1. TITLE PAGE

Title

Improvement of platelets after SVR among patients with chronic HCV infection and advanced hepatic fibrosis

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HCV; hepatitis C virus, HCC; hepatocellular carcinoma, SVR; sustained virological response; IQR; interquartile range, AUC; area under the curve, BMI; body mass index, CI; confidence interval, HR; hazard ratio, TPO; thrombopoietin, HVPG; hepatic venous pressure gradient,

Keywords

Chronic hepatitis C, cirrhosis, regression, platelet count, sustained virological response

Conflict of interest

Dr. van der Meer received financial compensation for lecture activities for MSD and Gilead. Drs. Maan received financial compensation for consultancy activities from AbbVie. Dr. Veldt received financial compensation for a board membership at GlaxoSmithKline and Janssen Therapeutics. Dr. Feld received financial compensation for consultancy, and

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Author contributions:

Dr. Adriaan J. van der Meer and Prof. Dr. Harry L.A. Janssen had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Van der Meer, Maan, Veldt, Feld, Wedemeyer, Dufour, Lammert, Duarte-Rojo, Manns, Zeuzem, Hofmann, De Knegt, Hansen, and Janssen.

Acquisition of data: Van der Meer, Maan, Veldt, Feld, Wedemeyer, Dufour, Lammert, Duarte-Rojo, and De Knegt.

Analysis and interpretation of data: Van der Meer, Maan, Veldt, Hansen, and Janssen.

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Critical revision of the manuscript for important intellectual content: Van der Meer, Maan, Veldt, Feld, Wedemeyer, Dufour, Lammert, Duarte-Rojo, Manns, Zeuzem, Hofmann, De Knegt, Hansen, and Janssen.

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2. ABSTRACT

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

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

Results. This study included 464 patients; 353 (76%) had cirrhosis and 187 (40%) attained sustained virological response (SVR). Among patients with SVR, median platelet count, increased by 35 $x10^9$/L (IQR 7-62, $p<0.001$). In comparison, patients without SVR showed a median decline of 17 $x10^9$/L (IQR -5-47, $p<0.001$). In a subgroup of 209 patients, median decrease in spleen size was 1.0 cm (IQR 0.3-2.0) for patients with SVR, while median spleen size increased with 0.6 cm (IQR -0.1-2.0, $p<0.001$) among those without SVR. The changes in spleen size and platelet count were significantly correlated ($R=-0.41$, $p<0.001$).

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

3. MANUSCRIPT

Introduction

The continuous inflammation in livers of patients with chronic hepatitis C virus (HCV) infection may cause hepatic fibrogenesis. Progression of this process may eventually lead to cirrhosis, at which stage patients have an unfavorable prognosis due to the elevated risk of hepatocellular carcinoma (HCC) and liver failure.(1) In one of the largest studies on the fibrosis progression rate, dating back to 1997, it was estimated that 33% of patients with chronic HCV infection develop cirrhosis within 20 years.(2) However, the number of patients who develop cirrhosis could be higher over a longer period of time as fibrosis development may not be linear. In fact, as the population with chronic hepatitis C is aging, it is expected that the incidence of HCV-related cirrhosis will increase during the upcoming years.(3)

The treatment of chronic HCV infection improved enormously during the last two decades. Even in case of advanced hepatic fibrosis, sustained virological response (SVR) rates over 90% can be achieved with combination regimens of direct-acting antiviral agents.(4-8) Several studies showed that hepatic fibrosis can regress once HCV is eradicated as causative agent of liver injury, also among patients with advanced hepatic fibrosis.(9-16) However, these studies are limited by a short follow-up duration or low number of patients with cirrhosis. Also, there is significant sampling error with respect to percutaneous liver biopsy, which remains an invasive procedure with potentially severe complications so that repeated assessments of liver histology are often not feasible.(16-18) The longitudinal pattern of hepatic fibrosis regression is thus difficult to study.

