Antiviral Therapy for Chronic HCV Infection
- Virological Response and Long-Term Outcome -

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Colofon

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ANTIVIRAL THERAPY FOR CHRONIC HCV INFECTION
- Virological Response and Long-Term Outcome -

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- virologische response en langetermijnuitkomsten -

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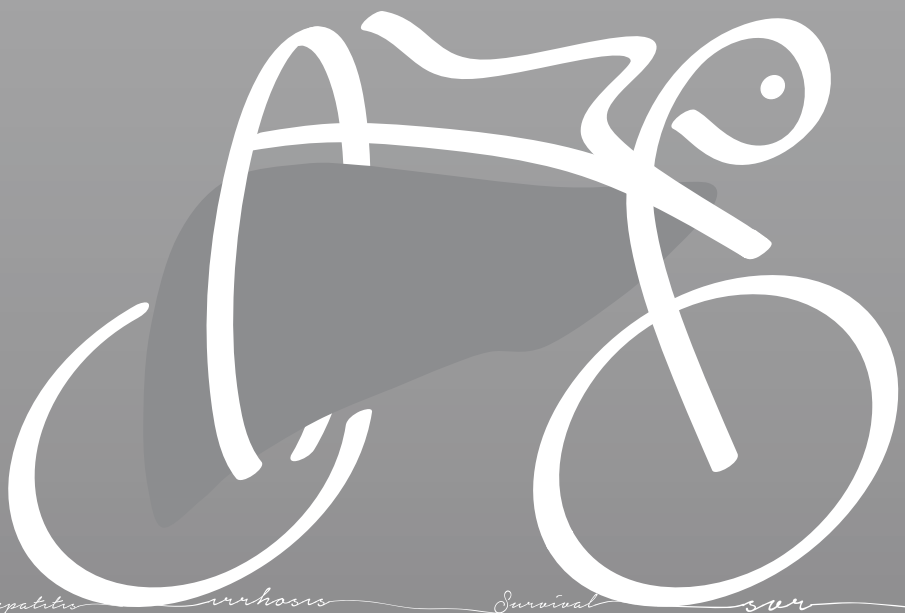
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GENERAL INTRODUCTION

Based on:
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EPIDEMIOLOGY

Hepatitis C is a major global health problem which is responsible for over 350,000 deaths each year.[1] In total, there are thought to be around 150 million hepatitis C virus (HCV) carriers, which comprise about 3% of the world population. The prevalence of HCV infection, however, shows a substantial geographical variation. In Europe the prevalence of HCV infection varies from 0.1 to 6.0%, with the highest occurrence in Southern and Eastern Europe.[2] The Netherlands represents a country in which HCV infection is not frequently observed. A recently performed Dutch epidemiological study indicated that the seroprevalence of anti-HCV antibodies was 0.3%, around the lowest in the world.[3] 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 the most important risk groups in the Netherlands.

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.[4,5] This so called non-A non-B hepatitis was sporadically found in absence of blood transfusion or receipt of other blood products as well.[6] 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.[7] 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.[8]

HCV is a small virus of approximately 55-65 nanometers in size and is the only member of the genus hepacivirus belonging to the family of *Flaviviridae*. The viral particle consists of an envelope, derived from host membranes, which surrounds a nucleocapsid containing a positive-sense, single-stranded RNA genome. The RNA genome is approximately 9600 nucleotides in length and has a single open reading frame flanked by highly conserved untranslated regions (UTR) at both the 5' and 3' termini. Translation results in a polypeptide of approximately 3000 amino acids. Cellular and viral proteases cleave this large protein into 10 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). The core protein is the major component of the nucleocapsid.[9] The glycoproteins E1 and E2 embedded in the envelope are responsible for binding and viral entry into the target host cell.[10] Protein

p7 is important for viral assembly and release, but unlike the other nonstructural proteins does not seem to facilitate HCV RNA replication.[11,12]

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.[13] A possible explanation for the hepatotropicity of HCV might be the distinct set of microRNAs, which are small non-coding regulatory RNAs, that are expressed by hepatocytes. MicroRNA-122 (miR-122) is the most abundant hepatocyte-derived microRNA, and actually constitutes about 70% of the total microRNA population in the liver.[14-17] As other tissues showed no or only minor expression of miR-122, this microRNA can be considered liver-specific.[14,16,17] Importantly, miR-122 seems to be essential for HCV RNA stability and propagation.[18,19] 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.[19-21] 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.[22] 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.[12] 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.[23,24] 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. 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.

ACUTE HCV INFECTION

Transmission of HCV

Transmission of HCV requires blood-blood contact. Presently, IDU who share needles have the highest risk of HCV infection.[2,25,26] Before 1992, due to a lack of screening tools, the virus was frequently transmitted through contaminated blood products.[27,28] While blood transfusion is no longer considered a significant risk factor, nosocomial HCV infections are still relevant today and proper hygienic standards are thus required.[29-32] Although the risk for sexual transmission is low among monogamous heterosexuals, this seems to be an emerging problem among human immunodeficiency virus (HIV) positive men who have sex with men.[33-35] Nevertheless, in 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.[26,36,37] 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.[28,38]

Natural history of acute HCV infection

Already 1 to 3 weeks after exposure, HCV RNA can be detected in the circulation and this represents the first manifestation of the disease.[39,40] Anti-HCV antibody seroconversion usually takes several weeks longer, and might be substantially delayed in patients with HIV co-infection or other immunocompromising comorbidities.[38,41,42] Serum alanine aminotransferase (ALT) levels can fluctuate in the early phase, but usually start to rise about 4 weeks after acquisition of HCV with peak levels often exceeding 10 times the upper limit of normal.[40,43] The rise in ALT is sometimes followed by clinical manifestations, which can persist for 2 to 12 weeks.[40,44] If present, symptoms such as fever, fatigue, nausea, loss of appetite, abdominal pain, myalgia and arthralgia are usually mild, and thus might not prompt patients to seek medical attention.[40,45,46] Jaundice can be regarded as the most specific sign of liver disease, which does facilitate diagnosing acute HCV infection. Consequently, there is a selection bias in studies reporting on acute hepatitis C. Indeed, while some reported that 50% of patients have an icteric course of disease, the actual occurrence of jaundice following HCV exposure is thought to be below 30%.[44-49] A severe course of acute HCV infection is rare.[38,50] While many asymptomatic cases remain undiagnosed, a retrospective multicenter study from Italy found only 2 (0.1%) deaths among 1536 registered patients with acute HCV infection.[51] Patient-related factors or HCV genotype do not seem to influence the clinical presentation during the early phase of infection.[45] Some reported that clinical manifestations less often occur among HIV positive patients who are exposed to HCV, but this might just be because the more intensive medical surveillance in this specific population facilitates the detection of asymptomatic cases.[52] Recently, patients with interleukin 28B (*IL28B*) rs12979860 C/C genotype, which has previously been associated with both spontaneous as well as treatment-induced HCV eradication, showed to experience jaundice more often as compared to patients with non-C/C genotype (33% versus 16%, respectively, p=.032).[53] This further supports that an icteric course of acute hepatitis C represents a stronger immunological response against the virus, which is needed to clear the HCV infection.

Spontaneous resolution of HCV infection

Spontaneous resolution of HCV infection may occur within 3-4 months after acquisition of the virus, but is unlikely to follow if HCV RNA is still detectable after 6 months. The long-term outcome of spontaneous resolved HCV infection is excellent, as was recently highlighted in a report from the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer (R.E.V.E.A.L.)-HCV study from Taiwan.[54] In this prospective natural history study among 19,636 HBsAg-seronegative participants who were followed for a mean duration of 16 years, those who spontaneously cleared HCV had a comparable survival to those who never acquired this virus. Similar results were found in Europe, although continuing risk behavior and other comorbidities in the specific population at risk for HCV infection in the Western world can negatively impact survival after HCV is cleared.[55]

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% (Figure i.1).[56,57] As discussed above, many studies on the natural history of acute HCV infection are expected to include a selection of patients more prone for viral clearance, as a symptomatic course of the acute phase of infection is more likely to result in self-limiting disease.[47,56,58,59] Other patient-related and virus-related factors that have been associated with spontaneous resolution of acute HCV infection include female gender, younger age, non-black race, HIV co-infection, HCV genotype, viral load, and *IL28B* genotype.[38,53,59-66] In addition, patients with spontaneous resolution of HCV infection had lower interferon-gamma-inducible protein-10 (IP-10) levels compared to patients who did not.[67,68] The rates of spontaneous clearance were 31%-32% among patients with an IP-10 level above 540 pg/mL and 50%-83% among patients with an IP-10 level below 540 pg/mL, depending on the *IL28B* genotype (p=.002).[67] Despite these associations it remains difficult to accurately differentiate between patients who will and patients who will not eradicate the HCV infection spontaneously. Monitoring HCV RNA kinetics during the early phase of infection may be one of the best ways to assess the likelihood of self-limiting disease, which is high for those with a rapid decline in viral load and low for those patients who are still HCV RNA positive at week 12.[69,70] Accurate prediction of spontaneous clearance is especially relevant in order to determine which patients need to start antiviral therapy in order to prevent chronicity of HCV infection.

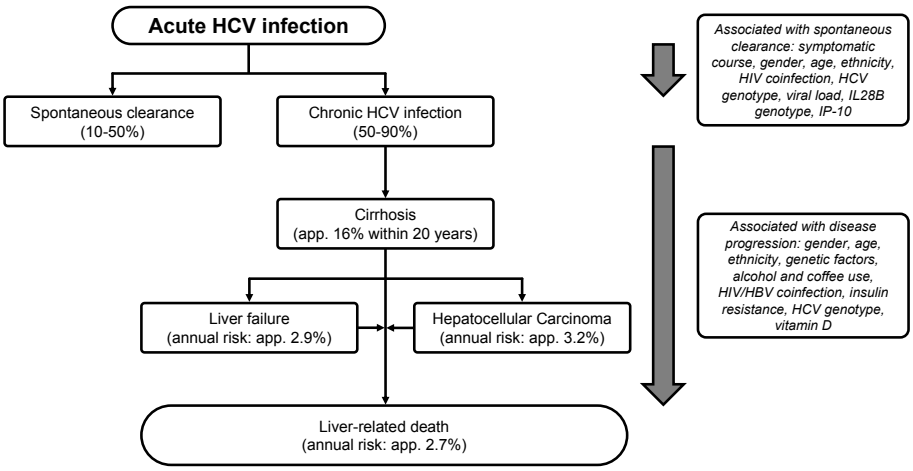

Figure i.1 Overview of the Natural History of HCV Infection
Abbreviations: app; approximately, HBV; hepatitis B virus, HCV; hepatitis C virus, HIV; human immunodeficiency virus, IP-10; interferon-gamma-inducible protein-10.

CHRONIC HCV INFECTION

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.[71] 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.[72] 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.

Symptoms and manifestations

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.[73] 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.[74] Importantly, the extrahepatic symptoms and manifestations may impact morbidity, treatment opportunities as well as mortality.[75]

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.[76] Patients with cirrhosis had fatigue more frequently and of more severe intensity, as was reported by a previous study as well.[77] 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.[77,78]

Hepatic consequences

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.[79] 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.[80,81] 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 vascularised 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.[82,83] 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 neovascularisation.[84] The vascular abnormalities within a cirrhotic liver are indeed though to play 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.[85,86] 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.[87] 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.[85,88-90]

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.[91] 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.[92,93]

Factors associated with disease progression

The prognosis among patients with chronic HCV infection varies extensively, even among those with cirrhosis who are most at risk to develop the clinical complications of their infection. Histological and clinical progression of HCV-induced liver disease may be influenced both by host factors and by viral factors (Figure i.1).

Age has a well-known association with the degree of hepatic fibrosis among patients with chronic HCV infection.[94] Among those infected in childhood, the course of disease seems to be milder.[95] Multiple studies including patients with chronic HCV infection and advanced hepatic fibrosis indicated that older patients are at increased risk of liver failure, HCC and death.[85,96-98] Female gender has been associated with a more benign course of disease, which seems to be related to the hormonal status. Indeed, the 25-year risk of liver-related mortality was only 0.5% among untreated female patients who were infected with the HCV in their twenties.[49] A cohort study including 472 female patients showed that the rate of fibrosis progression was higher in postmenopausal and nulliparous women.[99] Moreover, this study indicated lower fibrosis progression among postmenopausal women who received hormone replacement therapy. Among patients with HCV-related cirrhosis, females showed a two to three fold lower risk of HCC as compared to males.[96,98] Ethnical differences in disease progression rates might be related to genetic factors as well as to behavioral factors. Although studies performed in the United States indicated that African American patients may have less rapid fibrosis progression, their risk of HCC was two-fold higher than in white patients with chronic HCV infection.[100-102] Cohort studies from Japan reported substantial higher HCC incidence rates as compared Western studies, with HCC frequently occurring among Japanese patients with chronic HCV infection in absence of cirrhosis as well.[103-110]

Although most genetic studies in the field of HCV infection have focused on the association between single nucleotide polymorphisms (SNPs) around the *IL28B* gene and the virological response to antiviral therapy, there is increasing evidence that genetic factors influence the natural history of chronic HCV infection. For instance, it was described that if the virus is not cleared among patients with the favorable *IL28B* genotype, their stronger immunological response to the virus may increase their risk of hepatic fibrosis and cirrhosis-related complications.[111,112] However, not all studies found these associations and contradicting results have been published as well.[113,114] Recently, a panel of seven SNPs termed the 'Cirrhosis Risk Score' was associated with cirrhosis by Huang et al, and this score has also been validated by others thereafter.[115] Among cirrhotic patients with HCV infection, the Cirrhosis Risk Score did show a trend for an association with HCC occurrence, but this was not statistically significant.[116] Moreover, genome-wide association studies among patients with chronic HCV infection have linked SNPs in genes involved in the apoptosis pathway of hepatic fibrogenesis and a SNP in the gene encoding MICA, a membrane protein which may activate anti-tumor effects, to HCC development.[117,118]

Metabolic factors also seem to have a relevant influence on the progression of HCV-induced liver disease. In a large population based study from Taiwan, which included 1095 chronic HCV carriers, a body mass index (BMI) ≥30 kg/m2 was as-

sociated with a four-fold increased HCC risk.[119] Possibly this is explained by a higher prevalence of steatosis, which can be considered as the hepatic equivalent of the metabolic syndrome, among obese patients. Indeed, steatosis was found to be a risk factor of HCC independent of hepatic fibrosis or cirrhosis.[120,121] Within the same spectrum, diabetes mellitus also showed an approximately three-fold increase in the risk of HCC, and these metabolic factors seemed to strengthen each other.[119,122] Relevant here is that interactions between the virus and these metabolic pathways make chronic HCV-infected patients more prone for the development of diabetes mellitus.[123] In line with these findings, a post-hoc analyses of the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial indicated that a higher dietary cholesterol intake was associated with elevated risk of disease progression among patients with advanced HCV-related liver disease.[124] However, alcohol abuse probably remains the best-known behavioral factor to influence the natural history of chronic HCV infection. In a study comparing 90 patients with chronic HCV infection with alcohol abuse and 86 without alcohol abuse, Wiley et al. showed that 58% of the alcoholic patients had developed cirrhosis after 2 decades compared to only 10% of the non-alcoholic patients.[125] The relation between alcohol use and an unfavorable outcome is also evident for important clinical endpoints, such as liver failure, HCC and mortality.[85,98]

While the stage of liver disease is important to determine the risk of liver failure and HCC, the grade of disease has been associated with hepatic fibrosis progression. Approximately 25% of the patients with chronic HCV infection have persistently normal ALT levels, and these patients were indeed found to have a decelerated fibrosis progression rate.[126] However, even in case of normal ALT levels, cirrhosis may be present and for this reason treatment initiation should not be based on ALT levels only.[38]

A relatively novel marker with respect to the natural history of chronic HCV infection concerns vitamin D. The liver actually plays an important role in the metabolism of vitamin D, and vitamin D deficiency is indeed highly prevalent among patients with liver diseases.[127] In a study including 57 non-cirrhotic HCV patients, 22.8% (19/57) had mild, 52.6% (30/57) had moderate, and 14% (8/57) had severe vitamin D deficiency.[128] Low serum vitamin D levels were associated with more severe hepatic fibrosis.[129-131] The Swiss cohort study, a prospective follow-up study including 251 chronic HCV patients, showed that vitamin D receptor gene polymorphisms as well as low serum vitamin D levels were significantly associated with the rate of fibrosis progression.[132] The role of vitamin D in the development of liver failure or HCC has yet to be elucidated.

The most important viral factor is HCV genotype, with HCV genotype 3 as the most unfavorable variant. For instance, steatosis is more frequently observed among

patients infected with HCV genotype 3 and this might explain the frequently observed association between this genotype and more advanced hepatic fibrosis.[121,133] In the American Veteran study including almost 17,000 patients with chronic HCV infection, patients infected with HCV genotype 3 demonstrated an impaired overall survival as compared to those with other HCV genotypes.[134] In line with this observation, others have described a higher incidence of HCC among patients with HCV genotype 3 infection.[135] The viral load does not seem to have an effect on the long-term histological and clinical prognosis. In addition to HCV, there are other infectious agents such as HBV and HIV which may cause hepatitis. Co-infection with these viruses has shown to negatively impact disease progression. For instance, patients with a HCV and HBV or HIV co-infection have demonstrated an accelerated hepatic fibrosis progression as compared those with HCV mono infection.[136-138] Whether resolved HBV infection, evidenced by anti-HBc positivity and HBsAg negativity, has a negative influence on the prognosis of patients with chronic HCV infection is subject to debate.

Despite the relevant associations between the above-described factors and the natural history of chronic HCV infection, adequate assessment of an individual's prognosis remains difficult. Currently, reliable risk scores are lacking, while such predictive tools would be relevant to assess the need for costly antiviral therapy or intensive follow-up among patients with chronic HCV infection.

INFLUENCING THE NATURAL COURSE OF HCV INFECTION

Antiviral therapy for acute HCV infection

To date, acute HCV infection is treated with interferon-based therapy. Interferon alfa is an immunomodifying protein which regulates various cytokines and their receptors, whereby it primes the host immune response in order to provide an effective virological response. 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.[139] 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.[140] These symptoms further deteriorate the health-related quality of life of HCV-infected patients and interfere with patients' ability to perform daily activities.[141] Furthermore, interferon-based therapy causes bone marrow suppression so that neutropenia, thrombocytopenia en anemia may develop.

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.[142] The efficacy was found to decline when antiviral therapy was initiated longer after HCV exposure, so that early treatment may be advised.[143] However, immediate therapy may result in overtreatment of those patients who might also spontaneously resolve their HCV infection.[46,142] 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.[58,144,145] 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, 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).[146] 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. Possibly other factors like *IL28B* genotype might play a role in such decisions.[147,148]

Ribavirin is a nucleoside inhibitor which exerts antiviral activity against a broad-spectrum of RNA viruses. Although the mechanism(s) of action are still not well understood, ribavirin has been part of the standard-of-care in the treatment of chronic HCV infection since 1998. There is insufficient data to generally recommend the use of ribavirin for acute HCV infection. Indeed, randomized controlled trials comparing interferon plus ribavirin to interferon alone have not been performed. 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.[149] This was particularly seen among those patients who were expected to have a longer duration of infection and among those patients with unfavorable *IL28B* genotype. In some cases, adding ribavirin to pegylated interferon to treat acute HCV infection might thus be considered. 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 erythropoietin therapy or recurrent blood transfusions.

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.[150] Also, the rapid development of highly effective interferon-free treatment regimens should be considered. Among patients with chronic HCV infection these regimens have shown

high antiviral efficacy and very good safety profiles. As soon as these treatments become generally available, treatment of acute HCV infection might no longer be preferred as long as this remains pegylated interferon-based. At present, there are no data on the efficacy of direct-acting antivirals (DAAs) in acute hepatitis C, but studies are currently ongoing.

Lifestyle modifications for chronic HCV infection

There are only a limited number of lifestyle behaviors which may positively influence the natural course of chronic HCV infection. Based on the well-known negative impact of alcohol, patients with chronic HCV infection should be advised to limit or refrain from alcohol intake. Especially in case of advanced liver disease, alcohol abstinence must be advocated. More recent data indicated that the use of several cups of coffee a day can be beneficial. Indeed, coffee consumption was found to delay the progression of liver disease among patients with HCV infection. A cross-over randomized controlled trial has been performed in which one group of patients was assigned to drink four cups of coffee per day and a second group abstained from coffee intake. After 30 days, the groups were switched over for a second month. During coffee intake, oxidative cell DNA damage and collagen levels were significantly lower than during abstinence.[151] Another prospective study among 766 patients with HCV infection and advanced liver disease showed that drinkers of at least three cups of coffee per day had a 53% lower risk of liver disease progression than non-coffee drinkers.[152] Interestingly, coffee consumption has been associated with a reduced incidence of HCC as well.[153,154] Perhaps weight reduction can be useful for obese patients with chronic HCV infection, as this might reduce steatosis and improve the insulin sensitivity. Both have been associated with an unfavorable outcome among patients with chronic HCV infection. However, even though lifestyle modifications are relevant, the longstanding goal in the management of patients with chronic HCV infection has been to eradicate the viral infection.

Interferon-based 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. Extra-hepatic symptoms and manifestations of chronic HCV infection, which can reduce the health-related quality of life, represent another relevant reason to initiate antiviral therapy. Furthermore, some patients might choose to be treated out of fear for further HCV transmission.

Hoofnagle et al. were the first to treat HCV-infected patients with interferon alfa, at which time these patients were still described to have non-A non-B hepatitis as HCV had yet to be discovered.[155] Consequently, this study assessed the response to therapy by measuring ALT activity rather than HCV RNA levels. 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.[156] 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.[157] 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.[158] 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.[159]

In 1994 Brillanti et al. showed that adding ribavirin, a purine-nucleotide analogue, to interferon could be beneficial for patients with chronic HCV infection.[160] 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.[158,161,162] 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.[163-165] 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.[166] 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.

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.[163] Interferon dose reductions, however, negatively impact the virological efficacy of antiviral therapy.[167] 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.[168] 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.[169,170] 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.

Direct-acting antivirals

Over the last decades, our understanding of the HCV life cycle has significantly improved and this resulted in the development of DAAs for the treatment of HCV infection.[171] 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.

Telaprevir and boceprevir, both NS3/4A protease inhibitors, were the first DAAs to become available for the treatment of chronic HCV infection. These drugs can only be used in patients with HCV genotype 1 infection as their potency in other HCV genotypes was low.[172] Although the antiviral efficacy of the protease inhibitors is high, their genetic barrier to resistance is low.[173] Combination with pegylated interferon and ribavirin was therefore required, and these first triple therapy regimens only increased the side effects of antiviral treatment. 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.[174-178] 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 is 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.[179] More recently, the second generation NS3/4A protease inhibitor simeprevir

was approved by the Food and Drug Administration (FDA) as well as the European Medical Agency (EMA) for the treatment of chronic HCV infection. Unlike telaprevir and boceprevir, simeprevir can be dosed once daily and does not seem to add side-effects to those associated with pegylated interferon and ribavirin treatment. These advantages are likely to improve treatment adherence and thus optimize antiviral treatment efficacy. 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.[180,181] 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 $\log_{10}$ in HCV RNA after 12 weeks of therapy).[182,183] Patients infected with HCV genotype 1a should be screened for the presence of the resistance-associated Q80K variant, as this mutation largely reduces the efficacy simeprevir-containing triple therapy. Among patients chronically infected with HCV genotype 4, against which simeprevir is active as well, similar SVR results have been obtained.[184]

Even greater strides in the treatment of chronic HCV infection have been made by the development of sofosbuvir, which is a NS5B polymerase inhibitor with pan genotypic activity and a high barrier to resistance.[185] At the time this thesis was written, sofosbuvir was already available in the United States and several parts of Europe. 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.[186-190] Even in case of cirrhosis, 80% of patients were able to eradicate their chronic HCV infection with this short triple regimen. Because of its high barrier to resistance, sofosbuvir has been one of the primary candidates for interferon-free therapy, by which the notorious side effects of interferon can be avoided. 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.[188,191,192] 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.[192] For interferon-experienced HCV genotype 3-infected patients with cirrhosis this sofosbuvir and ribavirin combination therapy might not be the best option, however, so that addition of pegylated interferon should be considered as long as other interferon-free regimens are not available. Also for patients with

HCV genotype 1 infection sofobuvir and ribavirin combination therapy might be a valuable treatment option, but probably only in case of interferon-intolerance. Based on limited data, which are mainly derived from patients with unfavorable characteristics, around 65% of patients with HCV genotype 1 infection may attain SVR as long as ribavirin is optimally dosed.[193] At the cost of side effects, addition of pegylated interferon certainly increases the virological efficacy among these patients, although such data within treatment-experienced patients is lacking. Most recent results indicated that combining sofosbuvir with other DAAs is likely to represent a much more attractive alternative to optimize the virological efficacy as well as the safety profile of antiviral therapy.

Studies in which sofosbuvir was combined with ledispasvir (an NS5A inhibitor), daclatasvir (also an NS5A inhibitor) or simeprevir resulted in SVR rates around 95%, independent of the stage of hepatic fibrosis or prior treatment response.[194-199] Treatment duration may even be shortened to 8 weeks for specific subgroups.[196] In general, ribavirin does no longer seem to boost the virological efficacy of antiviral therapy with combinations of DAAs. Although based on a limited number of patients, especially the SVR rates up to 100% with the combination of sofosbuvir and simeprevir were important, as both these drugs are available or will become available during 2014.[197,199] This combination is thus likely to become the first all-DAA treatment regimen used in clinical practice. Efficacy results have been presented of a regimen which combines all three major DAA classes as well. Six phase 3 studies showed SVR rates of above 95% with a 12-week combination regimen including ritonavir-boosted ABT-450 (a NS3/4A protease inhibitor), ombitasvir (a NS5A inhibitor) and dasabuvir (a NS5B polymerase inhibitor), in treatment-naïve as well as treatment-experienced patients and in patients with as well as patients without cirrhosis.[200-204] However, for certain subgroups, such as prior null-responders with cirrhosis and/or HCV genotype 1a infection, treatment extension to 24 weeks and/or addition of ribavirin might optimize virological response rates.[202,203]

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.[205] It is important that these improvements in antiviral therapy seem to be broadly-carried, with many different combinations of DAAs showing excellent virological efficacy. Next to the above-described regimens there are many other potent combinations of DAAs in the anti-HCV drug pipeline. Hopefully this will stimulate competition among pharmaceutical companies and reduce the costs of interferon-free therapy. Although the price of most DAAs remains unknown at present, costs are expected to be high and this can certainly limit the availability of these drugs for the majority of patients around the globe. Pegylated interferon and ribavirin therapy is therefore likely to remain an

important treatment option for many. Real-world experiences with interferon-free treatment regimens will also need to confirm the high efficacy as was observed in the phase 3 trials. Nevertheless, we are currently standing on the verge of 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.

Clinical benefit of antiviral therapy for chronic HCV infection

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.[76,206] Histological studies showed that patients who attained SVR had regression of hepatic fibrosis, even in case cirrhosis was established prior to treatment initiation.[207] The regression of fibrosis following antiviral therapy among patients with HCV-induced cirrhosis has been related to improved clinical outcome.[208] Following SVR, the hepatic venous pressure gradient was found to decline as well.[209-211] 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.[96-98,212] However, data on the association between SVR and all-cause mortality, the most definite and robust clinical endpoint, remains scarce. In a large cohort of American veterans, which were followed for a median of 3.8 years, 5-year mortality rates of 6.7 to 8.0% among those with SVR and 14.4 to 24.4% among those without SVR were reported.[134] However, this study included almost solely men which had many comorbidities and the overall mortality rate was rather high. It was therefore questioned whether this study is representative for the general HCV-infected population. Moreover, only a limited number of patients with advanced liver disease were included, while these patients are at highest risk of the long-term clinical sequelae of their chronic viral infection. More data supportive of a clinical benefit among patients with advanced hepatic fibrosis is thus urgently needed in order to justify the high costs and side effects of antiviral therapy, especially as the HCV-infected population with cirrhosis is rapidly growing.[92,93]

SCOPE AND AIMS OF THIS THESIS

This thesis focuses on the clinical outcome of patients with chronic HCV infection and advanced liver disease. Among this patient population at risk for the clinical complications of their viral infection, the possible beneficial effects of successful antiviral therapy on overall survival were studied and discussed. Furthermore, the improvement of the clinical efficacy of antiviral therapy was assessed according to the increase in virological efficacy. Factors associated with clinical outcome were explored among patients with advanced hepatic fibrosis who did attain SVR as well as among those patients who did not attain SVR. Efforts to reduce HCV-related morbidity and mortality were made as well. First, in order to optimize the current pegylated interferon-based treatment regimes, the need for dose reductions because of interferon-induced thrombocytopenia or neutropenia was assessed in relation to the occurrence of on-treatment bleedings and infections. Next, the diagnostic value of the liver-specific and liver-abundant miR-122 was explored as a circulating marker of hepatocellular injury, in order to facilitate diagnosing patients with chronic HCV infection so that they can be treated. Finally, as miR-122 serves as an important host factor for HCV abundance in hepatocytes, the safety and virological efficacy of an antisense molecule targeting this specific microRNA was evaluated among patients with chronic HCV infection, in an attempt to improve antiviral therapy.

Aims

The aims of this thesis were to assess:

1. the association between successful interferon-based antiviral therapy and all-cause mortality among patients with chronic HCV infection and advanced hepatic fibrosis
2. 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.
3. the incidence of cirrhosis-related complications following antiviral therapy among patients with chronic HCV infection and advanced hepatic fibrosis with or without SVR
4. the diagnostic qualities of circulating microRNA-122 for chronic HCV infection as compared to that of ALT, which is the currently used standard screening test to detect hepatocellular injury.
5. whether microRNA-122, a host-factor which facilitates HCV abundance within hepatocytes, can be safely and effectively targeted with an antisense oligonucleotide.

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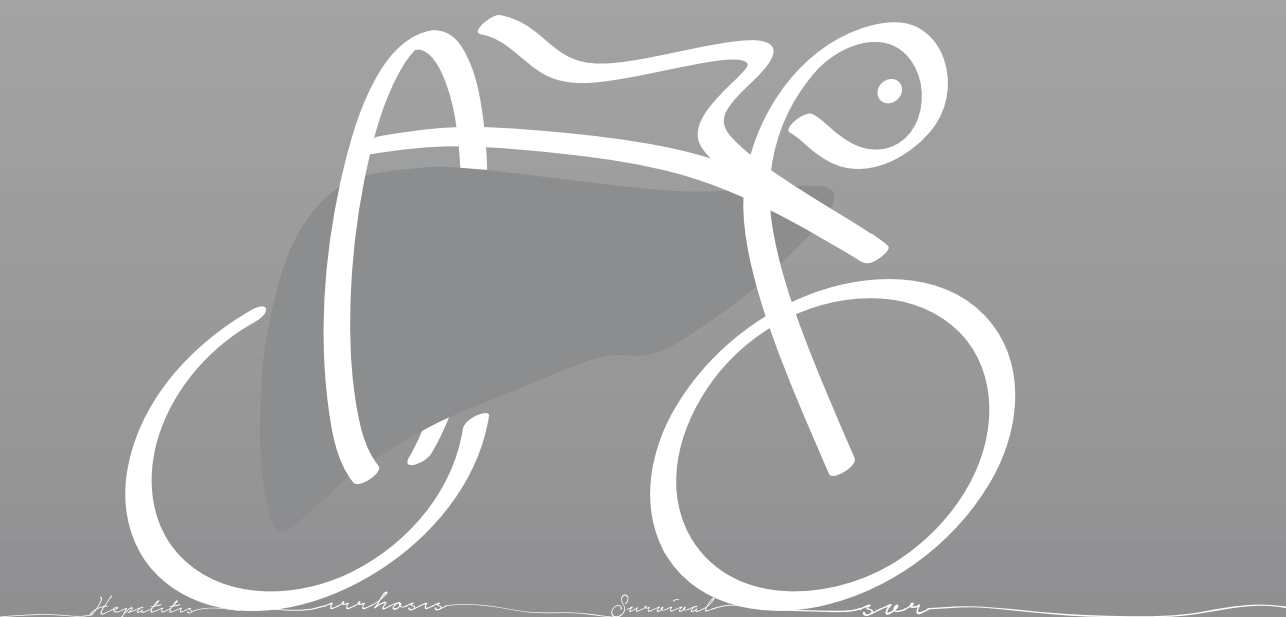
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CHAPTER 1

Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis

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ABSTRACT

Context

Chronic hepatitis C virus (HCV) infection outcomes include liver failure, hepatocellular carcinoma (HCC), and liver-related death.

Objective

To assess the association between sustained virological response (SVR) and all-cause mortality in patients with chronic HCV infection and advanced hepatic fibrosis.

Design, Setting, and Patients

An international, multicenter, long-term follow-up study from 5 large tertiary care hospitals in Europe and Canada of 530 Patients with chronic HCV infection who started an interferon-based treatment regimen between 1990 and 2003, following histological proof of advanced hepatic fibrosis or cirrhosis (Ishak score 4-6). Complete follow-up ranged between January 2010 and October 2011.

Main Outcome Measures

All-cause mortality. Secondary outcomes were liver failure, HCC, and liver-related mortality or liver transplantation.

Results

The 530 study patients were followed up for a median (interquartile range [IQR]) of 8.4 (6.4-11.4) years. The baseline median (IQR) age was 48 (42-56) years and 369 patients (70%) were men. The Ishak fibrosis score was 4 in 143 patients (27%), 5 in 101 patients (19%), and 6 in 286 patients (54%). There were 192 patients (36%) who achieved SVR; 13 patients with SVR and 100 without SVR died (10-year cumulative all-cause mortality rate: 8.9% [95% confidence interval (CI) 3.3-14.5] with SVR vs 26.0% [95% CI 20.2-28.4] without SVR, p<.001). In time-dependent multivariate Cox regression analysis, SVR was associated with reduced risk of all-cause mortality (hazard ratio [HR] 0.26, 95% CI 0.14-0.49, p<.001) and reduced risk of liver-related mortality or transplantation (HR 0.06, 95% CI 0.02-0.19, p<.001), the latter occurring in 3 patients with SVR and 103 without SVR. The 10-year cumulative incidence rate of liver-related mortality or transplantation was 1.9% (95% CI 0.0-4.1) with SVR and 27.4% (95% CI 22.0-32.8) without SVR (p<.001). There were 7 patients with SVR and 76 without SVR who developed HCC (10-year cumulative incidence rate: 5.1% [95% CI 1.3-8.9] vs 21.8% [95% CI 16.6-27.0], p<.001), and 4 patients with SVR and 111 without SVR experienced liver failure (10-year cumulative incidence rate: 2.1% [95% CI 0.0-4.5] vs 29.9% [95% CI 24.3-35.5], p<.001).

Conclusion

Among patients with chronic HCV infection and advanced hepatic fibrosis, sustained virological response to interferon-based treatment was associated with lower all-cause mortality.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major cause of cirrhosis, hepatocellular carcinoma (HCC), and end-stage liver disease. The incidence of HCV-related cirrhosis and its complications is expected to increase in upcoming years.[1,2] Davis et al. estimated that currently 25% of the approximately 3.5 million US patients with chronic HCV infection have cirrhosis and that the proportion of patients with cirrhosis is likely to increase up to 45% by 2030.

Pegylated interferon and ribavirin treatment is effective in 50% to 80% of patients.[3-5] Sustained virological response (SVR) is defined as absence of viremia 24 weeks after cessation of all antiviral medication.[6] Although SVR has long-term durability, data on the relationship with improved survival to support its use as a surrogate end point of antiviral therapy is scarce.[7] Demonstrating direct clinical benefits would better justify the use of intensive and costly antiviral therapy, such as expensive direct antiviral agents, which improve treatment efficacy when added to pegylated interferon and ribavirin for many patients with chronic HCV genotype 1 infection.[8-10]

Our group previously demonstrated that SVR is associated with a reduced occurrence of liver failure and liver-related deaths in patients with chronic hepatitis C (CHC) and advanced hepatic fibrosis.[11] Studies in other western populations confirmed these findings.[12-14] Whether these beneficial effects of SVR also result in a reduced all-cause mortality in the high risk population of patients with chronic HCV infection and severe hepatic fibrosis is currently not clear.

Because all-cause mortality is the most definite clinical end point with clear interpretation, knowledge about the effect of treatment on all-cause mortality is important in considering antiviral treatment. The Centers for Disease Control and Prevention recently recommended birth-cohort screening for HCV infection[15]; thus, scientific proof that SVR to interferon-based treatment is associated with lower all-cause mortality is also important for screening purposes.

With this large, international, multicenter, long-term follow-up study, we investigated whether achievement of SVR vs without SVR is associated with a prolonged overall survival in 530 patients with CHC and advanced hepatic fibrosis.

METHODS

Patients

All patients included in our international, multicenter cohort from 5 large hepatology units of tertiary care centers in Europe and Canada were reevaluated by

reviewing the medical charts.[11] This cohort included all consecutive patients with CHC who started interferon-based treatment between 1990 and 2003 if they had histological proof of advanced fibrosis or cirrhosis (Ishak score 4-6). Histologically, Ishak fibrosis score 4 is characterized by fibrous expansion of most portal areas with marked portal-to-portal as well as portal-to-central bridging; Ishak fibrosis score 5, by marked portal-to-portal and/or portal-to-central bridging with occasional nodules (early cirrhosis); and Ishak fibrosis score 6, by probable or definite cirrhosis.[16] The interobserver agreement concerning the degree of fibrosis is strong, especially regarding the presence or absence of cirrhosis.[17] Interferon-based therapy has been standard of practice since the beneficial effects of recombinant interferon alpha in patients with chronic HCV infection were described.[18,19] Patients coinfected with human immunodeficiency virus or hepatitis B virus and patients with a history of liver failure were excluded.

Compared with the 479 patients analyzed in our prior report, we extended this cohort with 51 additional patients who were eligible to be included in the analyses according to the same inclusion and exclusion criteria.[11] In contrast with the prior data collection, these patients now either had their medical chart available for data acquisition or had follow-up beyond 24 weeks after the end of treatment with a documented virological response.

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. According to the standards of the local ethics committees, written informed consent was obtained from patients visiting the outpatient clinics, and written or oral informed consent was obtained from patients contacted by telephone. If patients were not reached, the general practitioner of the patients was contacted by their treating physician without informed consent. The ethics committee approved the above described procedure as our study was considered a low-risk study using anonymized patient data.

Data were obtained on patient demographics (sex, age, height, and weight), severity of fibrosis (Ishak fibrosis score), antiviral treatment (type of medication, treatment period, virological response, previous treatment), and presence of diabetes mellitus or a history of severe alcohol use as stated in the chart by the treating physician. In the participating centers, the use of more than 50 gram/day of alcohol was considered severe alcohol use. Virology data (HCV genotype, anti-hepatitis B core antigen) and baseline laboratory data (platelet count, bilirubin, albumin, aspartate aminotransferase [AST], alanine aminotransferase [ALT]) within 6 months

before treatment were also registered. Liver biopsy samples were scored by local experienced pathologists who were unaware of virological or long-term clinical outcome after treatment.

Complete follow-up was defined as death or clinical follow-up beyond January 1, 2010, which was approximately half a year before the start of data collection. For patients without ongoing clinical follow-up to 2010, the patient or primary care physician was recontacted. Patients were invited for a single visit to obtain a detailed history and perform a physical examination, laboratory testing, and ultrasonographic evaluation. If the patient was unable to visit, the patient or primary care physician were asked to answer a structured questionnaire over the telephone. If the patient and primary care physician could not be reached, the patient was censored at the last available follow-up visit.

Clinical outcome measures

The primary outcome measure of the study was all-cause mortality. Secondary clinical outcome measures were liver failure, HCC, and liver-related mortality or liver transplantation. Liver transplantation events and liver-related mortality were analyzed as a combined endpoint. The cause of death was determined by the treating physician. 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 not liver related.

The definition of liver failure included an episode of either ascites confirmed by ultrasonography, bleeding varices, jaundice with a bilirubin level of more than 2.05 mg/dL (to convert to μmol/L, multiply by 17.104), or overt hepatic encephalopathy. The diagnosis of HCC was based on histopathological confirmation or 2 coincident imaging techniques (computed tomography, magnetic resonance imaging, or contrast-enhanced ultrasonography), showing a focal lesion of more than 2 cm with arterial-phase hyperenhancement or 1 imaging technique showing a focal lesion of more than 2 cm with arterial-phase hyperenhancement in the presence of an α-fetoprotein level of more than 400 ng/mL.[20]

Statistical analyses

At the initial design of the study in 2004, the power calculation indicated that 137 patients with SVR would be needed to show a quantitative survival benefit of 8.8% after 5 years with a power of 90%, and a level of significance of .05, assuming a 5-year mortality of 2.5% in patients with SVR based on mortality data from the general population and a 5-year mortality of 11.3% in patients without SVR based on a model assessing the natural history of chronic HCV infection.[21,22]

The baseline characteristics were compared between patients with and without SVR after the baseline treatment, using the Mann-Whitney test for continuous variables and the χ^2 test for categorical variables.

The association between SVR and all-cause mortality, liver-related mortality or liver transplantation, HCC, and liver failure was estimated with the Cox proportional hazards regression method. Twenty-four weeks after end of treatment was defined as time 0, because patients with undetectable HCV RNA at this time point were classified as having attained SVR, and others were classified as without SVR. Patients were not censored for any reason other than loss to follow-up for the all-cause mortality analyses. Deceased patients were censored at the time of death for the non-mortality outcomes. Patients experiencing liver failure were thus not censored in the analyses for HCC, or vice versa. Treated patients who were lost to follow-up or experienced a clinical event before 24 weeks after end of treatment were, per definition, not able to attain SVR status before dropout or reaching the event. For this reason, these patients were not included in the analyses. Because retreatment in patients without SVR could result in SVR, patients without SVR were able to switch from a non-SVR to a SVR status during the follow-up. To correct for patients who changed their response status, SVR was included as a time-dependent covariate in the Cox proportional hazards regression analyses. Other baseline variables that were considered included age, sex, body mass index (calculated as weight in kilograms divided by height in meters squared), genotype 1 vs no genotype 1, genotype 3 vs no genotype 3, Ishak fibrosis score, treatment naive vs treatment experienced, treatment duration, presence of diabetes, history of severe alcohol use, anti-hepatitis B core antigen positivity, platelet count, AST/ALT ratio, and the albumin and bilirubin level. Age, sex, SVR, and variables with $p<.20$ in univariate analyses were included in multivariate analyses. All multivariate analyses were adjusted for the year treatment started, and stratified by treatment center, to control for possible heterogeneity between centers. Akaike's Information Criteria was used to compare the goodness of fit between models. The proportionality assumption was checked graphically via the log-minus-log plots for categorical variables and by including an interaction term between the variable and log-transformed follow-up time for both continuous and categorical variables. Interactions between SVR and other baseline variables included in the final model were explored.

Survival curves for the SVR status were constructed by using a clock-reset approach. Patients who switched from the without SVR to the with SVR group were censored in the without SVR group at the time of SVR. The time of SVR was then reset as time zero for the patients' further follow-up in the with SVR group. The difference between the survival curves in the without SVR and with SVR groups was

assessed with univariate Cox proportional hazards regression analyses with SVR as a time-dependent covariate.

Sensitivity analyses using multiple imputation to impute missing values were performed.[23,24]

All statistical tests were 2-sided, and $p<.05$ was considered statistically significant. The significance level for interactions was set at $p=.01$ to correct for multiple testing. SPSS version 17.0.2 (SPSS Inc) was used for all statistical analyses.

RESULTS

Study population

In total, 546 patients with CHC and advanced hepatic fibrosis started an interferon-based regimen at the participating centers between 1990 and 2003. Despite our attempts to recontact all patients, 8 patients were lost to follow-up before reaching 24 weeks after end of treatment. Six of these 8 patients were HCV RNA positive. For the other 2 patients, HCV RNA was not documented, but both had an elevated ALT level at the last visit. These patients were excluded from the analyses. Three patients were diagnosed with HCC and 5 patients developed liver failure within 24 weeks after end of treatment, who were thus also excluded from the analyses. All these patients had showed virological nonresponse or relapse; therefore, the total study cohort consisted of 530 patients.

Overall, 192 patients (36%) achieved SVR and 338 patients (64%) did not. Of these, 125 patients (65%) achieved SVR after the baseline treatment and were thus considered sustained responders for the entire study period. A total of 204 patients (60%) with initial non-SVR were retreated and 67 (33%) of them achieved SVR after a median (interquartile range [IQR]) of 5.8 (3.1-8.5) years of follow-up. The patients with successful retreatment were considered as patients without SVR in the analysis until after successful retreatment, at which point they were treated as patients with SVR for the remainder of follow-up.

The baseline treatment consisted of interferon monotherapy (approved by the US Food and Drug Administration [FDA] in 1991) in 175 patients (33%), interferon and ribavirin (FDA approval in 1998) in 148 patients (28%), and pegylated interferon and ribavirin (FDA approval of pegylated interferon in 2001) in 176 patients (33%). A minority of patients were treated with pegylated interferon monotherapy (3% [n = 14]) or consensus interferon (FDA approval in 1997) with or without ribavirin (3% [n = 17]).

Table 1.1 shows the baseline characteristics according to the initial virological response. The overall median (IQR) age was 48 (42-56) years and the majority of patients were men (70% [n = 369]). The Ishak fibrosis score was 4 in 143 patients (27%),

Table 1.1 Baseline Characteristics According to Treatment Response [a]

Characteristics	Overall (n = 530)	With SVR (n = 125)	Without SVR (n = 405)	p-value [b]
Age, years, median (IQR)	48 (42-56)	47 (43-54)	48 (42-56)	.57
Male	369/530 (70)	94/530 (75)	275/530 (68)	.12
BMI, kg/m^2, median (IQR) (n = 401)	26.1 (23.6-29.3)	25.4 (23.1-28.5)	26.5 (24.0-29.5)	.06
Fibrosis score				.41
Ishak 4	143/530 (27)	38/125/125 (30)	105/405 (26)	
Ishak 5	101/530 (19)	26/125 (21)	75/405 (19)	
Ishak 6	286/530 (54)	61/125 (49)	225/405 (56)	
HCV genotype				<.001
1	340/502 (68)	50/118 (42)	290/384 (76)	
2	48/502 (10)	27/118 (23)	21/384 (5)	
3	88/502 (18)	36/118 (31)	52/384 (14)	
4	22/502 (4)	4/118 (3)	18/384 (5)	
Other	4/502(1)	1/118 (1)	3/384 (1)	
Type of treatment				<.001
Interferon monotherapy	175/530 (33)	9/125 (7)	166/405 (41)	
Interferon and ribavirin	148/530 (28)	35/125 (28)	113/405 (28)	
Pegylated interferon monotherapy	14/530 (3)	4/125 (3)	10/405 (2)	
Pegylated interferon and ribavirin	176/530 (33)	75/125 (60)	101/405 (25)	
Consensus interferon (+/- ribavirin)	17/530 (3)	2/125 (2)	15/405 (4)	
Treatment duration, weeks, median (IQR)	26.3 (21.5-48.0)	47.7 (24.4-50.4)	24.3 (17.1-47.3)	<.001
Laboratory markers of liver disease severity, median (IQR)				
Platelet count, x10^9/L (n = 457)	151 (114-200)	164 (133-207)	145 (109-195)	.009
Albumin, g/L (n = 423)	42 (39-44)	43 (40-45)	42 (39-44)	.12
Bilirubin, mg/dL (n = 442) [c]	0.76 (0.58-0.99)	0.64 (0.53-0.88)	0.82 (0.60-1.11)	<.001
AST/ALT ratio (n = 431)	0.70 (0.57-0.90)	0.67 (0.54-0.82)	0.71 (0.58-0.91)	.04
Treatment naïve	477/530 (90)	112/125 (90)	365/405 (90)	.87
Year treatment started, median (IQR)	1999 (1996-2002)	2002 (1999-2002)	1998 (1995-2001)	<.001
Diabetes mellitus	66/530 (12)	11/125 (9)	55/405 (14)	.16
History of severe alcohol use	119/494 (24)	31/120 (26)	88/374 (24)	.61
AntiHBc positivity	195/416 (47)	42/91 (46)	153/325 (47)	.88

Abbreviations: ALT; alanine aminotransferase, antiHBc; anti-hepatitis B core antigen, AST; aspartate aminotransferase, BMI; body mass index (calculated as weight in kilograms divided by height in meters squared), HCV; hepatitis C virus, IQR; interquartile range, SVR; sustained virological response.

[a] Data are presented as No./total No. (%) unless otherwise noted.

[b] Baseline characteristics were compared between patients with SVR and patients without SVR using the Mann-Whitney test for continuous and the χ^2 test for categorical variables.

[c] SI conversion: to convert bilirubin to μmol/L, multiply by 17.104.

5 in 101 patients (19%), and 6 in 286 patients (54%) and did not differ significantly between response groups (p=.41). As expected, patients without SVR were more often treated with non-pegylated interferon regimens and more frequently infected with HCV genotype 1.

Follow-up duration

The median (IQR) follow-up duration was 8.4 (6.4-11.4) years. The last follow-up encounter among patients who survived and had complete follow-up ranged between January 2010 and October 2011. Follow-up was shorter for patients with SVR (median 6.6 years, IQR 5.0-8.3) than for patients without SVR (median 8.1 years, IQR 5.7-11.1, p<.001) because SVR occurred more often near the end of the inclusion period due to introduction of more effective combination treatment with pegylated interferon and ribavirin. In total, 454 patients (86%) had complete follow-up. Complete follow-up percentage did not differ significantly between response groups (84% in SVR group and 86% in without SVR group, p=.53). During the entire study period, 18 patients (9%) with SVR and 169 patients (50%) without SVR experienced at least 1 clinical outcome event (Table 1.2).

Table 1.2 Clinical Events According to Treatment Response

Outcomes	With SVR			Without SVR			p-value [b]
	Events No.	Observation Period, Person-Years	Rate per 100 Person-Years (95% CI)	Events No.	Observation Period, Person-Years	Rate per 100 Person-Years (95% CI)	
Any event [a]	18	1260	1.43 (0.77-2.09)	169	2921	5.79 (4.91-6.66)	<.001
All-cause mortality	13	1283	1.01 (0.46-1.56)	100	3410	2.93 (2.36-3.51)	<.001
Liver-related mortality or liver transplantation	3	1283	0.23 (<0.01-0.50)	103	3120	3.20 (2.58-3.82)	<.001
Hepatocellular carcinoma	7	1270	0.55 (0.14-0.96)	76	3222	2.63 (1.83-2.89)	<.001
Liver failure	4	1271	0.31 (<0.01-0.62)	111	3066	3.62 (2.95-4.29)	<.001

Abbreviations: CI; confidence interval, SVR; sustained virological response

[a] Any event is the composite of all analyzed outcomes, to which only the first event contributed in case of multiple events in an individual patient.

[b] The p-value is based on unadjusted Cox proportional hazard regression analyses, including SVR as a time-dependent covariate.

