Long-term clinical outcome of treatment for chronic hepatitis C

Bart J. Veldt

Dit project is mogelijk gemaakt door

Financial support to the costs associated with the publication of this thesis, from Roche Nederland BV, Schering-Plough B.V., UCB Parma B.V. / Gilead, Zambon Nederland B.V., Tramedico, Astra Zeneca B.V., Abbott B.V., Novartis, Nederlandse Vereniging voor Hepatologie and Erasmus Universiteit Rotterdam, is gratefully acknowledged.

Cover graph: “Triangle” from the Euclidean Runes series, by George H. Cladis, Fine Arts Housing, New York.

Lay-out by Optima Grafische Communicatie, Rotterdam, The Netherlands.
Printed by Optima Grafische Communicatie, Rotterdam, The Netherlands.

Long-term Clinical Outcome Of Treatment For Chronic Hepatitis C

Lange termijn uitkomsten van de behandeling van chronische hepatitis C

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus
Prof.dr. S.W.J. Lamberts
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
Vrijdag 13 juni 2008 om 11.00 uur

door

Bartholomeus Johannes Veldt

geboren te Heerhugowaard

Promotiecommissie

Promotor:
Prof.dr. H.L.A. Janssen

Overige leden:
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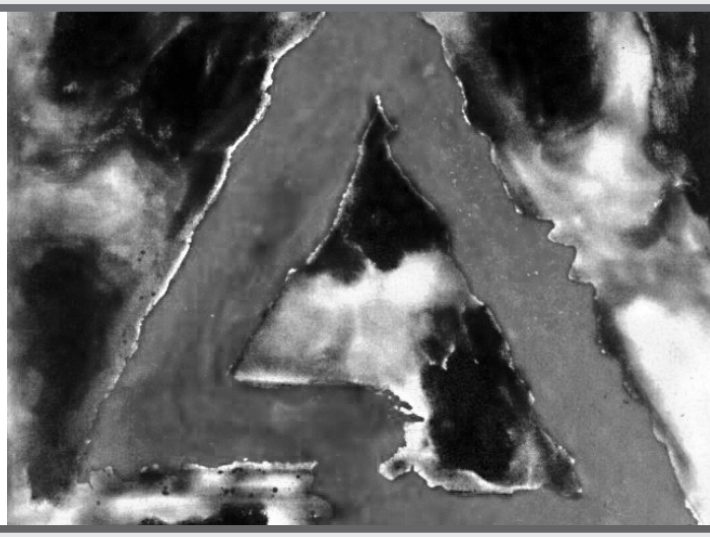
Chapter 1

General Introduction



The hepatitis C virus was discovered in 1989 (1). This virus is a small, enveloped, single-stranded, positive sense RNA virus in the *Flaviviridae* family which replicates within hepatocytes in the liver. There is some evidence that it might also replicate outside the liver, in peripheral blood mononuclear cells, in lymphoid cells and in neurons (2, 3). The hepatitis C virus species is classified into six genotypes with several subtypes within each genotype (4).

Hepatitis C can be transmitted by contact with infected blood, for example by blood transfusion or intravenous drug abuse. There is a small risk of transmission in case of birth from an infected mother, body piercing, tattoos and sharing tooth brushes or razors with an infected person (5-7). Sexual transmission of hepatitis C rarely occurs in monogamous heterosexual couples (8-10).

Acute infection with the hepatitis C virus is often subclinical, and only a minority of patients experience severe symptoms such as jaundice, for which they seek medical attention (11). Patients with an acute icteric hepatitis C have a more vigorous immune response and are therefore more likely to spontaneously clear the infection than patients with a subclinical infection (12). The virus persists in 60-85% of infected patients, leading to chronic inflammation of the liver. While early chronic hepatitis C usually only gives little and aspecific symptoms such as fatigue, it may take years or even decades before patients discover that they have chronic hepatitis C infection. Patients may be identified when routine blood tests show elevated liver enzymes or if there is a suspicion because of a history of blood transfusion before 1991 or intravenous drug abuse. Between one and two percent of the patients develop extrahepatic manifestations of hepatitis C, such as porphyria cutanea tarda, glomerulonephritis or vasculitis due to cryoglubulinemia (13, 14).

Hepatitis C infects an estimated 170 million people worldwide. The prevalence of chronic hepatitis C in Europe and North America is 1.0 to 1.7 percent. The prevalence is higher in Southern Europe, in Asia and in Egypt (15). Hepatitis C is most prevalent among haemophiliacs who received blood products before 1991, among intravenous drug users and among prison inmates.

Chronic hepatitis C causes hepatic inflammation which may lead to fibrosis or scarring of the liver. It has been estimated that it takes about 30 years before cirrhosis develops, but there are large differences in the outcomes of cohort studies describing the natural course of hepatitis C (16). Patients infected with hepatitis C are at risk of developing hepatic failure and hepatocellular carcinoma (HCC). For patients with hepatitis C cirrhosis the 5-year risk for development of HCC is about 2 to 10% (17, 18).

Interferon alpha was discovered to be effective for treating chronic hepatitis C. Although the mechanism of action has not been fully clarified, it has been shown

that interferon has both a direct antiviral effect as well as important interactions with the adaptive and innate immune responses (19). Clearance of hepatitis C is associated with the development and persistence of virus specific responses by cytotoxic and helper T cells (20).

Interferon causes many side effects, such as flu-like symptoms, cytopenia, dermatitis and mood disorders and the response rates in the first studies were as low as 10 to 15%.

Addition of the antiviral agent ribavirin significantly enhanced the reponse rates. Pegylated interferon is a polyethylene-glycol-conjugated derivative of conventional interferon alpha, which acts longer and allows for weekly dosing instead of thrice a week (21). The response rates following treatment with peginterferon and ribavirin are close to 90% for patients with genotype 2 or 3 and may be as high as 60% for patients with genotype 1 or 4 (22, 23).

Sustained virological response (SVR) to treatment is defined as undetectability of hepatitis C virus ribonucleic acid (HCV-RNA) in the serum by sensitive molecular tests at 24 weeks after the end of treatment.

For those patients not responding to treatment with pegylated interferon and ribavirin and for patients in whom this treatment is contraindicated, new treatment options are being developed (24). Patients with end stage liver disease due to hepatitis C may be candidates for liver transplantation. Chronic hepatitis C is one of the major indications for liver transplantation in Europe and North America (25). However, recurrence of infection is universal post-transplant.

CLINICAL OUTCOME AFTER TREATMENT

Hepatologists have to deal with the following dilemma: How can we avoid severe complications of hepatitis C using the current treatment of pegylated interferon and ribavirin, without unnecessarily subjecting patients with a favorable course of the disease to a therapy which is a burden for the individual (side effects) as well as to society (costs)?

It is difficult to investigate the long-term benefit of treatment as the natural course of the disease is not clearly defined. Furthermore, as there is a treatment available for hepatitis C, it is ethically impossible not to treat hepatitis C patients in order to further study this natural course of the disease. There has risen a high pressure, first from the pharmaceutical industries and later from the consensus guidelines of liver experts, to treat every patient. These guidelines are based on the chance of achieving a sustained virological response, which is a surrogate marker for clinical outcome.

Patients who achieved SVR are often referred to as being cured. However, it remains to be proven whether undetectability of hepatitis C virus ribonucleic acid (HCV-RNA) in the serum by sensitive molecular tests at 24 weeks after the end of treatment, indeed leads to a decreased risk of liver failure and liver cancer and leads to an improved survival. Some studies show that replicative parts of the hepatitis C virus can still be detected within patients who achieved SVR and a small percentage of patients with SVR may show a late relapse of the virus.

Reports of the long-term benefit of standard interferon therapy for patients with cirrhosis have been disappointing since few achieved SVR (26). Large studies from Japan indicate that maximum benefit of SVR is achieved among those treated prior to the development of cirrhosis (27). Since SVR rates following peginterferon plus ribavirin therapy are significantly higher than for interferon monotherapy, it has become feasible to evaluate the effect of therapy on solid clinical endpoints such as liver failure, HCC and survival and to establish whether SVR leads to an improved long-term outcome.

Cirrhotic patients with a sustained virological response may still develop hepatocellular carcinoma during long-term follow-up. Therefore, there may be other factors that increase the risk of hepatocellular carcinoma among patients with advanced fibrosis (28).

There is recent epidemiological evidence that the presence of diabetes mellitus increases the risk of hepatocellular carcinoma (HCC) (28). An explanation for this association may be that diabetes mellitus often occurs as part of the metabolic syndrome, which increases the risk of non-alcoholic steatohepatitis (NASH) and that HCC can be a late complication of NASH (29).

Diabetes mellitus is more prevalent among patients with chronic hepatitis C than in the general population (30). Liver disease contributes to insulin resistance as it leads to a progressive impairment of insulin secretion and it induces hepatic insulin resistance (31). Studies in transgenic mouse models with the hepatitis C core gene have shown that hepatic insulin resistance may be caused by elevated levels of tumor necrosis factor-alpha, which disturbs the tyrosine phosphorylation of insulin receptor substrate-1 (32).

Chronic hepatitis C virus infection itself also increases the risk of HCC. It leads to chronic inflammation of the liver, to liver fibrosis and eventually to cirrhosis. Therefore, among patients with both chronic hepatitis C and diabetes mellitus there are two potential ways in which HCC may develop: by the metabolic pathway and by the carcinogenic effect of the hepatitis C virus. This interaction between hepatitis C and diabetes mellitus in the development of HCC has been described in population based studies (33), but the risk of developing HCC over

time for patients with hepatitis C cirrhosis and diabetes mellitus has not been quantified.

COST EFFECTIVENESS OF TREATMENT

In order to limit side-effects and costs, current consensus guidelines recommend that treatment of chronic hepatitis C with peginterferon and ribavirin should not be continued when the probability of SVR is low. The recommendations about whether to continue treatment are based on measurements of the serum concentration of hepatitis C ribonucleic acid (HCV-RNA); if the HCV-RNA has not dropped a factor 100 by week 12 or below levels of detection at week 24 of treatment, the treatment should be stopped (25). However, testing HCV-RNA levels by real-time polymerase chain reaction (rtPCR) is logistically complicated, expensive and often not performed outside academic or referral centres.

Decrease in alanine aminotransferase (ALT) levels have been shown to reflect the drop in HCV-RNA which occurs during treatment (34, 35). This finding suggests that the ALT test, which is cheap and is easily performed in all hospitals, may provide an alternative basis for recommending the continuation of treatment. In addition, it is also useful to study the cost-per-cure in relation to the duration of therapy, as there is a nonlinear increase in the probability of cure over time, while the cost of medication increases proportionally to the duration.

DEVELOPMENT OF NEW TREATMENT OPTIONS FOR NON-RESPONDERS TO INTERFERON

For patients not responding to treatment with peginterferon and ribavirin, new treatment options should be explored. In Japan, glycyrrhizin has been propagated as an anti-inflammatory drug, capable of minimizing disease activity in the chronically infected liver. Placebo controlled trials have proven that the administration of glycyrrhizin leads to a significant reduction of ALT levels in chronic hepatitis C patients (36). The question remains whether this reduction of ALT levels leads to a reduced risk of liver-related morbidity and mortality.

In analogy with the immunomodulatory effects of interferon, α-galactosylceramide, a synthetic glycosphingolipid, has been shown to induce powerful antiviral immune responses (37-40). It can be recognized by CD1-restricted natural killer T cells which, upon stimulation, produce cytokines such as interferon-γ and tumour necrosis factor-α and enhance the development of "classical" T helper

1 type responses (41). It has recently been demonstrated that human circulating Vα24+Vα11+ natural killer T cells and CD1d-reactive intrahepatic lymhocytes induce profibrotic T helper 2 type cytokines during disease progression of chronic hepatitis C (42, 43). As the numbers of circulating natural killer T cells are comparable between chronic hepatitis C patients and healthy controls (44), this indicates that the quality rather than the quantity of Vα24+Vα11+ natural killer T cells is altered during chronic hepatitis C infection. α-Galactosylceramide may exert antiviral activity against hepatitis C by reversing the T helper 2-type cytokine profile of Vα24+Vα11+ natural killer T cells.

AIMS

In this thesis we aim to examine the clinical outcome (hepatic failure, HCC, mortality) of patients with a sustained virological response six months after therapy, as compared with non-responders and compared with the natural course of the disease, specified according to age, gender and stage of fibrosis. Based on these long-term effects, we will try to develop or modify treatment strategies.

We will also assess whether stopping rules during the treatment of chronic hepatitis C can be based on ALT levels and on cost-per-cure.

Finally, we aim to investigate the effect of concomitant factors such as overweight and diabetes mellitus on the clinical outcome of hepatitis C patients and to evaluate new treatment options for non-responders to (peg-)interferon and ribavirin.

OUTLINE OF THE THESIS

Chapter 1 Introduction.

Chapter 2 Natural history.
In this chapter, we use a mathematical model that corrects for factors such as age at infection, gender and comorbidity to explain the marked differences in clinical outcome of published cohort studies and to give insight in the natural course of the disease.

Chapter 3 Interferon and ribavirin treatment.
In chapter three we describe a placebo controlled randomized trial, that aims to evaluate the effect of ribavirin for the treatment of patients who did not respond to previous interferon monotherapy.

Chapter 4 Early treatment discontinuation.
In chapter four we present a large study that determines whether recommendations for continuing or discontinuing treatment of chronic hepatitis C can be based on ALT testing and on the cost-per-cure.

Chapter 5 Long-term clinical outcome of non-responders.
In co-operation with Japanese academical and large general hospitals we were able to identify a large cohort of patients treated for chronic hepatitis C. In this chapter we evaluate the long-term clinical outcome of non responders to interferon treatment. In addition, we evaluate the effect of glycyrrhizin, a compound used in Japan to lower the inflammatory activity in the liver, on the occurrence of hepatocellular carcinoma among these patients.

Chapter 6 Long-term clinical outcome of sustained virological responders.
In this chapter we determine the long term clinical outcome of sustained virological responders treated in protocolled studies.

Chapter 7 Long-term clinical outcome of patients with advanced fibrosis.
We evaluate the effect of therapy on solid clinical endpoints such as liver failure, HCC and survival and establish whether SVR leads to an improved long-term outcome in a high-risk population of patients with advanced fibrosis.

Chapter 8: Effect of treatment with peginterferon and ribavirin on graft survival in liver transplant patients infected with hepatitis C.
The aim of this study is to assess whether antiviral treatment improves graft survival in patients with recurrent hepatitis C after transplantation.

Chapter 9 Effect of diabetes mellitus on clinical outcome of patients with advanced fibrosis.
In this chapter we quantify the risk of HCC among patients with both diabetes mellitus and hepatitis C and we assess which factors are associated with the risk of diabetes mellitus among patients with advanced fibrosis.

Chapter 10 Development of new treatment strategies for non-responders.
In this chapter we describe an international multicenter randomized placebo-controlled trial investigating the safety and the antiviral activity of α-galactosylceramide for the treatment of chronic hepatitis C patients.

Chapter 11 Discussion.

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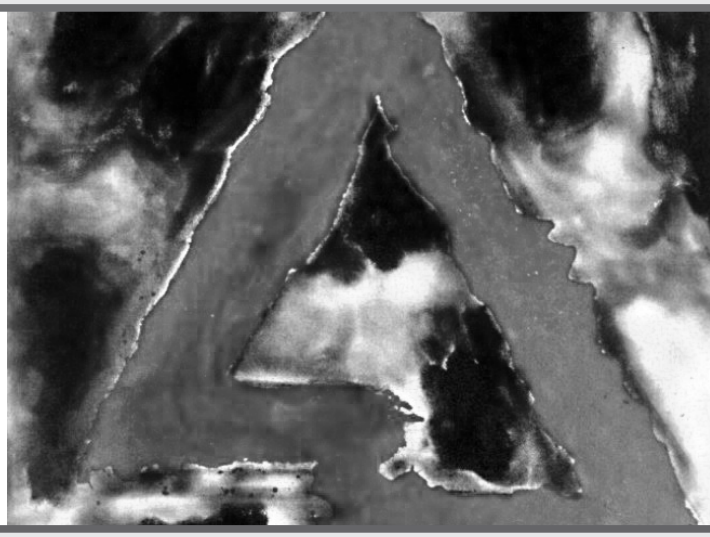
Chapter 2

Towards reliable estimates of life expectancy for untreated chronic hepatitis C patients



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ABSTRACT

Background/Aims: Cohort studies describing the natural history of hepatitis C show a great disparity in outcome. In this study we try to bridge these differences, making more precise estimations possible of the risk of liver-related mortality for untreated chronic hepatitis C patients, based on gender, age, fibrosis stage and age at infection.

Methods: We built a Markov-model, using two studies that were pivotal for the description of the natural history of chronic hepatitis C. The model calculates the percentage of patients with uncomplicated chronic hepatitis, cirrhosis, decompensated cirrhosis or hepatocellular carcinoma as well as the estimated mortality (both liver-related and non-liver related) during 30 years of follow-up, for males and females aged 15-60 years at infection.

Systematic review of the literature was performed to identify large cohort studies including >100 patients, describing the occurrence of liver related death among chronic hepatitis C patients.

Results: The rates of liver-related mortality predicted by the model fitted well with the outcome of the 9 published cohort studies identified by systematic literature review (correlation coefficient 0.783,p=0.013).

Conclusion: The model developed in this study explains the differences in occurrence of liver-related death observed in cohort studies and may help to individualize estimations of life expectancy.

INTRODUCTION

Chronic hepatitis C affects some 170 million patients world-wide (1). The infection may lead to inflammation of the liver, with subsequent fibrosis and eventually cirrhosis. The disease is usually slowly progressive. The National Institute of Health consensus on chronic hepatitis C states that the risk of developing cirrhosis during 20 years of hepatitis C infection is 10-15% and that there are 10,000 to 12,000 deaths yearly in the United States due to hepatitis C (2). Although it is mentioned that fibrosis progression rates may be variable according to sex and age at infection, no precise estimates of the occurrence of liver-related death are given for different subgroups of patients. The French consensus mentions that fibrosis may accelerate after the age of 50 to 60 years, but again precise estimates of the occurrence of liver-related death for hepatitis C patients are lacking (3).

The reason that the natural history is only described in such rough terms is that cohort studies describing the natural history of hepatitis C show a great variety in outcome. The outcome ranges from a 25-year risk of liver-related mortality of 0.5% for female patients infected in their twenties(4) to an 8-year risk of liver-related mortality of 16% for 61-year-old patients with cirrhosis (5).

As the treatment of hepatitis C goes along with significant side effects for the patients and high costs for society, the decision to start treatment depends highly on the estimated risk of liver-related morbidity and mortality if treatment were not given. Therefore, it is of major importance to develop more precise knowledge of the variations in natural history of chronic hepatitis C. In this study we try to explain the differences in outcome of cohort studies describing the natural history of chronic hepatitis C and to make more precise estimations of the risk of liver-related mortality for chronic hepatitis C patients, based on gender, age, fibrosis stage and age at infection.

PATIENTS AND METHODS

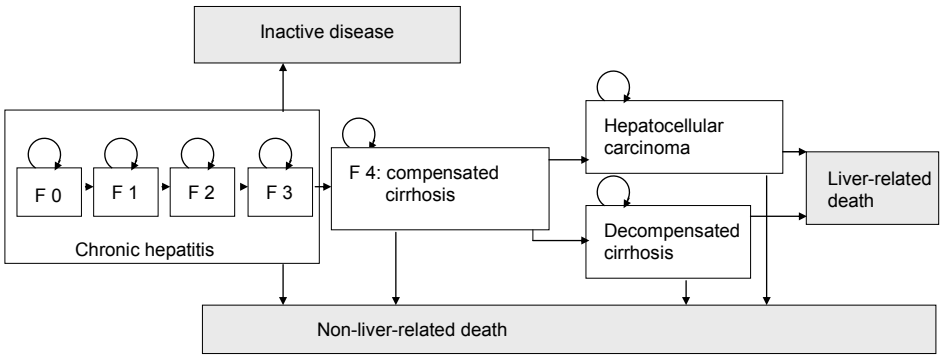
We built a Markov-model, using two studies that were pivotal for the description of the natural history of chronic hepatitis C (6, 7). We chose to use a Markov-model, since it is the standard method for actuaries to describe a disease that progresses in well-defined stages. In a Markov model, members of a cohort are divided among several mutually exclusive health states, and movements of the cohort across these states are modelled over time (8-10). In this study, at the end of every three months, the members of the cohort are reallocated across the health states, with these movements guided by transition probabilities that reflect

the natural history of the disease. The modelling of the natural history was done separately for men and women, for different ages at infection and for different fibrosis stages. In our model, the classical flow diagram for the natural history of hepatitis C was adapted to account for death from hepatitis C unrelated causes, and for spontaneous loss of the viral infection (figure 1).

The transition probabilities from chronic hepatitis to cirrhosis were derived from Poynard et al (6) (table 1).

Data from Fattovich (7) were used for the transition probabilities of cirrhosis to decompensated cirrhosis and hepatocellular carcinoma, respectively. The progression rates from decompensated cirrhosis to death and from hepatocellular carcinoma to death were also derived from the study of Fattovich et al. (figure 2) (7).

The transition probabilities from cirrhosis to HCC, decompensation and death were fitted in a formula using constant, linear and exponential factors. In almost all cases the exponential factor did not fit the data. Only the transition from decompensated cirrhosis to liver–related death was best described in an exponential way. The rate of loss of the hepatitis C infection in chronic hepatitis C was

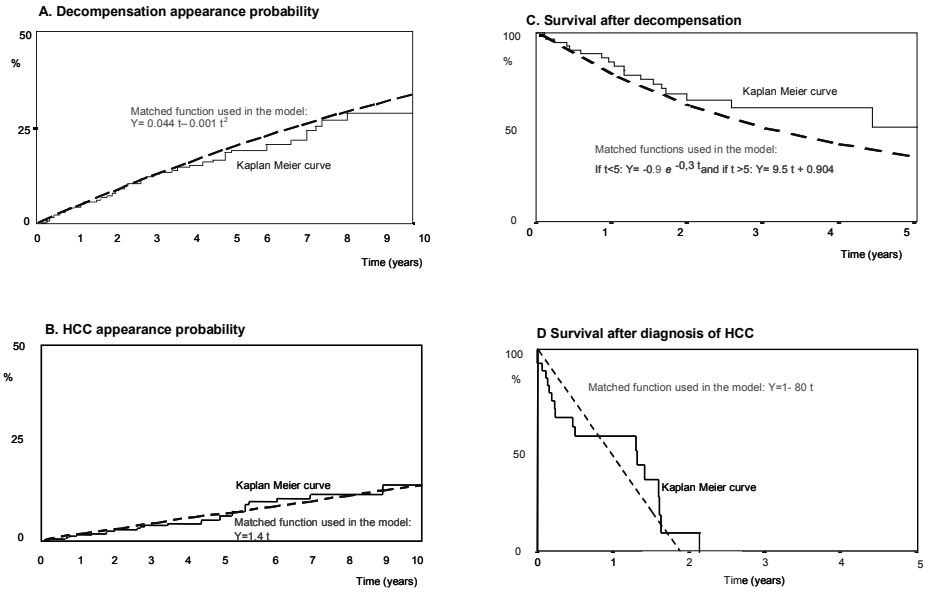


2.1: Overview of the disease stages in hepatitis C infection. Arrows indicate transitions from one stage to another. The Markov-model uses transition probabilities to calculate the number of patients moving across one stage to the next, every 3 months during 30 years of infection. Circular arrows indicate that there is also a possibility to stay in a certain disease stage.

Table 1.

Gender	Time of infection (years)	Transition probability, % per year, (95% CI)
Male	< 40	11.1 (9.1-13.0)
Male	> 40	30.1 (23.5-33.3)
Female	< 40	9.5 (8.8-10.0)
Female	> 40	20.0 (16.7-25.0)

Transition probabilities between the different fibrosis stages from METAVIR F0 to F1, F1 to F2, F2 to F3 and F3 to F4, according to gender and age at infection. Data derived from Poynard et al (6).



2.2: Kaplan meier curves derived from Fattovich et al. indicating the risk of decompensation over time (A) and the risk of occurrence of hepatocellular carcinoma over time (B) for patients with chronic hepatitis C cirrhosis, as well as the survival probability after occurrence of decompensation (C) or hepatocellular carcinoma (D). Dotted lines represent the mathematical functions, matched to the Kaplan Meier curves, that were used as transition probabilities in the Model.

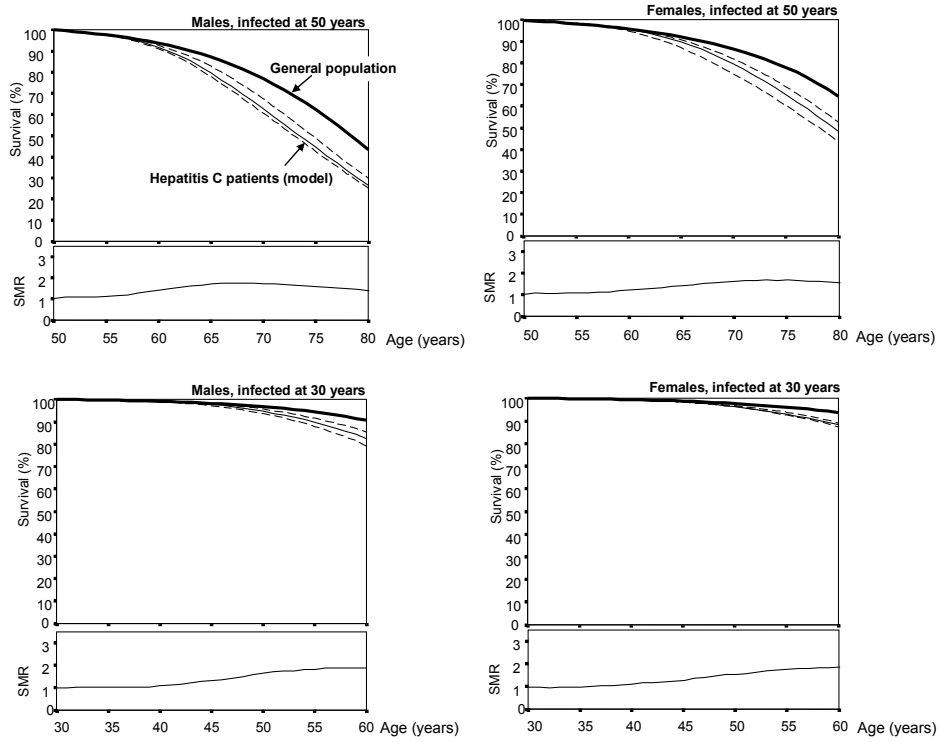
based on findings of Alter et al (11). The non-liver related mortality was derived from the mortality tables for the Dutch population over the period 1995-2000, which are published yearly (12). These mortality tables, which are similar to other European countries, describe the average mortality for each age and gender for all causes of death. Because the impact of hepatitis C on the average mortality is very low, it was not necessary to make a correction for hepatitis C related mortality in the overall population.

Sensitivity analysis:

In order to assess the reliability of our results, the lower limit and the upper limit of the 95% confidence intervals of the transition probabilities for fibrosis progression were subsequently entered in the model. These confidence intervals are given in figure 3.

Systematic literature review:

A Pubmed search was performed, using the key words “hepatitis C, natural history, cohort”. From 54 articles the abstracts were judged according to the following criteria: original data on liver related mortality, number of patients



2.3: Predicted survival of hepatitis C patients compared with the general population, matched for age and gender. Dotted lines represent the upper and lower limits of the confidence interval.
The standard mortality ratio (SMR) is given in the lower part of the figure and represents the predicted mortality among hepatitis C patients divided by the observed mortality in the matched general population.

> 100, subgroups excluded (co-infection, drug users, hemodialysis, transplant recipients). Fifteen papers were selected and all references of these fifteen selected papers were also screened and judged according to the same procedure. Selected papers were reviewed in detail and finally 9 articles fulfilled the criteria for validation of the model (5, 13-19). The incidence of liver-related mortality is expressed in percentages and the confidence intervals of the original study were used, if available. If no confidence intervals were given, they were calculated according to the formula: Standard Error = $\sqrt{}$ (p*(1-p))/n, where p is the risk of liver-related mortality and n is the number of patients included in the study.

RESULTS

Outcome of the model:

The model calculates the percentage of patients with uncomplicated chronic hepatitis, cirrhosis, decompensated cirrhosis or HCC as well as the estimated mortality (liver-related and non-liver related), for males and females aged 15 to 60 years at infection. Figure 3 shows the occurrence of liver-related mortality for males and females infected at 30 and 50 years respectively, as an example of the variety in outcome of hepatitis C according to gender, age and age at infection.

The impact of hepatitis C as a risk factor for death in comparison with the risk from other causes is best expressed by the age- and sex standardised mortality ratio.

The absolute risk of dying from a liver-related cause within 30 years after infection was estimated to be 8.5% (95% CI 5.3-11.8) for male patients and 5.5% (95% CI 4.7-6.5) for female patients infected at the age of 30 years. For these patients, the SMR starts to increase from the age of fourty on and increases up to two at the age of sixty (figure 3). Once cirrhosis is established, the standard mortality ratio increases markedly: in patients with established cirrhosis at 30 years of age, the SMR at ten years of follow-up is estimated to be 24.6 for males and 36.8 for females.

The progression of liver disease due to chronic hepatitis C is faster in patients infected after the age of fourty. The absolute risk of dying from a liver-related cause within 30 years after infection was estimated to be 28.3% (95% CI 21.9-30.8) for male patients and 20.6% (95% CI 15.1-26.8) for female patients infected at the age of 50 years. Despite this more agressive course in older patients, the estimated SMR remained below 2, due to a simultaneous increase in non-liver related mortality (figure 3). For patients diagnosed with cirrhosis at the age of fifty, the SMR at ten years of follow-up is estimated to be 4.5 for males and 6.8 for females.

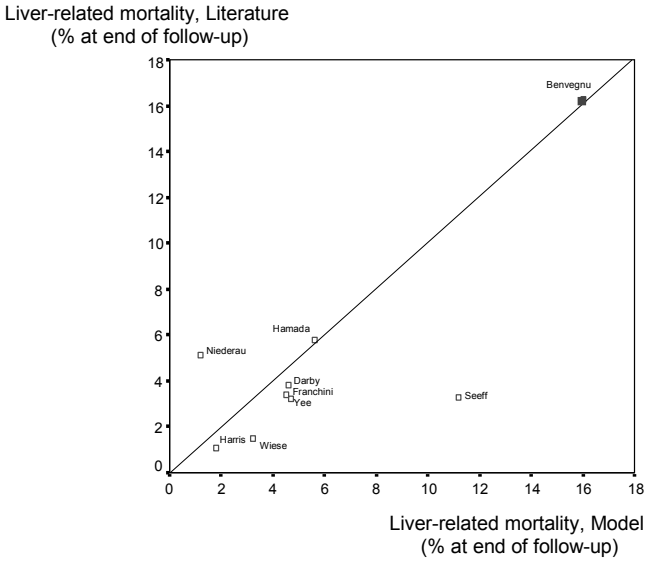
Comparison of the model to published cohort studies:

There were nine large cohort studies with more than 100 patients, describing the occurrence of liver related death among chronic hepatitis C patients (table 2). Three studies described cohorts of transfusion recipients, another three described cohorts of patients with bleeding disorders, one study described a community-based cohort and two studies described cohorts of patients referred to Hepatology units. In one of these cohort studies, patients were not followed-up from time of infection, but from time of development of cirrhosis. Benvegnu et al. describe a cohort of 254 patients with Child-Pugh A cirrhosis, followed up for 7.6 years (5).

Table 2.

Author	Journal	Year	nº	Cohort	Follow-up (years)	Liver-related mortality (95% CI)	Liver-related mortality predicted by the model[1] (95% CI)
Darby	Lancet	1997	4865[2]	Bleeding disorder	25	3.8 (1.4-10.6)	4.6 (2.8-6.6)
Wiese	J Hepatol	2005	1718	Community based	25	1.5 (0.0-2.4)	3.2 (2.3-3.5)
Harris	BMJ	2002	924	Transfusion recipients	10	1.1 (0.4-1.8)	1.8 (1.3-3.4)
Niederau	Hepatology	1998	838	Hepatology Unit	4.2	5.1 (3.6-6.6)	1.2 (0.5-2.0)
Seeff	NEJM	1992	568	Transfusion recipients	18	3.3 (1.8-4.8)	11.2 (5.4-13.7)
Hamada	Cancer	2002	445	Transfusion recipients	28	5.8 (3.6-8.0)	5.6 (3.8-7.1)
Benvegnu	Gut	2003	254	Hepatology unit	7.6	16.3 (5.1-27.5)	16.0 (11.4-20.6)
Yee	Gut	2000	185	Bleeding disorder	25	3.2 (0.7-5.7)	4.7 (2.9-6.8)
Franchini	Blood	2001	102	Bleeding disorder	25	3.4 (0.0-6.9)	4.5 (3.0-6.6)

Cohort studies including more than 100 patients, describing liver-related mortality among chronic hepatitis C patients.
[1]Corrected for age at infection, gender, fibrosis stage and duration of follow-up.
[2]Only those patients, aged 25-44 without HIV and with mild or moderate bleeding disorders were selected.



2. 4: Correlation between liver-related mortality described in cohort studies and the outcome of the model, corrected for gender, age, age at infection and fibrosis stage. Overall there was a good correlation, Pearson correlation coefficient 0.783 (p=0.013). The blue dot indicates a cohort consisting only of cirrhotics.

The model predicts a risk of liver related death of 16% for such a cohort, which matches exactly the 16.3% (95% CI 5.1-27.5) that was observed. In the other cohort from a Hepatology unit, 141 of 838 patients already had cirrhosis at the entry of the study. All 9 cohort studies are plotted in figure 4. The occurrence of liver-related mortality in these cohorts is compared to the outcome of the model, corrected for gender, age at infection, duration of follow-up and fibrosis stage at entry. The lowest rate of liver-related mortality, of 0.5% during 25 years of follow-up, occurred in a cohort of young females infected by contaminated batches of anti-D immunoglobulin, used for prophylaxis of anti Rhesus isoimmunization. In general, the rates of liver-related mortality predicted by the model fitted well with the published cohort studies (correlation coefficient 0.783; p=0.013 figure 4).

DISCUSSION

In this study, the natural history of chronic hepatitis C was modelled by computer simulation, based on data from two large cohorts of patients of various age and gender, covering the whole time-span of chronic hepatitis C. This approach, that links well-founded data from the 3 stages of chronic hepatitis C infection, may be complimentary to the most accurate means of determining the outcome of HCV infection, namely a prospective active surveillance of a large cohort of patients from the onset of chronic hepatitis C for a period of 30 or more years, without providing therapy. A few of such studies have been ongoing, however these studies are limited to subgroups with specific age and gender. Furthermore, since hepatitis C has become a treateable disease, further prospective observation of the natural course has become impossible.

While previous models on hepatitis C concentrated on describing the hepatitis C epidemic and discussed the possible consequences for health care policy, in this manuscript we focus on explaining the differences in outcome of large cohort studies (20-22).

Our model could be validated and predictions from the model are well in accordance with published cohort studies. The studies that matched the outcomes of the model the least well were the studies by Niederau et al. and Seeff et al. In the study described by Seeff et al. in 1992, the overall estimated mortality rates are higher than in our model (51% versus 26%), but the liver related mortality is lower. The explanation for this difference lies probably in the presence of comorbidity. The indication for the blood transfusions given to the fifty year old patients in Seeff's cohort are likely to have limited their life expectancy, causing them to die of other causes before they could develop end-stage liver disease.

Freeman et al. showed that the characteristics of the population that is studied play an important role in the prognosis, because of selection bias and referral bias (24). Indeed, the two cohorts of patients referred to liver clinics that were included in this study, consisted at least partially of patients who already had developed liver cirrhosis and these two studies reported the highest occurrence of liver-related mortality. The reason for the underestimation of liver-related mortality compared to the study by Niederau et al. may be that data on liver histology were present in only 580 (69%) of the patients, leading to an underestimation of the fibrosis stage at entry of the study. In the study by Benvegnu et al. we were able to make a solid prediction using our model, by correcting for fibrosis stage at entry.

We have tested the various assumptions the model is based on. One of the issues that were raised after publication of Poynard's results was that the linear fibrosis progression rates did not match clinical practice. In the Markov-model, this problem is overcome by translating the linear progression rates into transition probabilities and by including data on non-liver related mortality. As recently outlined by Kim et al., the competing risk of non-liver related mortality may lead to an observed non-linear increase in fibrosis progression, while the underlying biological mechanism may still be linear (25). This can be explained by the fact that patients with slowly progressive disease tend to disappear from the cohort due to mortality of other causes than hepatitis C as they get older.

Our model does not yet take into account concurrent factors such as HIV or hepatitis B co-infection, which probably also affect liver disease progression. If the present model is accepted, the complex matter of co-infections needs additional study in order to detangle the effect of chronic hepatitis C and of HIV or hepatitis B which in turn are affected by antiviral treatment.

Precise knowledge of the natural history of chronic hepatitis C provides the background for indications for therapy. The model developed in this study may help to individualize estimates on the long-term prognosis of untreated patients, based on gender, age, time of infection and fibrosis stage. Therefore, we aim to use the model as part of a European online consultation system for physicians treating patients with hepatitis C (26). Obviously, we aim for further refinement of the model by including specific subgroups of patients.

In conclusion, the model developed in this study explains the differences in occurrence of liver-related death observed in cohort studies and it may help to individualize estimations of life expectancy in the absence of treatment, based on gender, age, fibrosis stage and time of infection.

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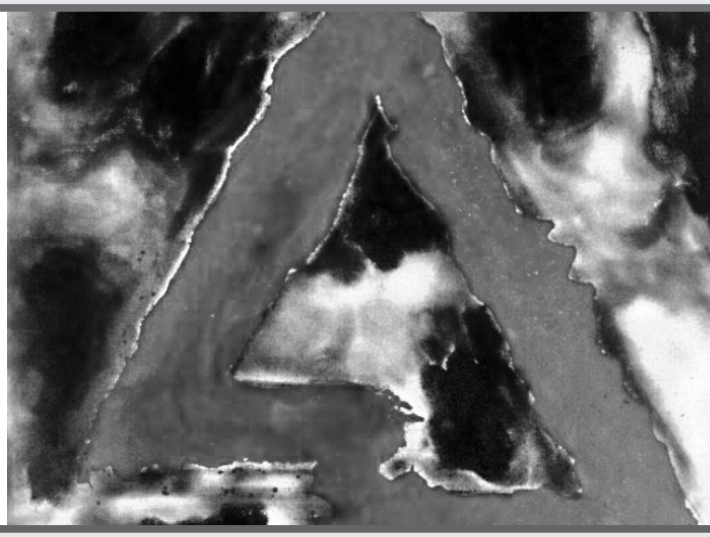
Chapter 3

Retreatment of hepatitis C non-responsive to Interferon. A placebo controlled randomized trial of Ribavirin monotherapy versus combination therapy with Ribavirin and Interferon in 121 patients in the Benelux



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BMC Gastroenterol. 2003 Aug 29;3:24.

ABSTRACT

Background

Evidence based medicine depends on unbiased selection of completed randomized controlled trials. For completeness it is important to publish all trials. This report describes the first large randomised controlled trial where combination therapy was compared to placebo therapy and to ribavirin monotherapy, which has not been published untill now.

Methods

One hundred and twenty one patients with chronic hepatitis C and elevated transaminases who did not respond to previous treatment with standard interferon monotherapy, were included from 16 centers in Belgium, the Netherlands and Luxembourg between 1992 and 1996. Patient poor-response characteristics were: genotype 1 (69%), HCV RNA above 2×10^6 copies/ml (55%) and cirrhosis (38%). Patients were randomized to 6 months combination therapy with interferon alpha-2b (3 MU tiw) and ribavirin (1000–1200 mg / day), 6 months ribavirin monotherapy (1000–1200 mg / day) or 6 months ribavirin placebo. The study was double blinded for the ribavirin / placebo component. One patient did not fit the entry criteria, and 3 did not start. All 117 patients who received at least one dose of treatment were included in the intention to treat analysis.

Results

At the end of treatment, HCV RNA was undetectable in 35% of patients on combination therapy and in none of the patients treated with ribavirin monotherapy or placebo. The sustained virological response rate at 6 months after therapy was 15% for patients treated with interferon and ribavirin.

During the 6 months treatment period 13% of patients on interferon ribavirin combination therapy, 13% of patients on ribavirin monotherapy and 11% of patients on placebo withdrew due to side effects or noncompliance. At 24 weeks of treatment the mean Hb level was 85% of the baseline value, which means a mean decrease from 9.1 mmol/l to 7.8 mmol/l. The Hb levels at the end of treatment were not significantly different from patients treated with ribavirin monotherapy ($p = 0.76$). End of treatment WBC was significantly lower in patients treated with combination therapy, compared to ribavirin ($p < 0.01$) as well as for patients treated with ribavirin monotherapy compared to placebo ($p < 0.01$).

Discussion

This belated report on the only placebo controlled study of interferon ribavirin combination therapy in non responders to standard doses of interferon mono-therapy documents the effectiveness, be it limited, of this approach as well as the dynamics of the effects on blood counts.

BACKGROUND

Until a decade ago, interferon was the only drug available for the treatment of hepatitis C. Only a minority of patients had a sustained response to standard doses of interferon monotherapy. However, non-responders might be still amendable to therapy by either high-dose daily interferon [1] or by standard interferon in combination with ribavirin [2]. This is a belated full report on the the first and probably only RCT where combination therapy was assessed in comparison to ribavirin monotherapy and to placebo therapy.