The platelet count is strongly related to the degree of hepatic histopathological abnormalities and portal pressure, especially among those patients with bridging fibrosis or cirrhosis.(19-26) Indeed, lower platelets have been repeatedly associated with a higher risk for cirrhosis-related morbidity and mortality, which supports that the platelet count is representative of the stage of liver disease.(1, 27-29) Importantly, the change in platelets correlated with the change in hepatic fibrosis following antiviral therapy among patients with

chronic HCV infection, including those who attained SVR.(30, 31) Changes in platelets thus represent a non-invasive alternative to assess the evolution of the stage of liver disease and portal pressure. Because splenic sequestration of blood cells as a direct result of elevated portal pressure causes the spleen to increase in size, splenomegaly is also considered a non-invasive marker of the degree of portal hypertension or presence of esophageal varices.(22-24)

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

Patients and Methods

All consecutive patients with chronic HCV infection and bridging fibrosis or cirrhosis (Ishak fibrosis score 4-6) who initiated interferon-based antiviral therapy between 1990 and 2003 were included from 5 large hepatogy units in Europe and Canada. The design of this retrospective cohort study have been described in detail previously.(29) For the current study, the patients were assessed from the last received interferon-based treatment course onwards. Hereby it was prevented that interferon-induced bone marrow suppression influenced the platelet counts during follow-up as a result of retreatment. Excluded were patients with a human immunodeficiency virus or hepatitis B virus co-infection, patients who had developed HCC or liver failure prior to start of follow-up or prior to the first available platelet count measurement during follow-up, and patients who received long-term low-dose pegylated interferon maintenance therapy. Patients without follow-up beyond January 1st 2010 were invited for a single visit to the outpatient clinic.

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

Outcome measures

Pre-treatment markers of liver disease severity closest to the start of therapy were included, as long as these were available within six months before the start of antiviral therapy. All available platelet count measurements were registered from 24 weeks after cessation of interferon-based antiviral therapy. A platelet count <150 $x10^9$/L was defined as thrombocytopenia. Measurements obtained after the diagnosis of HCC or liver failure were not considered. The diagnosis of HCC was based on histopathological confirmation or 2 coincident imaging techniques (computed tomography, magnetic resonance imaging or contrast-enhanced ultrasonography) showing a focal lesion larger than 2 centimeters with arterial-phase hyper-enhancement or 1 imaging technique showing a focal lesion larger than 2 centimeters with arterial-phase hyper-enhancement in the presence of an α-fetoprotein level greater than 400 ng/mL.(32) Liver failure was defined as an episode of ascites, bleeding varices, jaundice or overt hepatic encephalopathy. Only the first episode of liver failure was considered in case patients experienced multiple liver failure events. The last available spleen size measurement (in centimeters), as determined by radiological examination (ultrasound, computed tomography or magnetic resonance imaging), was registered. Spleen sizes which were measured after initiation of treatment for HCC or portal hypertension were not considered, and in such cases the last spleen size prior to these events was used.

Statistical analyses

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

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

Linear regression analysis was used to determine which baseline variables were associated with the change in platelets from baseline to last follow-up among the patients who attained SVR. Repeated measurement analyses with a random intercept and slope per patient and an unstructured covariance matrix were performed to analyze the evolution of platelets over time, correcting for potential non-linearity by including the squared time to the platelet count measurement into the model. As chronic HCV infection and interferon therapy can influence the platelets as well, 24 weeks after cessation of antiviral therapy was considered as time zero in the repeated measurement analyses.

All statistical tests were two-sided, and a *p*-value <0.05 was considered to be statistically significant. SPSS version 17.0.2 (SPSS Inc., Chicago, IL, USA) and SAS 9.2 PROC GENMOD (SAS institute, Cary, NC) were used for all statistical analyses.