All-cause mortality

Thirteen patients (7%) with SVR and 100 patients (30%) without SVR died after prolonged follow-up of our cohort, which was more than 4 times the number of deaths registered during the first data collection (n = 2 among patients with SVR and n = 24 among patients without SVR).[11] There was a significant difference in the cumulative 10-year mortality rate between patients with SVR (8.9%, 95% CI 3.3-14.5) and without

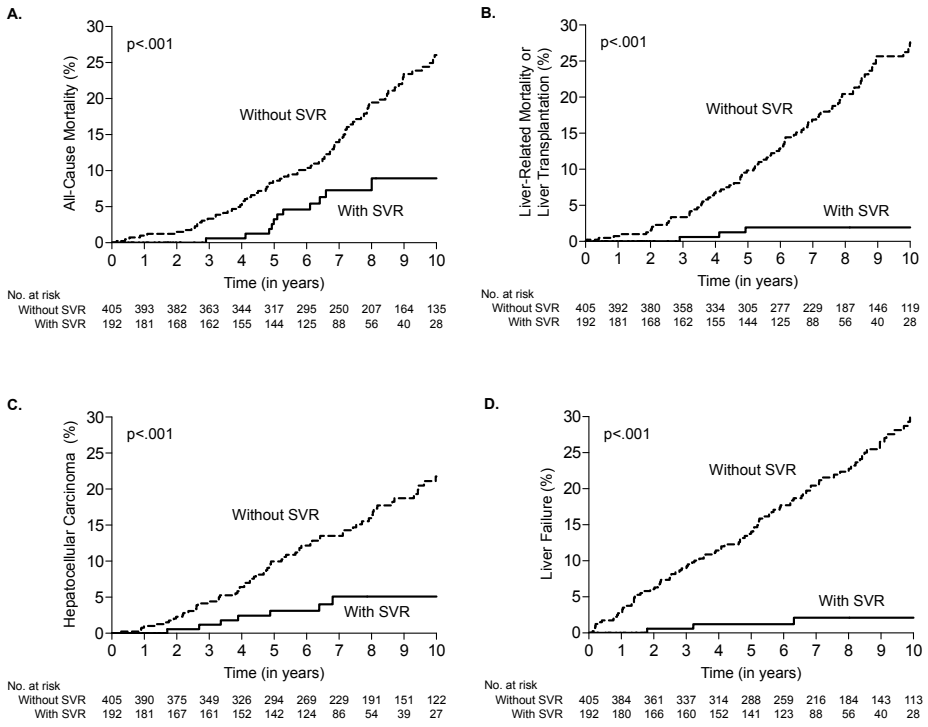

Figure 1.1 Survival Outcomes among Patients With Chronic HCV Infection and Advanced Hepatic Fibrosis With and Without Sustained Virological Response (SVR)

Survival curves for all-cause mortality (A), liver-related mortality or liver transplantation (B), hepatocellular carcinoma (C), and liver failure (D) were constructed using a clock-reset approach; patients who switched from the without SVR to the with SVR group due to successful retreatment during follow-up were censored in the without SVR group at the time of SVR. The time of SVR was then defined as time zero for their further follow-up in the with SVR group. Statistical significance between the survival curves in the without and with SVR groups was assessed with univariate Cox proportional hazards regression analyses, including SVR as a time-dependent covariate. The 10-year cumulative occurrence rates for all-cause mortality were 8.9% (95% CI 3.3%-14.5%) for with SVR and 26.0% (95% CI 20.2%-28.4%) for without SVR; for liver-related mortality or liver transplantation, 1.9% (95% CI 0.0%-4.1%) for with SVR and 27.4% (95% CI 22.0%-32.8%) for without SVR; for hepatocellular carcinoma, 5.1% (95% CI 1.3%-8.9%) for with SVR and 21.8% (95% CI 16.6%-27.0%) for without SVR; and for liver failure, 2.1% (95% CI 0.0%-4.5%) for with SVR and 29.9% (95% CI 24.3%-35.5%) for without SVR.

SVR (26.0%, 95% CI 20.2-28.4, p<.001) (Figure 1.1). Cox proportional hazards regression analysis showed that SVR was associated with a statistically significant reduction in the hazard of overall death (adjusted hazard ratio [HR] 0.26, 95% CI 0.14-0.49, p<.001) (Table 1.3, model 1).

Other baseline factors significantly associated with all-cause mortality in multivariate analysis were older age, HCV genotype 3 infection, higher Ishak fibrosis score, presence of diabetes, and a history of severe alcohol use. Patients with HCV genotype 3 infection were younger (median [IQR] age 44 [40-49] years) compared with patients without genotype 3 infection (median [IQR] 49 [43-57] years, p<.001), and after correction for age, the relationship with genotype 3 became apparent. Among 311 patients for whom HCV genotype and a probable transmission route was known, patients with HCV genotype 3 infection were more frequently infected through injection drug use (41 of 63 patients [65%] with genotype 3 infection vs 106 of 248 patients [43%] without genotype 3 infection, p=.002). The HR of HCV genotype 3 infection remained similar (HR 2.35, 95% CI 1.11-4.95, p=.03) when corrected for injection drug use as route of transmission, which showed a higher but not statistically significant risk for all-cause mortality itself (HR 1.51, 95% CI 0.71-3.21, p=.28).

The laboratory markers of liver disease severity were included in a second model. These laboratory markers were all available in a representative subgroup of 390 patients (74%) (Table 1.3, model 2). The estimated HR of SVR for all-cause mortality was essentially the same (HR 0.25, 95% CI 0.12-0.53, p<.001) in this analysis. Corrected for SVR, the all-cause mortality risk did not differ for patients with all 4 laboratory markers available compared with the patients who were missing at least 1 laboratory marker (HR 0.89, 95% CI 0.57-1.37, p=.59). Furthermore, also after multiple imputation for missing values, performed as sensitivity analyses, the HR of SVR for all-cause mortality remained statistically significant (adjusted HR 0.28, 95% CI 0.15-0.52, p<.001).

Subgroup analyses showed that the association between SVR and reduced all-cause mortality remained in patients with a history of severe alcohol use (adjusted HR 0.04, 95% CI 0.00-0.40, p=.006), in patients with most severe cirrhosis (adjusted HR 0.22, 95% CI 0.10-0.48, p<.001), as well as in patients older than 55 years (adjusted HR 0.28, 95% CI 0.11-0.71, p=.008). However, the interactions between SVR and these covariates were not statistically significant.

Liver-related mortality or liver transplantation

Of the 100 deaths in patients without SVR, the cause was liver-related in 70 patients (70%), not liver-related in 15 patients (15%), and unknown in another 15 patients (15%). A liver-related, not liver-related, or unknown cause of death was present in 3 patients (23%), 6 patients (46%), and 4 patients (31%) with SVR, respectively. Among

Table 1.3 Cox Proportional Hazard Regression Analyses for All-Cause Mortality [a]

	All-Cause Mortality									
	Model 1 (n = 493)					Model 2 (n = 368) [b]				
Characteristics	No. of Events	No. of Patients	HR	95% CI	p-value	No. of Events	No. of Patients	HR	95% CI	p-value
Virological response										
Without SVR	92	315	1	(Reference)		60	224	1	(Reference)	
With SVR [c]	13	178	0.26	0.14-0.49	<.001	9	144	0.25	0.12-0.53	<.001
Age, per year			1.09	1.06-1.12	<.001			1.08	1.05-1.11	<.001
Gender										
Female	30	147	1	(Reference)		18	102	1	(Reference)	
Male	75	346	1.52	0.93-2.48	.09	51	266	1.35	0.69-2.67	.38
HCV genotype										
Non-genotype 3	87	410	1	(Reference)		55	303	1	(Reference)	
Genotype 3	18	83	2.08	1.18-3.66	.01	14	65	2.68	1.37-5.25	.004
Diabetes mellitus										
No	84	432	1	(Reference)		52	318	1	(Reference)	
Yes	21	61	1.76	1.02-3.01	.04	17	50	2.46	1.28-4.72	.007
History of severe alcohol use										
No	76	380	1.00	(Reference)		48	276	1	(Reference)	
Yes	29	113	2.20	1.32-3.67	.002	21	92	2.38	1.21-4.68	.01
Fibrosis score					.09					.67
Ishak 4	14	134	1	(Reference)		6	93	1	(Reference)	
Ishak 5	14	96	1.29	0.60-2.77	.52	10	73	1.65	0.56-4.91	.37
Ishak 6	77	263	1.87	1.02-3.45	.04	53	202	1.38	0.54-3.50	.50
Platelet count, per $10x10^9$/L			-	-	-			0.90	0.85-0.96	.002
Bilirubin, per mg/dL			-	-	-			1.01	0.95-1.07	.83
Albumin, per g/L			-	-	-			0.99	0.92-1.07	.80
AST/ALT ratio, per 0.1			-	-	-			1.11	1.02-1.22	.02

Abbreviations: ALT; alanine aminotransferase, AST; Aspartate aminotransferase, CI; confidence interval, HCV; hepatitis C virus, HR; hazard ratio, SVR; sustained virological response.

[a] Multivariate Cox proportional hazards regression analyses to adjust the HR of SVR for all-cause mortality. Both models were adjusted for the year treatment started and stratified by treatment center to control for possible heterogeneity between centers.

[b] A complete case analyses was performed after inclusion of the laboratory markers of liver disease severity. In 26% of the patients either baseline platelet count, bilirubin, albumin or AST/ALT ratio was missing.

[c] Included as a time-dependent variable, to control for patients who changed their response status from without SVR to with SVR due to successful retreatment during the follow-up.

the total 21 non-liver-related mortalities, 8 patients died of extrahepatic malignancy, 4 of a cerebrovascular or cardiovascular event, 2 because of advanced pulmonary disease, and 7 of other not liver-related causes. None of the patients with SVR underwent liver transplantation in contrast with 46 patients without SVR, of whom 13 died during follow-up. Liver-related mortality or liver transplantation occurred in 103 patients (30%) without SVR and in only 3 patients (2%) with SVR (Table 1.2).

In comparison, after the previous data collection, we found that liver-related mortality or liver transplantation occurred in 34 patients without SVR and only 1 patient with SVR.[11] The 10-year cumulative incidence risk of liver-related mortality or liver transplantation was 1.9% (95% CI 0.0-4.1) in patients with SVR and 27.4% (95% CI 22.0-32.8) in patients without SVR (p<.001) (Figure 1.1). This resulted in a lower hazard in patients achieving SVR (adjusted HR 0.06; 95% CI 0.02-0.19, p<.001) (Table 1.4). In contrast with the overall mortality model, diabetes (HR 0.86, 95% CI 0.45-1.66, p=.66) and HCV genotype 3 infection (HR 1.18, 95% CI 0.62-2.27, p=.62) were not associated with liver-related mortality or liver transplantation. Having Ishak fibrosis score 5 or 6 was a risk factor (HR 4.02, 95% CI 1.67-9.69, p=.002 and HR 4.84, 95% CI 2.16-10.85, p<.001, respectively). Further adjusting for laboratory markers of liver disease severity showed that the HR of SVR for liver-related mortality or liver transplantation remained statistically significant (adjusted HR 0.05, 95% CI 0.01-0.22, p<.001).

Liver-related morbidity

Seven patients with SVR (4%) were diagnosed with HCC up to 6.8 years after SVR was achieved. In the without SVR group, HCC occurred in 76 patients (22%). One hundred fifteen patients, of whom only 4 had SVR, had liver failure with or without signs of portal hypertension (Table 1.2). Ascites was the most frequent first sign of liver failure occurring in 75 cases (65%), followed by 23 cases with variceal bleeding (20%), 11 with hepatic encephalopathy (10%), and 6 with jaundice only (5%). One patient reached SVR due to retreatment after the ascites had resolved. Three of 4 patients with SVR and liver failure had ascites and 1 patient was jaundiced.

After 10 years, the cumulative occurrence of HCC was 5.1% (95% CI 1.3-8.9) in patients with SVR and 21.8% (95% CI 16.6-27.0) in patients without SVR (p<.001) (Figure 1.1). The 10-year cumulative liver failure rate was 2.1% (95% CI 0.0-4.5) in patients with SVR vs 29.9% (95% CI 24.3-35.5) in patients without SVR (p<.001) (Figure 1.1). The risk of HCC (adjusted HR 0.19, 95% CI 0.08-0.44, p<.001) and the risk of liver failure (adjusted HR 0.07, 95% CI 0.03-0.20, p<.001) were reduced in patients with SVR (Table 1.4). More severe hepatic fibrosis, older age, and a history of severe alcohol use were risk factors for both HCC and liver failure. Male sex, presence of diabetes at baseline, and HCV genotype 3 infection were significantly associated with HCC occurrence

Table 1.4 Cox Proportional Hazard Regression Analyses for Secondary Clinical Outcomes [a]

Characteristics	Liver-Related Mortality or Liver Transplantation (n = 483)					Hepatocellular Carcinoma (n = 491)					Liver Failure (n = 498)				
	No. of Events	No. of Patients	HR	95% CI	p-value	No. of Events	No. of Patients	HR	95% CI	p-value	No. of Events	No. of Patients	HR	95% CI	p-value
Virological response															
Without SVR	96	309	1	(Reference)		68	312	1	(Reference)		102	317	1	(Reference)	
With SVR [b]	3	174	0.06	0.02-0.19	<.001	7	179	0.19	0.08-0.44	<.001	4	181	0.07	0.03-0.20	<.001
Age, per year			1.04	1.01-1.06	.005			1.09	1.06-1.12	<.001			1.05	1.02-1.07	<.001
Gender															
Female	22	143	1	(Reference)		14	145	1	(Reference)		29	149	1	(Reference)	
Male	77	340	1.50	0.90-2.52	.12	61	346	2.00	1.07-3.76	.03	77	349	1.11	0.70-1.75	0.67
HCV genotype															
Non-genotype 3	86	402	1	(Reference)		62	407	1	(Reference)		95	413	1	(Reference)	
Genotype 3	13	81	1.18	0.62-2.27	.62	13	84	2.07	1.06-4.05	.03	11	85	0.78	0.39-1.57	0.49
Diabetes mellitus															
No	73	425	1	(Reference)		61	431	1	(Reference)		92	437	1	(Reference)	
Yes	26	58	0.86	0.45-1.66	.66	14	60	2.01	1.07-3.80	.03	14	61	1.14	0.62-2.06	0.68
History of severe alcohol use															
No	73	373	1	(Reference)		54	379	1	(Reference)		72	385	1	(Reference)	
Yes	26	110	1.71	1.02-2.88	.04	21	112	2.20	1.23-3.94	.008	34	113	2.57	1.61-4.10	<.001
Fibrosis score					.001					.04					<.001
Ishak 4	8	131	1	(Reference)		8	134	1	(Reference)		7	134	1	(Reference)	
Ishak 5	21	93	4.02	1.67-9.69	.002	17	97	2.93	1.23-6.95	.02	23	98	5.64	2.35-13.51	<.001
Ishak 6	70	259	4.84	2.16-10.85	<.001	50	260	2.62	1.20-5.70	.02	76	266	6.30	2.82-14.11	<.001

Abbreviations: CI; confidence interval, HCV; hepatitis C virus; HR; hazard ratio, SVR; sustained virological response.

[a] Multivariate Cox regression models to adjust the hazard ratio of SVR. Both models were adjusted for the year treatment started and stratified by treatment center, to control for possible heterogeneity between centers.

[b] SVR was included as a time-dependent variable, to control for patients who changed their response status from non-SVR to SVR due to successful retreatment during the follow-up.

only (Table 1.4). The adjusted HR of SVR was 0.17 (95% CI 0.06-0.47, p=.001) for HCC and 0.06 (95% CI 0.02-0.21, p<.001) for liver failure, when adding laboratory markers of liver disease to the multivariate Cox proportional hazards regression models.

DISCUSSION

In our international, multicenter, long-term follow-up study, SVR was associated with prolonged overall survival. The risk of all-cause mortality was almost 4-fold lower in patients with SVR compared with patients without SVR. Our study with a long follow-up duration demonstrated a lower risk for all-cause mortality in patients with chronic HCV infection and advanced hepatic fibrosis who achieved SVR. In addition, we were able to further establish and quantify the risk reduction of HCC, liver failure, and liver-related mortality or liver transplantation in patients with SVR.

Although prior studies have described a clinical benefit of SVR in patients with CHC and severe hepatic fibrosis, most did not investigate all-cause mortality as a single outcome. A reduced liver-related mortality has been demonstrated, but this remains a suboptimal surrogate endpoint.[12-14] A reduction in liver-related death may not directly translate into an overall survival benefit, as liver-related mortality in patients without SVR could mask an overall deteriorating clinical condition leading to death due to indirect causes related to cirrhosis, such as increased risk of infections in patients with cirrhosis or increased risk of vehicle accidents in patients with low grade encephalopathy. Our finding of reduced all-cause mortality should be free of this bias.

In another partially prospective study, the association of SVR with all-cause death and liver transplantation as a combined endpoint was analyzed.[14] The adjusted cumulative proportion of patients who died or underwent liver transplantation after 7.5 years of follow-up was higher in patients not responding to pegylated interferon and ribavirin therapy (27.2%) compared with patients with virological breakthrough or relapse (4.4%) or SVR (2.2%). In a Spanish cohort of patients with cirrhosis, the 5-year mortality was 2% in patients with SVR vs 14% in patients without SVR.[25] Multivariate analysis for all-cause mortality was not reported, probably because of the limited number of deaths in the relatively short follow-up of 2.9 years. In a large and predominantly male population of US veterans followed up for a median of 3.8 years, 5-year mortality rates of 6.7% to 8.0% in patients with SVR vs rates of 14.4% to 24.4% in patients without SVR were reported.[26] Because only 9% to 16% of the included patients were registered as having cirrhosis, the relatively high death rate in the study of the US veterans may be due to other comorbidities in this patient population. Both of these studies included patients treated with pegylated interferon

and ribavirin combination treatment from 2001 onwards, but we included all consecutive patients with CHC with histological-proven advanced fibrosis from the first interferon treatment available. We performed time-dependent Cox proportional hazards regression analyses in which the virological response status could change from non-SVR to SVR during the follow-up, as is the case in the real-life setting.

A further new finding of our study was the approximately 2-fold increased risk of all-cause mortality and HCC in patients with HCV genotype 3 infection compared with patients without genotype 3 infection. Genotype 3 infection has been associated with more rapid fibrosis progression.[27] The association with genotype 3 infection remained after correction for fibrosis stage and laboratory markers of liver disease severity. A higher risk of HCC in patients with genotype 3 infection has been found previously and could be explained by hepatic steatosis.[28] Steatosis is more frequently observed in patients with HCV genotype 3 infection and is a risk factor for HCC, independent of cirrhosis.[29,30] Recognition of worse clinical outcome in patients with HCV genotype 3 infection should encourage clinicians to treat this population now rather than to await newer antiviral agents.

The development from interferon monotherapy to pegylated interferon and ribavirin combination therapy has led to an improvement of SVR rates. Accordingly, patients without SVR in our study were more frequently treated with interferon monotherapy and thus earlier during the inclusion period compared with patients with SVR. Duration of treatment was shorter in patients without SVR, both because treatment efficacy is reduced if discontinued early because of intolerability to interferon and because of recommended on-treatment stopping rules for nonresponse.[6] Because more advanced liver disease is associated with virological nonresponse, it was expected that the SVR group had a higher platelet count, lower bilirubin level, lower AST/ALT ratio, and lower prevalence of Ishak fibrosis score 6. Nevertheless, our extensive multivariate analyses including all the markers of liver disease severity showed that SVR was independently associated with reduced all-cause mortality and liver-related mortality as well as liver-related morbidity. There remains, however, the possibility of unmeasured confounding.

There are several limitations with our study. Due to improvements of antiviral treatment, it is inevitable that the follow-up duration was shorter in patients with SVR than in patients without SVR. It is unlikely, however, that this follow-up difference had a substantial effect on our results because the clinical events followed linear patterns over time. Furthermore, cohort studies can be susceptible to bias when many patients are lost to follow-up and this is associated with the end points that are studied. It was therefore important that we recontacted patients and achieved a very high complete follow-up percentage of 86%. Data on laboratory markers at baseline was expected to be missing at random and indeed availability of laboratory markers

was not associated with mortality. Furthermore, after using multiple imputation to impute missing values, the HR of SVR for all-cause mortality remained essentially the same. The retrospective nature of our study could have led to a selection of a relatively healthy cirrhotic HCV population, because patients with most severe clinical characteristics are usually not considered for antiviral treatment. Because of the long follow-up duration and high number of patients with severe cirrhosis (Ishak fibrosis score 6), we registered sufficient events to show a clear decrease in all-cause mortality and liver-related morbidity in patients with SVR.

In conclusion, our study indicates that SVR was associated with improved overall survival in patients with chronic HCV infection and advanced hepatic fibrosis.

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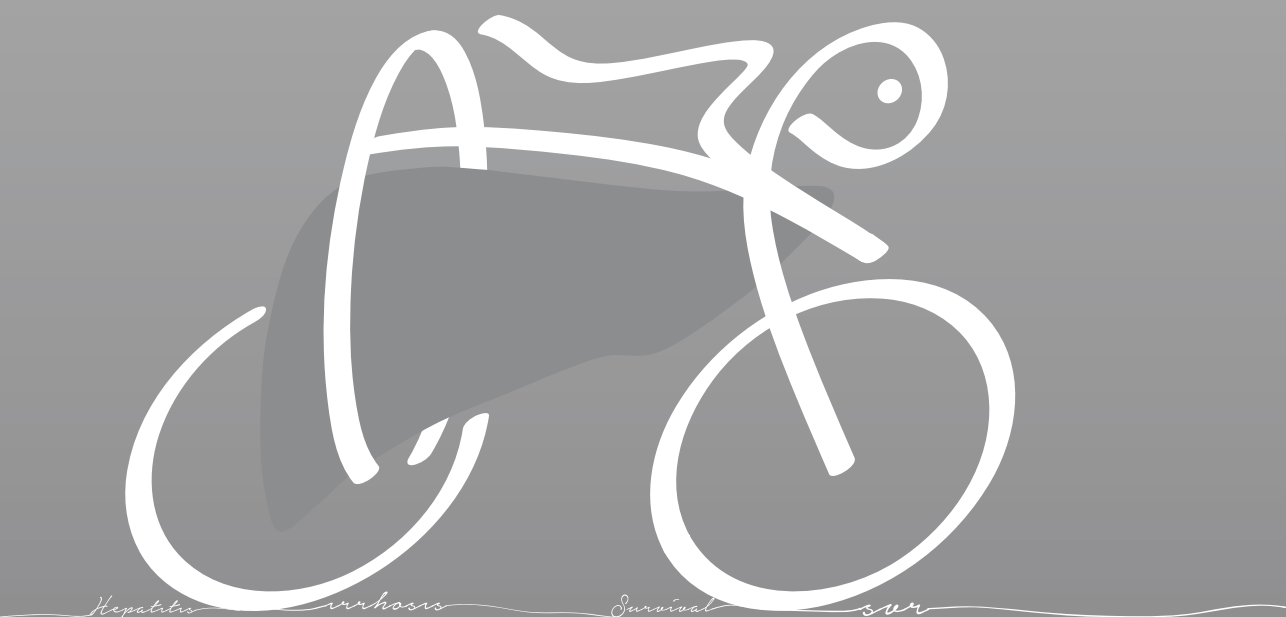
Hepatitis
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CHAPTER 2

Life expectancy in patients with chronic HCV infection and cirrhosis compared with a general population

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Submitted

INTRODUCTION

Almost 3 million people in the United States are chronically infected with the hepatitis C virus (HCV).[1] The life expectancy of patients with chronic HCV infection is reduced compared with the general population, largely attributable to the development of cirrhosis, liver failure and hepatocellular carcinoma.[2]

We previously showed that the hazard of all-cause mortality was four-fold lower among chronic HCV-infected patients with advanced hepatic fibrosis and sustained virological response (SVR) as compared to those without SVR.[3] Currently, little is known with respect to the remaining health risk among patients with SVR, especially in case of advanced liver disease. Therefore, to further substantiate the possible effect of attaining SVR, we aimed to compare the overall survival among patients with chronic HCV infection who already had bridging fibrosis or cirrhosis before therapy, with and without SVR, to that of the general population.

METHODS

The general study design and characteristics of the study population have been described in detail previously.[5] In short, all consecutive patients with chronic HCV monoinfection and biopsy-proven advanced hepatic fibrosis (Ishak fibrosis scores 4, 5 or 6) who initiated antiviral therapy between 1990 and 2003 were included from five large Hepatology Units in Europe and Canada. Follow-up started 24 weeks following cessation of antiviral treatment, at which time achievement of SVR (defined as HCV RNA negativity in the circulation 24 weeks after cessation of therapy) was determined with molecular assays. If the survival status was not known up to or beyond January 1 2010 with retrospective chart review, the patient and/or his primary care physician were re-contacted in an attempt to complete the follow-up beyond this date. If this was not possible, the follow-up was considered incomplete. Per virological response group, the observed overall survival was compared to the expected survival from matched age-, gender- and calendar time-specific death rates of the general population in the Netherlands, using the life table method and the Wilcoxon (Gehan) test. The statistical tests were 2-sided, and a $P<.05$ was considered statistically significant. SPSS version 21.0 (SPSS Inc) was used for the statistical analyses.

RESULTS

In total, 530 patients were followed for a median of 8.4 (interquartile range [IQR] 6.4-11.4) years. In 454 (86%) patients the follow-up was complete. Median age was 48 (IQR 42-56) years, 369 (70%) patients were male and 192 (36%) attained SVR. Thirteen patients with SVR died, resulting in a cumulative 10-year overall survival of 91.1% (95% confidence interval [CI] 85.5-96.7), which did not differ significantly from the age- and gender-matched general population (p=.57) (Figure 2.1). In contrast, 100 patients without SVR died. The cumulative 10-year survival was 74.0% (95%CI 71.6-79.8) among these patients, which was significantly lower compared to the matched general population (p<.001).

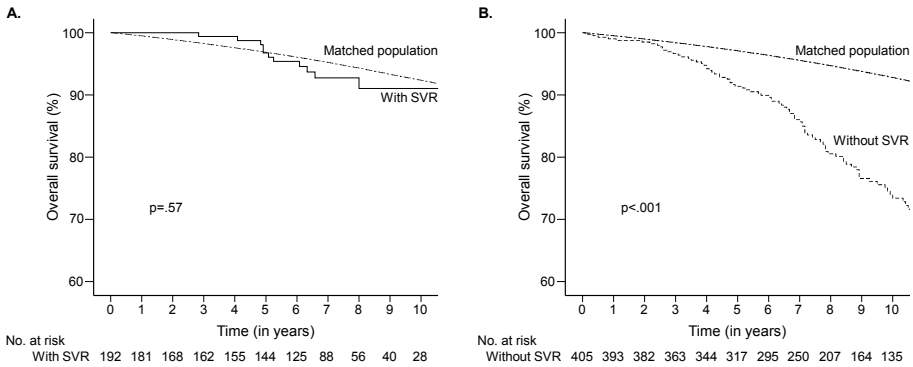

Figure 2.1 Overall Survival among Patients With Chronic HCV Infection and Advanced Hepatic Fibrosis With and Without Sustained Virological Response (SVR) as Compared to an Age- and Gender-Matched General Population

The survival curves among the patients with chronic HCV infection with SVR (A) and without SVR (B) were constructed using a clock-reset approach; patients who switched from the without SVR to the with SVR group due to successful retreatment during follow-up were censored in the without SVR group at the time of SVR. The time of SVR was then defined as time zero for their further follow-up in the with SVR group. Per virological response group, the survival was compared to matched age-, gender- and calendar time-specific death rates of the general Dutch population.

DISCUSSION

Among patients with chronic HCV infection and bridging fibrosis or cirrhosis, attaining SVR was associated with a survival comparable to that of the general population, while not attaining SVR was associated with reduced survival. The excellent survival among patients with advanced liver disease and SVR might be explained by the previously indicated associations between SVR and regression of hepatic inflammation and fibrosis, reduced hepatic venous pressure gradient, reduced occurrence of

hepatocellular carcinoma and liver failure, as well as reduced occurrence of diabetes mellitus, end-stage renal disease and cardiovascular events.[4,5] Although patients with cirrhosis and SVR remain at risk of HCC, their annual HCC incidence is low and the survival after HCC is substantially better among those with SVR as compared to those without SVR.[6] The limited impact of HCC occurrence on overall survival among patients with SVR could also be explained by competing risks.

Next to the retrospective nature, our study is limited by the fact that data regarding the general population were available for the Netherlands only. However, because the life-expectancy in the Netherlands is similar to that in the other participating countries this is not expected to have had a major influence on our results. Also, all patients received interferon-based therapy. Thus, these results need to be reconfirmed when interferon-free therapy is widely used, as these highly effective regimens may be administered to patients with more comorbidity and are able to cure patients with more advanced liver disease. For this reason our results do not vindicate the restriction of DAAs to those patients with most advanced liver disease only.

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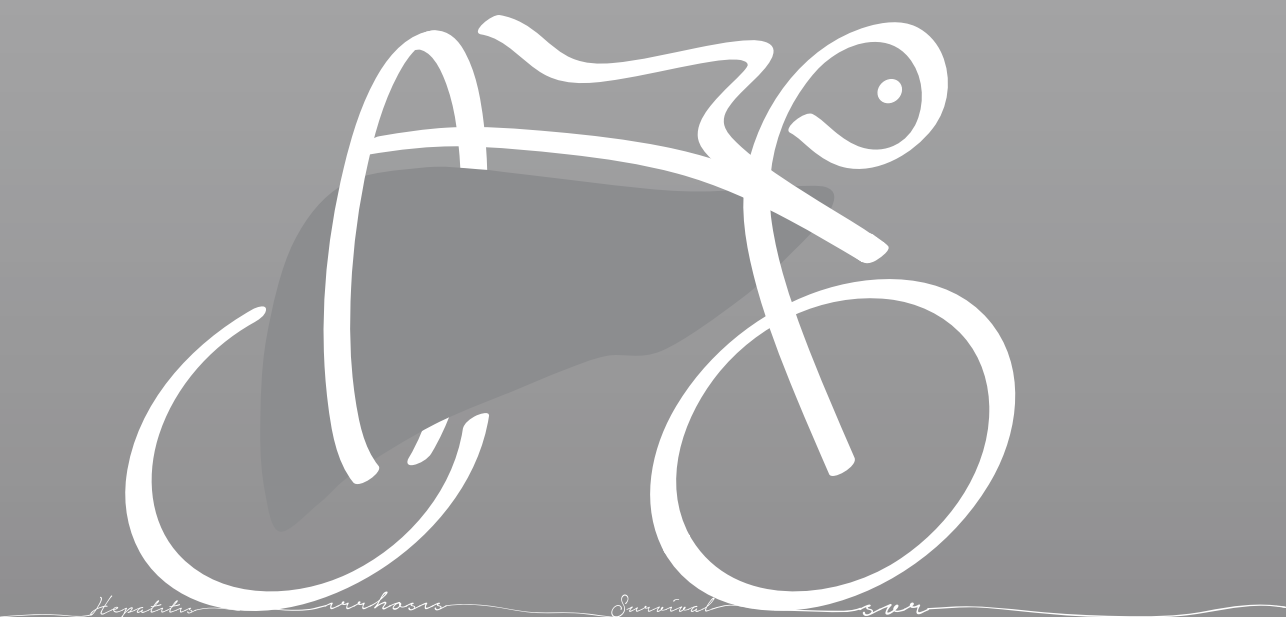
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CHAPTER 3

Improvement in platelet counts and spleen size following sustained virological response among patients with chronic hepatitis C virus infection and advanced hepatic fibrosis

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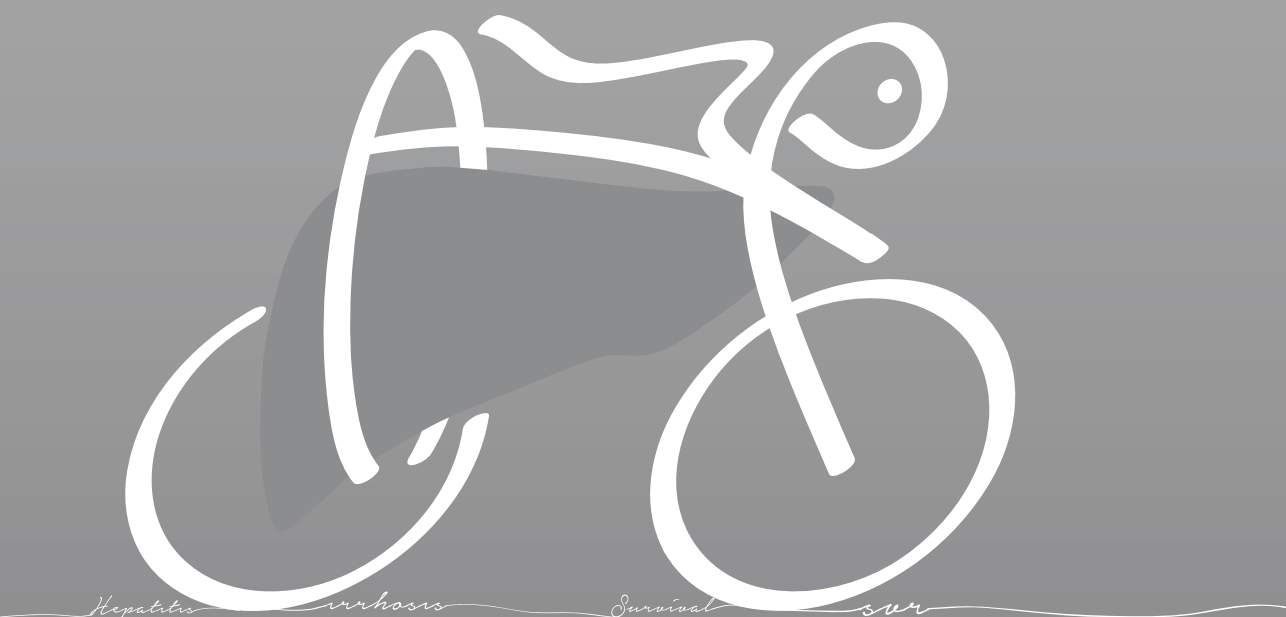
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CHAPTER 4

The number needed to treat to prevent mortality and cirrhosis-related complications among patients with cirrhosis and HCV genotype 1 infection

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ABSTRACT

Introduction

Cirrhotic patients with chronic hepatitis C virus (HCV) infection remain at risk for complications following sustained virological response (SVR). Therefore, we aimed to evaluate treatment efficacy with the number needed to treat (NNT) to prevent clinical endpoints.

Methods

Mortality and cirrhosis-related morbidity were assessed in an international multicenter cohort of consecutively treated patients with HCV genotype 1 infection and cirrhosis. The NNT to prevent death or clinical disease progression (any cirrhosis-related event or death) in one patient was determined with the adjusted (event-free) survival among patients without SVR and adjusted hazard ratio of SVR.

Results

Overall, 248 patients were followed for a median of 8.3 (IQR 6.2-11.1) years. Fifty-nine (24%) patients attained SVR. Among patients without SVR, the adjusted 5-year survival and event-free survival were 94.4% and 80.0%, respectively. SVR was associated with reduced all-cause mortality (HR 0.15, 95% CI 0.05-0.48, p=.002) and clinical disease progression (HR 0.16, 95% CI 0.07-0.36, p<.001). The NNT to prevent one death in 5 years declined from 1052 (95% CI 937-1755) at 2% SVR (interferon monotherapy) to 61 (95% CI 54-101) at 35% SVR (peginterferon and ribavirin). At 50% SVR, which might be expected with triple therapy, the estimated NNT was 43 (95% CI 38-71). The NNT to prevent clinical disease progression in one patient in 5 years was 302 (95% CI 271-407), 18 (95% CI 16-24) and 13 (95% CI 11-17) at 2%, 35% and 50% SVR, respectively.

Conclusion

The NNT to prevent clinical endpoints among cirrhotic patients with HCV genotype 1 has declined enormously with the development of antiviral therapy over the last two decades.

INTRODUCTION

Chronic infection with the hepatitis C virus (HCV) leads to ongoing inflammation in the liver and can consequently result in cirrhosis, hepatocellular carcinoma (HCC) and liver failure.[1] In both the United States as well as in Europe, it is expected that the incidence of HCV-induced cirrhosis and its related complications will increase substantially during the upcoming years.[2,3] Sustained virological response (SVR), defined as undetectable HCV RNA in the circulation 24 weeks after cessation of antiviral therapy, has been associated with reduced occurrence of liver failure, HCC and liver-related mortality.[4-7] Moreover, we recently showed that patients with chronic HCV infection and advanced hepatic fibrosis who attained SVR had a prolonged overall survival.[7] These findings support the use of SVR to evaluate the efficacy of interferon (IFN)-based antiviral therapy.

Over the last two decades, SVR rates for chronic HCV-infected patients have increased to approximately 60% with pegylated IFN (PegIFN) and ribavirin (RBV) combination therapy. However, cure rates differ extensively for various HCV genotypes and stages of hepatic fibrosis.[8,9] Especially for cirrhotic patients with HCV genotype 1 infection, the most common genotype in the Western world, the likelihood of SVR with IFN-based treatment has been disappointing.[10-12] In this difficult-to-cure subgroup, SVR rates were as low as 2% with IFN monotherapy and increased up to approximately 35% with PegIFN and RBV.[12,13] Recently, addition of a protease inhibitor to PegIFN and RBV showed to further enhance antiviral efficacy for HCV genotype 1 infection, also among patients with advanced liver disease.[14,15]

Achievement of SVR is, however, not the ultimate goal of antiviral therapy. Rather, we treat patients to prevent liver failure, HCC and, most importantly, prolong life expectancy. Instead in SVR rates, the efficacy of treatment could also be expressed in its ability to prevent, or rather postpone, these clinical outcomes. The number of patients that needs to be treated (NNT) to prevent an event in one patient is a useful parameter for such evaluation and has gained popularity as a clinical measure of efficacy.[16,17] It is not well known how many patients with cirrhosis and HCV genotype 1 infection, a group with unfavorable prognosis and frequent failure of antiviral therapy, need to be treated to prevent clinical events. Yet, this is relevant especially because cirrhotic patients who attained SVR are not free-warded from complications of their advanced liver disease.[4-7]

In this study, we aimed to assess how the improvement in virological efficacy of antiviral therapy influenced the NNT to prevent all-cause mortality or clinical disease progression among patients with chronic HCV genotype 1 infection and cirrhosis.

METHODS

Patients

All patients included in our international multicenter cohort, from five large hepatology units of tertiary care centers in Europe and Canada, were evaluated by reviewing the medical charts. The characteristics of the population and methodology have been previously described in detail.[7] Briefly, all consecutive patients with chronic HCV infection and histological proof of advanced hepatic fibrosis who initiated IFN-based therapy between 1990 and 2003 were included, also those patients treated outside of clinical trials. Patients co-infected with the human immunodeficiency virus or the hepatitis B virus were excluded. Complete follow-up was defined as death or clinical follow-up beyond January 1, 2010, and patients in whom follow-up was incomplete were invited for a single visit to the outpatient clinic. If the patient was unable to visit, the patient or primary care physician were asked to answer a structured questionnaire over the telephone. The current analyses were restricted to those patients with HCV genotype 1 infection and biopsy-proven cirrhosis because HCV genotype and fibrosis stage are two well-recognized factors influencing the SVR rate and clinical outcome, which affect the NNT as described below.[7-9]

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of good clinical practice. The study was 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 sought according to the local regulations. According to the standards of the local ethics committees, written informed consent was obtained from patients visiting the outpatient clinics, and written or oral informed consent was obtained from patients contacted by telephone.

Study endpoints and definition of events

All-cause mortality was the primary endpoint of this study. The secondary endpoint was clinical disease progression, which was a combined endpoint of liver failure, HCC, liver transplantation and death. In case multiple events occurred in a single patient, only the first clinical event was counted as clinical disease progression. Liver failure was defined as an episode of either ascites, confirmed by ultrasonography, bleeding varices, jaundice with a bilirubin level >35 μmol/L or overt hepatic encephalopathy. 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 hyperenhancement or one imaging technique showing a

focal lesion larger than 2 cm with arterial-phase hyperenhancement in the presence of an α-fetoprotein level >400 ng/mL.[18]

Statistical analyses

Baseline characteristics were compared between patients with SVR and patients without SVR after the first treatment, using the Mann-Whitney U-test for continuous and the $\chi 2$ test for categorical variables. To determine which variables were associated with time to death or clinical disease progression, Cox regression analysis was used. Twenty-four weeks after end-of-treatment was defined as time zero. Patients without detectable HCV RNA in serum at this time were classified as having SVR. Age, gender, SVR and variables with a p-value below 0.20 on univariate analyses were included in multivariate analyses to correct the association between SVR and clinical outcomes. Multivariate Cox models were further adjusted for the year treatment started as well as study site to control for possible heterogeneity between centres. As the response status of patients could change from without SVR to with SVR due to successful retreatment during follow-up, SVR was included as a time-dependent covariate in the Cox regression analyses to prevent an overestimation of the hazard ratio of SVR.[7] Complete case analyses were performed. Due to missing data, the final Cox model for each endpoint was extended with the laboratory markers of liver disease severity as a sensitivity analyses. A clock-reset approach was used to construct the survival curves for SVR status. Those patients who switched from the without-SVR group to the with-SVR group were censored in the without-SVR group at the time of SVR, which was then reset as time zero for their further follow-up in the with-SVR group.

The NNT to prevent death in one patient within [t] years was calculated with the adjusted survival in patients without SVR and the adjusted hazard ratio (HR) of SVR for all-cause mortality. Both parameters were derived from multivariate Cox regression analyses to reflect the average estimates in patients with HCV genotype 1 infection and cirrhosis.[19] The formula NNT = (1 / ((Survival$_{\text{without SVR}}$[t]$^{\text{HR of SVR}}$) - Survival$_{\text{without SVR}}$[t])) x (100/SVR rate) was used. The NNT to prevent clinical disease progression in one patient was calculated accordingly, with the adjusted event-free survival in patient without SVR and the adjusted HR of SVR for clinical disease progression. The NNT was calculated for all SVR rates on a continuous scale. The 95% confidence interval (CI) of the NNT was based on the 95% CI of the HR of SVR, as previously suggested.[19] The NNT to prevent one clinical outcome event can be calculated at every time point [t] during the follow-up. In the manuscript, the NNT to prevent one clinical outcome event within 5 years is reported, unless otherwise noted.

All statistical tests were two-sided, and a p-value <.05 was considered to be statistically significant. SPSS version 17.0.2 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Study population

In total, 257 patients with chronic HCV genotype 1 infection and cirrhosis started an IFN-based regimen at the participating centers between 1990 and 2003. Nine patients were excluded from the analyses as they could not be traced while being lost to follow-up prior to 24 weeks after the end-of-treatment (n = 4) or were diagnosed with HCC (n = 2) or liver failure (n =3) before this time. The total study cohort thus consists of 248 patients, of which 59 patients (24%) attained SVR and 189 patients (76%) did not. Of the 59 SVR patients, 33 (56%) achieved SVR after the initial treatment. Twenty-six (44%) patients achieved SVR due to retreatment after a median of 4.8 [interquartile range (IQR) 2.7-6.2] years. The median follow-up duration was 8.3 (IQR 6.2-11.1) years, and 213 (86%) patients had complete follow-up.

Table 4.1 shows the baseline characteristics according to the virological response on the initial treatment. The overall median age was 51 (IQR 44-57) years, and most patients were men (n =168, 68%). Sixty-seven (27%) patients had Ishak fibrosis score 5, and 181 (73%) patients had Ishak fibrosis score 6. The initial treatment consisted of IFN monotherapy in 82 patients (33%), IFN and RBV in 69 patients (28%) and PegIFN and RBV in 80 patients (32%). As expected, patients without SVR were less often treated with PegIFN and RBV (p<.001).

All-cause mortality

In total, 64 patients died during the follow-up, of which only three patients had attained SVR. There was a significant difference in the cumulative 10-year mortality rates between the patients without SVR (30.8%, 95% CI 23.2-38.4) and patients with SVR (8.9%, 95% CI 0.0-18.7, p<.001) (Figure 4.1). Univariate time-dependent Cox regression analysis showed that SVR was associated with a reduction in the hazard of all-cause mortality (HR 0.20, 95% CI 0.06-0.64, p=.007) (Table 4.2). In multivariate analyses, SVR remained significantly associated with reduced mortality (HR 0.15, 95% CI 0.05-0.48, p=.002) (Table 4.2, Model 1). In a sensitivity analysis, for which the multivariate Cox model was extended with the laboratory markers of liver disease severity, the HR of SVR remained similar (HR 0.13, 95% CI 0.02-0.99, p=.049) (Table 4.2, Model 2).

Table 4.1 Baseline Characteristics According to the Response at the Initial Treatment [a]

Characteristics	Overall (n = 248)	With SVR (n = 33)	Without SVR (n = 215)	p-value [b]
Age, years, median (IQR)	51 (44-57)	50 (45-59)	51 (44-57)	.932
Male	168/248 (68)	24/33 (73)	144/215 (67)	.511
BMI, kg/m^2, median (IQR) [c]	26.8 (24.1-29.0)	25.4 (23.2-29.0)	26.8 (24.5-29.1)	.272
Fibrosis score [d]				.194
Ishak 5	67/248 (27)	12/33 (36)	55/215 (26)	
Ishak 6	181/248 (73)	21/33 (64)	160/215 (74)	
Type of treatment				<.001
Interferon monotherapy	82/248 (33)	1/33 (3)	81/215 (38)	
Interferon and ribavirin	69/248 (28)	9/33 (27)	60/215 (28)	
Pegylated interferon monotherapy	7/248 (3)	0/33 (0)	7/215 (3)	
Pegylated interferon and ribavirin	80/248 (32)	22/33 (67)	58/215 (27)	
Consensus interferon (+/- ribavirin)	10/248 (5)	1/33 (3)	9/215 (4)	
Laboratory markers of liver disease severity, median (IQR) [c]				
Platelet count, x10^9/L	141 (105-180)	147 (118-205)	138 (102-177)	.101
Albumin, g/L	42 (38-44)	42 (40-43)	42 (38-44)	.801
Bilirubin, μmol/L	13 (10-19)	11 (9-14)	13 (11-19)	.010
AST/ALT ratio	0.73 (0.59-0.92)	0.67 (0.57-0.83)	0.73 (0.59-0.93)	.281
Treatment naïve	219/248 (88)	30/33 (91)	189/215 (88)	.617
Year treatment started, median (IQR)	1999 (1996-2002)	2002 (2000-2002)	1998 (1995-2001)	<.001
Diabetes mellitus	39/248 (16)	4/33 (12)	35/215 (16)	.541
History of severe alcohol use [e]	84/226 (22)	8/31 (22)	42/195 (26)	.595
AntiHBc positivity	84/194 (42)	13/24 (54)	71/174 (41)	.214

Abbreviations: ALT; alanine aminotransferase, antiHBc; anti-hepatitis B core antigen, AST; aspartate aminotransferase, BMI; body mass index (calculated as weight in kilograms divided by height in meters squared), IQR; interquartile range, SVR; sustained virological response.

[a] Data are presented as No./total No. (%) unless otherwise noted.

[b] The baseline characteristics were compared between patients with and without SVR after the baseline treatment, using the Mann-Whitney test for continuous variables and the $_x^2$ test for categorical variables.

[c] Baseline data on BMI was missing in 59 (24%) patients, on platelet count in 31 (13%) patients, on albumin in 50 (20%) patients, on bilirubin in 40 (16%) patients, and on AST/ALT ratio in 45 (18%) patients.

[d] Ishak fibrosis score 5 is characterized by marked portal-to-portal and/or portal-to-central bridging fibrosis with occasional nodules (early cirrhosis); and Ishak fibrosis score 6 by probable or definite cirrhosis.

[e] The use of more than 50 gram alcohol per day was considered severe alcohol use.

At 5 years of follow-up, the survival in patients without SVR, adjusted for age, sex, diabetes mellitus, history of severe alcohol use, Ishak fibrosis score, study site and year of treatment initiation, was 94.4% (Figure 4.2). The formula to calculate the adjusted NNT to prevent 1 death in 5 years among patients with HCV genotype 1 infection and cirrhosis was thus represented by: NNT = (1 / ((0.944$^{0.15}$) - 0.944)) x

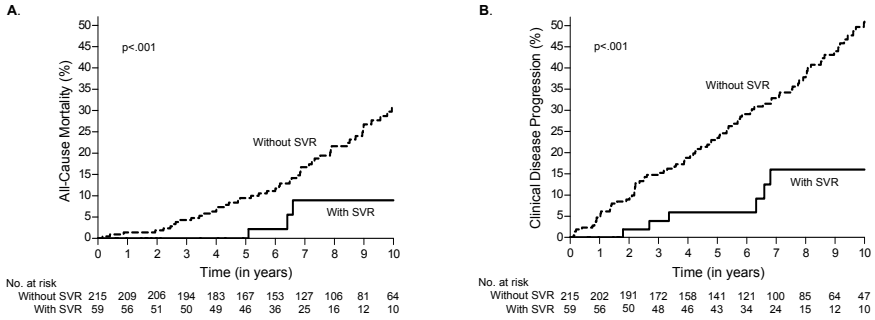

Figure 4.1 Survival Outcomes among Patients With Chronic HCV Infection and Advanced Hepatic Fibrosis With and Without Sustained Virological Response (SVR)

Survival curves for all-cause mortality (A), clinical disease progression (B) were constructed using a clock-reset approach; patients who switched from the without SVR to the with SVR group due to successful retreatment during follow-up were censored in the without SVR group at the time of SVR. The time of SVR was then defined as time zero for their further follow-up in the with SVR group. Statistical significance between the survival curves in the without and with SVR groups was assessed with univariate Cox proportional hazards regression analyses, including SVR as a time-dependent covariate.

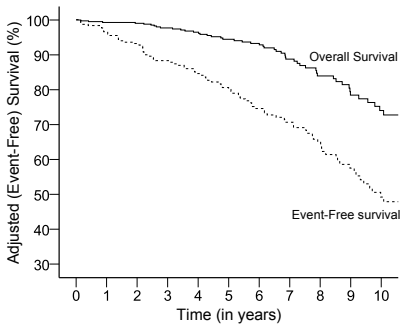

Figure 4.2 Adjusted Overall and Event-Free Survival among Patients Without SVR

The overall survival curve is adjusted for age, gender, diabetes mellitus, history of severe alcohol use, Ishak fibrosis score, study site and the year treatment started. Liver failure, hepatocellular carcinoma, liver transplantation and death were considered events for the event-free survival curve, which is adjusted for age, sex, history of severe alcohol use, Ishak fibrosis score, study site and the year treatment started.

Table 4.2 Cox Proportional Hazard Regression Analyses for All-Cause Mortality

Characteristics	All-Cause Mortality								
	Univariate			Multivariate Model 1 [b] (n = 248)			Multivariate Model 2 [b] (n = 182)		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
SVR [a]	0.20	0.06-0.64	.007	0.15	0.05-0.48	.002	0.13	0.02-0.99	.049
Age, per year	1.09	1.06-1.13	<.001	1.09	1.05-1.13	<.001	1.06	1.05-1.11	<.001
Male	0.90	0.53-1.52	.681	1.44	0.79-2.62	.237	1.28	0.69-2.67	.383
BMI, per kg/m^2	1.01	0.94-1.08	.874	-	-	-	-	-	-
Treatment naive	0.76	0.30-1.90	.556	-	-	-	-	-	-
Diabetes mellitus	1.81	0.98-3.35	.058	1.47	0.72-2.86	.259	3.71	1.56-8.81	.003
History of severe alcohol use [c]	1.89	1.06-3.36	.031	2.73	1.43-5.24	.002	4.10	1.65-10.65	.003
Ishak fibrosis score 6 [d]	3.03	1.38-6.67	.006	2.87	1.25-6.56	.013	2.54	0.23-28.10	.447
Laboratory markers of liver disease severity [e]									
Platelet count, per 10x10^9/L	0.89	0.84-0.95	<.001	-	-	-	0.92	0.84-1.01	.078
Bilirubin, per μmol/L	1.03	1.01-1.06	.016	-	-	-	1.02	0.98-1.06	.427
Albumin, per g/L	0.91	0.85-0.97	.004	-	-	-	1.05	0.94-1.16	.416
AST/ALT ratio, per 0.1	1.19	1.10-1.28	<.001	-	-	-	1.25	1.13-1.38	<.001
AntiHBc positivity	1.26	0.74-2.14	.404	-	-	-	-	-	-

Abbreviations: ALT, alanine aminotransferase; antiHBc, anti-hepatitis B core antigen; AST, aspartate aminotransferase; BMI; body mass index, CI; confidence interval, HR; hazard ratio, SVR; sustained virological response.

[a] SVR was included as a time-dependent variable.