METHODS

Participants

Patients between 18 and 70 years of age with chronic hepatitis C, no ALT normalisation during and after treatment and positive HCV RNA after treatment with interferon monotherapy, were elegible for the study. The previous treatment course consisted of three mega units interferon thrice weekly for 24 weeks or at least eight weeks of treatment with 6 mega units interferon thrice weekly or at least 4 weeks of 10 mega units interferon thrice weekly.

Patients with hepatitis of other viral origin, patients with inherited metabolic liver disease, patients with acquired metabolic liver disease related to alcohol, hepatotoxic drugs or obesity and patients with autoimmune hepatitis were excluded from the study. Other exclusion criteria were significant medical illness within the past five years, pregnancy or likelihood of becoming pregnant, immune modulating therapy (corticosteroids) within the past 6 months, inadequate levels of hemoglobin (< 6 mmol/l), platelet count (<50 × 10e9/l), white blood cells (< 3 × 10e9/l), signs of hepatocellular carcinoma or decompensated liver disease, recent drug or alcohol addiction and unlikeliness to attend regularly for treatment and follow up.

Data were collected in 16 centers in Belgium and in the Netherlands; central data collection was done in the Erasmus Medical Center in Rotterdam, the Netherlands. The study received ethics committee approval in all centers involved and all patients gave signed informed consent.

Interventions

Patients were randomised and assigned to receive either ribavirin (ICN pharmaceuticals, Bucks, United Kingdom) monotherapy or a matched placebo orally twice a day in a total dose of 1200 mg a day (body weight ≥ 75 kg) or 1000 mg a

day (body weight <75 kg) for 24 weeks, or interferon (Intron A, Schering Plough, Kenilworth, NJ, USA) 3 mega units thrice a week combined with ribavirin 1000 or 1200 mg according to body weight during 24 weeks.

All patients were assessed in an outpatient setting for safety, tolerance and efficacy at the end of weeks 2, 4, 8, 12, 16, 20 and 24 and every 4 weeks during follow up until week 48. Initial HCV-RNA was measured by bDNA with a lower detection limit of 2000 copies/ml, HCV-RNA negativity at week 24 and week 48 was measured by PCR. HCV-RNA and hematologic- and biochemical parameters were measured in the certified laboratories of the participating hospitals; results were corrected for local normal values. Liver biopsies, taken within 12 months before treatment were reviewed by the local pathologist for the presence or absence of cirrhosis.

Objectives

The objective of this study was to evaluate if interferon ribavirin combination therapy or ribavirin monotherapy would be a therapeutic option for non-responders to previous interferon therapy.

Outcomes

The primary endpoint for this study was defined as loss of detectable serum HCV RNA 24 weeks after the end of treatment. The secondary endpoint was normalisation of alanine aminotransferase 24 weeks after treatment.

Sample size

It was calculated that 120 patients (40 per treatment arm) were needed in order to have 90% chance of detecting a difference of 30% at the significance level of $p = 0.05$ (single sided testing) in disappearance of HCV RNA or normalization of ALT levels, assuming the frequency of these endpoints would be ten percent or less in the placebo group.

Criteria for 50% dose reduction of ribavirin were anemia with Hb < 5.4 mmol/l or excessive subjective symptoms such as myalgia, fatigue or dyspepsia interfering severely with daily activities. Criteria for a 50% dose reduction of the total weekly interferon dose included leucopenia < 1.5 ×10e9/l, thrombocytopenia < 40 ×10e9/l or excessive subjective symptoms interfering with daily activities.

Randomization

Randomization was performed centrally at the coordination center in Rotterdam, the Netherlands after receiving all entry data, by opening an envelope [3]. Patients were randomized to either combination therapy or monotherapy at a ratio

of 1:2 after stratification for the presence of cirrhosis. The ribavirin and placebo arms were double blinded. Patients allocated to monotherapy received blinded study medication containing either ribavirin or placebo.

Allocation concealment

The randomization code of the monotherapy arms of the study remained concealed until all patients had completed their follow up and the database had been verified and closed.

Statistical analysis

All statistical analyses were peformed using SPSS for Windows (Version 10 SPSS Inc, Chicago, IL, USA). The Kaplan Meier method was used to evaluate the time of ALT normalisation according to treatment using the log rank test to assess statistical significance. The Pearson Chi-square test was used to compare the differences in mean end of treatment and end of follow up response between the different groups. The Mann Whitney test was used to compare the differences in mean end of treatment blood counts.

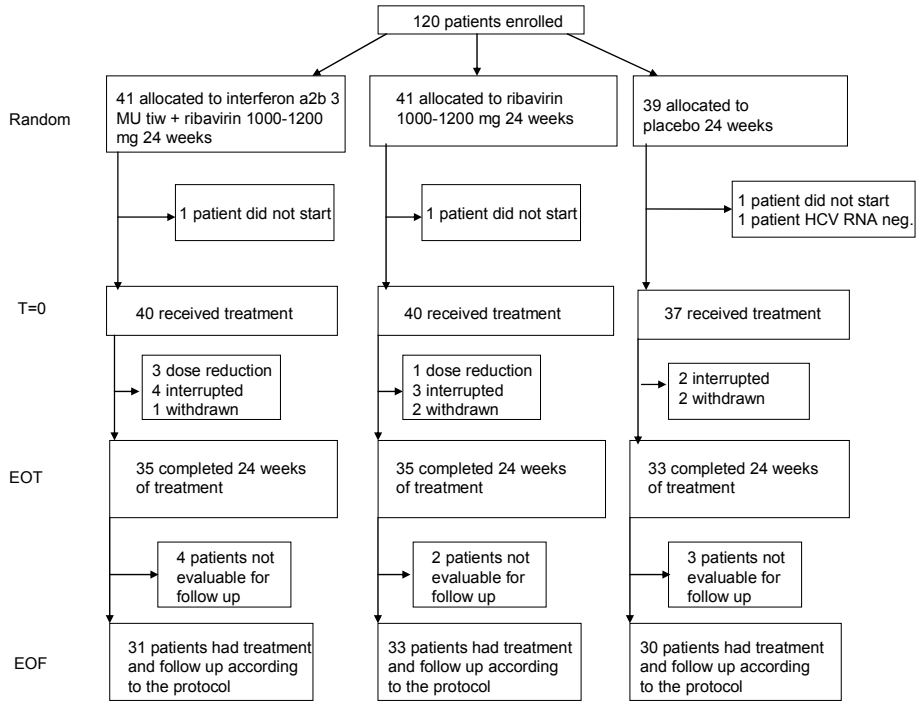

Figure 1: Trial profile. Patients who had a dose reduction following the protocol were included in all analyses.

RESULTS

Participant flow

121 patients were recruited from 16 university and affiliated hospitals in Belgium, the Netherlands and Luxembourg ('Benelux'). Seventy seven patients had been treated in a previous Benelux study on interferon mono therapy [4], the other 44 had been treated with a comparable treatment schedule of 6 MU interferon thrice a week for at least 12 weeks or 3 MU for at least 24 weeks.

One patient was excluded in view of a negative HCV RNA-test at baseline and three never started therapy. All remaining 117 patients were included in the analysis (fig 1).

Recruitment

Patients were recruited between 1992 and 1996.

Baseline data

There were no differences in patient characteristics between the 3 treatment groups (table 1). Patient poor-response characteristics were: genotype 1 (69%), HCV RNA above 2×10^6 copies/ml (55%) and cirrhosis (38%).

Numbers analyzed

The results are presented on the basis of patients who received at least one dosis of study medication, the intention to treat population. 40 patients received at least one dose of combination therapy, another 40 patients received at least one dose of ribavirin and 37 patients received at least one dose of placebo.

Table 1. Base-line characteristics of the patients (intention-to-treat population).

Characteristic*	Interferon and Ribavirin 6 mo (n=40)	Ribavirin 6 mo (n=40)	Placebo 6 mo (n=37)
Age – yr	47 ± 12	48 ± 12	46 ± 12
Sex – M/F	28/12	28/12	31/6
Serum ALT – times ULN	3.8 ± 2.0	3.8 ± 2.0	3.7 ± 1.6
Serum HCV RNA†			
Copies/ml – log10	6.2 ± 0.8	6.3 ± 0.8	6.3 ± 0.7
≥2 x 10^6 copies/ml – %	54	55	55
Genotype – n (%)			
1	29 (79)	24 (62)	28 (76)
2 or 3	6 (16)	11 (22)	8 (23)
Other	2 (5)	4 (6)	1 (3)
Cirrhosis – n (%)	18 (45)	13 (33)	14 (38)

*means ± SD.

†Assessed by bDNA in 68 patients (24 interferon+ribavirin, 22 ribavirin, 22 placebo).

Table 2. HCV RNA response at end of therapy and end of follow up, by therapy.

HCV RNA negativity – n (%)	Group A Interferon and Ribavirin 6 mo (n=40)	Group B Ribavirin 6 mo (n=40)	Group C Placebo 6 mo (n=37)
end of therapy	14 (35) [1]	0	0
end of follow-up	6 (15) [2]	0	0

[1] p<0.01 (Pearson Chi-square)
[2] p<0.01 (Pearson Chi-square)

Outcomes and estimation

Patients who received combination therapy had significantly better responses than patients treated with ribavirin monotherapy or placebo, both virologically (HCV-RNA negativity) as well as biochemically (ALT normalisation). At the end of treatment, HCV RNA was undetectable in 35% of patients on combination therapy and in none of the patients treated with ribavirin monotherapy or placebo. The sustained virological response rate at 6 months after therapy was 15% for patients treated with interferon and ribavirin (table 2).

18% of patients receiving combination therapy, 3% of patients receiving ribavirin monotherapy and 3% of patients receiving placebo treatment had persistently normal ALT levels during 24 weeks follow up (table 3).

Adverse events

Hemoglobin levels decreased in patients treated with combination therapy and in patients treated with ribavirin monotherapy (fig 3A).

After week four a plateau-phase was reached. There was a significant difference between the end of treatment Hb change of the patients receiving combination

Table 3. ALT response, by therapy group

ALT response N (%)	Group A Interferon and Ribavirin (n=40)	Group B Ribavirin (n=40)	Group C Placebo (n=37)
On therapy[1]	23 (58%)	9 (23%)	3 (8.1%)
End of treatment[2]	19 (48%)	4 (10%)	2 (5.4%)
End of follow up[3]	7 (18%)	1 (2.7%)	1 (2.5%)

[1]normal ALT on at least two occasions with at least 1 month interval, p<0.01 (Pearson Chi-square).
[2]normal ALT at week 24 and at least one month earlier, p<0.01 (Pearson Chi-square).
[3]persistently normal ALT levels during 24 weeks follow up, p=0.02 (Pearson Chi-square).

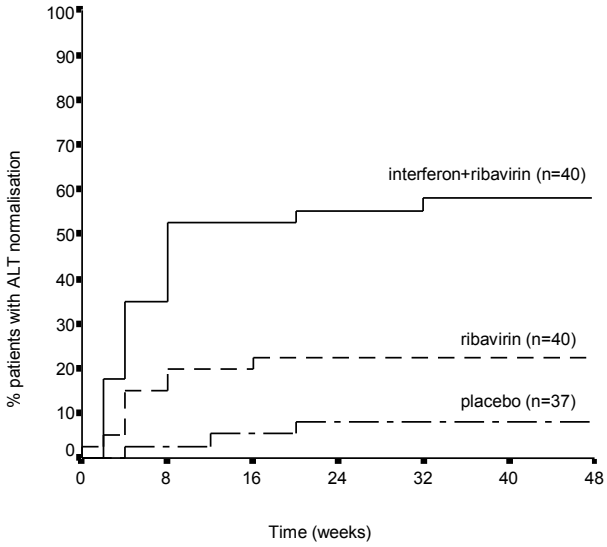

Figure 2: Cumulative probability of ALT response during treatment. Intention to treat population, per treatment group. Response was defined as normal ALT on at least two sequential occasions, the time of response was taken at the first normal ALT. p = 0,00 interferon + ribavirin vs placebo p = 0,02 ribavirin vs placebo p = 0,02 interferon + ribavirin vs ribavirin (Log rank test).

therapy or ribavirin monotherapy, compared to patients treated with placebo ($p < 0.01$). The difference in mean end of treatment Hb change between combination therapy and ribavirin monotherapy was not statistically significant ($p = 0.76$).

Hemoglobin levels returned to baseline values for both groups within 8 weeks after cessation of treatment. Placebo treatment did not significantly affect hemoglobin levels.

White blood counts decreased in patients treated with combination therapy with interferon and ribavirin (fig 3B). At week 12 the mean WBC for this group was 64% from the baseline value. After week 12 a plateau-phase was reached. Ribavirin monotherapy also caused a small, but significant decrease in mean WBC. Placebo treatment did not affect WBC levels.

The mean change in WBC at the end of treatment was greater for the patients treated with combination therapy than for patients treated with ribavirin monotherapy ($p < 0.01$). There was also a significant difference between the end of treatment WBC change in patients receiving ribavirin monotherapy compared to patients treated with placebo ($p < 0.01$).

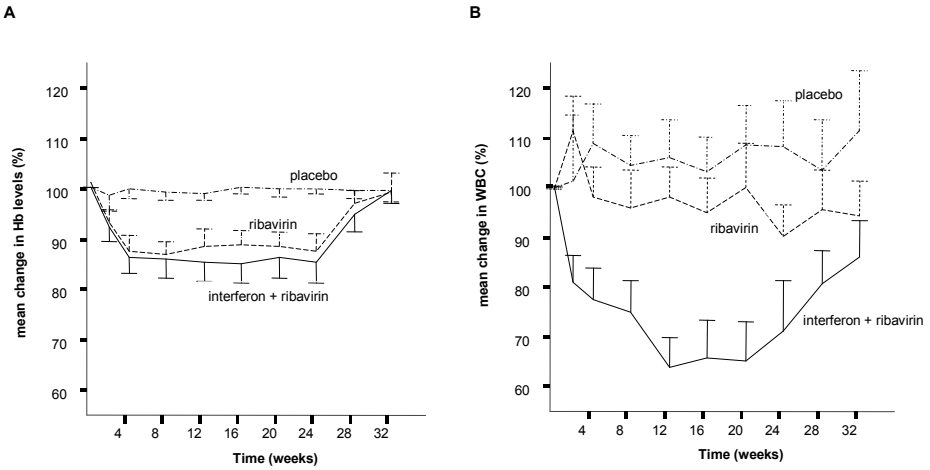

Figure 3: Changes in blood counts over time, by treatment. A: hemoglobin; B: total white blood cells.

Side effects

During the 6 months treatment period 13% of patients on interferon ribavirin combination therapy, 13% of patients on ribavrin monotherapy and 11% of

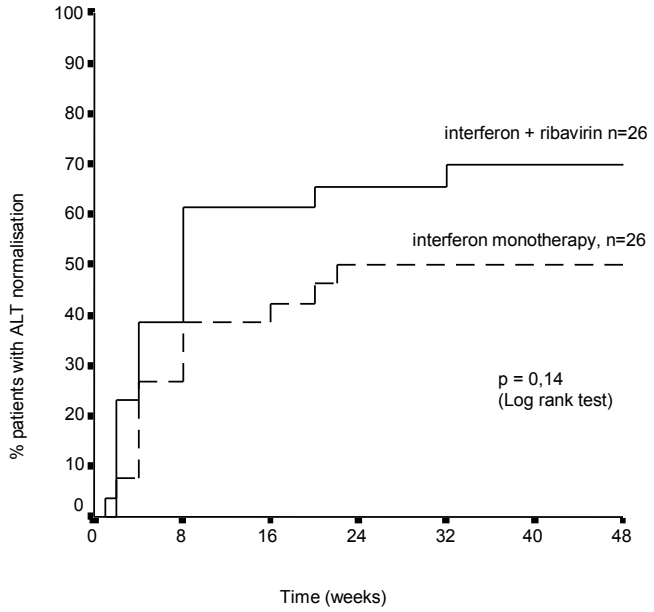

Figure 4: Cumulative probability of ALT response during treatment. Patients treated with combination therapy in this trial vs results of the same patients in a previous trial with interferon monotherapy [4]. There is a trend towards increased normalisation rate with interferon and ribavirin combination therapy.

patients on placebo withdrew due to side effects or noncompliance (figure 1).

In the group receiving combination treatment 3 patients needed dose reduction of interferon because of dyspnoea, depression and pyelonephritis One patient stopped interferon and had a dose reduction of ribavirin because of subjective side effects and anemia.

Among patients treated with ribavirin, one had a dose reduction because of anemia which gave rise to cardiovascular complaints. One interrupted therapy because of vasculitis.

Ancillary analyses

Figure 4 shows the rates of ALT normalisation of 26 patients who were treated in a previous trial with interferon monotherapy [4] compared to the rates of ALT normalisation the same patients showed in this trial on combination therapy. There is a trend towards increased ALT normalisation rate with interferon and ribavirin combination therapy.

DISCUSSION

In 1991 there were two main therapeutic options for patients not responding to standard doses of interferon monotherapy: treatment with high daily doses of interferon [1] or combination therapy with interferon and ribavirin.

To test this second option we started this randomised controlled trial, in which we compared combination therapy with interferon and ribavirin to ribavirin monotherapy and to placebo. This trial is scientifically sound, with high enough numbers treated to draw reliable conclusions. The feasibility of such a trial was, however, low because of the placebo arm included; consequently it took several years for inclusion of all patients.

Part of the results of this study were published in meta-analyses [5]. This full report can still be of value for meta-analysis by the Cochrane approach and can serve as a reference for ribavirin monotherapy in non-responders and for placebo therapy in non-responders.

This study documents a significant, though small effect of adding ribavirin to the treatment of non-responders. Since then various RCT have been published comparing combination therapy with interferon monotherapy [6-9]. Saracco et al. reported sustained response rates comparable to the 15% we found in this study in patients asssigned to a comparable treatment schedule [7]. In meta-analyses that have been published on this subject, also response rates of 14–16% have been reported [10-13]. One way to increase response rates in patients retreated with

combination therapy, is to make a stricter selection and to treat only subgroups of patients who are more likely to have a sustained response. Camma et al state that sustained response rates can be increased to 30% by selecting patients less than 45 years old with normal gammaglutamyltransferase levels and by treating them with high dose long course combination therapy [12]. Indeed Saracco et al have shown that non-responders benefit the most from prolonged treatment with high dose interferon, achieving response rates up to 23% in these patients.

In naive patients good results are obtained by treatment with pegylated interferon combined with ribavirin [14,15]. New studies are on their way evaluating the effect of pegylated interferon and ribavirin in non-responders to interferon.

This trial where combination treatment is compared to ribavirin monotherapy and placebo therapy, gives us the opportunity to determine which adverse effects can be expected from which medication. The decrease in WBC in combination therapy is mainly due to interferon, but also ribavirin causes a modest, though significant decrease in WBC.

The decrease in Hb levels seen during combination therapy is almost entirely caused by ribavirin, with only a marginal additional effect of interferon.

CONCLUSION

This belated report on the only placebo controlled study of interferon ribavirin combination therapy in non responders to standard doses of interferon monotherapy documents the effectiveness, be it limited, of this approach as well as the dynamics of the effects on blood counts.

COMPETING INTERESTS

Investigator initiated study, coordinated by the Foundation for Liver Research in Rotterdam, the Netherlands. ICN Pharmaceuticals and Schering Plough International provided free drug, placebo and financial support.

ACKNOWLEDGEMENTS

Participants in this study of the Benelux Study Group on treatment of chronic hepatitis C are: [1]M. Adler, [2]G.P van Berge Henegouwe, [1]N. Bourgeois, [3]J.T. Brouwer, [4]P. Buydens, [5]R.A.F.M. Chamuleau, [6]J. Delwaide,[7]Th.J.M. Ditzhuysen, [8]A.

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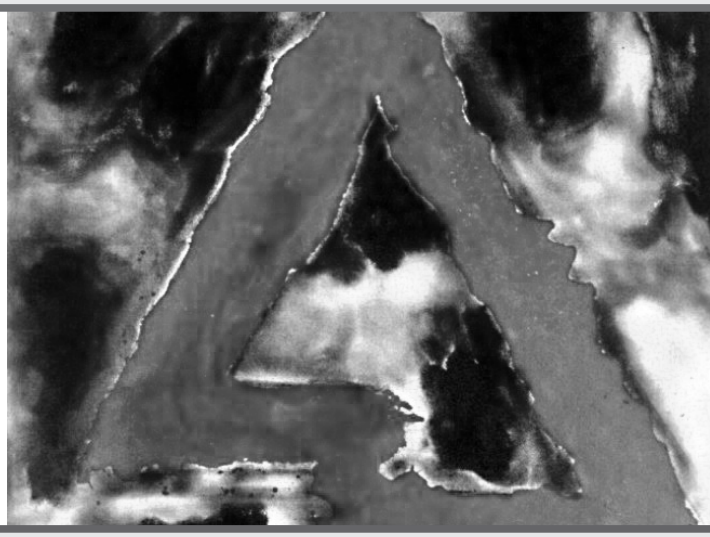
Chapter 4

Dynamic decision analysis to determine optimal treatment duration in chronic hepatitis C



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Aliment Pharmacol Ther. 2005 Mar 1;21(5):539-47.

SUMMARY

Background: Current guidelines for stopping treatment of chronic hepatitis C are based on hepatitis C ribonucleic acid measurements at 12 and 24 weeks.
Aim: To explore an alternative approach for making individualized recommendations about treatment duration, based on simple alanine aminotransferase tests and on cost-per-cure.
Methods: We analysed individual patient data from 13 randomized, controlled trials with interferon alone or combined with ribavirin. Using multiple logistic regression, we built a model that estimated the probability of sustained virological response for treatment durations of 24 and 48 weeks. Decisions to prolong treatment were based on an increase in probability of sustained virological response. If the increase was <10%, the cost-per-cure became decisive with a limit of 50 000 €.
Results: Noncirrhotics with genotype 2 or 3 did not benefit when treatment was continued beyond 24 weeks. Sustained virological response rates in cirrhotic patients increased by 14–47% if treatment was continued up to 48 weeks. In noncirrhotic genotype 1 or 4 patients who had elevated alanine aminotransferase levels at week 4, the probability of sustained virological response increased by <10% if treatment was continued up to 48 weeks; the cost-per-cure for these patients would exceed 50 000 €.
Conclusion: The dynamics of alanine aminotransferase levels and cost-per-cure provides a useful alternative to determine the duration of therapy in chronic hepatitis C.

INTRODUCTION

Chronic hepatitis C affects 170 million people worldwide (1). Some 10–15% of them eventually develop cirrhosis, thereby increasing their risk of hepatocellular carcinoma and liver-related death (2). Treatment with pegylated interferon and ribavirin eliminates the virus in 50–80% of the patients (3, 4). The prognosis after sustained virological response (SVR) is favourable (5).

In order to limit side-effects and costs, current consensus guidelines recommend that treatment should not be continued when the probability of SVR is low. The recommendations about whether to continue treatment are based on measurements of the serum concentration of hepatitis C ribonucleic acid (HCV-RNA); if the HCV-RNA has not dropped a factor 100 by week 12 or below levels of detection at week 24, treatment should be stopped(2). However, testing HCV-RNA levels by real-time polymerase chain reaction (rtPCR) is logistically complicated, expensive and often not performed outside academic or referral centres.

Decrease in alanine aminotransferase (ALT) levels have been shown to reflect the drop in HCV-RNA which occurs during treatment (6, 7). This finding suggests that the ALT test, which is cheap and is easily performed in all hospitals, may provide an alternative basis for recommending the continuation of treatment. In addition, it is also useful to study the cost-per-cure in relation to the duration of therapy, as there is a nonlinear increase in the probability of cure over time, while the cost of medication increases proportionally to the duration.

Therefore, we perform a study in a large cohort of patients to determine whether recommendations for continuing or discontinuing treatment can be based on ALT testing and on the cost-per-cure. While previous analyses of the cost-effectiveness of treatment for chronic hepatitis C were based on the overall patient population, we specify the cost-per-cure by age group and sex.

MATERIAL AND METHODS

We used a database containing individual patient data from 13 randomized, controlled trials from the Eurohep study group (8-17) and the Benelux study group (18-20). Patients who had received either interferon monotherapy or interferon and ribavirin for 24 weeks or 48–72 weeks were included.

Statistics

Logistic regression analysis was applied on data of all 1093 patients for the estimation of effect of different covariates on the chance of SVR. A model consisting of

treatment regimen, age, patient type (naïve, previous relapser or nonresponder), sex, genotype and presence of cirrhosis and their interactions was designed. A backward elimination procedure resulted in the final model. The likelihood ratio test, Akaike information criterion and Schwartz criterion were performed on every analysis to assess the overall model fit.

Subsequently, the model was tested in sub-populations of naïve patients, patients with cirrhosis and patients without cirrhosis. As the model over-estimated the chance of SVR in patients with cirrhosis, a separate model was used for this subgroup, containing the main effects of treatment regimen, age, sex, genotype and presence of cirrhosis, without interactions.

In order to estimate the predictive value of ALT levels during treatment, the models were run for time points t = 0, 4 and 12 weeks (Figure 1).

Results are expressed as odds ratios (OR). In addition, an estimate of the probability of SVR was also included in the analysis which was calculated for subgroups based on the presence of cirrhosis, genotype, sex, age and ALT levels during follow-up, using the principle of weighted means over different studies.

Analysis of cost-per-cure

The cost-per-cure was calculated in a dynamic model using the method of averaging out and folding back, using the predicted probabilities of SVR for the different subgroups. First the optimal treatment decision at week 12 was analysed, based on ALT levels at week 4 and 12. Subsequently, based on the outcomes, the optimal treatment decisions for week 4 and for the start of treatment were

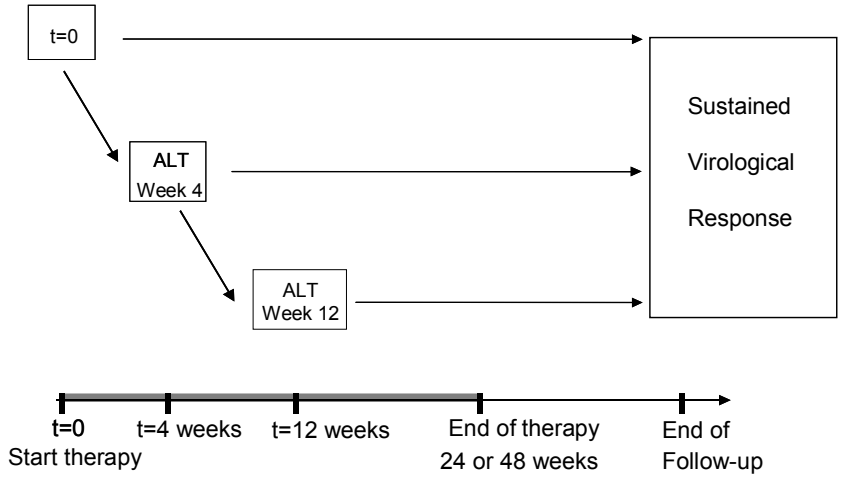

Figure 1: Methods. Multiple regression analyses were used to evaluate the probability of sustained virological response according to baseline variables and ALT-levels at t=4 and t=12 weeks.

Table 1: Costs of medication (per week) and diagnostic tests (per test).

Test:	Cost (Euro)	Test:	Cost (Euro)
Leukocytes	2.2	AntiHCV	13
Haemoglobin	1.8	HCV-RNA	190
Thrombocytes	2.2	HBsAg	12
Prothrombin time	5.0	AntiHBc	13
Creatinin	3.2	AntiHIV	13
Bilirubin	2.5	Liver biopsy + histology	210
ALT	4.3	Ultrasound	71
Gamma GT	4.3		
Albumin	2.5		
IgG	14		
Glucose	2.9		
TSH	11		
Ferritin	14	Vena punction	11
Alfa-fetoprotein (AFP)	14	Outpatient visit	77

Medication	Cost (Euro)
Interferon (Intron A ®)	
- per dose (3000000 IE)	27
- doses per week	3
costs per week	82
Ribavirin (Rebetol ®)	
- per dose (200 mg)	5.5
- dose per week	7 x 1000 mg (<80 kg)
	7 x 1200 mg (>80 kg)
- costs per week	191 (<80 kg)
	229 (>80 kg)
Pharmacy	6.0*
Cost per week	
- interferon	
medication	82
pharmacy	3.0 (week 1-2)
	0.50 (week 3 and further)
- interferon + ribavirin	
medication	272 (<80 kg)
	310 (>80 kg)
pharmacy	6.0 (week 1-2)
	1.0 (week 3 and further)

* In the Netherlands the pharmacist is paid the fixed amount of 6 Euro per prescription. In this analysis we assumed that the medication was prescribed for the first two weeks and then every three months.

assessed. Table 1 shows the costs of treatment and diagnostics, based on the rates in 2002 in The Netherlands (21,22).

The frequency of different tests performed during treatment and derived from a large randomized trial co-ordinated by the Erasmus Medical Centre in Rotterdam, was included in the analysis (18). In Table 2, the expected costs were

Table 2: Average total costs of treatment, per patient, adjusted for costs of diagnostic monitoring and occurrence of side effects.

Treatment	Expected according to protocol (Euro)	Observed due to correction for early discontinuation (Euro)
Interferon. 24 weeks	4,334	4,207
Interferon. 48 weeks	7,123	5,843
Interferon + Ribavirin. 24 weeks	9,575	8,993
Interferon + Ribavirin. 48 weeks	17,350	14,708

given for the four treatment modalities: interferon monotherapy for 24 or 48 weeks and interferon combined with ribavirin for 24 or 48 weeks. The right-hand column indicates the total cost of medication, including diagnostic tests, with a correction for early treatment discontinuation.

The number of patients with early treatment discontinuation was estimated by Kaplan–Meier analysis of the results of the above-mentioned multicentre study (18). These estimations included treatment discontinuation because of noncompliance, adverse events and nonresponse. Because of side-effects, three patients needed hospitalization, at a cost of 265 € per day, for a mean duration of 10 days. In addition, costs were calculated as of side-effects that did not require hospitalization. Overall, two patients suffered from psychiatric side-effects (psychiatric consultation costs 77 €), one patient suffered from pancreatitis (450 €), one patient from an atrio-ventricular block (heart catheterization 930 €, including nursing) and one patient had an abscess at the injection site (420 € including nursing). The total estimated cost for treating side-effects was 10 800 €, which is 54 € per patient. No costs were calculated for patients complaining of fatigue, anaemia or headache. In some cases, these symptoms led to treatment discontinuation.

Assumptions

To determine the optimal treatment schedule, the following nonconventional assumptions were made: if the probability of SVR is <5%, antiviral therapy should not be continued or started. If the probability of SVR is >10%, antiviral therapy should always be continued. If the probability of SVR is between 5 and 10%, continuation of treatment may be beneficial, but the decision should be based on an analysis of the cost-per-cure, limited at 50 000 €.

RESULTS

One thousand and ninety-three patients were included in the analysis. Eighty four percent (919/1093) were treatment naïve, 715 patients were males and 195 patients had cirrhosis. All patients included in this analysis had elevated ALT

levels at the start of treatment. Further patient characteristics are presented in Table 3. Although body weight may also be a baseline factor of influence (3) we did not assess its impact on the probability of SVR, because insufficient data on body weight were available.

Multiple logistic regression analysis showed that, in all patient groups, combination therapy was more effective than interferon monotherapy. The OR for 48-week of interferon monotherapy vs. 48-week interferon plus ribavirin treatment was 0.43 (95% CI 0.23–0.83, P= 0.01) for patients with genotype 1 or 4 without cirrhosis (Table 3). For patients with genotype 2 or 3 without cirrhosis, the OR was 0.22 (95% CI 0.13–0.35, P < 0.01) for 24 weeks of interferon monotherapy vs. 24 weeks of combination therapy (Table 4). Therefore, all further estimations of the probability of SVR are made for patients treated with combination therapy.

Table 3: Patient characteristics, number (%) by treatment schedule.

		Treatment and duration								Total	
		IFN 24 weeks		IFN 48 weeks		IFN+RBV 24 weeks		IFN+RBV 48 weeks		N	%
Covariates		n	%	N	%	n	%	n	%		
Patient type	Naive°	434	(87)	200	(100)	185	(63)	100	(100)	919	(84)
	Relapser#	30	(6)			30	(10)			60	(6)
	Non-responder*	33	(7)			81	(27)			114	(10)
Sex	Male	332	(67)	128	(65)	192	(65)	63	(63)	715	(66)
	Female	165	(33)	69	(35)	104	(35)	37	(37)	375	(34)
Age	<=30	80	(16)	30	(15)	41	(14)	11	(11)	162	(15)
	30-40	121	(24)	44	(22)	68	(23)	24	(24)	257	(23)
	40-60	226	(46)	90	(46)	146	(49)	39	(39)	501	(46)
	>=60	70	(14)	34	(17)	41	(14)	25	(25)	170	(16)
Genotype	1 or 4	284	(57)	119	(60)	192	(65)	71	(71)	666	(61)
	2 or 3	192	(39)	59	(30)	90	(30)	22	(22)	363	(33)
	Other	21	(4)	22	(11)	14	(5)	7	(7)	64	(6)
Cirrhosis	No	401	(82)	129	(84)	231	(78)	85	(85)	846	(81)
	Yes	91	(19)	25	(16)	64	(22)	15	(15)	195	(19)
ALT response week 4	No	278	(57)	110	(56)	121	(41)	37	(39)	546	(51)
	Yes	211	(43)	88	(44)	172	(59)	59	(62)	530	(49)
ALT response week 12	No	248	(51)	91	(46)	92	(32)	20	(21)	451	(42)
	Yes	236	(49)	105	(54)	198	(68)	75	(79)	614	(58)
Total		497	(100)	200	(100)	296	(100)	100	(100)	1093	(100)

° naive = Not previously treated

relapser = Patient with response to previous therapy and subsequent relapse

* non-responder = Patient with non-response to previous treatment

Missing data are not mentioned in the table, but can be deduced from the totals.

Table 4: Outcomes of the model for patients with and without cirrhosis.

Cirrhosis			
Effect	Contrast	Odds Ratio (95% CI)	p-value
Therapy	IFN 24 vs IFN + RBV 48	0.08 (0.02-0.30)	<0.01
	IFN 48 vs IFN + RBV 48	0.08 (0.02-0.30)	<0.01
	IFN + RBV 24 vs IFN + RBV 48	0.14 (0.04 – 0.44)	<0.01
	IFN 24 vs IFN + RBV 24	0.01 (0.00 – 0.08)	<0.01
	IFN 48 vs IFN + RBV 24	0.01 (0.00 – 0.08)	<0.01
	IFN 24 vs IFN 48	0.14 (0.04 - 0.44)	<0.01
Genotype	1 or 4 vs. 2 or 3	0.12 (0.03-0.42)	<0.01
ALT week 4	Abnormal vs. Normal	0.09 (0.02-0.47)	<0.01
ALT week 12	Abnormal vs. normal	0.78 (0.15-3.97)	0.77

No cirrhosis			
Effect	Contrast	Odds Ratio (95% CI)	p-value
Therapy in patients with Genotype = 1 or 4	IFN 24 vs IFN + RBV 48	0.13 90.06-0.27)	<0.01
	IFN 48 vs IFN+RBV 48	0.43 (0.23-0.83)	0.01
	IFN+RBV 24 vs IFN+RBV 48	0.59 (0.30-1.18)	0.14
	IFN 24 vs IFN + RBV 24	0.22 (0.13-0.35)	<0.01
	IFN 48 vs IFN + RBV 24	0.73 (0.37-1.44)	0.37
	IFN 24 vs IFN 48	0.29 (0.14-0.60)	<0.01
Therapy in patients with Genotype = 2 or 3	IFN 24 vs IFN+RBV 48	0.34 (0.16-0.71)	<0.01
	IFN 48 vs IFN+RBV 48	0.43 (0.23-0.83)	0.01
	IFN+RBV 24 vs IFN+RBV 48	1.59 (0.73-3.45)	0.24
	IFN 24 vs IFN+RBV 24	0.22 (0.13-0.35)	<0.01
	IFN 48 vs IFN+RBV 24	0.27 (0.14-0.54)	<0.01
	IFN 24 vs. IFN 48	0.79 (0.43-1.45)	0.44
Patient type	Relapser vs. Naive	0.33 (0.12-0.89)	0.03
	non-responder vs. Naive	0.13 (0.04-0.46)	<0.01
	non-responder vs. Relapser	0.39 (0.08-1.87)	0.24
ALT week 4, genotype=1 or 4	Abnormal vs. Normal	0.21 (0.10-0.42)	<0.01
ALT week 4, genotype=2 or 3	Abnormal vs. Normal	0.60 (0.32-1.13)	0.11
ALT week12	Abnormal vs. normal	0.34 (0.19-0.62)	<0.01

Patients with cirrhosis

In patients with cirrhosis, 48 weeks of combination treatment was more effective than 24 weeks (OR = 0.14; 95% CI 0.04–0.44, P < 0.01) (Table 3). The estimated probability of SVR in patients with cirrhosis increased by more than 10% if treatment was continued from 24 to 48 weeks, even in the absence of an ALT response regardless of the genotype.

In cirrhotic patients with genotype 1 or 4, the estimated probability of SVR increased from 2% (95% CI 1–8) to 16% (95% CI 5–39) if treatment was prolonged in patients without ALT response at week 4. If there was an ALT response, the estimated probability of SVR increased from 24% (95% CI 11–47) to 71% (95% CI 42–89). Therefore, patients with genotype 1 or 4 with cirrhosis should always be treated for 48 weeks.

For patients with genotype 2 or 3 and cirrhosis, the estimated probability increased from 17% (95% CI 7.0–39) to 61% (95% CI 27–87), even in the absence

Table 5. Estimated probability of sustained virological response, based on the model. Results are specified according to treatment duration and ALT at week 4, for patients with genotype 1 or 4 without cirrhosis.

ALT=normal at week 4				
Gender	Age	Probability of SVR (%), (95% CI) IFN + RBV 24 weeks	Probability of SVR (%), (95% CI) IFN + RBV 48 weeks	Increase in probability of SVR (%)
Female	<30	45 (28-63)	58 (37-76)	13
	30-40	51 (34-67)	63 (44-79)	12
	40-60	37 (25-51)	50 (32-67)	13
	>60	42 (23-63)	54 (34-73)	12
Male	<30	50 (34-65)	62 (43-78)	12
	30-40	29 (18-44)	41 (26-58)	12
	40-60	43 (30-56)	55 (39-70)	12
	>60	37 (20-58)	49 (27-72)	12

ALT=elevated at week 4				
Gender	Age	Probability of SVR (%), (95% CI) IFN + RBV 24 weeks	Probability of SVR (%), (95% CI) IFN + RBV 48 weeks	Increase in probability of SVR (%)
Female	<30	9 (4-21)	15 (6-33)	6
	30-40	12 (5.0-24)	18 (8-36)	6
	40-60	7 (3-14)	11 (5-23)	4
	>60	8 (3-20)	13 (5-28)	5
Male	<30	11 (5-22)	17 (8-34)	6
	30-40	5 (2-11)	8 (4-17)	3
	40-60	9 (4-17)	14 (7-26)	5
	>60	7 (3-17)	11 (4-27)	4

Table 6: Increase in estimated probability of SVR if treatment is prolonged from 24 weeks to 48 weeks in patients with genotype 1 or 4 without cirrhosis, according to ALT-response at week 4 and week 12.

Time point:	Week 4	Week12	Week 12	Week 4	Week 12	Week 12
ALT week 4	ALT4= Normal	ALT 4= Normal	ALT 4= Normal	ALT 4= Elevated	ALT 4= Elevated	ALT 4= elevated
ALT week 12		ALT 12= Normal	ALT 12= Elevated		ALT 12= Normal	ALT 12= elevated
Increase in estimated Probability of SVR	12*	13	10	5*	7	3

* Median value of the increase in estimated probability given in table 5.

of ALT response at week 4. Therefore we suggest that genotype 2 or 3 patients with cirrhosis should also be treated for 48 weeks.

Genotype 2 or 3 without cirrhosis

Patients with genotype 2 or 3 without cirrhosis did not benefit from continuing treatment up to 48 weeks: OR = 1.59 (95% CI 0.73–3.45, P = 0.24) for 24 vs. 48 weeks combination therapy. Therefore, prolongation of treatment beyond 24 weeks is not indicated.

Genotype 1 or 4 without cirrhosis

Although the outcome after 48 weeks of combination therapy tended to be better than that after 24 weeks for patients with genotype 1 or 4 without cirrhosis, the difference was not statistically significant: OR = 0.59 (95% CI 0.30–1.18, P = 0.14). Therefore, we estimated the probability of SVR for different subgroups, according to age, sex and ALT response in order to identify the patients most likely to benefit from continuing treatment up to 48 weeks. Table 4 shows the estimated probability of SVR after 24 and 48 weeks of treatment, according to age, sex and ALT response. In patients with elevated ALT levels at week 4, the increase in the estimated probability of SVR after continuing treatment up to 48 weeks was lower than 10%, even if there was an ALT response at week 12 (Tables 5 and 6).

Further analysis showed that the cost-per-cure would exceed 50 000 € if treatment was continued up to 48 weeks in these patients (Figures 2 and 3). We therefore suggest that the total treatment duration for noncirrhotic patients with genotype 1 or 4 should be 24 weeks in case the ALT levels are elevated at week 4 (Figure 4). For patients with normal ALT levels at week 4 and 12, the increase in estimated probability of SVR is higher than 10% if treatment is continued from 24 up to 48 weeks. Therefore, treatment should be continued up to 48 weeks in patients with normal ALT at week 4.