Results

Study population

Between 1990 and 2003, 546 patients with chronic HCV infection and histological proof of advanced hepatic fibrosis received interferon-based antiviral therapy. Eight patients who were lost-to-follow-up and 8 patients who developed HCC or liver failure before 24 weeks after their initial treatment course were excluded. Of the remaining 530 patients, 125(24%) attained SVR and 405(76%) did not. During follow-up 204 patients without SVR were retreated at least once, which resulted in SVR for another 67 patients. Among retreated patients, 7 experienced a cirrhosis-related complication before 24 weeks following

their last treatment course, 14 received long-term low dose pegylated interferon maintenance therapy, and 45 had no available platelet count measurement during follow-up. These patients were excluded as well, so that the total study cohort consisted of 464 patients; 187 with SVR and 277 without SVR (**Figure 1**). **Table 1** summarizes the baseline characteristics according to the virological response to the last antiviral treatment course.

Platelet count in relation to hepatic fibrosis

At baseline, platelet counts were associated with histological stage of fibrosis: the median platelet count was 186 $x10^9$/L (interquartile range [IQR] 143-226) among patients with Ishak fibrosis score 4, 160 $x10^9$/L (IQR 134-209) among patients with Ishak fibrosis score 5 and 133 $x10^9$/L (IQR 92-176) among patients with Ishak fibrosis score 6 (p<0.001). Accordingly, the percentage of patients with thrombocytopenia increased with higher Ishak fibrosis score (31%, 40% and 60% for Ishak fibrosis score 4, 5 and 6, respectively, p<0.001). The platelet count could largely discriminate patients with Ishak fibrosis score 4/5 from those with Ishak 6 (area under the curve [AUC] 0.70, 95% confidence interval [CI] 0.65-0.75, p<0.001). The AUC of the platelet count was similar to differentiate between Ishak 4 and 5/6 (AUC=0.69, 95%CI 0.63-0.74, p<0.001). The discriminating abilities of bilirubin, albumin or the ratio between the aspartate and alanine aminotransferase were lower (AUCs ranging from 0.56 to 0.62). Logistic regression analyses indicated that the platelet count was the only objective laboratory marker of liver disease severity that had an independent statistically significant association with the baseline stage of hepatic fibrosis (**Table 2**).

Changes in platelets following antiviral therapy

During the follow-up which started 24 weeks post-treatment, 3387 platelet count measurements were registered. The median interval between platelet count measurements was 0.45 years (IQR 0.13-0.79), which differed between patients with SVR (0.54 years, IQR 0.28-1.02) and patients without SVR (0.31 years, IQR 0.12-0.61, p<0.001). The last available platelet counts were measured after a median of 5.7 (IQR 2.1-7.6) years among

patients with SVR and after 4.4 (IQR 1.9-7.1) years among patients without SVR (p=0.111). The median last platelet count was 198 x10^9/L (IQR 166-248) in the group with SVR and 113 x10^9/L (IQR 73-167) in the group without SVR (p<0.001). In 426 patients a platelet count measurement was available both at baseline as well as during follow-up. Among those with SVR, 44(62%) of the patients with thrombocytopenia at baseline showed a normal platelet count at the time of the last measurement (*p*<0.001), while only 2(2%) patients with SVR and normal platelets at baseline had thrombocytopenia at the final measurement (**Table 3**). The platelet counts in these patients were 122 x10^9/L and 134 x10^9/L at the end of follow-up, respectively. Among patients without SVR, 47(43%) patients changed from a normal platelet count at baseline to thrombocytopenia at the end of follow-up (*p*<0.001), while 14 (10%) patients with thrombocytopenia at baseline had normal platelet counts at the time of the last measurement. From baseline to the last available measurement, the platelet count showed a median increase of 35 x10^9/L (IQR 7-62) among the patients with SVR and a median decrease of 17 x10^9/L (IQR -5-47) among patients without SVR (p<0.001; for the paired analyses within each response group as well as for the comparison between both response groups).

Linear regression analyses were performed to assess which factors were associated with improvement of platelets following SVR (**Table 4**). Higher body mass index (BMI) was negatively associated with the change in the platelet count (β=-1.59, standard error 0.78, *p*=0.043). These analyses were corrected for the baseline platelet count and the time from SVR to the last platelet count measurement.

Repeated measurement analyses showed a gradual and almost linear increase in platelets beyond the moment of SVR, while the platelet counts further declined among those patients who did not attain SVR (p<0.001; for the change within each response group as well as for the comparison between both response groups) (**Figure 2**).