[b] Multivariate Cox models are corrected for the year treatment started and study site.

[c] The use of more than 50 gram alcohol per day was considered severe alcohol use.

[d] Ishak fibrosis score 5 is characterized by marked portal-to-portal and/or portal-to-central bridging fibrosis with occasional nodules (early cirrhosis); and Ishak fibrosis score 6 by probable or definite cirrhosis.

[e] At least one of the baseline laboratory markers of liver disease severity (platelet count, bilirubin, albumin or AST/ALT ratio) was missing in 66 (27%) patients, which were thus only included in the second multivariate Cox model as a sensitivity analysis.

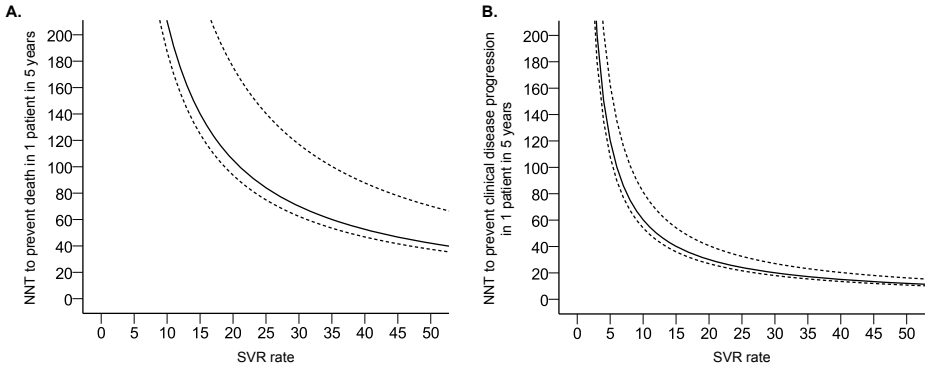

Figure 4.3 Adjusted Number Needed to Treat (NNT) to Prevent Death or Clinical Disease Progression in One Patient in 5 Years According to the SVR rate

The adjusted NNT to prevent death (A) or clinical disease progression (B) in one patient in 5 years. Clinical disease progression was defined as the occurrence of liver failure, hepatocellular carcinoma, liver transplantation or death. The adjusted NNT to prevent death or clinical disease progression is calculated for every SVR rate, using the adjusted 5-year overall or event-free survival probability in patients with chronic hepatitis C genotype 1 virus infection and cirrhosis who did not achieve sustained virological response (SVR), and the adjusted hazard ratio of SVR for all-cause mortality or clinical disease progression. The dashed lines represent the 95% confidence interval of the NNT, which are based on the 95% confidence interval of the adjusted hazard ratio of SVR for all-cause mortality or clinical disease progression.

(100/SVR rate). Figure 4.3A shows the NNT to prevent death in one patient in 5 years as a function of the SVR rate. The NNT decreased from 1052 (95% CI 937-1755) at a SVR rate of 2% to 61 (95% CI 54-101) at a SVR rate of 35%. At a SVR rate of 50%, which might be expected with triple therapy, the NNT was 43 (95% CI 38-71).

The NNT also declined if one death was to be prevented over a longer time period (Figure 4.4). To prevent one death in 10 years, 14 (95% CI 12-24) patients with HCV genotype 1 infection and cirrhosis needed to be treated with PegIFN and RBV combination therapy (assuming a SVR rate of 35%), and this was estimated to be 10 (95% CI 9-17) at 50% SVR.

Clinical disease progression

Clinical progression of liver disease occurred in 105 patients without SVR and six patients with SVR. Among the patients without SVR, the initial event of disease progression was liver failure in 60 patients, HCC in 35, liver transplantation in 2 and death in 8. Six patients with SVR showed clinical disease progression as a result of liver failure in 2, HCC in 3 and death in one patient(s). Univariate time-dependent Cox regression analysis showed that SVR was associated with a reduction in the hazard of clinical disease progression (HR 0.19, 95% CI 0.09-0.44, p<.001), which remained

Table 4.3 Cox Proportional Hazard Regression Analyses for Clinical Disease Progression

Characteristics	Clinical Disease Progression								
	Univariate			Multivariate Model 1 [b] (n = 248)			Multivariate Model 2 [b] (n = 182)		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
SVR [a]	0.19	0.09-0.44	<.001	0.16	0.07-0.36	<.001	0.16	0.06-0.45	.001
Age, per year	1.05	1.02-1.07	<.001	1.05	1.02-1.08	<.001	1.04	1.00-1.07	.040
Male	1.36	0.88-2.09	.165	1.76	1.11-2.80	.017	1.66	0.84-3.28	.147
BMI, per kg/m^2	0.99	0.94-1.05	.778	-	-	-	-	-	-
Treatment naive	1.13	0.62-2.07	.694	-	-	-	-	-	-
Diabetes mellitus	1.19	0.72-1.99	.496	-	-	-	-	-	-
History of severe alcohol use [c]	2.12	1.37-3.28	.001	2.36	1.48-3.78	<.001	2.92	1.54-5.54	.001
Ishak fibrosis score 6 [d]	1.54	0.96-2.46	.073	1.51	0.92-2.46	.100	0.59	0.14-2.50	.475
Laboratory markers of liver disease severity [e]									
Platelet count, per 10x10^9/L	0.88	0.84-0.92	<.001	-	-	-	0.93	0.88-0.99	.024
Bilirubin, per μmol/L	1.05	1.03-1.07	<.001	-	-	-	1.02	0.99-1.05	.241
Albumin, per g/L	0.89	0.85-0.94	<.001	-	-	-	0.94	0.86-1.03	.192
AST/ALT ratio, per 0.1	1.19	1.12-1.23	<.001	-	-	-	1.18	1.10-1.27	<.001
AntiHBc positivity	1.29	0.87-1.91	.215	-	-	-	-	-	-

Abbreviations: ALT, alanine aminotransferase; antiHBc, anti-hepatitis B core antigen; AST, aspartate aminotransferase; BMI; body mass index, CI; confidence interval, HR; hazard ratio, SVR; sustained virological response.

[a] SVR was included as a time-dependent variable.

[b] Multivariate Cox models are corrected for the year treatment started and study site.

[c] The use of more than 50 gram alcohol per day was considered severe alcohol use.

[d] Ishak fibrosis score 5 is characterized by marked portal-to-portal and/or portal-to-central bridging fibrosis with occasional nodules (early cirrhosis); and Ishak fibrosis score 6 by probable or definite cirrhosis.

[e] At least one of the baseline laboratory markers of liver disease severity (platelet count, bilirubin, albumin or AST/ALT ratio) was missing in 66 (27%) patients, which were thus only included in the second multivariate Cox model as a sensitivity analysis.

significant in multivariate analyses (HR 0.16, 95% CI 0.07-0.36, p<.001) (Figure 4.1 and Table 4.3).

Adjusted for age, sex, history of severe alcohol use, Ishak fibrosis score, study site and year of treatment initiation, the 5-year event-free survival rate was 80.0% (Figure 4.2). The adjusted NNT to prevent clinical disease progression in one patient in 5 years was calculated with the following formula: NNT = (1 / (($0.800^{0.16}$) - 0.800)) x (100/SVR rate). Figure 4.3B depicts the decline in the NNT to prevent clinical disease progression in one patient in 5 years as a function of the SVR rate. The NNT declined from 302 (95% CI 271-407) at a SVR rate of 2% to 18 (95% CI 16-24) at a SVR rate of 35%. At a SVR rate of 50%, the NNT was 13 (95% CI 11-17). To prevent clinical disease progression in one patient in 10 years, the NNT was 8 (95% CI 7-11) or 5 (95% CI 5-8) at 35% or 50% SVR, respectively (Figure 4.4).

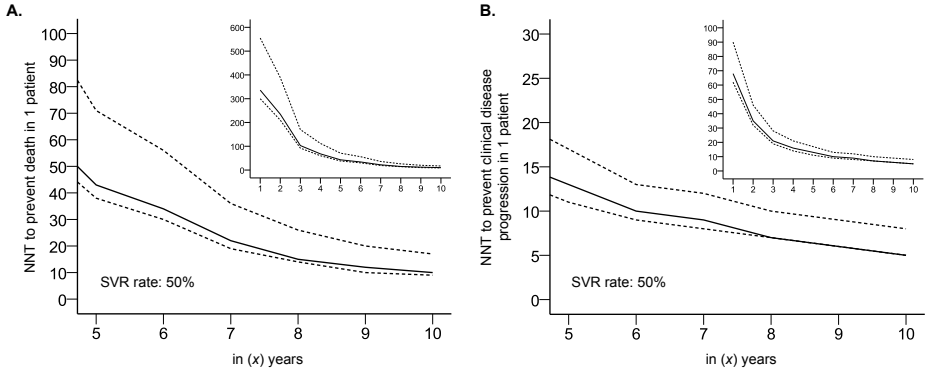

Figure 4.4 Adjusted Number Needed to Treat (NNT) to Prevent Death or Clinical Disease Progression in One Patient According to the Follow-Up Time

The adjusted NNT to prevent death (A) or clinical disease progression (B) in one patient according to various follow-up intervals in which 1 clinical outcome event needs to be prevented. Clinical disease progression was defined as the occurrence of liver failure, hepatocellular carcinoma, liver transplantation or death. The adjusted NNT to prevent death or clinical disease progression in one patient is calculated for each time interval, using the adjusted overall or event-free survival probability in patients with chronic hepatitis C genotype 1 infection and cirrhosis who did not achieve sustained virological response (SVR) at the specific time points, and the adjusted hazard ratio of SVR for all-cause mortality or clinical disease progression. The dashed lines represent the 95% confidence interval of the adjusted NNT, which are based on the 95% confidence interval of the adjusted hazard ratio of SVR for all-cause mortality or clinical disease progression. The SVR rate was set at 50%, which might be expected with current triple therapy. The small graph shows the NNT to prevent 1 clinical outcome event in a follow-up period from 1 to 10 years, while the large graph is the magnification of the 5- to 10-year follow-up period.

DISCUSSION

Among the patients with chronic HCV genotype 1 infection and biopsy-proven cirrhosis included in this international multicenter long-term follow-up study, SVR due to IFN-based treatment was associated with prolonged overall survival and reduced occurrence of cirrhosis-related complications. In this difficult-to-treat subgroup of patients with unfavorable prognosis, the risk of mortality and clinical disease progression was more than 6-fold lower among patients with SVR compared with those without SVR. Importantly, among cirrhotic patients with chronic HCV genotype 1 infection, the NNT to prevent death or clinical disease progression declined substantially with the increase in SVR rate due to the development of antiviral treatment. As prevention of solid clinical endpoints remains the most important goal of treatment for chronic HCV infection, the decline in the NNT is highly relevant when considering the improvement of antiviral therapy.

To our knowledge, this is the first study to show the increased efficacy of antiviral treatment with a clinical effect measure as the NNT to prevent solid clinical endpoints. Currently, HCV-treating physicians mostly rely on the SVR rate and relative risk reduction in SVR for clinical outcome when deciding to initiate antiviral treatment. Interestingly, the NNT combines both these parameters in a single absolute effect measure. Although there is no evidence to prefer one measure of effect over another, it is known that physicians more easily treat patients when considering relative risk reductions compared with absolute risk reductions.[20-22] Studies reporting on the NNT to prevent clinical events would thus enable better decision-making on treatment initiation in patients with chronic HCV infection.

The results presented in our study are estimates of the average NNT in patients with HCV genotype 1 infection and cirrhosis and thus provide valuable insights from particularly a public health perspective. The analyses were restricted to patients with HCV genotype 1 infection and cirrhosis, because both the SVR rate and prognosis were found to differ per HCV genotype and stage of fibrosis.[7-9,23-25] As can be derived from the formula to calculate the NNT, patient characteristics which influence both the SVR rate as well the baseline risk of clinical outcomes could lead to a varying NNT among individuals.[19,26] However, favorable patient characteristics increasing the chance to attain SVR often lower the risk of cirrhosis-related events, which have opposite effects on the NNT. Additional long-term follow-up studies including larger numbers of consecutively treated patients are required for further subgroup analyses which could lead to more individualized estimates.[19]

The estimated NNT to prevent death or clinical disease progression in one patient in 5 years further declined to 43 and 13, respectively, at a SVR rate of 50%, which may be expected from triple therapy in patients with chronic HCV genotype

1 infection and cirrhosis in a real-world setting.[27] It should, however, be recognized that the patients in our cohort were included at the time triple regimens were not available. As the incidence of newly acquired HCV infection has declined over the last two decades, a substantial part of the expected increase in HCV-induced cirrhosis will be attributable to patients who were infected decades ago, but who had less rapid progression of fibrosis.[28] It is currently unclear whether this will also affect the progression rate to clinical complications (and consequently the NNT) once cirrhosis has established in these patients, as at the stage of cirrhosis other factors due to formed vascular abnormalities play a role in disease progression as well.[29] Of course, clinical efficacy of antiviral therapy can only be determined in studies with long follow-up duration. Furthermore, any changes in average baseline risk of events would apply to all treatment regimens and thus not influence the pattern by which the decline in NNT relates to the increase in SVR rate. Relevant in this respect is that the major improvement in clinical efficacy has already been accomplished by the early development from IFN monotherapy to PegIFN and RBV combination therapy. In comparison, the addition of a protease inhibitor has only a modest effect. For justification of additional costs, it is thus also important that the future treatment regimens will increase the safety and tolerability of antiviral therapy.[30-32] Of specific interest are therefore cirrhotic patients with low platelet counts or albumin levels, who were identified to be at high risk for severe complications during antiviral therapy with PegIFN, RBV and a protease inhibitor.[33] Such unwanted severe side effects could very well hamper the increase in SVR rate with triple therapy in these subgroups, and further stratified SVR results thus need to be awaited to assess their gain in the NNT. However, as became clear from our results, in situations where SVR rates are low, even small increases in the virological efficacy of antiviral therapy may lead to large declines in the NNT to prevent clinical events.

To calculate the NNT, we set the improved prognosis in those with successful antiviral therapy against the occurrence of clinical outcomes in patients who failed to respond to therapy and corrected for the SVR rate. Prior Western studies in patients with chronic HCV infection and cirrhosis indeed showed similar outcomes for non-responders as compared to untreated patients with HCV infection and cirrhosis.[34,35] In our study, which included patients around the same time, the survival among patients without SVR was similar to that in a previously described large cohort of untreated patients with cirrhosis.[34] The patients without SVR in our study are thus likely to be representative of the natural history. Although it has been suggested that antiviral therapy might positively influence clinical outcome in the absence of SVR as well, two large randomized controlled trials did not find a clinical benefit of long-term PegIFN monotherapy in patients without SVR.[36,37] Of course, clinical efficacy as measured by the NNT is preferably determined in randomized trials comparing

antiviral therapy to no treatment. These trials are, however, no longer considered to be ethical as they would deny many patients a chance to attain SVR, which has been repeatedly associated with improved clinical outcome.[4-7] For evaluation of clinical efficacy, we thus need to rely on large retrospective studies which assessed interferon regimens that were not sufficiently effective to result in a highly selected group of uncured patients with unfavourable prognostic characteristics, as was the case in our study.

Several caveats should be taken into account calculating the NNT with time-to-event data.[38-40] First, a single NNT does not actually exist, as the NNT depends on the follow-up time. Therefore, we have reported the NNT for various time points.[41,42] Second, we have corrected our NNT analyses for varying follow-up times and censoring using the overall or event-free survival rates based on Cox regression survival analyses.[19,41] Finally, the width of the confidence intervals of the NNT remains difficult to define because the uncertainty of the survival in the control group (the patients without SVR in our case) cannot be taken into account.[19] We indicated the uncertainty of our NNT estimates by presenting confidence intervals based on the confidence interval of the HR for SVR as previously suggested.[19] Importantly, our finding of a major decline in the NNT to prevent clinical outcomes due to improvement of interferon-based antiviral therapy would not change if the confidence intervals were different.

In conclusion, the improvement of antiviral treatment efficacy over the last two decades has resulted in an enormous decline in the number of patients with HCV genotype 1 infection and cirrhosis that needs to be treated to prevent death or clinical disease progression. Our results clearly emphasize the clinical impact of the development of antiviral therapy for chronic HCV infection.

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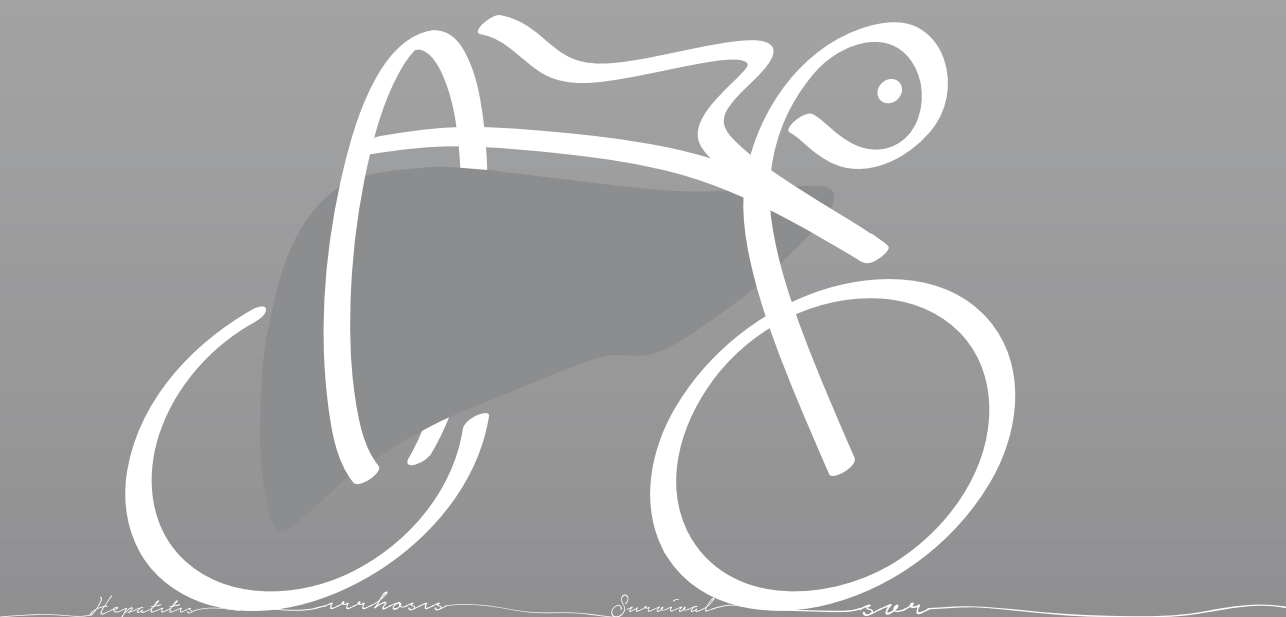
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CHAPTER 5

Is there sufficient evidence to recommend antiviral therapy in hepatitis C?

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SUMMARY

While patients with chronic hepatitis C virus (HCV) infection are treated in order to prevent liver-related morbidity and mortality, we rely on sustained virological response (SVR) as a virological biomarker to evaluate treatment efficacy in both clinical practice as well as in drug development. However, conclusive evidence for the clinical benefit of antiviral therapy or validity of SVR as surrogate marker, as derived from trials randomizing patients to a treatment or control arm, is lacking. In fact, the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) trial recently showed an increased mortality rate among interferon-treated patients compared to untreated controls. Consequently, the recommendation to treat patients with chronic HCV infection was challenged.

Here, we argue that the possible harmful effect of long-term low-dose pegylated interferon monotherapy, as was observed in the HALT-C trial cohort, cannot be extrapolated to potentially curative short-term treatment regimens. Furthermore, we discuss SVR as a surrogate biomarker, based on numerous studies which indicated an association between SVR and improvements in health-related quality of life, hepatic inflammation and fibrosis, and portal pressure as well as a reduced risk for hepatocellular carcinoma (HCC), liver failure and mortality.

INTRODUCTION

For the treatment of HCV infection we currently rely on interferon-based antiviral regimens. These therapies are very effective to prevent chronification of the acute infection, and also have good potential to eradicate HCV in those chronically infected.[1-4] Sustained virological response (SVR) is defined as absence of viremia 24 weeks after cessation of antiviral therapy, which showed long-term durability.[5] Consequently, antiviral therapy is considered successful and patients are usually considered 'cured' upon achievement of SVR. Although SVR may be the most widely used endpoint to evaluate antiviral treatment efficacy, it remains an indirect outcome measure. Indeed, the main reason to treat patients with chronic HCV infection is to improve their prognosis, by preventing cirrhosis-related morbidity and mortality. Are we convinced that the currently available treatment regimens achieve this goal so that we are right to recommend antiviral therapy to our patients?

FINDINGS OF A RECENT COCHRANE META-ANALYSIS

Recently, this discussion flared up due to the Cochrane review of Dr. Koretz and colleagues which aimed to assess the efficacy of interferon-based re-treatment on solid clinical endpoints.[6] Their study included only randomized controlled trials (RCT), in which patients with chronic HCV infection and nonresponse or relapse to a prior interferon-based treatment course were randomized to interferon re-treatment or no treatment. Extensive literature searches resulted in seven eligible trials for meta-analyses. For each endpoint of interest a subset of these trials was used, depending on the described endpoints in the original study reports. Only three trials reported on clinical outcomes, with all-cause mortality as most definite endpoint.[7-10] These three studies solely included patients with significant hepatic fibrosis or cirrhosis, so that the meta-analyses on clinical outcomes focused on a difficult-to-treat subgroup of patients with advanced liver disease and prior treatment failure. Combining the results of these trials indicated a higher mortality rate among interferon re-treated patients as compared to patients who did not receive further antiviral therapy, although this difference was not statistically significant (Odds Ratio [OR] 1.30, 95% Confidence Interval [CI] 0.95-1.79). However, in a sensitivity analysis including only the two largest trials with a low risk of bias, the disadvantage for patients who received interferon did reach statistical significance (OR 1.41, 95% CI 1.02-1.95). With respect to the other clinical endpoints, the occurrence of liver-related mortality, encephalopathy, ascites, spontaneous bacterial peritonitis, and hepatocellular carcinoma (HCC) was not found to differ significantly between re-treated patients and

controls. An exception was variceal bleeding, which occurred significantly less often among the patients who were randomized to interferon-based therapy (OR 0.26, 95% CI 0.09-0.71). A secondary aim was to assess the validity of SVR as a surrogate endpoint of antiviral therapy. Although four studies reported on this virological efficacy measure, only two trials included patients that actually attained SVR.[8,10] However, because of the difficult-to-treat patient population and the assessment of suboptimal treatment regimens for HCV eradication, the number of patients with SVR was very low. Nevertheless, and as expected, SVR occurred more often among the patients treated with interferon (OR 14.73, 95% CI 2.78-77.97). The meta-analyses thus found a discrepancy between the effect of interferon therapy on the surrogate outcome measure SVR and the clinical outcome measure all-cause mortality, as both occurred more frequently among actively treated patients. The harmful effect of interferon-based therapy on survival, which was found within the clinical scenario of the included trials, led to the conclusion that (pegylated) interferon is not an effective treatment option for patients with chronic HCV infection who failed a previous antiviral treatment course. Since this negative effect of interferon-based therapy was not captured by suppression of HCV RNA, SVR failed the criteria to be considered as a valid surrogate endpoint.[11-14] Based on these findings, the authors subsequently stated that their results caution physicians to stop advocating antiviral interventions of any kind. Extrapolating their recommendation to anti-HCV therapy in general was thus not discouraged by the fact that their meta-analyses regarding all-cause mortality and SVR were almost exclusively based on the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) trial. However, this important limitation warrants more careful interpretation of the results of this review in order to prevent unbalanced statements with potentially major consequences for the HCV-infected population.

HALT-C TRIAL

In brief, the HALT-C trial included 1050 patients with chronic HCV infection and advanced hepatic fibrosis, who were randomized to receive 3.5 years of 90 μg pegylated interferon alfa-2a weekly or no treatment. Interferon maintenance therapy was not found to slow down clinical and/or histological liver disease progression.[8] In fact, a post hoc analysis after prolonging the follow-up in this cohort indicated a poorer survival among patients in the interferon maintenance arm, as the cumulative 7-year mortality rate was 20% in treated and 15% in control patients (p=.049).[9] This impaired overall survival was predominantly caused by deaths of non-liver-related origin among patients with advanced hepatic fibrosis (but not yet cirrhosis). Based

on this study, there is reasonable consensus that interferon maintenance therapy has no regular place in the treatment of chronic HCV infection. The findings of the recent Cochrane meta-analysis further underline this general perception.

There are, however, several reasons not to withhold short-term interferon-based therapy with the potential to eradicate the chronic HCV infection based on the HALT-C trial results. First, the patients in the control arm of the HALT-C trial were not treatment naive. All included patients showed an insufficient virological response to a full-dose pegylated interferon and ribavirin treatment course just prior to randomization. The survival among patients who received interferon maintenance therapy for 3.5 years was thus significantly reduced compared to that of patients who received short-term interferon-based treatment, indicating that the possible harmful effects of long-term pegylated interferon monotherapy cannot be projected onto standard 24-48 week regimens. Second, the increase in mortality only began to arise after 3 years of pegylated interferon therapy, suggesting that the possible off-target treatment effects require long-term continuous interferon stimulation. Third, patients in the control arm of the HALT-C more frequently underwent liver transplantation, which can substantially prolong the survival. Consequently, the survival also becomes dependent on non-patient-related factors such as the availability of donor livers. In fact, allocation of donor liver grafts based on the Model for End-stage Liver Disease (MELD score) favors those patients with poorest prognosis.[15-17] In the HALT-C trial, the 7-year cumulative rate of all-cause mortality or liver transplantation as a combined endpoint was similar among the patients who received maintenance therapy (25%) vs those who did not (24%, p=.45).[9] Last, as mentioned in the Cochrane review, the excess mortality in a subgroup of the treated patients in the HALT-C study could be a chance finding. A significant increase in mortality due to interferon-based therapy was neither confirmed in another large RCT evaluating 5 years of maintenance therapy (Evaluation of PegIntron in Control of Hepatitis C trial), nor in smaller RCTs with shorter durations of interferon treatment.[7,10,18-24]

Furthermore, it can be questioned whether it is legitimate to assess the validity of SVR as a surrogate marker with a trial that did not aim to induce SVR and in fact assessed an interferon regimen almost unable to result in this virological endpoint. Indeed, the power was limited, as less than 4% of the treatment-experienced patients with advanced hepatic fibrosis attained SVR with the low-dose pegylated interferon maintenance regimen. Surely, these few patients with SVR could not significantly affect the clinical outcome of the entire treated study arm, whether or not a harmful effect would have been present.

SVR AS SURROGATE ENDPOINT

Currently, many clinical development trials aim to increase the SVR rate of anti-HCV therapy. In support, there are numerous arguments to consider SVR as a relevant endpoint. Treatment-induced viral clearance is important to prevent transmission of HCV and, even with the risk for re-infection among injecting drug users, antiviral therapy will decrease the prevalence of chronic HCV infection and the incidence of its sequelae.[25,26] Achieving SVR before liver transplantation in patients with advanced cirrhosis showed to eliminate the risk for post-transplant HCV recurrence, which is known to limit graft and overall survival.[27-29]

The majority of patients with chronic HCV infection are fortunate not to develop cirrhosis and the need for liver transplantation.[30] Although clinical outcome is often focused on solid cirrhosis-related endpoints such as hepatocellular carcinoma and mortality, it should be noted that the health-related quality of life is also impaired among patients with chronic HCV infection in absence of end stage liver disease.[31] Indeed, extrahepatic symptoms including fatigue, headaches, nausea, musculoskeletal and abdominal pain, and neuropsychiatric symptoms like depression and irritability can accompany the chronic infection.[32] Multiple studies indicated that the health-related quality of life, although further diminished for the duration of interferon-based treatment, improved compared to baseline in patients who attained SVR.[31,33-37] As the total burden of chronic HCV infection extends beyond the liver, the impact of SVR on patient-reported outcome measures covering physical, social as well as mental health should be appreciated.

Still, the predominant consequences of infection with HCV should be sought in the liver, where continuous inflammation can lead to fibrosis. Relevant are thus the many histological studies which showed regression of hepatic inflammation and fibrosis, as assessed by semi- quantitative grading and staging scores (Ishak and METAVIR), after interferon-induced eradication of HCV as the causative agent.[38-45] These histological improvements were frequently observed among patients who had already developed cirrhosis as well. In addition, the quantitatively measured total liver collagen content was also described to reduce upon achievement of SVR.[38,46,47] In fact, among patients with cirrhosis who did not show an improved METAVIR score in their post-SVR liver biopsy, the total amount of fibrosis was still significantly reduced.[38] Two prior Cochrane meta-analyses indicated that, compared to no treatment, interferon significantly improved liver histology, and that regression of hepatic fibrosis was more often achieved with interferon and ribavirin combination therapy compared to interferon therapy alone.[48,49] An important study by Mallet et al. linked the histological improvement following antiviral therapy to a favorable

clinical outcome, as the 'regression of cirrhosis' was associated with reduced occurrence of cirrhosis-related morbidity and prolonged overall survival.[40]

Improved histology could explain the reduction in portal pressure among patients with SVR, as measured by the hepatic venous pressure gradient (HVPG).[50-52] Importantly, the HVPG is one of the best validated surrogate markers within the field of hepatology, as higher HVPG levels are associated with worse clinical outcome and RCTs have indicated that interventions to reduce the portal pressure resulted in both decreased HVPG levels as well as improved clinical outcome.[12,53-55] Indeed, cirrhotic patients with chronic HCV infection who attained SVR did not develop esophageal varices or variceal bleeding, the most direct clinical complication of portal hypertension which is associated with substantial mortality.[50,56]

Several Western cohort studies assessed the relation between SVR and the occurrence of solid clinical endpoints such as liver failure, hepatocellular carcinoma, liver transplantation and death.[56-61] Our group was one of the first to show that patients with chronic HCV infection and advanced hepatic fibrosis had a reduced risk for liver failure as well as liver-related mortality already shortly after SVR.[62] Studies with longer follow-up confirmed that these events remained rare among successfully treated patients, and also indicated a strong association between SVR and reduced occurrence of HCC (hazard ratios [HR] varying from 0.19 to 0.38).[56-60] A partially prospective study with up to 7.5 years of follow-up found that all-cause mortality or liver transplantation, as a combined endpoint, occurred significantly less often among patients with SVR compared to those with virological nonresponse (HR 0.17, 95% CI 0.06-0.46, p<.001). In a multicenter study from Spain, which included 1599 patients with chronic HCV and human immunodeficiency virus co-infection who were followed for a median of approximately 5 years, SVR was independently associated with a reduced risk for non-liver-related, non-AIDS-related deaths (HR 0.35, 95% CI 0.13-0.93, p=.036).[63] Population-based studies indicated a favorable overall survival among HCV-exposed patients without detectable HCV RNA. A study, expected to include over 90% of all Danish patients tested for HCV RNA, found a significantly lower 5-year survival among patients with chronic HCV infection compared to those who cleared HCV RNA (86% vs 92%, respectively).[61] Recent data from the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer (R.E.V.E.A.L.)-HCV study, a prospective natural history study from Taiwan including 19,636 HBsAg-seronegative patients, indicated that the cumulative 18-year all-cause mortality rate was similar among anti-HCV seropositive patients with undetectable HCV RNA (12.4%) and anti-HCV seronegative patients (12.8%).[64] In contrast, the mortality rate was substantially higher among anti-HCV seropositive patients with detectable HCV RNA (30.1%, p<.001). Recently, important data have emerged regarding the association between SVR and reduced all-cause mortality as well. Multivariate

analyses, stratified for HCV genotype, indicated SVR was independently associated with reduced risk for death of any cause (HR 0.51-0.70, p<.01 for HCV genotypes 1, 2, and 3) among almost 17,000 American veterans with chronic HCV infection and varying stages of liver disease.[65] An update of our cohort, including 530 patients with HCV-induced advanced hepatic fibrosis or cirrhosis, resulted in a median follow-up duration of 8.4 years, and showed a 10-year cumulative all-cause mortality rate of 9% among patients with SVR compared to 26% among patients without SVR (p<.001).[56] Multivariate analyses indicated that SVR was the most important factor that was independently associated with improved survival, as patients with SVR had an approximately four-fold lower mortality risk compared to those without SVR (HR 0.26, 95% CI 0.14-0.49, p<.001). Together these large follow-up studies provide the most important data to endorse SVR as a relevant endpoint, as all showed similar and conclusive findings with strong adjusted hazard ratios for the association between SVR and improved clinical outcome.

RANDOMIZED CONTROLLED TRIALS REPORTING ON CLINICAL OUTCOME

It should, however, be recognized that cohort studies suggesting a clinical benefit of SVR share a similar limitation. Despite extensive multivariate analyses, the association between SVR and improved clinical outcome remains potentially influenced by unmeasured confounding factors.[66] In other words, observational studies cannot rule out the possibility that patients who have attained SVR are merely a selection of patients who would have a favorable natural history if left untreated as well. Indeed, several host and viral factors were related to a favorable long-term clinical outcome as well as to an adequate virological response to interferon-based therapy.[56,58,59,67,68] Thus, the frequently reported association between SVR and improved clinical outcome from cohort studies neither validates SVR as a surrogate endpoint nor confirms that antiviral therapy has clinical benefits. This requires RCTs to indicate that interferon therapy positively affects SVR as well as clinical outcome.[11-14] As discussed, this was not the case in the latest Cochrane meta-analysis.[6]

Since RCTs on solid clinical endpoints usually require long and costly prospective follow-up, especially in a slowly progressive disease as chronic hepatitis C, it is not surprising that only few have been performed. The trials that have been performed all exclusively included patients with advanced liver disease, probably because these patients are at highest risk for clinical events. Due to the restriction to interferon re-treatment, not all RCTs reporting on clinical outcome events were included in the recent Cochrane review. Unfortunately, however, most of the additional trials

are limited by a low number of included patients and the use of interferon-based regimens with relatively low antiviral efficacy.[10,18-24] Especially among patients with cirrhosis, SVR rates of the early interferon-based antiviral regimens have been poor.[69] Although several trials did report a clinical benefit of interferon-based antiviral therapy, the results varied and not all positive trials were without controversy.[10,18,22,23] Therefore, definite evidence for the clinical efficacy of interferon therapy was never established and SVR was never formally validated. The use of SVR as surrogate outcome measure thus remains with some uncertainty. Nevertheless, another recent Cochrane meta-analysis did indicate that the combination of interferon and ribavirin significantly reduced morbidity plus mortality, as a composite clinical endpoint, compared to interferon monotherapy.[49] This finding is in line with the increase in SVR rate due to the addition of ribavirin to interferon therapy.[70,71]

Presently, new treatment regimens and the introduction of protease inhibitors have substantially increased the antiviral efficacy of interferon-based therapy. Also for patients with cirrhosis, pegylated interferon and ribavirin combination therapy (with the addition of a protease inhibitor for those with HCV genotype 1) is likely to increase SVR rates to above 50%.[2-4,72-76] None of the RCTs on clinical efficacy have assessed a full-dose pegylated interferon and ribavirin treatment course, however, while this has been the standard of care over the last decade. Future interferon-free regimens are even expected to further enhance antiviral efficacy, while simultaneously reducing treatment duration and improving side effect profiles.[77,78] Thus, assuming the biologically plausible causal relation between HCV eradication and improved clinical outcome, RCTs with current antiviral regimens would have higher power to show a clinical benefit of antiviral therapy as well as to validate SVR as surrogate endpoint. However, the accumulated data suggesting patients benefit from SVR impedes justification of trials in which patients are denied a chance to eradicate their chronic HCV infection. Ethical concerns thus prevent us from performing the trials which could bring conclusive evidence regarding the clinical efficacy of antiviral therapy. Such trials should thus not be awaited for the decision to initiate antiviral therapy in the individual patient.

CONCLUSION

To conclude, we are aware that definite proof for the surrogacy of SVR and clinical benefit of interferon-based antiviral therapy is lacking. Nevertheless, SVR has been repeatedly associated with improvements in health-related quality of life, hepatic inflammation and fibrosis, and portal pressure as well as with a reduced occurrence of solid clinical endpoints such as hepatocellular carcinoma, liver failure and death.

Collectively, this strongly argues that SVR is a patient-relevant endpoint and reasonably likely to predict clinical benefit.[13] Furthermore, there is no clear evidence to suggest a long-term harmful effect of 24-48 weeks of interferon-based therapy, by which we usually attempt to achieve this virological outcome measure in our patients. With future triple therapy, a treatment duration of 12 weeks might even be sufficient.[79] The increased mortality rate in a subgroup of patients who received long-term interferon maintenance therapy is not representative for short-term antiviral therapy with the potential to result in SVR. Nevertheless, we do acknowledge that interferon-based therapy is accompanied by substantial side effects, which was also highlighted again by the recent meta-analysis.[6] Thus, careful patient selection remains a necessity at this time, and better tolerated interferon-free treatment regimens with combinations of direct-acting antiviral agents are urgently required. We oppose, however, that the results of the recent Cochrane meta-analysis, or more specifically the HALT-C study, should discourage physicians from treating their patients with chronic HCV infection in general.

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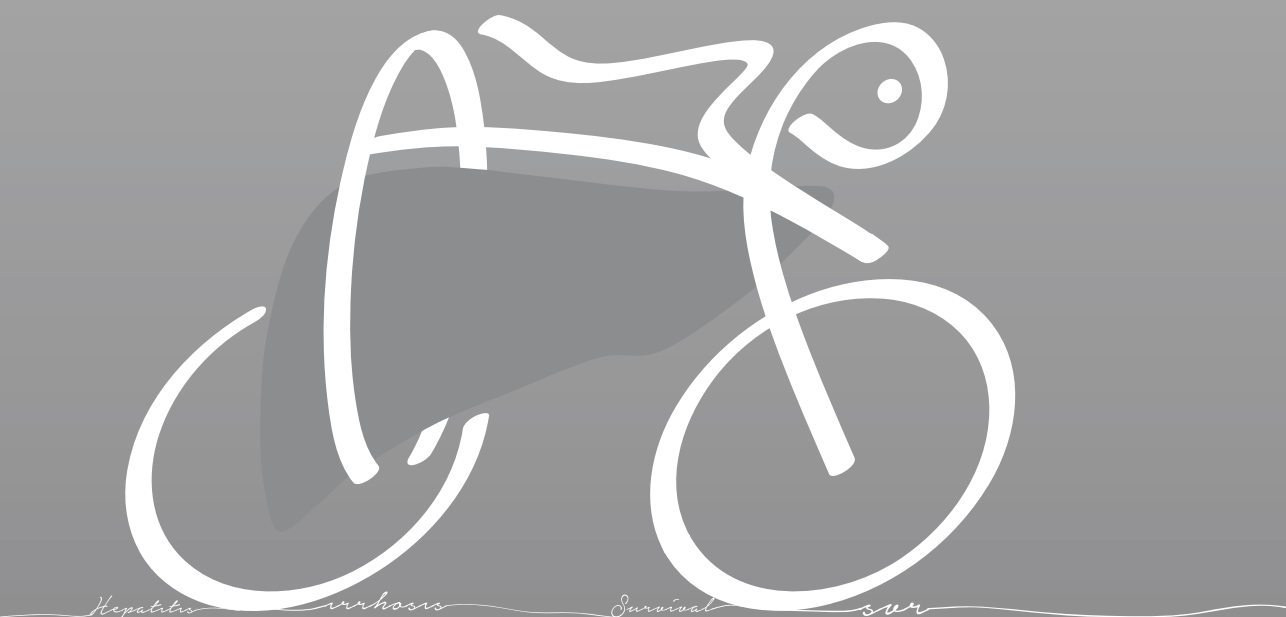
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CHAPTER 6

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Reply to: 'Evidence recommending antiviral therapy in hepatitis C'

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TO THE EDITOR:

We thank Dr. Koretz and colleagues for responding to our appraisal of their Cochrane meta-analysis.[1,2] The discussion on the clinical benefits of antiviral therapy for chronic hepatitis C virus (HCV) infection is important because physicians should be aware of the strengths of current evidence as well as of the remaining uncertainties.

Koretz et al. again highlight and explain that sustained virological response (SVR) is not a validated surrogate marker as substantial proof from randomized placebo-controlled trials that antiviral therapy improves clinical outcome is lacking. As was clearly discussed in our recent review, this is correct. We also mentioned that the repeatedly found association between SVR and reduced cirrhosis-related morbidity and mortality might potentially be subject to residual confounding. Indeed, this possibility cannot be excluded in the performed cohort studies. However, in light of the extensive multivariate analyses in which SVR remained the most important factor associated with beneficial clinical outcome, we agree with others that it is hard to think of a confounder which would completely annihilate this association.[3-5]

While recognizing that the possibility of residual confounding remains a scientific limitation, we have indeed challenged the statement that no kind of antiviral therapy can currently be advocated. One of the key arguments by which Koretz et al. try to substantiate this statement is the increased mortality rate among interferon-treated patients as compared to controls, which was observed in their meta-analysis. However, it should be clearly mentioned that this was only found in the extended follow-up analyses of the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) trial, which almost solely drove their meta-analyses on SVR and mortality.[6] Unfortunately, in their response letter, Koretz et al. do not share their thoughts on the fact that all controls in the HALT-C study received a regular pegylated interferon (PegIFN) and ribavirin treatment course just prior to randomization. Consequently, this study compared long-term PegIFN therapy to short-term PegIFN therapy rather than to no treatment.[7] The design of the HALT-C trial thus prohibits extrapolation of the increased mortality rate as observed with long-term maintenance therapy to the regular PegIFN regimens. Therefore, this study should not have been included in the meta-analyses.

Our review did discuss that patients treated with interferon and ribavirin combination therapy had a beneficial clinical outcome as compared to patients treated with interferon monotherapy. In fact, as the improved clinical outcome is in line with the improved SVR rate of combination therapy, we consider this to be another argument to strengthen our case. However, we acknowledge that the number needed to treat (NNT) to prevent cirrhosis-related events with these earlier interferon-based regimens was high. Awareness of this alternative measure of treatment efficacy is

desired, especially when considering the cost-benefit ratio of new treatment regimens or the allocation of limited treatment resources. Based on our analyses among patients with HCV genotype 1 infection and cirrhosis, we have recently described the enormous decline in the NNT to prevent cirrhosis-related morbidity or mortality with the development of antiviral therapy over the last two decades.[8]

Indeed, there is limited data available to assess the validity of SVR as surrogate endpoint, especially considering that trials assessing long-term low-dose PegIFN should be excluded. However, when validation of SVR is aimed, restriction to interferon monotherapy in treatment experienced patients is not needed. Still, randomized placebo-controlled trials on clinical endpoints are scarce and new trials, which might be able to settle this discussion, are unlikely to be executed. Recently, several phase 3 clinical studies showed SVR rates around 95% with 8-12 weeks of well-tolerated interferon-free regimens. These high response rates were independent of baseline characteristics, thereby excluding the unlikely possibility that we are only able to cure patients with a benign natural course of disease. First clinical data already suggest that viral suppression/eradication with these regimens is linked to an over proportionate treatment-related improvement in clinical outcome.[9,10] We are convinced that, in particular among patients with advanced liver disease, long-term follow-up assessment of these treated patient populations will further confirm the strong link between SVR and reduced mortality.

The data clearly show that treatment increases the rate of SVR. Although it is only now that data are emerging to validate the clinical importance of this longstanding and robust surrogate endpoint, we consider it to be unethical to generally withhold treatment and perform randomized studies, in which many patients are denied a good chance to eradicate their HCV infection, in order to confirm the well-supported and biologically plausible causal relation between SVR and improved clinical outcome.

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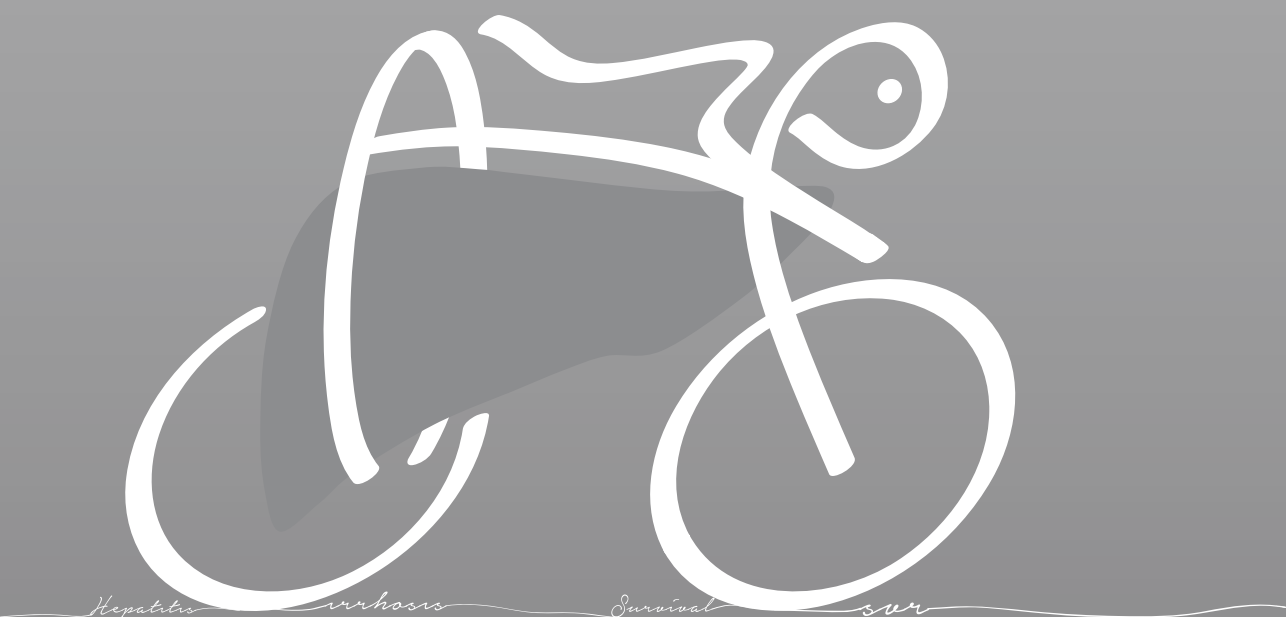
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CHAPTER 7

Reliable prediction of clinical outcome in patients with chronic HCV infection and compensated advanced hepatic fibrosis: a validated model using objective and readily available clinical parameters

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ABSTRACT

Objective

Reliable tools to predict long-term outcome among patients with well compensated advanced liver disease due to chronic HCV infection are lacking.

Design

Risk scores for mortality and for cirrhosis-related complications were constructed with Cox regression analysis in a derivation cohort and evaluated in a validation cohort, both including patients with chronic HCV infection and advanced fibrosis.

Results

In the derivation cohort, 100/405 patients died during a median 8.1 (interquartile range [IQR] 5.7-11.1) years of follow-up. Multivariate Cox analyses showed age (hazard ratio [HR] 1.06, 95% confidence interval [CI] 1.04-1.09, p<.001), male sex (HR 1.91, 95% CI 1.10-3.29, p=.021), platelet count (HR 0.91, 95% CI 0.87-0.95, p<.001) and $\log_{10}$ aspartate aminotransferase/alanine aminotransferase ratio (HR 1.30, 95% CI 1.12-1.51, p=.001) were independently associated with mortality (C statistic 0.78, 95% CI 0.72 to 0.83). In the validation cohort, 58/296 patients with cirrhosis died during a median of 6.6 (IQR 4.4-9.0) years. Among patients with estimated 5-year mortality risks <5%, 5-10% and >10%, the observed 5-year mortality rates in the derivation cohort and validation cohort were 0.9% (95% CI 0.0-2.7) and 2.6% (95% CI 0.0-6.1), 8.1% (95% CI 1.8-14.4) and 8.0% (95% CI 1.3-14.7), 21.8% (95% CI 13.2-30.4) and 20.9% (95% CI 13.6-28.1), respectively (C statistic in validation cohort 0.76, 95% CI 0.69-0.83). The risk score for cirrhosis-related complications also incorporated HCV genotype (C statistic 0.80, 95% CI 0.76-0.83 in the derivation cohort; and 0.74, 95% CI 0.68-0.79 in the validation cohort).

Conclusions

Prognosis of patients with chronic HCV infection and compensated advanced liver disease can be accurately assessed with risk scores including readily available objective clinical parameters.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major cause of liver cirrhosis, liver failure and hepatocellular carcinoma (HCC).[1] It is expected that the incidence of HCV-related cirrhosis and its complications will increase substantially during the upcoming years.[2,3] Among patients with chronic HCV infection and advanced liver disease, antiviral therapy resulting in sustained virological response (SVR) was associated with reduced occurrence of liver failure, HCC and mortality.[4-8] Unfortunately, many patients with advanced fibrosis or cirrhosis fail to attain SVR with current interferon-based treatment regimens, even with the addition of direct-acting antiviral drugs for those infected with HCV genotype 1.[9-13]

Patients with advanced liver disease who do not achieve SVR do not have a good prognosis. A recent meta-analysis indicated these patients have an overall annual risk of 2.9% of developing liver failure, 3.2% of progressing to HCC and 2.7% of dying of liver-related causes.[14] Clinical disease progression and survival, however, may vary considerably among patients with compensated advanced liver disease. Thus, reliable risk scores to assess an individual patient's long-term prognosis would be helpful for counselling and clinical decision making. Due to the rising costs of the new antiviral treatment regimens with high virological efficacy, these scores are relevant to assess the clinical efficacy of therapy. Especially with universally high SVR rates, the number needed to treat to prevent hard clinical endpoints is very much dependent on the baseline risk for events.[15] Costly antiviral therapy with high cure rates will thus have most clinical effect among patients at highest risk of cirrhosis-related events. Furthermore, although intensive monitoring is currently advised in all patients with HCV-induced cirrhosis, this may not be necessary or cost effective for those with lowest risks.[16,17] Validated predictive tools for mortality, as a definite and robust clinical endpoint, or cirrhosis-related complications in general are currently lacking.

We previously identified SVR as the strongest factor independently associated with reduced all-cause mortality and liver-related morbidity in our multicenter cohort of interferon-treated patients with chronic HCV infection and advanced hepatic fibrosis who were followed in the long term.[4] Patients with HCV-induced cirrhosis and SVR also showed reduction in portal pressure and regression of hepatic fibrosis, which were related to improved clinical outcome.[18-21] Together, these data suggest that patients with cirrhosis and SVR should be considered separately from patients with cirrhosis and ongoing HCV infection. Therefore, in this study we focus on patients who did not attain SVR. Among these patients numerous cirrhosis-related events and deaths occurred during follow-up, which enabled identification of prognostic baseline factors. The primary aim of this study was to develop prediction

scores based on objective and readily available clinical variables for mortality and clinical disease progression among patients with HCV-induced advanced hepatic fibrosis who failed to attain SVR. Next, we sought to validate the risk scores in an independent cohort of patients with chronic HCV infection and cirrhosis.

METHODS

Derivation cohort

The derivation cohort included all consecutive patients with chronic HCV infection who failed to attain SVR on their initial interferon-based treatment between 1990 and 2003 following histological proof of advanced hepatic fibrosis or cirrhosis (Ishak fibrosis score 4-6).[22] Patients were treated in five large hepatology units of tertiary care centers in Europe and Canada. Characteristics of this international multicenter cohort and study design have been described in detail previously.[4] Briefly, all included patients had compensated liver disease as patients with decompensated liver disease were not eligible for antiviral therapy. Coinfection with HIV or hepatitis B virus (hepatitis B surface antigen and/or HBV DNA positivity) was an exclusion criterion. Survival and occurrence of liver failure, HCC and liver transplantation was retrospectively assessed by reviewing medical charts. In case the follow-up was not complete, the patient was invited to visit the outpatient clinic or, if this was not feasible, the patient or primary care physician was asked to answer a structured questionnaire over the telephone. Baseline laboratory markers of liver disease severity (platelet count, bilirubin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT)) were registered if available 6 months prior to the start of treatment.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. Informed consent was obtained.