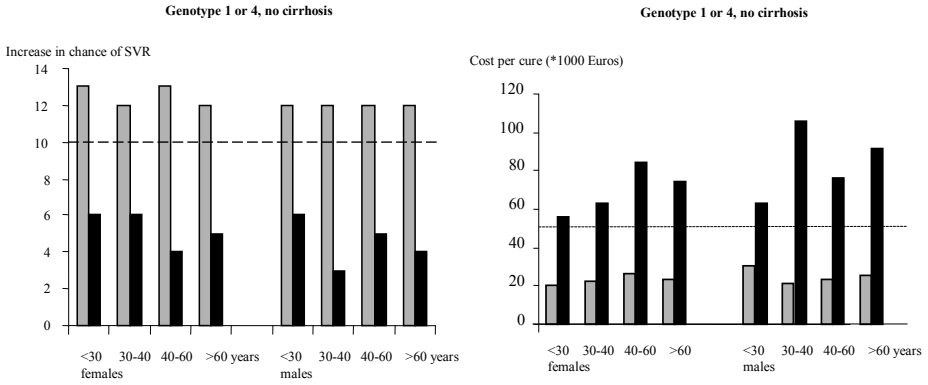

Figure 2 & 3: Estimated increase in sustained virological response if treatment is prolonged from 24 to 48 weeks, specified for age and sex.
For patients with abnormal ALT-levels at week 4 (black bars), the benefit of prolongation was less than 10%, whereas the benefit for patients with normal ALT-levels at week 4 (grey bars) was higher than 10%. **Figure 3**: Cost-per-cure for 48 weeks' treatment of patients with normal (grey bars) and abnormal (black bars) ALT levels at week 4, specified for age and sex. The cost-per-cure for a patient with elevated transaminases is higher than 50,000 Euro.

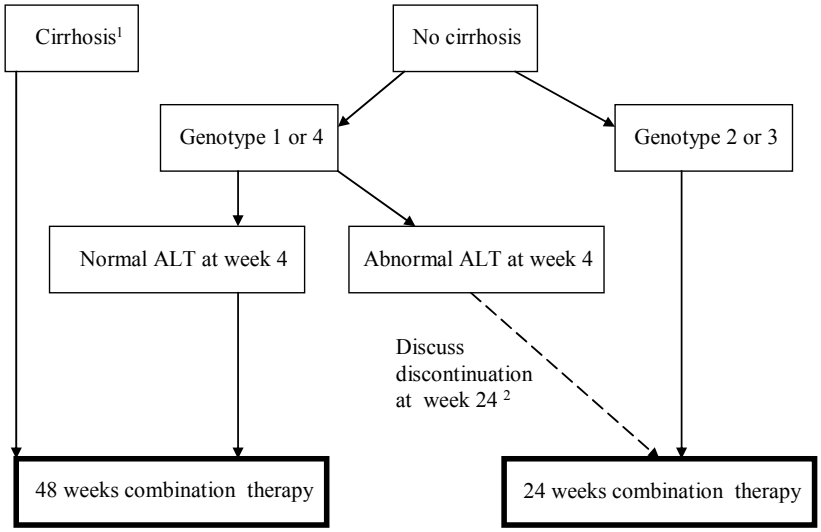

Figure 4: Flow-chart showing recommendations for the implementation of our results, using ALT-levels and cost-per-cure.
[1] The optimal treatment-duration for patients with cirrhosis is 48 weeks, regardless of genotype and ALT.
[2] If ALT is elevated at week 4, patients should be informed on the small additional chance of SVR by adding another 24 weeks of treatment, and the associated costs. Depending on the experienced side effects, the patient and physician may decide for a treatment duration of 24 weeks.

DISCUSSION

In this study, we present an unconventional approach to medical decision-making during the treatment of chronic hepatitis C. Treatment with interferon and ribavirin is associated with considerable side-effects and costs (23,24). The present analysis is based on the idea that the treatment should be extended to 48 weeks only if the expected benefit is substantial, in contrast to the current strategy of aiming at the highest possible sustained response rates regardless of side-effects and costs.

If SVR rates increase by only 3–7%, the side-effects and costs may outweigh the clinical benefit; we suggest that the cost-per-cure can be decisive in these situations.

This analysis of individual patient data of almost 1100 patients suggests that recommendations for interferon ribavirin treatment of noncirrhotic genotype 1 or 4 patients with chronic hepatitis C can be based upon ALT levels at 4 weeks after initiation of treatment. These results are in accordance with those of Ribeiro et al. (7) who compared the dynamics of ALT levels and HCV-RNA showing that the 4-week ALT decline correlates with the drop in HCV-RNA and the efficacy of therapy. Earlier, Brouwer et al. (6) showed that elevated ALT levels at week 4 correctly predict nonresponse in 96% of cases, compared with 93% at week 8 and 95% at week 12. HCV-RNA levels at week 12 have the highest predictive value. However, our findings show that, instead of waiting till week 12, a simple, low-cost and widely available ALT test 4 weeks after the start of treatment can provide a reliable prediction of the probability of clinically relevant increase in SVR rate with prolongation of therapy beyond 24 weeks.

The current guidelines for the treatment of chronic hepatitis C suggest that patients with genotype 2 or 3 should be treated for 24 weeks. Patients with genotype 1 or 4 are treated for 48 weeks, but treatment is stopped if the HCV-RNA has not dropped by a factor 100 at week 12 or if it is still detectable at week 24 (2). No specifications are provided for patients with cirrhosis.

Our analysis confirms that the optimal treatment duration for patients with genotype 2 or 3 without cirrhosis is 24 weeks. For noncirrhotic patients with genotype 1 or 4, we suggest that the treatment duration be 24 weeks if ALT levels are elevated at week 4 and that treatment only be extended up to 48 weeks if the ALT levels are normal at week 4.

Another important finding in this study concerns the optimal treatment duration for patients with cirrhosis. Current guidelines state that patients with genotype 2 or 3 should be treated for 24 weeks, with no exception for cirrhotics. This study suggests that the benefit of extending interferon ribavirin therapy up to 48 weeks

in cirrhotic patients is substantial, i.e. more than 10%. In the absence of specific information on pegylated interferon-ribavirin therapy in this patient category, extending therapy for another 12–24 weeks might be considered in patients whose tolerance of therapy at 24 weeks is still good.

In this analysis, we used a limit of 50 000 € per cure. However, the acceptable limit of cost-per-cure may differ by country or by organization. Recently, it has been estimated that an amount of 30 000–45 000 € (£20 000–30 000) per quality-adjusted life-years (QALY) would be acceptable for The National Institute of Clinical Excellence in the UK (25). As it has been estimated that one cure of chronic hepatitis C accounts for two QALYs gained (26) this would amount to 60 000–90 000 € per cure. In comparison, liver transplantation is an accepted treatment for end-stage liver disease, which costs 31 000 €–72 000 € (£21 000–£48 000) per QALY gained (27). In our analysis, it is possible to use a higher limit of costs. Changing this limit may influence the decision about continuing treatment for different subgroups of patients (Figure 3).

We based our analysis on the current cost of medication, hospital care and laboratory analyses in the Netherlands. In regions outside north-western Europe, our conclusion and recommendations should therefore be adjusted for different costs.

A limitation of this study is that patients were treated with interferon either singly or in combination with ribavirin, while the current standard therapy for chronic hepatitis C combines pegylated interferon and ribavirin. It remains to be established whether our conclusions also hold true for the latter treatment. However, this study – using dynamics of transaminase levels and cost-per-cure – presents an alternative approach for making individualized recommendations about treatment duration. The same methodology can be applied in future studies using data on pegylated interferon.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Dutch National Health Service (Ziekenfondsraad), OG-99-015.

The input of patient data by the following investigators from the Eurohep study group and the Benelux study group is greatly appreciated:

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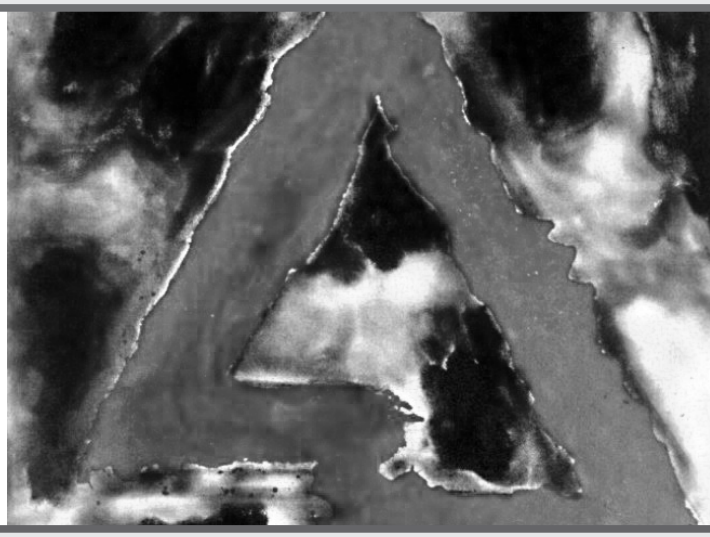
Chapter 5

Long term clinical outcome of chronic hepatitis C patients with sustained virological response to interferon monotherapy



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Gut. 2004 Oct;53(10):1504-8.

ABSTRACT

Background: The key end point for treatment efficacy in chronic hepatitis C is absence of detectable virus at six months after treatment. However, the incidence of clinical events during long term follow up of patients with sustained virological response is still poorly documented and may differ between the Eastern and Western world.

Aims: To assess clinical end points during long term follow up of European patients with a sustained virological response to interferon monotherapy.

Methods: Meta-analysis of individual patient data from eight European protocolled follow up studies of interferon treatment for chronic hepatitis C.

Results: A total of 286 sustained virological responders and 50 biochemical responders (detectable virus but normal alanine aminotransferase levels) were followed up for 59 months. Fifteen sustained virological responders (5.2%) had cirrhosis before treatment and 112 (39%) had genotype 1. The late virological relapse rate after five years of follow up was 4.7% (95% confidence interval (CI) 2.0–7.4) among sustained virological responders; all late relapses occurred within four years after treatment. Among sustained virological responders, the rate of decompensation after five years of follow up was 1.0% (95% CI 0.0–2.3) and none developed hepatocellular carcinoma (HCC). Survival was comparable with the general population, matched for age and sex, the standard mortality ratio being 1.4 (95% CI 0.3–2.5). Clinical outcome of patients with cirrhosis was similar to other sustained virological responders. For biochemical responders, the rates of development of decompensation and HCC during long term follow up were 9.1% (95% CI 0.5–17.7) and 7.1% (95% CI 0–15.0), respectively.

Conclusions: Five year survival of European sustained virological responders was similar to the overall population, matched for age and sex. No HCCs were detected during long term follow up.

INTRODUCTION

Chronic infection with the hepatitis C virus (HCV) can lead to decompensated liver cirrhosis and hepatocellular carcinoma (HCC). However, treatment of hepatitis C is based on surrogate end points, and evaluation of treatment for clinical end points has only slowly been forthcoming due to the slow course of the disease and the small number of clinical events in patients treated for hepatitis C.

Protocolled studies use sustained virological response as the key outcome measure for hepatitis C treatment. This sustained virological response is defined as no detectable HCV-RNA in serum at six months after treatment. The aim of this study was to determine the long term clinical outcome of sustained virological responders who had been treated in protocolled studies.

PATIENTS AND METHODS

Study design

All European centres that had published long term data on patients treated for chronic hepatitis C before 1997 were invited to participate in the protocol and to include patients with response to treatment.

Additional entry criteria were study follow up longer than one year and availability of HCV-RNA data. Nine centres met these criteria. Patients from eight European hepatology units were included in the study (1–12).

Patient selection

Data from 343 consecutive chronic hepatitis C patients with response to interferon monotherapy were obtained. All patients had participated in protocolled studies (clear cohort or randomised controlled trial). Data were collected on separate case record forms, one per patient, by the local investigator. The case record forms were sent to the coordination centre in Rotterdam where data were entered into a central database. Before the data were entered, they were checked and, in case of doubt, contact was made with the local investigator.

Data recorded

Information was obtained on demographics (date of birth, sex) and on details of treatment (initial dose, duration of treatment, and total dose of interferon). Virological data (genotype, viraemia) and biochemical data (platelet count, bilirubin, albumin, and transaminase levels) were measured in certified laboratories of participating hospitals and added to the case record form by the local investiga-

tor. Centrally, results were corrected for local normal values. Results of pre- and post-treatment liver biopsies were recorded using the HAI score for activity and the Knodell score for fibrosis (13). All centres used polymerase chain reaction (PCR) methods with a detection limit of 100 copies/ml, except for one centre where PCR with a detection limit of 1000 copies/ml was used before 1998. No late virological relapses were reported from this centre after introduction of a test with a sensitivity of 100 copies/ml.

Follow up data were recorded every six months and included alanine aminotransferase (ALT) levels, HCV-RNA, and the occurrence of clinical events (decompensation, HCC, death).

Patients were considered to have decompensation if they showed any of the following symptoms: ascites, bleeding varices, jaundice, or hepatic encephalopathy. Patients were classified as having developed cirrhosis on the basis of ultrasound (nodular contour, diminished hepatopetal flow, collaterals), serology (platelets <80000, albumin <35 g/l, clotting factors<50%), or liver biopsy. Patients were considered to have HCC if the αfetoprotein level was >400 and ultrasound confirmed a focal lesion, or if biopsy proved so. Death was classified as liver related or liver unrelated.

Sustained virological response was defined as no detectable HCV-RNA at the end of treatment and after six months of follow up. Patients with normal ALT levels at these time points, but with detectable HCV-RNA at the end of treatment or six months thereafter, were referred to as biochemical responders. Sustained virological responders were considered to have a late virological relapse if HCV-RNA was detectable on any occasion after six months of follow up, confirmed by either a second PCR or elevation of ALT levels above the upper limit of normal.

Statistical analysis

To evaluate factors of influence on late virological relapse, univariate and multivariate Cox regression analyses were performed. The Kaplan-Meier method was used to evaluate the five year late relapse rate and to determine the rate of occurrence of clinical events during five years of follow up. The number of expected deaths and the expected survival probability were calculated based on sex and age ranked mortality among the Dutch general population, which is similar to most European countries (14). The standard mortality ratio was calculated by dividing the observed number of deaths by the expected number of deaths.

We used multiple regression analysis to identify risk factors for fibrosis progression.

Statistical analyses were performed using SPSS for Windows (SPSS Inc, Chicago, Illinois, USA). All analyses were performed by the Meta-Analysis of Individual

Data group in Rotterdam (BEH, SWS, BJV), which is experienced in the conduct of such studies.

RESULTS

Study population

Data were obtained for 343 patients treated for chronic hepatitis C. A total of 286 patients had a sustained virological response and 50 had a biochemical response. Seven patients did not fit the entry criterion of HCV-RNA data availability at the end of treatment and after six months of follow up and were excluded from further analysis.

Characteristics of sustained virological responders and biochemical responders are shown in table 1. Patients had been treated with recombinant interferon a2a, a2b, or natural interferon monotherapy. Patients were treated for an average duration of 39 weeks (range 11–96). Patients with genotype 1 were treated longer (mean duration 41 weeks v. 38 weeks in other genotypes; $p<0.01$) and received a higher total dose of interferon (581 mega units (MU) v 525 MU in other genotypes; $p<0.01$, Mann-Whitney U test).

Sustained virological responders

Of 286 sustained responders, 15 patients had cirrhosis before the start of treatment, as determined by liver biopsy.

Two patients presented with decompensated cirrhosis after 30 and 60 months of follow up (fig 1). These patients were hepatitis B surface antigen negative and HCV-RNA negative, and no other risk factors for liver disease were reported. The latter patient died of decompensated cirrhosis. Five other patients died of non-liver related causes. One patient died of lung cancer and two patients died

Table 1: Patient characteristics.

	Sustained virological responders	Biochemical responders	P-values (Mann Whitney /χ^2)
Number	286	50	
Mean age (range)	41 (17-72)	45 (23-72)	0.04
Male (%)	169 (59)	26 (52)	0.35
Mean follow-up, months (range)	59 (12-120)	59 (6-96)	0.99
Mean total dose of interferon, MU (SD)	550 (283)	469 (290)	0.05
Genotype 1 (%)	112 (39)	21 (42)	0.71
Cirrhosis (%)	15 (5.2)	11 (22)	0.00

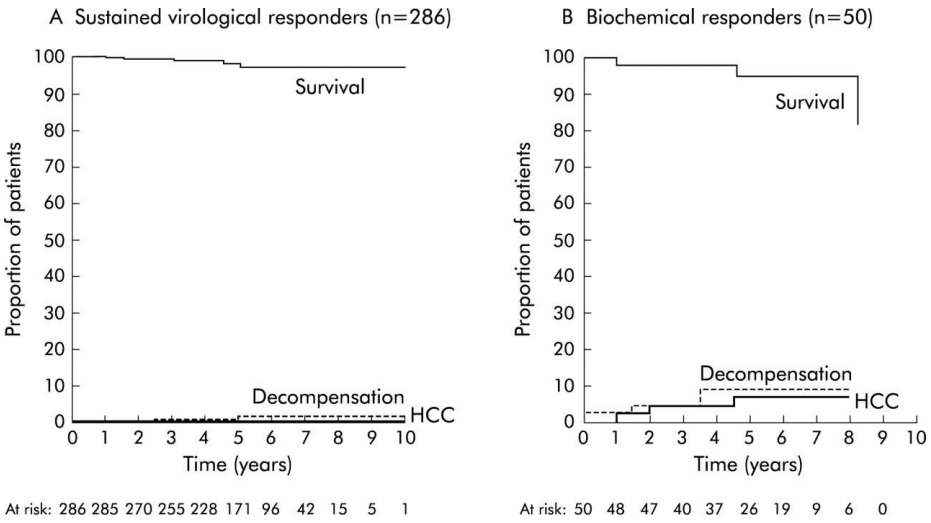

Figure 1: Kaplan-Meier curve showing survival and development of clinical events in sustained virological responders and biochemical responders. HCC=hepatocellular carcinoma.

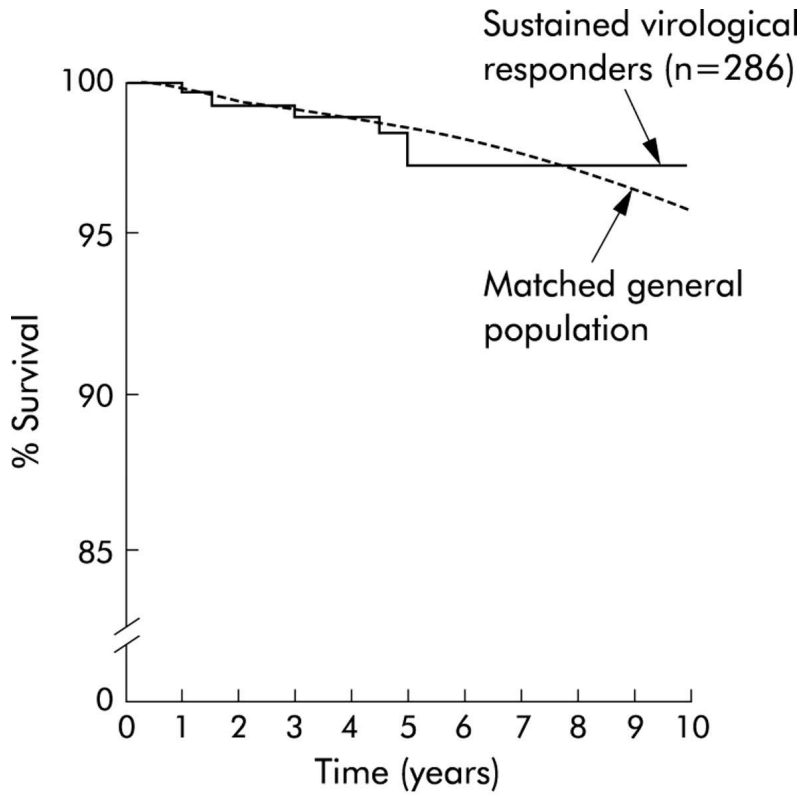

Figure 2: Kaplan-Meier curve showing survival of sustained virological responders compared with the age and sex matched general population.

Table 2: Standard mortality ratios for sustained virological responders and biochemical responders.

	N	Deaths	Deaths expected	SMR	95% CI
Sustained virological responders	286	6	4.3	1.4	0.3 - 2.5
Biochemical responders	50	3	0.5	5.6	0.0 - 12.6

of trauma; another patient died of cardiovascular complications and one patient died of a haemolytic uraemic syndrome.

The standard mortality ratio of sustained responders was 1.4 (95% confidence interval (CI) 0.3–2.5) (table 2), and there was no statistically significant difference in mortality between sustained virological responders and the general population, matched for age and sex (fig 2).

Biochemical responders

Fifty patients had normal transaminase levels at the end of follow up and six months after treatment while HCV-RNA was still detectable. Of these biochemical responders, three patients died during long term follow up, all of liver related causes. The occurrence of decompensation and HCC among biochemical responders was 9.1% (95% CI 0.5–17.7) and 7.1% (95% CI 0–15.0), respectively (fig 1). Biochemical responders were older and had a higher prevalence of cirrhosis. Although there was a trend to a higher standard mortality ratio (corrected for age and sex) in biochemical responders after five years of follow up, the difference did not reach statistical significance (table 2).

Liver histology

One hundred and twenty five patients (110 sustained virological responders and 15 biochemical responders) underwent liver biopsy both before and after treatment. Mean time between these two biopsies was 1.6 years (SD 0.8). Thirty two sustained virological responders (29%) and none of the biochemical responders showed regression of fibrosis. Progression of fibrosis was seen in six sustained virological responders (5%) and in three biochemical responders (20%) (table 3). Baseline characteristics of sustained virological responders and biochemical

Table 3: Change in fibrosis according to response to treatment in patients who underwent pre-treatment and post-treatment biopsies.

	SVR	BR
Number of patients	110	15
2 points progression	3 (3%)	1 (7%)
1 point progression	3 (3%)	2 (13%)
No change	72 (65%)	12 (80%)
1 point regression	23 (21%)	0 (0%)
2 points regression	9 (8%)	0 (0%)

Table 4: Multiple regression analysis assessing risk factors for fibrosis progression

	Odds Ratio	95% CI		p-value
		Lower	Upper	
Male sex	0.02	-0.29	0.34	0.86
Age	0.29 [b]	0.00	0.03	0.01
Fibrosis stage pre-treatment	-0.50 [a]	-0.52	-0.21	<0.01
Activity score pre-treatment	0.09	-0.04	0.12	0.37
Time between biopsies	0.08	-0.12	0.29	0.40
Biochemical Response to therapy versus Sustained Virological Response	0.31[c]	0.30	1.49	<0.01

[a]Higher pre-treatment fibrosis stage associated with a smaller chance of fibrosis progression, while older age [b] and biochemical response [c] were associated with a higher chance of fibrosis progression.

responders who underwent two biopsies were different, with sustained virological responders being younger (mean 39 (SD 13) v 47 (14) years), having a lower mean pretreatment fibrosis stage (1.75 (1.1) v 2.5 (1.4)), and having a shorter time between the two biopsies (1.5 (0.6) v 2.3 (1.5) years). Therefore, we performed a multiple regression analysis to determine independent risk factors for progression of fibrosis. Fibrosis progression was associated with older age, lower pretreatment fibrosis score, and biochemical response rather than sustained virological response (table 4).

Late relapsers

Twelve sustained responders had a late virological relapse (fig 3). In six patients (50%), late virological relapse was accompanied by an elevation in transaminase levels. Multivariate Cox regression analysis did not show any pretreatment factors to be associated with an increased risk for late virological relapse. None of the late relapsers developed decompensation or HCC during follow up.

DISCUSSION

This large European study allows, for the first time, an approximation of the incidence of clinical events during long term follow up of sustained virological responders in Europe. The most important finding is that clinical events are rare in this population, indicating that sustained virological responders have an excellent prognosis.

The largest European study to date describing clinical outcome in sustained responders to interferon treatment did not report any events among 74 patients followed up for 2.7 years (15). Two other European studies, involving seven and

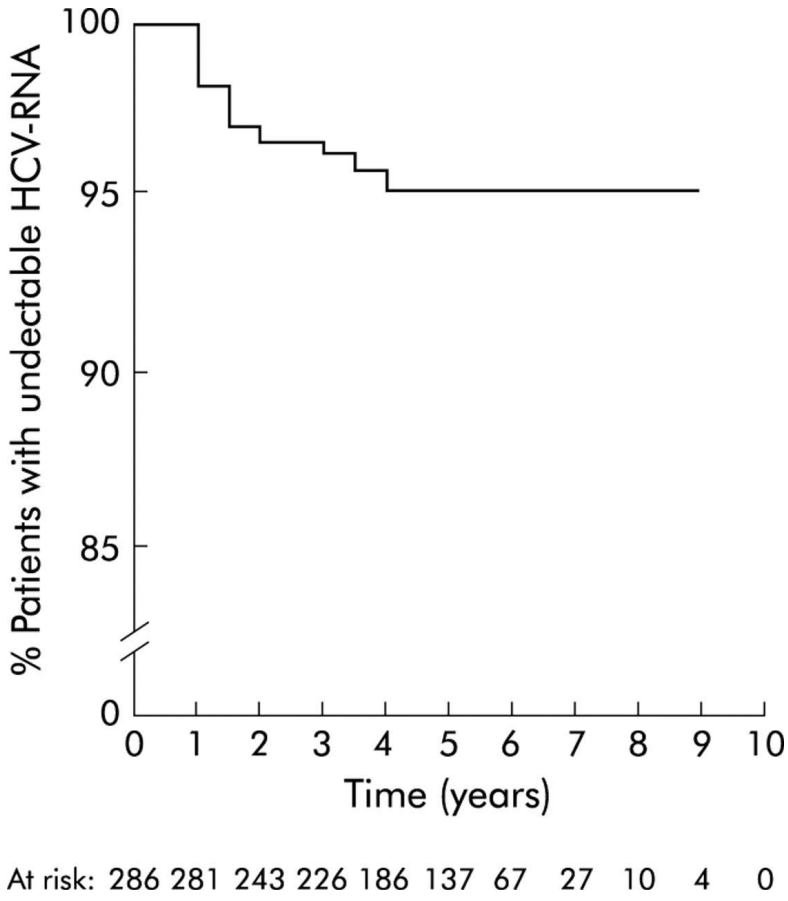

Figure 3: Kaplan-Meier curve showing late virological relapse among sustained virological responders. No late virological relapses were seen after four years of follow up, the maximal delay between the last negative polymerase chain reaction (PCR) and the first positive PCR result being 12 months.

56 sustained responders, also showed no clinical events during 4.6 and 5.2 years of follow up, respectively (8,16). Bruno et al described 32 sustained responders of whom one cirrhotic patient developed HCC (17). Although another HCC has been reported recently in a Western sustained responder (18), these cases seem to be rare and limited to patients with cirrhosis. In the present study, no HCCs occurred during long term follow up. According to several large studies, the yearly incidence of HCC among Japanese sustained virological responders still varies between 0.02% and 0.5% per year (19–26); the difference in the incidence of HCC between East and West apparently persists in conditions without detectable viral replication.

The lowest rates in Japan were reported by Yoshida et al, with one HCC among 817 sustained responders during 5.4 years of follow up (26). The highest incidence of HCC reported among sustained responders in Japan was by Kasahara et al who reported five HCCs among 313 sustained virological responders followed up for three years (22).

Fifteen cirrhotic patients were included in this study. Only two patients with decompensated cirrhosis were reported. Among untreated cirrhotics, occurrence of clinical events of 38% (28% decompensation and 10% HCC) would be expected, according to Fattovich and colleagues (27). These results suggest, but do not prove, a change in the natural course of chronic hepatitis C. Further studies, including more cirrhotics, will be necessary to investigate the effect of treatment on the natural course of chronic hepatitis C.

In this study, sustained virological response was associated with a decrease in fibrosis score. Similar findings have been reported for sustained responders to pegylated interferon (28,29). Previous studies have shown that regression of fibrosis can also occur in biochemical responders and non-responders to interferon. In common with our study, sustained virological responders show the highest rate of regression (30). Because of the large proportion of sustained virological responders that showed regression of fibrosis and the low incidence of clinical events in these patients, in our view, non-cirrhotic patients with a sustained virological response can be regarded as cured.

A limitation of our study is that all patients had been treated with interferon monotherapy whereas the current standard therapy for chronic hepatitis C is pegylated interferon with ribavirin. This current standard however dates from 2002 and long term follow up data of peginterferon and ribavirin were not available at the time of this study (31). In general, combination therapy leads to higher sustained virological response rates (32,33) and also the late relapse rate seems to decrease. In this study with data on interferon monotherapy, the late relapse rate was 4.7% (95% CI 2.0–7.4); Camma et al reported 8.7% in a meta-analysis of 14 trials with interferon monotherapy (34). After four years of follow up of treatment with interferon and ribavirin, late virological relapse rates of 3% (95% CI 1.4–4.6) and 1% (95% CI 0–2.0) have been reported for patients treated for 24 weeks and 48 weeks, respectively (18). After treatment with pegylated interferon with or without ribavirin, a late relapse rate of 0.8% was reported after four years of follow up (35). The possibility of reinfection could not be ruled out in our cohort as data on risk behaviour and concordance of genotypes were not available. However, introduction of more sensitive PCR methods may also have contributed to a decrease in late virological relapse over time. It is possible that

with an insensitive assay, patients with low viraemia are regarded as sustained virological responders.

As the late relapse rate seems to decrease with newer treatment regimens, long term clinical outcomes may be similar or even better than results obtained with interferon monotherapy. Therefore, in our opinion, the favourable clinical outcome of sustained virological responders is likely to hold true in the era of pegylated interferon and ribavirin.

In conclusion, the long term clinical outcome of patients with a sustained response to interferon is favourable. Five year survival of European sustained virological responders was similar to the general population, matched for age and sex, and no HCCs were detected during long term follow up.

ACKNOWLEDGEMENTS

The input of patient data by the following members of the Benelux Study Group on Hepatitis C was greatly appreciated:

M Adler, Erasme University Hospital, Belgium; R Brenard, Hospital St Joseph, Brussels, Belgium; JT Brouwer, Reinier de Graaf Hospital Group Delft, the Netherlands; J Delwaide, University Hospital Liège, Belgium; A Elewaut, University Hospital Gent, Belgium; ML Hautekeete and H Reynaert, Academic Hospital AZ-VUB, Brussels, Belgium; MHGM Houben, Rode Kruis Hospital Den Haag, the Netherlands; JBMJ Jansen, Academic Hospital St Radboud Nijmegen, the Netherlands; PP Michielsen, University Hospital Antwerpen, Belgium; C J Mulder, Rijnstaten Hospital Arnhem, the Netherlands; HCJ Weegink, Academic Medical Center Amsterdam, the Netherlands; PJ Wismans, Haven Hospital Rotterdam, the Netherlands.

This study was financially supported by a grant from the European Union (Biomed grant No BMMI-CT 92-0755, Eurohep), by an unrestricted grant from Schering-Plough International, Kenilworth, USA, and by the Foundation for Liver Research (SLO) Rotterdam.

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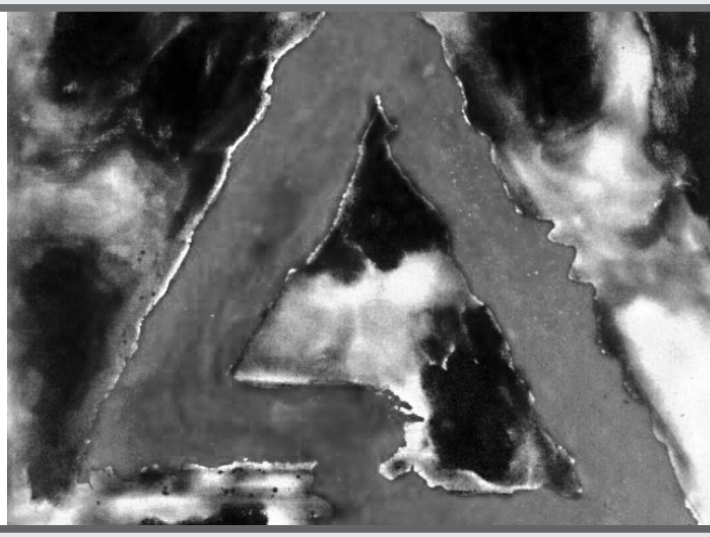
Chapter 6

Long-term clinical outcome and effect of glycyrrhizin in 1093 chronic hepatitis C patients with non-response or relapse to interferon



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Scand J Gastroenterol. 2006 Sep;41(9):1087-94

ABSTRACT

Objective. Patients with chronic hepatitis C who do not respond to interferon can be treated with glycyrrhizin to reduce disease activity. The objective of this study was to evaluate the effect of glycyrrhizin on the incidence of hepatocellular carcinoma (HCC) during long-term follow-up after non-response to interferon.
Material and methods. We analyzed individual patient data of all consecutive patients treated with interferon in 12 major Japanese hospitals between 1990 and 1995 who showed no sustained response.
Results. The study comprised 1093 patients. During a mean follow-up of 6.1±1.8 years, 107 patients developed HCC. The Cox regression analysis with time-dependent variables showed that older age, male gender, higher alanine aminotransferase (ALAT) and higher fibrosis stage were significantly associated with a higher risk of developing HCC. Response to glycyrrhizin, defined as ALAT < 1.5×upper limit of normal, was significantly associated with a decreased incidence of HCC: hazard ratio 0.39 (95% CI 0.21-0.72; $p<0.01$). G-estimation, used to correct for ALAT as the confounder, showed no significant benefit of glycyrrhizin in the overall study population.
Conclusions. This study provides some evidence to show that interferon non-responder patients with chronic hepatitis C and fibrosis stage 3 or 4 may have a reduced incidence of HCC if glycyrrhizin therapy leads to normalization of ALAT levels.

INTRODUCTION

Chronic hepatitis C is a major cause of liver disease world-wide. Infection with the hepatitis C virus may lead to chronic inflammation of the liver, which is manifested in elevated liver enzymes such as alanine aminotransferase (ALAT). This chronic inflammation may lead to fibrosis and subsequent cirrhosis. It has been estimated that the delay for developing cirrhosis is about 30 years, but the individual prognosis may vary substantially depending on factors such as age at infection, gender, alcohol abuse and co-infection with hepatitis B or the human immunodeficiency virus (HIV) (1).

Over the past 15 years, treatment regimens based on the administration of interferon have proven to be increasingly effective against hepatitis C. Combination treatment with pegylated interferon and ribavirin will lead to disappearance of the virus from the blood in 50% to 80% of patients (2,3). If the virus remains undetectable in the blood at 6 months after the end of treatment, we speak of a sustained virological response. Sustained virological response is almost always associated with normalization of serum ALAT and a survival similar to that for the overall population (4). There still remains a considerable proportion of patients who do not achieve a sustained virological response. These patients require other therapeutic approaches, and various long-term interferon-based regimens are being investigated (5,6).

In Japan, glycyrrhizin has been propagated as an anti-inflammatory drug, capable of minimizing disease activity in the chronically infected liver. Glycyrrhizin is extracted from the roots of the plant Glycyrrhiza glabra (liquorice) and is a conjugate of a molecule of glycyrrhetinic acid and two molecules of glucuronic acid. Although the mechanism of action remains to be elucidated, it has been suggested that glycyrrhizin acts in a cytoprotective manner by its ability to inhibit tumor necrosis factor (TNF) alpha-mediated apoptosis and/or via inhibition of anti-Fas antibody-induced hepatitis (7-9). Glycyrrhizin is hydrolyzed to a pharmacologically active metabolite, glycyrrhetic acid, which inhibits 11--hydroxysteroid dehydrogenase and other enzymes involved in the metabolism of corticosteroids. Although this may lead to increased cortisol levels in the kidneys and other mineralocorticoid-selective tissues, van Rossum et al. showed that patients with chronic hepatitis or compensated cirrhosis only show minor reversible symptoms of pseudo-aldosteronism after treatment with 1200 mg glycyrrhizin weekly for 4 weeks (10).

Placebo-controlled trials have proven that the administration of glycyrrhizin leads to a significant reduction of ALAT levels in chronic hepatitis C patients (11). The question remains whether this reduction in ALAT levels leads to a reduced

risk of liver-related morbidity and mortality. Ideally, a randomized, controlled trial with a prolonged follow-up of at least several years should be designed, in order to investigate the effect of glycyrrhizin on these clinical end-points. However, even if such a study were restricted to cirrhotics, based on the incidence of hepatocellular carcinoma (HCC), decompensation and mortality (12), it would take at least 5 years before we had an answer to whether glycyrrhizin is a beneficial drug or not. Therefore we performed a large retrospective multicenter study, in which data collected in Japan were analyzed independently, Japan being the only country, so far, where hepatologists have extensively used this compound. We were especially careful to minimize the various biases associated with retrospective studies and to apply the most sophisticated statistics designed for such studies.

The aim of this study was to evaluate the effect of glycyrrhizin treatment on the incidence of HCC among patients with chronic hepatitis C who did not respond to interferon monotherapy.

MATERIAL AND METHODS

Study design

Japanese academic hospitals and major general hospitals were invited to participate in this retrospective cohort study. Hospitals could participate if they had searchable databases with available data on previous treatment with interferon and on clinical outcomes.

All consecutive chronic hepatitis C patients who received interferon alpha treatment between 1 January 1990 and 31 December 1995 and who did not show a sustained virological response were included. Thus, the study population consisted of non-responders and relapsers to interferon monotherapy. Sustained virological response was defined as a normal ALAT level and negative HCV-RNA at the end of treatment and 6 months thereafter.

The ethics committees of all the participating centers approved the protocol. In order to safeguard the privacy of the patients, the treating physician replaced patients' names with a code before entry in the database.

Patient selection

Data on all consecutive patients with chronic hepatitis C with a non-response to previous interferon treatment were collected. Data were collected on separate case record forms, one per patient, by the local investigator. The case record

forms were sent to the co-ordination center in Rotterdam, where the data were entered in a central database. Before the data were entered, they were checked and if there was any doubt, the local investigator was contacted.

Data recorded

Information was obtained on demographics (age, gender) and on details of the interferon treatment (starting date, duration, total dose) as well as the glycyrrhizin treatment (starting date, duration, total dose). Virological data (genotype, viremia), hematological (platelet count) and biochemical data (aminotransferase levels, bilirubin and gamma glutamyltransferase) were measured in the certified laboratories of the participating hospitals and added to the case record form by the local investigator. Centrally, the results were corrected for local normal values. Liver biopsies were scored by local pathologists using the METAVIR fibrosis score.

Follow-up data were recorded every four weeks if available and included ALAT levels, start of glycyrrhizin treatment and the occurrence of HCC. Patients were considered to have HCC if proven by biopsy or if ultrasound or computed tomography showed a focal lesion in the presence of a serum alpha-fetoprotein of >400.

Statistics

A data analysis plan was developed before closure of the database. The Kaplan-Meier method was used to estimate the occurrence of HCC over time, according to baseline ALAT levels. Entry into the study started at 24 weeks after interferon treatment. The Cox regression analysis was applied to determine which factors were independently associated with development of HCC. The following baseline factors were considered: age, gender, fibrosis stage, ALAT levels, anti-HBc positivity, genotype, viral load and route of transmission. All variables were checked for interactions.

In order to analyze the hypothesis of an association between glycyrrhizin therapy and a reduced occurrence of HCC over time, glycyrrhizin therapy was considered as a time-dependent factor, since glycyrrhizin treatment was started at various follow-up times. This means that all patients enter at time 0 as untreated. At the time of glycyrrhizin treatment the patient is censored in the untreated group and the patient enters the glycyrrhizin group. In this way, the period that a patient lived as untreated is calculated as the "event-free survival period" in the Cox analysis. In order to avoid bias, cases were censored at the time of a second

interferon-based treatment. According to the data analysis plan, a second analysis was done to assess the effect of glycyrrhizin according to response. Response to glycyrrhizin was defined as ALAT levels < 1.5×upper limit of normal (ULN) at the first measurement 3 months after initiation of treatment.

Statistical analyses were performed using SPSS Windows version 11 (SPSS Inc., Chicago, Ill., USA). The findings showed a strong influence of fibrosis and ALAT elevation on the development of HCC. Therefore, an additional analysis was done in a more homogeneous group of patients with advanced fibrosis.

The outcome of simply adjusting for ALAT as a time-dependent covariate in a Cox model may be a biased estimate of the treatment effect, since higher ALAT levels were associated with a higher probability of developing HCC and also of starting glycyrrhizin therapy (Figure 1). In order to estimate the causal effect of time-dependent glycyrrhizin treatment in the presence of a time-dependent covariate, ALAT, we used the G-estimation described by Robins et al. (13). This method is designed to gain an unbiased estimate of a treatment effect in the presence of a confounding variable, which is also intermediate. The G-estimation estimates the factor ψ. We use the exponent of - ψ, further referred to as *E*, as the factor by which the time towards development of HCC would be expanded (or contracted should *E* be smaller than 1.0) if the treatment with glycyrrhizin were not given (Appendix 1). This G-estimation was carried out with a macro written in SAS (SAS Institute Inc., Cary, N.C., USA).