Spleen size

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

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

Discussion

With this study we showed that the platelet counts improved following eradication of chronic HCV infection among patients with bridging fibrosis or cirrhosis. With a repeated measurement analysis including over 3000 platelet count evaluations, a rather linear increase in platelets was observed from the moment of SVR onwards. In contrast, patients who did not attain SVR showed a further decline. The increase in platelets continued for

many years after SVR, suggesting that the histopathological abnormalities and portal pressure gradually improve among chronic HCV-infected patients with advanced liver disease who were successfully treated. This was further substantiated by the reduction in spleen size among the patients who had attained SVR.

Four previous studies have assessed the change in platelets after SVR among Western patients with chronic HCV infection.(10, 33-35) George et al. followed 150 patients with interferon-induced clearance of their chronic HCV infection for 5 years.(10) In this study the mean pre-treatment platelet count (232 $x10^9$/L) did not significantly differ from the mean last measured platelet count during follow-up (235 $x10^9$/L). However, only 16 (11%) patients with cirrhosis were included in this study, at which stage platelet counts are most affected. Although limited by the inclusion of only 10 (10%) patients with cirrhosis as well, another follow-up study did show that the mean platelets significantly increased from 209 $x10^9$/L at baseline to 239 $x10^9$/L at the final follow-up (which ranged from 1 to 22 years after successful therapy) among 100 patients with SVR.(34) The change in platelets following antiviral therapy was also assessed in the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) trial, which only included patients with at least Ishak F3 fibrosis.(28) In contrast to both above-described studies, a control group without SVR was included. As compared to pre-treatment levels, the mean platelet count was already significantly increased at the time SVR was attained (24 weeks after cessation of therapy). An additional post-hoc assessment among the patients with SVR after approximately 5 years of follow-up indicated that the platelet counts further increased with time. In line with our findings, the platelet counts declined among patients without SVR.

With repeated measurement analyses including all platelet count assessments during follow-up, we have showed the evolution of platelets according to the virological response following antiviral therapy among patients with bridging fibrosis or cirrhosis in more detail. Pre-treatment platelet counts were not included in these analyses as we started the follow-up at the time of SVR. This is important, because other mechanisms than splenic sequestration as a result of elevated portal pressure may reduce the platelet counts among

patients with chronic HCV infection as well, such as reduced thrombopoietin (TPO) production, HCV-mediated bone marrow suppression and the presence of autoantibodies, causing chronic immune thrombocytopenic purpura.(36) At the time of SVR, when HCV has been suppressed for more than 6 months, bone marrow suppression due to HCV or interferon-based therapy should no longer be of influence.(37, 38) Increasing TPO levels could continue to be relevant for the rise of platelets after SVR, since the production of TPO has been negatively correlated with the degree of hepatic fibrosis.(39, 40) In this case regression of hepatic fibrosis and improvement of liver function would thus remain the underlying cause for the improved platelet counts. Nevertheless, lowering of portal pressure and reversal of splenomegaly is likely to remain a predominant reason for the increase in platelets once HCV is eradicated as cause of liver injury. Indeed, the change in platelets and change in spleen size were correlated in our study, which has not been shown by any of the previous studies. A limitation of the current study, however, is that data on baseline spleen size were available in only a limited number of the included patients, also because we restricted the baseline period to 6 months prior to the start of antiviral therapy.

Another interesting result of our study was that higher BMI was negatively associated with the increase in platelet counts among the patients with SVR. Currently, risk factors for disease progression following viral eradication remain largely unknown. Our finding might be explained by the presence of hepatic steatosis and inflammation among patients with high BMI.(41, 42) Although data on the presence of steatosis is lacking in our cohort, its association with higher BMI is well known.(43) To what extend these or other aspects of the metabolic syndrome may impact the clinical course of cirrhosis after patients have cleared their chronic HCV infection requires further study. For now, life-style modifications to reduce obesity may be advocated.(44)

