MAJOR ARTICLE

Toward the Establishment of a Prediction System for the Personalized Treatment of Chronic Hepatitis C

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Background. Although several direct-acting antivirals (DAAs) are now available, the therapy regimen for chronic hepatitis C will continue to include pegylated interferon and ribavirin for the foreseeable future. Despite their improved rate of sustained virological response (SVR), DAAs pose increased risks of side effects and selection for antiviral resistance. Not all patients require DAA to achieve SVR, whereas others are unlikely to respond even to triple therapy. Therefore, a personalized approach to candidate selection is necessary.

Methods. In this retrospective study, data from 640 Japanese patients who were treated for chronic hepatitis C genotype 1, 2, or 3 with pegylated interferon plus ribavirin combination therapy was compiled to identify robust pretreatment predictive factors for SVR.

Results. A logistic regression model for personalized therapy was developed based on age, viral genotype, initial viral load, aspartate aminotransferase/alanine aminotransferase ratio, α-fetoprotein levels, and *IL28B* single-nucleotide polymorphism genotype. The area under the receiver-operating characteristic curve (AUC) was 0.85. The mean AUC following 10 rounds of 10-fold cross validation was 0.82, with a true positive rate of 78.2%.

Conclusions. A personalized approach to therapy may better inform treatment decisions and reduce incidence of side effects and antiviral resistance.

The hepatitis C virus (HCV) affects >100 million people worldwide and is a major global cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1–5]. The current standard of care, pegylated interferon (PEG-IFN) plus ribavirin combination therapy, is both expensive and poorly tolerated, and treatment efficacy is <50% for genotype 1b [6].Telaprevir and boceprevir, 2 direct-acting antiviral (DAA) protease inhibitors, have recently been approved for clinical use in the United States [7] and are expected to improve the rate of sustained virological response (SVR) to 65%–75% [8]. However, the addition of a DAA to the current standard of care increases the risk of side effects, including anemia and rash, and failure to achieve SVR may pose an increased risk of accumulating protease inhibitor–resistant viral strains that may be recalcitrant to future treatment [8]. Consequently, it may be advantageous to identify patients who are unlikely to respond to therapy, as well patients who are likely to achieve SVR under the current standard of care without requiring a DAA. Patients who are able to achieve at least a transient response (relapsers) under combination therapy are more likely to achieve a SVR under triple therapy, whereas patients who fail to respond to combination therapy are also less likely to respond to triple therapy [9]. Therefore, it may be possible for patients who are highly likely to respond to combination therapy to be spared the additional risks

Received 23 June 2011; accepted 1 September 2011; electronically published 28 November 2011.

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The Journal of Infectious Diseases 2012;205:204–10

DOI: 10.1093/infdis/jir726

and costs of triple therapy, but to determine the optimal treatment for each patient, reliable and inexpensive pretreatment predictors are needed for SVR.

A number of pretreatment predictors associated with SVR or nonresponse have been reported. Older female patients have been shown to respond poorly to therapy in Japan [10–12], and metabolic factors such as obesity [13], insulin resistance [13], hepatic steatosis [14], low-density lipoprotein (LDL) cholesterol levels [15, 16], and γ-glutamyl transpeptidase (γ-GTP) levels [17] have been shown to influence treatment outcome. Baseline virus titer is also an important predictor of treatment outcome [14, 18]. HCV genotypes differ in the response to interferon therapy, with genotypes 1 and 4 considered more difficult to treat than genotypes 2 and 3 [18, 19]. The importance of these and other factors in triple therapy remains unclear, although they may influence the effectiveness of interferon and ribavirin in suppressing emergence of resistant strains.

Genetic differences among patients also influence response to treatment and incidence of side effects. Genomewide association studies have reported common single-nucleotide polymorphisms (SNPs) predictive of response to interferon therapy. A set of linked SNPs within the *IL28B* locus on chromosome 19 has recently been shown to be the strongest predictor of sustained virological response as well as spontaneous viral clearance [20–26]. So far, the SNP also appears to be the strongest predictor for triple therapy [9, 27]. Other SNPs are associated with the occurrence of side effects. In particular, SNPs in the *ITPA* locus have been found to be associated with anemia in patients treated with PEG-IFN plus ribavirin combination therapy [28–30] and appear to be predictive of anemia in triple therapy as well [31]. Although there are currently few options for treating HCV, SNP genotyping may nonetheless help gauge expectations and help identify patients at risk for severe side effects that may disrupt the course of therapy.

Even though telaprevir and boceprevir are now available for use in clinical practice, DAAs must be coadministered with PEG-IFN and ribavirin, to prevent rapid selection for resistance mutations [32]. As a result, patients who respond poorly to PEG-IFN and ribavirin may not only fail to achieve SVR under triple therapy but may be more likely to encounter viral breakthrough, with confounding effects on future treatment efforts. Consequently, there remains a need to identify robust predictors for response to PEG-IFN and ribavirin to establish a personalized approach for treatment of chronic hepatitis C.

METHODS

Patients

Data from 640 patients who were treated with PEG-IFN plus ribavirin combination therapy for chronic HCV infection were compiled from hospitals belonging to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/e/) in Hiroshima, Japan. All patients were interferon treatment–naive and were infected with HCV genotype 1, 2, or 3. Study participants tested positive for HCV RNA over a span of >6 months, tested negative for hepatitis B and human immunodeficiency virus (HIV), and showed no evidence for other liver diseases. All patients gave written informed consent to participate in the study in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and according to the process approved by the ethical committees of Hiroshima University and the SNP Research Center at the Institute of Physical and Chemical Research in Yokohama. Patient profiles are shown in Table 1 and Figure 1.

PEG-IFN Plus Ribavirin Combination Therapy

Patients received weekly injections of PEG-IFN-α-2b at 1.5 μg/kg body weight for 48 weeks. Ribavirin was administered orally, and the dosage was determined based on the patient's body weight (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg), based on guidelines by the Ministry of Health, Labor, and Welfare of Japan [33]. The ribavirin dose was reduced when hemoglobin levels fell below 10 g/dL, and both PEG-IFN and ribavirin were discontinued when hemoglobin levels dropped to <8.5 g/dL.

Outcome of Therapy

We evaluated treatment success based on SVR, defined as undetectable HCV RNA levels 24 weeks after cessation of treatment. Some patients showed a transient response (relapsers), in which HCV RNA dropped to undetectable levels during treatment but then rebounded during the follow-up period. In nonresponders, HCV-RNA levels failed to decline by 2 $\log_{10}$ IU/mL by week 12 of treatment and never dropped below detectable levels. Histopathological diagnosis was made as described previously [34].

HCV RNA Levels

We monitored HCV RNA levels throughout the course of therapy using polymerase chain reaction (PCR)–based methods (the original Amplicor method, the high-range method, or the TaqMan real-time PCR test). The measurement ranges of these assays were 0.5–850 KIU/mL, 5–5000 KIU/mL, and 1.2–7.8 $\log_{10}$ IU, respectively. Samples exceeding the measurement range were diluted with phosphate-buffered saline and reanalyzed. All values were reported as $\log_{10}$ international units per milliliter.

