rekening houdend met de mogelijke bijwerkingen van de behandeling met interferon en de hoge kans op herbehandeling, kunnen de extra kosten per SVR van de DAAs beperkt zijn, vooral voor patiënten met de meest ernstige leverziekte. Natuurlijk moeten deze gegevens met terughoudendheid worden geïnterpreteerd en niet worden gebruikt om de hoge kosten van de nieuwe geneesmiddelen te rechtvaardigen. Om wereldwijd meer patiënten te kunnen behandelen, zouden deze kosten aanzienlijk moeten worden verminderd.

## HET NIEUWE LANDSCHAP: DIRECT-WERKENDE ANTIVIRALE MIDDELEN

Na de goedkeuring van telaprevir en boceprevir, de eerste DAAs, lieten verschillende studies zien dat belangrijke bijwerkingen werden gezien tijdens de behandeling, met name bij patiënten met gevorderde leverfibrose.[120 121] Gegevens van patiënten die niet worden behandeld in gerandomiseerde studies zijn, na de goedkeuring van nieuwe medicijnen, van heel groot belang. De werkzaamheid en veiligheid van deze behandelingen zijn namelijk gebaseerd op het behandelen van patiënten die aan de strenge criteria voldoen. In **hoofdstuk 7** onderzochten we de veiligheid en de effectiviteit van

DAA-gebaseerde antivirale therapie bij patiënten met cirrose. Gegevens van vier grote academische ziekenhuizen in Canada, Duitsland en Nederland werden gecombineerd. In totaal werden 433 patiënten met cirrose geïncludeerd, waaronder 114 patiënten met gedecompenseerde cirrose (gedefinieerd als een Child Pugh B of C). Het totale SVR percentage bedroeg 81% (350/433) en er was een trend naar een betere respons bij patiënten met CP A in vergelijking met patiënten met CP B/C (264/319 [82.8%] versus 86/114 [75,4% ], $p$ = 0,088). Wat nog belangrijker was, was dat bij 50 (11,5%) van de patiënten leverdecompensatie of een verergering van de bestande leverdecompensatie optrad tijdens antivirale therapie. Factoren die waren geassocieerd met het optreden van leverdecompensatie waren HCV genotype 3 (versus niet-genotype 3), Child Pugh B/C (vs Child Pugh A) en albumine <35 g/l. Wanneer Child Pugh score werd vervangen door MELD score, vonden we dat een MELD score ≥14 was geassocieerd met leverdecompensatie. Indien drie risicofactoren (HCV genotype 3, albumine <35 g / l en MELD score ≥14) aanwezig waren, was de cumulatieve incidentie voor leverdecompensatie tijdens de eerste 24 weken 75,0% (50,5-99,5%) terwijl de kans op het bereiken van SVR 67% bedroeg. Door het bepalen van deze drie parameters, kunnen artsen het risico op leverdecompensatie beoordelen, wat moet worden afgewogen tegen de kans op SVR. Men zou kunnen stellen dat, wanneer de kans op decompensatie min of meer gelijk is of hoger is dan de kans op het bereiken van SVR, het uitstellen van de therapie een betere optie zou zijn. Deze studie geeft tevens het belang van HCV genotype 3 aan. Eerdere gegevens hebben aangetoond dat HCV genotype 3 is geassocieerd met een snellere progressie naar cirrose, de ontwikkeling van HCC en een verminderde algehele overleving.[299 335] Onze gegevens tonen aan dat HCV genotype 3 nog steeds relevant is bij patiënten met gedecompenseerde cirrose, omdat we vonden dat het ook in verband gebracht kon worden met de ontwikkeling of verergering van leverdecompensatie. Gelukkig werden de uitkomsten van meer effectieve behandelingen voor HCV genotype 3 onlangs gepresenteerd, waarbij meerdere combinaties van nieuwe DAAs mogelijk zijn (sofosbuvir en velpatasvir, vooral met de toevoeging van GS-9857 en ABT-493 en ABT-530).[168 375-377] De goedkeuring van deze middelen moet hoge prioriteit krijgen.