Despite the limitations of percutaneous liver biopsy (ethical concerns regarding multiple measurements and the subjective character of hepatic fibrosis assessment), the impact of antiviral therapy on liver histology among patients with chronic HCV infection has been studied.(9, 10, 15, 16, 45) The largest study to date included almost 700 patients with

at least METAVIR F2 fibrosis at baseline from 4 randomized controlled trials.(15) Even though all patients were biopsied already 24 weeks after cessation of antiviral therapy, patients with SVR showed regression of hepatic fibrosis while patients without SVR had rather stable liver disease, with median estimated annual METAVIR fibrosis progression rates of -0.591 and 0, respectively. However, 24 weeks after cessation of antiviral therapy might too early to assess the true impact of successful antiviral therapy on liver histology. Indeed, a prior study including 183 patients with various degrees of HCV-induced hepatic fibrosis and SVR indicated that regression of hepatic fibrosis takes time, as the regression of fibrosis was more pronounced in case of longer post-SVR follow-up.(16) Our data, showing the continued increase in platelets for many years after SVR, is in line with this finding. A recent histological study including patients with HCV-related cirrhosis and SVR showed that 23 (61%) patients had a reduction of the METAVIR F4 fibrosis score in their liver biopsy obtained after a median of approximately 5 years following treatment cessation.(9) Interestingly, even though the METAVIR F4 score was not reduced in the remaining 15 (39%) patients, morphometric analyses indicated that their total liver collagen content was still significantly reduced. Because the semi-quantitative hepatic fibrosis scores, by which regression of hepatic fibrosis has been largely assessed so far, may thus be somewhat too crude, objective and continuous variables for the assessment of changes in hepatic fibrosis are relevant to fully appreciate the impact of SVR on liver disease severity. As the change in platelets has been linked to the change in hepatic fibrosis, the platelet count represents an easily accessible biomarker to assess histological improvement.(30, 31) We remained with the platelet count as outcome measure in our study because many other laboratory-based non-invasive fibrosis markers showed a poor accuracy for hepatic fibrosis assessment among patients with SVR.(46) Perhaps this is explained by the fact that most include parameters of hepatic inflammation, which is also affected by viral eradication.

Regression of hepatic fibrosis, especially in case of cirrhosis, was recently shown to be clinically relevant. In a pivotal study by Mallet et al. clinical outcome was superior among chronic HCV-infected patients with cirrhosis who showed a substantial reduction of their

post-treatment METAVIR score as compared to patients who did not regress.(11) In addition, other large cohort studies have indicated that SVR was also associated with a reduced occurrence of liver failure, HCC and mortality among patients with advanced liver disease.(27-29, 47-49) Only a few studies have directly measured the hepatic venous pressure gradient (HVPG) before and after antiviral therapy.(50-52) All included only a small number of patients, but showed that SVR was significantly associated with a reduction in HVPG, which remains one of the best validated surrogate markers in hepatology. Our finding of a decrease in spleen size following SVR in a large group of patients with bridging fibrosis or cirrhosis further substantiates the reduction of portal pressure once the chronic HCV infection is eradicated.

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

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Table 1. Baseline characteristics according to virological response