SNP Genotyping

We genotyped each patient for 2 SNPs: rs8099917 in the *IL28B* locus, which is associated with therapy outcome, and rs1127354 in the *ITPA* locus, which is associated with ribavirin-induced anemia. Although there are 2 SNPs associated with *ITPA* enzyme activity in white patients [28], 1 of these SNPs appears to

Table 1. Patient Profiles[a]

	Total (N = 640)	SVR (n = 388)	TR (n = 119)	NR (n = 119)
Age, years	59 (10–82)	56 (10–77)	63 (24–78)	63 (27–82)
Sex, M/F	327/313	221/167	47/72	52/67
BMI	22.09 (14–32)	21.95 (15–32)	22.22 (14–32)	22.62 (16–31)
Viral genotype, 1b/others	441/186	223/153	98/21	108/11
Virus titer, $\log_{10}$ IU/mL	6.1 (3.6–7.5)	6 (3.6–7.3)	6.25 (4–7.4)	6.4 (5.1–7.3)
Fibrosis, 0/1/2/3/4	7/213/156/74/17	4/140/99/41/4	1/44/28/15/3	2/26/26/17/9
Activity, 0/1/2/3/4	108/147/207/32/0	64/89/130/24/0	18/31/37/3/0	24/25/35/5/0
rs8099917, TT/GT, GG	476/161	330/57	90/29	46/72
rs1127354, CC/CA, AA	468/171	273/114	88/31	100/19
γ-GTP level, IU/L	40 (8–535)	38.5 (8–535)	34 (8–341)	52 (12–213)
Hemoglobin level, g/dL	14 (7.8–18)	14.15 (7.8–18)	13.6 (9.7–18)	13.8 (9.2–16)
ALT level, IU/L	50 (10–512)	53.5 (11–512)	48 (11–408)	46 (10–224)
AST level, IU/L	43 (0–312)	41 (0–312)	45 (16–197)	47 (0–142)
α-Fetoprotein level, μg/L	5 (0.8–262)	5 (0.8–262)	6.8 (1.9–152)	7.5 (1–244)
Ferritin level, μg/L	121.2 (0.56–1057)	127 (3–1057)	110.1 (0.56–1023)	135 (7.7–769)
LDL cholesterol level, mg/dL	173 (93–271)	171 (99–264)	178.5 (102–271)	171 (93–260)
Triglyceride level, mg/dL	93 (20–541)	90.5 (35–541)	99 (44–303)	104.5 (20–305)
HDL cholesterol level, mg/dL	57.5 (21–167)	57 (27–101)	57 (27–114)	63 (21–106)
Iron count, μ/dL	128.5 (11–339)	123 (11–339)	138 (61–305)	156.5 (31–286)
Fasting blood sugar level, mg/dL	98 (15–248)	96 (15–248)	99 (76–243)	101 (80–211)
White blood cell count, cells/mm^3	4800 (4.4–10 760)	4970 (4.4–10 760)	4600 (5.4–8780)	4460 (2160–8400)
Platelet count × 10^4 cells/mm^3	16.4 (4.8–246)	17.5 (5–229)	15.3 (5.6–246)	15.05 (5–116)
IFN reduction, Y/N	93/547	50/338	20/99	21/98
Ribavirin reduction, Y/N	208/432	114/274	50/69	43/76
Anemia reported, Y/N	158/482	83/305	44/75	30/89
Stopped, Y/N	15/610	0/382	0/115	1/113

Abbreviations: ALT, aspartate aminotransferase; AST, alanine aminotransferase; BMI, body mass index; γ-GTP, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; IFN, interferon; NR, nonresponse; SVR, sustained virological response; TR, transient response (relapse).

[a] All patients were interferon treatment–naive and were treated with pegylated interferon plus ribavirin combination therapy. Counts are listed for categorical values and the median and range are reported for continuous variables.

be fixed in the Japanese population, and so only rs1127354 was genotyped [29]. Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or the Invader or TaqMan assay, as described previously [35].

Statistical Analysis

All analysis was performed using the R statistical package (http://www.r-project.org). Nonparametric tests (χ^2 and Mann–Whitney U tests) were used to detect significant associations. All statistical analyses were 2 sided, and $P < .05$ was considered significant. Multiple logistic regression analysis with forward/backward stepwise selection of variables was used to identify independent factors associated with SVR. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each factor. Receiver-operating characteristic (ROC) curves and areas under the curve (AUC) were calculated for each model using the ROCR software package. CIs for predicted SVR probabilities were calculated over a range of ages and viral loads, and results were stratified by *IL28B* SNP genotype and viral genotype using the rms software package. Models were validated based on 10 rounds of 10-fold cross-validation using the WEKA data-mining package [36].

RESULTS

Patient Characteristics

Patient profiles are shown in Table 1. In total, 388 (61%) patients achieved SVR, 119 (19%) were transient responders, and 119 (19%) were nonresponders. The frequency of the deleterious allele for the *IL28B* SNP rs8099917 (G) was 0.14. A total of 476 patients had the favorable TT genotype, and 148 and 13 patients had the unfavorable GT and GG genotypes, respectively. Genotype data for rs12979860, another commonly reported *IL28B* SNP, were not available for all patients, but the 2 SNPs are in high linkage disequilibrium and genotypes are highly correlated (0.99). The frequency of the favorable allele for the *ITPA* SNP rs1127354 (A) was 0.15. A total of 468 patients had the anemia-susceptible CC genotype, and 152 and 19 patients had the protective AC and AA genotypes, respectively.

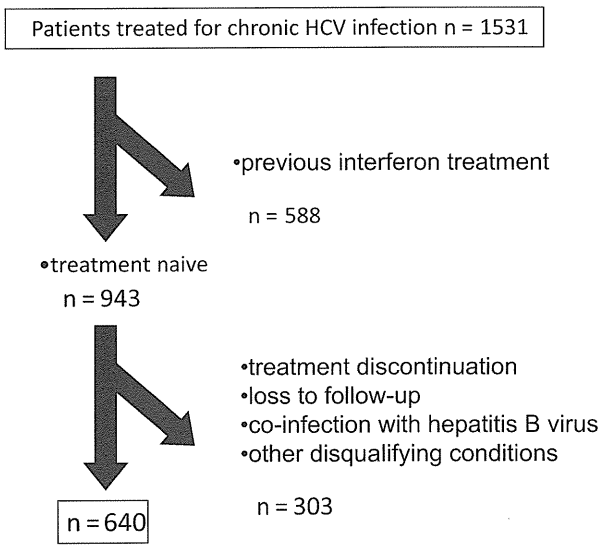

Figure 1. Patient-selection flowchart. Beginning with a database of 1531 patients treated for chronic hepatitis C virus (HCV) infection with pegylated interferon and ribavirin combination therapy, 588 patients were excluded due to prior history of interferon treatment and another 303 patients were excluded because of treatment discontinuation, loss to follow-up, or other disqualifications as described in the Methods, leaving 640 patients who were included in the analysis.

Predictive Factors for SVR

The following predictors were significantly associated with SVR using univariate analysis after Bonferroni correction for 26 tests: male sex, age, genotype 1b, initial viral load, aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio, rs8099917 TT genotype, diabetes mellitus, α-fetoprotein level, white blood cell count, platelet count, and hemoglobin level (Table 2). When all factors were included in multivariate analysis, age, genotype 1b, initial viral load, AST/ALT ratio, rs8099917 TT genotype, and α-fetoprotein were identified as independent predictors for SVR (Table 3) with an AUC of 0.85 (Figure 2). The mean AUC based on 10 rounds of 10-fold cross-validation was 0.82 with a true-positive rate of 78.2%, a false-positive rate of 21.8%, and a κ statistic of 0.53. Figure 2 shows predicted probabilities of achieving SVR for patients with genotype 1b stratified by the rs8099917 genotype and the initial viral load. The probability of SVR can be calculated using the following prediction formula:

log odds (SVR) = 11.7007–0.0479*age – 1.5417*genotype1b – 1.2804*log(viral load) – 0.7638*log(AST/ALT) + 1.6610* rs8099917TT – 0.3449*log(α-fetoprotein)

DISCUSSION

In this study, we present a simple predictive model for outcome of PEG-IFN plus ribavirin combination therapy for patients infected with HCV. Although the *IL28B* SNP is the best single predictor of SVR, not all patients with the favorable genotype achieved SVR, whereas some patients with an unfavorable genotype were able to achieve SVR. Inclusion of other viral and host factors is therefore expected to improve the accuracy of treatment-outcome predictions. Although a number of predictors have been reported, this study achieves relatively high accuracy using only a simple subset of pretreatment predictors, the most important of which are *IL28B* SNP genotype, age, viral genotype, and initial viral load.