Validation cohort

The validation cohort was derived from a second international multicenter cohort from Europe (EUROHEP cohort), which included all consecutive patients with non-A, non-B chronic hepatitis and compensated biopsy-proven cirrhosis between January 1982 and December 1992 from seven tertiary care centers. The general design of this study has been described previously.[23] The EUROHEP cohort included patients with cirrhosis who received interferon treatment during follow-up and patients who remained untreated. Both were included in the current study as these patients had similar clinical outcome.[23] A second data collection round updated the follow-up to

January 1, 1997, by review of medical charts, consultation of population registries and an invitation for all patients to return to the outpatient clinics for evaluation.[24]

To be included in the validation cohort, patients from the EUROHEP cohort were required to have confirmed anti-HCV antibodies, which were retrospectively tested. Patients were not included in the validation cohort if they tested negative for HCV RNA or in case the parameters incorporated in the primary risk score for mortality were not available. Furthermore, patients enrolled and followed at the Erasmus Medical Center in Rotterdam were not considered to prevent possible overlap with patients in the derivation cohort.

Study endpoints

The primary outcome of this study was all-cause mortality. Clinical disease progression was analyzed as a secondary endpoint, to which liver failure, HCC, liver transplantation or death contributed. In case of multiple events in an individual patient, only the first event was considered for this combined endpoint. Episodes of ascites, bleeding varices, jaundice or overt hepatic encephalopathy were considered as liver failure. The diagnosis of HCC was based on cytohistological confirmation, two coincident imaging techniques (ultrasonography, CT or MRI) showing a focal lesion larger than 2 cm with arterial hypervascularisation or one imaging technique showing a focal lesion larger than 2 cm with arterial hypervascularisation in the presence of an α-fetoprotein level greater than 400 ng/mL.[25]

Statistical analyses

In the derivation cohort, follow-up started 24 weeks after cessation of antiviral treatment, as at this time the distinction is made between sustained virological responders and nonresponders. Since we specifically aimed to assess survival and clinical disease progression among patients without SVR, patients who did attain SVR were not included. In the validation cohort, follow-up started at the time of diagnosis as not all patients received antiviral therapy. In both cohorts patients were censored at the time of SVR due to (re-)treatment, since viral eradication is likely to alter the natural course of advanced hepatic fibrosis.[4-7,18-21] Otherwise, if the clinical endpoint did not occur, patients were censored at the last follow-up visit.

In the derivation cohort, Cox proportional hazard regression analysis was used to assess the association between baseline factors and time to event. To construct objective risk scores, which can be reliably reproduced, only the objective variables age, sex, body mass index (BMI), HCV genotype, antiHBc status, platelet count, albumin, bilirubin and AST/ALT ratio were considered. These factors have been linked with clinical outcome in the past. To have sufficient power, a cut-off of minimally 10 events per variable was used.[26] Linearity of the association of continuous vari-

ables was assessed by including polynomial terms of the variables, which remained included in multivariate analyses in case these were statistically significantly associated with the outcome measure. To create parsimonious final models, which are most easily used and reproduced in clinical practice, variables that were no longer statistically significant in multivariate analyses were removed.[27] Potential confounding was checked. Bootstrapping with 1000 replications was performed as internal validation analyses to check the stability of variables included in the final models. Linear prediction equations, representing the risk scores, were derived from the final Cox model. For visualisation purposes, three risk groups were created based on the outcome of the risk scores in the derivation cohort. The highest quartile of patients represented the high-risk group, the lowest quartile the low-risk group and the remaining 50% of patients the intermediate-risk group. Cumulative incidence rates of mortality or clinical disease progression among these three groups were determined by Kaplan-Meier analyses and tested with the Log-Rank test in the derivation cohort. The absolute 2.5-year, 5-year and 7.5-year risks for mortality or clinical disease progression according to the outcome of the risk scores were determined with the baseline hazard as derived from the final Cox models.

In the derivation and the validation cohort the C statistic was used to assess the predictive accuracy of the risk scores.[28,29] Predicted mortality and clinical disease progression rates were compared with those observed.

To check the stability of the final model, sensitivity analyses using multiple imputation with replacement to impute missing values was performed to construct 10 complete datasets.[30,31] All statistical tests were two sided and a $p<.05$ was considered to be statistically significant. Interaction terms between the variables included in the final models were assessed in the complete case analyses and the multiple imputation analyses. The significance level for interactions was set at $p<.01$ to correct for multiple testing. The proportional hazard assumption was checked by assessing the interaction between the variables and time. SPSS version 17.0.2 (SPSS Inc., Chicago, Illinois, USA) and SAS version 9.2 PROC GENMOD (SAS institute, Cary, North Carolina, USA) were used for all statistical analyses.

RESULTS

Characteristics of the derivation cohort

The derivation cohort consisted of 405 patients out of the 546 patients who started an interferon treatment regimen, as 125 patients attained SVR and 8 patients were lost, 3 were diagnosed with HCC and 5 experienced liver failure before the start of follow-up (Figure 7.1). Baseline characteristics are shown in Table 7.1. Median follow-

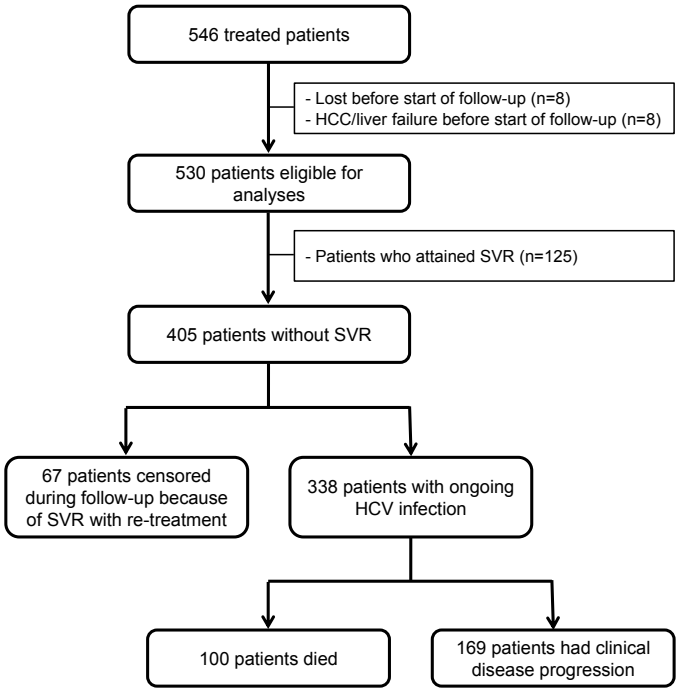

Figure 7.1 Study Flow Chart
Abbreviations: HCC; hepatocellular carcinoma, HCV; hepatitis C virus, SVR; sustained virological response

up duration was 8.1 (interquartile range [IQR] 5.7-11.1) years. Follow-up was complete in 359 (89%) patients. Retreatment during follow-up resulted in SVR for 67 additional patients after a median of 5.8 (IQR 3.1-8.5) years and these patients were censored at the time of achieving SVR.

In total, 100 patients died: 70 of liver-related causes, 15 of non-liver-related causes and for another 15 the cause of death was not known. The overall 5-year survival probability was 91.4% (95% confidence interval [CI] 88.5-94.3) (Figure 7.2). One patient experienced liver failure before initiating retreatment and died during this course of pegylated interferon and ribavirin due to severe infection. Two patients died within 6 months following cessation of a repeated treatment course with pegylated interferon and ribavirin. The first patient, who also had a history of liver failure prior to retreatment, discontinued therapy at week 15 because of a deteriorating liver function and died approximately 2 months later, shortly after being diagnosed with HCC. The other patient received a full 48-week treatment course and died 5 months after therapy following the onset of liver failure.

Table 7.1 Patient Characteristics [a]

Characteristics	Derivation cohort (n = 405)	Validation cohort (n = 296)
Age, years, median (IQR)	48 (42-56)	55 (48-61)
Male	275/405 (68)	162/296 (55)
BMI, kg/m^2, median (IQR) [b]	26.5 (24.0-29.5)	-
Stage of liver disease		
Bridging fibrosis	105/405 (26)	0 (0)
Cirrhosis	300/405 (74)	296 (100)
HCV Genotype		
1	290/384 (76)	169/230 (74)
2	21/384 (5)	49/230 (21)
3	52/384 (14)	10/230 (4)
4	18/384 (5)	1/230 (0)
Other	3/384 (<1)	1/230 (0)
Diabetes Mellitus	55/405 (14)	-
History of alcohol use [c]		
No	-	196/296 (66)
Any	-	100/296 (34)
Severe	88/374 (24)	-
AntiHBc positivity	153/325 (47)	96/275 (35)
Laboratory markers of liver disease severity, median (IQR) [d]		
Platelet count, x10^9/L	145 (109-195)	132 (99-173)
Albumin, g/L	42 (39-44)	42 (38-45)
Bilirubin, μmol/L	14 (10-19)	15 (12-20)
AST/ALT ratio	0.71 (0.58-0.91)	0.72 (0.56-0.92)

Abbreviations: ALT; alanine aminotransferase, antiHBc; anti-hepatitis B core antigen, AST; aspartate aminotransferase, BMI; body mass index (calculated as weight in kilograms divided by height in meters squared), HCV; hepatitis C virus, IQR; interquartile range.

[a] Data are presented as No./Total No. (%) unless otherwise noted.

[b] Baseline BMI was missing in 113 (28%) patients in the derivation cohort, and was not available in the validation cohort.

[c] In the derivation cohort patients were classified as having a history of severe alcohol use (the use of more than 50 grams of alcohol a day) or not. In the validation patients were classified as having a history of using any alcohol or not.

[d] In the derivation and validation cohorts, baseline platelet count was missing in 68 (17%) patients and 0 (0%) patients, albumin in 100 (25%) and 30 (10%), bilirubin in 81 (20%) and 13 (4%), and AST/ALT ratio in 90 (22%) and 0 (0%), respectively.

Clinical disease progression was experienced by 169 (42%) patients. The overall 5-year event-free survival was 77.6% (96% CI 73.5-81.7) (Figure 7.2). The first cirrhosis-related complication was liver failure in 87 (51%) patients, HCC in 60 (36%) and death in 19 (11%). Three (2%) patients underwent liver transplantation for progressive liver disease, although no clear event of liver failure or HCC could be registered.

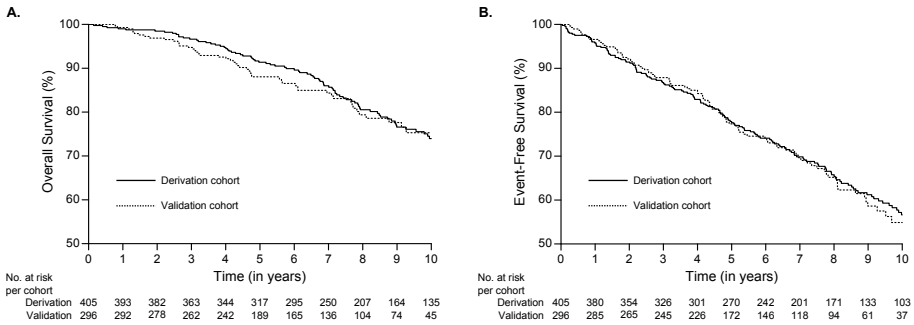

Figure 7.2 Overall and Event-Free Survival
Overall survival (A) and event-free survival (B) probability among patients with advanced liver disease and chronic hepatitis C virus infection in the derivation cohort (continuous line) and validation cohort (dashed line).

7

Construction of the mortality risk score

In the derivation cohort, higher age, lower platelet count, higher total bilirubin level, lower albumin level and higher AST/ALT ratio were significantly associated with mortality in univariate Cox regression analyses (Table 7.2). Multivariate analyses showed that bilirubin and albumin were not independently associated with mortality (Table 7.2, model A). Since HCV genotype was no longer significantly associated with mortality after bilirubin and albumin were excluded (HR 1.842, 95% CI 0.922-3.682, p=.084), it was removed to create the final model (Table 7.2, model B). Also after bootstrapping and multiple imputation analyses all variables included in the final model remained statistically significantly associated with mortality. The final analysis included 305 (75%) representative patients of the derivation cohort, for whom platelet count and AST/ALT ratio were available at baseline. The 305 patients included in the analyses did not differ significantly from the 100 patients who were not included in the analyses with respect to other baseline characteristics of liver disease severity, age, and survival (Log Rank test p=.957) (Figure 7.3 and Table 7.3).

The linear prediction equation for mortality as derived from the final Cox model was represented by: Rm = (6 x age in years) - (platelet count per 10^9/L) + (258.8 x $\log_{10}$(AST/ALT)) + (64.5 for male patients).

Table 7.2 Cox Proportional Hazard Regression Analyses for Mortality in the Derivation Cohort

Characteristics	Mortality											
	Univariate analyses			Multivariate analyses						Imputation analyses		
				Model A (n = 265 / 60 deaths)			Model B (final) [a] (n = 305 / 75 deaths)			Model B (final) (n = 405 / 100 deaths)		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p	HR	95% CI	p-value [b]
Age, per year	1.080	1.056-1.104	<.001	1.072	1.039-1.106	<.001	1.062	1.035-1.090	<.001	1.071	1.046-1.096	<.001
Male	0.988	0.644-1.516	.955	1.786	0.950-3.357	.072	1.907	1.104-3.292	.021	1.650	1.020-2.670	.041
BMI, per kg/m^2	0.991	0.937-1.047	.737	-	-	-	-	-	-	-	-	-
AntiHBc positive	1.090	0.703-1.688	.701	-	-	-	-	-	-	-	-	-
Platelet count, per 10*10^9/L	0.895	0.857-0.934	<.001	0.896	0.841-0.954	.001	0.907	0.865-0.952	<.001	0.936	0.896-0.978	.004
Albumin, per g/L	0.894	0.853-0.938	<.001	1.016	0.952-1.084	.638	-	-	-	-	-	-
Bilirubin, per μmol/L	1.029	1.006-1.052	.013	0.993	0.960-1.028	.692	-	-	-	-	-	-
Log_{10} AST/ALT, per 0.1	1.351	1.179-1.547	<.001	1.318	1.068-1.627	.010	1.295	1.112-1.509	.001	1.288	1.105-1.502	.001
HCV genotype 3	1.194	0.663-2.148	.555	2.089	1.019-4.280	.044	-	-	-	-	-	-

Abbreviations: ALT; alanine aminotransferase, antiHBc; anti-hepatitis B core antigen, AST; aspartate aminotransferase, BMI; body mass index, CI; confidence interval, HCV; hepatitis C virus, HR; hazard ratio.

[a] The mortality risk score based on the regression coefficients of the variables in model B was represented by Rm = (6 x age in years) – (platelet count per 10^9/L) + (258.8 x log_{10}(AST/ALT)) + (64.5 for males).

[b] The p-value is based on the maximum likelihood ratio test.

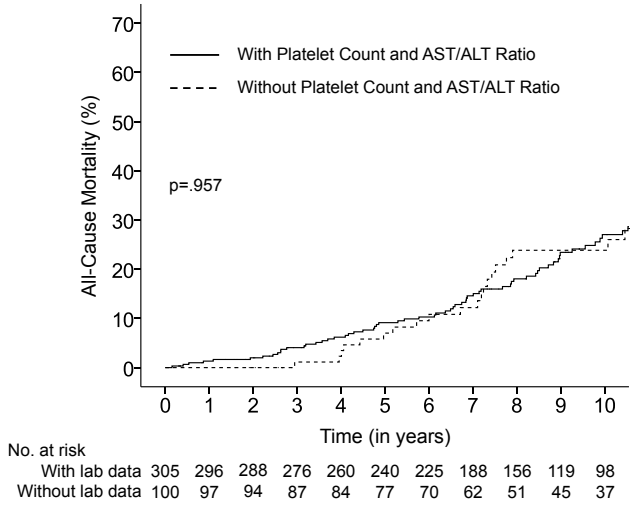

Figure 7.3 All-Cause Mortality in the Derivation Cohort According to the Availability of Laboratory Data

The all-cause mortality curves were constructed using the Kaplan Meier method. Twenty-four weeks after cessation of antiviral therapy was considered as time zero. Statistical significance between the survival curves in the group with and without available data on the platelet count and the ratio between the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was assessed with the Log Rank test.

The predictive accuracy of this risk score for mortality, as measured by the C statistic, was 0.78 (95% CI 0.72-0.83). To visualize cumulative mortality rates, patients were categorized into three risk groups according to the IQRs of Rm. After 5 years of follow-up the cumulative mortality rate was 0% in the low-risk group (Rm < 87.1), 7.2% (95% CI 2.9-11.5) in the intermediate-risk group (87.1 ≤ Rm ≤ 221.2) and 22.2% (95% CI 12.6-31.8) in the high-risk group (Rm > 221.2) (p<.001) (Figure 7.4).

To assess a patient's individual likelihood for mortality, Figure 7.4B illustrates the estimated 2.5-year, 5-year and 7.5-year mortality risk as a function of the mortality risk score. The baseline survival, as derived from the final Cox model, was 0.9965, 0.9870 or 0.9752 at 2.5 years, 5 years or 7.5 years, respectively (under the condition that Rm=0). Among the patients with an estimated 5-year mortality risk < 5% (n = 130 (43%), mortality risk score < 137), the observed 5-year cumulative mortality rate was 0.9% (95% CI 0.0-2.7) (Table 7.4). The observed cumulative 2.5-year and 7.5-year mortality rates were also in line with the predicted estimates (Table 7.4).

Validation of the mortality risk score

Of the 319 patients who were eligible to be included in the validation cohort, 23 were not considered because the baseline platelet count and/or AST/ALT ratio were missing. Table 1 summarizes the characteristics of the remaining 296 patients with

Table 7.3 Baseline Characteristics According to the Availability of Platelet Count and AST/ALT Ratio [a]

Characteristics	Overall (n = 405)	Platelet count and AST/ALT ratio available (n = 305)	Platelet count and AST/ALT ratio not available (n = 100)	p-value
Age, years, median (IQR)	48 (42-56)	47 (42-55)	49 (42-57)	.572
Male,	275 (68)	215 (70)	60 (60)	.051
BMI, kg/m^2, median (IQR) [b]	26.5 (24.0-29.5)	26.4 (23.8-29.5)	26.8 (24.8-29.4)	.329
Fibrosis score				.104
Ishak 4	105 (26)	71 (23)	34 (34)	
Ishak 5	75 (19)	58 (19)	17 (17)	
Ishak 6	225 (56)	176 (58)	49 (49)	
HCV Genotype [b]				.569
1	290 (76)	218 (75)	72 (77)	
2	21 (5)	16 (6)	5 (5)	
3	52 (14)	40 (14)	12 (13)	
4	18 (5)	14 (5)	4 (4)	
Other	3 (<1)	2 (1)	1 (1)	
Treatment naïve	365 (90)	271 (89)	94 (94)	.134
Type of treatment				.005
Interferon monotherapy	166 (41)	114 (37)	52 (52)	
Interferon and ribavirin	113 (28)	83 (27)	30 (30)	
Pegylated interferon monotherapy	10 (2)	6 (2)	4 (4)	
Pegylated interferon and ribavirin	101 (25)	87 (29)	14 (14)	
Consensus interferon (+/- ribavirin)	15 (4)	15 (5)	0 (0)	
Year treatment started	1998 ('95-'01)	1999 ('95-'01)	1998 ('95-'00)	.061
Diabetes Mellitus	55 (14)	46 (15)	9 (9)	.123

Table 7.3 Baseline Characteristics According to the Availability of Platelet Count and AST/ALT Ratio[a] (continued)

Characteristics	Overall (n = 405)	Platelet count and AST/ALT ratio available (n = 305)	Platelet count and AST/ALT ratio not available (n = 100)	p-value
History of severe alcohol use	88 (24)	70 (24)	18 (20)	.437
AntiHBc positivity	153 (47)	112 (46)	41 (49)	.712
Laboratory markers of liver disease severity, median (IQR) [b]				
platelet count, $x10^9$/L	145 (109-195)	144 (106-196)	157 (118-193)	.373
albumin, g/L	42 (39-44)	42 (39-44)	41 (38-44)	.316
bilirubin, μmol/L	14 (10-19)	14 (10-19)	13 (11-18)	.778
AST/ALT ratio	0.71 (0.58-0.91)	0.71 (0.58-0.92)	0.65 (0.55-0.75)	.238

Abbreviations: ALT; alanine aminotransferase, antiHBc; anti-hepatitis B core antigen, AST; aspartate aminotransferase, BMI; body mass index (calculated as weight in kilograms divided by height in meters squared), HCV; hepatitis C virus,

[a] Data are presented as No./Total No. (%) unless otherwise noted.

[b] In the derivation and validation cohorts, baseline platelet count was missing in 68 (17%) patients and 0 (0%) patients, albumin in 100 (25%) and 30 (10%), bilirubin in 81 (20%) and 13 (4%), and AST/ALT ratio in 90 (22%) and 0 (0%), respectively.

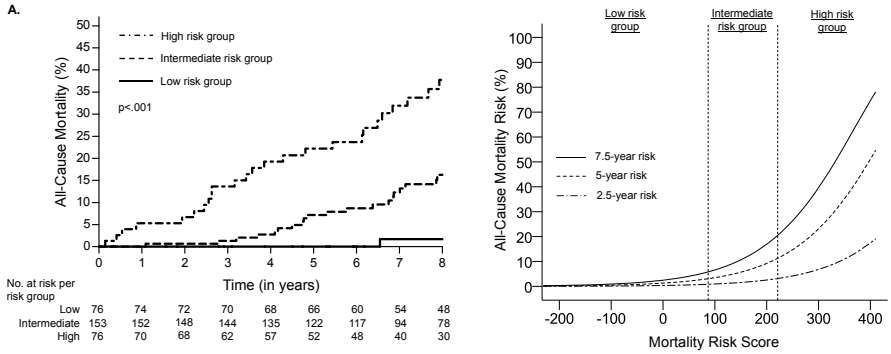

Figure 7.4 Mortality Assessment

The mortality risk score is represented by Rm=(6 x age in years) – (platelet count per 10^9/L) + (258.8 x $\log_{10}$(AST/ALT)) + (64.5 for male patients). Panel A shows cumulative mortality rates according to three risk groups based on the interquartile ranges of Rm in the derivation cohort. The low-risk group included 76 (25%) patients with Rm<87.1, the intermediate-risk group included 153 (50%) patients with Rm≥87.1 and ≤221.2, and the high-risk group included 76 (25%) patients with Rm>221.2. Mortality rates were determined by Kaplan Meier analysis and compared by the Log Rank test. Panel B shows the absolute risk for mortality at 2.5, 5 or 7.5 years as a function of the mortality risk score, which was estimated with the baseline survival derived from the final Cox model in the derivation cohort (absolute mortality risk=100 x (1 - (baseline survival$_{(t)}$^{x})); with x=exp$^{(Rm/100)}$).

cirrhosis. During a median follow-up of 6.6 (IQR 4.4-9.0) years, 166 (56%) patients underwent at least one interferon-based treatment course. Eight (5%) patients attained SVR. In total, 58 patients died and the overall 5-year survival probability was 88.1% (95% CI 84.2-92.0) (Figure 7.2). In the validation cohort, the C statistic of the mortality risk score was 0.76 (95% CI 0.69-0.83). Table 7.4 shows the predicted against the observed mortality rates, which were comparable to those in the validation cohort.

As a sensitivity analysis, 63 (21%) patients with non-A, non-B chronic hepatitis were excluded in whom HCV RNA was not assessed. In this subgroup the C statistic of the mortality risk score remained similar (0.75, 95% CI 0.65-0.84).

Construction of a risk score for clinical disease progression

With a similar approach a prediction score for clinical disease progression was constructed in the derivation cohort. Table 7.5 shows the results of univariate and multivariate Cox regression analyses. In contrast to mortality, the association between clinical disease progression and platelet count was nonlinear. Also, HCV genotype 3 versus non 3 remained an independent predictor of cirrhosis-related events and was thus included in the final model (Table 7.5, model B). All variables included in this model remained statistically significantly associated with clinical disease progression after bootstrapping and multiple imputation analyses.

Table 7.4 Predicted Against Observed Mortality

		2.5-year mortality rate			
		Derivation cohort (n = 305)		Validation cohort (n = 296)	
Predicted 2.5 year rate	**Risk score range**	No. of patients (%)	Observed cumulative mortality rate (95% CI)	No. of patients (%)	Observed cumulative mortality rate (95% CI)
<2.5%	< 197	202 (66)	0.0	161 (54)	0.6 (0.0-1.8)
2.5-5%	≥ 197 – 269	67 (22)	6.1 (0.2-12.0)	67 (23)	1.5 (0.0-4.4)
>5%	≥ 269	36 (12)	11.4 (1.0-21.8)	68 (23)	13.3 (5.3-21.3)
		5-year mortality rate			
		Derivation cohort (n = 305)		**Validation cohort (n = 296)**	
Predicted 5-year rate	**Risk score range**	No. of patients (%)	Observed cumulative mortality rate (95% CI)	No. of patients (%)	Observed cumulative mortality rate (95% CI)
<5%	< 137	130 (43)	0.9 (0.0-2.7)	94 (32)	2.6 (0.0-6.1)
5-10%	≥ 137 – 209	83 (27)	8.1 (1.8-14.4)	73 (25)	8.0 (1.3-14.7)
>10%	≥ 209	92 (30)	21.8 (13.2-30.4)	129 (43)	20.9 (13.6-28.2)
		7.5-year mortality rate			
		Derivation cohort (n = 305)		**Validation cohort (n = 296)**	
Predicted 7.5-year rate	**Risk score range**	No. of patients (%)	Observed cumulative mortality rate (95% CI)	No. of patients (%)	Observed cumulative mortality rate (95% CI)
<7.5%	< 113	105 (34)	3.5 (0.0-7.4)	72 (24)	3.6 (0.0-8.7)
7.5-15%	≥ 113 – 187	84 (28)	10.4 (3.0-17.8)	83 (28)	10.7 (3.1-18.3)
>15%	≥ 187	116 (38)	31.3 (21.1-40.5)	141 (48)	27.4 (19.0-35.8)

Abbreviation: CI; confidence interval.

Table 7.5 Cox Proportional Hazard Regression Analyses for Clinical Disease progression in the Derivation Cohort

Characteristics	Clinical Disease Progression											
	Univariate analyses			Multivariate analyses						Imputation analyses		
				Model A (n = 265 / 102 events)			Model B (final) [a] (n = 290 / 116 events)			Model B (final) (n = 405 / 169 events)		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value [b]
Age, per year	1.050	1.033-1.068	<.001	1.049	1.024-1.075	<.001	1.053	1.031-1.077	<.001	1.043	1.023-1.064	<.001
Male	1.094	0.782-1.530	.600	2.568	1.537-4.292	<.001	2.310	1.422-3.752	.001	1.830	1.218-2.750	.001
BMI, per kg/m^2	0.987	0.945-1.030	.540	-	-	-	-	-	-	-	-	-
AntiHBc positive	0.984	0.708-1.366	.921	-	-	-	-	-	-	-	-	-
Platelet count, per 10*10^9/L	0.871	0.840-0.903	<.001	0.760	0.665-0.870	<.001	0.754	0.666-0.853	<.001	0.817	0.712-0.937	<.001
Platelet count squared	-	-	-	1.005	1.001-1.009	.008	1.005	1.002-1.009	.005	1.003	0.999-1.007	.040
Albumin, per g/L	0.887	0.854-0.921	<.001	0.989	0.940-1.040	.662	-	-	-	-	-	-
Bilirubin, per μmol/L	1.041	1.024-1.059	<.001	0.995	0.971-1.019	.669	-	-	-	-	-	-
Log_{10} AST/ALT, per 0.1	1.477	1.329-1.642	<.001	1.469	1.262-1.710	<.001	1.431	1.259-1.626	<.001	1.393	1.235-1.571	<.001
HCV genotype 3	1.036	0.647-1.659	.883	1.850	0.994-3.442	.052	1.833	1.043-3.221	.035	1.612	0.945-2.749	.039

Abbreviations: ALT; alanine aminotransferase, antiHBc; anti-hepatitis B core antigen, AST; aspartate aminotransferase, BMI; body mass index, CI; confidence interval, HCV; hepatitis C virus, HR; hazard ratio.

[a] The clinical disease progression risk score based on the regression coefficients of the variables in model B was represented by Rc = (5.2 x age in years) – (2.8 x platelet count per 10^9/L) + (0.00517 x (platelet count per 10^9/L)^2) + (358.2 x $\log_{10}$(AST/ALT)) + (83.7 for males) + (60.6 in case of HCV genotype 3).

[b] The p-value is based on the maximum likelihood ratio test.

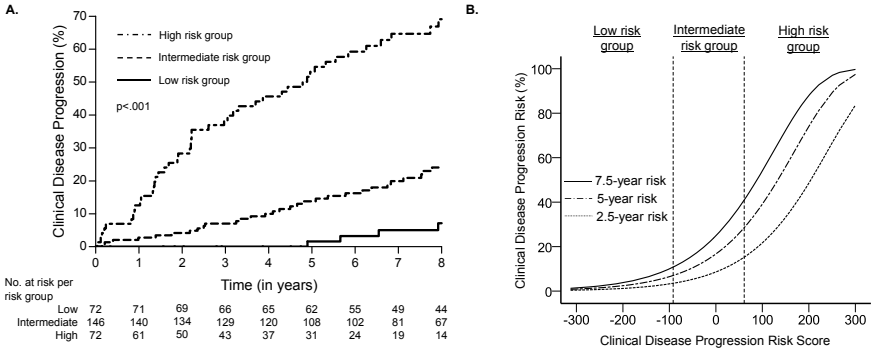

Figure 7.5 Assessment of Clinical Disease Progression

The risk score for clinical disease progression is represented by Rc=(5.2 x age in years) - (2.8 x platelet count per 10^9/L) + (0.00517 x (platelet count per 10^9/L)^2) + (358.2 x $\log_{10}$(AST/ALT)) + (83.7 for male patients) + (60.6 in case of HCV genotype 3). Clinical disease progression was defined as the occurrence of liver failure, hepatocellular carcinoma, liver transplantation or death. Panel A shows cumulative clinical disease progression rates according to three risk groups based on the interquartile ranges of Rc in the derivation cohort. The low-risk group included 72 (25%) patients with Rc<-91.9, the intermediate-risk group included 146 (50%) patients with Rc≥-91.9 and ≤61.0, and the high-risk group included 72 (25%) patients with Rc>61.0. Clinical disease progression rates were determined by Kaplan Meier analysis and compared by the Log Rank test. Panel B shows the absolute risk for clinical disease progression at 2.5, 5 or 7.5 years as a function of the clinical disease progression risk score, which was estimated with the baseline event-free survival derived from the final Cox model in the derivation cohort (absolute risk of clinical disease progression = 100 x (1 - (baseline event-free survival$_{(t)}^{x}$)); with x=exp$^{(Rc/100)}$).

The risk score for clinical disease progression is represented by: Rc = (5.2 x age in years) - (2.8 x platelet count per 10^9/L) + (0.00517 x (platelet count per 10^9/L)^2) + (358.2 x $\log_{10}$(AST/ALT)) + (83.7 for male patients) + (60.6 in case of HCV genotype 3). The C statistic of this model was 0.80 (95% CI 0.76-0.83). According to the interquartile ranges of Rc, the 5-year cumulative event rate was 1.6% (95% CI 0.0-4.7) in the low-risk group (Rc < -91.9), 13.8% (95% CI 8.1-19.5) in the intermediate-risk group (-91.9 ≤ Rc ≤ 61.0) and 53.1% (95% CI 41.3-64.9) in the high-risk group (Rc > 61.0) (p<.001) (Figure 7.5).

The individual 2.5-year, 5-year and 7.5-year risk for clinical disease progression according to the clinical disease progression risk score is illustrated in Figure 7.5B. The baseline event-free survival, as derived from the final Cox model, was 0.9139, 0.8330 or 0.7509 at 2.5-years, 5-years or 7.5-years, respectively (under the condition that Rc=0). Among the 54 (19%) patients with an estimated annual event rate below 1% during the first 5 years of follow-up (Rc < -127), no cirrhosis-related events were observed (Table 7.6). In these patients the observed cumulative rate with clinical disease progression was 2.5% (95% CI 0.0-7.4) after 7.5 years (Table 7.6).

The mortality risk score correlated strongly with the clinical disease progression risk score (Pearson's R 0.93, p<.001). However, the accuracy of the mortality risk

Table 7.6 Predicted Against Observed Clinical Disease Progression Rate

		2.5-year clinical disease progression rate			
		Derivation cohort (n = 290)		Validation cohort (n = 230)	
Predicted 2.5 year rate	**Risk score range**	No. of patients (%)	Observed cumulative event rate (95% CI)	No. of patients (%)	Observed cumulative event rate (95% CI)
<2.5%	< -127	54 (19)	0.0	36 (16)	0.0
2.5-5%	≥ -127 - <-56	57 (20)	1.9 (0.0-5.4)	48 (21)	4.2 (0.0-10.9)
>5%	≥ -56	179 (62)	18.9 (13.0-24.8)	146 (63)	9.8 (4.9-14.7)
		5-year clinical disease progression rate			
		Derivation cohort (n = 290)		Validation cohort (n = 230)	
Predicted 5-year rate	**Risk score range**	No. of patients (%)	Observed cumulative event rate (95% CI)	No. of patients (%)	Observed cumulative event rate (95% CI)
<5%	< -127	54 (19)	0.0	36 (16)	0.0
5-10%	≥ -127 - <-55	59 (20)	7.3 (0.4-14.2)	49 (21)	9.0 (0.6-17.4)
>10%	≥ -55	177 (61)	31.4 (24.3-38.4)	145 (63)	27.4 (19.8-35.0)
		7.5-year clinical disease progression rate			
		Derivation cohort (n = 290)		Validation cohort (n = 230)	
Predicted 7.5-year rate	**Risk score range**	No. of patients (%)	Observed cumulative event rate (95% CI)	No. of patients (%)	Observed cumulative event rate (95% CI)
<7.5%	< -130	53 (18)	2.5 (0.0-7.4)	32 (14)	4.5 (0.0-13.1)
7.5-15%	≥ -130 - <-57	58 (20)	9.5 (1.7-17.3)	51 (22)	14.1 (3.5-24.7)
>15%	≥ -57	179 (62)	41.7 (34.1-49.3)	147 (64)	36.9 (28.3-45.5)

Abbreviation: CI; confidence interval.

score for the prediction of clinical disease progression was lower (C statistic 0.78, 95% CI 0.74-0.82).

Prediction of clinical disease progression in the validation cohort

In the validation cohort, 103 patients showed clinical progression of their liver disease, with an overall 5-year event-free survival probability of 77.3% (95% CI 72.2-82.4) (Figure 7.2). The C statistic of the risk score for clinical disease progression was 0.74 (95% CI 0.68-0.79) in the validation cohort. The observed cumulative incidences of events corresponded very well to those predicted (Table 7.6).

DISCUSSION

In our multicenter follow-up study among patients with chronic HCV infection and advanced hepatic fibrosis or cirrhosis we found that, during a median follow-up of 8.1 years, 25% of the patients who did not attain SVR died and 42% experienced clinical disease progression. Readily available and objective variables were used to develop a reliable risk score for mortality, including the readily available clinical parameters age, sex, platelet count and AST/ALT ratio. This score accurately predicted the long-term mortality risk among patients with chronic HCV infection and advanced hepatic fibrosis who did not attain SVR. Importantly, the predictive accuracy was validated in a large and independent international cohort of patients with chronic HCV infection and cirrhosis.[23] Prediction of clinical disease progression was optimized with a comparable but separate risk score, for which the weighing of the variables was slightly adjusted. As expected, the risk score for clinical disease progression included similar variables as liver-related morbidity is closely related to mortality. Inclusion of HCV genotype was anticipated as well, as HCV genotype 3 has previously been associated with fibrosis progression and HCC.[32,33] Although the differences between the scores are modest, separate scores led to the highest predictive accuracies for the respective outcomes.

Our risk scores represent the first validated predictive tools to specifically assess long-term prognosis among patients with advanced but compensated HCV-induced liver disease. They will be useful for counselling patients regarding their prognosis and possibly to determine the benefit that might be expected from a repeated attempt to eradicate chronic HCV infection. Furthermore, the scores might be useful for the allocation of costly and extensive semi-annual surveillance with ultrasound for cirrhosis-related complications. Current guidelines state that such surveillance is only cost effective for patients with chronic HCV infection if the annual HCC inci-

dence exceeds 1.5%.[16] Careful allocation is relevant, especially as the population of patients with HCV-induced cirrhosis is rapidly growing.[2,3]

As it was specifically designed to assess long-term mortality in patients with compensated cirrhosis, the proposed mortality risk score represents a valuable prognostic tool in addition to the Child-Turcotte-Pugh (CTP) score and the Model for End-stage Liver Disease (MELD) which are mainly used for prediction of short-term mortality in patients with advanced or decompensated cirrhosis. Both these scores were originally developed to assess the mortality risk of invasive shunting procedures among patients with cirrhosis with severe portal hypertension.[34,35] Although the CTP score has shown predictive value for non-operative mortality in patients with cirrhosis, it was never actually validated to be used for this purpose.[36-39] In addition, the CTP score contains subjective components and lacks statistical weighting of the included variables. The more sophisticated MELD is free of these limitations and has replaced CTP for the allocation of donor liver grafts after it was validated to predict mortality in patients with end-stage liver disease.[34,40,41] However, this mainly concerns prediction of short-term mortality due to the high mortality rates in these validation studies as 2-21% of the patients in the study by Kamath et al and 12% of the patients in the study by Wiesner et al died within 3 months.[40,41] In comparison, among our derivation and validation cohort only 1% of the patients had died within 1 year of follow-up. The performance of MELD for long-term clinical outcome could not be reliably assessed in our cohort as baseline creatinine and INR were available for only a minority of the patients (23%). However, others indicated the predictive accuracy of MELD was limited among patients with compensated cirrhosis and for the assessment of mortality beyond 3 years.[42,43] In contrast to the CTP and MELD score, our risk scores do not include bilirubin or albumin as these markers were not independent predictors of long-term clinical outcome. Deteriorating liver function leading to death in patients with end-stage liver disease is often accompanied by elevated bilirubin and lowered albumin levels; however in patients with compensated advanced liver disease, who were included in this study, the liver function can remain stable over long periods of time. Our scores are thus meant to be used for the guidance of patients with compensated HCV-induced cirrhosis and preserved liver function. For patients with decompensated cirrhosis the MELD and CTP score remain the most useful tools for the assessment of mortality.

In a recent post-hoc analysis of the HALT-C trial, another objective prediction score was suggested to assess the likelihood of disease progression in patients with chronic HCV infection and advanced hepatic fibrosis who did not attain SVR.[44] While the prospective nature of this study is important, the risk score was never validated. Several other differences compared with our study are relevant to discuss as well. First, the HALT-C score was not solely based on clinical events. In fact, increases in

CTP score ≥7 contributed to 66% of the composite endpoints studied, while only 48% of these patients actually progressed to having liver failure with a delay of up to 3.1 years. As we are lacking data regarding increases in CTP score, it would not be valid to assess the performance of the HALT-C model in our cohort. Second, although early detection of HCC is one of the key reasons for surveillance, this important complication was excluded from the combined endpoint in HALT-C analyses. The endpoints assessed in our study might thus be considered to be clinically more relevant. Third, age was no independent predictor of disease progression in the HALT-C study, possibly because this trial included a selected population with narrow age range.[45] However, higher age has been repeatedly associated with worse clinical outcome.[5-7,23,46,47] In contrast, our derivation cohort included all consecutively treated patients, including many patients treated outside of clinical trials. Consequently, our risk score was based on patients who are representative of the general Western population with HCV-induced advanced liver disease.

Importantly, the risk scores performed very well in the validation cohort, despite several differences between the derivation and validation cohort, which indicates that our risk scores are consistent and robust. First, patients included in the validation cohort were older compared with the patients in the derivation cohort, and all patients in the validation cohort had cirrhosis, whereas the derivation cohort also included patients with advanced fibrosis. Second, in contrast to the derivation cohort, patients in the validation cohort were required to have abnormal ALT or AST levels. However, the proportion of patients with HCV-induced cirrhosis and normal aminotransferase levels is likely to be small.[48] Third, almost half of the patients in the validation cohort were not treated with interferon-based antiviral therapy, while interferon-based treatment was an inclusion criterion in the derivation cohort. A proportion of the untreated patients in the validation cohort are thus likely to have attained SVR with antiviral therapy, and these patients might have a beneficial prognosis. However, as only 5% of the treated patients with cirrhosis in the validation cohort cleared the virus, this is not likely to have influenced the current results. As the efficacy of the earlier interferon-based therapies has been limited among patients with advanced hepatic fibrosis, those patients without SVR in current long-term follow-up studies can be considered to represent the natural history.[23,49] Therefore, our risk scores also have the potential to perform well among untreated patients. Caution is currently required with respect to patients with specific comorbidities which contraindicate interferon therapy and also influence prognosis. Of course, further validation by independent groups and impact and implementation analyses will need to be conducted.[50] Assessing the predictive accuracy of our risk scores in patients with advanced hepatic fibrosis due to causes other than chronic HCV infection would be relevant as well. However, differences in the natural history prior

to the stage of decompensated cirrhosis might limit generalization of our risk scores to all chronic liver diseases.

There are certain limitations regarding the present study. Both cohorts included patients from tertiary referral centers, in which patients might have more advanced disease compared with the population with HCV-induced cirrhosis as seen in secondary care hospitals. However, patients with the most advanced liver disease were generally not treated with interferon-based therapy and were therefore not included in our study. The studied cohorts here are thus likely to be representative of the general population with chronic HCV infection and cirrhosis. As discussed above, for those patients with impaired liver function, the MELD and CTP score are probably more relevant. As we aimed to construct objective scores, which can be reliably assessed in daily practice, we did not consider several subjective factors which were previously found to be associated with clinical outcome.[4] Diabetes mellitus was not included, as this was not prospectively assessed and fasting glucose, haemoglobin A1c and/or insulin levels were not available for the majority of patients in our study. However, the need for patients to come in fasting would decrease the practicality of the risk score. Limited data concerning the effect of antidiabetic therapy on clinical outcome also supports the exclusion of insulin resistance as an objective risk factor at this time. Nevertheless, the presence of diabetes mellitus was significantly associated with mortality (HR 2.59, 95% CI 1.44-4.64, p=.001) and showed a trend for an association with clinical disease progression (HR 1.67, 95% CI 1.00-2.78, p=.051) when added to our final Cox models. These results could not be confirmed in the validation cohort due to a lack of data on insulin resistance. Self-reported alcohol use and histological degree of hepatic fibrosis, which requires invasive liver biopsy, were not considered as these variables are not objective. However, active alcohol use influences the AST/ALT ratio, and might thus be indirectly accounted for in our risk scores. It can, nevertheless, be anticipated that the clinical outcome is worse than estimated by our risk scores among patients who continue to abuse alcohol. As expected, in our study a history of alcohol abuse as indicated by the treating physician was also independently associated with mortality (HR 1.8, 95% CI 1.06-3.21, p<.032) and clinical disease progression (HR 2.1, 95% CI 1.34-3.33, p<.001). Of course, all patients in our derivation cohort were treated with interferon-based therapy, which is not generally initiated among those with active alcohol abuse. In such patients abstinence should be advocated to improve their prognosis, also because this might enable anti-HCV therapy.[51,52] As the AST and ALT levels fluctuate, regression dilution bias might have had an impact.[53] However, as we included the ratio between these two strongly correlating markers, this might be of lesser concern for the risk scores presented here. Missing data in the derivation cohort was another expected limitation as our study has a retrospective nature and included patients

from 1990 onwards. Also, blood works from before 6 months prior to the start of antiviral therapy were not considered. Importantly, the platelet count and AST/ALT ratio were missing at random (Figure 7.3 and Table 7.3), and we have performed imputation analyses. As for HCV genotype, the association between gender and clinical outcome was confounded in our cohort. Female patients were older and had a higher AST/ALT ratio compared with male patients. As expected, multivariate analyses confirmed the association between clinical outcome and HCV genotype or gender. Interestingly, liver stiffness was recently associated with clinical outcome in patients with cirrhosis and is objectively and non-invasively measured by transient elastography.[54] Future studies need to evaluate whether the predictive accuracy of our risk score could improve when extended with liver stiffness.

In conclusion, we have developed and validated risk scores for long-term mortality and clinical disease progression in patients with chronic HCV infection and advanced liver disease who failed to attain SVR. The risk scores are based on objective and readily available laboratory markers and patient characteristics, so that they can be easily and reliably reproduced in daily practice.

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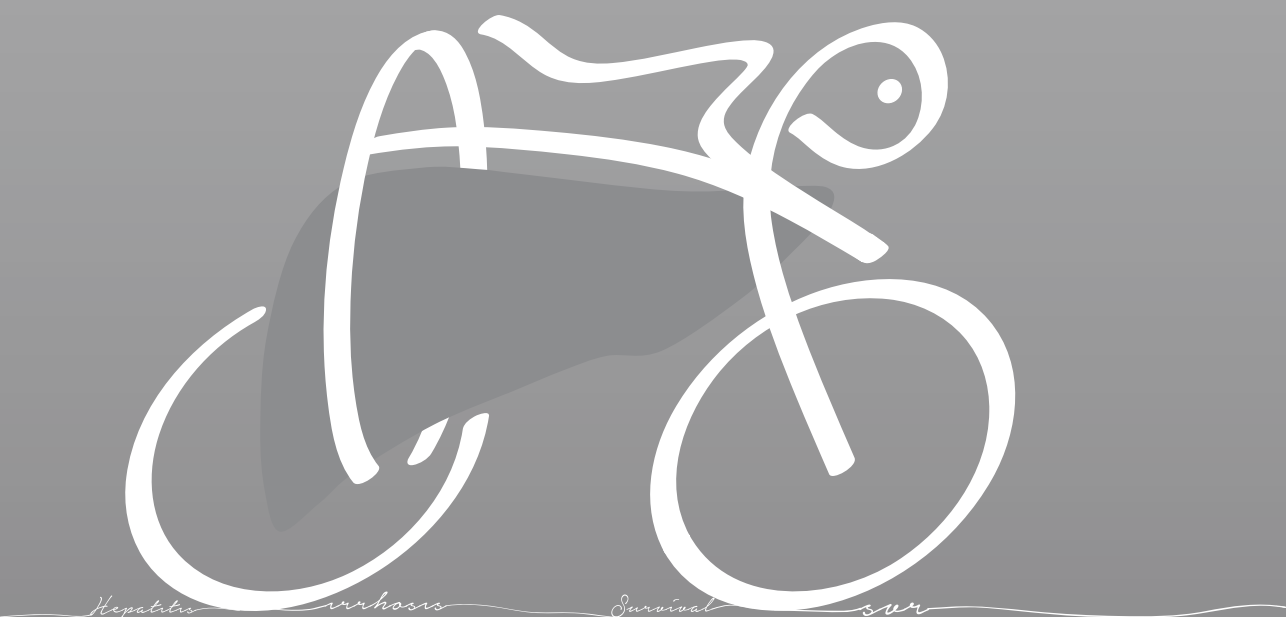
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CHAPTER 8

The risk of hepatocellular carcinoma among patients with chronic hepatitis C virus infection and advanced hepatic fibrosis following sustained virological response

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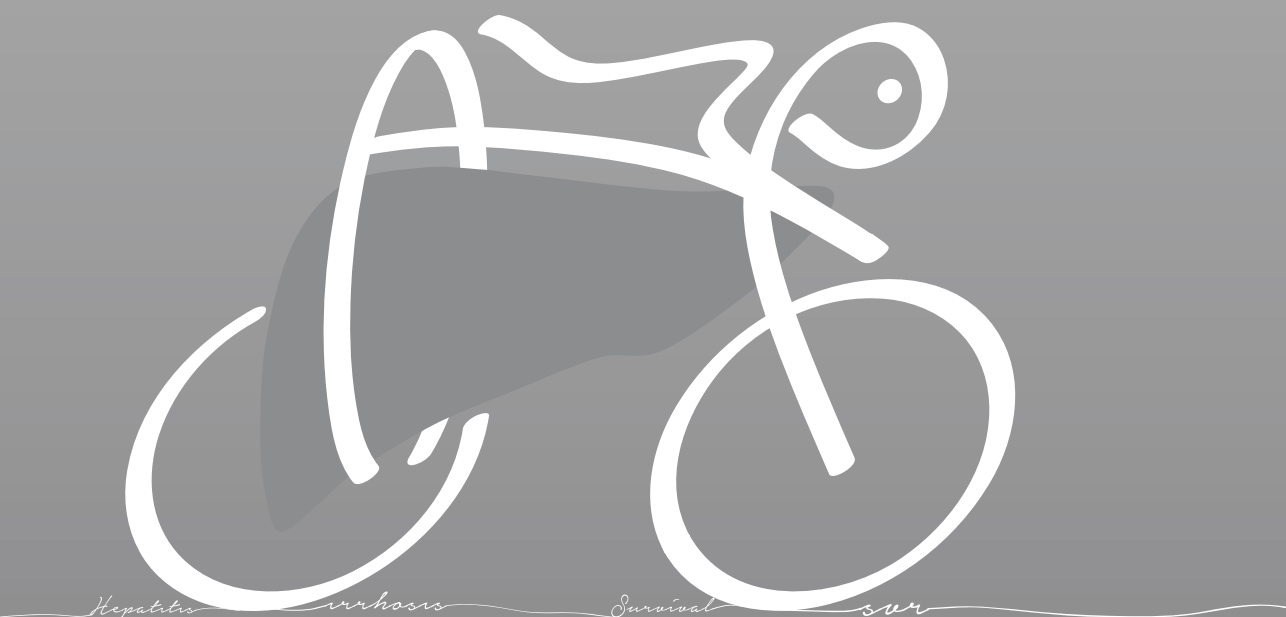
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CHAPTER 9

Risk of infections during interferon-based treatment in patients with chronic HCV infection and advanced hepatic fibrosis

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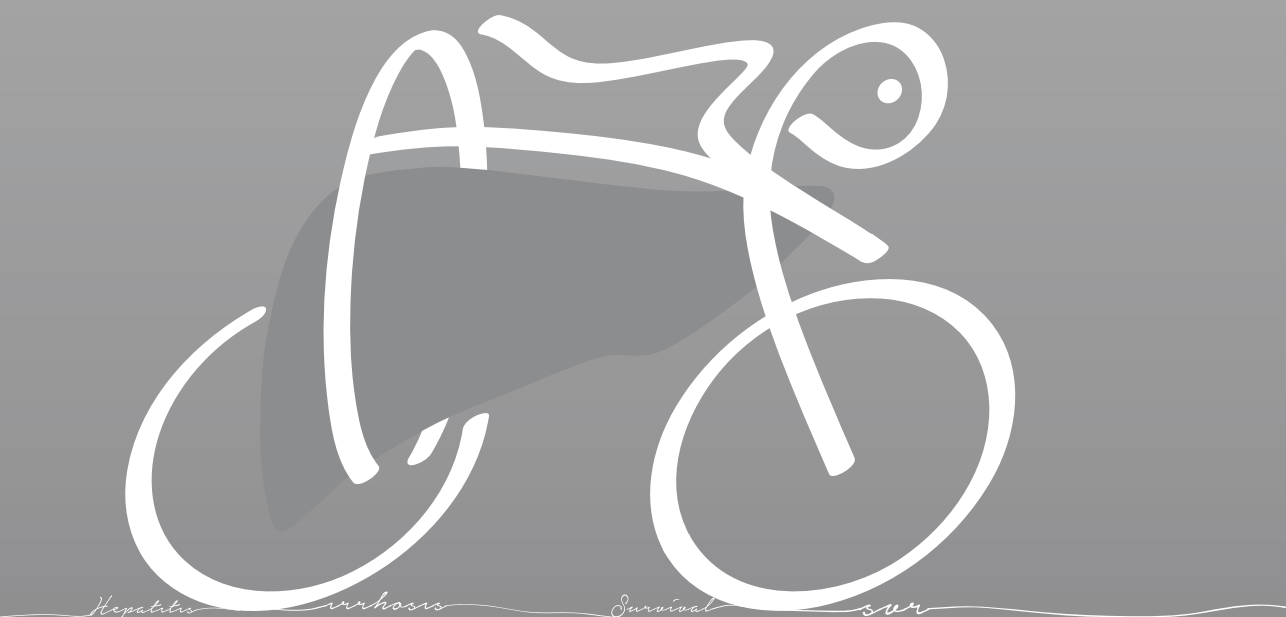
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CHAPTER 10

Effect of thrombocytopenia on treatment tolerability and outcome in patients with chronic HCV infection and advanced hepatic fibrosis

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ABSTRACT

Background

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 sustained virological response (SVR) and long-term clinical outcome.