	Overall (n = 464)	With SVR (n = 187)	Without SVR (n = 277)	*p*-value
Age, years	51 (44-57)	49 (44-57)	51 (44-58)	0.071
Male, *n/total* (%)	321/464 (69)	137/187 (73)	184/277 (66)	0.118
BMI, kg/m^2 [a]	25.9 (23.4-28.7)	25.6 (23.1-28.6)	26.1 (23.8-29.0)	0.135
Fibrosis score, *n/total* (%)				0.429
- Ishak 4	111/464 (24)	50/187 (27)	61/277 (22)	
- Ishak 5	91/464 (20)	33/187 (18)	58/277 (21)	
- Ishak 6	262/464 (56)	104/187 (55)	158/277 (57)	
HCV genotype, *n/total* (%)				<0.001
- 1	300/440 (68)	93/179 (52)	207/261 (79)	
- 2	42/440 (10)	32/179 (18)	10/261 (4)	
- 3	79/440 (18)	46/179 (26)	33/261 (13)	
- 4	15/440 (3)	6/179 (3)	9/261 (3)	
- Other	4/440 (1)	2/179 (1)	2/261 (1)	
Type of treatment, *n/total (*%)				<0.001
- IFN mono	70/464 (15)	9/187 (5)	61/277 (22)	
- IFN and RBV	110/464 (24)	49/187 (26)	61/277 (22)	
- pegIFN mono	12/464 (3)	4/187 (2)	8/277 (3)	
- pegIFN and RBV	253/464 (54)	118/187 (63)	135/277 (49)	
- ConsensusIFN (+/- RBV)	11/464 (2)	2/187 (1)	9/277 (3)	
- pegIFN and RBV and PI	8/464 (2)	5/187 (3)	3/277 (1)	
Laboratory markers of liver disease severity [b]				
- Platelet count, x10^9/L	150 (112-199)	162 (132-205)	142 (100-191)	<0.001
- Albumin, g/L	42 (39-44)	42 (40-44)	41 (38-44)	0.016
- Bilirubin, µmol/L	13 (10-18)	12 (9-15)	14 (10-19)	<0.001
- AST/ALT ratio	0.73 (0.59-0.92)	0.68 (0.55-0.82)	0.76 (0.62-0.97)	<0.001
Spleen size, cm	12.5 (11.0-14.3)	12.0 (10.6-13.8)	12.9 (11.2-14.8)	0.012
Treatment naïve, *n/total* (%)	267/464 (58)	109/187 (58)	158/277 (57)	0.789
Year treatment started	2001 (1998-2003)	2002 (2000-2003)	2001 (1998-2003)	0.003
Treatment duration, weeks	31 (22-48)	48 (25-49)	24 (16-48)	<0.001
Diabetes mellitus, *n/total* (%)	71/464 (15)	20/187 (11)	51/277 (18)	0.024
History of severe alcohol use, *n/total* (%) [c]	100/437 (23)	38/180 (21)	62/257 (24)	0.460
AntiHBc positivity, *n/total* (%)	168/364 (46)	63/141 (45)	105/223 (47)	0.654

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

[a] BMI was missing in 24 (13%) patients with SVR, and 61 (22%) patients without SVR.

[b] Platelet count was missing in 9 (5%) patients with SVR and 29 (10%) patients without SVR. Albumin was missing in 13 (7%) patients with SVR and 48 (17%) patients without SVR. Total bilirubin was missing in 14 (7%) patients with SVR and 36 (13%) patients without SVR. The AST/ALT ratio was missing in 15 (8%) patients with SVR and 37 (15%) patients without SVR. The spleen size was missing in 88 (47%) patients with SVR and 130 (47%0 patients without SVR.

[c] Severe alcohol use was defined as the use of more than 50 gram of alcohol per day.

Table 2. Logistic regression analyses for the stage of hepatic fibrosis

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

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

Table 3. Thrombocytopenia at baseline versus follow-up

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

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

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

Table 4. Linear regression analyses for the change in platelets among patients with SVR [a]

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

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

[a] The unstandardized coefficients are reported.

[b] The β indicates that for each unit increase of the predictor variable, the change in platelets will increase (in case of a positive β) or decrease (in case of a negative β) by β units. So, when specified to our multivariate analysis above, for every 1 kg/m^2 increase in BMI at baseline, the change in platelets will be 1.59 x10^9/L lower.