A prediction equation based on the coefficients in Table 3 was used to generate the predicted response over a range of ages and viral loads (Figure 3). For example, a 60-year-old patient with the favorable *IL28B* SNP genotype and HCV genotype 1b has a probability of SVR of 0.61, whereas the probability is only 0.23 for a patient with an unfavorable *IL28B* genotype. On the other hand, the probability increases to 0.80 for a 40-year-old patient or 0.88 for a patient with genotype 1a, 2, or 3. Based on this model, it appears that older patients who have high viral load for genotype

Table 2. Univariate Predictors for Sustained Virological Response

Variable	OR	(95% CI)	*P* Value	
Sex	1.93	(1.39–2.7)	.000101	***
Age	0.948	(.933–0.963)	1.66×10^{-12}	***
BMI	0.961	(.91–1.01)	.1035	
Genotype 1b vs others	0.202	(.127–.311)	1.09×10^{-13}	***
Virus titer, log IU/mL	0.39	(.285–.524)	7.12×10^{-9}	***
ALT	1.28	(1.01–1.63)	.07166	
AST/ALT ratio	0.311	(.177–.528)	5.97×10^{-6}	***
rs8099917 TT genotype	4.24	(2.9–6.26)	2.75×10^{-6}	***
rs1127354 CC genotype	0.64	(.432–.939)	.0237	(*)
γ-GTP	0.999	(.996–1)	.04023	(*)
Fibrosis	0.744	(.595–.929)	.02826	(*)
Activity	1.12	(.909–1.39)	.2868	
α-Fetoprotein	0.69	(.559–.844)	1.30×10^{-5}	***
LDL cholesterol	0.997	(.992–1)	.3184	
Triglycerides	0.998	(.995–1)	.04564	(*)
HDL cholesterol	0.993	(.976–1.01)	.558	
Iron	0.995	(.99–1)	.01145	(*)
Fasting blood sugar	0.989	(.981–.997)	.01578	(*)
White blood cells	1	(1–1)	.000859	***
Platelets	2.32	(1.46–3.82)	.000342	***
Hemoglobin	1.25	(1.11–1.41)	.000224	***
Core aa 70 substitution	1.35	(.517–3.69)	.548	
Core aa 91 substitution	1.24	(.491–3.19)	.6604	
ISDR substitutions (0–1 vs >1)	3.87	(1.4–12)	.01034	(*)
Hypertension	0.497	(.278–.878)	.01599	(*)
Diabetes mellitus	0.206	(.0841–.454)	6.90×10^{-5}	***

Abbreviations: ALT, aspartate aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; OR, odds ratio.

***$P < .001$; **$P < .01$; *$P < .05$; (*): $P < .05$ but not significant following Bonferroni correction for multiple testing (α: 0.05/26 = .0019)

Table 3. Multivariate Predictors for Sustained Virological Response

Variable	Coeff	OR	(95% CI)	*P* Value	
Age	−0.0479	0.953	(.93–.976)	9.71×10^{-5}	***
Genotype 1b vs others	−1.542	0.214	(.107–.405)	4.97×10^{-6}	***
Log viral load	−1.28	0.278	(.166–.445)	3.57×10^{-7}	***
AST/ALT ratio	−0.7638	0.466	(.226–.931)	.03396	*
rs8099917 TT genotype	1.661	5.26	(2.98–9.57)	2.16×10^{-8}	***
α-Fetoprotein	−0.3449	0.708	(.549–.91)	.007249	**

Abbreviations: ALT/AST, aspartate aminotransferase/alanine aminotransferase ratio; CI, confidence interval; Coeff, coefficient; OR, odds ratio.

***$P < .001$; **$P < .01$; *$P < .05$; (*): $P < .05$.

1b and lack the favorable *IL28B* genotype are less likely to benefit from combination therapy and possibly triple therapy, whereas young patients with the favorable *IL28B* genotype and low viral load have a high probability of achieving SVR with combination therapy alone and may not benefit from the addition of a DAA. The model presented here includes data from 640 patients and achieves an AUC score greater than 0.82 following 10-fold cross-validation using some pretreatment predictors. However, in future studies, this model should also be validated against external data sets based on patients from different populations and ethnic groups.

Several predictive models for outcome of combination therapy for HCV have been reported and have used a variety of different approaches. Several studies have used artificial neural networks [37–39] and support vector machines [40] to predict SVR, although these types of models are more difficult to interpret than regression-based methods and are less amenable to adoption in clinical use. Other studies have used decision trees [41] and classification and regression tree analysis [42], both of which provide an intuitive, flowchart-based approach to prediction. However, small changes can dramatically alter the topology of the tree, and individual paths through the tree make use of only a fraction of the available data. Medrano et al proposed a logistic-regression SVR prediction model for patients in Spain coinfected with HCV and HIV [43]. The model achieved high accuracy (AUC, 0.85–0.89) using 4 predictors: *IL28B* SNP genotype, liver stiffness, viral genotype, and viral load. For HCV-monoinfected patients, O'Brien et al [44] proposed a prediction model for SVR in European–American patients with genotype 1 based on *IL28B* SNP genotype, viral load, AST/ALT ratio, fibrosis score, and prior ribavirin treatment. The model proposed here is similar to the model proposed by O'Brien et al, differing mainly in patient ethnicity (Japanese vs European–American) and treatment history (prior ribavirin treatment vs treatment-naive), although patients in the model of O'Brien et al had more severe fibrosis (Ishak fibrosis score ≥3 vs 0–4), and were younger (median age, 49 vs 59 years) and more likely to be male (73% vs 51%). Both models had similar factors and AUC scores (0.79 vs 0.82), and the inclusion of various host and viral factors in both models underscores the variability in response to therapy and the limitations of *IL28B* SNP genotype alone in predicting the outcome of therapy. Presumably, future studies will introduce models geared specifically for response to triple therapy, but until additional data become available, predictions based on response to combination therapy may help guide patient selection.

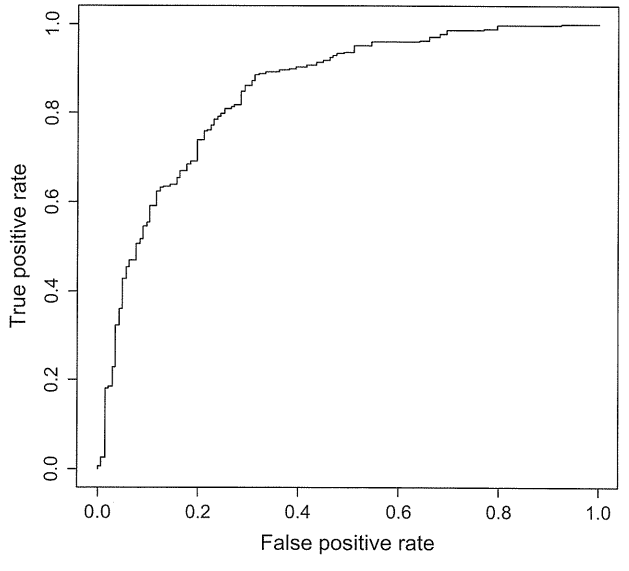

Figure 2. Receiver-operating characteristic curves for prediction of sustained virological response. The area under the curve was 0.85 for the full data set, and the mean of 10 rounds of 10-fold cross-validation was 0.82.

CONCLUSIONS

Pretreatment predictors based on clinical and viral factors may be used to predict the outcome of therapy. Regardless of the approach or the specific predictors analyzed, most prediction studies report a consistent set of important predictive factors, including viral genotype, *IL28B* SNP genotype, age, viral load, and ≥1 clinical factors reflecting liver function (eg, γ-GTP, LDL cholesterol, blood sugar, α-fetoprotein, and platelet count). By adopting a personalized approach to treatment, clinicians may be better able to determine the most appropriate course of therapy for individual patients while minimizing the risk of side effects.

Notes

Acknowledgments. We thank Mika Tsuzuno, Sakura Akamatsu, Sanae Furuya, and other members of the clerical staff and Yoshiiku Kawakami and other physicians at Hiroshima University Hospital for their help.

Financial support. This study was supported by the Ministry of Health, Labor, and Welfare of Japan (21390229).