Methods

All consecutive patients with chronic hepatitis C virus (HCV) infection and biopsy-proven advanced hepatic fibrosis (Ishak fibrosis score 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<.001); interferon was discontinued due to thrombocytopenia in 1 (<1%), 8 (3%), and in 8 (16%) treatments, respectively (p<.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<.001), hepatocellular carcinoma (p<.001), liver-related death or liver transplantation (p<.001), and all-cause mortality (p=.001) compared to patients without SVR.

Conclusion

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.[1] Cirrhosis and portal hypertension lead to splenomegaly and subsequent thrombocytopenia (platelet count <150 / 10^9/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.[2-5] Therefore, thrombocytopenia is a frequent manifestation in chronic liver disease and serves as an indicator of disease severity.[6]

Sustained virological response (SVR), i.e. HCV RNA negativity in blood 6 months after cessation of antiviral therapy, is the goal of antiviral therapy, with important clinical implications. Patients with advanced fibrosis who achieve SVR have less liver-related and all-cause mortality as well as a reduced risk of hepatocellular carcinoma.[7-9] 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.[10] 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.[11,12]

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.[13-17] 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 (≤100 x 10^9/L) or serum albumin <35 g/L.[18]

Current guidelines advise to reduce the dose of PegIFN when platelet counts fall below 50 x 10^9/L and to stop treatment when platelet counts fall below 25 x 10^9/L.[19,20] As a result patients with platelet counts below 75 x 10^9/L are often excluded from antiviral therapy or have to stop treatment prematurely.[19,20] However, patients with thrombocytopenia are those with most advanced liver disease, who are most likely to have clinical benefit from successful antiviral treatment.[7-9,21] 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.

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.[9,22] 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 150 x 10^9/L; moderate thrombocytopenia was defined as a platelet count of 75-149 x 10^9/L and severe thrombocytopenia as a platelet count below 75 x 10^9/L.[20,23] 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.[9,22]

Outcome

The primary outcome measures were thrombocytopenia-induced interferon (IFN) dose reductions, early treatment discontinuation, and bleeding complications dur-

ing 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 2 coincident imaging techniques (computed tomography, magnetic resonance imaging, or contrast-enhanced ultrasonography), showing a focal lesion of more than 2 cm with arterial-phase enhancement or 1 imaging technique showing a focal lesion of more than 2 cm with arterial-phase enhancement in combination with the presence of an a-fetoprotein level of more than 400 ng/mL.[24]

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 χ^2 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 $<50 \times 10^9$/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 analyse 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 <150 x 10^9/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 <.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 10.1). For 88 (10%) treatments there was no platelet count available within 6 months prior to treatment initiation (Figure 10.1).

Dynamics of platelet counts during treatment

The dynamics of the platelet counts during treatment are illustrated in Figure 10.2. The group with severe thrombocytopenia reached a platelet count <50 x 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 <50 x 10^9/L was respectively 1, 24, and 46 weeks, all p<.001) (Figure 10.3). The platelet count dropped below 50 x 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<.001) (Figure 10.4).

Table 10.1 Baseline Characteristics According to Baseline Platelet Count [a]

Characteristics	Treatments with normal platelets (n = 394)	Treatments with moderate TCP (n = 324)	Treatments with severe TCP (n = 53)
Male	281 (71)	222 (69)	37 (70)
Age, years, median (IQR) †	48 (42-55)	51 (45-58)	51 (44-59)
BMI, kg/m², median (IQR)	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)
Laboratory markers of liver disease severity, median (IQR)			
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 pegylated interferon and ribavirin	184 (47)	151 (47)	23 (43)
Treatment duration, weeks, median (IQR)	29 (21-48)	25 (17-48)	24 (14-47)
History of severe alcohol use	94 (24)	74 (23)	8 (15)
Diabetes mellitus †	39 (10)	60 (19)	8 (15)

Abbreviations: ALT; alanine aminotransferase, AST; aspartate aminotransferase, BMI; body mass index (calculated as weight in kilograms divided by height in meters squared), gGT; gamma-glutamyltransferase, HCV; hepatitis C virus, IQR; interquartile range, TCP; thrombocytopenia.

[a] Data are presented as No. (%), unless otherwise noted.

Variables that were significantly different among two groups were marked with # for moderate versus severe thrombocytopenia, † for moderate thrombocytopenia versus normal platelets and * for severe thrombocytopenia versus normal platelets.

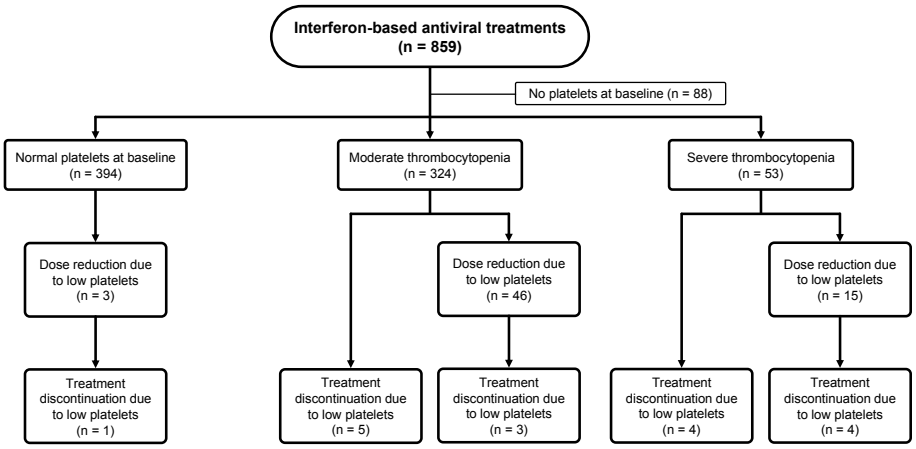


Figure 10.1 Study Flow Chart

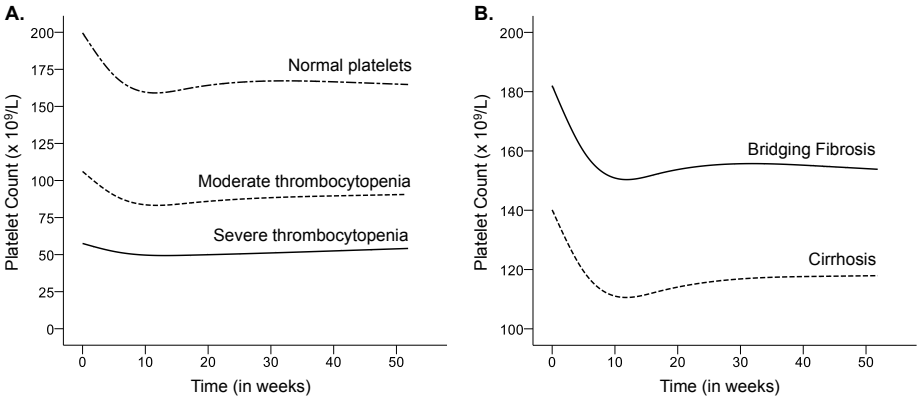


Figure 10.2 Dynamics of the Platelet Counts during Interferon-Based Therapy
Panel A shows the predicted mean platelet counts during interferon-based antiviral treatment among patients with chronic hepatitis C virus infection and a normal platelet count (≥150 x 10^9/L), a moderate thrombocytopenia (platelet count 75-149 x 10^9/L) or a severe thrombocytopenia (platelet count <75 x 10^9/L) at baseline. Panel B shows predicted mean platelet counts during treatment among patients with bridging fibrosis or cirrhosis.

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

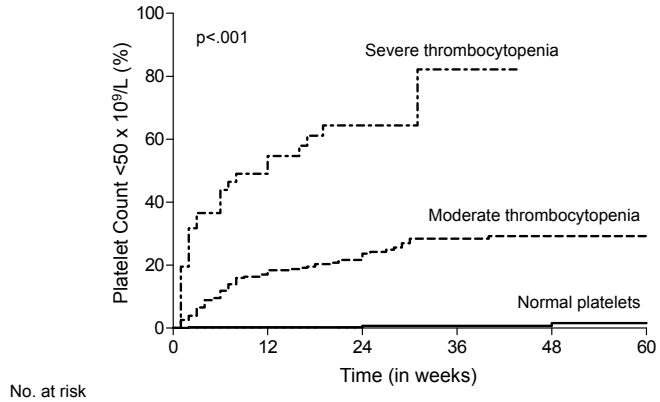


Figure 10.3 Time to a Platelet Count <50 x 10^9/L during Interferon-Based Therapy According to Baseline Platelet Counts

The incidence curves for platelet count <50 x 10^9/L during interferon-based antiviral treatment among patients with chronic hepatitis C virus infection were constructed using the Kaplan Meier method. Statistical significance was assessed with the Log Rank test. Abbreviations: TCP; thrombocytopenia, PLT; platelets.

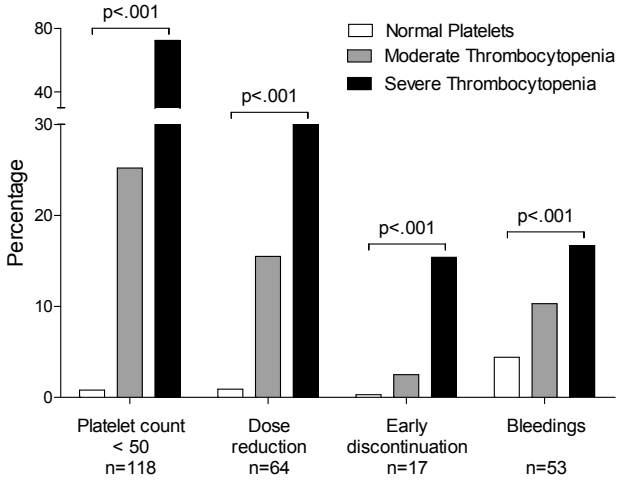


Figure 10.4 Occurrence of Primary Endpoints According to Baseline Platelet Counts

The figure shows the percentage of treatments during which a platelet count <50 x 10^9/L, a dose reductions due to thrombocytopenia, early treatment discontinuation due to thrombocytopenia, or a bleeding occurred, according to baseline platelet counts. Statistical significance was assessed with the χ^2 test.

for at least one (peg)interferon dose reduction (p<.001) (Figure 10.4). In univariate analysis, the use of PegIFN was associated with the occurrence of dose reductions due to thrombocytopenia (Odds ratio [OR] 2.16, 95% CI 1.25-3.72, p=.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<.001) (Figure 10.4).

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<.001). Further safety details are shown in Table 10.2.

Table 10.2 Adverse Events During Treatment [a]

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 [b]		
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)

Abbreviation: TCP; thrombocytopenia

[a] Data are reported as No. of treatments (percentage of the total number of treatment courses)

[b] Reasons for admission defined as adverse events, included a ruptured ovary cyst, stroke, renal impairment, atrial fibrillation, chest pain (no diagnosis), abdominal pain (no diagnosis), pleural effusion, cardiac decompensation and hematological side effects.

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 10.3. Eleven (11%) of 104 bleedings required treatment or further diagnostic procedures; sigmoidoscopy, gastroscopy, or consultation of another

Table 10.3 Details on Bleedings [a]

Type of bleedings	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

Abbreviation: TCP; thrombocytopenia.

[a] Data are reported as No. of treatments with a bleeding (percentage of total number of treatment courses during which a bleeding occurred).

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<.001) (Figure 10.4).

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), haematuria (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=.006), and a platelet count <50 x 10^9/L at the previous visit (OR 5.4, 95% CI 2.8-10.5, p<.001) were independently associated with the occurrence of on-treatment bleeding (Table 10.4). As a sensitivity analysis, only those patients treated with PegIFN were considered, showing that a platelet count <50 x 10^9/L remained independently associated with the occurrence of on-treatment bleeding (OR 4.5, 95% CI 2.3-9.0, p<.001, Table 10.4).

Effect of thrombocytopenia on SVR

In total, 193 (35%) of 546 patients attained SVR. Details on SVR rates are shown in Table 10.5. To assess the association between platelet counts and virological response, only the last treatment that a patient received was considered.

Table 10.4 Logistic Regression Analyses for On-Treatment Bleeding

Characteristics	Univariate analyses		Multivariate analyses		Multivariate analyses Pegylated Interferon Only	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age, per year	1.0 (0.97-1.03)	.867	0.98 (0.95-1.02)	.31	0.97 (0.94-1.00)	.09
Female sex	1.61 (0.82-3.19)	.169	1.61 (0.88-2.97)	.12	3.02 (1.46-6.25)	.003
BMI, per kg/m^2	1.03 (0.96-1.11)	.42	-	-	-	-
Cirrhosis	6.0 (2.0-17.7)	.001	4.64 (1.55-13.9)	.006	2.59 (0.75-8.98)	.135
Albumin, per g/L	0.93 (0.88-1.00)	.042	-	-	-	-
Bilirubin, per μmol/L	1.04 (1.00-1.08)	.035	-	-	-	-
Platelet count (at baseline) [a]						
Normal	1 (Reference)		-	-	-	-
Moderate thrombocytopenia	2.72 (1.31-5.64)	.007	-	-	-	-
Severe thrombocytopenia	4.32 (1.62-11.5)	.003	-	-	-	-
Weeks on treatment, per week	0.98 (0.97-1.00)	.057	-	-	-	-
Type of interferon (pegylated interferon vs standard interferon)	0.75 (0.38-1.48)	.41	-	-	-	-
Platelet count <50x10^9/L at the previous visit	6.90 (3.62-13.1)	<.001	5.38 (2.76-10.5)	<.001	4.51 (2.25-9.03)	<.001

Abbreviations: BMI; body mass index, CI; confidence interval, OR; odds ratio.

[a] Platelet counts ≥750 x10^9/L were considered normal, platelet counts from 500 to 749 x10^9/L were classified as moderate thrombocytopenia, a platelet counts <500 x10^9/L were classified as severe thrombocytopenia.

Table 10.5 SVR Rates According to HCV Genotype, Previous Response and Type of Interferon treatmen t[a]

	Interferon		Interferon and Ribavirin		Pegylated Interferon and Ribavirin [b]	
	TCP	Normal platelets	TCP	Normal platelets	TCP	Normal platelets
Treatment naïve (n = 430)						
HCV genotype 1 and 4	1/45 (2.2)	0/51	4/43 (9.3)	9/41 (22.0)	14/61 (23.0)	20/51 (39.2)
HCV genotype 2 and 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 unkown	3/14 (21.4)	2/8 (25.0)	1/3 (33.3)	1/2 (50.0)	0/1	1/1 (100)
Treatment experienced (n = 341)						
HCV genotype 1 and 4	0/14	0/19	5/34 (14.7)	6/34 (17.6)	15/72 (20.8)	22/87 (25.3)
HCV genotype 2 and 3	0/1	005	4/9 (44.4)	5/10 (50.0)	5/25 (20.0)	11/24 (45.8)
HCV genotype 5, 6 or unkown	-	0/1	0/3	1/1 (100)	1/1 (100)	0/1

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

[a] Platelet counts ≥750 x10^9/L were considered normal, thrombocytopenia was defined as platelet counts <750 x10^9/L.

[b] Included 27 treatments with pegylated interferon monotherapy.

Table 10.6 Logistic Regression Analysis for SVR at the Last Treatment

Characteristics	Univariate analyses		Multivariate analyses		Multivariate analyses Pegylated Interferon Only	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age, per year	0.98 (0.96-1.00)	.013	-	-	-	-
Males	1.31 (0.89-1.93)	.131	-	-	-	-
HCV genotype 2 or 3	3.58 (2.39-5.36)	<.001	3.04 (1.89-4.89)	<.001	2.40 (1.37-4.23)	.002
Cirrhosis	0.70 (0.46-1.06)	.091	-	-	-	-
BMI, per kg/m^2	0.96 (0.92-1.00)	.069	-	-	-	-
Diabetes Mellitus	0.45 (0.26-0.80)	.006	0.50 (0.26-0.97)	.041	0.40 (0.18-0.90)	.026
AST/ALT ratio, per 1.0	0.27 (0.14-0.53)	<.001	0.34 (0.15-0.77)	.009	0.39 (0.15-1.02)	.056
Albumin, per g/L	1.08 (1.03-1.12)	<.001	-	-	-	-
Bilirubin, per μmol/L	0.95 (0.93-0.98)	<.001	-	-	-	-
Thrombocytopenia [a]	0.41 (0.28-0.60)	<.001	0.49 (0.31-0.76)	.002	0.45 (0.27-0.77)	.004
Treatment year	1.11 (1.05-1.16)	<.001	-	-	-	-
Treatment naïve	1.21 (0.85-1.73)	.284	1.80 (1.16-2.79)	.009	3.02 (1.71-5.33)	<.001
Type of interferon treatment						
Pegylated interferon vs interferon	7.03 (3.42-14.4)	<.001	18.7 (5.57-63.0)	<.001	-	-
Pegylated interferon vs interferon and ribavirin	0.96 (0.63-1.46)	.856	0.95 (0.57-1.58)	.831	-	-
Interferon versus interferon and ribavirin	7.30 (3.37-15.8)	<.001	19.8 (5.64-69.6)	<.001	-	-

Abbreviations: ALT; alanine aminotransferase, AST; aspartate aminotransferease, BMI; body mass index, CI; confidence interval, OR; odds ratio.

[a] Patients were considered to have thrombocytopenia in case the platelet counts were <750 x10^9/L.

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=.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=.749).

Thrombocytopenia prior to treatment was inversely associated with SVR (OR 0.41, 95% CI 0.28-0.60, p<.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=.002, Table 10.6). 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=.004, Table 10.6).

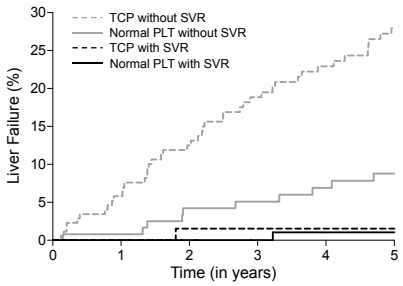
Effect of thrombocytopenia on long-term clinical outcome

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 (Figure 10.5). Among the 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

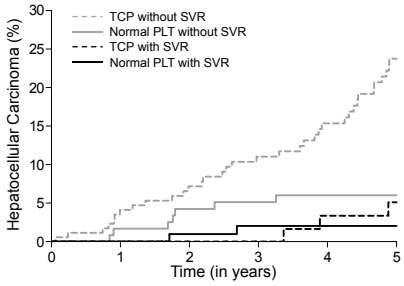
This large cohort study describes the course of antiviral treatment and post-treatment clinical outcome among patients with chronic HCV infection and advanced liver disease, including those with moderate and severe thrombocytopenia. These data are unique since severe thrombocytopenia normally is a contraindication 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

A.



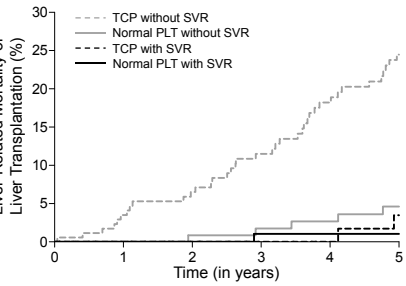
No. at risk						
TCP without SVR	176	157	141	124	110	98
Normal PLT without SVR	126	118	112	107	101	92
TCP with SVR	69	67	63	60	57	53
Normal PLT with SVR	113	105	98	95	90	84

B.



No. at risk						
TCP without SVR	176	160	149	132	115	98
Normal PLT without SVR	126	117	112	106	101	94
TCP with SVR	69	67	64	61	56	53
Normal PLT with SVR	113	105	97	94	90	84

C.



No. at risk						
TCP without SVR	176	162	153	138	120	107
Normal PLT without SVR	126	119	116	109	104	95
TCP with SVR	69	67	64	61	58	54
Normal PLT with SVR	113	105	98	95	91	85

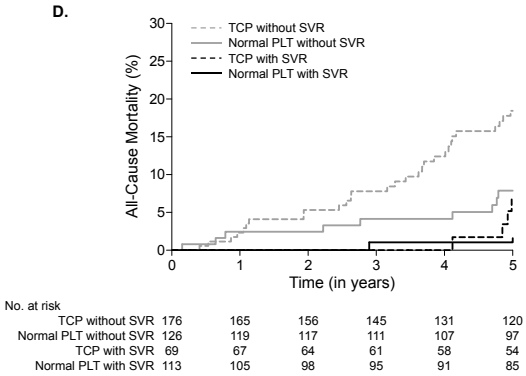


Figure 10.5 Survival Outcomes Following the Last Antiviral Treatment According to the Virological Response and Baseline Platelet Counts

The figures show the cumulative incidence of liver failure (A), hepatocellular carcinoma (B), liver-related mortality or liver transplantation (C), and all-cause mortality (D), which were constructed using the Kaplan Meier method. Twenty-four weeks after cessation of the last antiviral therapy during follow-up was considered as time zero. Statistical significance was assessed with the Log Rank test. Occurrence of liver failure over time: p=.001 for with sustained virological response (SVR) vs without SVR within patients with normal platelets (PLT; ≥150 x 10^9/L); p<.001 for with SVR vs without SVR within patients with thrombocytopenia (TCP; <150 x 10^9/L); p<.001 for TCP vs normal PLT within patients without SVR; and p=.606 for TCP vs normal PLT within patients with SVR. Occurrence of hepatocellular carcinoma over time: p=.008 for with SVR vs without SVR within patients with normal PLT; p<.001 for with SVR vs without SVR within patients with TCP; p<.001 for TCP vs normal PLT within patients without SVR; and p=.079 for TCP vs normal PLT within patients with SVR. Occurrence of liver-related death or liver transplantation over time: p=.008 for with SVR vs without SVR within patients with normal PLT; p<.001 for with SVR vs without SVR within patients with TCP; p<.001 for TCP vs normal PLT within patients without SVR; and p=.332 for TCP vs normal PLT within patients with SVR. Occurrence of all-cause mortality over time: p=.032 for with SVR vs without SVR within patients with normal PLT; p=.001 for with SVR vs without SVR within patients with TCP; p<.001 for TCP vs normal PLT within patients without SVR; and p=.260 for TCP vs normal PLT within patients with SVR.

infection, mainly patients with advanced liver disease.[25] Importantly, the proportion of patients with chronic HCV infection and cirrhosis is rapidly rising.[26]

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 moderate thrombocytopenia, in line with findings from earlier studies that excluded patients with platelets below 75 x 10^9/L.[27,28]

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 90 x 10^9/L.[29-31]

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 2 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.[32]

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 50 x 10^9/L is generally safe.[32] 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.[33]

Although several interferon-free regimens are currently in development, PegIFN 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.[34] Despite established cirrhosis, attaining SVR may reduce these risks and improve prognosis in terms of liver-related death and all-cause mortality.[7-9] We showed that clinical disease progression or death was more common in nonresponders with thrombocytopenia than in nonresponders 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.[9,22] 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.[35,36] In general, dose reductions will compromise treatment efficacy as seen in patients with HCV genotype 1 infection.[11,12] 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 2 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.[37-39] 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.[37]

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.[13-17] A recent report from the French early access program for use of protease inhibitors in patients with HCV-induced cirrhosis showed platelet counts below 50 x 10^9/L in 13% of the patients treated with telaprevir, PegIFN, and RBV and in 6% of the patients treated with boceprevir, PegIFN, and RBV.[18] 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 advanced hepatic fibrosis or cirrhosis treated with interferon-based regimens. Cirrhosis and a platelet count below 50 x 10^9/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.

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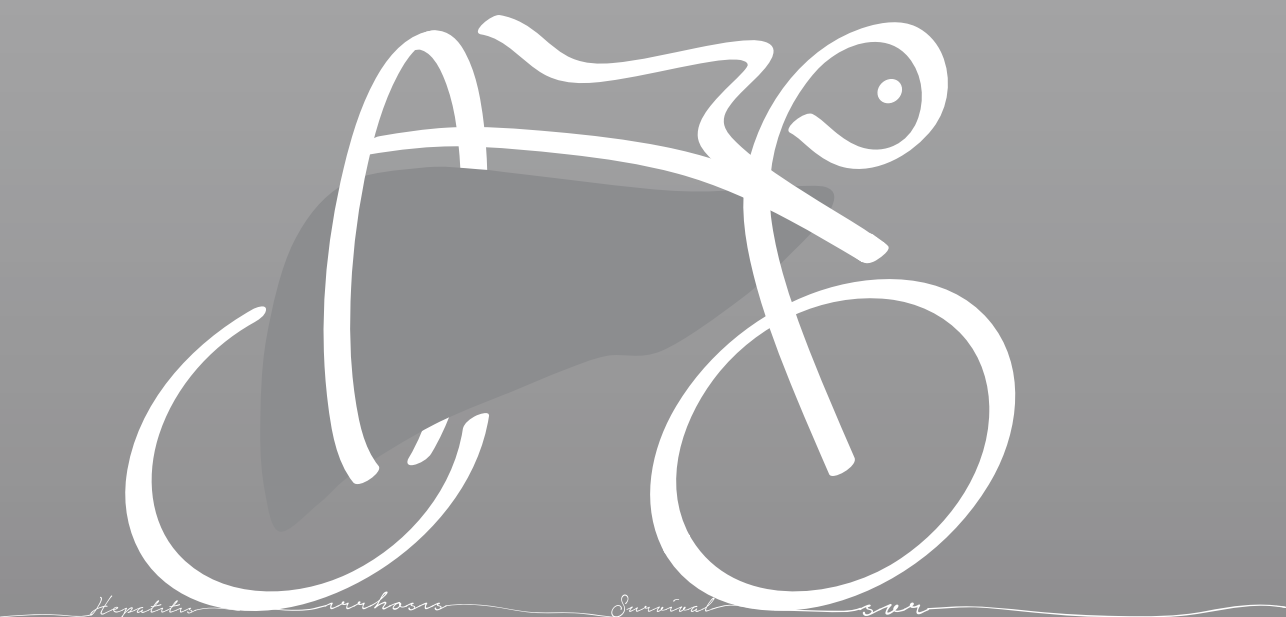
Hepatitis
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CHAPTER 11

Correspondence

Telaprevir for chronic HCV infection

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TO THE EDITOR:

McHutchison et al. and Hézode et al. (April 30 issue) found an important effect of adding telaprevir to current antiviral therapy.[1,2] Nevertheless, the results of the Protease Inhibition for Viral Evaluation (PROVE) trials (ClinicalTrials.gov numbers, NCT00336479 and NCT00372385) are disappointing, since they demonstrate the risk of serious side effects resulting from high dosing of a new molecule, with profound consequences for efficacy. Combined data from all telaprevir regimens in both trials show a significant difference in sustained virologic response between patients completing and those discontinuing treatment (78% and 25%, respectively, p<.001) (Table 11.1). Although telaprevir had an acceptable initial side effect profile, the extended administration of high doses of telaprevir was accompanied by a high rate of treatment discontinuation, mainly because of unexpected rash and more severe anemia.

Table 11.1 SVR Rates According to Treatment Adherence in the PROVE Trials [a]

		T12PR12	T12PR24	T12PR48	All T12arms
PROVE 1	overall	35% (6/17)	61% (48/79)	67% (53/79)	
	completed therapy	67% (6/9)	98% (41/42)	89% (48/54)	
	discontinued therapy	0% (0/8)	22% (8/37)	20% (5/25)	
PROVE 2	overall	60% (49/82)	69% (56/81)	-	
	completed therapy	64% (46/72)	77% (47/61)	-	
	discontinued therapy	30% (3/10)	45% (9/20)	-	
PROVE 1+2 COMBINED	overall	56% (55/99)	65% (104/160)		
	completed therapy	64% (52/81)	85% (88/103)	see data PROVE 1	**78% (189/243)**
	discontinued therapy	17% (3/18)	30% (17/57)		**25% (25/100)**
					p<.001 [b]

Abbreviations: P; pegylated interferon, PROVE; Protease Inhibition for Viral Evaluation, R; Ribavirin, SVR; sustained virological response, T; telaprevir.

[a] The SVR rates are shown in percentages, with the No./Total No. between brackets. All data are withdrawn from the PROVE 1 and PROVE 2 trials. Subdivision of SVR is based on treatment adherence, irrespective of the cause of discontinuation of anitiviral therapy. The T12PR12 group received 12 weeks of pegylated interferon, ribavirin and telaprevir. The T12PR24 received 24 weeks of pegylated interferon and ribavirin combined with telaprevir during the first 12 weeks. The T12PR48 group received 48 weeks of pegylated interferon and ribavirin combined with telaprevir during the first 12 weeks. The PROVE 2 trial did not have a T12PR48 treatment arm.

[b] Statistical significance assessed using the χ^2 test.

The first study of telaprevir showed similar initial viral declines with different dosages; viral breakthrough occurred in the lower dosing regimen.[3] The fear of selection of telaprevir-resistant variants can be negated, since mutant viruses are sensitive to peginterferon.[4]

We are concerned that major decisions in the development of new antiviral agents are primarily based on the reduction of hepatitis C virus RNA levels and on the highest tolerated doses in short phase 1 trials. Subsequent trials should incorporate lower, albeit effective, dosages to reduce the risk of adverse events and to enhance treatment adherence, which might improve efficacy further.

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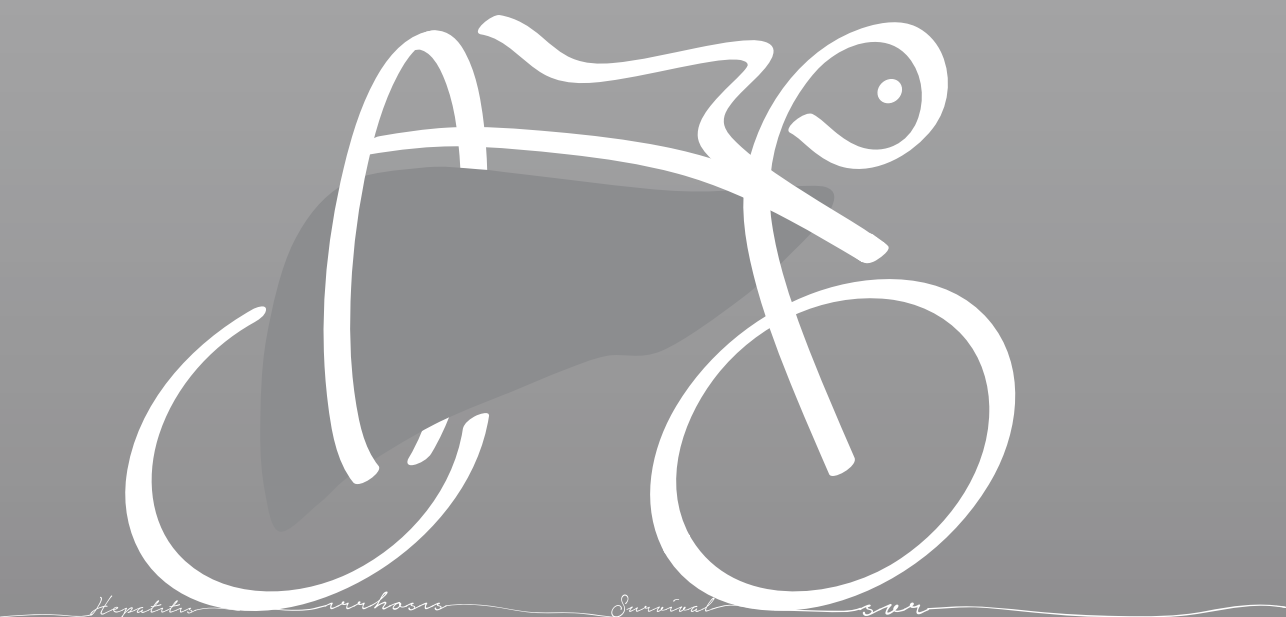
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CHAPTER 12

Treatment of HCV Infection by Targeting MicroRNA

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ABSTRACT

Background

The stability and propagation of hepatitis C virus (HCV) is dependent on a functional interaction between the HCV genome and liver-expressed microRNA-122 (miR-122). Miravirsen is a locked nucleic acid-modified DNA phosphorothioate antisense oligonucleotide that sequesters mature miR-122 in a highly stable heteroduplex, thereby inhibiting its function.

Methods

In this phase 2a study at seven international sites, we evaluated the safety and efficacy of miravirsen in 36 patients with chronic HCV genotype 1 infection. The patients were randomly assigned to receive five weekly subcutaneous injections of miravirsen at doses of 3 mg, 5 mg, or 7 mg per kilogram of body weight or placebo over a 29-day period. They were followed until 18 weeks after randomization.

Results

Miravirsen resulted in a dose-dependent reduction in HCV RNA levels that endured beyond the end of active therapy. In the miravirsen groups, the mean maximum reduction in HCV RNA level ($\log_{10}$ IU per milliliter) from baseline was 1.2 (p=.01) for patients receiving 3 mg per kilogram, 2.9 (p=.003) for those receiving 5 mg per kilogram, and 3.0 (p=.002) for those receiving 7 mg per kilogram, as compared with a reduction of 0.4 in the placebo group. During 14 weeks of follow-up after treatment, HCV RNA was not detected in one patient in the 5-mg group and in four patients in the 7-mg group. We observed no dose-limiting adverse events and no escape mutations in the miR-122 binding sites of the HCV genome.

Conclusions

The use of miravirsen in patients with chronic HCV genotype 1 infection showed prolonged dose-dependent reductions in HCV RNA levels without evidence of viral resistance. (Funded by Santaris Pharma; ClinicalTrials.gov number, NCT01200420.)

INTRODUCTION

Approximately 170 million persons worldwide are chronically infected with the hepatitis C virus (HCV).[1] Chronic HCV infection is a major cause of liver cirrhosis, liver failure, and hepatocellular carcinoma and is the leading indication for liver transplantation in many Western countries.[2] Sustained eradication of HCV infection has been associated with a reduced risk of liver-related morbidity and all-cause mortality.[3-5]

Despite the recent registration of protease inhibitors for the treatment of chronic HCV genotype 1 infection, current therapeutic regimens remain dependent on the administration of pegylated interferon and ribavirin for 24 to 48 weeks.[6,7] Thus, anti-HCV therapy continues to be associated with substantial side effects. In addition, there is the risk of drug interactions mediated by cytochrome P-450 3A, drug resistance, unknown sustainability of response, and reduced efficacy against certain HCV genotypes and subtypes.[8]

MicroRNAs (miRNAs) are small, endogenous, noncoding RNAs that direct post-transcriptional regulation of gene expression by binding to partially complementary sites within the 3′ untranslated region (UTR) of target messenger RNAs (mRNAs), resulting in translational repression or mRNA deadenylation and degradation.[9] MiRNAs have been implicated in the regulation of a wide range of important biologic processes, such as cellular growth and differentiation, developmental timing, apoptosis, and modulation of host response to viral infection.[10]

MicroRNA-122 (miR-122) is a highly abundant miRNA expressed in the liver and is essential to the stability and propagation of HCV RNA.[11,12] MiR-122 binds to two closely spaced target sites (S1 and S2) in the highly conserved 5′ UTR of the HCV genome, thereby forming an oligomeric miR-122–HCV complex that protects the HCV genome from nucleolytic degradation or from host innate immune responses.[12-14] A third potential miR-122 binding site in the 3′ UTR of the HCV genome does not appear to have any functional relevance.[11] The miR-122 binding sites are conserved across all HCV genotypes and subtypes.[15] MiR-122 could thus represent a host target for antiviral therapy.

Miravirsen is a 15-nucleotide locked nucleic acid–modified antisense oligonucleotide complementary to and with a high affinity and specificity for the 5′ region of mature miR-122. Miravirsen can sequester and thus inhibit miR-122 (Figure 12.1). The administration of miravirsen to chimpanzees with chronic HCV infection provided long-lasting viral suppression without evidence of resistant mutations at the two miR-122 binding sites of the 5′ UTR of the HCV genome, and no adverse events were observed in phase 1 studies in healthy volunteers.[16,17] Here, we report on the safety and activity of miravirsen in patients with chronic HCV infection.

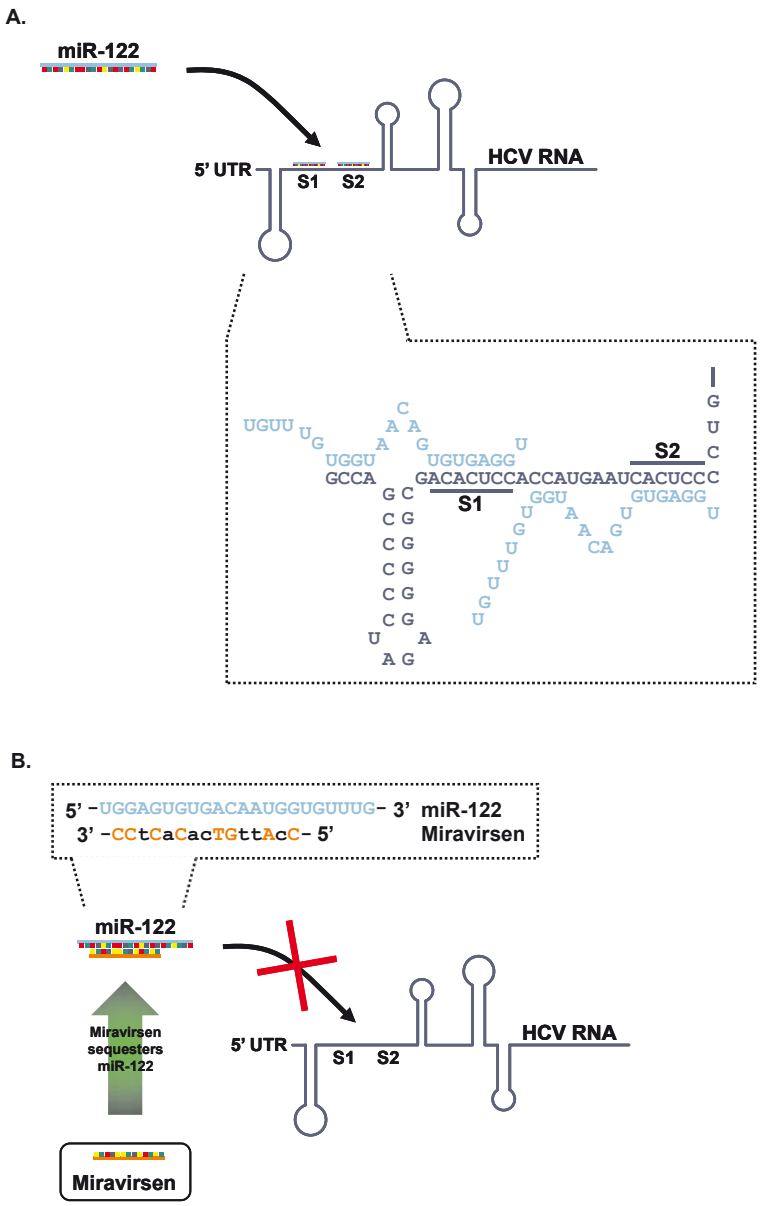

Figure 12.1 Mechanism of Action of Miravirsen

In Panel A, microRNA-122 (miR-122) binds to two closely spaced target sites (S1 and S2) in the 5′ untranslated region (UTR) of the hepatitis C virus (HCV) genome and thereby promotes the propagation of HCV RNA. In Panel B, miravirsen, a locked nucleic acid–modified antisense oligonucleotide, sequesters mature miR-122 in a highly stable heteroduplex, which results in the functional inhibition of miR-122.

METHODS

Study population

Patients who had not undergone previous therapy for chronic HCV genotype 1 infection were enrolled at seven international sites. Eligible patients were 18 to 65 years of age and were required to have compensated liver disease with a plasma HCV RNA level of more than 75,000 IU per milliliter. Patients with other causes of chronic liver disease, cirrhosis (as diagnosed on previous biopsy), or decompensated liver disease were excluded. Patients were also required to be seronegative for hepatitis B surface antigen and antibodies to human immunodeficiency virus and to have an absolute neutrophil count of 1500 or more per cubic millimeter, a platelet count of 100,000 or more per cubic millimeter, normal values for serum creatinine and for total and direct bilirubin, and alanine aminotransferase levels less than 3 times the upper limit of the normal range.

The study was approved by the institutional review board or ethics committee at each participating center and was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulations. All patients provided written informed consent before enrollment in the study.

Study design

From September 2, 2010, to November 16, 2011, we enrolled 36 patients in a randomized, doubleblind, placebo-controlled, sequential-series, ascending multiple dose–ranging study. The patients underwent central randomization with the use of a Web-based system in a 3:1 ratio to receive either miravirsen (in doses of 3 mg, 5 mg, or 7 mg per kilogram of body weight) or placebo. An independent review committee evaluated safety data for the 3-mg group before the study drug was administered to the 5-mg group; the same process was used for the 7-mg group.

Miravirsen was reconstituted to a concentration of 150 mg per milliliter and was administered subcutaneously in five weekly doses over a 29-day period (Figure 12.2). Placebo injections contained normal saline (0.9% sodium) and were administered at a volume equivalent to that for miravirsen. After the administration period, patients returned for weekly follow-up visits until week 8, for visits every 2 weeks until week 14, and for a final visit at week 18. At the investigators' discretion, patients were allowed to initiate therapy with pegylated interferon and ribavirin at study week 7 (for patients receiving 3 mg of miravirsen per kilogram) or week 10 (for those receiving 5 mg or 7 mg per kilogram).

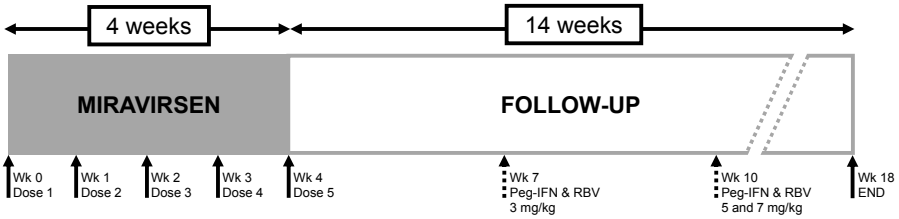

Figure 12.2 Study Design
Patients were administered 5 weekly subcutaneous miravirsen doses during the first 29 days of the study (week [wk] 0, 1, 2, 3, 4, and 5) and followed until 14 weeks after the last miravrisen administration. Pegylated interferon and ribavirin (Peg-IFN & RBV) therapy was allowed after week 7 in the 3 mg/kg treated cohort and after week 10 in the 5 mg/kg and 7 mg/kg treated cohort.

Study oversight

The study protocol (available at www.NEJM.org) was designed and developed by the sponsor, Santaris Pharma, along with the principal investigator at each study site and representatives of Duke Clinical Research Institute. All authors participated in the collection of the data, had complete access to data, participated in the data analysis, and were involved in the preparation and content review of the final manuscript. Duke statisticians performed the statistical analyses. The publication committee, consisting of the first author and several coauthors (including a representative of the sponsor), made the decision to submit the manuscript for publication. All authors vouch for the completeness and accuracy of this report as well as the fidelity of the report to the study protocol.

Efficacy, safety, and pharmacokinetics

At every patient visit, we measured the plasma HCV RNA levels using the Abbott RealTime HCV assay, with a reported lower limit of detection and quantification of 12 IU per milliliter. Samples with HCV RNA levels below the lower limit of detection or quantification were considered to have undetectable levels. The HCV RNA genotype was assessed by an in-house genotype-specific polymerase-chain-reaction (PCR) assay that had been developed with the use of the nucleotide sequence of the hypervariable region 1 of the E2 protein of HCV. Direct sequencing was used to confirm the genotype in the event that the result obtained on PCR assay was ambiguous.

Assessment of resistance-associated mutations was performed in all patients at baseline and at week 5 and at the time of viral rebound in the event that the HCV RNA level exceeded 1000 IU per milliliter and pegylated interferon and ribavirin were not initiated. Amplification and sequence analysis of miR-122 binding sites (S1, S2 and S3) within the 5′ and 3′ HCV RNA UTR was accomplished by site-specific primed RT-PCR followed by population-based sequencing. Briefly, RNA was isolated from

200 μL of serum using the QIAamp MiniElute virus Spin kit (Qiagen product number 57704; Valencia, CA), according to the manufacturer's instructions and eluted in 24 μL elution buffer. Following reverse transcription, target regions (S1, S2 and S3) were amplified by PCR and S1, S2 and S3 nucleotide sequences were determined by population-based sequencing analysis using an Applied Biosystems 3730xl Genetic Analyzer (Carlsbad, CA, USA). LBCM4279 HCV isolate (Genbank accession number HM043170, genotype 1) was used as a reference sequence for 5′ UTR analysis and HPCTQ3 HCV genomic RNA (Genbank accession number D63922, genotype 1b) was used as a reference sequence for 3′ UTR analysis.

We performed physical examinations and serum biochemical and hematologic laboratory tests at all study visits. We asked the patients open-ended questions to determine whether new adverse events, both general and according to organ system, had emerged since the previous visit; all adverse events were classified according to terms used in the *Medical Dictionary for Regulatory Activities*. Adverse events were considered to be mild if they were transient in nature and generally did not interfere with normal activities, moderate if they were sufficiently discomforting to interfere with normal activities, and severe if they prevented normal activities. Twelve-lead electrocardiography was performed at screening and periodically throughout the study. Plasma samples for miravirsen pharmacokinetic assessment were collected before the administration of each dose of a study drug and 2 hours after each dose. Miravirsen levels were determined with the use of a hybridization enzyme-linked immunosorbent assay.[17]

Statistical analyses

The primary analyses included all patients who underwent randomization and who received at least one dose of a study drug. We compared the rates at which HCV RNA levels declined using HCV RNA measurements obtained throughout the study period or until the initiation of therapy with pegylated interferon and ribavirin. To gauge the decline in viral load associated with miravirsen alone, we excluded from the analyses HCV RNA results after the initiation of therapy with pegylated interferon and ribavirin. To report all potential toxicity during or after the administration of miravirsen, we reported side effects and biochemical safety profiles on all data that were collected from baseline to week 18, regardless of whether patients were receiving pegylated interferon and ribavirin.

For each patient, we determined the maximum change in the HCV RNA level from baseline and used two-sample t-tests to compare the means of these values in each miravirsen-dose group with the placebo group. A p-value of .05 was considered to indicate statistical significance; all tests were two-sided, with no adjustment for multiple testing. We assessed the dose–response relationships to HCV RNA levels

using a linear trend test, assuming an equal space among the four study groups. All analyses were performed with the use of SAS software, version 9.2 (SAS Institute).

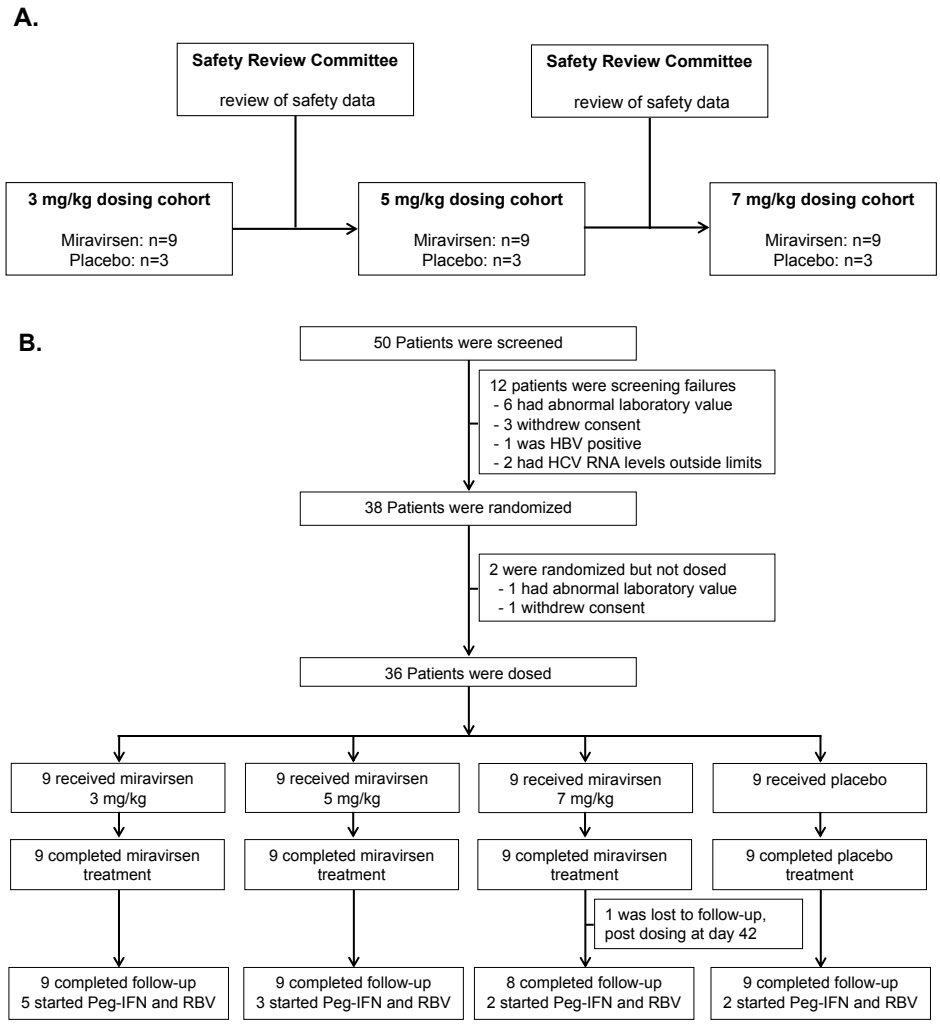

Figure 12.3 Study Flow Chart

Patients were included in three sequential dosing cohorts (A), and randomized to receive either active treatment (n = 9) or placebo (n = 3). The placebo patients of the three dosing cohorts are pooled (B). Patients who received all 5 subcutaneous miravirsen doses were classified as having completed treatment. Patients who were followed untill week 18 were classified as having completed the study, irrespective of the start of pegylated interferon and ribavirin therapy during follow-up.

RESULTS

Patients

A total of 36 patients with chronic HCV genotype 1 infection were enrolled in the study, with 9 patients in each miravirsen group (receiving 3 mg, 5 mg, or 7 mg per kilogram) and in the placebo group (Figure 12.3). Baseline characteristics were similar among the four study groups (Table 12.1). Of the 36 patients, 12 started therapy with pegylated interferon and ribavirin during follow-up.