De behoefte aan effectievere therapie voor HCV genotype 3 werd ook beschreven in **hoofdstuk 8**. Deze studie was onderdeel van een grote registratiestudie HCV-TARGET en includeerde 197 patiënten met HCV genotype 3 die werden behandeld met sofosbuvir en ribavirine met of zonder toevoeging van gepegyleerd interferon. Van de 178 patiënten die met sofosbuvir en ribavirine werden behandeld, bereikte 60% SVR, vergeleken met 84% van de 19 patiënten behandeld met sofosbuvir, gepegyleerd interferon en ribavirine. Na gecorrigeerd te hebben voor leeftijd en geslacht, bleken het ontbreken van cirrose, albumine ≥3.2 g/dl en bloedplaatjes >100x$10^9$/l geassocieerd te zijn met een grotere kans op SVR. Hoewel deze studie hier niet voor was opgezet, bleek dat het toevoegen van gepegyleerd interferon voor de behandeling van HCV genotype 3 de kans op SVR kan verhogen. Dit werd ook aangetoond in een gerandomiseerde gecontroleerde trial waarbij sofosbuvir en ribavirine gedurende 16 of 24 weken werd vergeleken met sofosbuvir, gepegyleerd interferon en ribavirine gedurende 12 weken bij patiënten met HCV genotype 3.[378] Deze studie toonde aan dat het toevoegen van gepegyleerd interferon aan sofosbuvir en ribavirine de kans op respons verhoogd binnen alle subgroepen, maar vooral bij patiënten met cirrose. Bij deze patiënten steeg de kans op SVR tot 82%, met sofosbuvir en ribavirine voor 24 weken, tot 91% met sofosbuvir, gepegyleerd interferon en

ribavirine bij de patiënten die niet eerder waren behandeld en van 77% tot 86% bij patiënten die al eerder waren behandeld. Gepegyleerd interferon werd goed verdragen en moest slechts bij één patiënt vroegtijdig worden gestopt. In onze studie werd hetzelfde gezien. Het aantal bijwerkingen was vergelijkbaar binnen de groep met en zonder gepegyleerd interferon en de SVR percentages waren hoger in de groep die werd behandeld met gepegyleerd interferon. Ondanks deze bemoedigende resultaten, werd binnen HCV-TARGET sofosbuvir, gepegyleerd interferon en ribavirine bij slechts 19 van de 197 (9,6%) patiënten met HCV genotype 3 infectie voorgeschreven. Het is duidelijk dat zowel artsen als patiënten terughoudend zijn om interferon-gebaseerde therapieën te gebruiken. Gelukkig hebben recente studies aangetoond dat sofosbuvir in combinatie met velpatasvir, een pangenotypische NS5A inhibitor, zeer effectief is voor patiënten met chronische HCV genotype 3 infectie, waardoor SVR percentages van 90% werden gevonden, zelfs bij patiënten met cirrose. Ribavirine lijkt ook niet langer nodig te zijn met deze nieuwe DAA combinatie.[168]