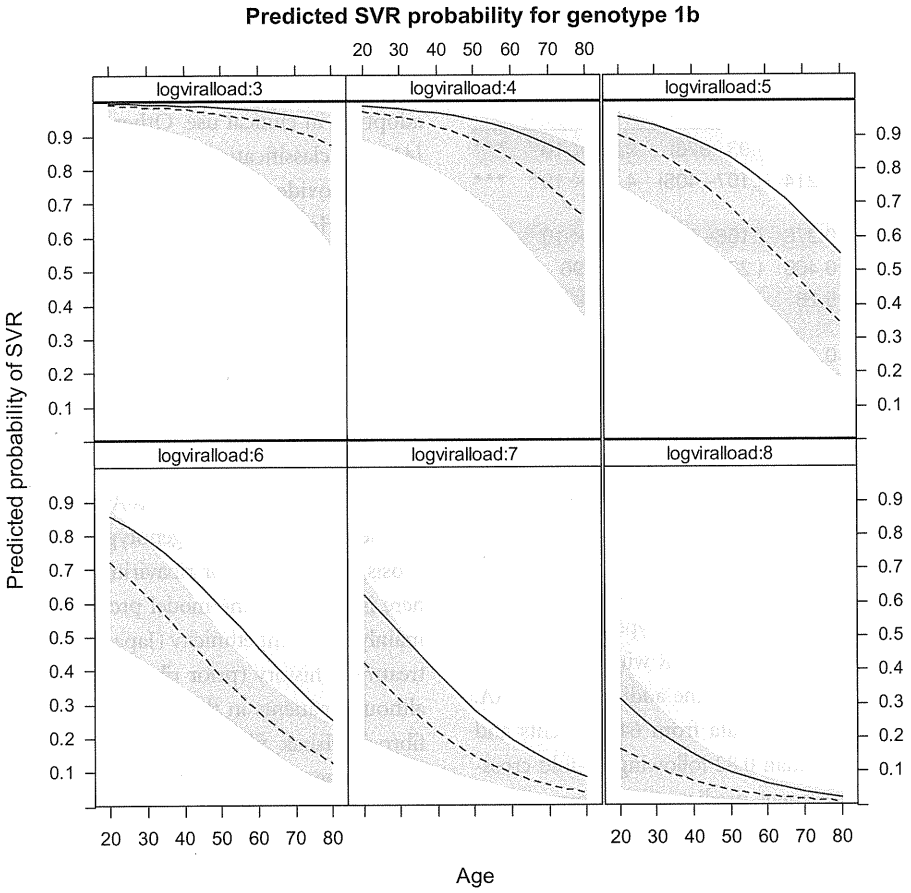

Figure 3. Predicted probabilities of sustained virological response (SVR) for patients with genotype 1b. Confidence intervals for predicted probability of SVR based on logistic regression by age, grouped by rs8099917 genotype and initial viral load for patients with genotype 1b, are shown. Solid lines represent the favorable rs8099917 TT single-nucleotide polymorphism genotype, and dashed lines represent the unfavorable GT or GG genotype.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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J Gastroenterol
DOI 10.1007/s00535-012-0679-8

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Risk factors for the exacerbation of esophageal varices or portosystemic encephalopathy after sustained virological response with IFN therapy for HCV-related compensated cirrhosis

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Received: 18 June 2012 / Accepted: 3 September 2012

Abstract
Background We aimed to identify risk factors contributing to the exacerbation of esophageal varices (EV) or portosystemic encephalopathy after hepatitis C virus (HCV) eradication with interferon (IFN) therapy in patients with compensated cirrhosis. Also, the prognosis after HCV eradication was analyzed.
Methods Fifty-two patients with sustained virological response to IFN treatment for HCV-related compensated cirrhosis were enrolled in this retrospective cohort study.
Results At the achievement of HCV eradication, in 31 of the 52 patients (60 %), feeding vessels for EVs (left gastric vein, posterior gastric vein, short gastric vein) were shown, and in 18 patients (35 %) there were extrahepatic portosystemic shunts (paraesophageal vein, paraumbilical vein, and splenorenal shunt). Although the HCV eradication was successful, significant improvements were not observed in portosystemic collateral vessels 1 year after HCV eradication, and EVs were exacerbated in 19 (36 %) patients. The cumulative 1- and 3-year rates of EV exacerbation were 13 % and 49 %, respectively. By multivariate analysis, the existence of feeding vessels for EVs at HCV eradication was an independent predictive factor for the exacerbation of EVs ($P = 0.009$). Seven patients who had an extrahepatic portosystemic shunt at HCV eradication developed portosystemic encephalopathy during follow up. The 1-, 3-, and 5-year incidences of portosystemic encephalopathy were 6, 21, and 34 %, respectively. The cumulative 5-year survival rate of the cohort was 81 %. Two patients died of hepatocellular carcinoma (HCC).
Conclusions Our findings suggest that the existence of radical portosystemic collateral vessels at successful HCV eradication increases the risk of the exacerbation of EVs and the incidence of portosystemic encephalopathy in patients with HCV-related cirrhosis.

Keywords HCV-related cirrhosis · Interferon therapy · Sustained virological response · Esophageal varices · Portosystemic encephalopathy · Portosystemic collateral vessels

Introduction

Chronic hepatitis C is a common disease that may progress to cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. Longitudinal evaluation of patients with compensated cirrhosis caused by HCV infection has clearly shown that the yearly development rate of HCC ranges between 2 and 4 %, and HCC is the most frequent liver-related complication in these patients [3]. Interferon (IFN)-based therapy in patients with chronic hepatitis C results in the amelioration of hepatic inflammation and fibrosis, improvement of serum alanine aminotransferase levels, decrease of circulating HCV-RNA levels, and a decrease in the incidence of HCC development. Accordingly, it has been considered that IFN therapy and HCV eradication would be an

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Published online: 05 October 2012

effective cancer chemoprevention strategy for patients with chronic hepatitis C [3–5].

A reduced incidence of HCC in HCV-associated cirrhosis brought about by IFN therapy has been reported by many investigators [6–10]. Also the prognosis of patients with cirrhosis has improved markedly in recent years through advances in clinical management, such as IFN therapy, long-term supplementation with branched-chain amino acids, and the use of a low protein and low salt diet [11–14]. In 2011, antiviral therapy using pegylated-interferonα2b (PEG-IFNα-2b) combined with ribavirin was accepted by the Japanese health insurance system for the treatment of liver cirrhosis. In the future, this therapy is expected to lead to benefits such as the regression of cirrhosis and the reduction and prevention of cirrhosis-related complications.

At present, hemorrhage from esophageal or gastric varices is still one of the main causes of death in patients with liver cirrhosis. The reported prevalence of esophageal varices (EVs) in patients with cirrhosis ranges from 80 to 90 % [15, 16], and, annually, 10–30 % of patients with EVs develop variceal hemorrhage [17]. Despite substantial improvements in the early diagnosis and treatment of variceal hemorrhage, the associated mortality remains high (20–35 %) [1, 12, 18]. Proper management of EVs would be expected to improve the prognosis of patients with liver cirrhosis.

The decreased incidence of HCC and the attenuation of liver fibrosis in cirrhotic patients with HCV eradication is now well documented [3–5], even though we often experience the development of HCC, exacerbation of EVs, and portosystemic encephalopathy in these patients. However, the risk factors contributing to the exacerbation of EVs and portosystemic encephalopathy after HCV eradication have not been fully investigated. In this study, we explored the risk factors that contribute to the exacerbation of EVs and portosystemic encephalopathy after HCV eradication in patients with liver cirrhosis, and we also analyzed the prognosis of the patients.

Patients, materials, and methods

Patients

We retrospectively reviewed 362 consecutive patients with hepatitis C virus (HCV)-related compensated cirrhosis who received IFN therapy at Hiroshima University Hospital between January 2001 and December 2010. HCV eradication was achieved in 63 of these 362 patients (17.4 %). In brief, the HCV eradication rate was 12.5 % in patients with genotype 1b, and 38.2 % in patients with genotypes 2a, 2b, and other genotypes. All patients were negative for hepatitis B surface antigen. Of the 63 patients with compensated cirrhosis after HCV eradication, 52 patients who received regular surveillance such as liver function tests, ultrasonography, dynamic computed tomography (CT), and endoscopic examinations periodically after IFN therapy were enrolled in this study.