Table 12.1 Baseline Characteristics of the Patients [a]

	Miravirsen			Placebo
Characteristics	**3 mg/kg (n = 9)**	**5 mg/kg (n = 9)**	**7 mg/kg (n = 9)**	**(n = 9)**
Age, years, median (range)	35 (26-66)	46 (33-65)	48 (31-61)	56 (42-66)
BMI, kg/m^2, median (range)	28 (18-31)	26 (19-38)	29 (21-38)	28 (18-37)
Males, No. (%)	5 (56)	8 (89)	6 (67)	3 (33)
Race, No. (%) [b]				
Caucasian	9 (100)	8 (89)	7 (78)	7 (78)
Black	0 (0)	1 (11)	1 (11)	2 (22)
Asian	0 (0)	0 (0)	1 (11)	0 (0)
ALT, IU/L	74.3 (±38.7)	69.1 (±21.4)	81.3 (±71.8)	92.7 (±37.0)
Bilirubin, μmol/L	10.2 (±5.1)	11.8 (±8.6)	9.9 (±3.6)	12.4 (±5.1)
Albumin, g/L	44.9 (±3.5)	43.0 (±2.8)	42.6 (±3.3)	43.3 (±2.7)
Platelet count, x10^9/L	267 (±70)	250 (±38)	245 (±51)	189 (±69)
Prothrombin time, seconds	11.1 (±0.4)	12.6 (±1.0)	12.2 (±1.4)	11.7 (±1.7)
HCV genotype 1 subtype, No. (%) [c]				
1a	5 (56)	7 (78)	5 (56)	6 (67)
1b	2 (22)	1 (11)	3 (33)	2 (22)
1a/1b	2 (22)	0 (0)	1 (11)	1 (11)
1a/3a	0 (0)	1 (11)	0 (0)	0 (0)
HCV RNA, $\log_{10}$ IU/ml	6.0 (±0.7)	6.2 (±0.3)	5.9 (±0.6)	6.2 (±0.4)
HCV RNA ≥ 800,000 IU/ml, No.(%)	5 (56)	8 (89)	6 (67)	7 (78)
IL28B-CC genotype, No. (%) [d]	2 (22)	4 (44)	4 (44)	2 (22)
IP-10, pg/mL	1209 (±1283)	1069 (±787)	798 (±501)	1390 (±881)

Abbreviations: ALT; alanine aminotransferase, BMI; body mass index (calculated as weight in kilograms divided by height in meters squared), HCV; hepatitis C virus, IP-10; interferon-inducible protein 10, SD; standard deviation.

[a] Data are reported as mean (± standard deviation), unless otherwise noted. There were no statistically significant differences among the groups.

[b] Race was self-reported.

[c] Several patients had a mixture of HCV genotype 1a and HCV genotype 1b. One patient had a mixture of HCV genotype 1a and HCV genotype 3a.

[d] The single nucleotide polymorphism at rs12979860 was genotyped.

Efficacy

The reduction in HCV RNA levels was dose-dependent and sustained beyond the administration period for miravirsen (Figure 12.4). In the miravirsen groups, the mean of the maximum reduction in HCV RNA levels (log_{10} IU per milliliter) from baseline was 1.2 (p=.01) for patients receiving 3 mg per kilogram, 2.9 (p=.003) for those receiving5 mg per kilogram, and 3.0 (p=.002) for those receiving 7 mg per kilogram, as compared with a decline of 0.4 in the placebo group. A reduction in the HCV RNA level of at least 2 log_{10} IU per milliliter occurred in one patient (11%) in the group receiving 3 mg of miravirsen per kilogram and in six patients each (67%) in the groups receiving 5 mg or 7 mg of miravirsen per kilogram, as compared with none of the patients in the placebo group. Corresponding dose-dependent pharmacokinetic profiles are shown in Figure 12.5.

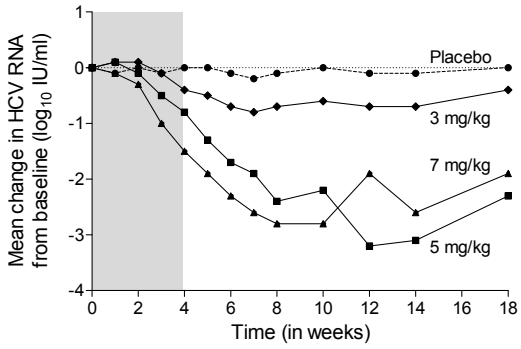

Figure 12.4 Change from Baseline in HCV RNA levels

Shown are the mean changes in hepatitis C virus (HCV) RNA levels from baseline for patients receiving 3 mg, 5 mg, or 7 mg of miravirsen per kilogram of body weight, as compared with placebo. Miravirsen was administered in five weekly subcutaneous injections during the first 29 days of the study (gray shading). The dashed line indicates no change from baseline. The HCV RNA levels during the use of pegylated interferon and ribavirin in some patients were not included in this analysis.

Figure 12.6 shows the reductions in HCV RNA levels among individual patients during the administration of miravirsen and after initiation of pegylated interferon and ribavirin. In five patients, the use of miravirsen alone resulted in undetectable HCV RNA (i.e., <12 IU per milliliter). Of these patients, one who received 5 mg of miravirsen per kilogram had undetectable HCV RNA at study week 14 but subsequently had an HCV RNA level of 3180 IU per milliliter at week 18. The other four patients, who received 7 mg of miravirsen per kilogram, had undetectable HCV RNA at study week 5 (in one patient), week 6 (in one patient), or week 14 (in two patients). In the two patients with undetectable HCV RNA at weeks 5 and 6, the HCV RNA levels remained undetectable up to and including week 10. Subsequently, one patient had

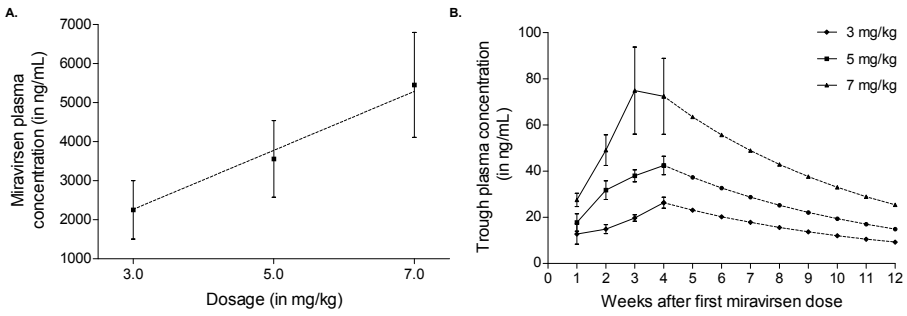

Figure 12.5 Pharmacokinetic Profile of Miravirsen

Panel A shows the mean plasma miravirsen concentration at 2 hours post-dose. The miravirsen concentration after each of the 5 doses for all 9 patients in the 3, 5 and 7 mg/kg treated cohorts was included in the analysis. The error bars represent the standard deviation. The mean plasma miravirsen concentrations measured 2 hours after subcutaneous injection increased linearly with the administered dose (R= 0.985), and were similar for each of the 5 doses. Panel B shows the mean plasma miravirsen trough concentrations at 168 hours (7days) post-dose. The mean miravirsen rough concentration after the first 4 doses is presented for the 3, 5 and 7 mg/kg treated cohorts. The error bars represent the standard deviation. The plasma miravirsen trough concentrations were 2-3 orders of magnitude lower compared to the 2 hour-post dosing time point. The trough concentrations remained dose proportional and increased with each dosage. The dashed lines represent the modelled miravirsen trough concentration after the 5th dose, which are based on the plasma terminal half-life of 32 days (Santaris Pharma data).

a detectable HCV RNA level of 6230 IU per milliliter at week 12, and the other started therapy with pegylated interferon and ribavirin at week 10 and remained without detectable HCV RNA through the end of study (week 18). Of the two patients with undetectable HCV RNA levels at week 14, one continued to have undetectable HCV RNA throughout the study, and the other had a low HCV RNA level of 80 IU per milliliter at week 18.

Virologic rebound, which is defined as an increase exceeding 1 $\log_{10}$ in the HCV RNA level over nadir, occurred after the discontinuation of miravirsen in 1 patient receiving 3 mg of the drug per kilogram (at week 18), in 5 patients receiving 5 mg of the drug per kilogram (3 patients at week 10, 1 at week 14, and 1 at week 18), and in 3 patients receiving 7 mg of the drug per kilogram (at weeks 12, 14, and 18). We did not detect resistance-associated mutations in the two miR-122 seed sites of the HCV genome in any of the 36 patients. We observed no apparent relationships between the declines in HCV RNA levels and factors that have previously been associated with such declines, including the IL28B genotype (rs12979860), baseline serum interferon-inducible protein 10 (IP-10) levels, baseline viral load, the presence of HCV genotypes 1a or 1b, and other baseline characteristics of the patients.

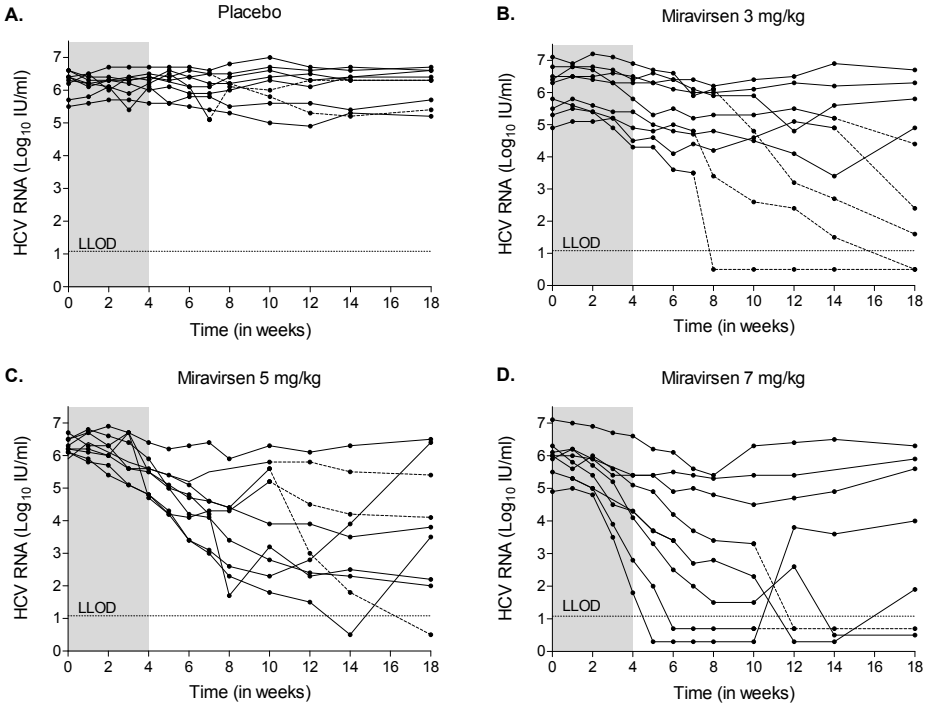

Figure 12.6 HCV RNA Levels for Individual Patients According to Study Group

Miravirsen or placebo was administered in five weekly subcutaneous injections during the first 29 days of the study (gray shading). The dashed curves in the data points represent hepatitis C virus (HCV) RNA levels after the initiation of therapy with pegylated interferon and ribavirin in 12 patients. The lower limit of detection (LLOD) was 12 IU (or 1.08 $\log_{10}$ IU) per milliliter. In the group receiving 3 mg of miravirsen per kilogram, two patients had decreases in HCV RNA levels below the LLOD after the initiation of pegylated interferon and ribavirin. In the group receiving 5 mg of miravirsen per kilogram, undetectable HCV RNA levels occurred at week 14 in one patient who was treated with miravirsen alone and at week 18 in one patient who started pegylated interferon and ribavirin after week 10. In the group receiving 7 mg of miravirsen per kilogram, four patients had undetectable HCV RNA levels with miravirsen alone: one at week 5, one at week 6, and two at week 14. Therapy with pegylated interferon and ribavirin was initiated in two patients receiving placebo (both after the week 7 visit), in five patients receiving 3 mg of miravirsen per kilogram (two after week 7, one after week 8, and two after week 14), in three patients receiving 5 mg of miravirsen per kilogram (all after the week 10 visit), and in two patients receiving 7 mg of miravirsen per kilogram (also all after the week 10 visit).

Safety

Of the 112 adverse events that were recorded for patients receiving miravirsen, 93 were grade 1, 17 were grade 2, and 2 were grade 3. Of the 31 adverse events recorded for patients receiving placebo, 23 were grade 1, 7 were grade 2, and 1 was grade 3 (thrombocytopenia, which was assessed by the investigator as moderate in severity).

Table 12.2 Adverse Events [a]

	Miravirsen			Placebo
Events	**3 mg/kg (n = 9)**	**5 mg/kg (n = 9)**	**7 mg/kg (n = 9)**	**(n = 9)**
Any adverse events				
No. of patients (%)	8 (89)	7 (78)	8 (89)	7 (78)
No. of events	29	32	51	31
Moderate or severe adverse events				
No. of patients (%)	2 (22)	1 (11)	2 (22)	2 (22)
No. of events	2	1	3	2
Most common adverse events, No. of patients (%) [b]				
Headache	3 (33)	2 (22)	4 (44)	3 (33)
Fatigue	1 (11)	3 (33)	4 (44)	3 (33)
Nasopharyngitis	3 (33)	2 (22)	1 (11)	2 (22)
Nausea	0	1 (11)	3 (33)	1 (11)
Rash	0	2 (22)	2 (22)	1 (11)
Diarrhea	2 (22)	0	0	1 (11)
Myalgia	0	2 (22)	1 (11)	1 (11)
Flu-like symptoms	2 (22)	0	1 (11)	1 (11)
Pruritus	0	1 (11)	2 (22)	1 (11)
Injection-site event [c]				
Classic reaction	0	0	2 (22)	0
Other event	1 (11)	0	3 (33)	0

[a] Listed are all adverse events that were reported throughout the 18-week study period regardless of whether patients were receiving pegylated interferon and ribavirin along with the study drug.
[b] The events listed in this category occurred in at least 15% of the patients in any study group.
[c] Classic reactions are those that are characteristic of oligonucleotide drugs; these include erythema, pruritus, persistent induration, and a burning sensation. Other events include any other injection-site reactions, including pain and hematoma.

There were no dose-limiting toxic effects or treatment discontinuations because of adverse events (Table 12.2).

During the 18-week study period, five patients in the miravirsen groups (two receiving 3 mg per kilogram, one receiving 5 mg per kilogram, and two receiving 7 mg per kilogram) and two patients in the placebo group had adverse events of moderate severity. These events included single occurrences of headache, otitis externa, pelvic bone injury after a fall, syncope, and flulike symptoms (after starting pegylated interferon and ribavirin) among the miravirsen-treated patients, and headache and a hand abscess among the placebo-treated patients. The only severe event during the study was loss of consciousness in one patient 9 weeks after the last dose of 7 mg of miravirsen per kilogram, which occurred after a fall and also resulted in pelvic bone

injury. This severe event was designated as a serious adverse event, since the patient was hospitalized overnight for observation.

Injection-site reactions that included a combination of erythema, pruritus, persistent induration, or a burning sensation are characteristic of oligonucleotide drugs and were reported in two patients in our study, both in the group receiving 7 mg of miravirsen per kilogram. These injection-site reactions were self-limited or resolved with minimal treatment. No systemic allergic reactions were observed. There were no deaths.

Among patients receiving miravirsen, biochemical safety profiles indicated a sustained decrease in levels of serum alanine aminotransferase (Figure 12.7), aspartate aminotransferase, and γ-glutamyl transpeptidase. Clinically insignificant increases in

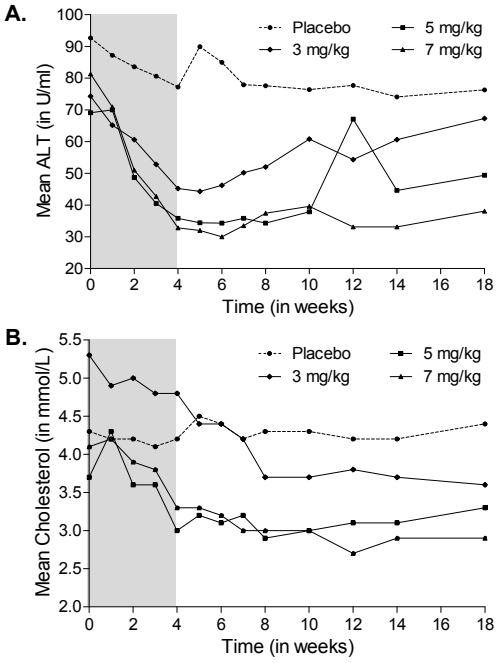

Figure 12.7 Alanine Aminotransferase and Total Cholesterol in Serum According to Study Group
This figures shows (A) alanine aminotransferase (ALT) and (B) total cholesterol levels during the study period for the placebo, 3, 5 and 7 mg/kg miravirsen-treated cohorts. Patients were administered with 5 weekly subcutaneous miravirsen doses during the first 29 days of the study (grey shaded background). Both ALT and total cholesterol showed a decrease in the three miravirsen treated cohorts, which lasted beyond the end of miravirsen treatment. The ALT and total cholesterol data were not removed in case pegylated interferon and ribavirin therapy was initiated. A small and clinically insignificant peak in the mean ALT level was seen at week 12 in the 5 mg/kg miravirsen treated cohort, which was caused by an ALT level of 251 U/L in a single patient who started Peg-IFN and RBV therapy after week 10. At week 14 the ALT level in this patient returned to normal (38 U/L).

levels of serum alkaline phosphatase and creatinine that were not dose-dependent were noted among most patients in all three groups receiving miravirsen. None of these increases exceeded the criteria for grade 1 toxicity, according to the Common Terminology Criteria for Adverse Events, version 4.0. No clinically significant changes in hemoglobin levels or total white-cell counts were noted during miravirsen administration. There were mean increases of 8 to 10% above baseline in platelet counts among patients receiving miravirsen, but there were no clinically significant changes in prothrombin time or activated partial thromboplastin time. No patient stopped miravirsen treatment or required a dose reduction because of laboratory abnormalities. As expected, a decrease in the serum total cholesterol level was found (Figure 12.7). There were no changes in the ratio of low-density lipoprotein cholesterol to high-density lipoprotein cholesterol.

DISCUSSION

In this study, five weekly injections of miravirsen, an antisense inhibitor of miR-122, produced a dose-dependent and prolonged decrease in HCV RNA levels in patients with chronic HCV genotype 1 infection. In some patients, undetectable HCV RNA levels were achieved. We observed no evidence of viral resistance.

Patients receiving miravirsen had reductions in aminotransferase levels, in contrast to the increased levels reported in those receiving phosphorothioate antisense compounds in previous studies.[18] Miravirsen treatment did not result in clinically significant changes in renal function or increases in the activated partial thromboplastin time. A gradual and prolonged non–dose-dependent reduction in cholesterol levels was observed in accordance with the effects of miR-122 antagonism on cholesterol homeostasis, consistent with data from previous studies in mice and nonhuman primates.[17,19-21]

Studies of miravirsen in animals have not indicated any adverse effects associated with the sequestration of miR-122 and subsequent upregulation of miR-122–regulated target mRNAs, suggesting that short-term inhibition of miR-122 is safe.[17,19,22] The degree of modulation of most miR-122–regulated target mRNAs is relatively small and could explain the good side effect profile. In contrast, the effects of miR-122 sequestration appear to result in a more substantial change in HCV RNA levels. The expression of several miR-122-regulated host genes has been implicated in the development of hepatocellular carcinoma, suggesting that miR-122 has tumor-suppressive effects. Although a direct causal relationship between sustained loss of miR-122 function and hepatocellular carcinoma remains to be determined, down-regulation of miR-122 has been described in hepatocellular carcinoma, with lower miR-122 levels cor-

relating with a poor prognosis.[23-26] However, Varnholt et al. have reported the up-regulation of miR-122 in HCV-induced hepatocellular carcinoma, suggesting that the role of miR-122 in HCV-derived hepatocellular carcinoma could be different from that in hepatocellular carcinomas not associated with HCV.[27] Regardless, short-term inhibition of miR-122 by miravirsen was shown to be reversible.

Reduced intrahepatic miR-122 levels, possibly related to higher expression of interferon-regulated genes, have been observed in patients who did not have a virologic response to interferon-based therapy.[28] However, other studies have not shown any association between intrahepatic interferon-regulated genes and miR-122 expression.[29] We did not find a clear association between virologic response to miravirsen and baseline IP-10 levels, IL28B genotype, or any other host and viral factors assessed in this study. However, the power of our study to identify factors associated with virologic response was limited because of the small sample size.

Our results are relevant to the consideration of miravirsen as a potential treatment for HCV infection. First, the miR-122 HCV binding sites are highly conserved, allowing the use of miravirsen in all HCV genotypes.[15] Second, we have not observed evidence of escape mutations in HCV RNA in primates or humans treated with miravirsen, indicating a high genetic barrier to resistance.[17] Third, the pharmacokinetic profile of miravirsen, with a gradual increase in trough levels representing hepatic accumulation and a prolonged tissue clearance half-life, allows once-monthly regimens, favoring patient compliance. Unlike the currently approved protease inhibitors, miravirsen is not a substrate for cytochrome P-450 and is therefore not expected to have significant drug-drug interactions.

Five patients receiving short-term miravirsen alone had undetectable HCV RNA levels, indicating the potential of miravirsen as monotherapy for chronic HCV infection. However, four of these five patients had a rebound in viral levels at the end of the study, indicating that four weeks of administration of miravirsen (at a weekly dose of 7 mg per kilogram) was insufficient to achieve a sustained virologic response in these patients. It is not clear whether regimens of miravirsen of longer duration could achieve a sustained virologic response; we are currently testing the effect of a 12-week regimen (ClinicalTrials.gov number, NCT01727934). A sustained virologic response has been achieved in several patients treated with an interferon-free regimen that combined direct-acting antiviral agents.[8,30] It is possible that miravirsen could be used as a host-targeting agent to increase the antiviral efficacy of such combination regimens by providing a continuous barrier to viral breakthrough, an approach that would seem worth testing, given the rapid clearance of direct-acting antiviral agents, potential issues of compliance with increased pill burden of current treatment regimens, and selection of resistance-associated mutations.

Because of the wide applicability of antisense therapy, the strategy we have described here may also be relevant for diseases other than chronic HCV infection. Within the field of hepatology, the inhibition of miR-122 has been associated with an improvement of steatosis in a mouse model of diet-induced obesity, suggesting a role for miR-122 antagonism in the treatment of nonalcoholic fatty liver disease.[20] Within other fields, therapeutic silencing of disease-associated miRNAs in preclinical studies of cancer and of cardiovascular and autoimmune disorders has delivered results that warrant clinical investigation.[31-33]

In conclusion, miravirsen administered in five weekly subcutaneous injections over 29 days to patients with chronic HCV infection resulted in significant virologic responses. With this study, we have shown a therapeutic effect by targeting a non-coding host miRNA.

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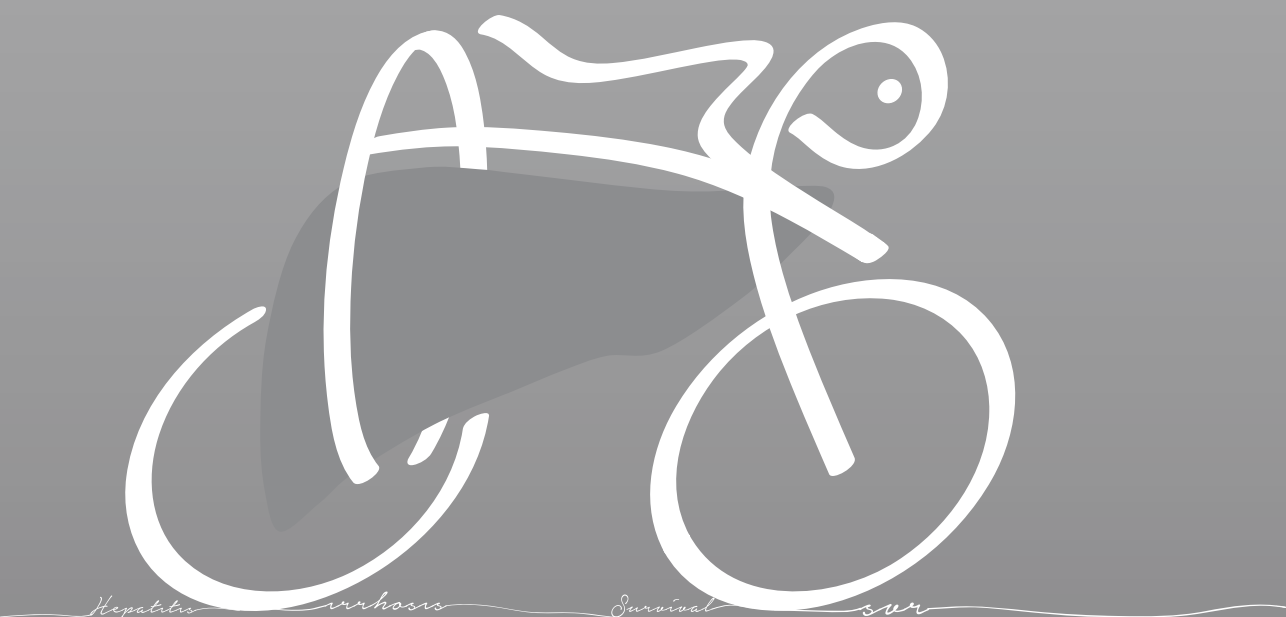
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CHAPTER 13

Long-term safety and efficacy of microRNA-targeted therapy in chronic hepatitis C patients

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ABSTRACT

Background

MicroRNA-122 (miR-122) is an important host factor for hepatitis C virus (HCV) and promotes HCV RNA accumulation. Decreased intra-hepatic levels of miR-122 were observed in patients with hepatocellular carcinoma (HCC), suggesting a potential role of miR-122 in the development of HCC. Miravirsen targets miR-122 and resulted in a dose-dependent and prolonged decrease of HCV RNA levels in chronic hepatitis C patients. The aim of this study was to establish the sustained virological response rate to peginterferon (PegIFN) and ribavirin (RBV) following miravirsen dosing and to assess long-term safety in patients treated with miravirsen.

Methods

In this multicenter, retrospective follow-up study we included 36 treatment naïve patients with chronic hepatitis C genotype 1 who received five weekly subcutaneous injections with miravirsen or placebo over a 29-day period in a phase 2a study. Patients were offered PegIFN and RBV therapy 3 weeks (3 mg/kg group) or 6 weeks (5 or 7 mg/kg group) after completion of miravirsen or placebo dosing.

Results

PegIFN and RBV therapy was started in 14/36 patients of whom 12 had received miravirsen. SVR was achieved in 7/12 patients previously dosed with miravirsen. All patients dosed with 7 mg/kg miravirsen who were subsequently treated with PegIFN and RBV achieved SVR. One patient had a prolonged undetectable HCV RNA period from week 14 to week 29 after baseline without subsequent antiviral therapy and relapsed thereafter. None of the patients treated with anti-miR-122 developed HCC or other liver-related complications.

Conclusion

No long-term safety issues were observed among miravirsen-treated patients. Targeting miR-122 may be an effective and safe treatment strategy for HCV infection.

INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded RNA virus and represents a major causative agent of chronic liver disease. Worldwide, 170 million people have a chronic HCV infection and are at risk to develop cirrhosis, leading to clinical complications such as hepatocellular carcinoma (HCC).[1,2] The aim of chronic hepatitis C treatment is to achieve a sustained virological response (SVR), which is associated with reduced occurrence of liver failure and HCC, and with prolonged overall survival.[3-5] Many highly potential direct-acting antivirals (DAA) are being assessed in clinical trials and various combinations of DAAs result in high SVR rates. While DAAs target viral proteins, such as NS3/4A protease and NS5A/B replication inhibitors, other drugs target host factors that are essential for HCV replication, such as cyclophilin A or microRNA-122 (miR-122).[6,7]

MicroRNAs (miRNAs) are small (19-24 nucleotides), non-coding, RNA molecules that are involved in various cellular processes by post-transcriptional suppression of gene expression.[8,9] MiR-122, a highly abundant miRNA expressed in the liver, regulates lipid metabolism and acts as a tumorsuppressor.[10-13] MiR-122 is also involved in HCV replication by binding to two highly conserved seed sites in the 5' UTR of the HCV genome and promotes HCV RNA accumulation by stabilizing the viral genome and stimulating its translation.[14,15] Furthermore, the miR-122-HCV complex protects the HCV genome from degradation and prevents induction of an innate immune response against HCV.[14,16] This discovery led to the development of the first successful miRNA-based therapeutic strategy wherein an anti-miR silences miR-122. In chimpanzees infected with HCV, silencing of miR-122 induced a potent and prolonged inhibition of HCV replication without viral resistance.[15] Recently, the results of the first study in which an anti-miR was administered to HCV-infected patients were presented.[7] In this phase 2a study, chronic HCV genotype 1 infected patients received five weekly injections of miravirsen, a locked nucleic acid-modified phosphorothioate oligonucleotide targeting miR-122. This resulted in a prolonged and dose-dependent decrease in HCV RNA, alanine aminotransferase (ALT) and cholesterol levels.[7] Patients were followed for an additional 14 weeks after the last dose of miravirsen and effects on HCV RNA and ALT could still be observed at the end of the study. The prolonged antiviral effect could be explained by the fact that miravirsen has a long tissue half-life (approximately 30 days) which suggests that the biological effect of miravirsen can last for weeks.

As earlier studies revealed that miR-122 has a tumor suppressive role and that mice lacking the gene encoding for miR-122 were at high risk to develop hepatosteatosis and HCC, it is of great importance to evaluate the long-term safety among the patients treated with this first anti-miR-122 therapy.[17,18] The primary objective of

this study was to assess the long-term safety and clinical efficacy of miR-122 targeted therapy among patients with chronic HCV genotype 1 infection. The secondary objective was to determine the virological response among those patients who subsequently received pegylated interferon (PegIFN) and ribavirin (RBV) therapy.

METHODS

Study population and design

This follow-up study was a retrospective analysis which assessed the long-term safety and clinical outcome of patients treated with different doses of miravirsen, with or without a subsequent course of PegIFN and RBV therapy. All 36 HCV genotype 1 infected, treatment naïve patients who previously participated in a multicenter, randomized, placebo-controlled, phase 2a study to assess the safety and efficacy of miravirsen were included.[7] In this study, patients were randomized in a 3:1 ratio to receive either miravirsen (in doses of 3 mg, 5 mg or 7 mg/kg) or placebo. Miravirsen was administered subcutaneously in five weekly doses over a 29-day period. After the administration period, patients returned for follow-up visits for a period of 14 weeks. Patients were allowed to start PegIFN and RBV therapy at the discretion of the investigator 3 weeks (patients dosed with 3 mg/kg) or 6 weeks (patients dosed with 5 or 7 mg/kg) after completion of miravirsen or placebo dosing. Patients were treated with PegIFN alfa-2a (dose 180 μg/0.5mL per week) and weight-based doses of ribavirin (1000 mg a day for those ≤75 kg and 1200 mg a day for those >75 kg). Treatment response was subdivided in virological breakthrough, virological relapse, nonresponse or SVR. Virological breakthrough refers to the reappearance of HCV RNA before treatment is completed. Virological relapse was defined as a decrease in HCV RNA below the limit of detection during treatment, but detectable HCV RNA after treatment was stopped. Nonresponse was defined as <2 $\log_{10}$ decline of HCV RNA at week 12 or HCV RNA positive HCV RNA at week 24 during treatment. SVR was defined as undetectable HCV RNA 24 weeks after treatment was stopped. A rapid viral response (RVR) was defined as undetectable HCV RNA at week 4 during treatment. Endpoints regarding safety were liver failure (including ascites, jaundice, variceal bleeding or hepatic encephalopathy), liver transplantation, HCC, hospitalization or death.

Data collection

We collected prolonged follow-up data to assess the long-term efficacy and safety. The obtained data included clinical safety data, local laboratory results, virological responses to PegIFN and RBV therapy, side effects and stage/grade of liver disease

(fibroscan or liver biopsy). The aspartate aminotransferase (AST) to platelet ratio index (APRI) score was calculated by the formula: (AST / reference AST) / (platelets x 100).

Ethics

The study was approved by the Medical Ethics Review Committee of the Academic Medical Center Amsterdam and was carried out in compliance with the protocol, the principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonised Tripartite Guideline for Good Clinical Practice and the local national laws governing the conduct of clinical research studies.

Statistical analyses

To compare the baseline characteristics and outcome measures of the study groups we used the Student's t-, one-way ANOVA, Kruskal Wallis, and χ^2 tests. A p-value of <.05 was considered statistically significant. All analyses were performed with the use of SPSS, version 20.

RESULTS

Patient characteristics

This study included 36 patients of whom 27 had received various doses of miravirsen and nine received placebo. Baseline characteristics were similar among the four study groups (Table 13.1). PegIFN and RBV therapy was initiated in 14 (39%) patients.

Virological response

Five subcutaneous injections with miravirsen resulted in a prolonged and dose-dependent decrease in HCV RNA levels.[7] The mean of the maximum reduction in HCV RNA levels (log_{10} IU/mL) from baseline was 1.2 (p=.01) for patients receiving 3 mg/kg, 2.9 (p=.003) for those receiving 5 mg/kg, and 3.0 (p=.002) for those receiving 7 mg/kg, compared with a decline of 0.4 in the placebo arm. Undetectable HCV RNA was achieved in one patient in the 5 mg/kg group and in four patients in the 7 mg/kg group. Levels of HCV RNA rebounded in most patients who were not treated with PegIFN and RBV therapy. One patient, a 43 year old female with fibrosis stage F0-F1 and HCV genotype 1b infection who was dosed with miravirsen 7mg/kg, became HCV RNA negative at study week 14 and remained this for a period of at least 15 weeks without the initiation of PegIFN and RBV therapy (Figure 12.1). This patient was followed-up frequently and experienced a virological relapse 44 weeks after miravirsen dosing, at which time the HCV RNA level (log_{10} IU/mL) was 4.37 and

Table 13.1 Baseline Characteristics [a]

	Miravirsen			Placebo
Characteristics	**3 mg/kg (n = 9)**	**5 mg/kg (n = 9)**	**7 mg/kg (n = 9)**	**(n = 9)**
Age, years, median (range)	35 (26-66)	46 (33-65)	48 (31-61)	56 (42-66)
Males, No. (%)	5 (56)	8 (89)	6 (67)	3 (33)
Race, No. (%) [b]				
Caucasian	9 (100)	9 (89)	7 (78)	7 (78)
Black	0	1 (11)	1 (11)	2 (22)
Asian	0	0	1 (11)	0
HCV RNA, $\log_{10}$ IU/mL	6.0 (±0.7)	6.2 (±0.3)	5.9 (±0.6)	6.2 (±0.4)
IL28B CC genotype, No. (%)	2 (22)	4 (44)	4 (44)	2 (22)
Subtype of HCV, No. (%)				
1a	5 (56)	7 (78)	5 (56)	6 (67)
1b	2 (22)	1 (11)	3 (33)	2 (22)
1a/1b	2 (22)	0 (0)	1 (11)	1 (11)
1a/3a	0 (0)	1 (11)	0 (0)	0 (0)
ALT, U/L	74.3 (±38.7)	69.1 (±21.4)	81.3 (±71.8)	92.7 (±37.0)
APRI score [c]	0.36 (±0.13)	0.34 (±0.07)	0.44 (±0.30)	0.93 (±0.76)
Fibrosis score, No. (%) [d]				
METAVIR F0-F1	3 (33)	6 (67)	7 (78)	4 (44)
METAVIR F2-F3	2 (22)	2 (22)	1 (11)	2 (22)
METAVIR F4	0	0	0	1 (11)
Unknown	4 (44)	1 (11)	1 (11)	2 (22)

Abbreviations: ALT; alanine aminotransferase, APRI; aspartate aminotransferase to platelet ratio index, HCV; hepatitis C virus, SD; standard deviation.

[a] Data are given as mean (± standard deviation), unless otherwise noted.

[b] Race was self-reported.

[c] APRI score was calculated by the formula: ((aspartate aminotransferase / reference aspartate aminotransferase) / platelet count [x 10^9/L] x 100);

[d] The METAVIR fibrosis score was determined by liver biopsy or liver elastography.

the ALT level (IU/L) was 109. Two weeks after the virological relapse, the HCV RNA level decreased to 3.83 with a simultaneous decrease in ALT level to 62. However, three months later, the viral load and ALT were back at the pre-treatment levels, with a HCV RNA level of 6.12 as compared to 5.92 at baseline and an ALT level of 78 compared to 82 at baseline. Population sequencing showed no nucleotide changes in the 5' UTR or amino acid differences in NS3, NS5A and NS5B regions.

Patients treated with PegIFN and RBV therapy

PegIFN and RBV therapy was started in 14 of the 36 patients of whom 2 received placebo, 5 received 3 mg/kg, 4 received 5 mg/kg and 3 received 7 mg/kg of miravirsen

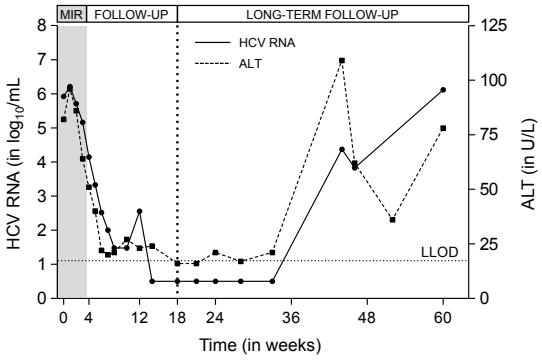

Figure 13.1 HCV RNA and ALT levels for a Single Patient with a Prolonged Antiviral Effect of Anti-miR-122 Therapy

This figure shows the prolonged antiviral effect of anti-miR-122 therapy in an individual patient. The lower limit of detection (LLOD) is 12 IU/mL (or 1.08 $\log_{10}$ IU/mL). This patient was treated with the highest dose of miravirsen (7 mg/kg) and became HCV RNA negative 14 weeks after the first dose. At week 18 the regular follow-up during the prospective phase 2a trial ended. Extended follow-up shows that this patient remained HCV RNA negative up to 29 weeks after miravirsen dosing. At 44 weeks after the first dose of miravirsen this patient had a virological relapse, with a simultaneous increase in the ALT level.

Table 13.2 Treatment Outcome of Patients Treated with Pegylated Interferon and Ribavirin [a]

	Miravirsen			Placebo
	3 mg/kg (n = 5)	**5 mg/kg (n = 4)**	**7 mg/kg (n = 3)**	**(n = 2)**
Time until start PegIFN and RBV, weeks, median (range)	4 (3-10)	6 (6-15)	7 (7-16)	3 (3-3)
Duration of PegIFN and RBV, weeks, median (range)	48 (47-48)	20 (13-47)	24 (23-24)	17 (11-22)
HCV RNA at the start of PegIFN and RBV, $\log_{10}$ IU/mL, mean (±SD)	4.2 (±1.9)	4.8 (±1.6)	2.8 (±1.2)	6.3 (±0.3)
IL28B genotype				
CC	0	0	1 (33)	0
CT	3 (60)	3 (75)	2 (67)	1 (50)
TT	2 (40)	1 (25)	0	1 (50)
SAE's [b]	1 (20)	0	1 (33)	0
RVR	1 (20)	0 [c]	3 (100)	0
SVR	3 (60)	1 (25)	3 (100)	0
Nonresponders	0	2 (50)	0	2 (100) [d]
Virological breakthrough	1 (20)	0	0	0
Virological relapse	1 (20)	1 (25)	0	0

Abbreviations: HCV; hepatitis C virus, PegIFN; pegylated interferon, RBV; ribavirin, RVR; rapid virological response (HCV RNA negative after 4 weeks of PR therapy), SAE; serious adverse event, SD; standard deviation, SVR; sustained virological response (HCV RNA negativity 24 weeks after cessation of PegIFN and RBV therapy).

[a] Data are presented as No. (%) unless otherwise noted.

[b] Hospitalization due to bronchopneumonia and trauma capitis, both during PegIFN and RBV therapy.

[c] Unknown in 3/4 patients.

[d] PegIFN and RBV therapy was stopped due to bad tolerability and insufficient viral response.

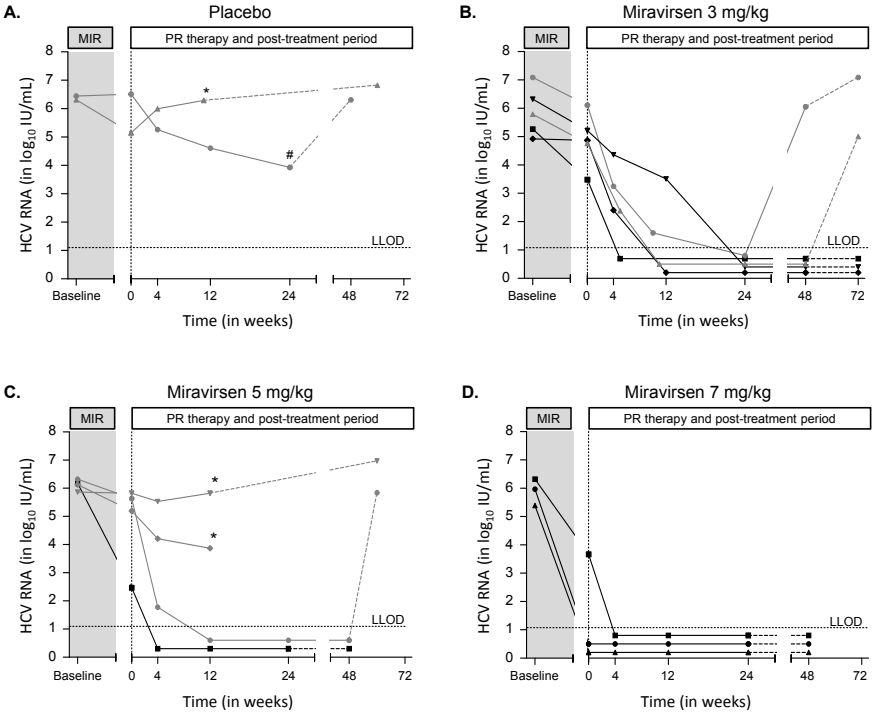

Figure 13.2 HCV RNA Levels for Individual Patients Treated with Pegylated Interferon and Ribavirin
Before the start of pegylated interferon (PegIFN) and ribavirin (RBV) therapy patients were dosed with miravirsen 3, 5, 7 mg/kg or placebo. The difference between hepatitis C virus (HCV) RNA levels at baseline and start of PegIFN and RBV therapy is simplified and illustrated in the gray shading. PegIFN and RBV therapy is started at week 0 and the treatment duration differs between the individual patients. The solid line represent the HCV RNA measurements during PegIFN and RBV therapy, and the dashed line the HCV RNA measurements post PegIFN and RBV therapy. Black lines represent all patients who achieved a sustained virological response (n = 7), and gray lines represent patients who failed on PegIFN and RBV therapy (n = 7). The lower limit of detection (LLOD) is 12 IU/mL (or 1.08 $\log_{10}$ IU/mL).
* PegIFN and RBV therapy was stopped due to nonresponse (< 2 $\log_{10}$ decline in HCV RNA at week 12).
# PegIFN and RBV therapy was stopped due to nonresponse (positive HCV RNA assay at week 24 during therapy).

(Table 12.2). The dose of RBV was reduced in two patients during treatment due to anemia and gingival bleeding. SVR was achieved in 7 (58%) of the patients previously treated with different doses of miravirsen. All patients (n = 3) who received the highest dose of miravirsen (7 mg/kg) and were treated with PegIFN and RBV therapy achieved RVR and SVR. Of these patients, 2 had undetectable HCV RNA at the start of PegIFN and RBV therapy (Figure 12.2). The median treatment duration of patients who achieved SVR was 24 weeks (interquartile range [IQR] 14-48), compared to 47 weeks (IQR 24-48) in patients without SVR (p=.01). Mean HCV RNA levels ($\log_{10}$

IU/mL) at the start of PegIFN and RBV therapy were significantly lower for patients achieving SVR compared to patients who did not achieve SVR, respectively 3.1 versus 5.2 (p=.029). The interleukin-28B (IL28B) genotype distribution of patients achieving SVR was CC (n = 1), CT (n = 4) and TT (n = 2). Therapy failed in five patients which was due to nonresponse (n = 2), virological relapse (n = 2), and virological breakthrough after therapy cessation due to hospitalization for a pneumonia (n = 1) (Table 12.2). The IL28B genotype distribution of patients who failed PegIFN and RBV therapy was CT (n = 4) and TT (n = 1). Two serious adverse events occurred during PegIFN and RBV therapy. One patient was hospitalized due to bronchopneumonia and one patient was observed overnight in the hospital due to loss of consciousness that occurred after a fall. Both events were considered unrelated to miravirsen dosing.

Long-term safety

Patients were followed up to 35 months after the start of miravirsen therapy, with a median duration of 24 months (IQR 14-28). None of the patients were diagnosed with HCC or other cirrhosis-related complications. There were no clinically relevant events or hospitalizations during follow-up, other than the serious adverse events that occurred during PegIFN and RBV therapy. None of the patients died. Mean ALT levels (U/L) at follow-up were lower compared to baseline in patients who achieved SVR, respectively 50 at baseline versus 24 at the end of follow-up (p=.03). Mean ALT levels were comparable between baseline and end of follow-up among patients who did not achieve SVR or did not start PegIFN and RBV therapy, 78 vs 67 (p=.45) and 101 vs 100 (p=.97), respectively. Median APRI score of patients treated with miravirsen was comparable between baseline and follow-up, 0.34 vs 0.32 (p=.97), respectively. There was no significant difference between baseline and end of follow-up median APRI score in patients who achieved SVR, 0.32 vs 0.15 (p=.11), respectively, or in patients who did not achieve SVR, 0.44 vs 0.48 (p=.57), respectively.

DISCUSSION

Here we present the results of the first study to assess long-term safety of miR-targeted therapy in humans. No long-term safety problems were observed among the chronic HCV-infected patients that were treated with miravirsen, up to 35 months following therapy. None of the patients treated with anti-miR-122 developed HCC or cirrhosis-related morbidity such as ascites or variceal bleeding. In addition, antiviral therapy with PegIFN and RBV following miravirsen resulted in SVR in 58% of HCV genotype 1, treatment-naïve patients.

MiR-122 is believed to have a tumor suppressive role and has been related to the development of HCC. In vitro studies showed that miR-122 levels were reduced in human HCC cells compared to normal hepatocytes, and that restoration of miR-122 in HCC cells reversed their malignant phenotype and tumorigenic properties.[10,19] Short-term inhibition of miR-122 using antisense oligonucleotides for 5 weeks was well-tolerated in adult mice, and these mice did not develop HCC.[11] In an obesity mouse model induced by a high fat diet, miR-122 inhibition led to a reduction of steatosis.[11] In contrast, mice lacking the gene encoding for miR-122 developed microsteatosis and inflammation of the liver that progressed to steatohepatitis and HCC later on in life.[17,18] It was postulated that hepatocarcinogenesis was initiated by activation of several oncogenic pathways and the production of pro-tumorigenic cytokines.[17] However, the biological and clinical effect of transient inhibition of miR-122 and the subsequent long-term risk of HCC development in humans is still unknown and should be carefully studied in future drug development trials assessing miR-122 inhibiting agents.

It was demonstrated that the serum miR-122 level is a sensitive marker for inflammatory activity in the liver and strongly correlates with serum ALT activity.[20,21] Furthermore, several studies showed that the expression of miR-122 was related to the progression of hepatic fibrosis and that serum and hepatic miR-122 levels decreased significantly if the stage of hepatic fibrosis progressed.[22,23] In this study we compared baseline and end of follow-up fibrosis stage of patients treated with miravirsen using the APRI score. We demonstrated that patients treated with miravirsen showed no difference in APRI score between baseline and end of follow-up. This finding suggests that there is no increase in fibrosis in patients treated with anti-miR-122 therapy. In addition, it was suggested that miR-122 could predict treatment response to PegIFN and RBV therapy in chronic hepatitis C patients. Several studies demonstrated that low pre-treatment levels of hepatic and serum miR-122 were associated with a poor virological response to PegIFN and RBV therapy, although another study did not confirm this finding.[24-27] Furthermore, it is established that patients with a pre-activated interferon system, which thus express hundreds of interferon-stimulated genes (ISGs) at high levels before treatment, are poor responders to interferon-based therapies.[28] A reduced hepatic miR-122 level was inversely correlated with a high ISG expression in nonresponders.[25,29,30] MiR-122-targeted therapy in HCV-infected chimpanzees induced a down-regulation of ISGs in their livers, and thus reverted the activation of the endogenous interferon system.[15] In the current study, one-third of the patients previously dosed with miravirsen started PegIFN and RBV therapy. We demonstrated that patients treated with miravirsen had similar responses rates to PegIFN and RBV therapy as would be expected in treatment-naïve patients with chronic HCV genotype 1 infection. In fact, all patients who were treated with the

highest dose of miravirsen achieved RVR and subsequent SVR with a short-term treatment course of 24 weeks of PegIFN and RBV. This favorable treatment response might be explained by the low baseline HCV RNA levels at the time PegIFN and RBV was initiated, however a possible relationship with normalization of ISG levels, permitting the endogenous interferon pathway to respond to therapy, should also be considered.

Compared to DAAs, which have a half-life of several hours, miravirsen has a long tissue half-life of approximately four weeks. It was shown that miravirsen does not only target mature miR-122, but also suppresses the biogenesis of miR-122 at the primary- and precursor-miRNA levels in vitro, which could explain this prolonged antiviral effect.[31] In this context, the single patient who remained HCV RNA negative for more than 7 months after the last dose of miravirsen is illustrative. The possibility of infection with a new virus or the development of viral resistance was excluded by population sequencing.

A limitation of this study is the small number of patients, which is due to the fact that this study was the first to administer an anti-miR to humans. Furthermore, there was only one patient with fibrosis stage F4 included, which made it difficult to evaluate the clinical effect of miR-122 inhibition in relation to cirrhosis. Another limitation of this study was that the extended follow-up was not part of the prospective study design, which led to a variation in follow-up duration. Nevertheless, the current long-term clinical safety evaluation remains of great importance, especially with respect to the potential risk of HCC. In fact, this theoretical risk to induce HCC by miR-122 suppression is the main reason why the Food and Drug Administration now requests a total follow-up duration of five years for patients treated with anti-miR-122 therapy. Since the initial follow-up period of these patients was 18 weeks, this study is the first to provide long-term safety data on anti-miR-122 therapy in humans. Currently, a clinical trial assesses 12 week regimen of miravirsen monotherapy is ongoing. This trial will also evaluate the virological efficacy and safety of miR-122 inhibition as a therapeutic target for HCV infection. In general, the rapid progression of miRNA research and the possible clinical implications of miR-targeted therapy outside of the field of hepatitis C may lead to comparable trials in other diseases. Our long-term safety experiences with the first miR-targeted therapy may be of relevance for these future drug development trials as well.

In conclusion, no long-term safety problems were observed in miravirsen-treated patients and targeting of miR-122 may be an effective treatment strategy for HCV-infected patients.

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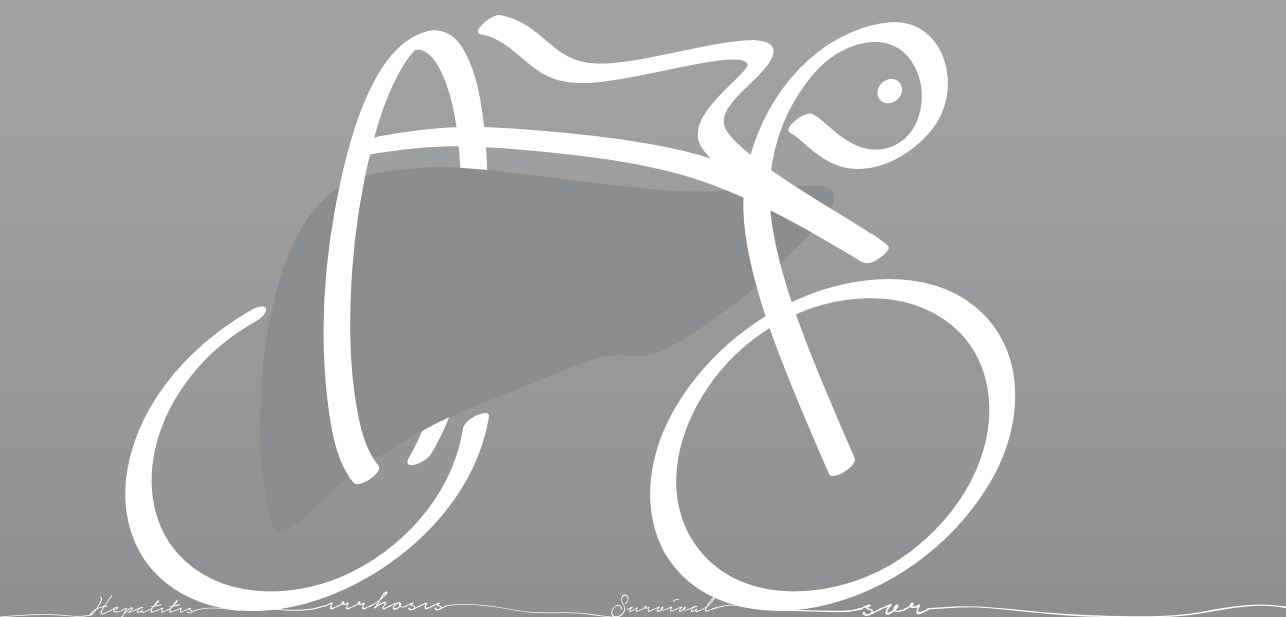
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CHAPTER 14

Sensitive detection of hepatocellular injury in chronic hepatitis C patients with circulating hepatocyte-derived microRNA-122

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ABSTRACT

Introduction

As chronic hepatitis C patients with progressive disease can present themselves with normal alanine aminotransferase (ALT) levels, more sensitive biomarkers are needed. MicroRNAs are newly discovered small noncoding RNAs that are stable and detectable in the circulation. We aimed to investigate the association between hepatocyte-derived microRNAs in serum and liver injury in patients with chronic hepatitis C.