Ondanks het feit dat sofosbuvir als zeer veilig wordt beschouwd, is het gebruik ervan niet aanbevolen bij patiënten met een geschatte glomerulaire filtratiesnelheid (eGFR) <30 ml/min/1,73m$^2$ of bij patiënten met eindstadium nierziekte. Dit komt omdat de belangrijkste actieve metaboliet van sofosbuvir, GS331007, wordt uitgescheiden door de nieren. Hierdoor worden hogere metaboliet concentraties gevonden bij patiënten met een geschatte glomerulaire filtratiesnelheid (eGFR) <30 ml/min/1.73m$^2$ of eindstadium nierziekte die hemodialyse ondergaan.[346] Hoewel de belangrijkste klinische studies over de veiligheid en werkzaamheid van sofosbuvir een uitstekend veiligheidsprofiel hebben aangetoond met betrekking tot de nier is het nog niet bekend of dat ook geldt in de dagelijkse klinische praktijk. Eerder meldden de registratiestudies van telaprevir en boceprevir ook een relatief goed renaal veiligheidsprofiel, terwijl kort na de goedkeuring belangrijke gegevens werden gepubliceerd over de toxiciteit van de nieren.[344 345] In **hoofdstuk 9** werd het optreden van nierfunctiestoornissen bekeken tijdens sofosbuvir-gebaseerde antivirale therapie. Deze grote studie omvatte 426 patiënten met chronische HCV infectie die werden behandeld met sofosbuvir-gebaseerde (n=233) of telaprevir/ boceprevir-gebaseerde (n=193) behandelingen in twee tertiaire centra in Noord-Amerika. Het risico op een nierfunctiestoornis was lager voor patiënten die werden behandeld met sofosbuvir-gebaseerde behandeling in vergelijking met telaprevir/ boceprevir-gebaseerde behandeling. Hoewel nierinsufficiëntie werd waargenomen bij 11% van de patiënten behandeld met sofsobuvir-gebaseerde therapie, heeft het een goed renaal veiligheidsprofiel, aangezien nierinsufficiëntie in bijna alle gevallen reversibel was. Patiënten met ascites en patiënten die NSAID's gebruikten, hadden een verhoogd risico op het ontwikkelen van een nierfunctiestoornis tijdens sofosbuvir-gebaseerde antivirale therapie. Controle van de nierfunctie en de standaard beschermende maatregelen voor de nieren worden aanbevolen bij het gebruik van sofosbuvir-gebaseerde regimes, vooral bij patiënten met gevorderde cirrose en portale hypertensie of bij patiënten met comorbiditeit met mogelijke gevolgen voor de nieren. Gelukkig hebben twee nieuwe behandelopties veelbelovende resultaten aangetoond bij patiënten met nierfalen. Op basis van hun beperkte renale excretie zijn grazoprevir en elbasvir en de combinatie van paritaprevir/ ritonavir, ombitasvir en dasabuvir geëvalueerd bij patiënten met gevorderde chronische nierziekte of hemodialyse en het blijkt dat deze middelen uiterst veilig en zeer effectief zijn.[158 162]

## CONCLUSIES

Met de goedkeuring van meerdere orale DAAs hebben artsen nu de mogelijkheid om een zeer effectieve en een zeer veilige antivirale therapie te starten bij patiënten met chronische HCV infectie, zelfs bij patiënten met de meest ernstige leverziekte. Hoewel de kosten wereldwijde toegang tot deze geneesmiddelen momenteel beperken, heeft een recent onderzoek naar het gebruik van generieke DAAs veelbelovende resultaten aangetoond.[379] Tot die tijd kan gepegyleerd interferon worden gebruikt, gelukkig met een kortere duur dan vóór 2014. Met de juiste controle kunnen zelfs patiënten met gecompenseerde cirrose veilig worden behandeld met gepegyleerd interferon. Indien nieuwe DAA combinaties niet beschikbaar zijn, kunnen artsen overwegen interferon-gebaseerde antivirale therapie te starten, met name bij patiënten met HCV genotype 3, die daadwerkelijk kunnen profiteren van deze toevoeging. Chronische HCV infectie is bij uitstek een ziekte die, wat betreft de behandeling, een patiënt-specifieke aanpak nodig heeft. Terwijl er bijna geen beperkingen meer zijn voor het gebruik van DAAs, hebben sommige patiënten wellicht geen klinisch voordeel van succesvolle antivirale therapie, aangezien het natuurlijke beloop van hun ziekte niet meer kan worden teruggedraaid. Met de aankomende pangenotypische behandelingen worden behandelingsbesluiten vereenvoudigd, hoewel dit niet voor elke patiënt zo zal zijn. Nauwe samenwerking tussen artsen, gezondheidsautoriteiten en de farmaceutische bedrijven is dringend nodig om de enorme ziektelast van chronische HCV infectie te verminderen. Patiënten moeten worden gediagnosticeerd, verwezen en behandeld, zodat het virus volledig uitgeroeid kan worden, vooral omdat we nu een goede kans op genezing kunnen bewerkstelligen.