The diagnosis of cirrhosis was based on histological findings and/or clinical findings. Compensated cirrhosis was defined as cirrhosis with no history of ascites, jaundice, hepatic encephalopathy, or variceal bleeding at entry into the study.

Endoscopic examination for assessing EVs

The endoscopic findings of EVs were evaluated according to the classification system of the Japanese Society for Portal Hypertension and Esophageal Varices [19]. The form (F) of EVs was classified as complete eradication after treatment (F0), small straight (F1), enlarged tortuous (F2), and large coiled-shaped (F3). The red color sign (RC) was also classified based on the criteria of the Japanese Society for Portal Hypertension and Esophageal Varices [19]. Endoscopic examinations were carried out at least annually. Worsening of the F and the RC sign compared with baseline findings on follow-up endoscopy was defined as exacerbation of EVs.

Computed tomography (CT) examination

CT examinations were done in the high-quality scanning mode, with 1.25-mm slice thickness, and reconstruction intervals of 0.625-mm for portal venous phase images. The first and second acquisitions were used for hepatic artery phase images, the third acquisition for portal venous phase images, and the fourth acquisition for hepatic venous phase images. The left gastric vein, posterior gastric vein, short gastric vein, paraesophageal vein, paraumbilical vein, and splenorenal shunt were considered as portosystemic collateral vessels. Evaluation of portosystemic collateral vessels was done by dynamic CT. The diameters of the portosystemic collateral vessels were measured, with the largest portion of the vessel being recorded in all cases. Cutoff diameters of 6, 4, 2, 4, 3, and 13 mm, respectively, represented the median values of these vessels. All patients received regular annual surveillance by dynamic CT, to check for extrahepatic portosystemic shunt and HCC.

Diagnosis of HCC

Hepatocellular carcinoma was diagnosed based on a hypervascular staining pattern in the arterial phase and a hypovascular staining pattern in the portal phase, shown by dynamic CT, magnetic resonance imaging, and/or

angiography. Tumors without enhancement upon imaging were diagnosed by fine-needle biopsy.

Follow up of enrolled patients

All 52 patients received regular surveillance, including annual blood examinations, endoscopic examinations, and dynamic CT scans after HCV eradication. Monitoring for the presence of hepatic encephalopathy and HCC was precisely evaluated at each examination. The rate of exacerbation of EVs, the incidence of portosystemic encephalopathy and HCC, and the prognosis after HCV eradication were evaluated based on these findings.

Statistical analysis

Statistically significant differences in quantitative data were determined using the Mann–Whitney *U*-test and the Wilcoxon rank-sum test, when applicable. Overall survival and EV exacerbation rates were calculated by the Kaplan–Meier method. Multivariate analysis was conducted with a Cox proportional hazard model using the stepwise selection of variables or two logistic analyses. All statistical analyses were performed using an SPSS software package (version 12.0 for Windows; SPSS, Chicago, IL, USA), with $P < 0.05$ denoting statistical significance.

Table 1 Clinical characteristics of 52 patients before interferon (IFN) therapy

Age (years, range)	58 (39–73)[a]
Sex (male/female)	36/16
Genotype 1b/others	36/16
Child–Pugh grade A/B	45/7
Diabetes mellitus (with/without)	19/33
Alcohol intake (with/without)	19/33
Total bilirubin (mg/dl, range)	0.9 (0.4–3.9)[a]
AST (IU/l, range)	62 (29–265)[a]
ALT (IU/l, range)	64 (22–321)[a]
Albumin (g/dl, range)	3.9 (2.8–5.0)[a]
Platelet count ($\times 10^4/\mu l$, range)	7.3 (3.4–24.6)[a]
Prothrombin time activity (%, range)	85 (36–121)[a]
Total cholesterol (mg/dl)	151.2 (90–2275)[a]
Body mass index (kg/m^2, range)	22.5 (16.3–30.4)[a]
Past history of HCC (with/without)	26/26
Esophageal variceal form (F1/F2)	22/5

[a] Median

Alcohol intake, ≥80 g/day for more than 5 years

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *HCC* hepatocellular carcinoma, *Variceal form* form according to the criteria of the Japanese Society for Portal Hypertension and Esophageal Varices

Results

Clinical characteristics of enrolled patients

The clinical characteristics of the 52 patients are shown in Table 1. The median age of the patients was 58 years (range 39–73 years); 36 patients were men and 16, women; 36 patients had HCV genotype 1b; and 45 patients were classified as Child–Pugh grade A before IFN therapy. Before the IFN therapy, 25 (48 %) patients did not have EVs, and among the 27 patients with EVs, those in 22 patients (42 %) were classified as F1 and those in 5 patients (10 %) as F2. The RC was not observed in any of the enrolled patients (Table 1). In this study, in regard to portosystemic collateral vessels, the left gastric vein, posterior gastric vein, and short gastric vein were defined as feeding vessels for EVs, and the paraesophageal vein, paraumbilical vein, and splenorenal shunt were defined as extrahepatic portosystemic shunts. The portosystemic collateral vessels are listed in Table 2.

Changes in portosystemic collateral vessels after HCV eradication

Among the 52 patients, 31 patients had feeding vessels for EVs and 18 patients had an extrahepatic portosystemic shunt at HCV eradication. We compared portosystemic collateral vessels at HCV eradication with those seen 1 year after HCV eradication. Significant improvements were not observed in the portosystemic collateral vessels (Fig. 1).

We also compared changes in portosystemic collateral vessels between those seen at HCV eradication and those seen 1 year after HCV eradication in patients who showed exacerbation of EVs and those who did not show exacerbation, and in patients with and without portosystemic hepatic encephalopathy. The diameter of the portosystemic collateral vessels had worsened in some patients. However,

Table 2 Portosystemic collateral vessels at the time that hepatitis C virus (HCV) eradication was achieved

Feeding vessels for EVs (number)	$n = 31/52$
Left gastric vein	18
Posterior gastric vein	20
Short gastric vein	28
Extrahepatic portosystemic shunt (number)	$n = 18/52$
Paraesophageal vein	13
Paraumbilical vein	9
Splenorenal shunt	9