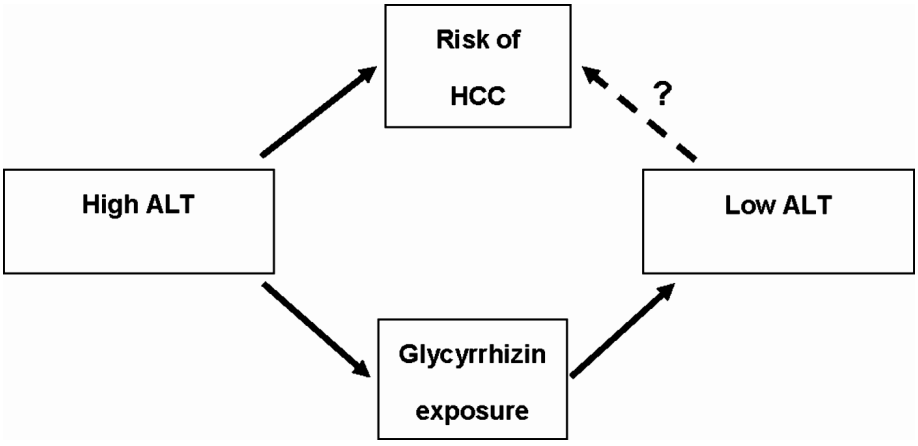

Figure 1: Elevated alanine aminotransferase (ALAT) levels during follow-up were associated with a higher probability of receiving glycyrrhizin, but also lead to a higher probability of developing hepatocellular carcinoma (HCC). As ALAT levels are lowered by glycyrrhizin treatment, ALAT is regarded as a time-dependent covariate which is both a confounder and an intermediate. In order to investigate whether glycyrrhizin reduces the risk of developing HCC by lowering ALAT levels (dotted arrow), sophisticated statistical analyses were required and a G-estimation was performed.

RESULTS

Descriptives:

A total of 1093 chronic hepatitis C patients with non-response to previous interferon therapy were included in the study. Follow-up started at 6 months after the end of treatment. During a mean follow-up of 6.1 years (SD 1.8) 26,450 visits were recorded. The mean duration of follow-up was 6.3 years (SD 1.8) for patients who were treated with glycyrrhizin and 6.0 years (SD 1.8) for patients who were not. Fifty-eight percent of the patients were males and the median age at time of inclusion was 52 years (range 17-81 years). Forty percent of the patients had acquired hepatitis C through blood transfusions. Further patient characteristics are presented in Table I.

Table I. Descriptives.

	Overall	Glycyrrhizin	No glycyrrhizin	*p*-value* (Chi-square/Mann-Whitney)
Number	1093	465	628	
M/F (%)	628/455 (58/42)	262/198 (56/43)	366/257 (58/41)	0.67
Age, mean (range)	52.2 (17-81)	53.9 (29-80)	50.9 (17-81)	<0.01
Genotype				<0.01
1 (%)	750 (69)	338 (73)	412 (66)	
2 (%)	214 (20)	90 (19)	124 (20)	
3 (%)	9 (0.8)	6 (1.3)	3 (0.5)	
4 (%)	4 (0.4)	0 (0)	4 (0.6)	
Fibrosis stage				<0.01
1 (%)	451 (41)	117 (25)	334 (53)	
2 (%)	372 (34)	181 (39)	191 (30)	
3 (%)	203 (19)	135 (29)	68 (11)	
4 (%)	54 (5)	29 (6)	25 (4)	
ALAT at t = 0				<0.01
< 1×ULN (%)	319 (29)	81 (17)	238 (38)	
1 - 1.5×ULN (%)	225 (21)	68 (15)	157 (25)	
1.5 - 2×ULN (%)	161 (15)	65 (14)	96 (15)	
2 - 3×ULN (%)	159 (15)	82 (18)	77 (12)	
> 3×ULN(%)	222 (20)	167 (36)	55 (9)	

Abbreviations: ALAT = alanine aminotransferase; ULN = upper limit of normal.
* p-value of the difference between patients treated or not treated with glycyrrhizin.

Four hundred and sixty-five patients received intravenous glycyrrhizin therapy, given as Stronger Neo Minophagen C (SNMC), which was started at various follow-up times; 164 of these patients had advanced fibrosis. The mean treatment duration with glycyrrhizin was 4.1 years (SD 2.6); 79% of the patients received treatment for 3 years or longer. The patients received a mean dose of 506 mg glycyrrhizin (191 ml SNMC) per week (range 106-1855 mg). Six patients stopped treatment because of side effects. Other treatments given to the interferon non-responders were interferon plus ribavirin (n=23), ursodeoxycholic acid (n=310 in the glycyrrhizin-treated group and n=347 in the untreated group) and herbal medicines (n=48 in the glycyrrhizin-treated group and n=46 in the untreated

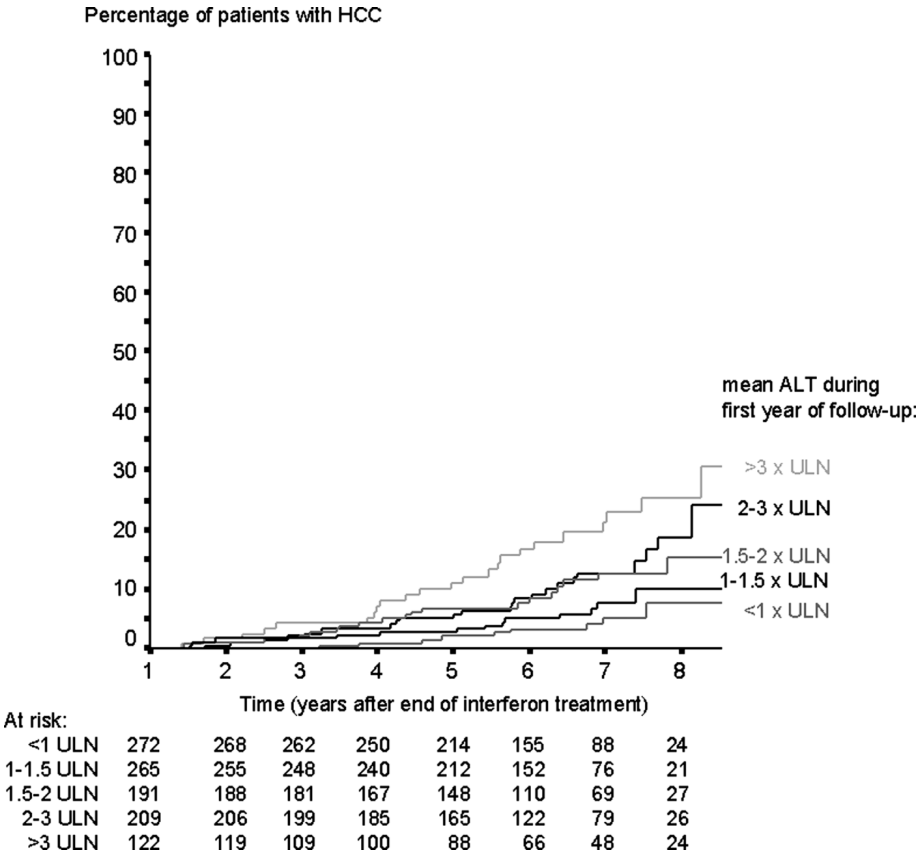

Figure 2: Kaplan-Meier curve showing the development of hepatocellular carcinoma (HCC) over time, according to mean alanine aminotransferase (ALAT) during the first year after interferon therapy. As the mean ALAT was calculated over the first year, the time-scale starts at one year of follow-up. Patients who did not fulfill one year of follow-up (n=7) and patients who developed HCC within the first year of follow-up (n=27) were excluded from this analysis.

group). The patients receiving interferon plus ribavirin were censored at the start of this treatment.

Events:

One hundred and seven patients developed HCC. We performed a Kaplan-Meier analysis in order to investigate the influence of raised ALAT levels on the risk of developing HCC (Figure 2). In patients with normal ALAT levels during the first year of follow-up, the 5-year incidence of HCC was 3.1% (95% CI 0.8-5.5). The incidence of HCC increased to 4.9 (95% CI 2.0-7.8) for ALAT levels between 1 and 1.5×ULN, 8.3% (95% CI 4.1-12.5) for ALAT levels between 1.5 and 2×ULN and 8.3% (95% CI 4.2-12.3) for ALAT levels between 2 and 3×ULN. The highest occurrence of HCC was seen in patients with ALAT levels three times above the ULN during the first year of follow-up: 16.6% (95% CI 9.3-24.0).

The time-dependent Cox regression analysis showed that older age, male gender, higher fibrosis stage and non-response to glycyrrhizin were significantly associated with a higher risk for developing HCC (Table II). The 5-year occurrence of HCC was 1.2% (95% CI 0.1-2.2) for patients with fibrosis stage 1 and 3.8% (95% CI 1.7-5.9) for patients with fibrosis stage 2. The occurrence of HCC was highest among patients with fibrosis stage 3 and 4: 13.7% (95% CI 8.6-18.8) and 26.6% (95% CI 13.9-39.4), respectively.

Subgroup analysis of patients with fibrosis stages 3 and 4 showed a trend towards less development of HCC among patients with a response to glycyrrhizin (hazard ratio = 0.50 (95% CI 0.22-1.12, p=0.09).

Seventy-four percent (343/465) of the patients treated with glycyrrhizin had ALAT levels above 1.5×ULN at the start of therapy and 66% (228/343) of these responded with decreased ALAT levels. In comparison, the rate of spontaneous ALAT normalization in patients with elevated ALAT levels at start of follow-up who were not treated with glycyrrhizin was 33% (114/344) at 3 months after inclusion in the study. In an analysis of all 465 treated patients, those with an ALAT response had a significant, lower risk of developing HCC than non-responders; hazard ratio 0.39 (95% CI 0.21-0.72, p<0.01) (Table III). The Cox regression analysis for untreated patients (patients censored at start of glycyrrhizin therapy) showed that spontaneous normalization of ALAT levels at 4 months after start of follow-up, though two times less common than normalization after initiation of glycyrrhizin, also tended to be associated with a lower risk of developing HCC (hazard ratio 0.44 (95% CI 0.19-1.02, p=0.06).

Table II. Time-dependent Cox regression analysis assessing risk factors for HCC (n=1093).

	Hazard ratio	95% CI	*p*-value
Gender			
Male	1		
Female	0.31	0.19-0.51	<0.01
Age	1.08	1.05-1.11	<0.01
ALAT levels at t = 0			
< 1.5×ULN	1		
> 1.5×ULN	1.58	0.92-2.70	0.10
Alcohol			
< 50 g/day	1		
> 50 g/day	1.15	0.64-2.04	0.65
Fibrosis stage			
Fibrosis stage 1	1		
Fibrosis stage 2	4.04	1.66-9.83	<0.01
Fibrosis stage 3	8.75	3.56-21.5	<0.01
Fibrosis stage 4	15.2	5.82-39.7	<0.01
Glycyrrhizin			
No glycyrrhizin	1		
Glycyrrhizin, no ALAT response	2.03	1.21-3.42	0.01
Glycyrrhizin, ALAT response	0.81	0.41-1.60	0.54

Abbreviations: HCC = hepatocellular carcinoma; ALAT = alanine aminotransferase; ULN = upper limit of normal. The hazard ratios with their 95% CIs and p-values associated with these factors are given. Hazard ratio < 1.0 indicates a decreased risk for HCC. Older age, male gender, higher fibrosis stage and non-response to glycyrrhizin treatment were significantly associated with a higher risk of developing HCC. Gender, ALAT, alcohol intake, fibrosis stage and glycyrrhizin treatment were entered as categorial values. A hazard ratio of 1 indicates the reference value. Age was entered as a continuous value.

G-estimation:

The G-estimation performed for the overall study population, showed that the time towards development of HCC was not significantly influenced by glycyrrhizin treatment (*E*=0.96 (95% CI 0.76-2.10).

There was a trend towards a prolonged time to development of HCC among patients with fibrosis stage 3 or 4 if they received glycyrrhizin; *E*=1.17 (95% CI 0.65-2.29). Among patients with fibrosis stage 1 or 2, no beneficial effect of glycyrrhizin was seen during the observation period, but the number of events was too small to make a reliable estimate in this subgroup.

Table III. Time-dependent Cox regression analysis assessing risk factors for HCC in patients who received glycyrrhizin treatment (n=465).

	Hazard ratio	95% CI	*p*-value
Gender			
Male	1		
Female	0.23	0.12-0.42	<0.01
Age	1.09	1.05-1.13	<0.01
ALAT levels at start of treatment			
< 1.5×ULN	1		
> 1.5×ULN	0.44	0.17-1.14	0.09
Fibrosis stage			
1	1		
2	2.41	0.89-6.50	0.08
3	3.35	1.26-8.92	0.02
4	7.95	2.71-23.3	<0.01
Response to glycyrrhizin			
No	1		
Yes	0.39	0.21-0.72	<0.01

Abbreviations: HCC = hepatocellular carcinoma; ULN = upper limit of normal; ALAT = alanine aminotransferase. The hazard ratios with their 95% CIs and p-values associated with these factors are given. Hazard ratio < 1.0 indicates a decreased risk for HCC. Older age, male gender and advanced fibrosis were significantly associated with a higher risk of developing HCC. Patients with an ALAT response to glycyrrhizin had a significantly decreased chance of developing HCC, compared with non-responders. Gender, ALAT, fibrosis stage and glycyrrhizin treatment were entered as categorial values. A hazard ratio of 1 indicates the reference value. Age was entered as a continuous value.

DISCUSSION

The aim of this study was to evaluate the effect of glycyrrhizin treatment on the incidence of HCC among patients with chronic hepatitis C who did not respond to interferon monotherapy.

During follow-up 107 patients developed HCC. This is concordant with data published by Yoshida et al., who presented the rates of development of HCC by age, gender and fibrosis stage in their population of non-sustained responders. Applying these rates to our data set would lead to an expected number of 117 HCCs (95% CI 99-139) during 6.1 years of follow-up (14). The incidence of HCC in our cohort is high, the 5-year incidence of HCC among patients with F1 and F2 fibrosis being 1.2% and 3.8%, respectively. However, we investigated a selected group of interferon non-responders and the incidence of HCC is much higher in Japan than in Europe. In our cohort the overall yearly incidence of HCC was 1.6%. Previous large cohort studies found a yearly incidence of 0.3% to 2.7%

per year in Japanese non-sustained responders to interferon treatment (14-16). In the literature, lower rates of HCC development are described for patients who relapsed after an initial response and for patients with persistently low ALAT levels (17). Similarly, in our cohort, patients with lower baseline ALAT levels had a smaller probability of developing HCC.

As chronic hepatitis C progresses slowly, it is difficult to evaluate the efficacy of treatment on clinical outcomes such as mortality and development of HCC in randomized controlled trials. Therefore, "best" information should be derived from cohort studies. However, cohort studies are only reliable if the drop-out rate is low compared to the events. In retrospective cohort studies the risk of introducing bias is even greater. Incomplete capture of early clinical events, confounding bias and compliance bias have been described as possible confounders in retrospective studies (18). In large randomized trials this problem is usually avoided, as unmeasured confounders are likely to be equally divided over the groups by randomization.

We executed this retrospective cohort analysis with great care to preclude these biases. Incomplete capture of clinical events could not play a role in our analysis as the development of HCC was monitored during the whole follow-up period. Secondly, confounding bias may have played a role, as raised ALAT levels increased both the chance of receiving glycyrrhizin treatment and the risk of developing HCC. Sophisticated statistical analyses were used to correct for this confounder (13,19-20).

Finally, compliance bias may have played a role in this study, as patients who are willing to attend the hospital several times a week for intravenous injections of glycyrrhizin are possibly also more likely to adhere to other protective types of behavior. However, the fact that the follow-up of patients who did not receive glycyrrhizin was similar to those who did, suggests that they were equally compliant in their hospital visits.

A previous study on the effect of glycyrrhizin on clinical outcome showed a significant protective effect on development of HCC (21). In our study we refined the methodology by using an intention-to-treat approach. All patients who received glycyrrhizin were included, even those who were treated for a short time. In this way we sought to avoid the exclusion of patients who stopped their glycyrrhizin early because they had died of HCC.

In the present study, there were significant differences in baseline demographics between treated and non-treated patients concerning genotype distribution, mean age and fibrosis stage. Therefore, we first of all used a multivariate Cox regression analysis to assess the effect of glycyrrhizin. Overall, there was no significant effect, but in patients with fibrosis stages 3 and 4 there was a trend

towards a protective effect on development of HCC. An intention-to-treat analysis of all patients treated with glycyrrhizin showed that patients responding by decreased ALAT levels had a significantly lower probability of developing HCC. A G-estimation was performed to address the problem of confounding by ALAT levels. The latter analysis failed to show an overall beneficial effect of glycyrrhizin, but in patients with fibrosis stage 3 or 4 at the start of follow-up, there was a trend towards a protective effect.

In conclusion, this study provides some evidence to suggest that interferon non-responder patients with chronic hepatitis C and fibrosis stage 3 or 4 may have a reduced incidence of HCC if glycyrrhizin therapy leads to normalization of ALAT levels.

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APPENDIX 1

The method of G-estimation by J. M. Robins offers a solution to estimate the causal effect of time-dependent glycyrrhizin treatment on the development of HCC, in the presence of a time-dependent covariate, ALAT, that is both a confounder and an intermediate variable.

G-estimation of the parameter of a nested structural model estimates the expansion or contraction parameter ψ of the time to event (HCC) due to the exposure to glycyrrhizin treatment. If, for instance, the exponent of $-\psi$ (referred to as *E* in the text) = 1.20, the time to HCC is expanded by 20%, corresponding with a beneficial effect.

Fundamental to this approach is the assumption of no unmeasured confounders. This means that all covariates influencing both the decision to use glycyrrhizin and the HCC-free survival time should be measured. This means that, given the covariates, the decision to start treatment is independent of the patient's (possibly counterfactual) HCC-free survival time under any treatment regimen.

A pooled logistic regression analysis over all visits was applied, with glycyrrhizin therapy at visit κ as outcome. This means that each subject contributed with multiple observations, one for each visit, until development of HCC or censoring. Covariates considered for inclusion in the model are baseline factors (age, gender, fibrosis stage, ALAT and gamma glutamyltransferase at the start of the study) and the covariate history before visit κ (ALAT, glycyrrhizin treatment and concomitant medication at the two visits prior to visit κ). Furthermore, the number of weeks since the prior visit and the number of weeks since the start of the study were included in the model.

The parameter ψ is G-estimated by extending the logistic model with sets of imaginary (counterfactual) HCC-free survival times, had glycyrrhizin treatment never been given. Weights have been calculated to adjust for patients who are lost to follow-up or who are censored at a second interferon-based treatment.

Data description and annotation

T_i=Observed failure time for subject *i*.

U_i=Time to failure (HCC) for subject *i* if never exposed to glycyrrhizin (=counterfactual failure time)

$Glycyrrhizin_i(t)$ = The treatment status of subject *i* at time-point t.

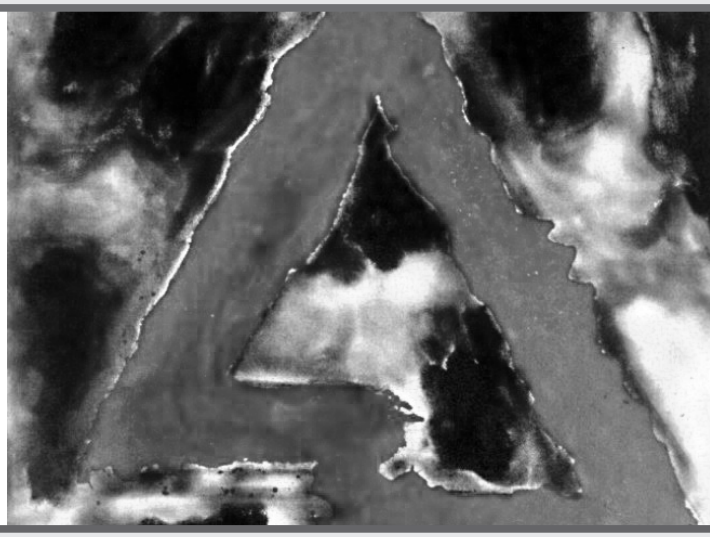
The model that relates the observed data T_i and $glycyrrhizin_i(Ti)$ to the counterfactual failure time U_i is assumed to be:

$$Ui(\psi) = \int_0^{T_i} \exp(\psi\ Glycyrrhizin_i(t))\ dt$$

The model of U as a function of ψ describes the relation between the counterfactual failure time, the observed failure time and the use of glycyrrhizin over time.

Chapter 7

Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis



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Ann Intern Med. 2007 Nov 20;147(10):677-84

ABSTRACT

Background: Clinical outcomes of chronic hepatitis C infection in patients with advanced fibrosis include liver failure, hepatocellular carcinoma, and death.
Objective: To investigate whether sustained virologic response to treatment for hepatitis C is associated with improved clinical outcomes.
Design: Retrospective cohort study.
Setting: 5 hepatology units of tertiary care centers in Europe and Canada caring for patients with chronic hepatitis C treated between 1990 and 2003.
Patients: Consecutively treated patients with chronic hepatitis C who had biopsy-proven advanced fibrosis or cirrhosis (Ishak score, 4 to 6).
Measurements: Sustained virologic response, defined as absence of detectable hepatitis C virus RNA at 24 weeks after the end of treatment, and clinical outcomes, defined as death (liver-related or non-liver-related), liver failure, and hepatocellular carcinoma.
Results: Of 479 patients, 29.6% had sustained virologic response and 70.3% did not. Median follow-up was 2.1 years (interquartile range, 0.8 to 4.9 years). Four patients with and 83 without sustained virologic response had at least 1 outcome event. Sustained virologic response was associated with a statistically significant reduction in the hazard of events (adjusted hazard ratio, 0.21 [95% CI, 0.07 to 0.58]; P = 0.003). The effect was largely attributable to a reduction in liver failure, which developed in no patients with and 42 patients without sustained virologic response (5-year occurrence, 0% vs. 13.3% [CI, 8.4% to 18.2%]; unadjusted hazard ratio, 0.03 [CI, 0.00 to 0.91]).
Limitations: Because few events occurred in the sustained virologic response group, the study had limited ability to detect differences between groups in individual outcomes. In addition, the study was retrospective; selection and survival biases may therefore influence estimates of effect.
Conclusion: Sustained virologic response to treatment is associated with improved clinical outcomes, mainly prevention of liver failure, in patients with chronic hepatitis C and advanced fibrosis.

INTRODUCTION

Worldwide, there are 170 million people chronically infected with the hepatitis C virus (1). Patients with chronic hepatitis C may develop decompensated liver disease and hepatocellular carcinoma (HCC). This risk is highest in patients with advanced fibrosis.

The treatment of chronic hepatitis C is usually evaluated by the number of patients reaching a sustained virological response (SVR), which is a surrogate marker. Several large studies have suggested that successful treatment with peginterferon and ribavirin may halt and even reverse hepatic fibrosis. Camma et al. assessed 1013 chronic hepatitis C patients with pre-treatment and post-treatment liver biopsies, treated with interferon or with pegylated interferon, and showed that SVR was associated with a reduction in fibrosis (2). Poynard et al. showed that among 3010 patients with pre-treatment and post-treatment biopsies, reversal of fibrosis was found in 12% of the patients treated for 24 weeks with standard interferon and in up to 24% of the patients treated with an optimal schedule of peginterferon and ribavirin (3). Although Poynard et al. observed regression of cirrhosis in 49% of their patients after successful treatment, it remains to be elucidated whether there is a clinical benefit of peginterferon treatment for patients who have already developed advanced fibrosis or cirrhosis.

Until now, reports of the long-term benefit of standard interferon therapy for patients with cirrhosis have been disappointing since few achieved SVR (4). Large studies from Japan indicate that maximum benefit of SVR is achieved among those treated prior to the development of cirrhosis (5). Since SVR rates following peginterferon therapy plus ribavirin therapy are higher than for interferon monotherapy, it is time to evaluate the effect of this therapy on solid clinical endpoints such as liver failure, HCC and survival and to establish whether SVR leads to an improved long-term outcome in this high risk population.

Therefore, the aim of this study is to investigate whether SVR, as compared to non-response, leads to an improved clinical outcome for patients with chronic hepatitis C and advanced fibrosis.

METHODS

Design:

International multicenter retrospective cohort study.

Participants:

All consecutive chronic hepatitis C patients with biopsy-proven advanced fibrosis or cirrhosis (Ishak score 4 to 6), treated with an interferon-based regimen between 1990 and 2003 in five large Hepatology units of tertiary care centers in Europe and Canada, were included in the study. Patients were excluded if they were co-infected with hepatitis B virus or human immunodeficiency virus. Patients with decompensated liver disease were not eligible for treatment, and were therefore not included in the study.

Approval was obtained from the ethics committees of each participating study site. Local investigators identified all eligible patients and the principal investigator (BJV) then visited each center to enter the individual patient data from the chart into a central database in a standardized and predefined way.

Data were obtained on patient demographics (gender, age) and details of the treatment (duration, interferon or pegylated interferon, ribavirin, treatment naive or previous non-responder). Age was assessed at the start of the last treatment. Virological data (genotype, baseline viral load, anti-hepatitis B core antigen positivity), biochemical data (bilirubin, albumin) and haematological data (platelet count, prothrombin time/Quick time) were measured in the certified laboratories of participating hospitals and were corrected centrally for local normal values. Liver biopsies were scored by local pathologists, who all had a large experience in scoring liver biopsies from patients with viral hepatitis. To assess HCV-RNA, all participating centers used commercial polymerase chain reaction tests from Roche diagnostics with a detection limit of 100-500 IU/ml. Before these tests became commercially available in 1994, nested in-house polymerase chain reaction tests were used where plasma HCV RNA was analyzed in duplicate by polymerase chain reaction using two sets of primers derived from the 5′ noncoding region followed by a hybridization assay, as described previously for the different centers (6-9). These sensitive HCV RNA tests were also used to define the absence of detectable HCV RNA at 24 weeks after the end of treatment. Non-responders or relapsers with detectable virus at this time point are referred to as non-responders.

Follow-up:

Patients were considered to have liver failure if they met any of the following criteria: ascites confirmed by ultrasound or computed tomography, bleeding oesophageal varices, jaundice with bilirubin > 35 µmol/L or hepatic encephalopathy. Patients were considered to have a hepatocellular carcinoma if the diagnosis was cyto-histologically confirmed, or if two coincident imaging

techniques (ultrasound, computed tomography or magnetic resonance imaging) showed a focal lesion > 2 cm with arterial hypervascularization, or if one imaging technique showed a focal lesion > 2 cm with arterial hypervascularization in the presence of an alpha-fetoprotein > 400 ng/ml. For the occurrence of HCC, the date was used when the diagnosis was confirmed by histology or radiography. Liver transplantation and mortality were recorded; the latter was classified as liver-related or non liver-related. If the follow-up was incomplete, the treating physician contacted the patient. In case the patient could not be reached, the treating physician contacted the patient's general practitioner in order to complete the follow-up.

Statistical analysis:

Logistic regression was used to analyze which baseline factors were associated with response to interferon-based treatment; the results are reported as odds ratios with 95% confidence intervals. The following factors were considered: Age, gender, previous non-response, treatment duration, treatment including ribavirin, treatment with peginterferon versus standard interferon, fibrosis stage, genotype, pre-treatment bilirubin, platelet count and albumin, viral load, treatment period (1990-1997/1998-2005) and treatment center. Multiple collinearity was observed between fibrosis stage and the covariates bilirubin, platelet count and albumin. Both the fibrosis stage and these laboratory parameters reflect the severity of liver disease. Therefore, two separate models were developed for either inclusion of fibrosis in the model or inclusion of the covariates bilirubin, platelet count and albumin. The model including the covariates bilirubin, platelet count and albumin had the lowest AIC score and provided the best fit to the data. The regression model was reliable (Hosmer and Lemeshow goodness-of-fit test, $p > 0.35$) and discriminated well between patients with and without sustained virological response to treatment (area under the receiver-operating characteristic curve 0.83).

The Kaplan-Meier method was used to estimate the effect of SVR on clinical events i.e. liver failure, HCC and liver-related death over time. Groups were compared using log-likelihood tests. Individuals could have more than one event, but could only contribute one to the any event analysis. For the analysis of overall death we included patients who had died either from a liver-related or a non-liver-related cause. Liver transplantation was considered as liver-related death at the time of transplantation. However, patients who were alive after liver transplantation were regarded as being alive in the analysis of overall death.

Since the definition of SVR is undetectable serum HCV-RNA by sensitive molecular tests at 24 weeks after the end-of-treatment, we used this time point as time 0 for classifying response versus non-response.

Separate Cox proportional hazards models were developed to determine which baseline factors were associated with development of HCC, liver failure, liver-related death, overall death and any event. Given our limited number of events, we were unable to adjust for all available risk factors. Instead, we adopted the following model selection strategy. First, factors that were highly correlated with each other resulting in multicollinearity were identified. Secondly, different models were compared with the overall-score test (SAS, PROC PHREG, with the subcommand BEST SUBSET) and finally the AIC method was applied. The following covariates were included in the final proportional hazards model: SVR, age, gender, previous non-response, bilirubin, albumin, platelet count and treatment center. The final proportional hazard model was stratified by treatment period (1990-1997/1998-2005), to represent evolution in the evaluation and treatment of hepatitis C since 1990. Treatment with or without ribavirin and treatment with peginterferon versus standard interferon were not included in the final proportional hazards model because of multiple collinearity with treatment period. Similar multiple collinearity was observed between fibrosis stage and the covariates bilirubin, platelet count and albumin. In the same manner as we did for the logistic regression model, we developed two separate models for either inclusion of fibrosis in the model or inclusion of the covariates bilirubin, platelet count and albumin. The model including the covariates bilirubin, platelet count and albumin had the lowest AIC score and provided the best fit to the data. The reported hazard ratios are the relative increases in hazard associated with increases of 10 µmol/l for the covariate bilirubin, 10 g/l for albumin and $10*10^9$/l for platelet count. The covariate "treatment duration" was not a risk factor for any of the endpoints in the univariate proportional hazards analysis and did not improve the multivariable model fit either. Therefore, treatment duration was not considered in the final model. The same accounts for genotype. The viral load at start of therapy was missing in 31.5% of cases and with such spare data was not considered for the analysis. Furthermore, SVR reflects the most recent measure of the viral load. Anti-hepatitis B core antigen positivity was a risk factor for HCC, however the hazard of HCC was not proportional over time. Therefore, the analysis for risk of development of HCC was stratified for serum anti-hepatitis B core antigen positivity. For other covariates than anti-hepatitis B core antigen positivity, the assumption of proportionality was not violated. Results are reported as relative hazards with 95% confidence intervals.

Results were the same when response to treatment was modeled as a time-dependent covariate to represent the ability of patients undergoing more than one treatment course to change their status from non-responders to responders in sequential courses.

Since either baseline bilirubin, albumin or platelet count were missing in 28.5% of the cases, multiple imputation was used to impute missing values (10, 11): the Markov Chain Monte Carlo method (MCMC) with single chain (PROC MI in SAS) was applied to construct 10 complete datasets. All baseline factors and time related factors and events related to the analyses were entered into the imputation procedure and if necessary transformed to conform to the multivariate normality assumption. Cox analysis (PROC PHREG) or logistic regression analysis (PROC LOGISTIC) was then run on each dataset and the results and inference combined using the PROC MIANALYZE in SAS. All statistical analyses were performed using SAS (version 9.1.3, SAS Institute Inc, Cary, NC, USA).

Role of the funding source: The sponsor did not have any influence on the design and conduct of the study; nor on collection, management, analysis, and interpretation of the data; nor on preparation, review, and approval of the manuscript.

RESULTS

Study population:

Five hundred and forty-one patients with advanced fibrosis were treated with an interferon-based regimen in the participating centers. Fifty-two patients were excluded from the study as they had not reached 24 weeks after end-of-treatment and ten patients were excluded because they had developed a clinical event within 24 weeks of follow-up after their last treatment.

The study cohort thus consists of 479 patients, 142 (30%) of whom were sustained virological responders and 337 (70%) were non-responders. Seventy-two percent of the patients had a complete follow-up until 1-1-2005, six months prior to the data acquisition.

One hundred and thirty-one patients (27%) were treated with interferon monotherapy, 130 (27%) with interferon and ribavirin, 10 (2.1%) with peginterferon monotherapy and 208 (43%) with peginterferon and ribavirin (table 1). One hundred and forty-three patients (30%) were non-responders to a previous course of interferon-based treatment, 72 of these patients (15%) received 2 treatment courses and 70 of these patients (15%) received 3 treatment courses. The median time interval between two treatment courses was 4.2 years (inter quartile

Table 1 Patient characteristics at the start of the last treatment.

	Overall (n=479)	Sustained virological responders (n=142)	Non-responders (n=337)	p-value (Mann Whitney U / χ^2)
Age (years) [1]	48 (43-56)	48 (42-56)	49 (43-56)	0.45
Male (n (%))	332 (69)	104 (73)	228 (68)	0.23
Genotype 1 (n (%))[2]	280 (59)	56 (39)	224 (67)	<0.01
Anti-Hepatitis B core antigen positive (n (%))	141 (30)	38 (27)	103 (31)	0.46
Treatment:				<0.01
-Interferon (n (%))	131 (27)	14 (10)	117 (35)	
-Interferon + ribavirin (n (%))	130 (27)	41 (29)	89 (26)	
-Peginterferon (n(%))	10 (2.1)	4 (2.8)	6 (1.8)	
-Peginterferon + ribavirin (n (%))	208 (43)	83 (59)	125 (37)	
Previous non-response (n (%))	143 (30)	35 (25)	108 (32)	0.11
Duration of treatment (weeks) [1]	26 (21-48)	47 (25-52)	24 (16-47)	<0.01
Treatment period				<0.01
-1990-1997 (n (%))	134 (28)	16 (11)	118 (35)	
-1998-2005 (n (%))	345 (72)	126 (89)	219 (65)	
Follow-up (years) [1]	2.1 (0.8-4.9)	1.1 (0.3-2.9)	2.8 (1.2-5.9)	<0.01
Viral load (IU/ml)[1,3]	8.1 10^E5 (4.0 10^E5-2.5 10^E6)	8.5 10^E5 (2.7 10^E5-3.9 10^E6)	8.0 10^E5 (4.4 10^E5-2.3 10^E6)	0.75
Bilirubin (µmol/l) [1]	9.8 (7.3-12.0)	9.2 (7.3-12.4)	10.2 (7.5-14.0)	0.02
Albumin (g/l) [1]	41 (38-43)	41 (38-44)	41 (38-43)	0.16
Platelet count (*10^9/l) [1]	160 (116-207)	166 (129-210)	151 (110-204)	0.07
Fibrosis:				0.45
-Ishak-score 4 (n (%))	120 (25)	41 (29)	79 (23)	
-Ishak-score 5 (n (%))	94 (20)	27 (19)	67 (20)	
-Ishak-score 6 (n (%))	265 (55)	74 (52)	191 (57)	

[1]Median (inter quartile range)

[2]Genotype was missing in 12% of the patients.

[3]Viral load was measured by local hybridization or polymerase chain reaction assays and could be retrieved in 68% of the patients.

range (IQR) 1.8-6.4). Overall, the median treatment duration was 26 weeks (IQR 21-48). Fifty-one patients received less than 12 weeks of treatment of whom 2 nevertheless achieved an SVR. The median treatment duration was 24 weeks for non-responders versus 47 weeks for sustained virological responders (table 1). Among non-responders, genotype 1 was predominant.

Multiple regression analysis showed that the following factors were associated with SVR: genotype non-1 (OR 2.65; 95% confidence interval (CI) 1.79-3.92), treatment naïve versus previous non-response (OR 1.31; CI 1.01-1.71), treatment including ribavirin (OR 1.96; CI 1.38-2.79) and treatment duration of more than 35 weeks versus less than 20 weeks (OR 3.83; CI 2.63-5.58). The associations

with peginterferon versus standard interferon (OR 1.14; 95% CI 0.87-1.49), pre-treatment serum bilirubin (OR 0.63; CI 0.39-1.02), platelet count (OR 0.98; CI 0.94-1.03) and albumin (OR 1.31; CI 0.62-2.77) were not statistically significant. There was no statistically significant effect of treatment center on outcome of treatment (p=0.10).

Overall follow-up was shorter for sustained virological responders than for non-responders, since patients who were treated after the introduction of ribavirin in 1998 exhibited higher response rates. Twelve percent (16/134) of the patients treated before 1998 achieved sustained virological response versus 37% (126/345) of the patients treated during or after 1998 (table 1).

We performed an analysis of the subgroup of patients treated from 1998, when ribavirin was introduced. In this subgroup of 345 patients, 122/126 sustained virological responders (97%) and 213/219 non-responders (97%) received ribavirin. The median follow-up time was 0.9 years (IQR 0.2-2.3) for sustained virological responders and 1.6 years (IQR 0.7-3.2) for non-responders, and the difference in occurrence of any clinical events remained statistically significant (HR 0.20; CI 0.05-0.86, p=0.031). Since almost every patient from 1998 was treated with ribavirin, we did not adjust for ribavirin in this analysis.

Events:

Four patients with SVR and 83 non-responders had at least one of the described events (i.e. hepatic failure, HCC, liver-related death or non-liver-related death). The proportion of patients experiencing events at 5 years was statistically significantly different between groups (figure 1a), and SVR was associated with a statistically significant reduction in the hazard of events (adjusted HR 0.21; CI 0.07-0.58, p=0.003) (table 2). Estimates of association between other variables and clinical outcomes are summarized in table 3.

Overall mortality:

Two patients with SVR and 24 non-responders died during follow-up. Five non-responders and one sustained virological responder died of non-liver-related causes. There was a trend towards improved survival of patients with SVR compared to non-responders at 5 years (figures 1b and 1c), and SVR was associated with a reduction in the hazard of mortality (adjusted HR 0.31; CI 0.07-1.38, p=0.124) (table 2).

Liver-related death and transplantation:

One patient with SVR and 16 non-responders died of a liver-related cause and 18 patients, who were all non-responders, underwent orthotopic liver transplanta-

Table 2: Clinical outcomes by response to treatment

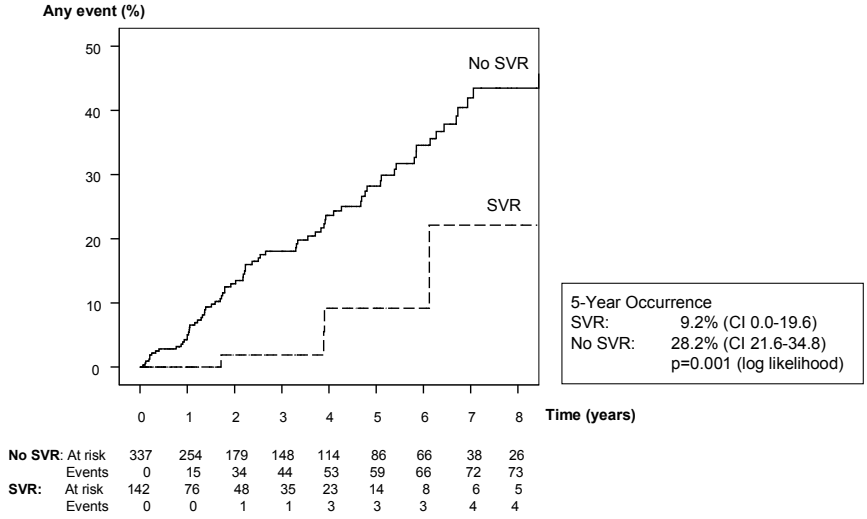
	Sustained Virological Response			No Sustained Virological Response			Unadjusted Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI) [1]	P (for adjusted)
	Events (n)	Observation time (patient-years)	Rate (Events per 10,000 patient-years), (95% CI)	Events (n)	Observation time (patient-years)	Rate (Events per 10,000 patient- years), (95% CI)			
Any Event	4	280	143 (2-283)	83	1107	750 (594-905)	0.20 (0.07-0.55)	0.20 (0.07-0.58)	0.003
Deaths	2	281	71 (0-170)	24	1243	193 (116-270)	0.44 (0.10-1.87)	0.31 (0.07-1.38)	0.124
Liver-related deaths[2]	1	281	36 (0-106)	34	1200	283 (189-377)	0.14 (0.02-1.03)	0.19 (0.02-1.44)	0.107
Non-liver related deaths[3]	1	281	36 (0-106)	5	1245	40 (5-75)	1.21 (0.14-10.6)	-	-
Liver failure[4]	0	281	0 (0-2)	42	1150	365 (257-474)	0.03 (0.00-0.91)	-	-
Hepatocellular carcinoma	3	280	107 (0-228)	32	1157	277 (182-371)	0.46 (0.14-1.52)	0.46 (0.12-1.70)	0.245

[1]Analyses are adjusted for the following covariates: age, gender, previous non-response, bilirubin, albumin, platelet count, treatment center and treatment period (1990-1997/1998-2005). The analysis for risk of hepatocellular carcinoma was also adjusted for anti hepatitis B core antigen positivity.

[2]"Liver-related death" includes 17 deaths (1 in a patient with sustained virological response, 16 in non-responders) and 18 liver transplantations (all in non-responders). Liver transplantations are counted as "Liver-related death", but not as overall "Death", unless a patient died after liver transplantation (3 among the non-responders).

[3]There were not enough non-liver-related deaths to assess the effect of sustained virological response on non-liver-related death in multivariable analysis.

[4]The effect of sustained virological response on liver failure could not be quantified in multivariable analysis, since none of the sustained virological responders developed liver failure during follow-up.