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Uiteraard wil ik ook iedereen bedanken met wie ik menig lief en leed heb gedeeld op de befaamde 'dakpoli'. Zonder de geweldige activiteiten, zoals wintersporten, BDDLs, feestjes, vrijdagmiddagborrels, koffiemomenten en bruggenlopen zou het leven van een promovendus toch een stuk saaier zijn. Ik ga mij er niet aan wagen om alle namen te noteren van collega's, want ik ben bang mensen te vergeten en dat verdient niemand. Wel wil ik stilstaan bij de hepa's Heng, Wim, Lisanne (Plompen), Ludi en Michelle. Bedankt voor de leuke congressen en etentjes. Marjolein, voorlopig de laatste HCV promovenda, bedankt voor de leuke samenwerking. Na mijn verdediging heb ik alle tijd voor jouw projecten! Lieve Els, ouwe Feyenoord-hooligan, jouw enthousiasme en interesse zijn ongekend. Succes met het afronden van alles. Lieve Maren, wat hebben we een geweldige tijd gehad. Met name de weken in Toronto waren heel tof. Jij bent het schoolvoorbeeld van "*Work hard, play harder*"! Laten we snel weer een etentje plannen.

Niemand kan zonder kamergenoten. Milan, wie had gedacht dat we elkaar na Wolfert op CA-419 zouden treffen! Bedankt voor de gezelligheid en input tijdens de afgelopen jaren. Helaas hebben we maar kort bij elkaar op de kamer gezeten, maar de congressen hebben een hoop goed gemaakt. Mede dankzij jouw speurwerk, belandden we altijd op de beste feestjes!
Lisanne (Holster), jouw gedrevenheid en werkethos werkten zeer aanstekelijk. Altijd tijd voor een koffie/thee-moment, waar we de meest uiteenlopende zaken bespraken. Helaas zien we elkaar niet zo vaak, maar daar zal snel verandering in komen als we weer samen in het EMC terechtkomen.
Lieve Margo, de jongste telg van CA-419 (inmiddels ook ex-CA-419). Wat hebben we een mooie tijd gehad op de kamer, maar ook op congressen. Ik wens je veel succes met het afronden van je promotie. Blijf zoals je bent!
Tot slot, lieve Eline, *het orakel*, wat hebben we een geweldige tijd gehad op CA-419 aka de toffe perenkamer. Je bent een geweldig mens en ik weet zeker dat je een hele goede huisarts gaat worden. Jouw organisatorisch vermogen en betrokkenheid zijn ongeëvenaard. Wat hebben we een hoop meegemaakt in de afgelopen jaren. Bedankt dat je er altijd voor me was.

Collega's uit het IJsselland Ziekenhuis, *YSL Masters*, in korte tijd voel ik mij al helemaal thuis bij jullie. Vanaf nu heb ik meer tijd voor die vrijdagmiddagborrel, zodat we niet alleen maar koffie met elkaar drinken.

Willem Pieter, wat geweldig dat jij, ongeveer een jaar na jouw eigen grote dag, nu achter mij plaatsneemt. Vanaf dag één wist ik dat ik niet alleen een nieuwe collega had, maar ook een nieuwe dierbare vriend. Bedankt voor alle geweldige momenten tot nu toe. Ik weet zeker dat er nog veel meer mooie momenten gaan volgen, ook samen met onze kleine mannen.

Ad, jouw invloed en drive zijn aanstekelijk en hebben een hele grote rol gespeeld bij de ontwikkeling van dit proefschrift. Mijn dank is ontzettend groot, je bent een geweldige en unieke vent en ik weet zeker dat we de basis hebben gelegd voor een prachtige toekomst, zowel op zakelijk als privé gebied. Onze gedeelde passie, ambitie en humor staat centraal in onze vriendschap. Vanzelfsprekend sta jij vandaag achter mij.