EVs esophageal varices

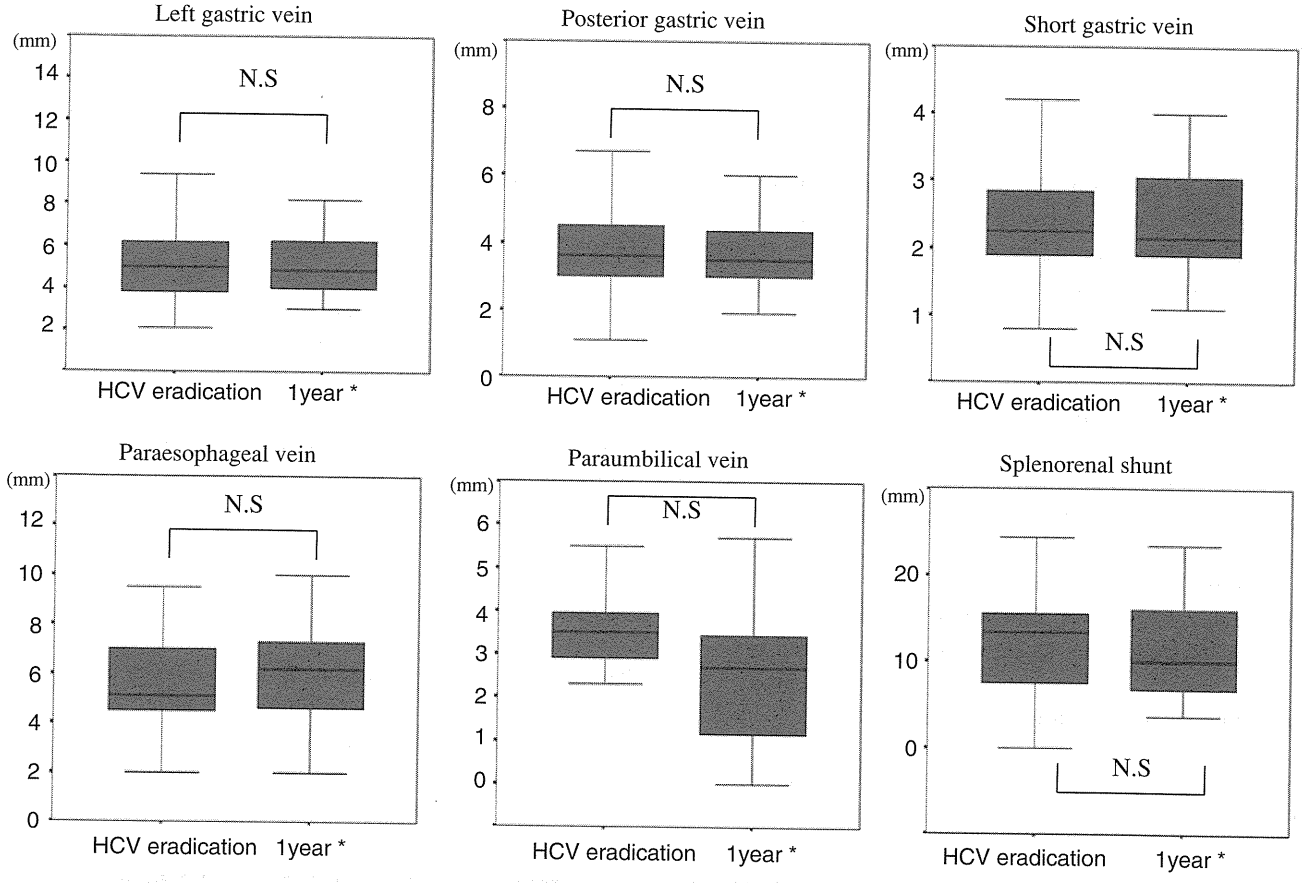

Fig. 1 Changes in the diameters of portosystemic collateral vessels in patients at hepatitis C virus (*HCV*) eradication and 1 year after HCV eradication. *1 year** 1 year after HCV eradication. *N.S Not significant*

significant improvements were not observed in portosystemic collateral vessels at 1 year after HCV eradication.

Risk factors for exacerbation of EVs after HCV eradication

Esophageal varices were exacerbated in 19 patients. The median period until exacerbation after HCV eradication was 13 months (range 4–36 months). The cumulative 1- and 3-year exacerbation rates were 13 and 49 %, respectively (Fig. 2). One year after HCV eradication, EVs were exacerbated from F1 to F2 in 13 patients, from F1 to F3 in 2 patients, and from F2 to F3 or development of the RC sign in 4 patients; a representative case is shown in Fig. 3. By univariate analysis, serum albumin <4.0 g/dl, past history of HCC, recurrence or emergence of HCC, and existence of feeding vessels for EVs at HCV eradication were significantly associated with the exacerbation of EVs during the follow-up period (Table 3). By multivariate analysis, the existence of feeding vessels for EVs at HCV eradication was an independent predictive factor for the exacerbation of EVs (risk ratio 3.719, 95 % confidence interval [CI] 1.387–89.975, $P = 0.009$).

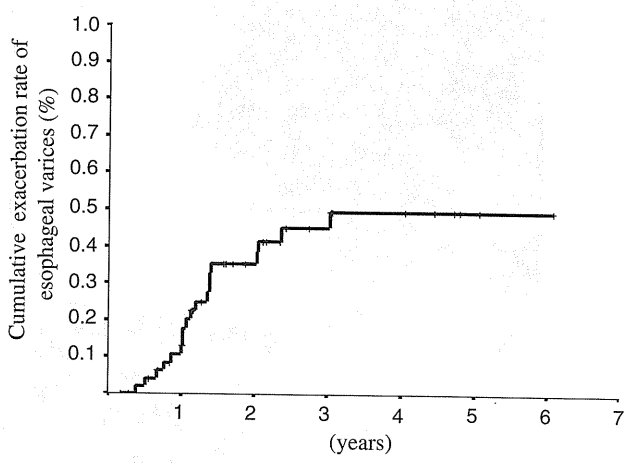

Fig. 2 The cumulative exacerbation rate of esophageal varices (EVs) after HCV eradication with interferon (IFN) therapy

Incidence of portosystemic encephalopathy after HCV eradication

Among the 52 patients, 7 patients who had an extrahepatic portosystemic shunt at HCV eradication developed portosystemic encephalopathy during follow up. The clinical

characteristics of these patients are shown in Table 4. Six of these patients were Child–Pugh grade A. All seven patients had a splenorenal shunt as the extrahepatic portosystemic shunt at the study entry. The 1-, 3-, and 5-year incidences of portosystemic encephalopathy were 6, 21, and 34 %, respectively (Fig. 4). The median period from HCV eradication until the development of portosystemic encephalopathy was 15 months (range 8–59 months). Representative changes in an extrahepatic portosystemic shunt are shown in Fig. 5.

Comparison of liver function in patients before IFN therapy and 1 year after HCV eradication

Liver function was compared between the patients with or without feeding vessels for EVs and extrahepatic portosystemic shunt before IFN therapy and 1 year after HCV eradication. Regardless of the presence of portosystemic collateral vessels, liver function was improved at 1 year after HCV eradication, as shown in Tables 5 and 6.

Development of HCC after HCV eradication

Among the 26 patients with no history of previous HCC, 3 (12 %) patients developed HCC after HCV eradication. The median period from the time of HCV eradication to HCC development was 53 months (range 21–56 months), and the cumulative 1-, 3-, and 5-year development rates were 0, 6, and 34 %, respectively. Sixteen of the 26 patients with a past history of HCC before IFN therapy developed tumor recurrence after HCV eradication.

Overall survival after HCV eradication

The median observation period after HCV eradication was 21 months (range 1–79 months). The cumulative 5-year survival rate was 81 % (Fig. 6). Two patients died of HCC and four patients died of extrahepatic complications (two from septic shock, one from cancer of the maxilla, and one from heart failure). No patients died from hemorrhage from EVs or from liver failure.

Discussion

The prognosis of patients with HCV-related cirrhosis is greatly affected by the development of HCC, hemorrhage from esophageal or gastric varices, and hepatic encephalopathy, especially during the compensation period. A previous report by Kasahara et al. [4] demonstrated that the