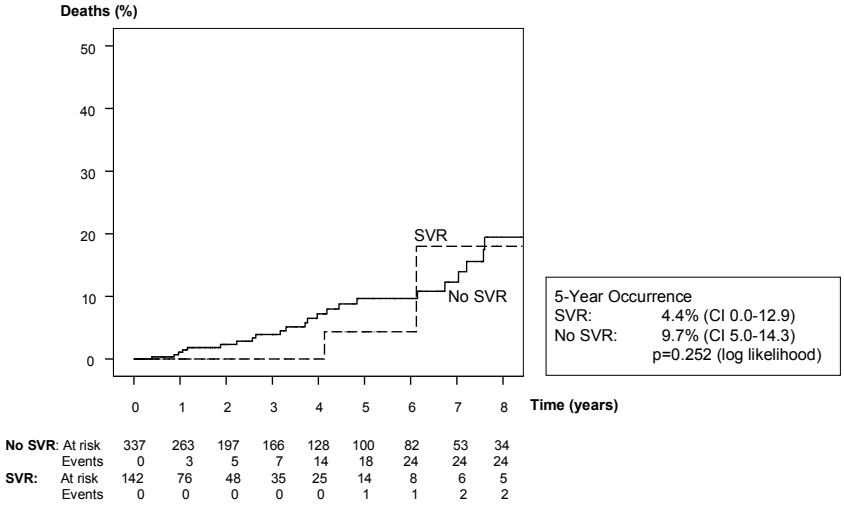
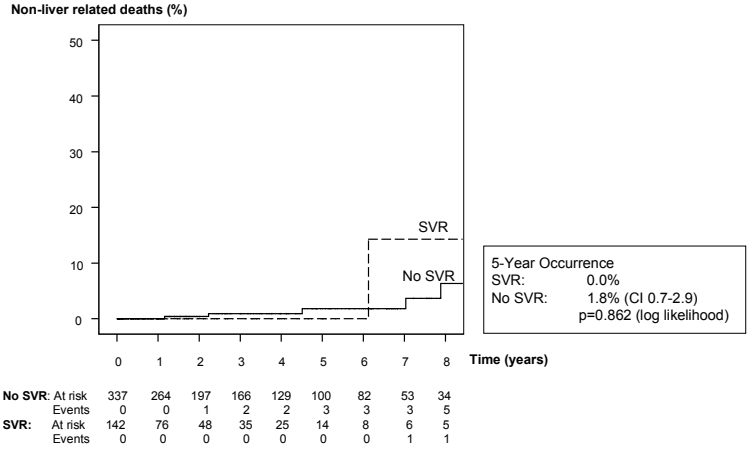


Figure 1: Kaplan-Meier curves showing the occurrence of (a) any event, (b) overall mortality, (c) non-liver-related mortality, (d) liver-related mortality, (e) hepatic failure and (f) hepatocellular carcinoma over time among sustained virological responders (SVR) versus non-responders (NR).



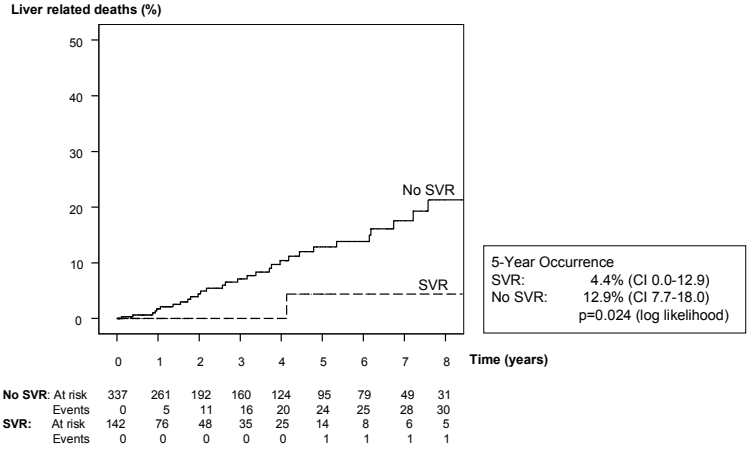
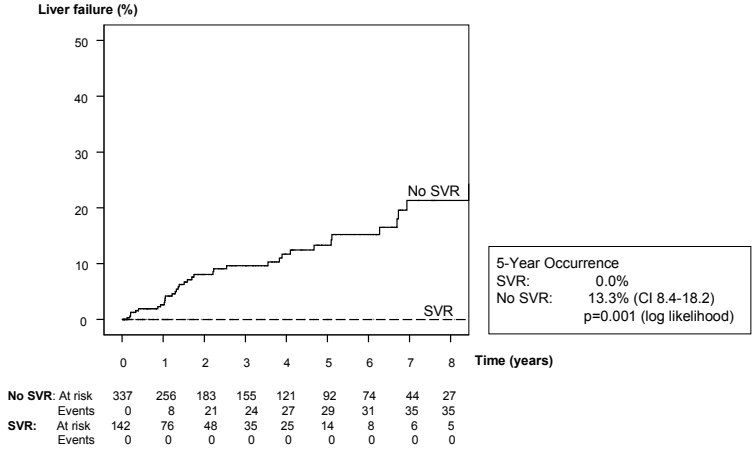


Figure 1 C en D: Kaplan-Meier curves showing the occurrence of (a) any event, (b) overall mortality, (c) non-liver-related mortality, (d) liver-related mortality, (e) hepatic failure and (f) hepatocellular carcinoma over time among sustained virological responders (SVR) versus non-responders (NR).



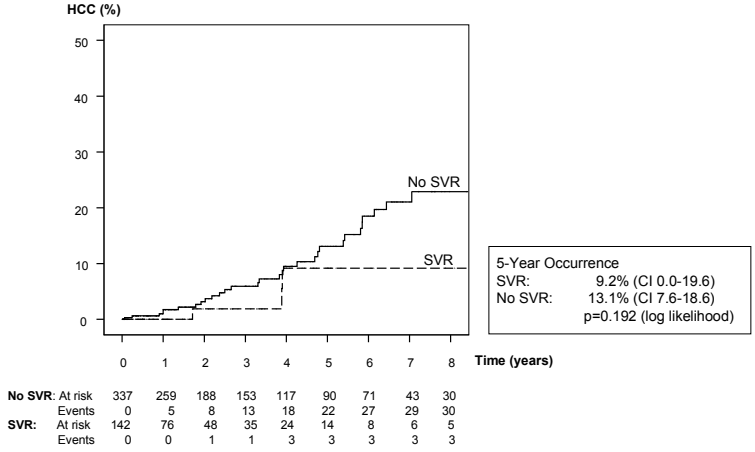


Figure 1 E en F: Kaplan-Meier curves showing the occurrence of (a) any event, (b) overall mortality, (c) non-liver-related mortality, (d) liver-related mortality, (e) hepatic failure and (f) hepatocellular carcinoma over time among sustained virological responders (SVR) versus non-responders (NR).

Table 3 Clinical outcomes by response to treatment

	Sustained Virological Response			No Sustained Virological Response			Unadjusted Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)	P (for adjusted)
	Events (n)	Observation time (patient-years)	Rate (Events per observation time)	Events (n)	Observation time (patient-years)	Rate (Events per observation time)			
Any Event	4	280	143	83	1107	750			
SVR	---	---	---	---	---	---	0.20 (0.07-0.55)	0.20 (0.07-0.58)	0.003
Age	---	---	---	---	---	---	1.05 (1.03-1.08)	1.57 (1.20-2.06)	0.001
Male sex	---	---	---	---	---	---	1.32 (0.83-2.13)	1.71 (0.99-2.93)	0.052
Previous non-response	---	---	---	---	---	---	0.93 (0.57-1.53)	0.51 (0.23-1.11)	0.090
Treatment duration	---	---	---	---	---	---	0.99 (0.98-1.01)	*	
Peginterferon	---	---	---	---	---	---	0.45 (0.24-0.86)	*	
Ribavirin	---	---	---	---	---	---	1.56 (0.93-2.63)	*	
Fibrosis stage	---	---	---	---	---	---	1.96 (1.40-2.74)	*	
Genotype 1	---	---	---	---	---	---	1.13 (0.63-2.02)	*	
Bilirubin	---	---	---	---	---	---	2.42 (1.77-3.32)	1.33 (0.98-1.80)	0.069
Albumin	---	---	---	---	---	---	0.01 (0.00-0.08)	0.61 (0.27-1.38)	0.228
Platelet count	---	---	---	---	---	---	0.11 (0.05-0.23)	0.90 (0.85-0.96)	0.001
Viral load	---	---	---	---	---	---	1.08 (0.70-1.66)	*	
Center 1 (ref)	---	---	---	---	---	---	1.00	1.00	
Center 2	---	---	---	---	---	---	3.07 (1.67-5.64)	4.97 (2.03-12.2)	0.001
Center 3	---	---	---	---	---	---	3.07 (1.60-5.91)	1.56 (0.71-3.41)	0.269
Center 4	---	---	---	---	---	---	1.78 (1.02-3.11)	1.00 (0.54-1.84)	0.994
Center 5	---	---	---	---	---	---	0.66 (0.16-2.74)	1.13 (0.24-5.45)	0.875
Treatment Period	---	---	---	---	---	---	1.40 (0.83-2.34)		

tion. There was a trend towards a decreased incidence of liver-related death of patients with SVR compared to non-responders at 5 years (figure 1d), and SVR was associated with a reduction in the hazard of liver-related mortality (adjusted HR 0.19; CI 0.02-1.44, p=0.107) (table 2).

Liver failure:

None of the patients with SVR developed signs of liver failure during follow-up. Among the non-responders 42 patients developed liver failure. There was a decreased incidence of liver failure among patients with SVR compared to non-responders at 5 years (figure 1e), and SVR was associated with a reduction in the hazard of liver failure (unadjusted HR 0.03; CI 0.00-0.91) (table 2). The effect of SVR on liver failure could not be quantified in multivariate Cox regression analysis, as none of the patients with SVR developed liver failure during follow-up.

Hepatocellular carcinoma:

Three patients with SVR and 32 non-responders developed HCC. Twenty-two cases (63%) had a histological diagnosis. In all but one of these cases, HCC was confirmed in the explant after transplantation. The one patient whose HCC was not confirmed in the explant had had ethanol injection of the tumor prior to transplantation.

The three sustained virological responders with HCC were still serum HCV-RNA-negative at the time of HCC diagnosis. Baseline radiologic imaging was available for all three patients and did not show any signs of HCC and pre-treatment alpha fetoprotein levels were within the normal range. One patient developed HCC 1.7 years after achieving SVR and two developed HCC 3.9 years after achieving SVR. Although SVR was associated with a reduction in the hazard of HCC (adjusted HR 0.46; 0.12-1.70, p=0.245) (table 2), the proportion of patients developing HCC at 5 years was not statistically significantly different between response groups (figure 1f).

The analysis for the risk of HCC development was stratified for serum anti-hepatitis B core antigen status, since anti-hepatitis B core antigen positivity was a risk factor for HCC (overall HR 2.24; CI 1.08-4.62), yet it was not proportional over time. The effect of anti-hepatitis B core antigen positivity decreased over time, (the first 2.5 year the effect was HR 4.21; CI 1.25- 14.2, and after 2.5 year of follow-up the effect was HR 1.61; CI 0.68-3.80).

DISCUSSION

This study shows that in a Western population with chronic hepatitis C and advanced fibrosis, SVR to antiviral therapy leads to a reduction in complications of liver disease. In contrast to studies from Japan, where the benefit of interferon treatment lies mainly in the prevention of development of hepatocellular carcinoma (12, 13), we showed that in our population there is a major reduction in the development of liver failure.

This is an important finding since the incidence of liver failure among untreated cirrhotics in Europe has been estimated to be four times as high as the incidence of HCC (14). Although it should be noted that patients with more severe liver disease are more likely to be both non-responders and to have subsequent decompensation, the observation that in a Western population there is a long-term clinical benefit for patients with an SVR to therapy may alter the attitude towards screening of persons at risk for hepatitis C infection. Until now, the lack of data on long-term outcomes after treatment has been one of the main reasons that such a screening program has not been implemented in the United States (15).

In our study population, the decrease in incidence of HCC was not statistically significant and less pronounced than the decrease in incidence of liver failure. Among 142 sustained virological responders, there were still 3 patients who developed HCC.

A recent report from Italy has shown that the incidence of HCC was 0.7% per year among sustained virological responders with cirrhosis (16). This is lower than the 5-year incidence of HCC of 9.2% found in our study, corresponding to an annual incidence of 1.8%. In a previous study we found no HCC among sustained virological responders followed for up to 4.9 years, but this cohort included a relatively small number of patients with advanced fibrosis: 53 patients had severe fibrosis (F3 according to the METAVIR fibrosis scoring system (17)) and 15 had cirrhosis (18). Although we observed no statistically significant reduction in the development of HCC among sustained responders compared to non-responders in the present study, most of the HCCs occurred among the non-responders. If the follow-up time had been longer, the differences in HCC development between sustained virological responders and non-responders might well have been more pronounced. The difference between sustained virological responders and non-sustained responders might also have been larger if we would have excluded relapsers from the study. Since relapsers had an undetectable viral load while being under treatment, their time towards complications may be somewhat prolonged compared to true non-responders.

In two other previous reports in the literature on Western patients who developed HCC after achieving SVR, both patients had cirrhosis (19, 20). This may imply that with further progression of fibrosis the liver may undergo irreversible changes, leading to an elevated risk of carcinogenesis, even when the original noxious factor has been removed. Another explanation may be that there are other risk factors present in these patients. Of the three sustained virological responders who developed HCC in our cohort, two were diabetics and anti-hepatitis B core antigen-positive. Both of these factors have previously been suggested to cause an increase in the risk for HCC (21), although the effect of anti-hepatitis B core antigen positivity on development of HCC remains controversial (22-24). Finally, another reason that HCC occurs in sustained virological responders may be that a small, yet undetectable HCC was already present before SVR was achieved.

Previous Japanese studies reported a yearly incidence of HCC of 0.7 to 2.5% per year among sustained virological responders with advanced fibrosis or cirrhosis (5, 13, 25, 26). The lowest rate was reported by Okanoue et al. who found 4 HCCs among 86 patients with advanced fibrosis (n=82 F3 and n=4 F4 according to the METAVIR fibrosis scoring system(17)) during 6 years of follow-up. The highest rate was reported by Shiratori et al. who described 11 HCCs during 6.8 years of follow-up of 64 sustained virological responders with cirrhosis.

Although we investigated a large cohort of patients, there are limitations to this study. Not all the genotype 1 patients in our retrospective cohort received 48 weeks of treatment, which is nowadays regarded as optimal. Therefore, the SVR rates that we report may be lower than in studies where all genotype 1 patients received 48 weeks of treatment. However, this does not jeopardize our finding that sustained virological responders have an improved clinical outcome compared to non-responders.

Another limitation of the study is that the number of events in the SVR group was scant and that follow-up time was relatively short. This is also reflected by our finding that the effect of SVR was only statistically significant in the combined outcome of “any event”, which may combine potentially disparate effects from the individual outcomes. Due to ongoing improvements in the treatment of chronic hepatitis C, patients who have been treated more recently have a higher chance of SVR than patients who have been treated earlier. Therefore, in this retrospective cohort study it is inevitable that sustained virological responders have a shorter follow-up than non-responders. However, since patients are censored at the time of their last visit in the Cox-regression analysis, a difference in follow-up time would only be relevant if the rate of occurrence of clinical events would change over time, e.g. if the incidence of HCC increases dramatically more than

2 years post-treatment and most of the sustained virological responders do not reach 2 years of follow-up. The Kaplan Meier curves shown in figure 1 contradict this and indicate instead that the rate of occurrence of clinical events is almost linear. Moreover, subgroup analysis of the patients who were treated after 1998 with combination therapy shows that the difference in occurrence of clinical events between sustained virological responders and non-responders remains, while their follow-up time is comparable. We realize that due to the few events in the SVR group, the strength of our analysis also depends on the modeling assumptions, the covariates included and non-informative censoring. Therefore, we carefully selected all relevant covariates to be used in the analysis and verified our findings. The fact that our results did not change when the data were analyzed in a different manner, modeling response to treatment as a time-dependent covariate, strengthens our belief that the data we present are solid.

Unfortunately, there are no samples of the patients treated in our study available for HCV RNA retesting. However, the risk of misclassification of sustained virological responders due to the fact that early polymerase chain reaction tests were less sensitive is very small. Previous studies have shown that when samples are retested with recent assays, there is complete concordance in the end-of-follow up samples (27, 28). The patients in our study are all classified according to their virological response at the end-of-follow up. Therefore, in our opinion, the patients included in our study represent true sustained virological responders.

Finally, the retrospective nature of the study may have led to selection bias. It is likely that some patients with severe cirrhosis were not considered for treatment and were therefore not included in our study. However, despite the fact that this bias could imply that we investigated a group of early cirrhotics, the incidence of clinical events was high enough to show that SVR leads to a decrease in the risk of developing events indicative of liver failure.

In conclusion, SVR to interferon or peginterferon with or without ribavirin is associated with an important reduction in clinical events for patients with chronic hepatitis C and advanced fibrosis.

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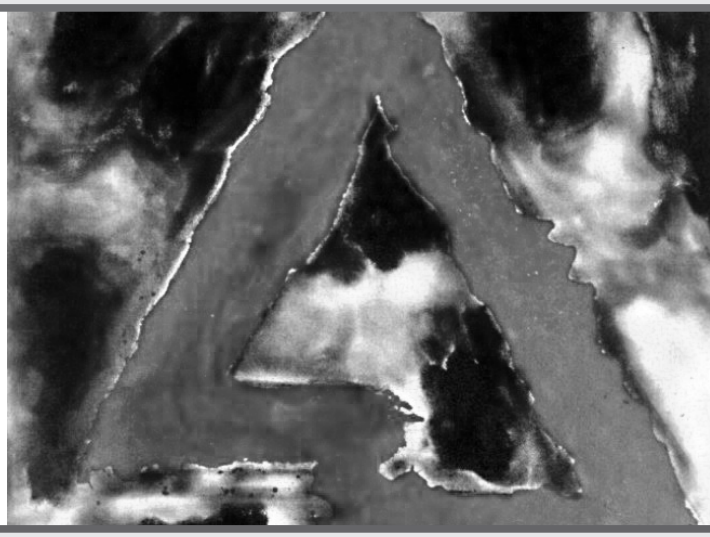
Chapter 8

Impact of pegylated interferon and ribavirin treatment on graft survival in liver transplant patients with recurrent hepatitis C infection



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Submitted to American Journal of Transplantation

ABSTRACT

Background: Recurrent hepatitis C virus (HCV) infection is a major cause of morbidity and mortality after liver transplantation for HCV-related end stage liver disease. Although previous studies have shown a short-term effect of interferon-based treatment on fibrosis progression, it is unclear whether this translates into an improved graft survival.
Aims: To evaluate whether treatment of recurrent HCV leads to an improved graft survival.
Methods: Cohort study including consecutive HCV patients who underwent liver transplantation between 1-1-1995 and 1-1-2005 in the Mayo Clinic, Rochester.
Results: Two hundred and fifteen patients were included in the study. During a median follow-up of 4.4 years (inter quartile range 2.2-6.6) 165 patients (77%) had biopsy-proven recurrent HCV infection, confirmed by serum HCV ribonucleic acid testing. Initiation of treatment depended on patient and physician preference and 78 patients were treated. Importantly, there were no differences in MELD-score, fibrosis stage or time towards HCV recurrence between treated and untreated patients at time of recurrence. The incidence of graft failure was lower for patients treated within 6 months of recurrence compared to patients not treated within this time-period (log rank p=0.002). Time-dependent multivariate Cox regression analysis showed that treatment of recurrent HCV infection was statistically significantly associated with a decreased risk of overall graft failure (Hazard Ratio 0.34; CI 0.15-0.77, p=0.009) and a decreased risk of graft failure due to recurrent HCV (Hazard Ratio 0.24; CI 0.08-0.69, p=0.008).
In conclusion, treatment of recurrent HCV infection after liver transplantation is associated with a reduced risk of graft failure.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is the most common indication for liver transplantation in the United States and Europe (1). Recurrence of HCV infection after transplantation is a serious cause of morbidity and mortality, resulting in death, graft loss or cirrhosis in one third of HCV-infected recipients by the fifth postoperative year (2). Although treatment with interferon or peginterferon and ribavirin is feasible in the post-transplant setting, sustained virological response (SVR) rates are lower than in non-transplant patients (3).

Two randomized controlled trials have reported the histological effects of antiviral therapy on treated and untreated recipients with biopsy proven recurrence of HCV infection (4, 5). Both studies noted improvement in fibrosis score among treated patients. In addition, Bizollon et al. found that sustained virological responders treated for post-transplant recurrent HCV infection experienced a lower incidence of cirrhosis during long-term follow-up than non-responders (6). It is, however, unknown whether antiviral therapy for recurrence of HCV infection confers improved graft survival.

As the relative contributions of direct and immune mediated graft injury to the course of posttransplant graft survival are not known, untill now antiviral treatment is typically offered to patients with clear evidence of HCV-mediated graft injury. However, with the current lack of scientific evidence on the optimal timing of antiviral treatment after liver transplantation for chronic hepatitis C, some physicians use a lower threshold for treatment than others. Moreover, patient related factors such as recovery from post-transplant complications and psychological status may play a role in the decision to start treatment.

The aim of this study is to assess whether antiviral treatment improves graft survival in patients with recurrent hepatitis C after transplantation, using a minimal definition of HCV recurrence.

Methods

Design: Single center, retrospective cohort study.

Participants: All consecutive chronic hepatitis C patients who underwent liver transplantation between 1-1-1995 and 1-1-2005 in the Mayo Clinic in Rochester, Minnesota. Both patients receiving their first graft and patients who underwent retransplantation could enter the study.

The study protocol was approved by the Institutional Review Board of the Mayo Clinic and was carried out in accordance with institutional guidelines. Patients gave informed consent for their data to be used in the study.

Data assembly:

Data were obtained on patient demographics (gender, ethnicity, age at transplantation), anthropomorphics (height, weight), donor demographics (donor age) and transplant procedure (cold and warm ischemic time). The body mass index (BMI) was calculated as weight divided by height squared.

Treatment characteristics (peginterferon / standard interferon, ribavirin, dose reductions, use of erythropoietin, treatment duration and response to treatment) were documented.

SVR is defined as undetectable serum HCV ribonucleic acid (RNA) at 24 weeks after the end-of-treatment.

Analytical procedures:

Virological data (genotype, viral load), biochemical data (creatinine, bilirubin, glucose, sodium, AST, ALT, gamma GT, cholesterol, triglycerides) and hematological data (hemoglobin, platelet count, INR) were measured in the certified Mayo Clinic laboratories. The Model for End-Stage Liver Disease (MELD)-score was calculated as previously described (3.8*log(bilirubin) + 11.2*log(INR) + 9.57*log(creatinine) + 6.43) (9).

Follow-up:

Re-transplantation and mortality were recorded. Graft failure was defined as occurring at the time of re-transplantation or death.

HCV Recurrence and Allograft Histology:

Liver biopsies were routinely performed at 1, 3 and 5 years after transplantation and when clinically indicated. A minimal definition of recurrence of HCV infection was applied. HCV recurrence required allograft histology showing lymphocytic infiltrates suggestive of recurrent HCV infection as determined by an experienced pathologist (absence of other Banff criteria for acute cellular rejection, i.e. absence of endotheliitis and cholangitis), confirmed by the presence of HCV RNA in the serum (positive qualitative HCV RNA test or quantitative HCV RNA test showing $>10^3$ IU/mL).

Statistics:

Baseline characteristics were compared using Mann Whitney and $\chi 2$ tests.

The Kaplan-Meier method was used to assess overall graft survival and to estimate the effect of treatment on graft survival after recurrence. In the latter analysis, but not in the Cox regression analysis, patients who had severe recurrence resulting in graft failure or death within 6 months after recurrence were

excluded by using 6 months after recurrence as time 0. Patients were classified according to history of treatment at 6 months to avoid survivor-treatment bias (10). Groups were compared using log-rank tests.

Univariate Cox regression analyses and backward and forward stepwise analyses were applied to build a multivariate proportional hazards model assessing risk factors for graft failure. In this analysis, which was performed on an intention-to-treat basis, treatment was modeled as a time-dependent covariate to represent the ability of patients undergoing treatment during follow-up to change their status from "untreated" to "treated". Several models were fit, the final model including the covariates with the best fit to the data, according to Chi-square test (11). The following factors were considered: gender, age, donor age, treatment, diabetes mellitus, BMI, MELD-score, fibrosis stage, genotype, platelet count, INR, AST, bilirubin, creatinine, albumin, ALT, cholesterol, triglycerides, HCV RNA, warm ischemia, cold ischemia. Importantly, in all models treatment was consistently significantly associated with decreased graft failure.

The final model for overall graft failure that provided the best fit to the data included the following covariates: albumin, MELD-score, BMI, platelet count and treatment. The final model for graft failure due to hepatitis C included the following covariates: albumin, MELD-score and treatment. The reported hazard ratios are the relative increases in hazard associated with increases of 10 g/dL for the covariate albumin and 10 years for donor age. Since the definition of SVR is undetectable serum HCV-RNA by sensitive molecular tests at 24 weeks after the end-of-treatment, we used this time point as time 0 for classifying response versus non-response. The results are reported as hazard ratios with 95% confidence intervals.

RESULTS

Study population: Between 1-1-1995 and 1-1-2005, 220 liver transplantations were performed. Five patients did not give informed consent and the study thus describes 215 liver transplantations. One hundred seventy-eight patients (83%) had a complete follow-up until 1 December 2006, 6 months prior to closure of the database.

During a median follow-up of 4.4 years (inter quartile range (IQR) 2.2-6.6) 165 patients (77%) had evidence of recurrent HCV infection, the median time from OLT to diagnosis of recurrence being 1.0 year (IQR 0.3-2.2). Twenty-four patients were diagnosed with recurrent HCV after protocolled biopsy at 1 year after OLT, 12 after protocolled biopsy at 3 years after OLT and 2 patients after protocolled

Table 1: Baseline characteristics at time of recurrent hepatitis C virus (HCV) infection [§].

	Overall	Treated	Not treated	p-value Mann Whitney / χ^2
Number of patients	165	78	87	
Male gender (%)	104 (63)	58 (74)	46 (53)	0.004
Age, years	52 (47-59)	52 (48-58)	51 (46-61)	0.85
Donorage, years	46 (30-59)	45 (28-54)	48 (31-61)	0.088
Diabetes mellitus (%)	35 (21)	16 (21)	19 (22)	0.84
Body mass index, kg/m^2	27 (23-31)	27 (24-30)	27 (23-32)	0.96
MELD-score [¶]	7.5 (6.6-9.2)	7.6 (6.7-8.8)	7.5 (6.4-9.5)	0.92
Fibrosis stage (%)				0.82
0	94 (57)	43 (55)	51 (65)	
1	30 (18)	15 (19)	15 (17)	
2	23 (14)	10 (13)	13 (15)	
3	15 (9)	9 (12)	6 (7)	
4	3 (2)	1 (1)	2 (2)	
Genotype (%)				0.79
1	99 (77)	53 (74)	46 (81)	
2	15 (12)	10 (14)	5 (9)	
3	10 (8)	6 (8)	4 (7)	
4	5 (4)	3 (4)	2 (4)	
Hemoglobin, g/dL	13 (11-14)	13 (11-14)	12 (11-14)	0.88
Platelet count, ×10^{9}cells/L	132 (103-177)	131 (107-166)	139 (100-185)	0.63
International normalized ratio	1.0 (0.9-1.1)	1.0 (0.9-1.1)	1.0 (0.9-1.1)	0.63
Sodium, mmol/L	140 (138-142)	140 (139-142)	140 (136-142)	0.109
Glucose, mg/dL	111 (97-136)	117 (98-136)	106 (95-136)	0.35
Aspartate aminotransferase, U/L	112 (42-227)	113 (47-222)	111 (40-230)	0.55
Bilirubin, mg/dL	1.1 (0.7-1.9)	1.0 (0.7-1.7)	1.1 (0.7-2.9)	0.47
Creatinine, mg/dL	1.3 (1.1-1.6)	1.3 (1.1-1.6)	1.3 (1.0-1.6)	0.53
Albumin, g/dL	3.8 (3.4-4.1)	3.9 (3.6-4.2)	3.6 (3.2-4.0)	0.001
Alanine aminotransferase, U/L	123 (55-262)	152 (68-289)	110 (49-241)	0.118
Cholesterol, mg/dL	152 (128-178)	151 (122-173)	152 (134-182)	0.53
Triglycerides, mg/dL	139 (105-203)	137 (98-193)	142 (108-208)	0.37
HCV ribonucleic acid (×10^6 IU/ml)	3.2 (0.5-7.7)	4.9 (1.1-7.7)	2.3 (0.5-6.5)	0.106
Warm ischemia, min	44 (33-59)	48 (37-60)	41 (31-57)	0.070
Cold ischemia, min	449 (380-513)	450 (391-513)	445 (364-513)	0.381
Interval between liver transplantation and HCV recurrence, years	1.0 (0.3-2.5)	1.0 (0.3-2.4)	1.0 (0.3-2.7)	0.61
Pre-transplant HCC [¥] (%)	45 (27)	23 (29)	22 (23)	0.55
Pre-transplant alcohol abuse (%)	55 (33)	24 (31)	31 (36)	0.51
Immunosuppression:				0.159
Tacrolimus	139 (84)	69 (88)	70 (80)	
Cyclosporine	26 (16)	9 (12)	17 (20)	

[§] Continuous variables are expressed as medians (inter quartile range).

[¶] MELD = Mayo Model for End-Stage Liver Disease

[¥] HCC = Hepatocellular carcinoma

biopsy at 5 years after OLT. The remaining 127 patients were diagnosed after biopsy for clinical indications such as elevated liver enzymes. There were no differences in occurrence of graft failure among patients diagnosed with recurrent HCV after protocolled biopsy or after biopsy for elevated liver enzymes (5-year occurrence 82%; CI 68-97 versus 73%; CI 62-85 for protocolled versus not protocolled respectively, Log Rank p=0.42. Of the 50 patients without proven recurrence, 10 were treated before transplantation and were HCV RNA negative, 21 had no signs of recurrence on either biopsy or by HCV RNA testing and 19 had signs of recurrence on either biopsy (n=8) or by HCV RNA testing (n=11), but not on both tests.

Treatment was initiated in 78 patients; 17 patients were treated with standard interferon and 61 patients were treated with pegylated interferon. Sixty-nine patients (88%) received combination therapy with ribavirin. Dose reductions were needed in 57 patients (73%) and 39 patients (50%) needed erythropoietin treatment to maintain hemoglobin levels. The median duration of treatment was 46 weeks (IQR 27-57).

Twenty-six patients (34%) achieved SVR and 45 patients (58%) did not acieve SVR: 40 patients were non-responders and 5 patients had an end-of-treatment response but relapsed. The remaining 7 patients could not be evaluated for SVR: 2 completed their therapy in another center, 4 died before they reached the end-of-follow-up and 1 was treated recently and had not reached end-of-follow-up yet.

Table 1 shows the baseline characteristics at the time of recurrence for treated and untreated patients. Although treated patients had higher serum albumin levels at baseline, there were no differences in severity of recurrence as determined by MELD-score or fibrosis stage. Also, the time between liver transplantation and diagnosis of recurrence was similar for treated compared to untreated patients (p=0.61, table 1). Eleven out of 87 untreated patients and 10/78 treated patients developed CMV infection post OLT (p=0.99). Twelve patients who received treatment for recurrent HCV infection and 24 untreated patients had received high dose prednisone treatment for rejection (800 mg on 3 alternating days) in the first month after OLT (p=0.058).

In order to test the hypothesis that treated patients could have been selected based upon their compliance, we compared the mean serum levels of tacrolimus and cyclosporine which were monitored closely during the first month after transplantation, between treated and untreated patients as a measure for previous treatment compliance. No statistically significant differences were found in mean serum levels of tacrolimus and cyclosporine and their standard deviations (SD). The mean tacrolimus levels were 9.9 ng/mL (SD 2.8) in treated versus 9.6 ng/mL

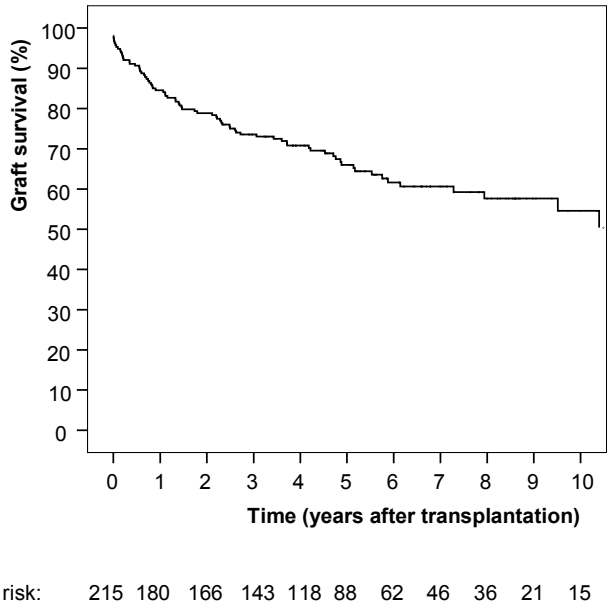


Figure 1: Kaplan Meier analysis showing overall graft survival after transplantation.

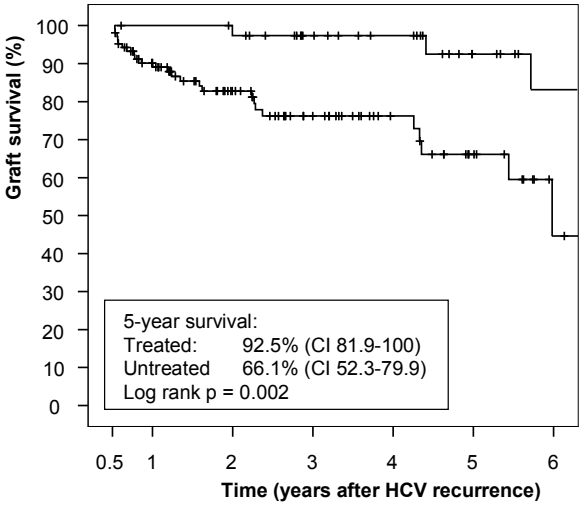
(SD 2.4) in untreated patients (p=0.59). The mean cyclosporine levels were 240 ng/mL (SD 103) in treated patients versus 196 ng/mL (SD 87) in untreated patients (p=0.29).

Effect of treatment on survival:

Figure 1 shows the overall graft survival of all patients included in the study. Seventy-eight patients were treated and 87 patients remained untreated, despite of HCV recurrence. The median time between recurrence of HCV infection and start of interferon-based treatment was 0.23 years (IQR 0.02-1.5).

Re-transplantation or death due to recurrent HCV infection occurred in 5 treated patients (6.4%) and in 22 untreated patients (25%), while the occurrence of graft failure due to other causes was similar in these two groups (table 2).

Figure 2 shows that overall graft survival was longer for patients treated within 6 months of HCV recurrence compared to patients with recurrent HCV infection not treated within this time-period (log rank p=0.002). Time-dependent multivariate Cox regression analysis showed that treatment of recurrent HCV was statistically significantly associated with a decreased risk of graft failure due to recurrent HCV (HR 0.24; CI 0.08-0.69, p=0.008) (table 2) as was serum albumin (HR 0.11; CI 0.04-0.29, p<0.001). Higher MELD-scores at the time of recurrence were associated with a higher risk of graft failure due to recurrent HCV (HR 1.31; CI 1.14-1.50, p<0.001).



At risk:							
Treated:	40	39	37	29	24	14	9
Events:	0	0	1	1	1	2	3
Untreated:	105	83	55	36	23	13	3
Events:	0	10	16	20	20	23	25

Figure 2: Kaplan Meier analysis showing graft survival in treated versus untreated patients with hepatitis C virus (HCV) recurrence. In this analysis, 6 months after recurrence is used as time 0 and patients are classified according to history of treatment at 6 months. Forty of 78 treated patients were treated within 6 months of HCV recurrence.

Table 2. Causes of graft failure among patients with recurrent hepatitis C virus (HCV) infection [§] (n=165):

Cause	Treated (n=78)	Untreated (n=87)
Liver related (overall) (%)	5 (6.4)	27 (31)
Liver-related (specified) (%)		
Recurrent HCV (%)	*5 (6.4)*	*22 (25)*
HCC (%)	*0 (0)*	*2 (2.3)*
Other liver-related ¶ (%)	*0 (0)*	*3 (3.4)*
Non-liver-related (overall) (%)	7 (9.0)	13 (15)
Non-liver-related (specified) (%)		
Sepsis (%)	*3 (3.8)*	*7 (8.0)*
Cardiac (%)	*0 (0)*	*1 (1.1)*
Renal (%)	*1 (1.3)*	*0 (0)*
Accident (%)	*1 (1.3)*	*1 (1.1)*
Non-liver malignancies (%)	*1 (1.3)*	*3 (3.4)*
Other / unknown (%)	*1 (1.3)*	*1 (1.1)*

[§] As proven by liver biopsy and confirmed by HCV ribonucleic acid testing

[¶] Includes hepatorenal syndrome and hepatic artery thrombosis.

Table 3. Graft survival by treatment status [§].

	Treated			Untreated			Unadjusted Hazard Ratio	95% confidence interval	Unadjusted p-value	Adjusted Hazard Ratio	95% confidence interval	Adjusted p-value
	Events	Patient years	Events / 10.000 patient years	Events	Patient years	Events / 10.000 patient years						
Overall Graft Failure	12	192	625	40	364	1099	0.39	0.19-0.78	0.008	0.34 [¶]	0.15-0.77	0.009
Graft Failure due to Recurrent Hepatitis C	5	192	260	22	364	604	0.23	0.08-0.63	0.005	0.24 [¥]	0.08-0.69	0.008

[§] In all Cox regression analyses treatment was modeled as a time-dependent covariate.

[¶] Adjusted for albumin levels, Mayo Model for End-Stage Liver Disease (MELD)-score, body mass index and platelet count.

[¥] Adjusted for albumin levels and MELD-score (see also *Statistics*).

Treatment of recurrent HCV infection (HR 0.34; CI 0.15-0.77, p=0.009), serum albumin levels (HR 0.14; CI 0.07-0.26, p<0.001) and BMI (HR 0.93; CI 0.89-0.97, p=0.001) were statistically significantly associated with a decreased probability of overall graft failure. The following factors were associated with an increased probability of overall graft failure: MELD-score (HR 1.23; CI 1.12-1.36, p<0.001) and platelet count (1.01; CI 1.00-1.01, p=0.030).

We performed an additional analysis in which patients who were retransplanted were censored at the time of retransplantation (n=17), instead of being re-included in the analysis. The effects of treatment on overall graft failure (HR 0.39; CI 0.18-0.88, p=0.023) and on graft failure due to recurrent HCV (HR 0.27; CI 0.09-0.86, p=0.027) remained essentially unchanged.

DISCUSSION

The effectiveness of treatment for chronic HCV infection is usually evaluated by the number of patients achieving SVR, defined by HCV RNA which is persistently undetectable for at least six months following completion of antiviral therapy (12). Although SVR has been shown to be associated with an improved clinical outcome in non-transplant patients, liver transplant recipients have more complex considerations, including the potential effects of chronic activation of the interferon stimulated response element through exogenous interferon administration. As the relative contributions of direct (cytopathic) and immune mediated graft injury to the course of posttransplant graft failure are not known, antiviral treatment is typically offered to patients with clear evidence of HCV-mediated graft injury. In order to demonstrate a beneficial effect of HCV treatment on survival, ideally, a cohort of treated patients would be compared to a similar but untreated control group.

Because of the limited success of interferon-based treatment in the posttransplant setting, the initiation of treatment for recurrent HCV infection at our center has depended on patient and physician preference over the past decade, more than on disease severity. A prospective protocol for obtaining liver biopsies together with a minimalist definition for recurrence of HCV, provided us with a unique opportunity to assess the effect of HCV treatment on graft survival, studying comparable groups of treated and untreated patients. Importantly, our study shows for the first time that treatment of recurrent HCV infection is independently associated with an improved graft survival.

Several smaller studies have previously reported a probability of improvement in fibrosis score after treatment varying between 0 and 31% (6, 13-17). A

recent study by Carrion et al. showed that regression of fibrosis after treatment for recurrent HCV infection goes along with an improvement in portal pressure, as measured by hepatic venous pressure gradient, which may contribute to the effect of treatment on graft survival (4).

A study by Bizolon et al., including 25 treated patients with paired liver biopsies and 21 untreated controls, suggests that improvement in fibrosis score does not necessarily depend on achievement of SVR (19). This means that there likely is some benefit of treatment, even if no SVR is achieved. However, SVR remains an important prognostic indicator. Picciotto et al. showed in univariate analysis that survival was better for sustained virologic responders than for non-responders after treatment of recurrent HCV (8).

Our study has several limitations. The non-randomized nature of the study may have led to selection bias. Patients with severe recurrence may not have been eligible for treatment, because they were too sick, or in contrast, more severe recurrence may have been an indication for treatment. However, the MELD-score and the fibrosis stage at the time of recurrence were similar for both groups, suggesting that the severity of recurrence was similar for both treated and untreated patients. Furthermore, when we started the Kaplan Meier survival analyses at 6 months after recurrence, thus excluding patients in whom severe recurrence led to graft failure or mortality before treatment could be initiated, the difference in survival between treated and untreated patients was essentially unchanged and remained statistically significant.