BVV six, cinq, quattro, dank voor alle uren van relativering en ontspanning. Ik beloof dat jullie de rest van dit seizoen de 'oude Maan' weer te zien krijgen. 'Dakterrastriviantje', bedankt voor jullie interesse in de 'vergeten ziekte'. De avondjes triviant, waarbij soms niet alle laatjes zich openden, zorgden voor de nodige uurtjes vermaak. Laten we snel weer een nieuwe avond plannen!

Lieve vrienden, bedankt voor alle interesse tijdens de afgelopen jaren. Jullie zijn geweldig! Lieve Jeroen, onze onvoorwaardelijke vriendschap heeft ons al veel gebracht. Ik ben trots op je en jouw succes inspireert mij enorm. De gesprekken tijdens onze vele lunches zorgden voor een welkome afleiding tijdens mijn promotie. Lieve Bob, jij bent het voorbeeld dat je met genoeg discipline kan bereiken wat je wilt. Onze vriendschap is mij zeer dierbaar. De week dat jullie in Toronto waren was geweldig! Lieve Koen, ook al zien we elkaar niet meer op regelmatige basis, de basis die wij in het

verleden hebben gelegd zorgt ervoor dat we nog steeds mooie momenten kunnen delen. Lieve Joost, de uurtjes boksen en gezellige avondjes op de Witte de With waren geweldig. Ik beloof weer meer tijd vrij te maken voor deze belangrijke momenten. Lieve Rick, wat hebben we al veel mooie dingen meegemaakt, van vakken vullen tot voetballen. Jouw eeuwige vrolijkheid is een voorbeeld voor iedereen.

Lieve Niek en Nique, wat ben ik blij dat we zulke goede vrienden zijn! Tot nu toe hebben we veel mooie herinneringen kunnen maken en ik weet zeker dat er nog veel meer mooie herinneringen gaan volgen. Het was een hele eer om jullie grote dag te regelen en ik kijk er naar uit dat jullie dit in 2017 voor ons kunnen doen. Lieve Hadewych, het moment dat je mij kwam uitzwaaien op Schiphol vergeet ik nooit meer. Je bent een schat!

Dear Canadian family, thank you so much for all the great moments during my visits to Toronto. I would never considered going to Toronto if you wouldn't be around. Thank you, for the great interest in my work and for taking care of me. I am sure we will visit Toronto some day!

Lieve familie, bedankt voor al jullie interesse in mijn werkzaamheden. Lieve opa Frans en oma Wip, ook al kunnen jullie vandaag niet meer aanwezig zijn, ik weet zeker dat jullie, net als bij de uitreiking van mijn artsenbul, heel trots geweest zouden zijn. Bedankt voor alle liefde en steun die jullie mij in het verleden hebben gegeven. Lieve opa Klaas, ook u zou heel trots zijn geweest op deze prestatie. Esmee heeft mij nog steeds aardig onder de duim. Bedankt voor alle mooie momenten. Lieve oma Bep, wat geweldig dat u dit nog mee kunt maken. Uw eigen doctor in de familie!

Lieve Henny, bedankt voor alle steun en interesse tijdens de afgelopen jaren. Ik heb heel veel respect voor de manier waarop jij alles doet en in het verleden hebt gedaan. Ik ken geen vrouw die sterker is dan jij. Lieve Jerry, ik weet zeker dat jij het prachtig zou hebben gevonden om hier vandaag te zijn. Ook al was het ons niet gegund om elkaar heel goed te leren kennen, toch denk ik dat we in die korte tijd genoeg hebben opgebouwd voor de rest van het leven. Lieve Ronald, bedankt voor alle interesse en goede gesprekken. Met name de gesprekken over hoe om te gaan met het temperament van de Kuppens zijn verhelderend. Je bent een topvent! Lieve Chloé, ik ben ontzettend trots op mijn kleine zusje. Geweldig om te zien hoe jij door blijft leren, respect!