Methods

The hepatocyte-derived microRNA-122 (miR-122) and microRNA-192 (miR-192) were analyzed in sera of 102 chronic hepatitis C virus (HCV)-infected patients and 24 healthy controls.

Results

Serum levels of miR-122 and miR-192 correlated strongly with ALT (R=0.67 and R=0.65, respectively, p<.001 for both). Median levels of miR-122 and miR-192 in HCV-infected patients were 23 times and 8 times higher as in healthy controls (p<.001 for both). Even within the HCV-infected patients with a normal ALT (n = 38), the levels of miR-122 and miR-192 were 12 times and 4 times higher compared with healthy controls (p<.001 for both). Multivariate logistic regression analyses showed that only miR-122 was a significant predictor of the presence of chronic HCV infection (p=.026). Importantly, miR-122 was also superior in discriminating chronic HCV-infected patients with a normal ALT from healthy controls compared with the ALT level (area under the curve [AUC]=0.97 vs AUC=0.78, p=.007).

Conclusion

Our study confirmed that liver injury is associated with high levels of hepatocyte-derived microRNAs in circulation and demonstrated that in particular miR-122 is a sensitive marker to distinguish chronic hepatitis C patients from healthy controls. More sensitive blood markers would benefit especially those patients with minor levels of hepatocellular injury, who are not identified by current screening with ALT testing.

INTRODUCTION

MicroRNAs (miRNAs), a class of small noncoding regulatory RNA molecules of approximately 22 nucleotides in size, are involved in various cellular processes by post-transcriptional suppression of gene expression.[1,2] Hepatocytes express a distinct set of miRNAs of which microRNA-122 (miR-122) is most abundant as it constitutes approximately 70% of the total miRNA population in the liver.[3-6] MiR-122 has been implicated in the hepatic cholesterol metabolism, iron homoeostasis and was shown to be an important host factor for efficient replication of the hepatitis C virus (HCV).[7-11] Decreased levels of miR-122 in liver tissue have been associated with cirrhosis, (advanced) hepatocellular carcinoma (HCC) and a poor virological response to pegylated interferon and ribavirin treatment in patients with chronic hepatitis C (CHC).[12-15]

Recently, miRNAs were shown to be detectable and highly stable in the circulation, after which their potential as easily accessible biomarkers was shown in different tumour types and nonmalignant diseases.[16-18] The release of hepatocyte-derived miRNAs (HDmiRs) into the circulation was first described for drug-induced acute liver injury in rodents.[19,20] Importantly, the elevation of miR-122 in blood occurred prior to or in the absence of increasing alanine aminotransferase (ALT) levels or histopathological changes.[19,20] Furthermore, in contrast to ALT, serum miR-122 was shown to be specific for liver injury as other tissues have no or only minor expression of this miRNA.[3,5,6] Whereas the serum ALT level increased because of skeletal muscle injury, miR-122 remained unaffected.[19,21]

Although a chronic infection with HCV is a major cause of cirrhosis, HCC and end-stage liver disease, many patients with CHC experience none or only nonspecific symptoms.[22] As a consequence, the infection is frequently diagnosed only after routine blood tests show an elevated ALT level. However, also in the absence of elevated ALT levels, patients can present with significant fibrosis or even cirrhosis.[23-25] Diagnosing CHC patients with normal ALT is an important clinical challenge as disease severity and subsequently the need for antiviral therapy should be evaluated irrespective of the ALT level.[26] To overcome the lack in sensitivity of the currently used surrogate markers for liver injury, new biomarkers that improve diagnostics are needed.

The aim of this study was to investigate the association between circulating levels of HDmiRs and liver injury, and their potential to serve as sensitive diagnostic biomarkers in the setting of CHC.

METHODS

Patient samples

Stored serum samples (-80 °C) from 102 patients with CHC were retrospectively analyzed, 38 with a normal and 64 with an elevated ALT level. All patient samples have been randomly collected at the outpatient clinic of our tertiary referral hospital. The diagnosis of CHC was based on the detection of anti-HCV antibodies and consistent detection of HCV RNA, for at least 6 months. Patients were not included in case of co-infection with hepatitis B virus or the human immunodeficiency virus or when treated for their CHC infection within 6 months prior to the available serum sample. Twenty-four voluntary blood donors without a relevant medical history and without obesity, use of medication and/or alcohol abuse were studied as a healthy control (HC) reference population; all had a normal ALT. Additionally, 23 patients with a sustained virological response (SVR) were analyzed.

Serum ALT and miRNA levels were all assessed in samples obtained within a maximum of 3 months from the time of liver biopsy (if available). The upper limit of normal (ULN) for the ALT level was defined as 40 U/mL for men and 30 U/mL for women, consistent with the cut-off level in our hospital's clinical laboratory. Available liver biopsy samples were assessed by a single experienced pathologist blinded to ALT and miRNA levels, using the Ishak inflammation and fibrosis score.[27]

All subjects gave informed consent, and ethical approval was obtained from the Ethics Committee for Medical Research in Rotterdam, the Netherlands, in accordance with the declaration of Helsinki.

RNA isolation

Total RNA was extracted from 200 μL serum with the miRNeasy Mini kit (Qiagen, Hilden, Germany) using a modified protocol. For isolation, 1.5 mL (7.5 x volume) of Qiazol lysis reagent was added and extensively mixed by vortexing. Chloroform (300 μL) was added, and after centrifugation (15 min, 16.000 RCF), 800 μL of an aqueous RNA-containing layer was obtained, which was further processed according to the manufacturer's protocol (Qiagen). We normalized for initial serum input, as RNA extracted from serum could not be quantified because of its low concentration and validated normalization methods are currently lacking.

Reverse transcription and real-time polymerase chain reaction (RT-PCR)

The TaqMan microRNA Reverse Transcription kit (Applied Biosystems, Carlsbad, CA, USA) was used to prepare cDNA, for multiple miRNAs in one reaction, using a modified protocol. Every multiplex cDNA reaction consisted of 0.4 μL dNTP mix, 1.35 μL Multiscribe RT enzyme, 2.0 μL 10 x RT buffer, 0.25 μL RNase inhibitor, 1.0 μL

of each RT primer and 7.5 μL of diluted template RNA. A total reaction volume of 20 μL was obtained by adding nuclease-free water. Two miRNAs reported to be highly expressed in hepatocytes, miR-122 and microRNA-192 (miR-192), were selected for cDNA synthesis and quantitative RT-PCR. MicroRNA-191 (miR-191) is not expressed in the liver but highly expressed in blood and was therefore selected to serve as a control for quality. The cDNA reactions were performed according to the manufacturer's instructions, and the obtained cDNA was diluted to a total volume of 200 μL (1:10 dilution).

All PCRs were carried out in duplicate and according to the manufacturer's protocol. Each reaction consisted of 10 μL Taqman universal PCR mastermix, 0.5 μL miRNAspecific PCR primer (Applied Biosystems) and 5.0 μL of the previously diluted cDNA. The total volume of each PCR was adjusted to 20 μL by adding 4.5 μL of nuclease-free water. Serum miRNA level was calculated with the 2^{-Ct} method, where Ct is the threshold cycle. The miRNA level is expressed as the fold change, relative to the lowest level in our cohort.

Statistical analyses

Comparative statistics between two or more groups were generated using the Mann-Whitney U or the Kruskal-Wallis test. Correlations were analyzed using the Spearman's rank correlation coefficient. Influence of baseline characteristics on the miRNA level was examined by linear regression. Logistic regression models and receiver operating characteristic curve analyses were used to assess the accuracy of the different biomarkers to detect the presence of CHC. All statistical tests were two-sided, and a p-value <.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

Patient characteristics

The characteristics of the subjects are shown in Table 14.1. Of the 102 patients with CHC, 66 (64.7%) were men and 76 (74.5%) were infected with HCV genotype 1. For 91 of 102 (89%) patients, a viral load was available and 60 (58.8%) had a viral load above 600.000 IU/mL. Except for the ALT level, none of the differences in the characteristics between the CHC patients' groups with a normal or elevated ALT reached statistical significance. Although all below the ULN, the HC had a significant 1.5-fold lower mean ALT level (19.1, standard deviation [SD] ± 10.3) compared with the normal ALT CHC patients (29.1, SD ± 6.5, p<.001).

Table 14.1 Patient Characteristics [a]

Characteristics	CHC patients normal ALT (n = 38)	CHC patients elevated ALT (n = 64)	Healthy Controls (n = 24)	SVR patients (n = 23)
Age, years [b]	49.9 (±10.9)	47.9 (±10.0)	35.3 (±11.5)	51.3 (±8.2)
Males (%)	76.3	57.8	54.2	52.2
BMI, kg/m^2	25.4 (±4.0)	26.6 (±5.1)	24.3 (±3.7)	26.1 (±3.8)
HCV genotype 1/2/3/4/5/6 (%)	76/5/11/5/0/3	73/8/17/3/0/0	-	-
HCV RNA >600,000 IU/mL (%)	53.1	72.8	-	-
ALT, in U/L [b]	29.1 (±6.5)	101.1 (±69.2)	19.1 (±10.3)	21.2 (±7.1)
Cirrhosis (%)	26.3	14.3	-	-

Abbreviations: ALT; alanine aminotransferase, BMI; body mass index (calculated as weight in kilograms divided by height in meters squared), CHC; chronic hepatitis C.

[a] Data are presented as mean (±standard deviation), unless otherwise noted.

[b] p<.001 among the groups of patients.

Patients with chronic hepatitis C have elevated serum levels of HDmiRs

Both miR-122 and miR-192 as well as the control miR-191 were detectable in the serum samples from patients with CHC and healthy individuals. As shown in Figure 14.1, the levels of miR-122 and miR-192 were significantly higher in serum of patients with CHC compared with the HC. In the whole group of patients with CHC, the median level of miR-122 was 23.4 times higher than in HC (levels were 368.4, interquartile range [IQR] 169.0-707.9, vs 15.8, IQR 8.2-35.8, p<.001). Interestingly, the subgroup of CHC patients with an ALT level below the ULN already showed an 11.6-fold higher median miR-122 level compared with HC (182.6, IQR 90.0-372.9, p<.001). Although less pronounced, similar findings were also observed for miR-192. Patients with CHC (71.8, IQR 33.8-120.9) had an 8.3-fold higher and the subgroup of normal ALT patients (35.9, IQR 22.7-82.2) a 4.1-fold higher median miR-192 level compared with HC (8.7, IQR 1.9-20.9, p<.001 for both). Median miR-191 levels, tested to control the quality of RNA isolation, varied to a lesser extent, but patients did show higher levels (29.9, IQR 16.4-45.1) than HC (10.1, IQR 2.8-22.2, p<.001).

Linear regression analyses, adjusted for the presence of HCV infection, showed no significant association between the age, body mass index or gender and the circulating level of both HDmiRs. Within the patients with CHC, the viral load and HCV genotype (genotype 1 vs non-1) were also not associated with serum miR-122 or miR-192 levels.

HDmiR serum levels correlate with liver injury markers

Both miR-122 and miR-192 showed a strong correlation with the ALT level (R=0.67, p<.001 and R=0.65, p<.001, respectively) (Figure 14.1). The levels of the two HDmiRs were strongly linked with each other (R=0.80, p<.001). Similar results were found

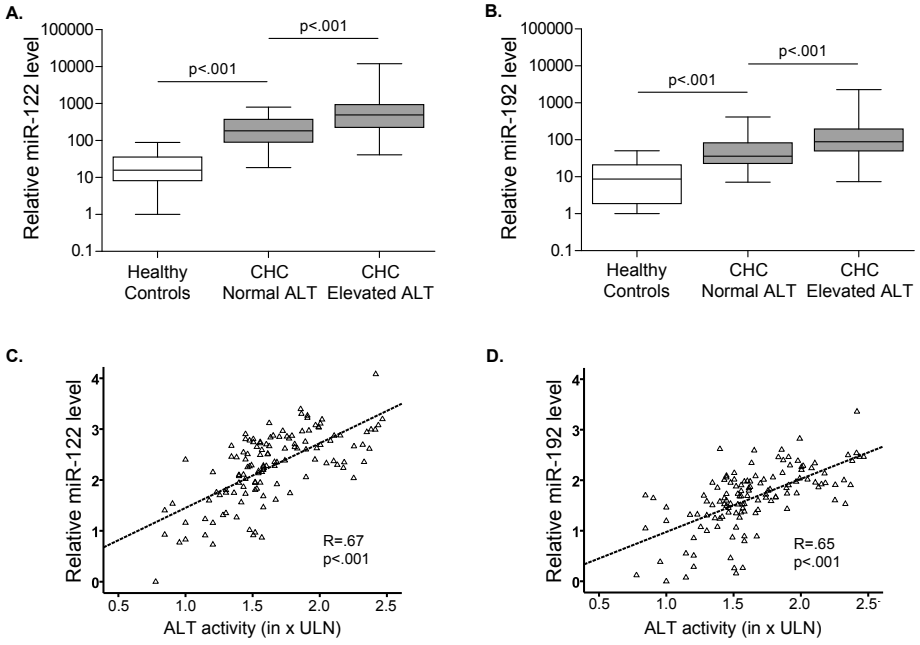

Figure 14.1 Hepatocyte-Derived MicroRNAs and ALT

Serum levels of hepatocyte-derived microRNAs in relation to ALT in patients with chronic hepatitis C (CHC). The median relative level of miR-122 (A) was 11.6-fold higher in sera of CHC patients with a normal ALT and 31.5-fold higher in sera of CHC patients with elevated ALT, as compared with healthy controls. For miR-192 (B), a similar trend was found, with a 4.1-fold higher level in normal ALT patients and a 10.2-fold higher level in elevated ALT patients. The boxes represent the median and quartiles, and the whiskers represent the 1st and 99th percentile of the relative microRNA levels. Both miR-122 (C) and miR-192 (D) showed a strong correlation with alanine aminotransferase (ALT) (data log-transformed). The correlation coefficient (R) is calculated using the Spearman's correlation test.

for aspartate aminotransferase (AST), with a Spearman's correlation coefficient of R=0.62 for miR-122 and R=0.63 for miR-192 (p<.001 for both). In contrast, the control miR-191 – which is not expressed in hepatocytes – did not show any correlation with ALT (R=0.02, p=.808) or AST (R=0.10, p=.304).

Additional liver-related blood tests were available for the patients with CHC only. The gamma-glutamyltransferase level was found to correlate moderately with miR-122 (R=0.26, p=.013) and miR-192 (R=0.33, p=.001), while alkaline phosphatase only showed a significant association with miR-192 (R=0.28, p=.006). Serum albumin and bilirubin levels did not show any correlation with miRNA levels, and neither did the viral load.

The Ishak inflammation score was blindly assessed in 52 CHC patients with an available liver biopsy around the time of the serum sampling. The majority of the liver biopsies belonged to patients with an elevated ALT level (49 of 52, 94%), and the

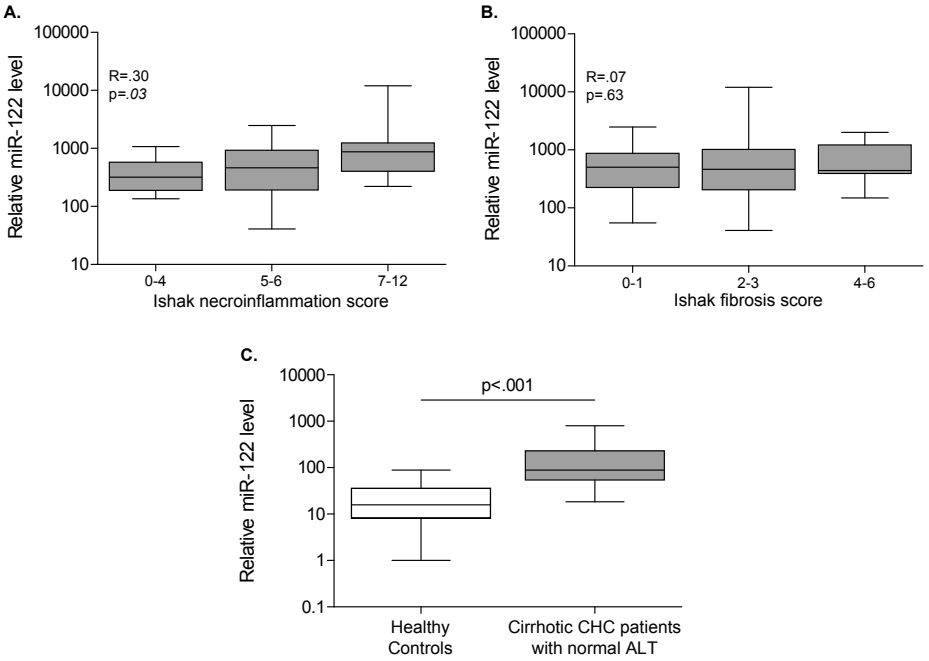

Figure 14.2 Relation Between MiR-122 Serum Levels and Liver Histopathology
Correlation between the relative serum levels of miR-122 and the Ishak necroinflammation (A) and Ishak fibrosis (B) score in the 52 blindly assessed liver biopsies. The correlation coefficient (R) is calculated using the Spearman's correlation test. Although cirrhosis was associated with a lower serum miR-122 levels, the median relative serum miR-122 level was significantly higher in the 10 cirrhotic chronic hepatitis C (CHC) patients with a normal ALT compared with healthy controls (C). The boxes represent the median and quartiles, and the whiskers represent the 1st and 99th percentile of the relative miR-122 level.

median Ishak inflammation score was 6.0 (IQR 4.3-7.0). MiR-122 showed a significant positive correlation with the inflammation score (R=0.30, p=.031), while miR-192 did not (Figure 14.2).

Presence of cirrhosis is associated with lower circulating levels of miR-122

The median Ishak fibrosis score in the 52 assessed liver biopsies was 3 (IQR 1-4) and did not correlate with either HDmiR level (Figure 14.2). Next, the association between serum miR-122 levels and the presence of cirrhosis was assessed by linear regression analysis. In total, 19 patients were classified as cirrhotic by liver biopsy, liver elastography or presence of oesophageal varices in the absence of portal vein thrombosis. Corrected for age, gender and ALT group (normal vs elevated), cirrhosis was associated with lower circulating miR-122 levels (β=-0.229, p=.037) but not with miR-192 (β=-0.120, p=.216). Importantly, within the normal ALT group, 10 patients were classified as having cirrhosis, and the median serum miR-122 level (89.1, IQR

54.1-228.8) in these 10 patients was still 5.6-fold higher than within the HC (p<.001) (Figure 14.2).

Serum miR-122 is superior to ALT in predicting the presence of chronic HCV infection

Corrected for age, the odds for the presence of CHC increased with rising ALT, miR-192 or miR-122 levels (Table 14.2). Importantly, miR-122 remained the only serum marker significantly associated with CHC in the multivariate model, without a significant additional benefit of miR-192 or ALT.

The power to discriminate patients with CHC from HC was significantly higher for miR-122 (area under the curve [AUC]=0.99, 95% CI 0.97-1.00) compared with ALT (AUC=0.91, 95% CI 0.85-0.97, p=.015) or miR-192 (AUC=0.91, 95% CI 0.86-0.97, p=.010). As expected, the performance of ALT declined in repeated receiver operating characteristics (ROC) curve analyses among those CHC patients with normal ALT

Table 14.2 Logistic Regression Analyses for the Presence of Chronic HCV Infection [a]

	Univariate marker analysis		Multivariate marker analysis	
	Exp(β)	p-value	Exp(β)	p-value
miR-122	1.094	.003	1.070	.026
miR-192	1.133	<.001	1.076	.252
ALT	1.141	<.001	1.063	.353

Abbreviations: ALT; alanine aminotransferase, HCV; hepatitis C virus.

[a] All analyses are corrected for age, as this significantly differed between the patients with CHC and healthy controls. All three biomarkers were included as continuous variables.

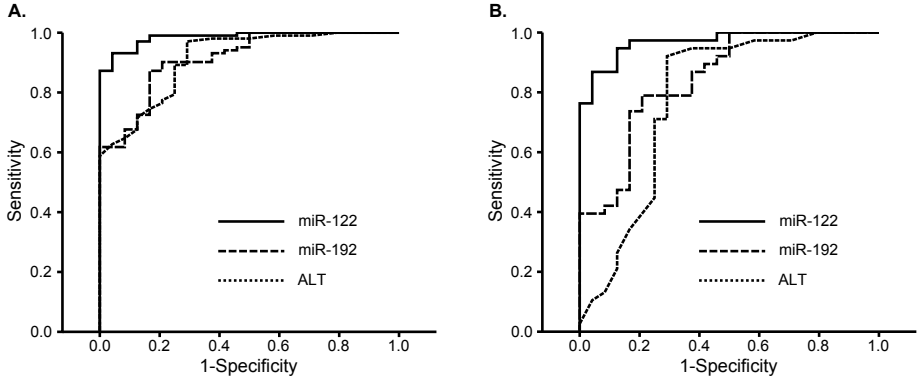

Figure 14.3 Receiver Operating Characteristic Curves

Receiver operating characteristic curves of serum miR-122, miR-192 and alanine aminotransferase (ALT) for discriminating healthy controls from all the patients with chronic hepatitis C (A) and only those patients with a normal ALT level (B).

(AUC=0.78, 95% CI 0.64-0.92). This large decline was not seen for miR-122, which retained its strong discriminating ability (AUC=0.97, 95% CI 0.92-1.00, p=.007) (Figure 14.3).

MiR-122 remains elevated shortly after SVR

Although liver inflammation and fibrosis can remain for quite some time after SVR, the ALT levels in general normalize quickly. We therefore tested the HDmiRs in 23 SVR patients. Serum samples were obtained after a median of 1.5 years (IQR 0.5–1.7) following SVR. Indeed, there was no significant difference in the ALT level between SVR patients (mean 21.2, SD ± 7.1) and HC (19.1, SD ± 10.3, p=.292) (Table 1, Figure 14.4). However, the median miR-122 serum level in these SVR patients (102.7, IQR 33.0-197.1) was 6.5 times higher compared with HC (p<.001), but 3.8 times lower compared with actively infected patients (p<.001) (Figure 14.4). Adding the SVR patients did not significantly alter the correlation between miR-122 and ALT (R=0.66, p<.001).

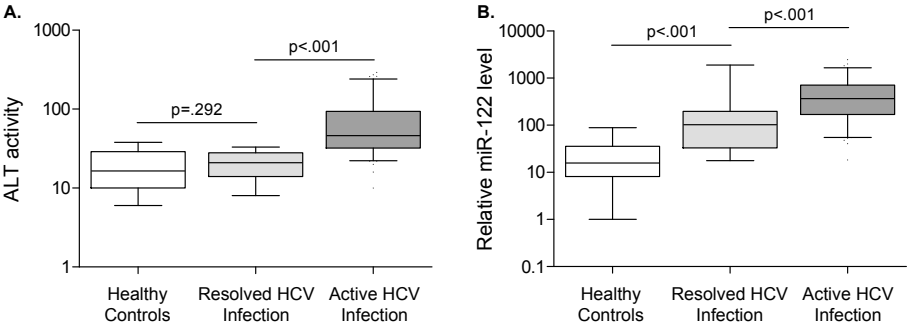

Figure 14.4 Liver Injury Markers in Patients with a Sustained Virological Response
Patients with a sustained virological response (resolved hepatitis C virus [HCV] infection) showed similar ALT (A) levels but higher serum levels of miR-122 (B) compared to healthy controls. The boxes represent the median and quartiles, and the whiskers represent the 1st and 99th percentile of the ALT and relative miR-122 levels.

DISCUSSION

New sensitive biomarkers are necessary to improve screening for liver disorders, especially for those diseases with low levels of liver injury. Indeed, most patients with chronic viral hepatitis present without (specific) symptoms, and many have normal ALT levels. Lack of diagnosis is one of the main reasons for the low treatment rate and the subsequent limited overall effect of antiviral therapy on liver-related deaths in patients with CHC.[28] In this study, we assessed the potential of the hepatocyte-

derived miR-122 and miR-192 to serve as diagnostic or screening biomarkers for the presence of chronic HCV infection. We demonstrate that circulating levels of the liver abundant miR-122 and miR-192 are detectable and elevated in serum of patients with CHC. Based on the correlation with the biochemical and histopathological degree of liver damage, these two miRNAs can be considered markers of liver injury. Our findings are in concordance with prior results in patients with chronic viral hepatitis as well as other forms of liver injury and contribute to the concept that HDmiRs may be used as biomarkers for many liver diseases.[20,29,30]

A key finding in our study concerns the elevation of serum miR-122 and miR-192 in CHC patients with normal ALT levels, suggesting these miRNAs to be more sensitive to detect HCV-induced liver injury. This is in concordance with prior results of our group in the setting of liver transplantation, which showed patients with low ALT to have an eightfold higher miR-122 in serum compared with HC.[31] The ROC analyses showed that miR-122 has an improved sensitivity for the detection of CHC over ALT, even in the subset of CHC patients with a normal ALT level. Importantly, as a new liver injury marker would ideally detect those patients who are currently not identified, miR-122 retained its high discriminating ability in the normal ALT subset of our CHC cohort. An improved sensitivity is further suggested by our finding of elevated miR-122 but similar ALT serum levels in SVR patients. This could be explained by the fact that liver histopathology is not immediately normalized upon achievement of SVR.[32] Unfortunately, data on liver inflammation were not available for SVR patients. The median 1.5 years following SVR in our study might be too short for serum miR-122 to return to similar levels as in HC.

Another study in patients with CHC by Bihrer et al. showed, in contrast to our study, comparable circulating miR-122 levels in normal ALT patients and healthy volunteers.[33] This difference might partly be explained by a different selection of patients and controls. The investigators restricted their inclusion to persistently normal ALT patients and do not provide data on the ALT level within the small group of healthy volunteers. The improved sensitivity of miR-122 is supported by the initial drug-induced liver injury studies, describing elevated miR-122 levels before ALT elevations or histopathological changes.[19,20] Recently, inactive chronic hepatitis B virus carriers and hepatitis B e antigen-negative patients with normal ALT were shown to have significantly elevated miR-122 levels compared with HC as well.[34] Our results are also in line with a previous study which found that serum miR-122 was superior to ALT in discriminating patients with chronic hepatitis B from HC.[30]

When restricting our analyses to subjects with an ALT level below the ULN, patients had a significantly higher mean ALT level compared with HC and ROC analysis showed ALT to maintain some discriminating power for CHC. The sensitivity of ALT to identify patients with CHC could indeed be increased by lowering the current

suboptimal cut-off level.[35] Our conclusion of an improved sensitivity of miR-122 over ALT is, however, based on logistic regression and ROC analyses which both do not take any cut-off for continuous variables into consideration. Furthermore, lowering the ULN for ALT would also decrease the specificity, which is thought to translate into many false positive results with major unwanted impacts on our healthcare system.[36] This discordance between the presence of a liver disease and the serum ALT level can, at least in part, be explained by apoptosis of hepatocytes.[37] Apoptosis is a genetically programmed form of cell death that prohibits the general release of intracellular constituents and is considered to be a major pathophysiological process in chronic viral hepatitis.[37,38] Although the M30 antigen is a specific apoptosis marker that indeed showed an increased sensitivity over ALT for the detection of patients with CHC, its serum level was also found to be elevated because of injury of other organs than the liver.[39-41] As miR-122 is almost exclusively expressed in the liver, organ specificity is not believed to be a major issue.[3-6] Important for a screening biomarker on the other hand, circulating miR-122 does not seem specific for one type of liver disease, as the blood level of miR-122 appears to rise because of liver injury of various aetiologies.[29-31,42]

The patients with CHC in our cohort were significantly older than the HC, but this age difference is unlikely to be the cause of the elevated serum levels of both HDmiRs because age was not associated with miRNA levels by linear regression analyses. The presence of cirrhosis did have a significant influence and lowered the circulating miR-122 level. This finding is in line with the described inverse relation between the expression of miR-122 in the liver and fibrosis stage, possibly due to a decreased number of functional hepatocytes in a cirrhotic liver.[12]

MiR-191, which is expressed in blood, was detectable in all samples, indicating proper RNA extraction without the need to exclude samples from the analysis. Although not correlated with the currently used liver injury markers, miR-191 did show slightly elevated levels in sera of patients with CHC. Factors associated with miR-191 levels are insufficiently elucidated, and the reason for this elevation remains speculative. It could be caused by an upregulation of miR-191 in the livers of patients with CHC as miR-191 reduces apoptosis and stimulates proliferation.[43] The presence of a chronic infection could also influence the level of miR-191 in lymphocytes and subsequently in serum.[44]

Apart from its diagnostic potential, miR-122 received even more attention for its role as a therapeutic target in the field of CHC. Targeting this miRNA was explored after the HCV was found to have multiple seed regions for miR-122 to bind the virus, by which the viral replication was facilitated.[10,45] Blocking miR-122 with a short term-dosed, locked nucleic acid–modified phosphorothioate oligonucleotide (Miravirsen, Santaris Pharma) resulted in a long-lasting viremic decline in HCV-infected

chimpanzees and humans.[11,46] Despite this vital interaction between host and virus, we found comparable miR-122 tissue levels between patients with HCV infection and various other aetiologies of advanced liver disease in a series of explanted livers (n = 23) (data not shown). This further underlines that the elevated miR-122 serum level in patients with CHC is not merely a consequence of an upregulated hepatic expression.

In conclusion, our study suggests that the hepatocyte abundant miR-122 may represent an easily accessible and more sensitive biomarker than the currently used ALT to identify patients with liver injury because of chronic HCV infection. A more sensitive marker would be beneficial for especially those patients with minor levels of hepatocellular damage, who are currently at risk not to be identified by routine blood tests. Although further validation is required before clinical application can be considered, evidence is accumulating that miR-122 is elevated in blood because of liver injury of various aetiologies. Serum miR-122 may have the potential to improve first-line screening for many liver diseases.

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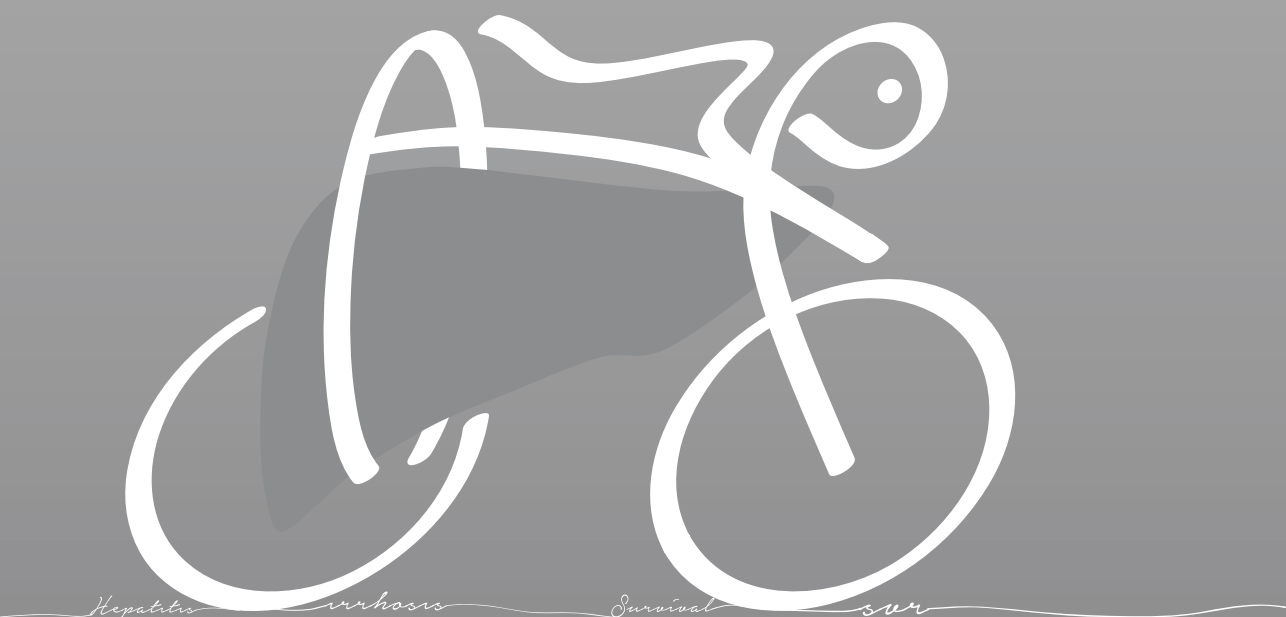
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GENERAL DISCUSSION AND CONCLUSIONS

BACKGROUND

Chronic infection with the hepatitis C virus (HCV) can lead to hepatic fibrosis development. Eventually, this can result in cirrhosis, at which stage patients are at the highest risk to develop the clinical complications of their chronic viral infection. A recent meta-analysis indicated that the annual risk of liver failure was 2.9% and that the annual risk of hepatocellular carcinoma (HCC) was 3.2% among patients with chronic HCV infection and advanced liver disease.[1] Both liver failure and HCC may necessitate liver transplantation and can result in liver-related death. The annual rate of liver-related death among patients with cirrhosis was 2.7%.[1] Recently, a large natural history study highlighted that patients with chronic HCV infection have a substantially impaired overall survival as compared to the general population which has never been in contact with HCV.[2] With an estimated 150 million patients with chronic HCV infection around the world, this infectious disease thus represents a major global health problem.[3]

For more than 20 years antiviral therapy has been available and applied, as interferon-based treatment showed the potential to eradicate chronic HCV infection.[4-6] Treatment is considered successful in case of a sustained virological response (SVR), which is determined by HCV RNA negativity in the circulation 24 weeks following cessation of therapy. Indeed, SVR attained with interferon-based therapy has a long-term durability.[7] While accompanied by substantial side effects, the cure rates with especially the earlier interferon regimens have been poor. Fortunately, the virological efficacy of antiviral therapy has increased substantially. Standard interferon was replaced by pegylated interferon, ribavirin was added and direct acting antivirals (DAAs) were designed.[5, 6, 8-10] Particularly within the last three years the antiviral treatment developments followed each other at a remarkable pace. The latest major success concerns the many different interferon-free treatment strategies, which combine multiple classes of DAAs. All these regimens showed an excellent virological efficacy with short treatment durations and hardly any side effects.[11-21] Even among patients who failed to attain SVR with prior pegylated interferon therapy or among patients with cirrhosis, two subgroups which used to be most difficult-to-cure, SVR rates around 95% have been described. Due to these impressive results we currently stand on the verge of a true paradigm shift with respect to the treatment of HCV infection.

VIROLOGICAL RESPONSE AND LONG-TERM OUTCOME

Policy-makers around the world will need to decide whether or not the new treatment regimens should be made available for all patients. For this reason it is currently debated if cure of HCV infection would be cost-effective. While it is thus needed to indicate the clinical relevance of SVR, data on the long-term clinical outcome following antiviral therapy among patients with chronic HCV infection are scarce. In 2007, Veldt et al. were the first to show that patients with HCV-induced advanced hepatic fibrosis and SVR had a reduced risk of liver failure and liver-related mortality as compared to those without SVR.[22] These results were confirmed by two independent groups from Italy and France, which also indicated that HCC occurred significantly less often among successfully treated patients with cirrhosis.[23, 24] More recently, SVR has been associated with a lower risk of all-cause mortality, as most definite and reliable clinical endpoint.[25] This study was performed in a specific male patient population of American veterans, however, with many comorbidities and a relatively high mortality rate. The representability of this study was thus questioned. Also, no data were presented for those patients with cirrhosis, who are at the highest risk to die from their chronic HCV infection. In our cohort, all consecutive patients with chronic HCV infection and histological proof of advanced hepatic fibrosis who were treated with interferon-based therapy from 1990 to 2003 in five large hepatology units in Europe and Canada were included.[22] As described in **chapter 1**, the 10-year overall survival in this patient population with advanced liver disease was 91% among those with SVR and 74% among those without SVR. Extensive multivariate analyses, adjusting for many important markers of liver disease severity, showed that SVR was the factor which was most strongly association with reduced all-cause mortality (adjusted hazard ratio 0.26). Others have recently confirmed this finding in a cohort of more than 300 patients with HCV-induced cirrhosis from Sweden.[26] To further substantiate the clinical relevance of SVR, we presented a related analysis in **chapter 2**, which shows that the overall survival among the patients with SVR in our cohort was similar to that of the age- and sex-matched general Dutch population. The comparable survival was an important finding, especially because all patients with SVR had bridging fibrosis or cirrhosis before treatment initiation. After updating the follow-up in our international cohort, we were also able to further establish the markedly reduced risk of liver failure, HCC and liver-related mortality among patients with SVR. Together, these results contribute to our understanding of the clinical outcome following antiviral therapy, especially with respect to the growing population with advanced hepatic fibrosis.[27, 28] Physicians and patients may be supported by our findings when initiation of antiviral therapy is considered, especially if this includes pegylated interferon or ribavirin and their associated side effects.

Our results may also be of interest to policy-makers, who need to be convinced that access to antiviral therapy is necessary for all patients at need. Last, also the institution on HCV screening programs, which could result in an important increase in treatment uptake, depends on the long-term benefits of antiviral treatment. Also based on the results of our study, the U.S. Preventive Services Task Force recently adapted the recommendation of the Centers for Disease Control and Prevention to screen all persons born between 1945 and 1965 for the presence of HCV infection.[29] Currently, there is no Dutch national HCV screening program. Further studies to assess for which subgroups this might be cost-effective in a low prevalence country such as the Netherlands are warranted.

Due to the retrospective nature of our study it was not possible to conclude a causal relation between (successful) antiviral therapy and reduced all-cause mortality. This actually remains a limitation of the entire field of hepatitis C, since adequate (placebo-)controlled trials on hard clinical endpoints which randomize patients with chronic HCV infection to treatment or no treatment are lacking.[30] Nevertheless, as extensively discussed in **chapters 5 and 6**, antiviral therapy for chronic HCV infection can still be recommended on the basis of an extensive body of evidence suggesting SVR is a highly relevant surrogate endpoint. 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.[31-44] Evaluating liver histology or the hepatic venous pressure gradient requires invasive procedures, however, so that multiple assessments are difficult to accomplish. In **chapter 3** 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.[45-47] Previously, changes in platelet count following antiviral therapy have been linked to changes in hepatic fibrosis. [48, 49] With a repeated measurement analysis we observed a significant increase in platelet counts following achievement of SVR, which showed to be rather linear. This finding suggests a gradual improvement in portal pressure and liver histology over many years following eradication of chronic HCV infection. The change in platelet count correlated with the change in spleen size, which represents another marker of portal pressure. Variceal bleeding, a severe and often fatal complication of portal hypertension, was not observed among patients with SVR. In contrast, the platelet counts further declined during follow-up among patients who were not successfully treated, and several of these patients did experience variceal bleeding.

CONTINUING HEALTH-RISKS AMONG PATIENTS WITH SVR

Although their risk is substantially lower, patients with HCV-induced advanced liver disease and SVR are not free-warded from cirrhosis-related complications. Especially the risk of HCC was found to remain following viral eradication. In our long-term followed cohort, seven of the 192 patients were diagnosed with HCC up to approximately seven years following achievement of SVR, and others have made similar observations.[23, 24, 26, 50] In order to substantiate the incidence of HCC following SVR as well as to assess which factors are associated with HCC occurrence following HCV eradication, we have combined the data of 1000 European and Canadian patients with HCV-induced cirrhosis and SVR for meta-analyses on the individual patient level. As described in **chapter 8**, patients with cirrhosis and SVR had an annual risk of HCC of almost 1%. Their annual risk of liver failure, HCC or death as a combined endpoint was actually twice as high. Because of their association with increasing age and severity of liver disease, physicians should expect to encounter these cirrhosis-related complications after SVR more frequently as soon as interferon-free therapy becomes widely used. Indeed, these new regimens will cure patients with more advanced liver disease. Based on these results we recommend that patients with HCV-related cirrhosis who have attained SVR should not be dismissed from intensive follow-up including ultrasonographic HCC surveillance.

The continuing health-risks following SVR also formed the rationale to consider alternative efficacy measures of antiviral therapy such as the number needed to treat (NNT) to prevent cirrhosis-related complications. The NNT represents an absolute clinical efficacy measure with a clear interpretation for both physicians and patients. It combines the risk of cirrhosis-related complications, the SVR rate of antiviral therapy as well as the expected benefit of SVR (i.e. the hazard ratio of SVR for a particular outcome) into a single efficacy measure. Interestingly, all these parameters are normally considered separately when deciding on initiating antiviral therapy. Whereas antiviral treatment improvements are usually evaluated with the virological efficacy (i.e. the SVR rate), our aim in **chapter 4** was to describe the development of antiviral therapy in terms of clinical efficacy (i.e. the NNT). For the difficult-to-cure subgroup of patients with chronic HCV genotype 1 infection and cirrhosis, we found that the NNT to prevent one death within five years declined from over 1000 at the time of interferon monotherapy (with a SVR rate of 2%) to 61 with pegylated interferon and ribavirin combination therapy, which is expected to result in SVR in 35% of these patients.[51, 52] At the costs of side effects, triple therapy with telaprevir or boceprevir boosted the SVR rate to approximately 50% and this further reduced the NNT to prevent one death in five years to 43.[53-55] The NNT to prevent one cirrhosis-related event in general within five years was 302, 18, and 13, at SVR rates of 2, 35, and 50%,

respectively. These massive declines in the NNT to prevent solid clinical endpoints highlight the enormous impact of antiviral treatment development for chronic HCV infection over the last two decades. More recent trials even indicated that SVR rates of 95% may be realistic with combinations of DAAs, also among those with HCV genotype 1 infection and cirrhosis.[11, 12, 17, 56, 57] When extrapolating our calculations to these interferon-free treatment strategies, the NNT would be as low as 13 to prevent one death and 7 to prevent one case with any cirrhosis-related event in five years' time (data not shown in this thesis). However, here we assume that the impact of SVR on clinical outcome is similar among all stages of cirrhosis. While this is supported by the lack of significant interaction terms between SVR and markers of liver disease severity in our Cox regression analyses in chapter 1, future studies will need to explore the clinical efficacy of interferon-free therapy in more detail. Yet, these studies might be difficult to accomplish if there are hardly any patients left without SVR.

RISK OF CIRRHOSIS-RELATED COMPLICATIONS AMONG PATIENTS WITHOUT SVR

Chapter 4 also describes that the NNT to prevent clinical endpoints is largely dependent on the patient's risk for the event which we want to avoid. However, reliable tools to assess the risk of liver failure, HCC, or death among patients with advanced but still compensated liver disease are lacking. Also within hepatology, the impact of prediction scores is well known. Today, the Model End-stage Liver Disease and the Child-Turcotte-Pugh score are frequently used to assess the survival among patients with decompensated cirrhosis.[58, 59] The Model End-stage Liver Disease even guides the allocation of donor liver for those in need of a liver transplantation.[60] Hereto, as described in **chapter 7**, we aimed to developed risk scores for all-cause mortality or liver failure, HCC and mortality as combined endpoint (i.e. clinical disease progression) among patients with advanced but compensated liver disease, based on readily available and objective clinical and laboratory parameters. We found that the risk of these events could be reliably assessed with the patient's age, gender, platelet count and ratio between the aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The predictive accuracy of the risk score for clinical disease progression was optimized by the addition of HCV genotype, as HCV genotype 3 was significantly associated with an unfavorable outcome. This finding was not unexpected, as HCV genotype 3 was previously associated with accelerated hepatic fibrosis progression and HCC development.[61, 62] These associations might be explained by the increased prevalence of steatosis among patients with chronic HCV genotype 3 infection.[63, 64] Importantly, the robustness of our risk scores was confirmed with validation analyses

in an independent European cohort of patients with HCV-related cirrhosis. In daily practice, these tools could be relevant when counseling patients with compensated HCV-related cirrhosis regarding their health risks and need for antiviral therapy. If further validated, the scores might also be useful for allocating intensive and costly HCC surveillance. While bi-annual liver ultrasonography is currently advised for all patients with HCV-related cirrhosis, this is only deemed to be cost-effective if the annual HCC risk exceeds 1.5% in these patients.[65] A calculator for our risk scores is freely available online through www.gut.bmj.com.

OPTIMIZING PEGYLATED-INTERFERON THERAPY

In 2014, pegylated interferon was still the backbone of the treatment for chronic HCV infection. Although various combinations of DAAs showed overwhelming SVR rates and excellent safety profiles, the high costs of these new drugs may limit their availability. Indeed, also in high-income countries the access to DAAs is currently subject to considerable debate. Pegylated interferon is therefore expected to remain a valuable treatment option for many patients with chronic HCV infection around the world. Optimizing the use of pegylated interferon in order to maximize the chance of SVR is thus still relevant. Especially among patients with advanced liver disease awaiting future interferon-free treatment options is not an option, as these patients are in urgent need to eradicate their chronic HCV infection. As was experienced with the first generation protease inhibitors, however, it may take more time than anticipated before new drugs become available.

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.[66-69] The importance of treatment adherence was again highlighted by the first phase 2 trials which assessed the addition of telaprevir to pegylated interferon and ribavirin therapy, as discussed in **chapter 11**.[70, 71] Due to a deteriorated safety profile of the telaprevir-containing regimens, about a third of patients prematurely discontinued therapy and this had a profound impact on the SVR rate. Rather than adjusting telaprevir dosages, it is now clear that the second generation DAAs are the solution. Indeed, these improved drugs no longer seem to be hampered by additional adverse-events. [72-76] Of course, with these triple therapies the pegylated interferon-associated side effects remain, of which thrombocytopenia and neutropenia are among those which are most commonly reported. Interferon-induced cytopenias were actually the most frequent causes of pegylated interferon dose reductions.[8] Out of fear for infections and bleedings, current guidelines and product labels recommend to reduce the dose of pegylated interferon when the ab-

solute neutrophil count (ANC) drops below 750/μL or when the platelet count drops below 50x10^9/L.[77] Treatment is even advised to be discontinued in case of a ANC below 500/μL or a platelet count below 25x10^9/L. It has been suggested, however, that these recommendations are overly cautious and could unnecessarily impede treatment success.[78, 79] Although chronic HCV-infected patients are at an increased risk of bacterial infections during interferon-based therapy, these infections were generally considered mild.[79-85] Moreover, a relation between the infection rate and interferon-induced neutropenia was never found. The only study which assessed the risk of on-treatment bleedings did describe a relation with interferon-induced thrombocytopenia, but all registered bleedings were deemed minor expect for one which occurred in a patient without thrombocytopenia.[78]

Yet, the above-cited studies included only a limited number of patients with advanced hepatic fibrosis, while patients with cirrhosis can be considered as immunocompromised and at highest risk of infections.[86] Because of an increase in portal pressure and a reduced thrombopoietin production patients with cirrhosis are most prone for thrombocytopenia, which may already be present in absence of interferon-based therapy.[87] Cirrhosis was found to be a risk factor for bleeding episodes during pegylated interferon therapy.[78] In **chapters 9 and 10**, we therefore investigated the risk of infections and bleedings during interferon-based antiviral therapy among the patients with chronic HCV infection and bridging fibrosis or cirrhosis included in our cohort.

Registered were 113 infections during 88 (12%) of the 723 interferon-based treatments which could be included in the analyses with respect to infections. 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. In a sensitivity analysis including only those regimens containing pegylated interferon this finding could not be confirmed, which is in line with other studies solely included patients treated with pegylated interferon and ribavirin.[79, 80, 82, 85] Importantly, the majority of infections which occurred during interferon-based therapy were mild, both in our study as well in those of others. As expected, we found that cirrhosis and diabetes mellitus were independent risk factors for on-treatment infections. In total, 104 bleedings 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 <50x10^9/L at the previous visit were significantly associated with bleeding episodes.[78] However, nearly all registered bleedings were considered to be minor, and remained without serious clinical consequences. The two (2%) bleedings which were considered to be severe both concerned variceal bleedings for which the patients were hospitalized. Antiviral therapy was not dis-

continued in these patients. Considering the natural history of HCV-related cirrhosis, two variceal bleedings in such a large cohort of patients with advanced liver disease might also be expected outside of the scope of interferon-based therapy.[88]

With respect to infections and bleedings, 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 bleedings 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 25x10^9/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 25x10^9/L in 2% of treatments), it should be appreciated that interferon dose reductions were at the discretion of the treating physicians. Prospective studies would of course be warranted to validate these alternative recommendations, especially when regimens of 12 to 24 weeks can be used.

MICRORNA-122 AND CLINICAL OUTCOME

Although optimizing the use of pegylated interferon may be relevant to maximize the chance of SVR, alternative treatment options are needed to substantially increase the cure rate and improve the safety-profile of antiviral therapy. At the time we conducted the drug-development study described in **chapter 12**, we had just learned that telaprevir and boceprevir induced a strong decline in HCV RNA and increased the SVR rate of antiviral therapy.[54, 55, 89-92] However, because of resistance associated variants among the HCV quasispecies, these two protease inhibitors needed to be added to pegylated interferon and ribavirin in order to prevent virological breakthrough.[92] Since both these drugs had their own side effects, triple therapy became even harder to endure. Furthermore, telaprevir and boceprevir were only effective against HCV genotype 1 infection and were subject to multiple drug-drug interactions mediated by cytochrome P-450 3A. The development of treatment strategies with pan-genotypic activity directed at novel host targets was thus thought to be worthwhile. Shortly before, the hepatocyte-specific microRNA-122 (miR-122) was shown to be essential for the stability and propagation of HCV RNA, and thus critical for HCV abundance in the liver.[93, 94] By binding of miR-122 to two seed sites on the highly conserved 5′ UTR of the HCV genome an oligomeric miR-122–HCV complex is formed by which the virus is thought to be protected from nucleolytic degradation and host innate immune responses.[94-96] Based on this interaction, miravirsen (a

locked nucleic acid–modified antisense oligonucleotide complementary to the 5′ region of miR-122) was developed to sequester and thus inhibit miR-122. After a preclinical chimpanzee study showed that miravirsen induced a long-lasting decline in HCV RNA, and healthy volunteer studies indicated that this drug could be safely dosed to humans, we conducted a phase 2a study to assess the safety and virological efficacy of miravirsen among patients with chronic HCV genotype 1 infection.[97] Five weekly subcutaneous miravirsen injections were well-tolerated and induced dose-dependent reductions in HCV RNA levels. The mean maximum declines in HCV RNA (in $\log_{10}$ IU per milliliter) were 1.2, 2.9 and 3.0 in the 3, 5 and 7 mg/kg groups, respectively. Based on the long half-life of this drug, the HCV RNA reductions lasted well beyond the dosing period and were actually not even back to baseline levels and the end of the study's follow-up period. Although the virological responses varied heavily, for which a clear explanation was not found, multiple patients reached undetectable HCV RNA levels with miravirsen therapy alone.