Although we corrected in multivariable analysis for baseline factors shown to influence survival, the result of non-randomized studies such as this might still be influenced by unmeasured variables (20). Since most of the patients were referred from other centers, we did not have reliable data on previous interferon-based treatment before transplantation. Thus, patients in the untreated group may have been selected for previous non-response. Non-responders to interferon-based treatment are usually characterized by a higher proportion of genotype 1, male gender, higher fibrosis stage and higher viral load. Notably, there was no difference between these variables for the treated compared to the untreated patients at the time of recurrence, except that male gender was more prevalent in the treated group. This suggests, but does not prove, that previous non-response was not more frequent in the untreated group. Another potential source of selection bias may be patient compliance. It is possible that those patients who were presumed to be most compliant, were more likely to be treated. However, the serum levels of immunosuppressive medication over the first month and their standard deviations were similar for treated and untreated patients, suggesting that they were at least equally compliant in taking their immunosuppressive medication.

Moreover, all liver transplant patients undergo a thorough pre-transplant evaluation and are only eligible for transplantation if they are compliant and adherent at that time. One of the strengths of our study is that our study cohort is larger than most studies among liver transplant patients with hepatitis C, which likely contributes to the reliability of the results.

In conclusion, our study suggests that treatment of recurrent HCV infection after liver transplantation is associated with improved graft survival, when a minimal definition of recurrence is used. Future randomized studies are needed to confirm our observations.

ACKNOWLEDGEMENTS

BJV received a stipend from the Netherlands Organisation for Health Research and Development (ZonMw, project number 1900120283) for his work as a clinical research trainee. During his stay at the Mayo Clinic in Rochester, Minnesota he was also supported by a travel grant from the Trustfund of the Erasmus University Rotterdam, the Netherlands (grant number 97030.10/07.0275).

The sponsors did not have any influence on the design and conduct of the study; nor on collection, management, analysis and interpretation of the data; nor on preparation, review or approval of the manuscript.

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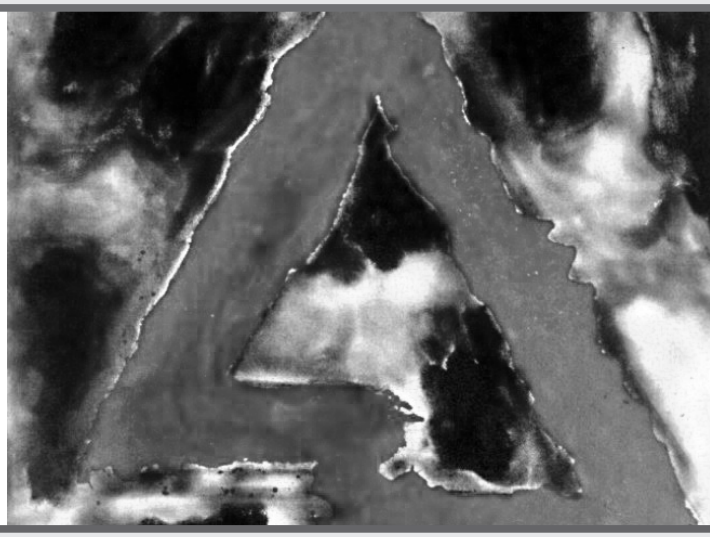
Chapter 9

Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus



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Hepatology 2008 in press

ABSTRACT:

Recent studies suggest that diabetes mellitus increases the risk of developing hepatocellular carcinoma (HCC). The aim of this study is to quantify the risk of HCC among patients with both diabetes mellitus and hepatitis C in a large cohort of patients with chronic hepatitis C and advanced fibrosis.

We included 541 patients of whom 85 (16%) had diabetes mellitus. The median age at inclusion was 50 years. The prevalence of diabetes mellitus was 10.5% for patients with Ishak fibrosis score 4, 12.5% for Ishak-score 5 and 19.1% for Ishak-score 6. Multiple logistic regression analysis showed an increased risk of diabetes mellitus for patients with an elevated body mass index (Odds ratio (OR) 1.05; 95% confidence interval 1.00-1.11, p=0.060) and a decreased risk of diabetes mellitus for patients with higher serum albumin levels (OR 0.81; 95% confidence interval 0.63-1.04, p=0.095). During a median follow-up of 4.0 years (inter quartile range 2.0-6.7), 11 patients (13%) with diabetes mellitus versus 27 patients (5.9%) without diabetes mellitus developed HCC, the 5-year occurrence of HCC being 11.4% (95% confidence interval 3.0-19.8) and 5.0% (95% confidence interval 2.2-7.8), respectively (p=0.013). Multivariate Cox regression analysis of patients with Ishak 6 cirrhosis showed that diabetes mellitus was independently associated with the development of HCC (Hazard ratio 3.28; 95% confidence interval 1.35-7.97, p=0.009).

Conclusion: For patients with chronic hepatitis C and advanced cirrhosis, diabetes mellitus increases the risk of developing HCC.

BACKGROUND:

Recent epidemiological studies suggest that the presence of diabetes mellitus increases the risk of hepatocellular carcinoma (HCC) (1, 2). An explanation for this association may be that diabetes often occurs as part of the metabolic syndrome, which increases the risk of non-alcoholic steatohepatitis (NASH) and that HCC can be a late complication of NASH (3).

Diabetes mellitus is more prevalent among patients with chronic hepatitis C than in the general population (4). Liver disease contributes to insulin resistance as it leads to a progressive impairment of insulin secretion and it induces hepatic insulin resistance (5). Studies in transgenic mouse models which harbored the hepatitis C core gene have shown that hepatic insulin resistance may be caused by elevated levels of tumor necrosis factor-alpha, which disturbs the tyrosine phosphorylation of insulin receptor substrate-1 (6).

Chronic hepatitis C virus infection itself also increases the risk of HCC. It leads to chronic inflammation of the liver, to liver fibrosis and it may eventually progress to cirrhosis. For patients with hepatitis C cirrhosis the risk for development of HCC is 0.54 – 2.0% per year (7, 8).

Therefore, among patients with both chronic hepatitis C and diabetes mellitus there are two potential ways in which HCC may develop: by the metabolic pathway and by the carcinogenic effect of the hepatitis C virus.

The aim of this study is to quantify the risk of HCC among patients with both diabetes mellitus and chronic hepatitis C in a large cohort of patients with chronic hepatitis C and advanced fibrosis.

PATIENTS AND METHODS:

All consecutive patients with chronic hepatitis C and biopsy proven advanced fibrosis or cirrhosis (Ishak score 4 to 6), treated between 1990 and 2003 in five large Hepatology units in Europe and Canada, were included in the study. Patients were not eligible if they were infected with hepatitis B (HBsAg positive) or human immunodeficiency virus.

Approval was obtained from the ethics committees of each participating center. Local investigators identified all eligible patients and the principal investigator (BJV) then visited each center to enter the individual patient chart review data in a central database in a standardized and predefined way (9).

Data recorded:

Data were obtained on patient demographics (gender, age), on body weight and height and on the presence of diabetes. Body mass index (BMI) was calculated as weight divided by height squared. Overweight is defined as BMI 25-30 kg/m2, obesity is defined as BMI 30-35 kg/m2 and morbid obesity as BMI >35 kg/m2. For patients of Asian descent we used an adapted scale of BMI 23-27.5 kg/m2 for overweight, BMI 27.5-32.5 kg/m2 for obesity and BMI >32.5 kg/m2 for morbid obesity (10).

Patients with recent alcohol abuse were not eligible for treatment and therefore not included in the study.

We recorded whether patients had any diagnosis of diabetes mellitus at baseline; either by elevated fasting serum glucose (> 6.1 mmol/L) or a positive glucose tolerance test or if patients used antidiabetic medication. Biochemical data (bilirubin, albumin), haematological data (platelet count, prothrombin time) and virological data (HCV genotype and viral load, anti hepatitis B core antigen (HBc) positivity) were measured in the certified laboratories of participating hospitals and were corrected centrally for local normal values. Fibrosis was assessed in liver biopsies scored by local pathologists, who all had a large experience in scoring liver biopsies from patients with viral hepatitis. Finally, details of hepatitis C treatment were recorded (duration of treatment, interferon or pegylated interferon, monotherapy or combination treatment with ribavirin).

Follow-up:

All participating centers had a protocol for follow-up of patients with advanced fibrosis or cirrhosis. Patients with advanced fibrosis were followed up with yearly visits, including physical examination, serum alanine aminotransferase (ALT), HCV ribonucleic acid (RNA) and alpha fetoprotein testing and abdominal ultrasound, computed tomography or magnetic resonance imaging. Patients with cirrhosis (Ishak score 5 or 6) were evaluated every 6 months. The protocol was similar for patients with and without diabetes mellitus. Patients were considered to have a HCC if the diagnosis was histologically confirmed, or if two coincident imaging techniques (ultrasound, computed tomography or magnetic resonance imaging) showed a new focal lesion > 2 cm with arterial hypervascularization, or if one imaging technique showed a new focal lesion > 2 cm with arterial hypervascularization in the presence of an alpha-fetoprotein > 400 ng/mL. For the occurrence of HCC, the date was used when the diagnosis was confirmed by histology or radiography.

If the follow-up was incomplete, the treating physician contacted the patient. In case the patient could not be reached, the treating physician contacted the patient's general practitioner in order to complete the follow-up.

Statistical analysis:

To identify factors associated with the presence of diabetes mellitus and to evaluate whether the presence of diabetes mellitus affects the response to treatment of chronic HCV infection, univariate and multivariate logistic regression analyses were used. All variables were checked for interactions. Results are expressed as odds ratios and their 95% confidence intervals.

The Kaplan Meier method was used to estimate the occurrence of HCC over time. Entry into the study started at the initiation of treatment. Cox regression analysis was applied to determine which factors were independently associated with the development of HCC. The following baseline factors were considered: age, fibrosis stage, diabetes mellitus, genotype, gender, BMI, anti-HBc positivity, bilirubin, albumin and platelet count.

Anti-HBc positivity is a risk factor for HCC, however the hazard of HCC was not proportional over time. Therefore, the analysis for risk of development of HCC was stratified for serum anti-HBc positivity. For covariates other than anti-HBc positivity, the assumption of proportionality was not violated. Multiple collinearity was observed between fibrosis stage and the covariates bilirubin, platelet count and albumin. Both the fibrosis stage and these laboratory values reflect the severity of liver disease. Therefore, two separate models were developed for either inclusion of fibrosis in the model or inclusion of the covariates bilirubin, platelet count and albumin. The model including the covariates bilirubin, platelet count and albumin had the lowest Akaike information criterion (AIC) score, which is a measure of the goodness of fit of a statistical model. The reported hazard ratios are the relative increases in hazard associated with increases of 1 µmol/L bilirubin, 1 g/L albumin, 10^9 cells/L platelets and 1 mmol/L glucose.

In order to assess whether the effect of diabetes mellitus on HCC could be confounded by response to interferon-based treatment, we performed an additional analysis where response to treatment was modeled as a time-dependent covariate to represent the ability of patients undergoing more than one treatment course to change their status from non-responders to responders in sequential courses. Since the definition of sustained virological response is undetectable serum HCV-RNA by sensitive molecular tests at 24 weeks after the end-of-treatment, we used this time point as time 0 for classifying response versus non-response.

Results are reported as relative hazards with 95% confidence intervals.

Since either baseline bilirubin, albumin or platelet count were missing in 28.5% of cases, multiple imputation was used to impute missing values (11, 12): the Markov Chain Monte Carlo method (MCMC) with single chain (PROC MI in SAS) was applied to construct 10 complete datasets. All baseline factors and time related factors and events related to the analyses were entered into the imputation procedure and if necessary transformed to conform to the multivariate normality assumption. Cox analysis (PROC PHREG) or logistic regression analysis (PROC LOGISTIC) was then run on each dataset and the results and inference combined using the PROC MIANALYZE in SAS. All statistical analyses were performed using SAS (version 9.1.3, SAS Institute Inc, Cary, NC, USA).

RESULTS:

Five hundred and forty-one patients were eligible and were included in the study, 85 (16%) of them had a diagnosis of diabetes mellitus at the time of inclusion.

The median age at inclusion was 50 years, the patients with diabetes mellitus being older than those without diabetes (median 51 versus 49 years) (table 1).

Patients with diabetes mellitus had more severe fibrosis, lower mean albumin levels and lower mean platelet counts. The prevalence of diabetes mellitus was 10.5% among patients with an Ishak fibrosis score of 4, 12.5% for Ishak fibrosis score 5 and 19.1% for Ishak fibrosis score 6. Of the 85 patients with a diagnosis of diabetes mellitus, 20 used subcutaneous insulin, 34 used oral antidiabetic medication and 31 had dietary measures only. The mean fasting glucose was 8.3 mmol/l (SD 3.1) for patients on treatment (oral or insulin) and 7.2 mmol/l (SD 2.4) for patients on diet. The prevalence of diabetes mellitus was similar among the European and the Canadian patients enrolled in the study (15.9% versus 15.5%, p=0.91). Seventy-two percent of the patients had a complete follow-up until 1-1-2005, six months prior to the data acquisition.

Effects of Body Mass Index (BMI) and previous alcohol use:

One hundred and forty-four patients without diabetes mellitus (39%) and thirty-two patients with diabetes mellitus (44%) were overweight, fifty-eight (16%) versus eleven (15%) were obese and twenty-two (6%) versus ten (14%) had morbid obesity. Patients with diabetes mellitus tended to have higher median BMIs than patients without diabetes mellitus (28 kg/m2 vs 26 kg/m2), p=0.25 (table 1). Multiple logistic regression analysis showed a strong trend towards a higher risk of diabetes mellitus among patients with an elevated body mass index (BMI) (OR 1.05; 95% CI 0.99 - 1.11, p=0.060), and a trend towards a lower risk

Table 1: Baseline characteristics

	Overall	DM	no DM	p-value (Mann Whitney U / χ^2)
Number of patients	541	85	456	
Age (y) [1]	50 (44-57)	51 (45-60)	49 (44-57)	0.09
Male gender (n (%))	370 (68)	59 (69)	311 (68)	0.83
BMI (kg/m^2)[1]	26 (24-29)	28 (25-31)	26 (23-29)	0.01
Fibrosis:				0.05
Ishak-score 4 (n (%))	134 (25)	14 (17)	119 (26)	
Ishak-score 5 (n (%))	104 (20)	13 (15)	91 (20)	
Ishak-score 6 (n (%))	303 (56)	58 (68)	245 (54)	
Duration of infection (y)[1]	3.7 (1.2-8.0)	3.8 (1.4-7.7)	3.3 (1.1-8.2)	0.95
Bilirubin (µmol/L) [1]	9.9 (7.2-13.6)	9.4 (7.2–13.8)	9.9 (7.2-13.6)	0.90
Albumin (g/L) [1]	41 (38-43)	40 (36-42)	41 (38-43)	0.02
Platelet count (*10^9/L) [1]	156 (117-210)	129 (102-186)	159 (120-213)	<0.01
HCV genotype (n (%))[2]				0.09
1	316 (66)	51 (65)	265 (67)	
2	49 (10)	10 (13)	39 (10)	
3	87 (18)	10 (13)	77 (19)	
4	19 (4)	4 (5)	15 (4)	
Other	5 (1)	3 (4)	2 (1)	
HCV Viral load[1, 4]	7.9 10^{E}5 (3.5 10^{E}5–2.4 10^{E}6)	9.8 10^{E}5 (2.6 10^{E}5–2.8 10^{E}6)	7.2 10^{E}5 (3.7 10^{E}5–2.4 10^{E}6)	0.70
Anti HBc positive (n (%))	152 (28)	22 (27)	130 (29)	0.74

[1]median (inter quartile range)

[2] Genotype was missing in 12% of the patients.

[3] Duration of infection was defined as the time since the first diagnosis of hepatitis C (HCV) and was known in 17 patients with diabetes mellitus (DM) and 69 patients without DM.

[4] Viral load was measured by local hybridization or polymerase chain reaction assays and could be retrieved in 68% of the patients.

of diabetes mellitus for patients with higher serum albumin levels (OR 0.81; 95% CI 0.63 - 1.04, p=0.095) and higher platelet count (OR 0.95; 95% CI 0.88-1.02, p=0.181). The effects of age, gender, fibrosis stage and bilirubin levels were not significant.

Twenty patients had a history of previous alcohol abuse and 1 of them developed HCC. Three patients both had a history of alcohol abuse and had diabetes mellitus, but none of these 3 patients developed HCC. Past alcohol abuse was not associated with development of HCC in univariate Cox regression analysis (HR 0.77; CI 0.11-5.58, p=0.79).

Diabetes Mellitus and Development of HCC: HCC developed more frequently among patients with diabetes mellitus. During a median follow-up of 4.0 years (IQR 2.0-6.7), 11 patients (13%) with diabetes developed HCC, versus 27 patients (5.9%) without diabetes mellitus. Twenty-four cases (65%) had a histological diagnosis.

The median time interval between the last imaging without tumor (ultrasound / CT or MRI) and the confirmation of the diagnosis of HCC was similar for patients with and without diabetes mellitus (0.9 years (IQR 0.1-2.1) versus 0.9 years (IQR 0.5-1.7), respectively (p=0.68). The 5-year occurrence of HCC was 11.4% (95% CI 3.0- 19.8%) for chronic hepatitis C patients with diabetes mellitus and 5.0% (95% CI 2.2 - 7.8%) for patients without diabetes mellitus (p=0.013). Univariate Cox regression analysis showed that there were no statistically significant differences in the risk of developing HCC between patients using insulin or oral antidiabetic medication and patients on diet (HR 1.11; CI 0.32-3.79, p=0.87). Among patients with diabetes mellitus, there was a trend towards a higher risk of HCC as fasting glucose levels increased (HR 1.22; CI 0.98-1.52, p=0.082).

Multivariate Cox regression analysis of the overall study population showed that male gender (HR 2.97; 95% CI 1.20 – 7.39, p=0.019) and older age (HR

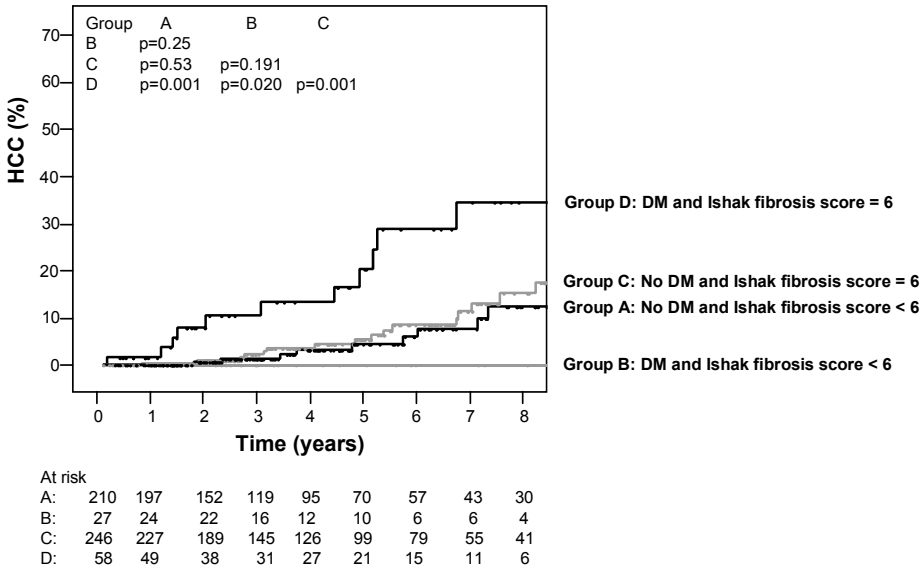

Figure 1: Kaplan-Meier curve showing the occurrence of hepatocellular carcinoma (HCC) over time, according to the presence of diabetes mellitus (DM) and of Ishak fibrosis score 6.
Group A: no DM, Ishak score<6; group B: DM, Ishak score <6; group C: no DM, Ishak score = 6; group D: DM, Ishak score = 6.
P-values (Log Rank) are given for pairwise comparisons between groups.

1.07; 95% CI 1.03 - 1.11, p=0.001) were significantly associated with an elevated risk of developing HCC. In addition, there was a strong trend towards a higher incidence of HCC among patients with diabetes mellitus (HR 2.07; 95% CI 0.95 – 4.47, p=0.066). The associations with BMI, platelet count, bilirubin and albumin levels were not statistically significant.

All eleven diabetic patients who developed HCC had Ishak fibrosis score 6 (figure 1). We performed a subgroup analysis on patients with Ishak fibrosis score 6 (n=303), in which diabetes mellitus was significantly associated with the development of HCC (HR 3.28; 95% CI 1.35 – 7.97, p=0.0087). Other factors predictive of HCC among patients with Ishak fibrosis score 6 were male gender (HR 2.91; 95% CI 1.03 – 8.26, p=0.044) and older age (HR 1.07; 95% CI 1.02 - 1.12, p=0.007) (table 2).

Effect of HCV treatment: All patients were treated for chronic hepatitis C with (peg)interferon with or without ribavirin. Twenty patients with diabetes mellitus (24%) and 139 patients without diabetes mellitus (30%) reached sustained virological response after one or more treatment courses. Logistic regression analysis showed no statistically significant effect of the presence of diabetes mellitus on the chance of responding to HCV treatment, in this population with advanced liver disease (OR 0.70; 95% CI 0.41-1.20, p=0.20).

When response to treatment was entered as a time-dependent covariate in the Cox regression model, it had no statistically significant effect on development

Table 2: Factors associated with the risk of diabetes mellitus.
The relative risks (odds ratios with their 95% confidence intervals) and p-values associated with these factors are given. Fibrosis stage was entered in the model as a categorial variable and the odds ratio gives the relative risk of diabetes mellitus compared to the reference value. BMI and albumin were entered in the model as quantitative variables. The odds ratio gives the odds of DM associated with the increase of one unit in the variable.

	Odds ratio	95% Confidence Interval		p-value
		Lower	Upper	
Age	1.01	0.98	1.04	0.43
Male gender	1.34	0.77	2.32	0.29
Fibrosis				
Ishak 4	1.00			
Ishak 5	1.00	0.44	2.30	0.99
Ishak 6	1.44	0.72	2.90	0.30
BMI	1.05	1.00	1.11	0.06
Bilirubin (xULN)	0.95	0.88	1.03	0.24
Albumin (xLLN)	0.81	0.63	1.04	0.10
Platelet count (xLLN)	0.95	0.88	1.02	0.18

ULN = upper limit of normal
LLN = lower limit of normal

Table 3: Prognostic factors associated with development of hepatocellular carcinoma (HCC) among patients with Ishak fibrosis score 6.
Cox regression analysis showed that diabetes mellitus, male gender and older age were independent prognostic factors associated with the development of HCC. The relative risks (hazard ratios with their 95% confidence intervals) and p-values associated with these factors are given. Age is entered in the model as a quantitative variable.

	Hazard ratio	95% confidence interval		p-value (Log Likelihood)
		Lower	Upper	
Age	1.08	1.03	1.13	< 0.01
Male Gender	2.78	1.04	7.69	0.04
Diabetes Mellitus	2.70	1.24	5.88	0.01
Platelet count (xLLN)	0.44	0.10	1.91	0.27

LLN = lower limit of normal

of HCC (HR 0.54; CI 0.16-1.88, p=0.33). Moreover, after adjusting for response to treatment, the effect of diabetes mellitus on HCC development remained essentially unchanged: HR 1.77; CI 0.77-4.09, p=0.182 for the overall study population and HR 3.35; CI 1.28-8.74, p=0.014 for patients with Ishak fibrosis score 6.

DISCUSSION:

This large cohort study gives, for the first time, a quantification of the risk of developing HCC over time for patients with chronic hepatitis C, advanced fibrosis and diabetes mellitus. The 5-year risk of developing HCC is 11.4% for patients with both diabetes mellitus and chronic hepatitis C with advanced fibrosis. Patients without diabetes mellitus included in our study had a lower risk of HCC with occurrence of HCC in 5.0% after 5 years, which is consistent with the 2.7 to 10% which has been described in the literature for patients with cirrhosis due to hepatitis C (7, 8).

A Japanese study of 279 chronic hepatitis C patients without cirrhosis, showed that the 5-year incidence of HCC was 9.5% for diabetics and 2.0% for non-diabetics (2). Although in a Western population development of HCC is very infrequent among patients without cirrhosis, the rate of diabetics versus non-diabetics developing HCC is similar.

All patients in our cohort have been treated with an interferon-based regimen. A previous study in the same cohort of patients showed that 2 out of 3 sustained virological responders who developed HCC during follow-up had diabetes mel-

litus and this study failed to show a statistically significant effect of treatment response on the risk of developing HCC (9).

In the present study, advanced liver disease itself turned out to be one of the factors determining the risk of diabetes mellitus. This concurs with previous reports, showing that advanced liver disease can lead to insulin resistance, independent of the cause of cirrhosis (13).

In subgroup analysis of patients with Ishak fibrosis score 6, diabetes mellitus was an independent predictor of HCC. Although there probably is a range for true severity of cirrhosis within fibrosis stage 6, diabetes mellitus was still an independent predictor of HCC if we corrected for other markers of advanced liver disease such as platelet count, bilirubin and albumin levels. This suggests that the presence of diabetes mellitus is more than just a marker for more severe liver disease with a greater propensity to progress to HCC (14). Moreover, a large population-based case-control study in the United States has shown that diabetes mellitus was independently associated with a threefold increase of the risk of HCC even for subjects without concomitant disease such as chronic hepatitis C (15).

The effect of diabetes mellitus on HCC development may be partly explained by behavioral factors such as alcohol use, tobacco smoking and high fat diet (16). It is well established that high BMI is a risk factor for diabetes mellitus and steatohepatitis. In addition, tobacco smokers have a 1.5 times increased risk of developing diabetes mellitus (17, 18) and although moderate alcohol consumption may have a protective effect on the development of diabetes mellitus, it clearly is an additional risk factor for steatohepatitis (19).

Alcoholic or non-alcoholic steatohepatitis (NASH) superimposed on chronic inflammation due to hepatitis C may lead to an elevated risk of carcinogenesis. A cohort study in an Asian population of patients with hepatitis C suggested that steatosis is an independent risk factor for development of HCC (20). A recent Australian case-control study found similar amounts of steatosis in patients with chronic hepatitis C who subsequently developed HCC and matched controls. However, the size of this study was limited (21). Among chronic hepatitis C patients, genotype 3 is most commonly associated with liver steatosis (22). In our cohort we found no effect of hepatitis C genotype 3 on the risk of HCC (table 1). Furthermore, the prevalence of obesity was high, both among patients with and without diabetes mellitus, suggesting that obesity is frequent among all chronic hepatitis C patients with advanced fibrosis.

Unfortunately we did not have data about tobacco use in our study population. Our cohort includes patients treated for HCV and because recent alcohol abuse

was a contra-indication for treatment, no patients with active alcohol abuse were included. We found no increased risk of HCC among patients with a past history of alcohol abuse.

Interestingly, among patients with diabetes mellitus, there was a trend towards a higher risk of HCC as fasting glucose levels increased. Higher fasting glucose levels are associated with higher compensatory insulin levels in patients with diabetes mellitus (23) and since previous in vitro studies have shown that hyperinsulinaemia enhances the proliferation of human hepatocellular cancer cells (24, 25), we hypothesize that the presence of hyperinsulinemia might further explain the increased risk of HCC among patients with diabetes mellitus.

In conclusion, for patients with chronic hepatitis C and cirrhosis, diabetes mellitus increases the risk of developing HCC.

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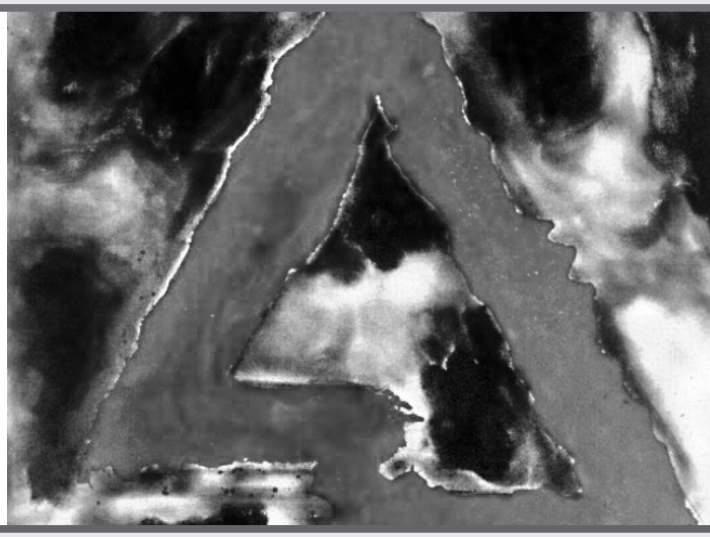
Chapter 10

Randomized placebo controlled phase I/II trial of α-galactosylceramide for the treatment of chronic hepatitis C



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ClinicalTrials.gov Identifier: NCT00352235

J Hepatol. 2007 Sep;47(3):356-65.

ABSTRACT

Background/Aims: The glycosphingolipid α-galactosylceramide has been shown to activate invariant natural killer T cells when presented in the context of CD1d and induces powerful antiviral immune responses via the production of inflammatory cytokines, including interferons and tumour-necrosis-factor α.

The aim of this study was to investigate the safety and the antiviral activity of α-galactosylceramide as a novel class of treatment for chronic hepatitis C patients.

Methods: International multicentre dose-escalating randomized placebo-controlled phase I/II trial.

Results: Fourty patients were allocated to a dose of 0.1 μg/kg(n=9), 1 μg/kg(n=9), 10 μg/kg(n=11) or to placebo(n=11). α-Galactosylceramide was well tolerated and no patients were withdrawn because of side-effects. Although most patients showed a decrease in invariant natural killer T cells after administration, no clinically relevant suppression of viral replication was observed. Only one patient, a previous non-responder to peginterferon and ribavirin with high baseline invariant natural killer T cell levels, showed profound signs of immune activation, accompanied by a transient 1.3 log decrease in HCV-RNA and a concomitant increase in ALT after the first administration.

Conclusion: α-galactosylceramide used as monotherapy for interferon-refractory patients in doses of 0.1-10 μg/kg is safe, however in its current form it has no significant effect on HCV RNA levels.

INTRODUCTION

Worldwide 170 million patients are chronically infected with the hepatitis C virus (HCV)(1). Treatment with pegylated interferon-α and ribavirin leads to a sustained virological response in as much as 60% of the patients with genotype 1 and close to 90% of the patients with genotype 2 or 3(2, 3). For those patients not responding to treatment with peginterferon-α and ribavirin, new treatment options should be explored.

Interferon-α has been shown to have a direct antiviral effect and to be involved in important interactions with the innate and adaptive immune system(4). Clearance of HCV is associated with the development and persistence of virus specific T cell responses(5). In analogy with these immunomodulatory effects of interferon, α-galactosylceramide (α-GalCer, KRN7000) has been shown to induce powerful antiviral immune responses(6-9).

α-GalCer is a synthetic glycosphingolipid, originally derived from the marine sponge *Agelas mauritianus*(10, 11). It can be recognized by CD1d-restricted invariant natural killer T (iNKT) cells which, upon stimulation, have the capacity to rapidly produce large amounts of both T helper (Th)1 and Th2 type cytokines which subsequently enhance the development of "classical" T cell responses(12).

The immunoregulatory iNKT cells have been highly conserved through evolution. They display an extremely restricted T cell receptor repertoire which in humans consists of a Vα24 chain preferentially paired to Vβ11 and they recognize glycolipid antigen in the context of the monomorphic CD1d antigen-presenting molecule(13). iNKT cells play crucial roles in various immune responses, including antitumor, autoimmune, allergic, and antimicrobial immune responses(13,14).

iNKT cells activated by α-GalCer have been shown to directly inhibit viral replication via the production of IFNγ and IFNα/β in hepatitis B virus transgenic mice(9). In addition, they induced a decrease in viral replication during chronic HCV infection in chimpanzees (K.Ushida, personal communication).

Since it was shown that human circulating iNKT cells and CD1d-reactive intrahepatic lymhocytes can induce profibrotic Th2 type cytokines during disease progression of chronic HCV(15, 16), α-GalCer may exert antiviral activity against HCV by reversing this Th2-type cytokine profile of iNKT cells. Previous clinical trials using α-GalCer demonstrated signs of immune activation only in patients with normal pretreatment iNKT cell levels(17). Therefore, it was important that a previous study demonstrated comparable numbers of circulating iNKT cells in chronic HCV patients compared to healthy controls(18).

A previous phase I clinical study in cancer patients suggested that weekly administration of α-GalCer may lead to a complete depletion of iNKT cells from the

circulation(17). As a subsequent phase I study among healthy volunteers demonstrated an initial decrease but a subsequent recovery of circulating iNKT cell numbers within 4 weeks after the administration of α-GalCer (Nishi N personal communication), we used this dose interval in the present study.

The aim of this study was to investigate the safety and the antiviral activity of α-GalCer in the treatment of chronic hepatitis C patients.

PATIENTS AND METHODS

Patients: Male and female patients aged 18-70 years with chronic HCV infection and previous non-response, relapse or contraindication to interferon-based therapy were eligible for enrolment. In eligible subjects serum alanine transaminase(ALT) levels had to be elevated at least 1.2 times the upper limit of normal(ULN), documented on 2 occasions within 8 weeks before initiation of treatment. The ULN for ALT was 40 U/l for males and 30 U/l for females. A liver biopsy obtained within 12 months before starting treatment, consistent with chronic HCV without cirrhosis as determined by the local pathologist, was required. The presence of HCV viremia $>10^5$ copies/mL was confirmed twice in serum samples obtained within 2 weeks before initiation of treatment and at baseline.

Patients were excluded if they were positive for HBsAg or if they had antibodies to HIV. Further exclusion criteria were histological evidence of cirrhosis or decompensated liver disease, as marked by bilirubin>17 μmol/L, serum albumin<35 g/L, prothrombin time prolonged by>3 seconds or Quick-time<70%, history of bleeding esophageal varices, ascites or hepatic encephalopathy; history of autoimmune disease; clinically significant or major other illnesses; pregnancy; inability to practice adequate contraception; systemic interferon-α treatment, systemic antiviral agents or another investigational drug within 3 months prior to enrolment in the study; immune suppressive treatment; pre-existing bone marrow suppression (white blood cell count<3*10^9/L, hematocrit<34% or platelets<100*10^9/L); evidence of hepatocellular carcinoma (α-fetoprotein>50 ng/ml and/or ultrasound or computed tomography demonstrating a mass suggestive of liver cancer); other acquired or inherited causes of liver disease.

Since there have been no psychiatric side-effects reported during treatment with α-GalCer thus far, patients with a history of psychosis or major depression, which is a contraindication to interferon-treatment, could enrol in the study.

The study was approved by the ethics committees at the participating centers according to the Declaration of Helsinki, and all patients gave written informed consent before enrolment.

Study design: This study was a multicenter double-blind randomized placebo-controlled phase I/II dose-escalation trial. The protocol was conducted in The Netherlands, Belgium and Germany. Patients with chronic hepatitis C who met the inclusion criteria were assigned to receive α-GalCer(KRN7000((2*S*, 3*S*, 4*R*)-1-*O*-(α-D-galactopyranosyl)-*N*-hexacosanoyl-2-amino-1,3,4-octadecanetriol), Kirin Pharmaceutical Co.,Ltd.,Gunma,Japan) or placebo intravenously, thrice with intervals of 4 weeks. Cohorts of patients were entered at each of the 3 dose levels (0.1, 1 and 10 μg/kg body weight). Three patients per dose level were randomized to the placebo arm. Computer generated randomization was performed centrally by an independent clinical research organization (IMRO-TRAMARKO,Berghem,The Netherlands),

Dose escalation to the next cohort was decided after evaluation by a safety review board (2 hepatologists,1 pharmacologist) of all the safety data collected on all the patients who had completed 3 weeks after the first injection in the preceding dose cohort. After completion of 8 weeks of treatment, with injections at 0, 4 and 8 weeks, patients were monitored without further therapy for 16 weeks.

Study objectives: The objective of the study was to evaluate and compare the safety and tolerability of 3 ascending doses of α-GalCer. The primary efficacy parameter was the response at the end of treatment, based on serum HCV ribonucleic acid(HCV RNA) levels. As a secondary efficacy parameter serum ALT levels were evaluated. Further objectives of the study were to evaluate the effect of α-GalCer on serum cytokines IFNγ,IFNα,IL-5 and TNFα and on iNKT cells.

Virology and immunology testing: All HCV RNA determinations were performed using the Amplicore HCV test(Roche-diagnostics).

Phenotyping was performed in a whole blood analysis. Lymphocyte numbers were determined by adding fixed volumes of FlowCount™ fluorospheres(Beckman-Coulter,Miami,USA) to the leucocytes after erythrocyte lysis just before flowcytometric evaluation. Lysing solution and monoclonal antibodies(CD3,CD4,CD8α,CD14,CD19,CD16/56,CD 45,CD69,mouse IgG1 and IgG2a) were purchased from Becton-Dickinson Biosciences(San Jose,USA). FITC-labeled anti-human Vβ11(clone C21) and PE-labeled anti-CD8β from Immunotech(Marseille,France) and PE-labeled anti-CD56 from IQ products. For staining with α-GalCer or vehicle loaded CD1d-tetramers(Gemini Science Inc.,San Diego,CA), 150 μL of whole blood was incubated with the tetramers(10 minutes at 37ºC), then with CD3 and Vα24-moAbs(10 minutes at 37ºC) and subsequently the erythrocytes were lysed.

iNKT cell subsets were further characterized from frozen PBMC samples, using CD4,CD8α and CD8β. Flowcytometric analysis was performed on a FACSCalibur using CELL Quest software(BD Biosciences). Lymphocytes were characterized by monoclonal antibodies or relevant isotype controls: iNKT cells were measured as $V\alpha24^+V\beta11^+CD3^+$ and α-GalCer-loaded CD1d-tetramer$^+CD3^+V\alpha24^+$ cells, natural killer cells as $CD16/56^+CD3^-$ and T cells as $CD3^+$lymphocytes. T helper cells, cytotoxic T cells and activated cells were characterized using CD4,CD8 and CD69.

Serum IFNγ,IFNα,TNFα and IL5 levels were measured by ELISA (R&D Systems,Abingdon,UK (IFNγ,IFNα) and Minneapolis,USA (TNF-α), and BD-Pharmingen (IL5)).

Statistical analyses: The study was exploratory in nature and sample size was based on previous experience with similar agents. Paired and unpaired Student T tests, Wilcoxon matched pairs test, repeated measures ANOVA and Pearson's correlation coefficient were used as appropriate. All analyses were performed on a modified intention-to-treat basis, including all patients who received at least 1 dose of study medication. P-values <0.05 were considered statistically significant.

RESULTS

Study patients:

Between August 2003 and October 2004, 47 patients were screened and 40 patients (28 males,12 females) met the criteria for enrolment into the study: 9 were allocated to a dose of 0.1 μg/kg (dose level 1), 9 were allocated to 1 μg/kg (dose level 2), 11 were allocated to 10 μg/kg (dose level 3) and 11 to placebo (figure 1). One patient showed signs of cirrhosis and was withdrawn from the study after 1 administration of study medication. Another patient withdrew from the study before the first injection.

The mean age at inclusion was 47 years. The mean ALT levels and HCV RNA levels at baseline were 108 IU/L (SD 83) and 8.8*10^6 copies/mL (SD 8.4*10^6). Further baseline characteristics are given in table 1.

Virological and biochemical response to treatment:

We frequently observed small decreases in HCV RNA directly following the first administration of α-GalCer (figure 2). Two days after the first administration the decrease in mean HCV RNA was 2.2 ±3.3*10^6 copies/mL from baseline for the patients in dose level 1 (p=0.10 vs placebo), 2.5 ±5.5*10^6 copies/mL from

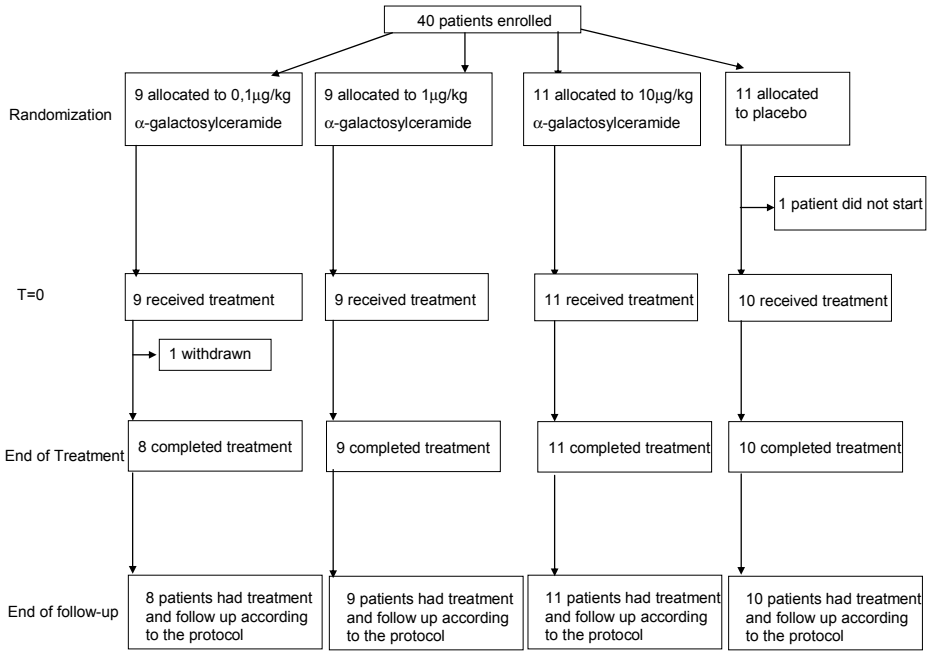

Figure 1: Trial profile.