Lieve Anoek, wat ben ik trots op je en wat ben ik blij dat jij mijn zus bent. Dankjewel dat je altijd interesse toont in alles wat ons bezighoudt. We hebben een geweldige tijd gehad in Toronto, waar ik met veel plezier aan terugdenk. Ik bewonder jouw doorzettingsvermogen en de manier waarop jij belangrijke keuzes maakt. Ik leer heel veel van je! Lieve David, ik had mij geen betere zwager kunnen bedenken. Jouw pragmatische manier van leven is een voorbeeld voor iedereen. Dankjewel voor de gezellige spelletjesavonden en de vele voortreffelijke maaltijden. Ik zou het je niet na kunnen doen!

Lieve papa en mama, bedankt voor alle steun en onvoorwaardelijke liefde die jullie mij geven. Jullie staan altijd voor ons klaar! Zonder jullie, zou ik niet alles uit het leven kunnen halen. Ik houd van jullie.

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Lieve Esmee, mijn allerliefste, ik weet niet hoe ik jou moet bedanken. Het geduld dat jij hebt gehad en de onvoorwaardelijke steun en liefde die je me hebt gegeven zijn niet in woorden uit te drukken. Jij stelde mij in staat om op de meest idiote tijden te kunnen werken en zonder jou was dit proefschrift er nooit gekomen. We kunnen nu ook deze mijlpaal aan al onze mooie en minder mooie momenten toevoegen. Vanaf vandaag gaan we onze grote dag voorbereiden. Ik kan niet wachten tot de dag komt, dat ik jou mijn vrouw mag noemen.

Lieve Morris, wat ben jij een verrijking. Ons leven is nu nog mooier en leuker dan het al was. Door jou sta ik iedere dag met een grote glimlach op. De liefde die jij ons geeft, is niet te beschrijven. We gaan een mooie toekomst tegemoet.

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# BIBLIOGRAPHY


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19 Berden FA, **Maan R**, de Knegt RJ, Kievit W, Drenth JP. Bleeding episodes during interferon-based triple therapy in hepatitis C: a nationwide cohort study. *Submitted*

# CURRICULUM VITAE

# CURRICULUM VITAE

Raoel Maan werd geboren op 3 december 1985 te Barendrecht. In 2004 behaalde hij zijn tweetalig VWO diploma aan het Wolfert van Borselen te Rotterdam. Hierna startte hij met zijn studie Geneeskunde aan de medische faculteit van de Universiteit Leiden. Hij verkreeg zijn doctoraal in 2008 nadat hij zijn afstudeeronderzoek naar de waarde van het electrocardiogram bij een myocard infarct had afgerond op de afdeling cardiologie van het Leids Universitair Medisch Centrum. Na het doorlopen van zijn coschappen, in onder andere het Tygerberg Ziekenhuis te Kaapstad, Zuid-Afrika, behaalde hij in 2010 zijn artsexamen. Vervolgens werkte hij als basisarts op de afdeling Interne Geneeskunde van het Medisch Centrum Haaglanden te Den Haag. Anderhalf jaar later startte hij een promotieonderzoek naar de veiligheid van antivirale therapie voor chronische hepatitis C. Dit deed hij op de afdeling Maag-, Darm-, en leverziekten onder supervisie van prof.dr. H.L.A. Jannsen en dr. R.J. de Knegt. Tijdens deze periode heeft hij een half jaar gewerkt in het Toronto Western Hospital te Toronto, Canada, als research-fellow onder begeleiding van prof.dr. H.L.A. Janssen en J.J. Feld. Sinds juli 2016 is hij in opleiding tot Maag-, Darm-, en Leverarts in het Erasmus MC (opleider prof.dr. R.A. de Man). Zijn vooropleiding Interne Geneeskunde volgt hij in het IJsselland Ziekenhuis, te Capelle aan den IJssel (opleider: dr. H.E. van der Wiel). Hij woont samen met Esmee Kuppen en hun zoon Morris in Rotterdam.