One patient actually remained without a detectable viral load for at least 7 months following the last miravirsen dose, as shown in **chapter 13**. Because miravirsen is largely cleared from the body by that time, some sort of induced immune control over the virus might explain this observation. Chapter 13 further describes the long-term outcome of the first patients with chronic HCV infection who were treated with miravirsen. Although limited by the low number of patients and the retrospective study design, it should be considered as an important finding that none of these patients were diagnosed with HCC during a median of 2 years following miravirsen therapy. Scientists have expressed their fear for HCC when miR-122 is sequestered, as miR-122 levels were found to be decreased in HCC, low miR-122 levels in HCC were related to a poorer clinical outcome, and miR-122 knock-out mice showed to be prone for HCC development.[98-103] However, in these animal studies germline deletion of miR-122 led to HCC through the pathway of steatohepatitis, while short-term miR-122 sequestration in an adult obesity mice model previously showed to reduce the degree of steatosis.[98, 99, 104] Although liver histology could not be assessed among the patients included in the phase 2a study, the ALT levels declined rapidly following the start of miravirsen therapy. This suggests a reduced rather than an increased inflammatory activity in the livers of miravirsen-treated patients. If short-term and reversible miR-122 inhibition shows to be able to eradicate HCV infection, it would reduce the risk of HCC as shown in this thesis. Until then, however, regulatory authorities warrant investigators to follow-up patients who are being exposed to miR-122 sequestration for at least 5 years in order to assess the long-term safety of this new class of drugs. In light of the extremely high SVR rates with DAAs and the varying virological responses miravirsen, it might be questioned whether anti-HCV therapy can be further improved with treatment strategies targeting miR-122.

Nevertheless, miR-122 might still be relevant to decrease HCV-related morbidity and mortality. While even the best antiviral treatment regimens need to be administered to patients in order to have a beneficial effect, treatment rates across Europe are generally below 5%.[105] Underdiagnosis seems to be the main reason for undertreatment.[106] In Europe it is thought that up to 90% of patients are unaware of their chronic viral infection.[107] Because of the mainly asymptomatic course of disease, patients with HCV infection are frequently referred to hepatology units on the basis of an abnormal ALT level during routine blood works. Yet, ALT levels are persistently normal in about a third of those with chronic HCV infection. While diagnosing patients thus is a major clinical challenge, modelling data clearly showed that we need to treat more patients rather than increase the SVR rate in order have a true impact on HCV-related mortality.[28, 108] Here, there might be a role for miR-122, as microRNAs are stable and detectable in the circulation and animal studies suggested that miR-122 blood levels are highly sensitive biomarker of liver injury.[109-112] In **chapter 14** we therefore investigated the diagnostic qualities of serum miR-122 levels for the detection of hepatocellular damage among patients with chronic HCV infection. Importantly, miR-122 levels correlated with ALT and hepatic inflammatory activity. With receiver operating curve characteristic analyses we showed that the power to discriminate patients with chronic HCV infection from healthy controls was higher for miR-122 than for ALT. Especially among those patients with normal ALT levels, a subgroup of patients at risk for being missed with routine laboratory investigations, the miR-122 blood level maintained a high discriminating ability. Because miR-122 is almost exclusively expressed in the liver, the organ specificity of an elevated miR-122 serum level is not thought to be a major issue.[113-116] Liver injury of other etiologies showed to elevate miR-122 blood levels as well, indicating that miR-122 might be a sensitive first-line screening biomarker of hepatocellular damage in general.[110, 112, 117-120]

CONCLUSIONS

Achievement of SVR is associated with a reduced risk of liver failure, HCC and all-cause mortality among patients with chronic HCV infection and advanced hepatic fibrosis. Improvement of liver histology following HCV eradication is likely to play a role in this beneficial clinical outcome. The continuous development of antiviral treatment over the last two decades has resulted in a substantial decline in the NNT to prevent liver failure, HCC or death. Especially if the risk of the cirrhosis-related complications increases, achievement of SVR should be pursued without any delay. For these patients awaiting future interferon-free is not an option, so that the cur-

rently available pegylated interferon-based therapy should be used optimally. Of course, even the best antiviral treatment regimens will not have a beneficial effect as long as they are not administered. In order to reduce HCV-related morbidity and mortality, it is thus crucial that we start diagnosing and treating more patients with chronic HCV infection. Important to realize is that the risk of cirrhosis-related complications among patients with cirrhosis is not eradicated in case of successful antiviral therapy. Despite SVR these patients should therefore remain included in follow-up programs incorporating ultrasonographic HCC surveillance.

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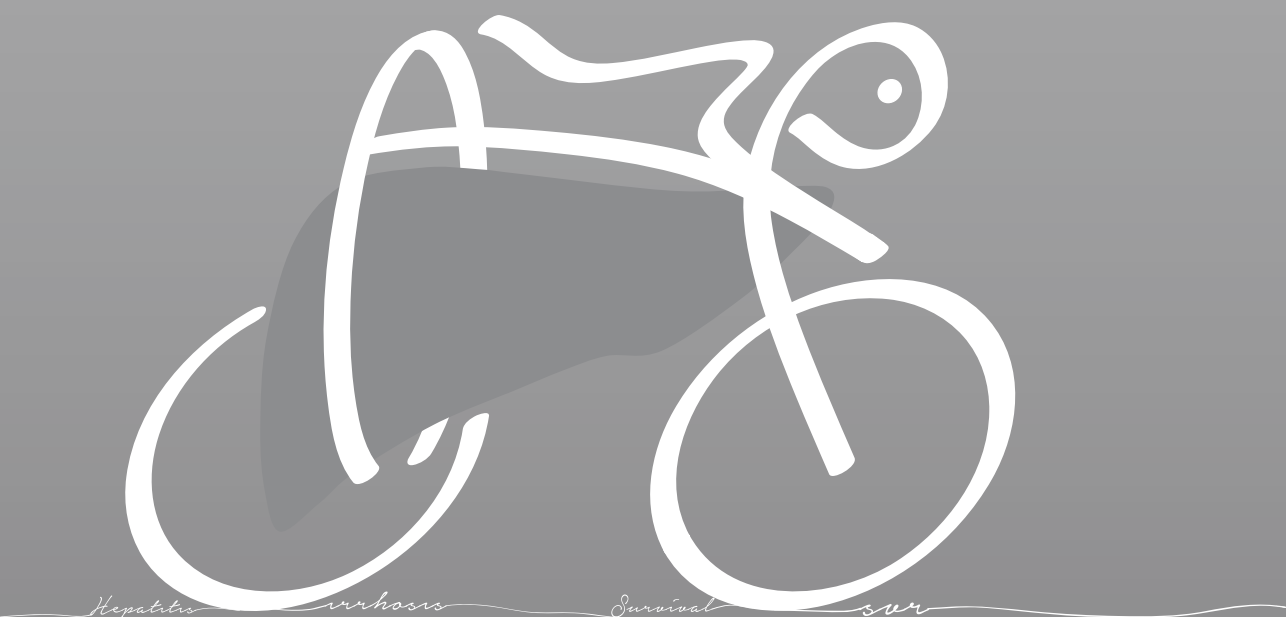
Hepatitis
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SAMENVATTING

ACHTERGROND

Personen die geïnfecteerd raken met het hepatitis C virus (HCV), hebben ongeveer 75% kans om chronische hepatitis C te ontwikkelen. Het gevolg hiervan is een continue laaggradige ontsteking van de lever, waardoor deze kan beschadigen. Dit ontstekingsproces kan namelijk leiden tot het ontstaan van leverfibrose, ofwel verlittekening van de lever. Bij toenemende beschadiging ontstaat er uiteindelijk cirrose, een situatie waarbij de normale architectuur van het leverweefsel geheel is verstoord. Cirrose wordt zodoende als het eindstadium van vele chronische leverziekten beschouwd, hoewel zelfs in dit stadium de leverfunctie nog lange tijd stabiel blijft. Wel lopen chronische hepatitis C patiënten met ernstige leverfibrose of cirrose een jaarlijks risico van ongeveer 3% dat de lever zijn functies in het lichaam niet meer voldoende uit kan oefenen, wat we gedecompenseerde cirrose noemen. Aangezien er geen leverfunctievervangende behandelingen bestaan, is levertransplantatie bij verdere achteruitgang de enige optie om overlijden te voorkomen. Patiënten met HCV-gerelateerde cirrose lopen eveneens risico op het ontwikkelen van leverkanker, genaamd het hepatocellulair carcinoom (HCC). Ook HCC wordt jaarlijks bij ongeveer 3% van deze patiënten gediagnosticeerd. Deze vorm van kanker heeft een hoge mortaliteit. Het doel van de behandeling van chronische hepatitis C is om de bovengenoemde complicaties van cirrose en HCV-gerelateerde sterfte te voorkomen.

De combinatie van gepegyleerd interferon (peginterferon), ribavirine en medicijnen die direct op het virus inwerken (DAA; *direct-acting antiviral*) geven tegenwoordig een goede kans op genezing van de chronische HCV infectie. We beschouwen patiënten als genezen indien er 24 weken na het staken van de antivirale therapie geen virus meer in het bloed aantoonbaar is. Dit noemen we een blijvende virologische response (SVR; *sustained virological response*). In zowel klinische studies als de dagelijkse praktijk is SVR het eindpunt van succesvolle behandeling. Echter, ook de verbeterde antivirale behandeling van tegenwoordig resulteert niet in alle patienten in SVR. Dit is problematisch, mede omdat de gebruikte antivirale medicijnen forse bijwerkingen kennen welke de kwaliteit van leven gedurende de behandeling negatief beïnvloeden. Toekomstige behandeling met combinaties van DAAs zonder peginterferon kunnen het aantal bijwerkingen minimaliseren, terwijl de virologische effectiviteit van deze interferon-vrije therapieën uitstekend lijkt. In de eerste studies behaalde ongeveer 95% van de patiënten SVR met deze behandelingen, zelfs in geval cirrose aanwezig was. Echter, interferon-vrije therapie is momenteel nog niet beschikbaar en de benodigde DAAs lijken uitzonderlijk prijzig te worden. De discussie over het nut van SVR en de kosteneffectiviteit van antivirale therapie voor chronische HCV infectie is zodoende opnieuw aangewakkerd. Om zowel de bijwer-

kingen als kosten te rechtvaardigen, is een goed beeld van de mogelijke klinische voordelen van antivirale therapie van essentieel belang. Dit geldt in het bijzonder voor chronische hepatitis C patiënten die al ernstige leverfibrose of cirrose hebben ontwikkeld, aangezien het niet duidelijk is of cirrose reversibel is en deze patiënten het hoogste risico lopen op de klinische complicaties van hun virale infectie. Echter, bij aanvang van dit promotieonderzoek was nog maar weinig bekend over de langetermijnuitkomsten na antivirale therapie bij patiënten met ernstige leverschade, zeker wat betreft hun algehele overleving in relatie tot de virologische response.

VIROLOGISCHE RESPONSE EN LANGETERMIJNUITKOMSTEN

In **hoofdstuk 1** vergeleken wij de algehele overleving tussen patiënten met een chronische HCV infectie die wel en zij die niet SVR behaalden met interferon-gebaseerde behandeling. Hiervoor hebben wij 530 opeenvolgende patiënten met chronische hepatitis C en ernstige leverfibrose of cirrose vanuit 5 grote hepatologie centra in Europa en Canada geïncludeerd. De 10-jaarsoverleving van 91% die we onder de patiënten met SVR vonden was significant hoger dan de 74% onder de patiënten zonder SVR. Ook gecorrigeerd voor vele factoren die zowel van invloed zijn op de virologische response als op de klinische uitkomsten op lange termijn, bleef SVR de sterkste factor die onafhankelijke geassocieerd was met een verbeterde overleving. Gedurende de lange mediane opvolgperiode van meer dan 8 jaar in onze studie, was het risico op overlijden bijna 4 keer lager in geval dat de patiënt van zijn chronische HCV infectie was genezen. In **hoofdstuk 2** tonen wij dat de overleving onder patiënten met SVR zelfs vergelijkbaar is aan die van de algemene Nederlandse bevolking, ondanks de aanwezigheid van ernstige leverfibrose of cirrose voorafgaand aan de start van de succesvolle antivirale behandeling. Tevens toonden wij in hoofdstuk 1 aan dat SVR gerelateerd was aan een sterk verminderd voorkomen van gedecompenseerde cirrose, HCC en lever-gerelateerde sterfte. In **hoofdstuk 5** worden onze resultaten uitgebreid bediscussieerd in het licht van de beschikbare literatuur, maar ook de ontbrekende studies op dit gebied.

Hoewel leverfibrose lange tijd als irreversibel werd beschouwd, hebben een aantal kleinschalige studies aangetoond dat de mate van leverfibrose kan verminderen nadat de chronische HCV infectie succesvol is geëlimineerd, zelfs in patiënten met cirrose. In **hoofdstuk 3** hebben wij de verandering in bloedplaatjes en miltgrootte na antivirale behandeling in ons studiecohort geanalyseerd. Een verminderd aantal bloedplaatjes en grotere milt zijn markers voor ernstigere leverfibrose en een verhoogde druk in het portale vaatbed. Tevens zijn het belangrijke voorspellers van cirrose-gerelateerde complicaties. Wij vonden een geleidelijke verbetering van het

aantal bloedplaatjes na het behalen van SVR, welke voor vele jaren aanhield. Op termijn bleek dat ook de miltgrootte was afgenomen onder de patiënten die waren genezen. Bloedingen vanuit spataderen in de slokdarm, een belangrijke complicatie van portale hypertensie met aanzienlijke mortaliteit, werden niet waargenomen onder patiënten die SVR hadden behaald. Deze bloedingen traden echter wel op bij patiënten zonder SVR, bij wie de bloedplaatjes en miltgrootte verder verslechterden.

BLIJVENDE GEZONDHEIDSRISICO'S NA SVR

Hoewel het risico substantieel verlaagd is, zijn chronische hepatitis C patiënten met ernstige leverschade na het behalen van SVR niet gevrijwaard van cirrose-gerelateerde complicaties. Vooral het risico op HCC lijkt te blijven bestaan. In ons cohort werden 7 van de 192 genezen patiënten gediagnosticeerd met HCC tot ongeveer 7 jaar na SVR. Om een beter beeld te krijgen van de continuerende gezondheidsrisico's na succesvolle antivirale behandeling, hebben we in **hoofdstuk 8** de gegevens van 1000 Europese en Canadese patiënten met HCV-geïnduceerde ernstige leverschade en SVR samengevoegd. Uit deze studie bleek dat het jaarlijkse risico op HCC voor Westerse patiënten met cirrose en SVR bijna 1% is. Het risico op decompensatie, HCC of overlijden (als gecombineerd eindpunt) was zelfs 2 keer zo hoog. Het optreden van deze cirrose-gerelateerde complicaties was geassocieerd met hogere leeftijd en ernstigere leverziekte, zodat we erop bedacht moeten zijn dat deze complicaties vaker na SVR gezien gaan worden in nabije toekomst. De verbeterde effectiviteit en veiligheid van de toekomstige antivirale behandelingen maken het namelijk mogelijk om ook oudere patiënten en patiënten met ernstigere leverziekte te genezen.

Vanwege de blijvende gezondheidsrisico's na SVR, is het percentage patiënten dat SVR behaalt eigenlijk een suboptimale effectiviteitsmaat om de antivirale behandeling goed te kunnen waarderen. Voor de subgroep van patiënten met HCV genotype 1 infectie en cirrose, welke de afgelopen jaren het moeilijkste te genezen was, beschrijven we in **hoofdstuk 4** de klinische effectiviteit van antivirale therapie. Op basis van de resultaten in onze cohortstudie, berekenden wij dat het aantal patiënten dat moest worden behandeld om 1 overlijden in 5 jaar tijd te voorkomen daalde van meer dan 1000 met interferon monotherapie (de eerste antivirale behandeling met een SVR percentage van maar 2%) tot 61 met peginterferon en ribavirine combinatie therapie (waarmee naar verwachting 35% van deze patiënten SVR behaalt). Ten koste van additionele bijwerkingen, verhoogt de toevoeging van telaprevir of boceprevir het SVR percentage naar ongeveer 50% voor patiënten met HCV geno-

type 1 infectie en cirrose. Het aantal patiënten dat moet worden behandeld om in 5 jaar tijd 1 overlijden te voorkomen daalt hiermee verder naar 43. Meer recente studies tonen zelfs SVR percentages rond de 95%, ook voor patiënten met cirrose. Wanneer we onze resultaten toepassen op deze toekomstige behandelingen daalt het aantal te behandelen patiënten om in 5 jaar tijd 1 overlijden te voorkomen zelfs naar 13 (resultaten niet gerapporteerd in dit proefschrift). Om in 5 jaar tijd 1 geval van gedecompenseerde cirrose, HCC of overlijden te voorkomen, moeten 302, 18, 13 of 7 patiënten worden behandeld met antivirale therapie met een SVR percentage van 2, 35, 50, of 95%. Deze enorme verbetering in de klinische effectiviteit benadrukt de belangrijke impact van de ontwikkeling van de antivirale therapie voor chronische HCV infectie in de afgelopen 20 jaar.

RISICO OP CIRROSE-GERELATEERDE COMPLICATIES ONDER PATIËNTEN MET CHRONISCHE HEPATITIS C

Mede omdat de hierboven beschreven klinische effectiviteit sterk afhankelijk is van het risico dat de patiënt loopt, hebben we in **hoofdstuk 7** getracht om het individuele risico op overlijden of cirrose-gerelateerde complicaties in te schatten voor chronische hepatitis C patiënten met ernstige leverfibrose of cirrose. Het doel van deze studie was om risicoscores te ontwikkelen met alleen objectieve variabelen, welke gemakkelijk beschikbaar zijn in de dagelijkse praktijk. Het bleek dat het risico op overlijden betrouwbaar kan worden geschat met behulp van de leeftijd van de patiënt, het geslacht, het aantal bloedplaatjes en de verhouding tussen het aspartaat aminotransferase en alanine aminotransferase (twee leverenzymen die vrijkomen bij levercelverval, waarvan de verhouding geassocieerd is met de ernst van leverfibrose). Voor het voorspellen van cirrose-gerelateerde complicaties was het HCV genotype ook nog toegevoegde waarde. De stabiliteit van de ontwikkelde risicoscores werd bevestigd door validatie-analyses in een tweede onafhankelijk Europees cohort bestaande uit chronische hepatitis C patiënten met cirrose. Dergelijke risicoscores zijn relevant voor de begeleiding van patiënten op de polikliniek. Patiënten met een hoger risico op de complicaties van ernstige leverziekte zijn vermoedelijk het meest gebaat bij snelle en succesvolle behandeling. Deze scores kunnen, na verdere validatie, wellicht ook van een rol spelen bij het beoordelen van de noodzaak van periodieke HCC surveillance met echografisch onderzoek voor de individuele patiënt.

HET OPTIMALISEREN VAN DE PEGINTERFERON BEHANDELING

Ondanks de veelbelovende resultaten van interferon-vrije therapie, is peginterferon in 2014 nog altijd de hoeksteen van de antivirale behandeling van chronische HCV infectie. Door de hoge kosten van de DAAs blijft peginterferon in de komende jaren vermoedelijk een belangrijke behandeloptie voor vele chronische hepatitis C patiënten wereldwijd. Het optimaliseren van het gebruik van dit medicament om de kans op succesvolle behandeling te maximaliseren, is daarom nog altijd relevant. De bijwerkingen van peginterferon limiteren namelijk de therapietrouw en kunnen aanleiding geven om de dosis peginterferon te verlagen of om de behandeling zelfs geheel te staken. Hiermee is de kans op SVR beduidend lager.

Interferon-geïnduceerde neutropenie (lage neutrofielen) en trombocytopenie (lage bloedplaatjes) zijn veelvoorkomende bijwerkingen van peginterferon en vormen de belangrijkste reden voor dosisreducties en het stoppen van de behandeling. Uit angst voor infecties (neutrofielen zijn witte bloedcellen die een rol in het afweersysteem hebben) en bloedingen (bloedplaatjes zijn belangrijk voor de bloedstolling) wordt in de huidige richtlijnen geadviseerd om de dosis peginterferon te reduceren indien het absoluut aantal neutrofielen onder de 750/μL daalt of wanneer het aantal bloedplaatjes onder 50x10^9/L daalt. Behandeling zou zelfs gestaakt moeten worden bij neutrofielen onder de 500/μL of bloedplaatjes onder de 25x10^9/L. Eerdere studies hebben echter gesuggereerd dat deze richtlijnen mogelijk te voorzichtig zijn, en daarmee dus de kans op SVR onnodig verkleinen. Omdat deze studies relatief weinig patiënten met ernstige leverziekte includeerden, terwijl dit een risicofactor voor infecties en bloedingen is, hebben wij in **hoofdstuk 9 en 10** het optreden van infecties en bloedingen gedurende de interferon-gebaseerde behandelingen in ons cohort van chronische hepatitis C patiënten met ernstige leverfibrose of cirrose onderzocht. In 12% van de behandelingen werd een infectie gerapporteerd, welke over het algemeen mild waren. Gedurende 23 behandelingen daalde het absoluut aantal neutrofielen onder de 500/μL, maar in slechts 3 van deze behandelingen trad een infectie na het ontstaan van dergelijk lage neutrofielen op. In tegenstelling tot de situatie bij alle geregistreerde interferon-gebaseerde behandelingen, waren neutrofielen <500/μL niet significant geassocieerd met het optreden van infecties tijdens peginterferon behandeling. Bloedingen kwamen in 8% van de behandelingen voor. Het hebben van bloedplaatjes <50x10^9/L bij het vorige bezoek was significant geassocieerd met het optreden van bloedingen. Echter, bijna alle bloedingen die werden geregistreerd, waren klinisch niet relevant. De 2 ernstige bloedingen die optraden, betroffen beide bloedingen vanuit spataderen in de slokdarm ten gevolge van portale hypertensie, welke ook verwacht kunnen worden in afwezigheid van antivirale therapie.

Ten aanzien van infecties en bloedingen bevestigden onze studies dat peginterferon behandeling over het algemeen veilig is. Omdat het belang van SVR groot is onder chronische hepatitis C patiënten met ernstige leverziekte, kan overwogen worden om de dosis peginterferon niet te verlagen zolang het absolute aantal neutrofielen stabiel boven de 500/μL blijft en het aantal trombocyten stabiel boven de $25x10^9$/L. Uiteraard zijn nieuwe studies nodig om deze alternatieve aanbevelingen te valideren.

MICRORNA-122 EN LANGETERMIJNUITKOMSTEN

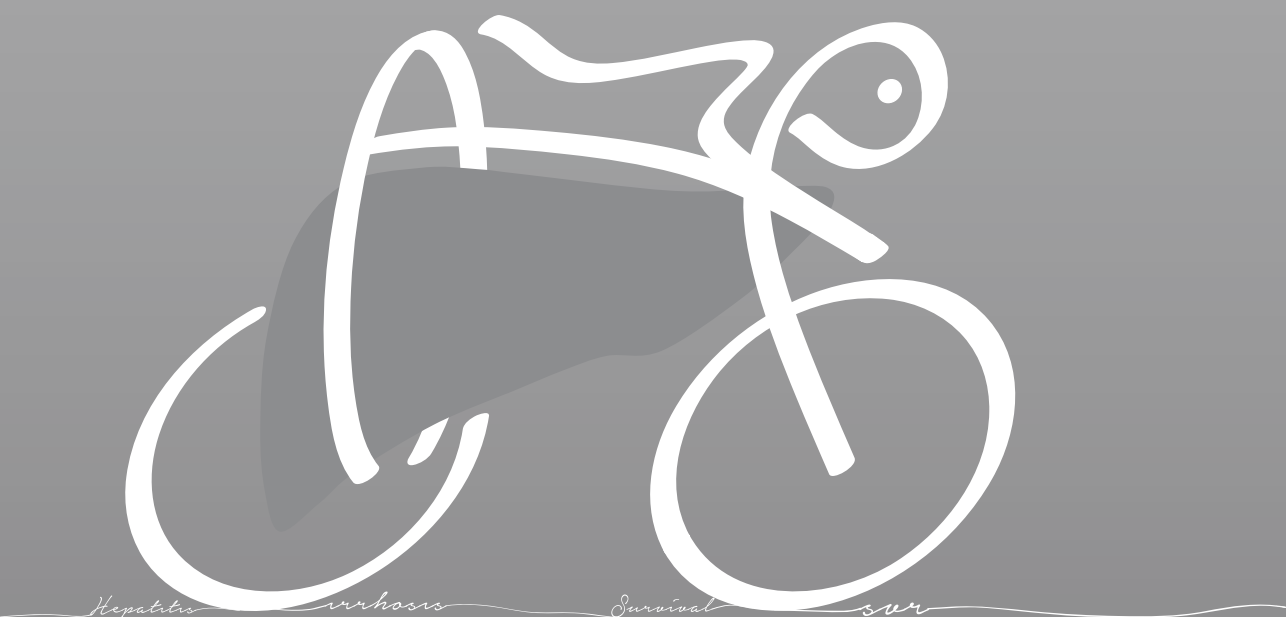
MicroRNA-122 (miR-122) is klein stukje RNA (genetisch materiaal) dat specifiek door levercellen tot expressie wordt gebracht. Het reguleert de activiteit van verschillende eiwitten in de lever. Tevens bleek miR-122 essentieel te zijn voor HCV om zich in de lever te kunnen nestelen. Zodoende werd miravirsen ontwikkeld, een medicijn dat miR-122 kan wegvangen. Het doel van de studie in **hoofdstuk 12** was om de virologische effectiviteit en veiligheid van miravirsen behandeling bij patiënten met chronische hepatitis C te beoordelen. Patiënten met chronische HCV genotype 1 infectie kregen elke week een dosis miravirsen onder de huid toegediend. De 5 doses die de patiënten in totaal kregen werden goed getolereerd en induceerde een dosis-afhankelijke daling van het HCV RNA in het bloed. De gemiddelde maximale daling in HCV RNA (in $\log_{10}$ IU per milliliter) was 1.2, 2.9 en 3.0 in de, respectievelijk, 3, 5 en 7 mg/kg groep. In meerdere patiënten was het HCV RNA na de miravirsen behandeling voor een korte periode zelfs niet meer aantoonbaar. Echter, de virologische respons op de behandeling varieerde sterk, zonder dat daar een duidelijke verklaring voor werd gevonden. Zoals in **hoofdstuk 13** staat beschreven, hebben we ook de langetermijnuitkomsten van de patiënten die aan deze studie hebben deelgenomen onderzocht. Hoewel gelimiteerd door het beperkte aantal patiënten en de exclusie van patiënten met ernstige leverschade, is het relevant dat geen van de miravirsen-behandelde patiënten HCC heeft ontwikkeld. Eerder werd namelijk gesuggereerd dat miR-122 het ontwikkelen van kanker onderdrukt, zodat het wegvangen van dit microRNA in theorie met een groter risico op HCC gepaard zou gaan. Echter, mede door tegenstrijdige resultaten in de literatuur is het zeker nog niet duidelijk of deze angst reëel is voor het kortdurend blokkeren van miR-122. Tot die tijd zullen patiënten bij wie miR-122 in toekomstige studies wordt geremd lang vervolg moeten worden. Omdat deze studie de eerste was die aantoonde dat het blokkeren van een microRNA tot een klinisch effect kan leiden, zijn de resultaten niet alleen van belang voor het veld betreffende chronische hepatitis C. Deze techniek zou ook toegepast kunnen worden binnen andere ziekten waar microRNAs bij betrokken

zijn. In het licht van de overweldigende resultaten met combinaties van DAAs die na het uitvoeren van dit onderzoek zijn verschenen, lijkt het blokkeren van miR-122 geen voor de hand liggende behandelstrategie voor chronische hepatitis C te zijn.

Ondanks dat zeer effectieve antivirale behandelingen beschikbaar zijn/komen, ligt het percentage chronische hepatitis C patiënten dat in Europa wordt behandeld over het algemeen onder de 5%. De voornaamste reden voor deze onderbehandeling is onderdiagnostiek. Mede vanwege de beperkte symptomen is naar schatting meer dan 90% van de patiënten nog niet op de hoogte van zijn/haar chronische virale hepatitis. Veel patiënten worden gediagnosticeerd op basis van een verhoogd ALT bij routinematig bloedonderzoek, maar dit leverenzym is niet verhoogd bij een aanzienlijk deel van de patiënten. Hier is mogelijk wel een rol voor miR-122, omdat werd aangetoond dat dit levercel-specifieke RNA in het bloed aantoonbaar is. In **hoofdstuk 14** hebben we de diagnostische kwaliteiten van de concentratie miR-122 in het bloed binnen chronische hepatitis C onderzocht. In vergelijking met gezonde vrijwilligers, was de miR-122 spiegel significant hoger in patiënten met een chronische HCV infectie, zelfs in de subgroep van patiënten waarbij het ALT normaal was. In vergelijking met het ALT was de miR-122 concentratie inderdaad beter in staat om patiënten van gezonde vrijwilligers te onderscheiden. Aangezien anderen hebben aangetoond dat de miR-122 bloedconcentratie ook stijgt wanneer levercellen worden beschadigd door andere schadelijke stimuli, zoals toxische stoffen of het hepatitis B virus, kan dit mogelijk tot een gevoelige bepaling voor leverontsteking in het algemeen worden ontwikkeld.

CONCLUSIES

Patiënten met chronische hepatitis C en ernstige leverschade die SVR behalen hebben een lager risico op het ontwikkelen van gedecompenseerde cirrose, HCC, en overlijden. De verbeterde behandeling voor chronische hepatitis C heeft geleid tot een enorme afname van het aantal patiënten met cirrose dat moet worden behandeld om deze complicaties te voorkomen. Zeker indien het risico op het optreden van decompensatie, HCC en overlijden hoog is, dient bij patiënten met chronische HCV infectie het behalen van SVR zo snel mogelijk nagestreefd te worden. Om de sterfte aan chronische hepatitis C terug te dringen, is het nu noodzaak dat er aandacht komt om patiënten actief op te sporen aangezien zelfs de beste antivirale behandelingen geen klinisch voordeel bieden als ze niet aan patiënten gedoseerd worden. Van belang is wel om te realiseren dat patiënten met ernstige leverschade ook na het genezen van de chronische HCV infectie risico blijven houden op de cirrose-gerelateerde complicaties. Ook na het behalen van SVR dienen zij daarom poliklinisch vervolgd te worden.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DANKWOORD

DANKWOORD

Hier is het dan, eindelijk! Dank voor jullie geduld.

Vele betrokkenen, zowel binnen de muren van het ziekenhuis als ver daarbuiten, hebben bijgedragen aan de voltooiing van dit proefschrift. Dankzij hun inzet en steun mag ik vandaag promoveren.

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Beste André (dr. P.A. Boonstra), dank voor de verfrissende blik die je als basaal onderzoeker op onze klinische studies hebt. Ik heb genoten van onze wetenschappelijke als ook niet-wetenschappelijke discussies. Beste Luc (dr. L.J.W. van der Laan) en Waqar, dank voor jullie onmisbare begeleiding rondom ons serum microRNA-122 project, één van de leukste van mijn proefschrift. Dank ook aan alle medewerkers van het MDL-lab die mij wegwijs hebben gemaakt tussen de pipetten, reagentia en erlenmeyers. Anthonie, jij specifiek veel dank voor alle ´tips´ man!

Marion en Margriet, zonder jullie is de hepatologie in Rotterdam kansloos. Dank voor de vrolijke doch strakke ondersteuning en het ferme toezicht in de laatste fase van mijn promotietraject.

Lieve Lucille, dank voor jouw ongeëvenaarde werkethos! Ik verheug me op ons weerzien in de kliniek. Heleen, Melek en Wilma, hepatitis C poli's zonder jullie inzet zijn uiteraard niet denkbaar. Voor de essentiële logistieke ondersteuning dank ik ook de collega's op de MDL-poli, en Irene, Edith en Judith van het *Clinical Research Bureau*.

Collega's uit het IJsselland Ziekenhuis (*YSL Masters*), dank voor jullie geduld tijdens de eerste maanden van mijn assistentschap en de gelegenheid die jullie mij hebben gegeven om mijn proefschrift af te ronden en andere wetenschappelijke bezigheden te continueren.

Fabian, dank voor je continue interesse in de voor jou minder bekende wereld van het ziekenhuis. Fijn om te weten dat wij elkaar nooit uit het oog zullen verliezen. Zoals je zelf wel hebt gezegd: "Je zult altijd mijn vriend zijn... je weet teveel van me!"

Hans en Hilco, dank voor de fantastische tijd op de Aelbrechtskade! Ik had dat nooit willen missen. Hilco, jij dient voor mij als voorbeeld om met vertrouwen je eigen ideeën en idealen na te streven. Hans, jouw vrolijke ontspannenheid en gouden hart zijn bewonderenswaardige eigenschappen die ook ik graag nastreef. Afrika vergeet ik nooit. Joost, dank dat jij die twee na mijn vertrek hebt opgevangen. Geen idee wat er anders van ze geworden was.

Jorn (dr. Fiere?), het werd pijnlijk duidelijk dat we elkaar veel te lang niet hadden gesproken. Gelukkig hebben we dat in Toronto in kunnen halen. Dank voor die enorm leuke maand.

Paul, veel plezier beleef ik aan jouw vermogen om zelfs de grootst mogelijke onzin kritisch te analyseren en becommentariëren. Dank dat ik altijd bij je terecht kan voor een eerlijk en doordacht advies. Koffie?

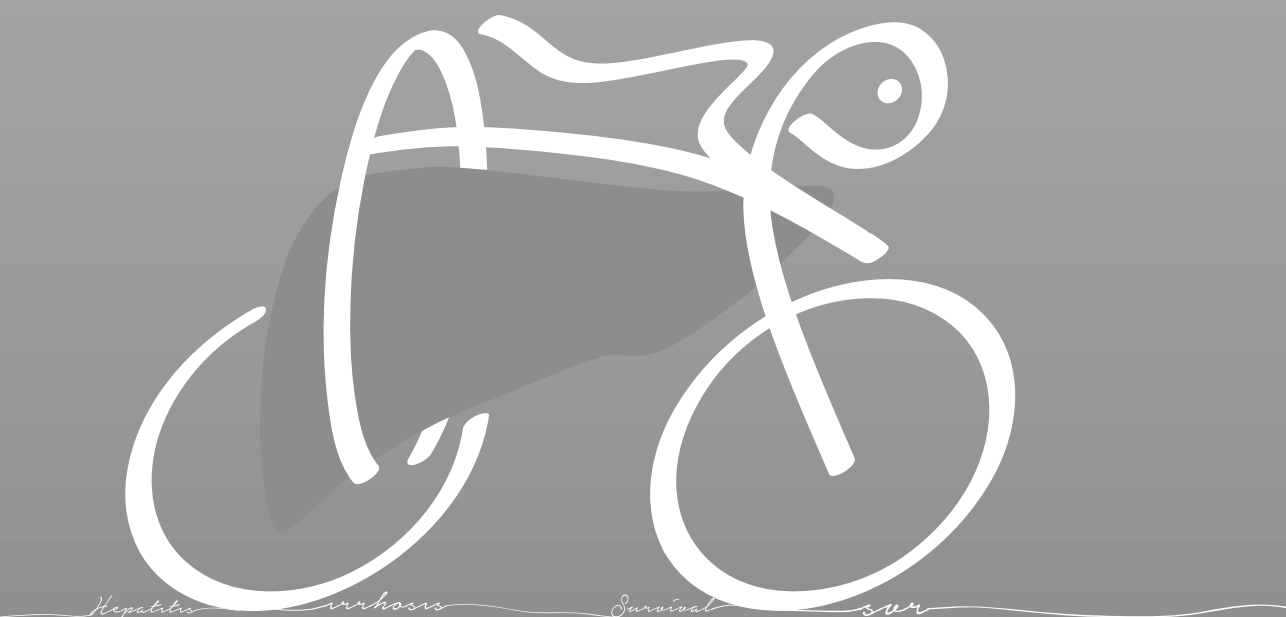
Lieve familieleden, dank julllie interesse in mijn wetenschappelijke activiteiten. Ook veel dank voor jullie begrip wanneer ik hierdoor niet bij al onze bijeenkomsten aanwezig kon zijn.

Lieve Flora en Chin Kie, dank voor de onzelfzuchtige ondersteuning die jullie mij, en ook Elaine, in de afgelopen jaren hebben geboden. Lieve Lizette, het doorzettingsvermogen dat jij de afgelopen jaren getoond hebt, is uitzonderlijk. Dank dat je altijd voor me klaarstaat, zeker wanneer er weer eens een digitaal probleem de kop opsteekt. Heel veel succes met jouw promotie.

Lieve Femke, ik ben ontzettend trots op de manier waarop jij jezelf blijft ontwikkelen door constant nieuwe uitdagingen aan te gaan. Ik neem daar graag voorbeeld aan. Dank voor al die keren dat ik een beroep op je mocht doen. Robert, jouw gedrevenheid, hoewel binnen een andere tak van sport, werkt enorm aanstekelijk en volg ik met veel interesse. Geweldig dat jij vandaag mijn paranimf wilt zijn! Jongens, Elaine en ik hebben enorm genoten van onze tijd als buren. We missen de spontane zondagmiddagborrels op het balkon.

Joop en Wietske, mijn lieve ouders, ontzettend veel dank voor jullie onvoorwaardelijke steun, interesse, trots en liefde. Jullie hebben mij altijd gestimuleerd het beste uit mijzelf te halen. Mam, dank voor je onuitputtelijke aandacht en behulpzaamheid. Je bent natuurlijk veel te goed voor me. Pa, ik neem maar weinig grote beslissingen zonder jou te consulteren. Jouw mening zal altijd belangrijk voor mij zijn.

En tot slot, Elaine, lieve schat, mijn dank aan jou is niet in een paar regels te beschrijven. Meer nog dan alle anderen heb jij voor mij klaar gestaan de afgelopen jaren, meer nog dan alle anderen heb jij geduld met mij gehad. Zonder jou zou ik de belangrijke balans tussen werk, verplichtingen en plezier geheel verliezen en was dit boekje er zeker nooit geweest. Nu is eerst de beurt aan jou, daarna is die aan ons! Ik verheug me er ontzettend op.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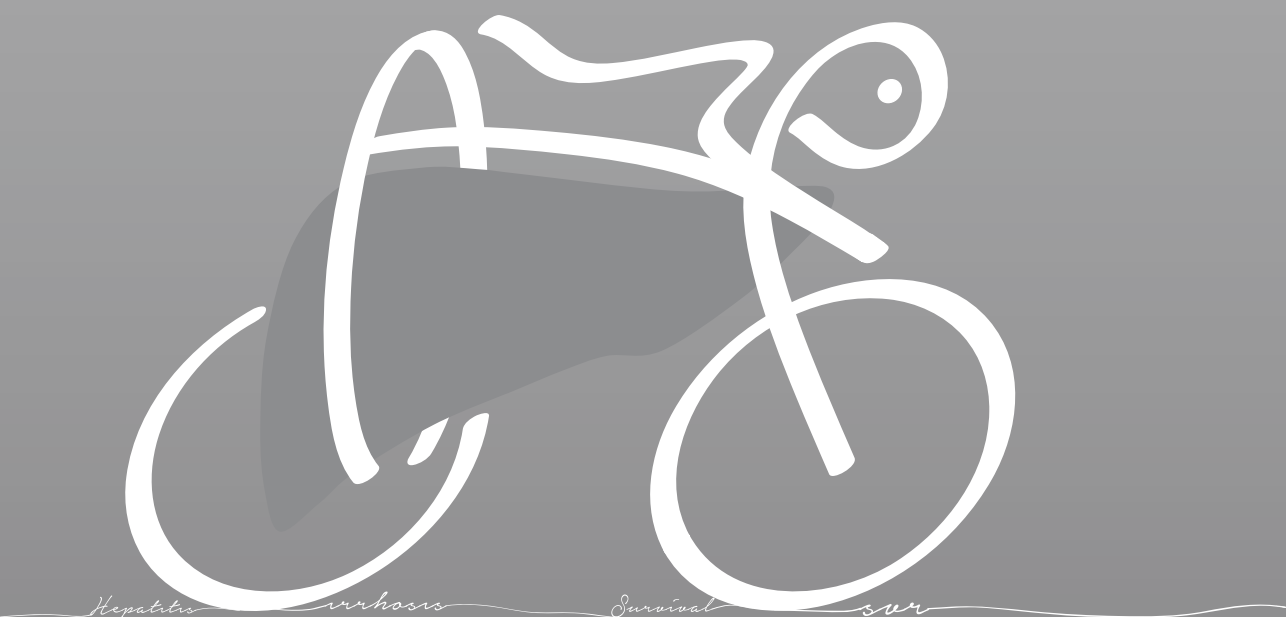
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CURRICULUM VITAE

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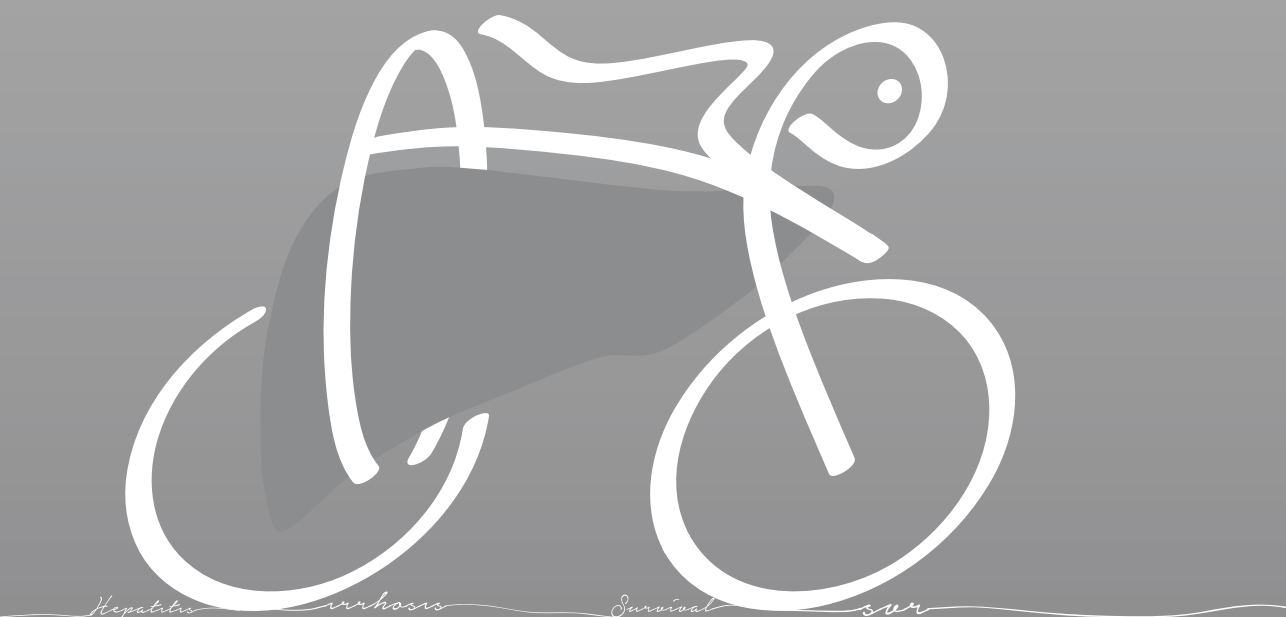
Adriaan Johannes Pieter van der Meer, de auteur van dit proefschrift, werd geboren op 5 november 1983 te Rotterdam. Na het behalen van zijn gymnasium diploma aan het Comenius College te Capelle aan den IJssel in 2002, startte hij met de studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. Gedurende zijn studie was hij in het Erasmus Medisch Centrum werkzaam als student-assistent op de afdeling Longziekten. Het onderwerp van zijn afstudeeronderzoek, waarmee hij in 2007 het doctoraal examen behaalde, betrof de prevalentie van *Schistosoma haematobium* infecties in Msambweni, Kenia. In 2009 behaalde hij het artsexamen na het doorlopen van de coschappen in de omgeving van Rotterdam. Hieropvolgend ving hij zijn promotieonderzoek naar de langetermijnuitkomsten van chronische hepatitis C aan, onder begeleiding van prof. dr. H.L.A. Janssen, op de afdeling Maag- Darm- en Leverziekten van het Erasmus Medisch Centrum te Rotterdam. In maart 2013 ontving hij van de Nederlandse Vereniging voor Hepatologie (NVH) de 'NVH Young Hepatologist Award' voor het beste klinisch hepatologisch wetenschappelijk artikel van Nederlandse bodem in 2012. Sinds juli 2013 is hij in opleiding tot specialist in de maag-, darm- en leverziekten (opleider: dr. R.A. de Man). De tweejarige 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 Elaine Utomo.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PhD PORTFOLIO

PHD PORTFOLIO

Summary of PhD training and teaching

Name PhD student: Adriaan J.P. van der Meer
PhD period: March 2009 – June 2013
Erasmus MC Department: Gastroenterology and Hepatology
Promotor: prof. dr. H.L.A. Janssen

1. PhD training	Year	Workload
General courses		
Basiscursus Regelgeving en Organisatie van Klinisch onderzoek, Erasmus MC, Rotterdam	2009	24 hours
Courses in methodology and biostatistics		
Introduction to data analysis, Nihes institute, Erasmus MC, Rotterdam	2010	24 hours
Regression analysis, Nihes institute, Erasmus MC, Rotterdam	2010	40 hours
Survival analysis, Nihes institute, Erasmus MC, Rotterdam	2010	40 hours
Oral presentations		
Sustained virological response improves overall survival in chronic hepatitis C patients with advanced fibrosis. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2011	12 hours
Improved platelet count and smaller spleen size long after sustained virological response in chronic hepatitis C patients with advanced fibrosis. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2011	24 hours
Circulating hepatocyte-derived microRNAs are highly sensitive biomarkers associated with the necroinflammation level in chronic hepatitis C patients. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2011	12 hours
Circulating hepatocyte-derived microRNAs as sensitive biomarkers for liver injury in chronic hepatitis C. Bioscience & Medicine, International Summer Academic Exchange Program, Shanghai Jiao Tong University School of Medicine, Shanghai, China	2011	36 hours
Sustained virological response improves overall survival in chronic hepatitis C patients with advanced fibrosis. 62nd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, United States of America.	2011	36 hours
Prediction of clinical disease progression in chronic hepatitis C virus infected patients with advanced hepatic fibrosis using objective variables. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2012	12 hours

Number of patients needed to treat to prevent death in genotype 1 chronic hepatitis C cirrhosis: the impact of improved interferon-based therapy. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2012	12 hours
Number of patients needed to treat to prevent death in genotype 1 chronic hepatitis C cirrhosis: the impact of improved interferon-based therapy. 63rd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America.	2012	36 hours
Miravirsen, an oligonucleotide targeting microRNA-122, induces a decline of interferon-gamma inducible protein-10 in treatment-naïve chronic hepatitis C genotype 1 patients. 63rd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America.	2012	36 hours
Long-term Follow-up of the Veldt-study. 9th Expert Summit on Viral Hepatitis, Frankfurt am Main, Germany	2013	24 hours
The risk for hepatocellular carcinoma among patients with chronic HCV infection and advanced hepatic fibrosis following sustained virological response. 64th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Washington, DC, United States of America	2013	36 hours
Clinical outcome of patients with chronic hepatitis C virus infection and advanced hepatic fibrosis – The benefits of antiviral therapy. 28th Erasmus Liver day, Rotterdam, The Netherlands	2013	12 hours
Achieving SVR: which impact does it have on further outcome of liver disease? The 3rd world congress on controversies in the management of viral hepatitis, Berlin, Germany	2014	12 hours
Link SVR to favourable outcome – why do we need to get them? From care to cure – chronic HCV symposium, Amsterdam, The Netherlands	2014	8 hours

Poster presentations

Liver-derived microRNAs in serum are sensitive biomarkers for hepatic injury in chronic hepatitis C. 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America.	2010	32 hours
Improved platelet count and smaller spleen size long after sustained virological response in chronic hepatitis C patients with advanced fibrosis. 62nd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, United States of America.	2011	32 hours
Circulating hepatocyte-derived microRNAs are highly sensitive biomarkers associated with the necroinflammation level in chronic hepatitis C patients. 62nd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, United States of America.	2011	24 hours
Factors associated with hepatocellular carcinoma in chronic hepatitis C patients with advanced liver fibrosis. 47th Annual meeting of the European Association for the Study of the Liver (EASL), Barcelona, Spain.	2012	32 hours
Prediction of long-term survival in chronic hepatitis C patients with advanced fibrosis using standard laboratory tests. 47th Annual meeting of the European Association for the Study of the Liver (EASL), Barcelona, Spain.	2013	32 hours
Gradual increase in platelets following sustained virological response among patients with HCV-induced advanced hepatic fibrosis. 64th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Washington DC, United States of America.	2013	24 hours

Comparison of the overall survival between patients with HCV-induced advanced hepatic fibrosis and the general population. 64th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Washington DC, United States of America.	2013	24 hours

Attended (inter)national conferences

Clinical pharmacology of hepatitis therapy, Boston, MA, United States of America.	2009	16 hours
The Liver Meeting 2009, 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America.	2009	28 hours
45th Annual Meeting of the European Association for the Study of the Liver (EASL). Vienna, Austria.	2010	28 hours
The Liver Meeting 2010, 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America.	2010	28 hours
46th Annual Meeting of the European Association for the Study of the Liver (EASL). Berlin, Germany.	2011	28 hours
Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, the Netherlands.	2011	12 hours
Bioscience & Medicine International Summer Academic Exchange Program, Shanghai Jiao Tong University School of Medicine, Shanghai, China	2011	36 hours
The Liver Meeting 2011, 62nd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). San Francisco, CA, United States of America.	2011	28 hours
47th Annual Meeting of the European Association for the Study of the Liver (EASL). Barcelona, Spain.	2012	28 hours
The Liver Meeting 2012, 63rd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). San Francisco, CA, United States of America.	2012	28 hours
9th Expert Summit on Viral Hepatitis, Frankfurt am Main, Germany	2013	12 hours
48th Annual Meeting of the European Association for the Study of the Liver (EASL). Amsterdam, The Netherlands.	2013	28 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

Awards

Full bursary from the European Association for the Study of the Liver (EASL) awarded for the best abstracts by young investigators	2012
Young Hepatologist Award 2013 from the Netherlands Association of Hepatology awarded for the best Dutch clinical hepatology paper in 2012	2013

Attended seminars and workshops

24th Erasmus Liver day. Rotterdam, The Netherlands	2010	6 hours
7th Post-AASLD symposium. Rotterdam, The Netherlands	2009	2 hours

3e Lagerhuisdebat Hepatitis B en C. Utrecht, The Netherlands	2010	2 hours
25th Erasmus Liver day. Rotterdam, The Netherlands	2010	6 hours
8th Post-AASLD symposium. Rotterdam, The Netherlands	2010	2 hours
26th Erasmus Liver day. Rotterdam, The Netherlands	2011	6 hours
9th Post-AASLD symposium. Rotterdam, The Netherlands	2011	2 hours
27th Erasmus Liver day. Rotterdam, The Netherlands	2012	6 hours
10th Post-AASLD symposium. Rotterdam, The Netherlands	2012	2 hours
4e Lagerhuisdebat Hepatitis B en C. Utrecht, The Netherlands	2012	3 hours
28th Erasmus Liver day. Rotterdam, The Netherlands	2013	6 hours

Reviewing for scientific journals

Including Hepatology, Gut, Journal of Hepatology, The Journal of Infectious Diseases, Liver International, Journal of Antimicrobial Chemotherapy, AP&T		40 hours

2. Teaching

Lecturing

Young Investigator Educational Seminar: Get going on the statistical highway. 46th Annual meeting of the European Association for the Study of the Liver (EASL), Berlin, Germany.	2010	6 hours
Diagnostics and treatment of chronic hepatitis C, Masterclass viral hepatitis, Erasmus Medical Center, Rotterdam, The Netherlands	2011	6 hours
Chronic HCV infection, 2nd year curriculum Medicine, Erasmus University Rotterdam, Rotterdam, The Netherlands	2012	6 hours