Table 1 Baseline characteristics.

	Placebo (n=11)	0.1 µg/kg (n=9)	1 µg/kg (n=9)	10 µg/kg (n=11)
Male gender (%)	6 (55)	6 (67)	6 (67)	9 (82)
Age, years*	49 (4.0)	43 (9.2)	43 (10)	51 (9.3)
Weight, kg*	82 (17)	82 (23)	82 (11)	77 (14)
Genotype				
1 (%)	6 (55)	9 (100)	5 (56)	9 (82)
2 (%)	1 (9)	0 (0)	0 (0)	0 (0)
3 (%)	3 (27)	0 (0)	3 (33)	0 (0)
4 (%)	1 (9)	0 (0)	1 (11)	2 (18)
Fibrosis				
None-mild / Moderate-severe	4 / 7	7 / 2	5 / 4	3 / 7
Previous treatment				
None (%)	3 (27)	2 (22)	3 (33)	2 (18)
Interferon + ribavirin	3 (27)	4 (44)	0 (0)	3 (27)
Peginterferon + ribavirin	5 (45)	3 (33)	6 (67)	6 (55)
Baseline ALT, IU/L*	124 (89)	78 (45)	115 (101)	113 (89)
Baseline HCV RNA, 6 Log_{10} copies/mL*	8.7 (5.5)	6.3 (5.5)	13.6 (13.9)	7.1 (5.7)
Baseline TNFα, pg/mL*	1.4 (1.1)	1.3 (0.5)	1.3 (0.7)	1.8 (1.1)
Baseline IFNγ, pg/mL*	21 (13)	26 (25)	18 (7.4)	48 (107)
Baseline NKT cells/10^6 T cells*	970 (1066)	858 (811)	1098 (2207)	382 (643)

* mean (standard deviation)

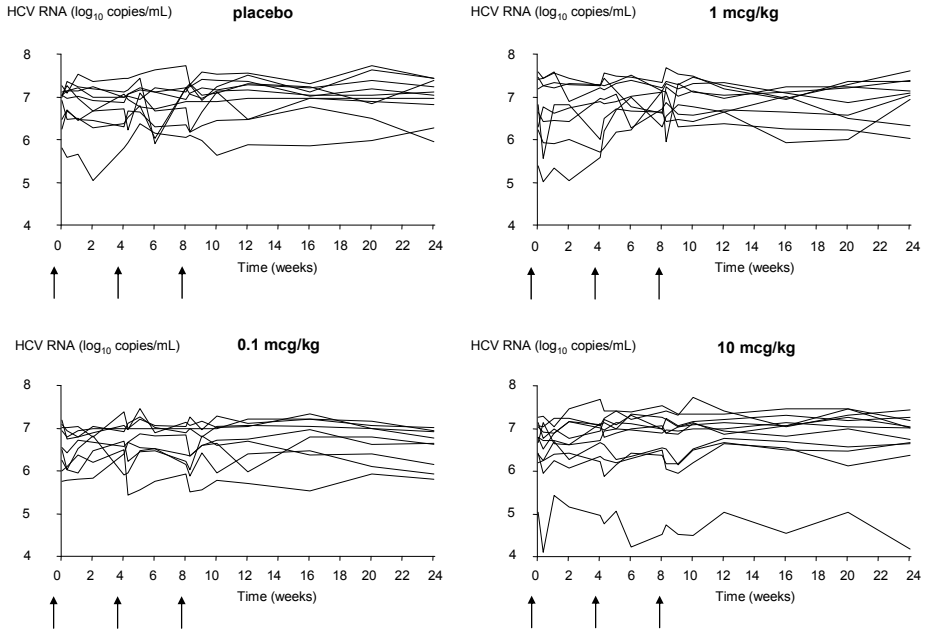

Figure 2: Serum HCV RNA levels of individual patients treated with α-GalCer or placebo. Patients with chronic hepatitis C received either 0.1, 1 or 10 μg/kg α-GalCer or placebo intravenously at week 0, 4 and 8 (arrows). Subsequently, patients were monitored without further therapy for an additional 16 weeks.

baseline in dose level 2 (p=0.14 vs placebo) and 0.8 ±3.9*10^6 copies/mL from baseline in dose level 3 (p=0.32 vs placebo). There were no decreases in HCV RNA in the placebogroup. At the end of treatment and at the end of follow-up no statistically significant changes in HCV RNA were observed in either group (figure 2).

According to the data analysis plan, we separately analyzed the data of patients with high baseline iNKT cell levels. Among those patients with baseline iNKT cells >1000 NKT cells/10^6 T cells, of whom 6 received α-GalCer, we found no statistically significant changes in HCV RNA.

There were no significant changes in mean ALT levels among the treated patients compared to the placebo group. At the end of follow-up, one patient in dose level 1 and one patient in the placebo group had normal ALT levels.

Serum cytokine levels:

The type 1 cytokines IFNα and IL5 were detectable in some of the patients, however, IFNα and IL5 were seldomly elevated simultaneously in the same patient (1 patient in each dose level) and there was no significant effect of α-GalCer on

Table 2: Efficacy data[2]. Changes in ALT, HCV RNA, %NKT cells and % of CD69+ cells of T cells during treatment with α-GalCer as well as changes in IFNγ, TNFα, IFNα and IL-5 before and after administration of α-GalCer.

Dose 0 (placebo)							
Time (day)	**0**	**2**	**28**	**30**	**56**	**58**	**168**
ALT IU/l	124 (89)	137 (116)	112 (78)	112 (70)	90 (52)	88 (52)	102 (61)
Log HCV RNA (copies/ml)	6.8 (0.5)	6.8 (0.5)	6.8 (0.5)	6.8 (0.5)	6.9 (0.5)	6.9 (0.5)	7.0 (0.5)
Ln NKTcells/10^6T cells	6.0 (1.6)	5.5 (2.0)	6.0 (1.7)	5.5 (2.3)	5.7 (1.7)	5.9 (1.5)	6.1 (1.4)
%CD69$^+$ of T cells	0.7 (0.5)	0.9 (1.1)	0.4 (0.4)	0.4 (0.4)	0.5 (0.5)	0.2 (0.2)	0.2 (0.3)
Time (day)1	**0 (before)**	**0 (after)**	**28 (before)**	**28 (after)**	**56 (before)**	**56 (after)**	
Ln IFNγ (pg/ml)	2.9 (0.4)	2.8 (0.2)	2.8 (0.3)	2.9 (0.3)	2.9 (0.3)	3.0 (0.4)	
Ln TNFα (pg/ml)	0.1 (0.6)	0.2 (0.6)	0.1 (0.6)	0.2 (0.8)	0.4 (0.7)	0.1 (0.6)	
Ln IFNα (pg/ml)	1.7 (2.1)	1.7 (2.1)					
Ln IL5 (pg/ml)	0 (0.4)	0 (0.4)					
Dose 0.1 mcg/kg							
Time (day)	**0**	**2**	**28**	**30**	**56**	**58**	**168**
ALT IU/l	78 (45)	77 (44)	74 (46)	74 (48)	76 (49)	76 (46)	74 (32)
Log HCV RNA (copies/ml)	6.5 (0.5)	6.4 (0.5)	6.7 (0.5)	6.5 (0.6)	6.6 (0.5)	6.4 (0.6)	6.5 (0.5)
Ln NKTcells/10^6T cells	6.2 (1.5)	3.9 (1.8)	5.7 (1.4)	3.6 (1.8)	4.3 (2.2)	3.1 (1.6)	5.2 (0.9)
%CD69$^+$ of T cells	0.3 (0.3)	0.4 (0.3)	0.6 (0.5)	0.9 (0.7)	1.3 (2.2)	0.5 (0.2)	0.5 (0.5)
Time (day) 1	**0 (before)**	**0 (after)**	**28 (before)**	**28 (after)**	**56 (before)**	**56 (after)**	
Ln IFNγ (pg/ml)	3.1 (0.6)	3.1 (0.6)	3.1 (0.6)	3.1 (0.6)	3.0 (0.5)	3.1 (0.7)	
Ln TNFα (pg/ml)	0.1 (0.5)	0.0 (0.5)	0.1 (0.5)	0.4 (0.7)	0.2 (0.4)	0.3 (0.3)	
Ln IFNα (pg/ml)	1.7 (2.2)	1.7 (2.1)					
Ln IL5 (pg/ml)	0.4 (0.9)	1.6 (2.0)					
Dose 1 mcg/kg							
Time (day)	**0**	**2**	**28**	**30**	**56**	**58**	**168**
ALT IU/l	115 (101)	122 (118)	111 (115)	110 (107)	95 (66)	103 (86)	86 (60)
Log HCV RNA (copies/ml)	6.8 (0.7)	6.6 (0.9	6.7 (0.7)	6.9 (0.6)	6.9 (0.4)	6.9 (0.5)	7.0 (0.5
Ln NKTcells/10^6T cells	5.8 (1.5)	2.9 (1.4)	4.6 (2.5)	2.3 (2.7)	4.5 (2.4)	2.8 (2.2)	3.5 (2.8)
%CD69$^+$ of T cells	0.8 (0.7)	0.9 (0.6)	0.7 (0.4)	0.5 (0.4)	0.5 (0.5)	0.3 (0.3)	0.3 (0.2)
Time (day) 1	**0 (before)**	**0 (after)**	**28 (before)**	**28 (after)**	**56 (before)**	**56 (after)**	
Ln IFNγ (pg/ml)	2.8 (0.3)	2.9 (0.4)	3.2 (0.6)	3.4 (0.7)	3.3 (0.7)	3.1 (0.7)	
Ln TNFα (pg/ml)	0.1 (0.4)	0.6 (1.1)	0.0 (0.4)	0.6 (1.7)	0.0 (0.3)	0.4 (1.5)	
Ln IFNα (pg/ml)	1.0 (1.2)	1.1 (1.2)					
Ln IL5 (pg/ml)	0.6 (0.9)	0.6 (0.9)					
Dose 10 mcg/kg							
Time (day)	**0**	**2**	**28**	**30**	**56**	**58**	**168**
ALT IU/l	113 (89)	107 (70)	95 (44)	93 (47)	88 (51)	91 (53)	97 (70)
Log HCV RNA (copies/ml)	6.6 (0.6)	6.4 (0.9)	6.7 (0.7)	6.7 (0.8)	6.7 (0.8)	6.7 (0.8)	6.7 (0.9)
Ln NKTcells/10^6T cells	5.1 (1.3)	1.2 (1.5)	2.8 (1.9)	2.3 (1.4)	2.6 (1.6)	2.0 (1.5)	2.9 (1.1)

Dose 0 (placebo)							
%CD69+ of T cells	0.5 (0.5)	0.7 (0.5)	0.4 (0.4)	0.3 (0.4)	0.2 (0.2)	0.2 (0.2)	2.6 (7.1)
Time (day) 1	**0 (before)**	**0 (after)**	**28 (before)**	**28 (after)**	**56 (before)**	**56 (after)**	
Ln IFNγ (pg/ml)	3.0 (1.0)	3.0 (0.8)	3.0 (0.9)	3.0 (0.8)	3.0 (0.9)	3.0 (0.9)	
Ln TNFα (pg/ml)	0.4 (0.6)	1.0 (0.8)	0.5 (0.5)	0.4 (0.6)	0.4 (0.5)	0.4 (0.5)	
Ln IFNα (pg/ml)	0.0 (0.1)	0.0 (0.1)					
Ln IL5 (pg/ml)	2.8 (3.3)	2.8 (3.4)					

[1]Before or 4 hours after administration of α-GalCer. [2]Numbers are given as mean (SD)

IFNα and IL5 levels. No statistically significant changes in serum levels of IFNγ were observed in any of the treatment groups analyzed as a whole. Similarly, no statistically significant changes in serum levels of TNFα were observed in dose level 1,2 and the placebo group (table 2). In dose level 3 there was a small overall increase in serum TNFα levels after 4 hours (mean change ±SD from baseline: 1.6 ±2.4 pg/ml (p=0.05)) that returned to baseline levels at day 2 (p=0.07). As is illustrated in figure 3, α-GalCer induced a reproducible increase in IFNγ and TNFα levels in several individual patients (for IFNγ: 1 patient in dose level 1, 2 patients in dose level 2; for TNFα: 2 patients in dose level 2).

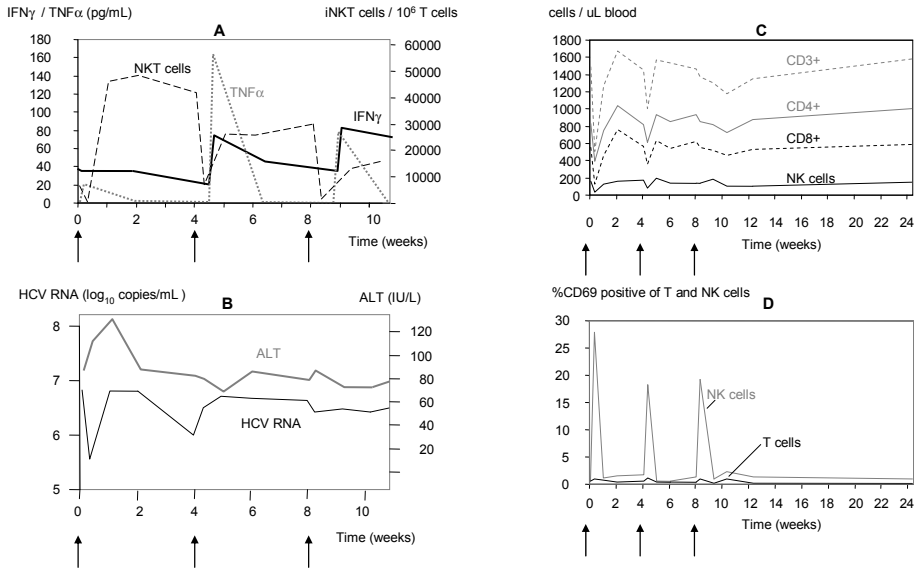

Figure 3: Serum levels of IFNγ, TNFα, iNKT cells (**A**); HCV RNA and ALT (**B**); CD3+,CD4+, CD8+ and NK cells (**C**) and NK and T cell CD69 expression (**D**) of 1 patient with a high baseline iNKT cell level, who showed evidence of immune activation after the administration of α-GalCer. IFNα and IL-5 levels were not detectable in this patient. The patient received 1μg/kg α-GalCer at week 0, 4 and 8 (arrows). 10.4: Percentage change in serum levels of iNKT cells of individual patients treated with α-GalCer or placebo.

The maximum increase in serum TNFα occurred in the patient with the highest baseline iNKT cell count (6885 iNKT cells/10^6 T-cells). This patient had a substantial increase in serum TNFα after each administration of α-GalCer, peaking at 163 pg/mL after the second administration. The α-GalCer-induced increases in serum TNFα levels in this patient were accompanied by marked increases in IFNγ levels (figure 3). Moreover, this was the only patient who showed a marked decrease in HCV RNA of 1.3 log compared to baseline after the first administration of α-GalCer. This decrease in HCV RNA was accompanied by a rise in serum ALT levels, suggestive of an immune response to HCV-infected hepatocytes. However, although the second administration of α-GalCer also led to high serum levels of both TNFα and IFNγ, no reduction in HCV RNA load was observed after the second administration, and only a minor reduction was observed after the third administration (figure 3).

Analyses of circulating iNKT cells: An almost perfect correlation (r = 0.999) was found between iNKT assessment with α-GalCer CD1d-tetramer staining and with monoclonal antibodies against the TCR Vα24 and Vβ11 chains (evaluated in 531 blood samples). As illustrated in figure 4, the first administration of α-GalCer resulted in a rapid decrease in circulating iNKT cells in all dose levels (Wilcoxon matched pairs tests; p=0.003 (dose level 1), p<0.0001 (dose level 2 and 3) which was followed by a recovery of iNKT cell numbers. Importantly, in the placebo group no statistically significant changes in iNKT cell numbers were observed.

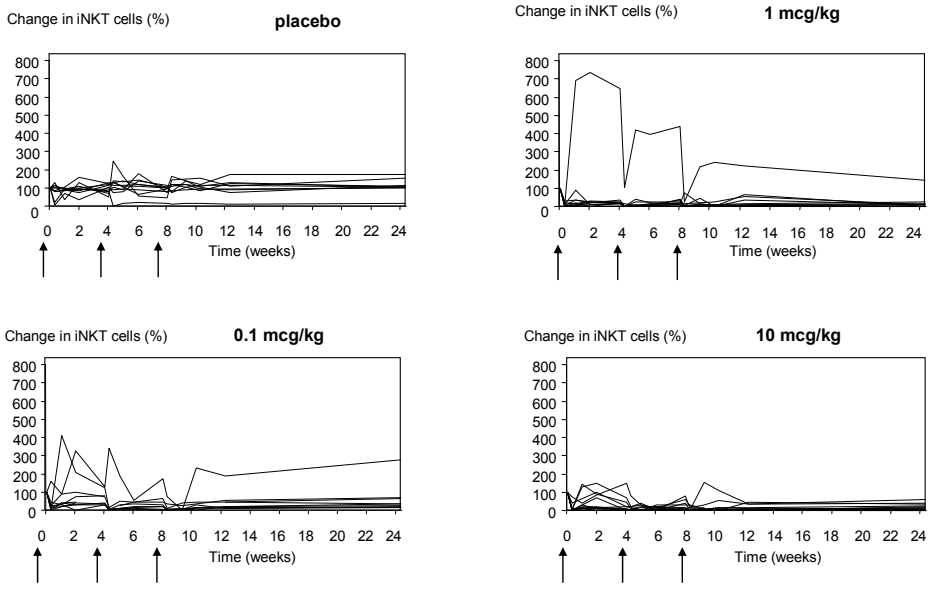

Figure 4

At baseline, the distribution of each iNKT cell subset was as follows: 37.6±21.6% $CD4^+$, 5.9±6.6% $CD8^+$, 36.5±19.2 $CD4^-CD8^-$double negative (DN). Interestingly, the first administration of α-GalCer did not significantly alter the proportion of $CD4^+$ iNKT cells (36.8 ±21.6% at day 28, p=0.63, paired Student t-test), but resulted in a significant decrease in the proportion of DN iNKT cells (22.2 ±19.3% at day 28, p=0.01), and a significant increase in the proportion of $CD8^+$ iNKT cells (15.4 ±14.2, p=0.03). The second and third administration of α-GalCer did not result in any significant changes in the contribution of each iNKT cell subset to the total iNKT cell pool.

Analyses of circulating T and NK cells:

Interestingly, we only observed a significant effect on circulating numbers of T cells among patients with above median iNKT cell numbers. Both the first and second administration of α-GalCer, but not placebo, resulted in a significant de-

Table 3: Most common adverse events, defined as those occurring in at least 2 patients of any treatment group or in at least 3 patients of all 4 dose groups.
All adverse events were scored grade I.

Category	Adverse event	All patients (n=39)	placebo	0,1µg/kg	1µg/kg	10µg/kg
Flu-like	Fatigue	13 (33%)	2	3	3	5
	Headache	21 (53%)	5	5	3	8
	Fever / Chills	20 (50%)	4	2	6	9
	Myalgia	10 (25%)	3	1	1	5
	Arthralgia	6 (15%)	2	-	1	3
	Back pain	7 (18%)	2	-	-	5
Gastrointestinal	Nausea	5 (13%)	2	-	2	1
	Vomiting	3 (7.5%)	2	-	1	-
	Abdominal pain	2 (5.0%)	1	-	1	3
	Diarrhea	6 (15%)	2	-	2	2
	Anorexia	4 (10%)	-	1	1	2
Respiratory	Rhinitis	10 (25%)	3	2	-	5
	Pharyngitis	4 (10%)	-	3	-	1
	Epistaxis	2 (5.0%)	-	2	-	-
	Cough	3 (7.5%)	2	1	-	-
Infectious	Herpes simplex	3 (7.5%)	-	1	2	-
	Wound infection	2 (5.0%)	-	2	-	-
CNS	Dizziness	7 (18%)	-	2	3	2
Dermatologic	Dermatitis	2 (5.0%)	-	2	-	-
	Pruritus	4 (10%)	1	3	-	-
Total number of adverse events		134	31	30	26	51

crease in the number of circulating T cells from 1345 ±397 to 1073 ±336 cells/ul after the first administration (p=0.02) and from 1366 ±509 to 1159 ±366 cells/ul after the second administration (p=0.03). The decrease in circulating T cells was caused by a decrease in both $CD4^+$ (p=0.02 and p=0.03 after the first and second administration) and $CD8^+$ T cells (p=0.02 and p=0.01 after the first and second administration). We found no statistically significant changes in the expression of CD69 on T cells, although we did note α-GalCer-induced upregulation of CD69 in several patients showing a substantial decrease in circulating T cell numbers.

Safety:
The most frequent clinical adverse events seen during treatment with α-GalCer were headache, fatigue, rhinitis, myalgia, fever and chills, back pain and dizziness (table 3). All reported adverse events were scored grade I. None of the patients discontinued treatment because of adverse events and no serious adverse events related to the study drug occurred. Table 4 shows the levels of creatinin, white blood cells (WBC), neutrophils, ALT, AST and γGT before and two days after administration of α-GalCer and at end of follow-up.

DISCUSSION

The results of this randomized placebo-controlled trial provide a unique insight into the activation of iNKT cells following treatment with α-GalCer.

As expected from previous clinical experience, a rapid and substantial reduction in the size of the circulating iNKT cell pool was found upon administration of α-GalCer. However, iNKT cell numbers recovered before the second injection, probably due to the increased interval of 4 weeks between sequential α-GalCer administrations in the current study.

Of interest, although not observed after subsequent injections, the first administration of α-GalCer resulted in an increase in the proportion of $CD8^+$ iNKT cells which preferentially produce IFN-γ. This may reflect a shift in the capacity of the iNKT cell population towards a more pronounced Th1 phenotype (19). Although overall no statistically significant changes in serum levels of IFN-γ or TNF-α were induced by α-GalCer, we did note increased serum levels of these cytokines in several patients, and most prominently in the patient with the highest baseline iNKT cell count. Interestingly, a 1.3 log decrease in HCV RNA after administration of α-GalCer was observed in this patient. Indeed, since the decrease in HCV RNA was accompanied by a temporary increase in ALT levels, these data do suggest that α-GalCer is capable of inducing an immune response against HCV-infected

Table 4: Safety data. Levels of creatinin, white blood cells (WBC), neutrophils, ALT, AST and γGT before (day 0,28 and 56) and two days after administration of α-GalCer and at end of follow-up (day 168). Numbers are given as mean (standard deviation).

Time (day)	0	2	28	30	56	58	168
Dose 0 (placebo)							
Creatinin mmol/l	76 (16)	-	78 (16)	-	78 (14)	-	77 (12)
WBC *10^9/l	5.2 (1.8)	4.6 (0.9)	4.9 (1.3)	5.1 (1.2)	5.3 (1.9)	4.8 (1.0)	5.2 (1.4)
Neutrophils *10^9/l	3.0 (1.5)	2.5 (0.7)	2.7 (0.9)	2.8 (1.0)	3.1 (1.7)	2.6 (0.9)	2.9 (1.2)
ALT IU/l	124 (89)	137 (116)	112 (78)	112 (70)	90 (52)	88 (52)	102 (61)
AST IU/l	83 (56)	72 (39)	66 (33)	69 (36)	61 (37)	62 (35)	62 (29)
γGT IU/l	85 (48)	88 (51)	82 (47)	81 (45)	78 (46)	76 (46)	81 (59)
Dose 0.1 mcg/kg							
Creatinin mmol/l	73 (12)	-	72 (13)	-	75 (12)	-	74 (14)
WBC *10^9/l	6.3 (1.5)	5.7 (1.3)	5.9 (1.1)	5.0 (1.2)	5.7 (1.1)	5.6 (14)	6.2 (1.6)
Neutrophils *10^9/l	3.7 (1.3)	3.2 (1.0)	3.4 (1.1)	2.9 (1.0)	3.1 (1.0)	3.0 (1.2)	3.6 (1.3)
ALT IU/l	78 (45)	77 (44)	74 (46)	74 (48)	76 (49)	76 (46)	74 (32)
AST IU/l	53 (25)	54 (25)	52 (21)	52 (21)	53 (20)	54 (20)	55 (20)
γGT IU/l	121 (72)	120 (74)	108 (57)	110 (56)	106 (61)	109 (68)	118 (63)
Dose 1 mcg/kg							
Creatinin mmol/l	81 (14)	-	83 (14)	-	82 (13)	-	85 (12)
WBC *10^9/l	5.6 (1.3)	5.4 (1.5)	5.9 (1.4)	5.7 (1.6)	5.8 (1.7)	5.9 (1.8)	5.8 (1.5)
Neutrophils *10^9/l	3.2 (1.0)	3.0 (1.2)	3.5 (1.2)	3.3 (1.5)	3.4 (1.5)	3.4 (1.6)	3.4 (1.3)
ALT IU/l	115 (101)	122 (118)	111 (115)	110 (107)	95 (66)	103 (86)	86 (60)
AST IU/l	63 (38)	66 (44)	62 (42)	60 (32)	56 (27)	59 (34)	55 (21)
γGT IU/l	108 (109)	160 (193)	116 (129)	116 (125)	109 (97)	112 (102)	91 (67)
Dose 10 mcg/kg							
Creatinin mmol/l	79 (15)	-	84 (15)	-	80 (15)	-	84 (15)
WBC *10^9/l	5.2 (1.3)	5.2 (1.4)	5.5 (1.5)	5.3 (1.5)	5.4 (1.5)	5.6 (1.5)	5.1 (1.2)
Neutrophils *10^9/l	2.7 (1.0)	2.7 (0.9)	2.7 (1.1)	2.5 (0.9)	2.8 (1.2)	2.8 (1.1)	2.4 (0.7)
ALT IU/l	113 (89)	107 (70)	95 (44)	93 (47)	88 (51)	91 (53)	97 (70)
AST IU/l	86 (70)	77 (53)	75 (42)	68 (37)	69 (44)	69 (44)	73 (55)
γGT IU/l	125 (120)	128 (114)	126 (107)	122 (102)	120 (118)	126 (131)	123 (126)

hepatocytes. It is also possible that CD1d+ HCV-infected hepatocytes are lysed by α-GalCer-activated iNKT-cells, which would explain the immediate effect on HCV RNA levels. Although we cannot be sure that the rise in ALT is not a sign of toxicity, overall there was no significant increase in ALT or AST levels after administration of α-GalCer.

Importantly, iNKT cell activation in this patient resulted in prominent signs of immune activation as determined by increased serum levels of IFNγ and TNFα, decreased levels of circulating T and NK cells with a concomitant upregulation of the activation marker CD69 on residual circulating T and NK cells, suggesting cross-talk between iNKT cells and conventional lymphocytes. Since this patient

was a previous non-responder to treatment with pegylated interferon and ribavirin, iNKT cell mediated immunomodulation might be a potential way of treating non-responders to pegylated interferon and ribavirin, or perhaps, enhance the efficacy when added to the current standard treatment with pegylated interferon and ribavirin. However, it is likely that the effect may only be substantial in patients with high pre-treatment iNKT cell levels. Although no severe side effects of α-GalCer occurred in this study, the risk of drug interactions increases with the addition of any new drug.

A possible explanation for the lack of effect of α-GalCer on HCV RNA levels in the present study may be that iNKT cell levels in human liver are substantially lower than in mice(15). Since administration of α-GalCer in the context of dendritic cells has been shown to be more potent than α-GalCer by itself in both preclinical and clinical tumor studies, this strategy might prove to be substantially more potent(20-22). Studies that have been conducted so far suggest that the amount of α-GalCer that has been administered in our study should be sufficient and that even in lower dose-levels immune responses are observed in patients with a high iNKT cell level(17). We can only expect a limited dose-response effect, since the number of NKT cells that is activated is limited and the activation initiates a chain-reaction (stimulation of NK cells and T cells).

A drawback of the present study is that the treatment is targeted to the liver, whereas all readouts are from the circulation. It would be interesting to know whether there was a cytokine response within the liver itself, and to know which cell types infiltrate the liver after administration of α-GalCer.

In conclusion, α-GalCer used as monotherapy in doses of 0.1 to 10 μg/kg is safe. It has no significant effect on HCV RNA levels, although changes in cytokine production and in HCV RNA levels are observed in a single patient. Further research is needed to investigate and refine the concept of iNKT cell activation as monotherapy or combination treatment for chronic viral hepatitis.

ACKNOWLEDGEMENTS

This study was supported by an unrestricted grant from Kirin Pharmaceutical Co., Ltd., Tokyo, Japan.

We thank prof. F. Nevens from the University Hospital of Leuven, Belgium, for his contribution to the study and we thank Dr. S. Kato (Gemini Science Inc., San Diego, CA) for providing the CD1d-tetramer.

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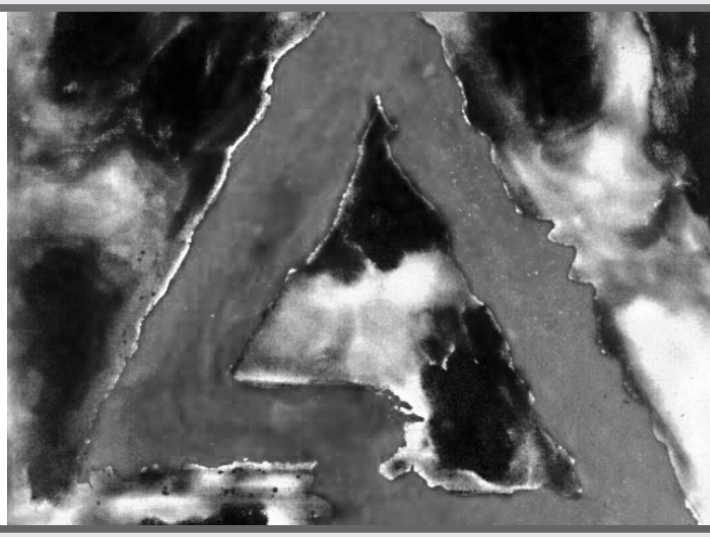
Chapter 11 general discussion:

The long-term clinical outcome of (peg)interferon treatment for patients with chronic hepatitis C



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INTRODUCTION

Hepatitis C is a single-stranded RNA virus which infects an estimated 170 million people worldwide. The prevalence of chronic hepatitis C in Northern Europe and North America is 1.0 to 1.7 percent. The prevalence is higher in Southern Europe, in Asia and in Egypt (1).

Chronic hepatitis C causes hepatic inflammation which may lead to fibrosis of the liver. It has been estimated that it takes about 30 years before cirrhosis develops, but there are large differences in the outcomes of cohort studies describing the natural course of hepatitis C (2). Patients with hepatitis C cirrhosis are at risk of developing hepatic failure and hepatocellular carcinoma (HCC). For patients with hepatitis C cirrhosis the 5-year risk for development of HCC is about 2.7 to 10% (3, 4). Patients with end stage liver disease due to hepatitis C may be candidates for liver transplantation and chronic hepatitis C is one of the major indications for liver transplantation in Europe and North America (5). However, recurrence of infection is universal post-transplant.

Interferon alpha was discovered to be effective for treating chronic hepatitis C. Although the mechanism of action has not been fully cleared up, it has been shown that interferon has both a direct antiviral effect as well as important interactions with the adaptive and innate immune responses (6). Clearance of hepatitis C is associated with the development and persistence of virus specific responses by cytotoxic and helper T cells (7). Interferon causes many side effects, such as flu-like symptoms, cytopenia, dermatitis and mood disorders and the response rates in the first studies were as low as 10 to 15%.

Addition of the antiviral agent ribavirin significantly enhanced the reponse rates. Pegylated interferon is a polyethylene-glycol-conjugated derivative of conventional interferon alpha, which acts longer and allows for weekly dosing instead of thrice a week (8). The response rates following a 24-week treatment course with peginterferon and ribavirin are close to 90% for patients with genotype 2 or 3. Genotype 1 and 4 of the virus are more difficult to treat; response rates up to 60% have been reported after 48 weeks of treatment (9, 10). Sustained virological response (SVR) to treatment is a surrogate endpoint defined as undetectability of hepatitis C virus ribonucleic acid (HCV-RNA) in the serum by sensitive molecular tests at 24 weeks after the end of treatment.

In this article we aim to review the literature about the long-term clinical outcome of chronic hepatitis C patients treated with (peg)interferon and/or ribavirin. Furthermore we will discuss treatment recommendations based on these long-term clinical findings.

LONG-TERM CLINICAL OUTCOME AFTER TREATMENT

In Europe, clinical events such as liver failure, HCC and liver related death are rare among sustained virological responders with mild to moderate fibrosis. In a large cohort study of 286 sustained virological responders, the rate of hepatic failure after five years of follow up was 1.0% (95% CI 0.0–2.3) and none of the patients developed HCC. Survival was comparable with the general population, matched for age and sex, the standard mortality ratio being 1.4 (95% CI 0.3–2.5). This indicates that sustained virological responders have an excellent prognosis. Another European study did not report any events among 74 patients followed up for 2.7 years (11) and two studies, involving seven and 56 sustained responders, also showed no clinical events during 4.6 and 5.2 years of follow up, respectively (12, 13). According to several large studies, the yearly incidence of HCC among Japanese sustained virological responders still varies between 0.2% and 0.5% per year (14-21). The lowest rates in Japan were reported by Yoshida et al, with one HCC among 817 sustained responders during 5.4 years of follow up (21). The highest incidence of HCC reported among sustained responders in Japan was by Kasahara et al who reported five HCCs among 313 sustained virological responders followed up for three years (17).

There are two studies that reported a European sustained virological responder developing HCC (22)-(23), but these cases seem to be rare and limited to patients with cirrhosis.

ADVANCED FIBROSIS AND CIRRHOSIS

In order to further investigate the clinical outcome of patients with advanced fibrosis or cirrhosis we performed a large multicenter study focused on patients with advanced disease. Hepatic failure did not occur among sustained virological responders and the five-year occurrence among non-responders was 13.3% (95% CI 8.4-18.2) (Log Likelihood p=0.001). The five-year occurrence of hepatocellular carcinoma was 9.2% (95% CI 0.0-19.6) among sustained responders versus 13.1% (95% CI 7.6-18.6) among non-responders (Log Likelihood p=0.192); the five-year occurrence of liver-related death was 4.4% (95% CI 0.0-12.9) among sustained responders versus 12.9% (95% CI 7.7-18.0) among non-responders (Log Likelihood p=0.024).

If the pre-treatment albumin levels were below the lower limit of normal of 35 g/L, the probability of achieving SVR dropped below 30 percent while the

risk of developing early liver failure, during or within 24 weeks after treatment, increased from 2 (normal albumin) to 15 percent (low albumin) (figure 1).

In contrast to studies from Japan, where the benefit of interferon treatment lies mainly in the prevention of development of hepatocellular carcinoma (21, 24), we showed that in a Western population there is a major reduction in the development of liver failure. This is an important finding since the incidence of liver failure among untreated cirrhotics in Europe has been estimated to be four times as high as the incidence of HCC (4).

In our study, there were still 3 patients who developed HCC, the incidence of HCC being 107 per 10,000 patient years, which means an annual incidence of 1.1%. A recent report from Italy has shown an incidence of HCC of 0.7% per year among sustained virological responders with cirrhosis (25).

Previous Japanese studies reported a yearly incidence of HCC of 0.7 to 2.5% per year among sustained virological responders with advanced fibrosis or cirrhosis (14, 15, 18, 24). The lowest rate was reported by Okanoue et al. who found 4 HCCs among 86 patients with advanced fibrosis (n=82 METAVIR-score F3 and

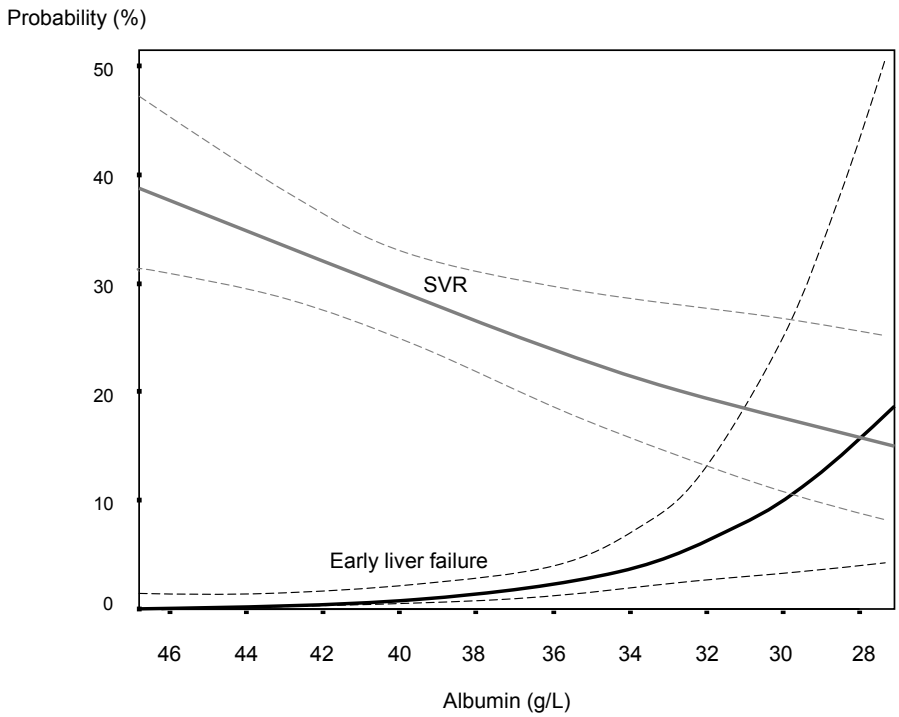

Figure 1: Probability (%) of SVR and early liver failure according to pre-treatment albumin levels. Dotted lines represent 95% confidence intervals. Note that the X-axis goes from high to low albumin levels.

Table 1: Overview of the literature describing clinical events among chronic hepatitis C patients with SVR to interferon-based treatment.

Author	Journal + year	Number of sustained responders	Patients with advanced fibrosis / cirrhosis	HCC	Decompensation	Liver related death	Follow-up (years)	HCC % / year (95% CI)
HCC								
Nishigushi	Lancet 1995	7	7	0			2-7	0
Mazzella	J Hepatol 1996	74	74	0			2.7	0
Okanoue	J Hepatol 1999	316	75	3			3.4	0.3 (0.0-1.2)
Imai	Ann Int Med 1998	120		1			4.0	0.2 (0.0-0.6)
Bruno	J Hepatol 2001	36	6	1			8.5	0.4 (0.0-1.1)
Kasahara	Hepatology 1998	313 [1]		5			3.0	0.5 (0.0-1.0)
Yoshida	Ann Int Med 1999	789	216	10			4.3	0.3 (0.1-0.5)
Tsuda	J Med Virol 2004	38		4			6.8	1.5 (0.0-5.5)
Iwasaki	Liver Int 2004	792	160	23			5.1	0.6 (0.0-1.1)
HCC and Liver related death								
Tanaka	Int J Cancer 2000	175 [1]		3		0	5.0	0.3 (0.0-0.7)
Imazeki	Hepatology 2003	116		1		1	8.2	0.1 (0.0-0.3)
Okanoue	Hepatol Res 2002	425	89	4		0	5.6	0.2 (0.0-0.3)
Shiratori	Ann Intern Med 2005	64	64	11		0	6.8	2.5 (0.0-6.5)
HCC, Decompensation and Liver related death								
Bruno	Hepatology 2007	124	124	7	0	2	8.0	0.7 (0.3-1.9)
Camma	J Hepatol 1998	62 [1]	5	0	0	0	5.2	0
Gramenzi	Gut 2001	7	7	0	0	0	4.6	0
Veldt	Gut 2004	286	68	0	2	1	4.9	0
Soriano	Antivir Ther 2004	77		0	0	0	4.8	0
Veldt	Ann Intern Med 2007	142	142	3	0	1	3.6	1.1 (0.0-2.3)
HCC and Decompensation								
Fattovich	J Hepatol 1997	14	14	0	0		5.0	0
Lau	Hepatology 1998	5	1	0	0		6-13	0
Liver related death								
Bjoro	Q J Med 1999	3				0	15	
Yoshida	Gastroenterology 2002	817	215			2	5.4	

[1]sustained biochemical response.

n=4 F4) during 6 years of follow-up. The highest rate was reported by Shiratori et al. who described 11 HCCs during 6.8 years of follow-up of 64 sustained virological responders with cirrhosis.

DIFFERENCES IN HCC OCCURRENCE BETWEEN WESTERN COUNTRIES AND ASIA

There are several possible explanations for the high incidence of hepatocellular carcinoma among chronic hepatitis C patients in Japan, as compared to Western countries.

The first explanation is that the hepatitis C epidemic started earlier in Japan, causing a greater spread and a higher prevalence of the infection. It has been estimated that HCV genotype 1 first appeared in Japan in around 1882, whereas emergence in the U.S. was delayed until around 1910. Furthermore, the major spread time for HCV infection in Japan probably occurred in the 1930s, whereas widespread dissemination of HCV in Europe and the U.S. occurred in the 1960s (26, 27). Patients in Japan may in general have a longer duration of infection, which increases their risk of developing HCC.

Secondly, dietary factors such as aflatoxins in the food or a high alcohol intake may have a carcinogenic effect and finally there may be genetic factors that account for the elevated incidence of HCC in Japan.

In order to investigate which factors may explain the elevated incidence of HCC in Japan, compared to Western countries, we analyzed the data of 382 individual European and Canadian chronic hepatitis C patients and 257 individual Japanese chronic hepatitis C patients, included in two large cohort studies (28, 29). These patients were all non-responders to interferon or peginterferon and they all had advanced fibrosis or cirrhosis.