# PHD PORTFOLIO

# SUMMARY OF PHD TRAINING AND TEACHING

| | |
|---|---|
| Name PhD student: | Raoel Maan |
| PhD period: | June 2012 – June 2016 |
| Erasmus MC Department: | Gastroenterology and Hepatology |
| Promotor: | prof. dr. H.L.A. Janssen |
| Co-promotor: | dr. R.J. de Knegt |

| 1. PhD training | Year | Workload |
|---|---|---|
| **Courses in Methodology and Biostatistics** | | |
| Introduction to clinical research, Netherlands Institute for Health Sciences, Rotterdam, The Netherlands | 2013 | 24 hours |
| Regression analysis for clinicians, Netherlands Institute for Health Sciences, Rotterdam, The Netherlands | 2013 | 40 hours |
| 10th Course on SNPs & Human Diseases, Molecular Medicine Postgraduate School | 2013 | 40 hours |
| **Research Integrity and Workshops** | | |
| Basiscursus Regelgeving en Organisatie van Klinisch onderzoek, Erasmus MC, Rotterdam | 2013 | 30 hours |
| UHN Good Clinical Practice – Principles, Toronto Western Hospital, Toronto, Canada | 2015 | 24 hours |
| Scientific Writing course, Molecular Medicine Postgraduate School | 2013 | 40 hours |
| **Oral Presentations** | | |
| Effect of thrombocytopenia on treatment tolerability and outcome in patients with chronic HCV infection and advanced hepatic fibrosis. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands. | 2013 | 12 hours |
| Assessment of noninvasive hepatic fibrosis markers among patients with chronic HCV infection and advanced liver disease. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands. | 2014 | 24 hours |
| Liver biopsy for the diagnosis of disease severity among patients with chronic HCV infection. 2nd HCV Symposium, Amsterdam, the Netherlands | 2014 | 12 hours |
| Benefit of attaining SVR. National Hepatitis Day, Amsterdam, the Netherlands. | 2015 | 12 hours |
| New insights in the treatment of chronic HCV infection; relevance for Nephrology. 25th Workshop Nephrology, Papendal, the Netherlands. | 2015 | 36 hours |

**Poster Presentations**

| | | |
|---|---|---|
| Effect of thrombocytopenia on treatment tolerability and outcome in patients with chronic HCV infection and advanced hepatic fibrosis. 64th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Washington, DC, United States of America. | 2013 | 32 hours |
| ITPA polymorphisms are associated with hemoglobin decline during pegylated interferon and ribavirin therapy in chronic hepatitis C. 64th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Washington, DC, United States of America. | 2013 | 32 hours |
| Incidence and risk factors for infections during interferon-based treatment of chronic hepatitis C patients with advanced hepatic fibrosis. 49th Annual Meeting of the European Association for the Study of the Liver (EASL), London, United Kingdom. | 2014 | 32 hours |
| DDRGK1 variants are associated with platelet decline during peginterferon and ribavirin therapy in Caucasian patients with chronic HCV infection. 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America. | 2014 | 32 hours |
| Assessment of noninvasive hepatic fibrosis markers among patients with chronic HCV infection and advanced liver disease. 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America. | 2014 | 32 hours |
| Epidemiological trends among patients with chronic HCV infection in a tertiary center in the Netherlands. 50th Annual Meeting of the European Association for the Study of the Liver (EASL), Vienna, Austria. | 2015 | 32 hours |
| Hepatocellular carcinoma has a more aggressive disease course in patients with HCV genotype 3 compared to other genotypes. 66th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, United States of America. | 2015 | 32 hours |
| Safety of DAA-based therapy for the treatment of patients with chronic hepatitis C virus infection and cirrhosis: results from an international multicenter cohort study. 51st Annual Meeting of the European Association for the Study of the Liver (EASL), Barcelona, Spain. | 2016 | 32 hours |
| HCV therapy selector webapp: an uniquely simple tool for evidence-based therapy selection for patients with chronic hepatitis C virus infection. 67th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America. | 2016 | 32 hours |