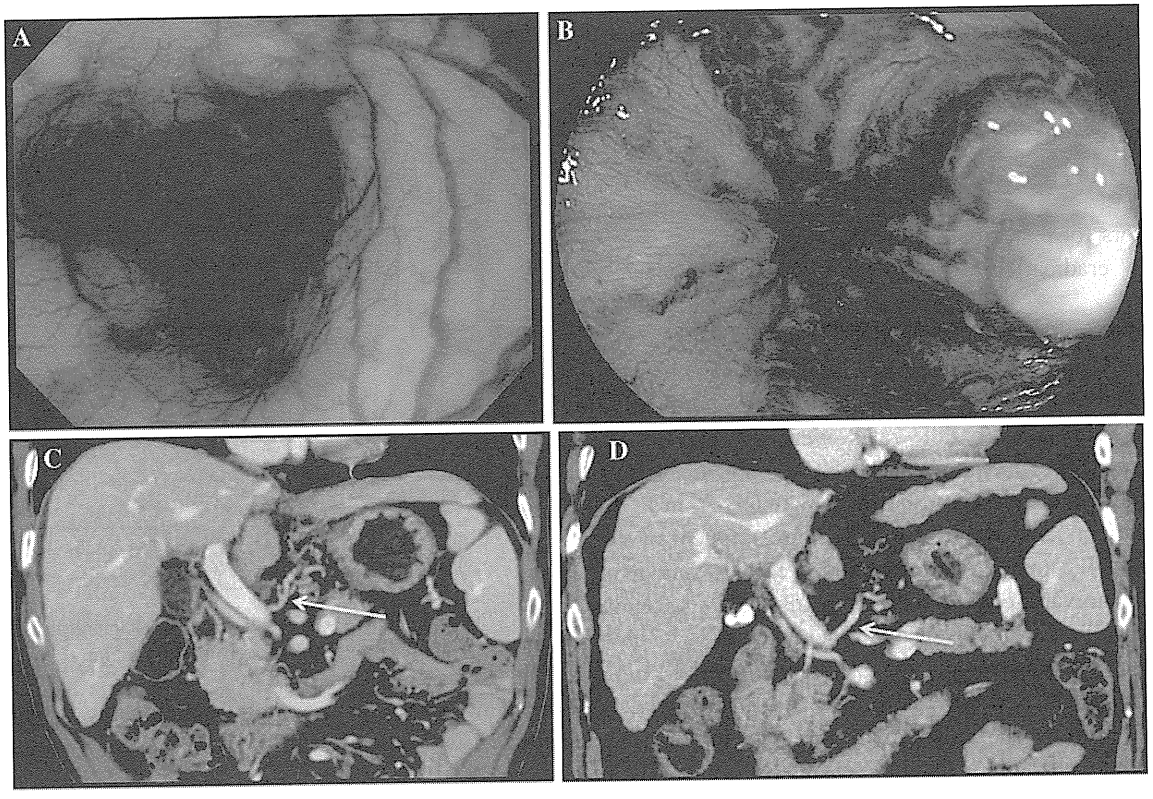

Fig. 3 Representative case: findings in a 56-year-old man with compensated HCV-related cirrhosis. Gastroendoscopic and dynamic computed tomography (CT) findings at the study entry (**a**, **c**) and 1 year after HCV eradication (**b**, **d**). EVs were exacerbated from F1 to F2. *Arrows* show the left gastric vein, and this was the feeding vessel responsible for the exacerbation

development of HCC in patients with chronic hepatitis C was suppressed by IFN therapy. Likewise, HCC was reported to be significantly inhibited by IFN therapy in patients with HCV-related cirrhosis [6–10]. However, the risk factors contributing to the exacerbation of EVs, the incidence of portosystemic encephalopathy, and the overall survival rates after HCV eradication in cirrhotic patients have not been fully elucidated. Accordingly, the present study aimed to elucidate these points.

In our study, no improvement of portosystemic collateral vessels was observed at 1 year after HCV eradication, but exacerbation of EVs was observed in 37 % of the patients. The cumulative 1- and 3-year exacerbation rates were 13 and 49 %, respectively. The existence of feeding vessels for EVs at HCV eradication was the only independent predictive factor for the exacerbation of EVs shown by multivariate analysis (risk ratio 3.719, 95 % CI 1.387–89.975, $P = 0.009$). Thirteen percent of all patients developed portosystemic encephalopathy after HCV eradication. They all had a splenorenal shunt as the extrahepatic portosystemic shunt. The 1-, 3-, and 5-year incidences of portosystemic encephalopathy after HCV eradication were 6, 21, and 34 %, respectively.

Regarding liver function, several studies have reported that IFN therapy in patients with HCV-related cirrhosis

Table 3 Univariate analysis of risk factors associated with the exacerbation of esophageal varices after HCV eradication with IFN therapy

At HCV eradication	Category	*P* value
Age (years)	≥60/<60	0.8297
Sex (male/female)	Male/female	0.7322
Genotype 1b/others	1b/others	0.7271
Diabetes mellitus	With/without	0.0666
Alcohol intake	With/without	0.2465
Child–Pugh grade	A/B	0.1192
Past history of HCC	With/without	0.0097
Recurrence or emergence of HCC	With/without	0.0112
Total bilirubin (mg/dl)	≥1.0/<1.0	0.1402
AST (IU/l)	≥50/<50	0.9826
ALT (IU/l)	≥50/<50	0.7608
Albumin (g/dl)	≥4.0/<4.0	0.0343
Platelet count ($\times 10^4/\mu l$)	≥12/<12	0.7238
Prothrombin time activity (%)	≥80/<80	0.2028
Total cholesterol (mg/dl)	≥180/<180	0.7429
Body mass index (kg/m^2)	≥25/<25	0.4416
Feeding vessels for EVs	With/without	0.0010
Extrahepatic portosystemic shunt	With/without	0.5156

Alcohol intake, ≥80 g/day for more than 5 years

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *HCC* hepatocellular carcinoma, *EVs* esophageal varices

Table 4 Clinical characteristics of the 7 patients who developed portosystemic encephalopathy

Case no.	Sex	Age (years)	Child–Pugh score	At HCV eradication					At the time portosystemic encephalopathy occurred						
				T-bil (mg/dl)	Alb (g/dl)	Plt ($\times 10^4/\mu l$)	PT (%)	Shunt	Period (months)[a]	Child–Pugh score	T-bil (mg/dl)	Alb (g/dl)	Plt ($\times 10^4/\mu l$)	PT (%)	NH_3 ($\mu g/dl$)
1	F	70	5	0.6	4.2	23.3	89	PE, SR	8	8	0.6	43.3	19.8	59	131
2	M	77	5	0.9	3.0	10.5	74	PE, SR	12	9	0.9	2.5	15.0	42	99
3	M	66	7	1.3	2.9	6.8	85	PU, SR	13	8	1.3	3.6	5.5	51	62
4	M	77	5	0.9	4.5	13.4	86	SR	15	5	0.6	4.3	11.3	96	127
5	M	56	5	1.2	4.1	6.5	80	PE, PU, SR	23	5	1.0	4.2	8.5	54	116
6	M	70	5	1.0	3.7	9.6	93	PE, SR	36	5	1.0	4.1	10.0	97	82
7	F	60	5	1.2	3.5	7.3	85	PE, SR	59	5	1.5	3.9	10.3	73	109

T-bil total bilirubin, *Alb* albumin, *Plt* platelet count, *PT* prothrombin time activity, NH_3 ammonia, *PE* paraesophageal vein, *PU* paraumbilical vein, *SR* splenorenal shunt

[a] Period from time of HCV eradication to development of portosystemic encephalopathy

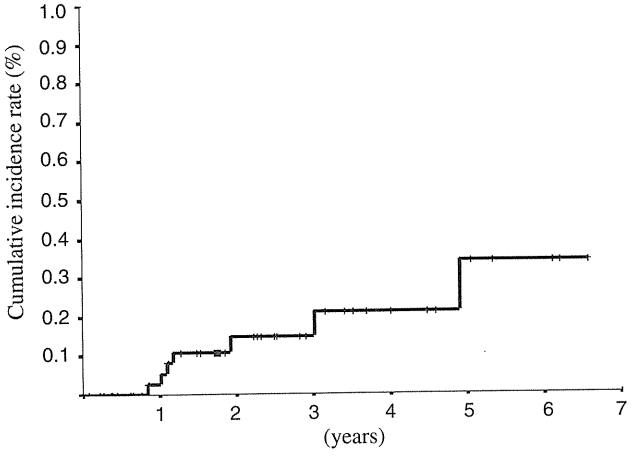

Fig. 4 The incidence rate of portosystemic encephalopathy after HCV eradication with IFN therapy

significantly reduces fibrosis and significantly improves liver function [20–22]. Similarly, we found a significant improvement of liver function in patients with cirrhosis with IFN therapy 1 year after HCV eradication. These improvements were observed irrespective of the existence of portosystemic collateral vessels. These results indicated that HCV eradication did not influence the improvement of portosystemic collateral vessels that already existed at the time of HCV eradication.