Although the incidence of HCC was indeed 1.7 times as high for Japanese non-responders than for European or Canadian non-responders, table 2 shows that after correction for baseline factors the only factors predictive of HCC were age, male gender and low platelet count and not the place of origin. This suggests that genetic and dietary factors might only play minor roles in the high incidence of HCC among Asian chronic hepatitis C patients.

Table 2: Factors predictive of development of HCC

	Hazard ratio	95% confidence interval		p-value
		lower	Upper	
Female Gender	0.18	0.07	0.48	0.001
Age	1.07	1.03	1.11	0.002
Bilirubin	1.32	0.59	2.93	0.503
Platelet count	0.16	0.05	0.52	0.002
Japanese vs European/ Canadian	0.66	0.30	1.44	0.297
Anti HBc positivity	1.32	0.67	2.63	0.426
Disease duration				
< 10 years	1 (reference)			
10-20 years	2.27	0.25	20.70	0.468
>20 years	2.30	0.30	17.37	0.420

The hazard ratios with their 95% confidence intervals and p-values associated with these factors are given. Hazard ratio <1.0 indicates a decreased risk for HCC. Older age, male gender and low platelet counts were significantly associated with a higher risk of developing HCC. Gender, Japanese vs European/Canadian, anti-HBc positivity and disease duration were entered as categorial values. Age, bilirubin and platelet count were entered as continuous values

COMPARISON OF TREATED PATIENTS VERSUS NATURAL COURSE

It is difficult to investigate the long-term benefit of chronic hepatitis C treatment since the natural course of the disease is not clearly defined. The National Institute of Health consensus on chronic hepatitis C states that the risk of developing cirrhosis during 20 years of hepatitis C infection is 10-15% and that there are 10,000 to 12,000 deaths yearly in the United States due to hepatitis C (30). Although it is mentioned that fibrosis progression rates may be variable according to sex and age at infection, no precise estimates of the occurrence of liver-related death are given for different subgroups of patients. The French consensus mentions that fibrosis may accelerate after the age of 50 to 60 years, but again precise estimates of the occurrence of liver-related death for hepatitis C patients are lacking (31).

The reason that the natural history is only described in such rough terms is that cohort studies describing the natural history of hepatitis C show a great variety in outcome. The outcome ranges from a 25-year risk of liver-related mortality of 0.5% for female patients infected in their twenties (32) to an 8-year risk of liver-related mortality of 16% for 61-year-old patients with cirrhosis (33).

Furthermore, as there is a treatment available for hepatitis C, it is ethically impossible not to treat hepatitis C patients in order to further study this natural course of the disease.

Therefore, we developed a Markov model to simulate the natural course of the disease. In a Markov model, members of a cohort are divided among several mutually exclusive health states, and movements of the cohort across these states

are modelled over time (34-36). In this study, at the end of every three months, the members of the cohort are reallocated across the health states, with these movements guided by transition probabilities that reflect the natural history of the disease. The model is based on 10 year old patient data, from a time when no curative treatment for chronic hepatitis C was available. The outcomes of the model fit well with cohort studies published in the literature, describing the long-term outcome of chronic hepatitis C infection (37).

Figure 2 shows that the occurrence of hepatocellular carcinoma and hepatic failure is decreased among patients treated for chronic hepatitis C, compared to a similar cohort of patients, corrected for age, gender, age at infection and fibrosis stage, according to the model described above. However, the survival curves of treated and untreated patients are comparable. The mean follow-up of 3.6 years for the treated patients may have been too short for the reduced incidence of HCC and hepatic failure to be translated into a survival benefit.

Moreover, our Markov model realistically takes into account the competing risk of other diseases. This means that a patient in whom a HCC is prevented may still die of lung cancer or a heart attack. This may explain why the effect on overall survival is smaller that we might expect and why we probably need a longer follow-up or an even larger cohort for a survival difference to become apparent.

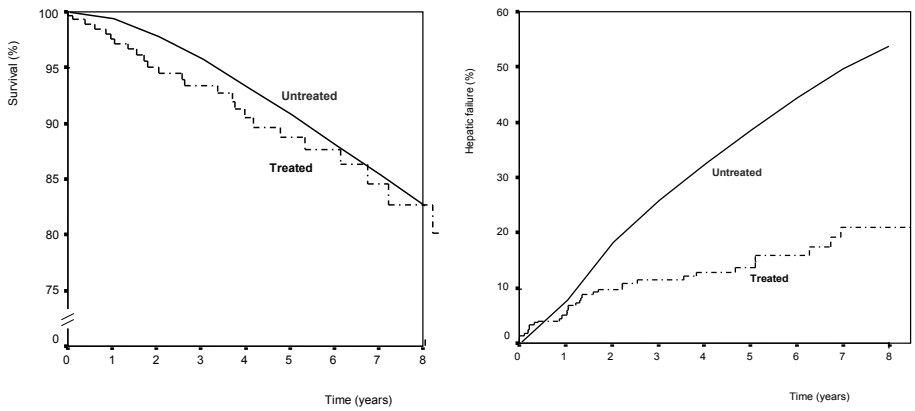

Figure 2: Survival (figure 2a), occurrence of hepatic failure (figure 2b) and of HCC (figure 2c) among patients with advanced fibrosis, treated with (peg)interferon and / or ribavirin, compared to the natural course of chronic hepatitis C according to the Markov-model.

CONCLUSION AND TREATMENT RECOMMENDATIONS BASED ON LONG-TERM OUTCOMES

In the absence of risk factors such as coinfection with hepatitis B or HIV or heavy alcohol use, disease progression is usually slow for patients without fibrosis or with mild fibrosis. These patients may be best off monitored without treatment. However, since shorter, though equally effective treatment schedules are now being developed for patients with genotype 2 or 3 and SVR rates are high in this group, motivated patients may still be candidates for treatment. For chronic hepatitis C patients with moderate fibrosis, survival after achieving SVR is comparable to the matched general population. Patients with moderate fibrosis are therefore the best candidates for treatment.

Treatment is also recommended for patients with advanced fibrosis due to chronic hepatitis C, since treatment is likely to reduce the incidence of hepatic failure and hepatocellular carcinoma. However, one should be cautious treating patients with albumin levels below the lower limit of normal due to advanced liver disease, as in this subgroup the risk of early hepatic failure is high. For these patients, pretreatment evaluation by a liver transplant team and careful monitoring during and after treatment is warranted.

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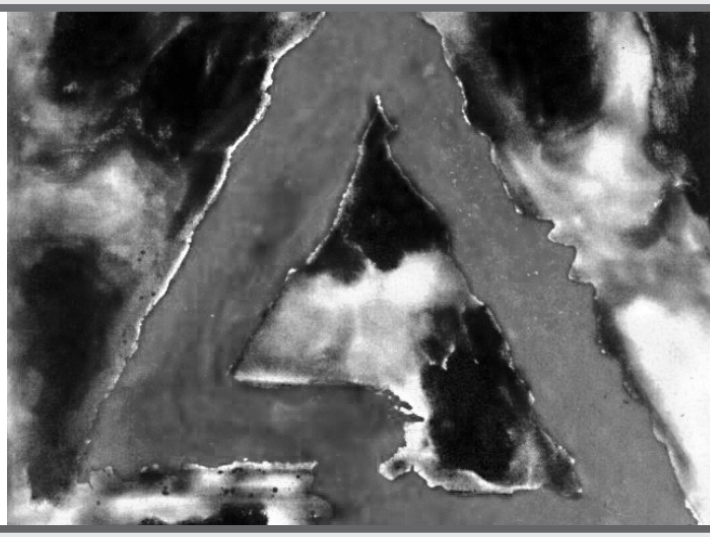
Chapter 12: Nederlandse samenvatting

De lange termijn uitkomsten van de behandeling van patiënten met chronische hepatitis C



INTRODUCTIE

Hepatitis C is een virus waar wereldwijd zo'n 170 miljoen mensen mee besmet zijn. In Noord Europa en Noord Amerika komt hepatitis C bij 1,0 tot 1,7 procent van de bevolking voor. In Zuid Europa, Azië en Egypte is het aantal ziektegevallen hoger (1). Het hepatitis C virus wordt onderverdeeld in 6 genotypes.

Chronische hepatitis C infectie leidt tot ontsteking van de lever en tot bindweefsel vorming (fibrose). Men schat dat het bij een chronische hepatitis C infectie ongeveer 30 jaar duurt voordat de lever verbindweefseld is en er sprake is van cirrose, maar er zijn grote verschillen in uitkomst tussen verschillende studies die het natuurlijk beloop van hepatitis C infectie beschrijven (2). Patiënten met chronische hepatitis C en levercirrose lopen risico op leverfalen en op het ontwikkelen van leverkanker.

Het risico om leverkanker te ontwikkelen is 2 tot 10 procent per 5 jaar voor deze patiënten (3, 4). Patiënten met een eindstadium leverziekte door hepatitis C kunnen in aanmerking komen voor levertransplantatie. Chronische hepatitis C infectie is een van de belangrijkste indicaties voor levertransplantatie in Europa en de Verenigde Staten (5). Na transplantatie raakt de nieuwe lever echter altijd weer opnieuw geïnfecteerd.

Men heeft ontdekt dat interferon-alfa een effectief middel is om chronische hepatitis C te behandelen. Hoewel het precieze werkingsmechanisme niet bekend is, is wel gebleken dat interferon een direct antiviraal effect heeft en dat het belangrijke interacties heeft met het aangeboren en het adaptieve immuunsysteem (6). Klaring van een hepatitis C infectie gaat gepaard met de ontwikkeling van virus specifieke responsen door witte bloedcellen, te weten cytotoxische T-cellen en T-helper cellen (7).

Interferon heeft echter veel bijwerkingen zoals griepachtige verschijnselen, tekort aan bloedplaatjes en witte bloedcellen, huidontstekingen en stemmingsstoornissen en de responspercentages in de eerste studies waren slechts 10 tot 15%. Door het toevoegen van het antivirale middel ribavirine werden deze responspercentages hoger. Bovendien is het zo dat als je patiënten, die eerder niet reageerden op interferon alleen, herbehandelt met interferon in combinatie met ribavirine, ze een kans hebben van 15% om alsnog een blijvende virologische respons te bereiken (hoofdstuk 3).

Gepegyleerd interferon, kortweg peginterferon, is een afgeleide van het gewone interferon waarbij het interferon gekoppeld is aan een keten van polyethyleen glycol. Peginterferon heeft een langere werkingsduur en hoeft maar 1 maal per week toegediend te worden in plaats van 3 maal per week (8).

De responspercentages na een behandeling van 24 weken met peginterferon en ribavirine zijn bijna 90% voor patiënten die geïnfecteerd zijn met hepatitis C genotype 2 of 3. Genotype 1 en 4 van het virus zijn moeilijker te behandelen: na 48 weken behandeling behaalt tot 60% van de patiënten een blijvende virologische respons (9, 10). Om de kans op een blijvende virologische respons in te schatten tijdens een interferon behandeling, kun je gebruik maken van de waarden van het leverenzym alanine aminotransferase (ALAT), gemeten in week 4 van de behandeling. Ook kan het de moeite waard zijn om de kosten per genezing mee te nemen in de overweging hoe lang een behandeling zou moeten duren (hoofdstuk 4).

Een blijvende virologische respons is een surrogaat eindpunt, dat gedefinieerd is als het niet detecteerbaar zijn van het ribonucleïnezuur van het hepatitis C virus (HCV RNA) in het serum door middel van gevoelige moleculaire tests op 24 weken na het einde van de behandeling.

Het doel van dit proefschrift is om de klinische uitkomsten van de behandeling van chronische hepatitis C te bepalen, zowel voor als na levertransplantatie. De uitkomsten na behandeling worden vergeleken met het natuurlijk beloop van de ziekte. Daarnaast wordt onderzocht of factoren zoals overgewicht en insuline resistentie een rol spelen bij de ziekteprogressie van chronische hepatitis C.

Wij zullen proberen de vraag te beantwoorden of patiënten die een blijvende virologische respons hebben na behandeling, daadwerkelijk minder risico lopen op het ontwikkelen van leverfalen en leverkanker en of zij een betere overleving hebben. Naar aanleiding hiervan zullen behandelingsadviezen besproken worden die gebaseerd zijn op deze lange termijn uitkomsten.

LANGE TERMIJN UITKOMSTEN NA BEHANDELING

I. Patiënten met een blijvende virologische respons.

In Europa komen leverfalen, leverkanker en lever-gerelateerde sterfte nauwelijks voor bij patiënten met milde tot matige fibrose van de lever die een blijvende virologische respons hebben bereikt. In een grote studie waarin we een cohort van 286 patiënten met een blijvende virologische respons volgden (hoofdstuk 5), ontwikkelde 1% van de patiënten leverfalen en geen van de patiënten ontwikkelde leverkanker. De overleving was vergelijkbaar met de algemene bevolking, aangepast voor leeftijd en geslacht. De standaard mortaliteitsratio was 1,4 (betrouwbaarheidsinterval (BI) 0,3-2,5). Dit betekent dat de overleving van

patiënten met een blijvende virologische respons niet significant verschillend is ten opzichte van de algemene bevolking.

We vergeleken onze resultaten ook met die van andere onderzoeksgroepen. Een andere Europese studie rapporteerde geen klinische problemen zoals leverfalen, leverkanker of lever-gerelateerde sterfte in 74 patiënten met een blijvende virologische respons die gedurende 2,7 jaar gevolgd werden (11) en twee studies met respectievelijk 7 en 56 patiënten met een blijvende virologische respons lieten ook geen klinische problemen zien gedurende de 4,6 en 5,2 jaar die ze gevolgd werden (12, 13). Uit enkele grote studies blijkt dat het aantal nieuwe gevallen van leverkanker onder Japanse patiënten met een blijvende virologische respons nog altijd 0,2 tot 0,5% per jaar is (14-21). Twee studies rapporteerden een Europese patiënt met een blijvende virologische respons die toch leverkanker ontwikkelde, maar deze gevallen lijken zeldzaam en beperkt tot patiënten die al een levercirrose hebben (22, 23).

II. Lange termijn uitkomst van non-responders.

Wij hebben de data geanalyseerd van 1093 Japanse patiënten die geen blijvende virologische respons hadden op behandeling met interferon (hoofdstuk 6). Risicofactoren voor het ontwikkelen van leverkanker waren oudere leeftijd, mannelijk geslacht, hoger fibrose stadium en hogere ALAT waarden. In Japan wordt bij non-responders op interferon veelvuldig het middel glycyrrhizine voorgeschreven, omdat dit de ALAT-waarden doet dalen. In onze studie populatie toonden we echter geen beschermend effect van glycyrrhizine aan op de ontwikkeling van leverkanker. Alleen patiënten met ernstige fibrose, die op de behandeling reageren met een daling van het ALAT, hebben mogelijk baat bij het middel.

Net als interferon kan α-galactosylceramide een sterke respons van het immuunsysteem teweeg brengen. α-Galactosylceramide kan namelijk herkend worden door bepaalde afweercellen, de invariante natural killer T-cellen (iNKT-cellen), die bij stimulatie cytokines produceren zoals interferon-γ en tumor necrose factor-α.

Bij een chronische hepatitis C infectie kunnen iNKT-cellen echter ook cytokines produceren die leiden tot fibrose, bijvoorbeeld interleukine 4 en interleukine 13. Omdat bij chronische hepatitis C patiënten het aantal iNKT-cellen hetzelfde is als bij mensen zonder hepatitis C, is waarschijnlijk niet het aantal iNKT-cellen maar de werking van de iNKT-cellen van belang.

Wij onderzochten of α-galactosylceramide antivirale werking heeft tegen het hepatitis C virus bij patiënten die eerder niet reageerden op een behandeling met

interferon, en of α-galactosylceramide veranderingen in het cytokine-profiel van de iNKT-cellen tweeg brengt.

Na toediening van α-galactosylceramide bij patiënten met chronische hepatitis C trad er zoals verwacht tijdelijk een forse daling op van het aantal circulerende iNKT-cellen. Na de eerste toediening nam het percentage CD8-positieve iNKT-cellen, die met name interferon-γ produceren, toe. Alleen in de patiënten met de hoogste aantallen iNKT-cellen in hun bloed zagen we echter een toename van de interferon-γ en tumor necrose factor-α spiegels. Slechts bij één patiënt, degene met het hoogste iNKT-cel aantal, was er een evidente daling (1,3 log) te zien van het HCV-RNA. De behandeling met α-galactosylceramide bleek wel veilig, maar was dus te weing effectief.

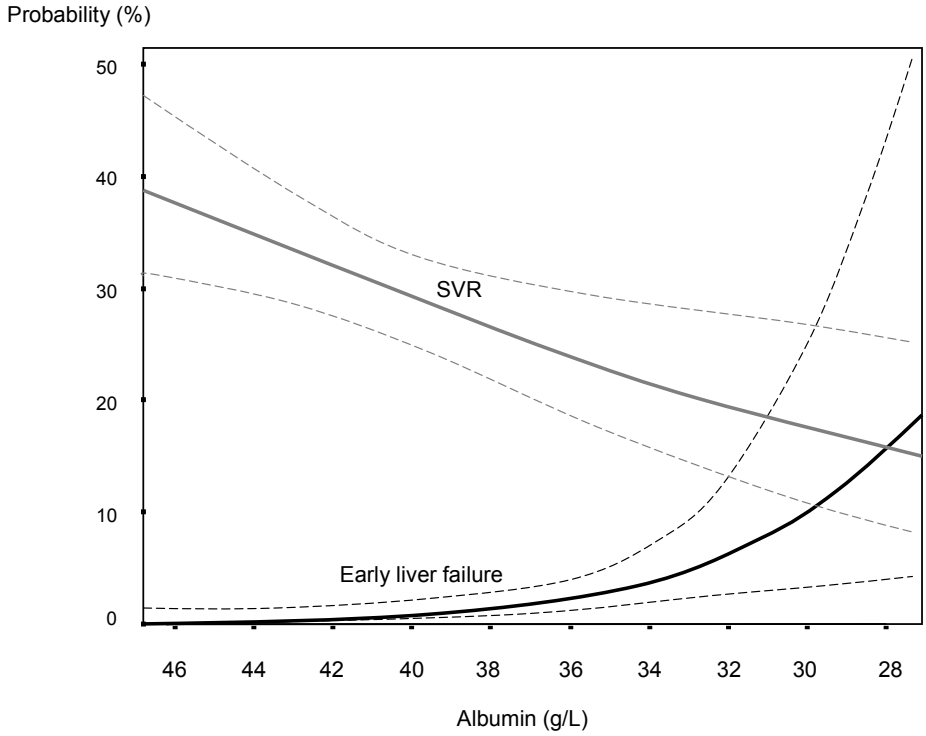
ERNSTIGE FIBROSE EN CIRROSE

In een leverbiopt kunnen verschillende stadia van leverfibrose onderscheiden worden, toenemend van fibrose stadium 0 (F0=geen fibrose) naar F1 (milde fibrose), F2 (matige fibrose) tot stadium F3 (ernstige fibrose) en uiteindelijk F4 (cirrose).

Om de lange termijn uitkomsten van patiënten met ernstige leverfibrose en cirrose nader te onderzoeken, voerden we in samenwerking met verschillende grote buitenlandse ziekenhuizen een grote studie uit waarin we ons richtten op patiënten met gevorderde ziekte (hoofdstuk 7). Voor deze studie werden op een systematische manier en volgens een tevoren vastgesteld protocol de data van alle opeenvolgende chronische hepatitis C patiënten, die behandeld werden met interferon of peginterferon, verzameld.

Geen van de patiënten die een blijvende virologische respons bereikten ontwikkelde leverfalen. Van de patiënten die niet respondeerden op de behandeling ontwikkelde daarentegen 13,3% leverfalen (p=0.001). Na 5 jaar ontwikkelde 9,2% van de patiënten met een blijvende virologische respons leverkanker ten opzichte van 13,1% van de patiënten die niet respondeerden (p=0,192). Sterfte gerelateerd aan de leverziekte trad gedurende 5 jaar op in 4,4% van de patiënten met een blijvende virologische respons vergeleken met 12,9% van de patiënten die niet respondeerden (p=0,024).

Als de albumine waarden in het bloed voorafgaand aan de behandeling lager waren dan 35 g/L, dan daalde de kans op het bereiken van een blijvende virologische respons met 30% terwijl het risico op het ontwikkelen van leverfalen gedurende de behandeling of kort daarna toenam van 2 naar 15% (figuur 1).



Figuur 1: Kans (%) op het behalen van een blijvende virologische respons (SVR) en risico op leverfalen ("early liver failure") bij verschillende albumine waarden. De gestippelde lijnen geven het betrouwbaarheidsinterval weer. De X-as loopt van hoog naar laag albumine.

In tegenstelling tot studies uit Japan, waar het gunstige effect van interferon behandelingen met name ligt in het voorkómen van het ontstaan van leverkanker (21, 24), lieten wij zien dat het in een Westerse populatie met name zorgt voor een belangrijke reductie van het ontwikkelen van leverfalen. Dit is een belangrijke bevinding omdat bij onbehandelde patiënten met cirrose in Europa vier keer zo vaak leverfalen optreedt als dat er leverkanker ontstaat (4).

In onze studie waren er nog steeds 3 patiënten met een blijvende respons die leverkanker ontwikkelden, hetgeen een incidentie betekent van 107 gevallen per 10.000 patiënt-jaren (1,1% per jaar).

Een recente studie uit Italië liet een incidentie zien van 0,7% per jaar bij patiënten met cirrose die een blijvende virologische respons hadden bereikt (25). Eerdere studies uit Japan rapporteerden een incidentie van 0,7 tot 2,5% per jaar voor patiënten met een blijvende respons en met ernstige fibrose of cirrose (14, 15, 18, 24).

Opvallend is dat 2 van de 3 patiënten uit onze studie die leverkanker ontwikkelden ondanks het feit dat ze een blijvende virologische respons hadden, suikerziekte (diabetes mellitus) hadden. Diabetes mellitus komt vaker voor bij hepatitis C patiënten dan in de algemene bevolking (4). De aanwezigheid van een leverziekte verhoogt de kans op het ontwikkelen van diabetes mellitus, omdat het de insuline regulatie negatief beïnvloedt. In een aanvullende analyse bleek echter dat na correctie voor de ernst van de leverziekte, de aanwezigheid van diabetes mellitus nog steeds een risicofactor was voor het ontwikkelen van leverkanker (hoofdstuk 9).

Het effect van diabetes mellitus op het ontwikkelen van leverkanker zou deels verklaard kunnen worden door leefstijlfactoren zoals roken, alcohol gebruik en vet eten (16). Een hoge body mass index (BMI) is een risicofactor voor diabetes mellitus en voor steatohepatitis, een leverontsteking die ontstaat door te veel vet in de lever. Verder hebben rokers een grotere kans om diabetes mellitus te ontwikkelen (17, 18) en hoewel gematigde alcohol consumptie een beschermend effect zou kunnen hebben op het ontwikkelen van diabetes mellitus, is alcohol-gebruik een risicofactor voor leververvetting (19).

Alcoholische of niet-alcoholische steatohepatitis bovenop een chronische ontsteking door hepatitis C kan leiden tot een verhoogd risico op leverkanker.

Bij patiënten met diabetes mellitus zagen we ook dat het risico op leverkanker toe leek te nemen als zij hogere nuchtere glucose waarden hadden (hoofdstuk 9). Het is aangetoond dat er een verband is tussen hogere nuchtere glucose waarden en hogere insuline waarden in patiënten met diabetes mellitus (23). Omdat bovendien uit laboratorium onderzoeken blijkt dat insuline de groei van leverkankercellen stimuleert (24, 25), denken we dat het verhoogde risico op leverkanker bij patiënten met diabetes mellitus verder verklaard zou kunnen worden door hogere insulinewaarden in het serum.

BEHANDELING VAN HEPATITIS C PATIËNTEN NA TRANSPLANTATIE

Patiënten met een eindstadium leverziekte door chronische hepatitis C infectie kunnen in aanmerking komen voor levertransplantatie. Na transplantatie treedt echter altijd weer reïnfectie op van de donorlever. Het was tot nu toe nog niet duidelijk of behandeling met peginterferon en ribavirine van zo'n reïnfectie leidt tot een betere overleving van het transplantaat. Daarom onderzochten wij alle patiënten die tussen 1995 en 2005 een levertransplantatie ondergingen vanwege hepatitis C in de Mayo Clinic te Rochester, Verenigde Staten (hoofdstuk 8). Hondervijfenzestig patiënten hadden een reïnfectie, die bewezen was met

leverbiopsie en met HCV RNA tests. Achtenzeventig van deze patiënten werden behandeld met peginterferon en ribavirine. Er waren geen verschillen in fibrose stadium, tijdsduur tussen transplantatie en reïnfectie en in ernst van de leverziekte tussen behandelde en onbehandelde patiënten. Met een multivariate analyse toonden we aan dat patiënten die een behandeling ondergingen voor hun reïnfectie, een significant betere overleving hadden van hun transplantaat. Dit betekent dat zij minder vaak een nieuwe transplantatie hoefden te ondergaan of overleden vanwege falen van de donorlever.

VERGELIJKING VAN DE UITKOMSTEN VAN BEHANDELDE PATIËNTEN MET HET NATUURLIJK BELOOP VAN DE ZIEKTE

Het is moeilijk om te onderzoeken wat de voordelen zijn op lange termijn van de behandeling van chronische hepatitis C, omdat het natuurlijk beloop van de ziekte niet duidelijk omschreven is. De consensus verklaring van het Amerikaanse National Institute of Health verklaart dat het risico op het ontwikkelen van cirrose na 20 jaar hepatitis C infectie 10 tot 15% is en dat er jaarlijks in de Verenigde Staten 10.000 tot 12.000 mensen aan de gevolgen van hepatitis C infectie overlijden (26). Hoewel zij aangeven dat de snelheid van zieteprogressie variabel kan zijn en afhangt van het geslacht en van de leeftijd ten tijde van de infectie, worden er geen precieze schattigen gegeven van het risico op lever-gerelateerde sterfte voor verschillende groepen patiënten.

De Franse consensus verklaring noemt dat de fibrose snelheid toe kan nemen na de leeftijd van 50 à 60 jaar, maar ook hier ontbreken gerichte schattingen van het risico op sterfte door de leverziekte (27).

De reden dat het natuurlijk beloop van de ziekte alleen in zulke algemene termen beschreven wordt is dat verschillende studies waarin cohorten onbehandelde hepatitis C patiënten werden onderzocht, verschillende uitkomsten laten zien. De uitkomsten variëren van een risico op lever-gerelateerde sterfte van 0,5% gedurende 25 jaar voor vrouwen die op hun twintigste geïnfecteerd raakten (28) tot een risico op lever-gerelateerde sterfte van 16% in 8 jaar voor 61 jaar oude patiënten met cirrose (29).

Nu er een behandeling bestaat voor hepatitis C is het ethisch niet meer verantwoord om patiënten niet te behandelen teneinde het natuurlijk beloop te bestuderen. Daarom ontwikkelden we een rekenmodel om het natuurlijk beloop van de ziekte te simuleren (hoofdstuk 2). In dit Markov-model worden patiënten die deel uit maken van een bepaald cohort verdeeld over verschillende ziekte stadia. De bewegingen van het ene naar het andere ziekte stadium worden

berekend over de tijd (30-32). In deze studie worden patiënten uit het fictieve hepatitis C cohort na elke 3 maanden opnieuw naar ziekte stadia toegewezen, waarbij de overgang van het ene naar het andere stadium bepaald wordt door een overgangskans die het natuurlijk beloop van de ziekte reflecteerd. Het model is gebaseerd op 15 jaar oude patiëntendata, uit een tijd waarin er nog geen behandeling voor hepatitis C bestond. De uitkomsten van dit model komen goed overeen met cohort studies uit de wetenschappelijke literatuur, die het natuurlijk beloop van hepatitis C beschrijven (33).

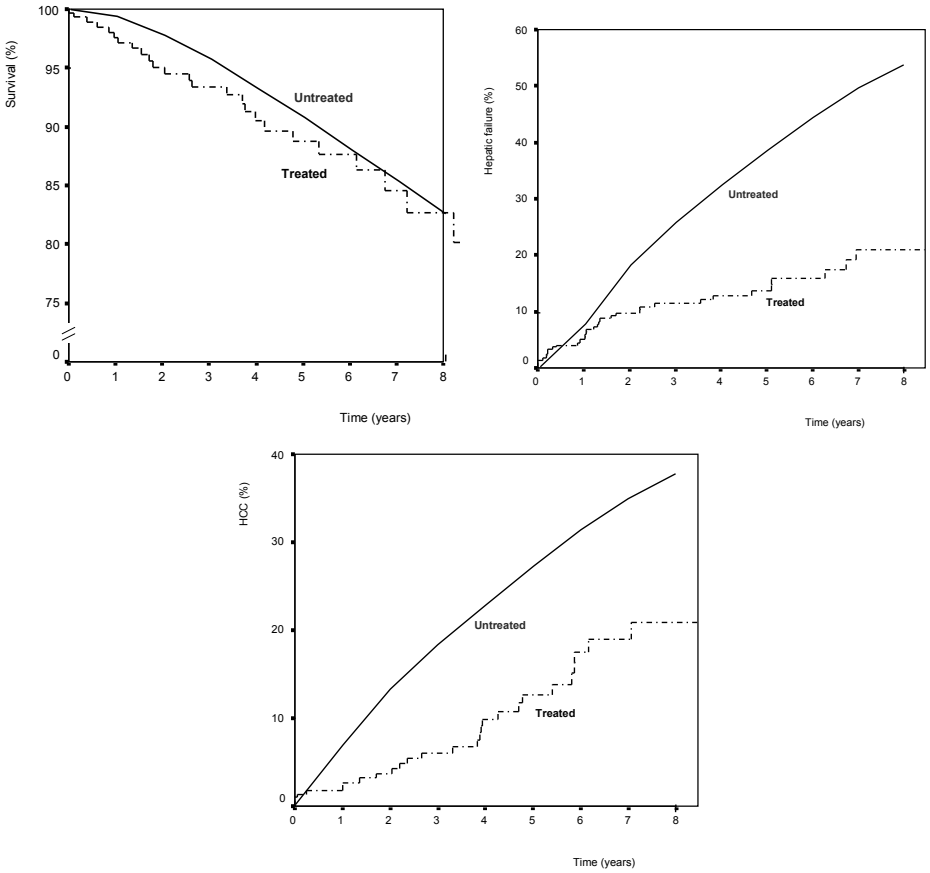
Figuur 2 laat zien dat er minder leverkanker en leverfalen optreedt bij patiënten die behandeld zijn voor hepatitis C ten opzichte van een vergelijkbare groep patiënten, gecorrigeerd voor leeftijd, geslacht, leeftijd bij infectie en fibrose stadium, berekend volgens bovenstaand model. We zien echter geen verschil in de overlevingscurves. De gemiddelde duur van 3,6 jaar waarin patiënten gevolgd zijn is mogelijk nog te kort om de verschillen in het ontstaan van leverkanker en leverfalen vertaald te zien in een betere overleving. Verder neemt het Markov-model ook sterfte aan andere oorzaken in beschouwing. Dat houdt in dat een patiënt met leverkanker of met leverfalen ook kan overlijden aan iets anders dan de leverziekte, bijvoorbeeld aan een hartaanval. Dit zou kunnen verklaren waarom het effect op algemene overleving kleiner is dan we wellicht zouden verwachten en waarom we waarschijnlijk een nog grotere groep of een langere observatie periode nodig zouden hebben om dit effect te kunnen zien.

CONCLUSIE EN BEHANDELINGSADVIEZEN GEBASEERD OP DE LANGE TERMIJN UITKOMSTEN

Als er geen sprake is van co-infectie met hepatitis B of met HIV of van alcohol-misbruik, is de ziekteprogressie meestal langzaam bij patiënten zonder fibrose of met milde fibrose. Deze patiënten zijn dus het beste af als zij gevolgd worden zonder dat er behandeling hoeft plaats te vinden. Tegenwoordig worden er echter wel kortere, effectieve behandelingen ontwikkeld voor patiënten met genotype 2 en 3. Omdat de kans op het behalen van een blijvende virologische respons groot is voor deze groep, kunnen gemotiveerde patiënten toch kandidaat zijn voor behandeling.

Voor patiënten met matige fibrose is de overleving vergelijkbaar met de algemene populatie als zij een blijvende virologische respons bereiken. Deze patiënten zijn daarom de beste kandidaten voor behandeling.

Behandeling wordt ook geadviseerd aan patiënten met ernstige fibrose door chronische hepatitis C infectie, omdat de behandeling het riscio op leverfalen



Figuur 2: Overleving (figuur 2a), optreden van leverfalen (figuur 2b) en optreden van leverkanker (HCC) (figuur 2c) bij patienten met ernstige fibrose, die behandeld zijn met (peg)interferon met of zonder ribavirine ("treated"), vergeleken met het natuurlijk beloop van de ziekte volgens het Markov-model ("untreated").

en het ontstaan van leverkanker waarschijnlijk omlaag brengt. Men moet echter uitkijken met het behandelen van patiënten met vergevorderde ziekte. Zo is het risico op het ontwikkelen van leverfalen gedurende de behandeling of kort daarna groot als het albumine-gehalte vanwege de leverziekte lager is dan de ondergrens van normaal. Voor deze patiënten geldt dat zij geëvalueerd dienen te worden door een levertransplantatie team voordat er met de behandeling begonnen wordt.

Wanneer reïnfectie met hepatitis C optreedt na transplantatie leidt behandeling met peginterferon en ribavirine tot een betere transplantaat overleving, zelfs als er geen blijvende virologische respons wordt bereikt.

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Dankwoord

Promoveren is teamwork en er zijn veel mensen die een bijdrage geleverd hebben aan dit proefschrift.

Allereerst wil ik de hepatitis C patiënten noemen die hebben deelgenomen aan de verschillende studies. Om de kennis over hun ziekte te vergroten hebben zij vaak verschillende aanvullende bezoeken aan het ziekenhuis afgelegd en extra bloed afgestaan gedurende hun behandeling. De manier waarop deze mensen zo dapper de vaak zware behandelingen ondergaan verdient respect.

Bij de uitvoering van de klinische studies was de hulp van de studie verpleegkundigen goud waard: Cockie, Heleen en Anneke bedankt! Ook de polikliniek assistenten en met name Wilma en Ronald hebben veel geholpen bij de poli voor de hepatitis C trials.

Verder wil ik Elke, Wanda, Eva en Gaalda van het trial-bureau bedanken voor hun onmisbare ondersteuning en het waken over onze *good clinical practice*.

Marion en Margriet hebben altijd klaargestaan voor secretariële ondersteuning, om te helpen bij het submitten van manuscripten en bij vele andere organisatorische zaken, dank jullie wel!

Bettina Hansen heeft een belangrijke bijdrage geleverd aan dit proefschrift. Dank je wel voor je enorme inzet en hulp bij het analyseren van alle onderzoeksgegevens. Ook al blijf ik bij je aankloppen voor hulp bij revisies en nieuwe analyses, je bent altijd even vriendelijk en geinteresseerd. Ik heb veel van je geleerd. Het mooie is dat niets jou te gek is: hebben we een meer dan ingewikkelde dataset? Dan reizen we gewoon af naar Boston om bijvoorbeeld de G-estimation te leren!

Professor Schalm, beste Solko, jij hebt geholpen dit promotie onderzoek op te zetten. Vanwege je grootse ideeën, jarenlange ervaring, vele internationale contacten, het vertrouwen dat je me altijd hebt geschonken, maar vooral vanwege je enorme enthousiasme is het altijd een groot plezier om met je samen te werken. Ik hoop nog nauw betrokken te blijven bij je nieuwste online project.

Professor Janssen, beste Harry, jij nam de begeleiding over dit onderzoek over en op een doortastende manier hielp je me niet alleen met publiceren, maar introduceerde je me *en passant* ook in één van de grootste onderzoekscentra van Amerika. Super! Nu ik weer terug ben in het Erasmus MC hoop ik nauw te blijven samenwerken met jou en je indrukwekkende onderzoeksteam.

Dr. de Man, beste Rob, toen ik in het Erasmus MC begon, hielp je om een traject uit te zetten met een keuze co-schap op de afdeling MDL, gevolgd door een

aanstelling als arts-onderzoeker. Het is fijn om opnieuw onder jouw vriendelijke en zorgvuldige begeleiding te komen gedurende de opleiding tot specialist.

Professor Kuipers, beste Ernst, dank voor het vertrouwen en voor de ondersteuning bij mijn AGIKO-traject. Ik ben heel content dat ik mijn opleiding tot MDL-arts onder jouw hoede op deze florerende afdeling kan doen.

Mijn collega's uit het Ikazia Ziekenhuis wil ik hartelijk danken voor de collegialiteit en flexibiliteit als ik weer eens op pad moest voor mijn onderzoek. Qua planning was het niet altijd eenvoudig, maar ik ben heel blij dat ook opleider Dr. Ad Dees mij de ruimte heeft gegeven om tijdens mijn vooropleiding nog 4 maanden vrij te maken voor het onderzoek.

Mijn directe collega's tijdens het onderzoek, in het bijzonder het hepatitis C team met (in wisselende samenstelling) Jilling Bergmann, Geert Bezemer, André Boonstra, Hans Brouwer, Mark Claassen, Rob de Knegt, Hans Orlent, Robert Roomer, Claudia Verveer en Jan Maarten Vrolijk; mijn kamergenoten Hayo Flink, Els Verschuur, Jesse Sarneel en later Erik Buster, maar ook alle andere collega's van de dakpoli, de flexplek en het MDL-lab: Het is heel leuk om deel uit te maken van deze grote groep jonge mensen die zich allemaal enthousiast met onderzoek bezig houden, en die bovendien ook zorgen voor een hoop gezelligheid tijdens congressen en borrels.

Many thanks to all the co-authors of the different studies published in this thesis. Dank aan onze collega's uit de VU voor hun input op immunologisch vlak tijdens de KIRIN studies, en aan Henk van Broekhoven voor zijn berekeningen aan het hepatitis C model.

I would also like to thank our colleagues from Germany (Dr. Heiner Wedemeyer, Dr. Peter Hofmann, Prof. Dr. Michael Manns and Prof. Dr. Stefan Zeuzem), Switzerland (Prof. Dr. Juerg Reichen) and Canada (Prof. Dr. Jenny Heathcote) who hosted me during my visits for the advanced fibrosis study and who contributed to the success of this study.

Many thanks also to Prof. Dr. Michael Charlton and his co-workers Kim and Anu for their hospitality during my stay in Rochester and for involving me in their research on insulin resistance, steatohepatitis and on liver transplantation for viral hepatitis.

Mijn vrienden en familie ben ik dankbaar voor hun interesse in mijn onderzoek, maar ook voor alle leuke dingen die we buiten werk en studie om ondernemen. Verder wil ik mijn ouders speciaal bedanken voor hun onvoorwaardelijke steun en vertrouwen.

Mijn paranimfen Carla en Eelco: het is super om 2 goede vrienden èn ervaren post-docs te hebben die mij bijstaan bij de verdediging van mijn proefschrift.

Lieve Petra, ik vind het geweldig om met je samen te zijn. Fantastisch ook dat we samen voor ons onderzoek naar het buitenland konden gaan. Ik kijk uit naar ons huwelijk, 2008 is een heel speciaal jaar voor ons!

Curriculum Vitae

De auteur van dit proefschrift werd op 20 oktober 1976 geboren te Heerhugowaard. Van 1989 tot 1995 volgde hij de opleiding voorbereidend wetenschappelijk onderwijs aan de Openbare Scholengemeenschap te Schagen.

In 1995 startte hij met de studie geneeskunde aan de Universiteit van Leiden. Hij was tijdens zijn studie als student-assistent werkzaam bij de afdeling fysiologie en hij verrichtte zijn afstudeeronderzoek aan de universiteit van Rennes, Frankrijk, waar hij onder leiding van Dr. R. Moirand en professor P. Brissot de indicatie voor levertransplantatie onderzocht bij patienten met alcoholische levercirrose. Tijdens zijn studie was hij actief als bestuurslid bij studentenvereniging SSR-Leiden.

Na het cum laude behalen van zijn artsexamen in 2002, begon hij als arts-onderzoeker in het Erasmus MC te Rotterdam aan het onderzoek naar de lange termijn uitkomsten van de behandeling van chronische hepatitis C, dat beschreven is in dit proefschrift (supervisor Prof.dr. S.W. Schalm, in 2006 opgevolgd door Prof.dr. H.L.A. Janssen). In 2003 werd hem een AGIKO-beurs toegekend door ZonMw, de Nederlandse organisatie voor gezondheidsonderzoek en zorginnovatie, die hem in staat stelt om zijn opleiding tot maag-, darm- en leverarts te combineren met zijn werk als arts-onderzoeker. Van januari 2005 tot mei 2007 deed hij de vooropleiding Interne Geneeskunde (opleider Dr. A. Dees) in het Ikazia Ziekenhuis te Rotterdam. Onder leiding van Prof. M.R. Charlton verrichtte hij vervolgens gedurende 7 maanden onderzoek naar het effect van insuline resistentie op progressie van leverfibrose bij hepatitis C patiënten, in de Mayo Clinic te Rochester, Verenigde Staten. Vanaf december 2007 tot heden vervolgt hij zijn opleiding tot maag-, darm- en leverarts in het Erasmus MC te Rotterdam (opleider Dr. R.A.de man en afdelingshoofd Prof.dr. E.J. Kuipers). In oktober 2008 trouwt hij met Petra Kok.