**Attended (inter)national conferences**

| | | |
|---|---|---|
| Twice annual meeting of the Dutch Association of Hepatology, Zeist, the Netherlands. | 2012 | 12 hours |
| The Liver Meeting 2012, 63rd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America. | 2012 | 28 hours |
| 48th Annual Meeting of the European Association for the Study of the Liver (EASL). Amsterdam, The Netherlands. | 2013 | 28 hours |
| Twice annual meeting of the Dutch Association of Hepatology, Veldhoven, the Netherlands. | 2013 | 12 hours |
| The Liver Meeting 2013, 64th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Washington, DC, United States of America. | 2013 | 28 hours |
| 49th Annual Meeting of the European Association for the Study of the Liver (EASL). London, United Kingdom. | 2014 | 28 hours |
| The 3rd world congress on controversies in the management of viral hepatitis. Berlin, Germany. | 2014 | 20 hours |
| Twice annual meeting of the Dutch Association of Hepatology, Veldhoven, the Netherlands. | 2014 | 12 hours |
| The Liver Meeting 2014, 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America. | 2014 | 28 hours |
| 50th Annual Meeting of the European Association for the Study of the Liver (EASL). Vienna, Austria. | 2015 | 28 hours |
| Twice annual meeting of the Dutch Association of Hepatology, Veldhoven, the Netherlands. | 2015 | 12 hours |
| The Liver Meeting 2015, 66th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). San Francisco, CA, United States of America. | 2015 | 28 hours |
| The Liver Meeting 2016, 67th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America. | 2016 | 28 hours |

**Attended seminars and workshops**

| | | |
|---|---|---|
| 4th Lagerhuisdebat Hepatitis B en C. Utrecht, The Netherlands | 2012 | 3 hours |
| 28th Erasmus Liver day. Rotterdam, The Netherlands | 2013 | 6 hours |
| 11th Post-AASLD symposium. Rotterdam, The Netherlands | 2013 | 2 hours |
| 5th Lagerhuisdebat Hepatitis B en C. Utrecht, The Netherlands | 2013 | 3 hours |
| 29th Erasmus Liver day. Rotterdam, The Netherlands | 2014 | 6 hours |
| 12th Post-AASLD symposium. Rotterdam, The Netherlands | 2014 | 2 hours |
| 6th Lagerhuisdebat Hepatitis B en C. Utrecht, The Netherlands | 2015 | 3 hours |

| | | |
|---|---|---|
| 30th Erasmus Liver day. Rotterdam, The Netherlands | 2015 | 6 hours |
| 3rd Post-EASL symposium, Amsterdam, The Netherlands | 2016 | 3 hours |

**Reviewing for scientific journals**

| | | |
|---|---|---|
| Including Hepatology, Journal of Hepatology, Liver International, AP&T, Drugs, PLoS One, Infectious Diseases, European Journal of Gastroenterology and Hepatology, Expert Review of Gastroenterology & Hepatology | | 40 hours |

| **2. Teaching** | **Year** | **Workload** |
|---|---|---|
| **Lecturing** | | |
| Diagnostics and treatment of chronic hepatitis C, Masterclass viral hepatitis, Erasmus Medical Center, Rotterdam, The Netherlands | 2013 | 6 hours |
| Diagnostics and treatment of chronic hepatitis C, Masterclass viral hepatitis, Erasmus Medical Center, Rotterdam, The Netherlands | 2015 | 6 hours |
| Chronic HCV infection, 2nd year curriculum Medicine, Erasmus University Rotterdam, Rotterdam, The Netherlands | 2016 | 6 hours |

**Supervision**

| | | |
|---|---|---|
| Supervision of graduation thesis of Esther Zoutendijk, Master of Health Sciences, Infectious Diseases and Public Health | 2014 | 60 hours |