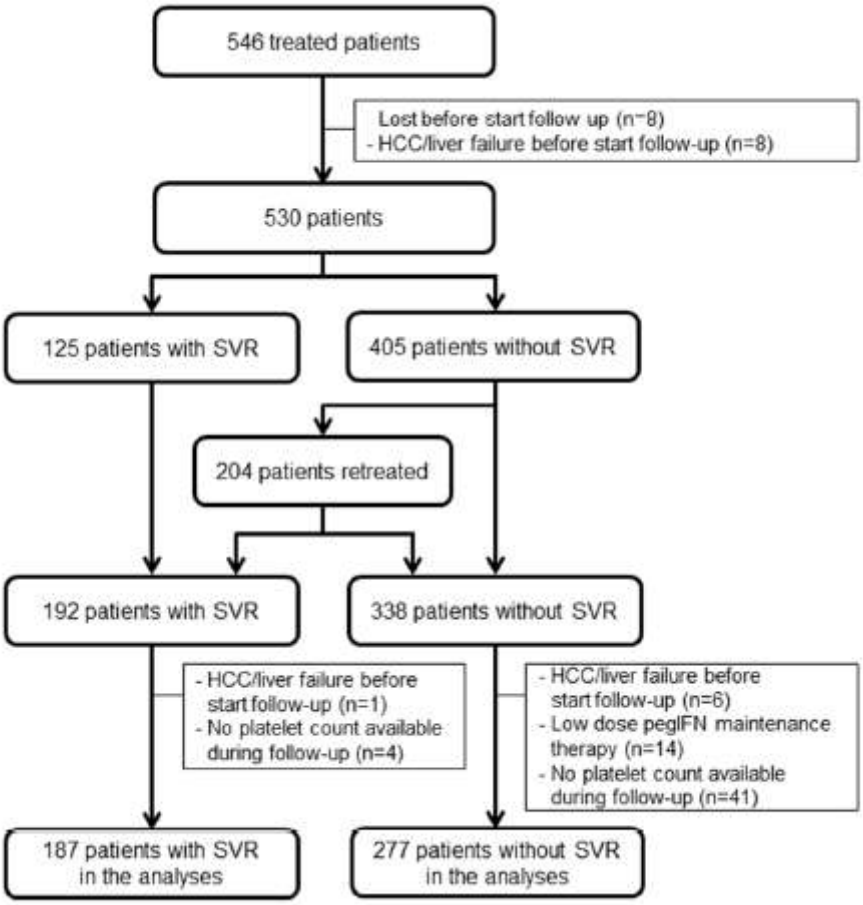

Figure 1. Study flow chart

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

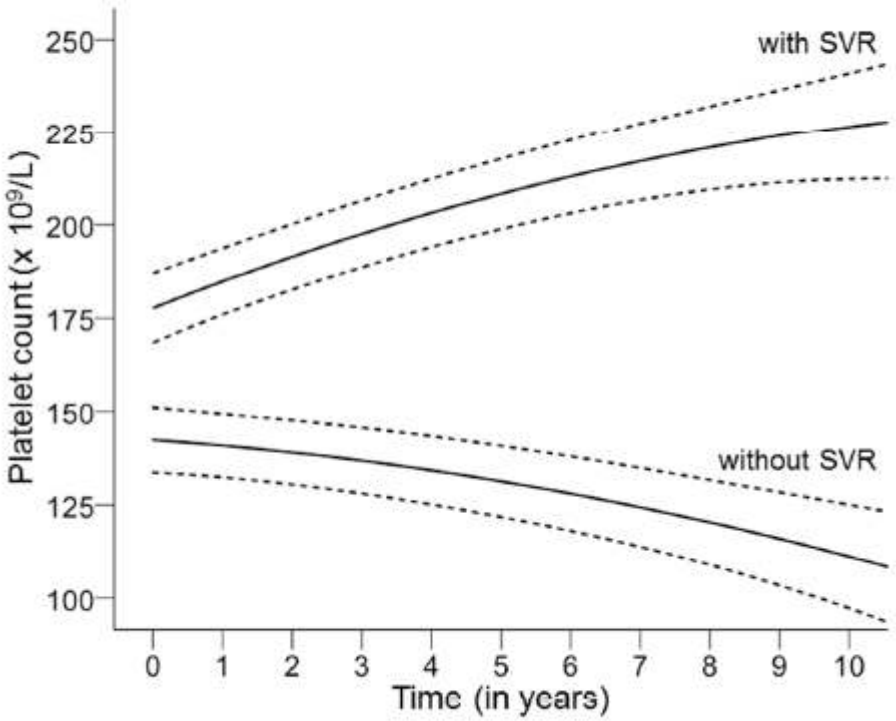

Figure 2. Evolution of platelet counts according to virological response

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