IFN therapy may produce clinically significant reductions in the hepatic venous pressure gradient (HVPG) in patients with HCV-related cirrhosis after HCV eradication [23, 24]. IFN therapy is reported to reduce the progression of hepatic fibrosis irrespective of the virological response [25–27]. The authors of these reports speculated that the reduction of portal vein pressure via a reduction of the

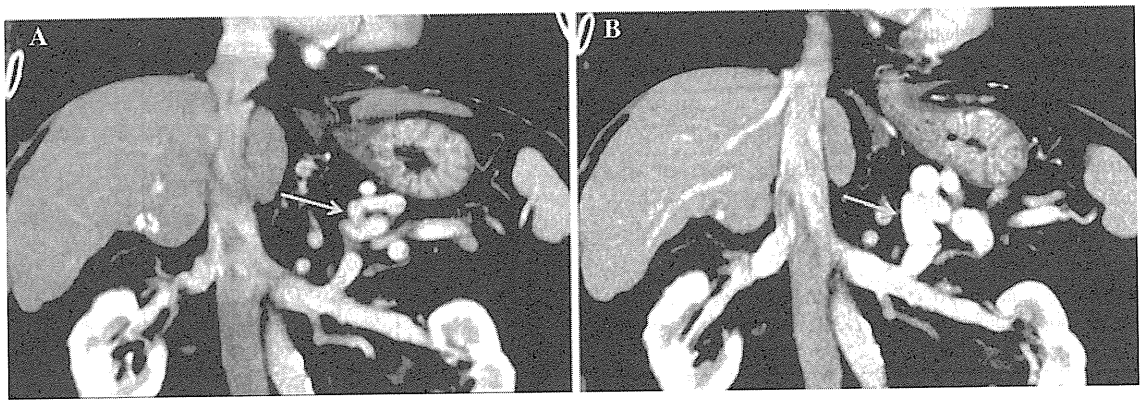

Fig. 5 Findings in a 77-year-old man who developed hepatic encephalopathy 15 months after HCV eradication with IFN therapy for HCV-related compensated cirrhosis (case 4 in Table 4). Dynamic CT showed exacerbated splenorenal shunt (*arrow*) 15 months after HCV eradication (**b**), compared with findings at HCV eradication (**a**)

Table 5 Liver function test results before IFN therapy and 1 year after HCV eradication in patients with and without feeding vessels for EVs

	Feeding vessels for EVs					
	With ($n = 31$)			Without ($n = 21$)		
	Before IFN therapy	1 Year after HCV eradication	*P* value	Before IFN therapy	1 Year after HCV eradication	*P* value
Total bilirubin (mg/dl)	1.4 ± 1.8	0.9 ± 0.5	0.258	1.0 ± 0.4	0.8 ± 0.4	0.034
AST (IU/l)	68 ± 46.0	51 ± 28.5	0.002	83 ± 60.0	45 ± 30.2	0.002
ALT (IU/l)	68 ± 52.8	42 ± 23.3	0.001	97 ± 73.8	47 ± 32.1	<0.001
Albumin (g/dl)	3.8 ± 0.8	4.0 ± 0.6	0.024	3.9 ± 0.7	4.5 ± 0.4	<0.001
Platelet count ($\times 10^4/\mu l$)	8.1 ± 3.0	11.6 ± 5.6	0.003	9.5 ± 4.3	13.0 ± 5.3	0.001
Prothrombin time activity (%)	86 ± 8.9	80 ± 16.5	0.192	90 ± 11.0	96 ± 11.3	0.043
Total cholesterol (mg/dl)	151 ± 41.5	180 ± 39.0	0.001	157 ± 32.3	177 ± 31.0	0.012

Values are means ± SD

AST aspartate aminotransferase, *ALT* alanine aminotransferase

P value, Wilcoxon rank-sum test

Table 6 Liver function test results before IFN therapy and 1 year after HCV eradication in patients with and without extrahepatic portosystemic shunt

	Extrahepatic portosystemic shunt					
	With ($n = 18$)			Without ($n = 34$)		
	Before IFN therapy	1 Year after HCV eradication	*P* value	Before IFN therapy	1 Year after HCV eradication	*P* value
Total bilirubin (mg/dl)	0.9 ± 2.1	0.9 ± 0.6	0.458	1.1 ± 0.7	0.7 ± 0.4	<0.001
AST (IU/l)	56 ± 48.2	34 ± 23.1	0.020	55 ± 47.3	31 ± 28.5	<0.001
ALT (IU/l)	57 ± 50.5	33 ± 17.8	0.018	61 ± 50.5	32 ± 29.9	<0.001
Albumin (g/dl)	3.8 ± 0.7	4.1 ± 0.6	0.045	3.9 ± 0.7	4.6 ± 0.5	0.001
Platelet count ($\times 10^4/\mu l$)	8.7 ± 3.3	10.9 ± 4.9	0.029	7.5 ± 3.3	11.8 ± 5.2	<0.001
Prothrombin time activity (%)	83 ± 14.6	86 ± 16.5	0.055	88 ± 12.4	92 ± 15.7	0.067
Total cholesterol (mg/dl)	132 ± 45.5	179 ± 49.5	0.007	153 ± 33.8	195 ± 43.6	0.001

Values are means ± SD

AST aspartate aminotransferase, *ALT* alanine aminotransferase

P value, Wilcoxon rank-sum test

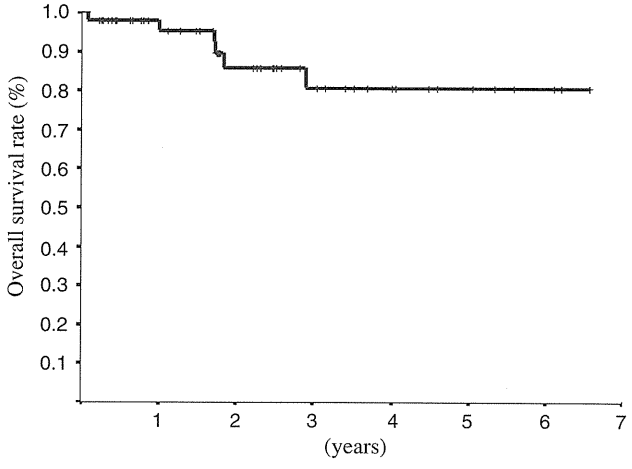

Fig. 6 Overall survival rate after HCV eradication with IFN therapy

sinusoidal space and a reduction of pressure in the peripheral branch of the portal vein led to an improvement of the fibrosis activity grade [25–27]. With our findings, similar these reported results, we expected that portosystemic collateral vessels would be improved by the IFN therapy. However, our results showed no improvement of cirrhosis-related complications such as EVs and portosystemic encephalopathy. These findings might be attributable to the existence of advanced fibrosis and/or increased blood flow volume in the portosystemic collateral vessels before the IFN therapy. Accordingly, we would recommend assessing portosystemic collateral vessels in cirrhotic patients before instituting IFN therapy; this assessment can be done by using CT portography, which would provide beneficial findings, as previously reported [28, 29].

The cumulative development rates of HCC at 5, 10, and 15 years were reported to be 32.5, 59.6, and 77.4 %, respectively, in Japanese patients with HCV-related cirrhosis [30]. In our study, 12 % of cirrhotic patients with no history of previous HCC developed HCC after HCV eradication. The cumulative 1-, 3-, and 5-year HCC development rates in these patients were 0, 6, and 34 %, respectively. The rate of development of HCC was not changed by HCV eradication in our patients. These findings indicate the need for long-term surveillance for HCC even after HCV eradication.

The cumulative 5-year survival rate in our study was 81 % and this was similar to the rate in previous reports of similar patient cohorts [31, 32]. Two patients in our study cohort died of HCC, and four others died of extrahepatic complications.

In our patient group, the results of treatment with endoscopic injection sclerotherapy (EIS) and/or surgical ligation for exacerbated EVs and portosystemic encephalopathy were adequate, probably because of the well-maintained liver function achieved by HCV eradication. As a result, there were no deaths from EV hemorrhage or liver failure in the studied patients during follow up.

In conclusion, our findings indicate that the existence of portosystemic collateral vessels increases the risk of the exacerbation of EVs and the incidence of portosystemic encephalopathy in patients with HCV-related cirrhosis, even after successful HCV eradication. We suggest that portosystemic collateral vessels should be evaluated in cirrhotic patients before IFN therapy is instituted.

Conflict of interest The authors declare that they have no conflicts of interest.

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JOURNAL OF VIRAL HEPATITIS
JVH

Journal of Viral Hepatitis, 2012, **19**, e126–e133 doi:10.1111/j.1365-2893.2011.01516.x

Serum PAI-1 is a novel predictor for response to pegylated interferon-α-2b plus ribavirin therapy in chronic hepatitis C virus infection

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Received April 2011; accepted for publication June 2011

SUMMARY. Obesity and insulin resistance have been reported as negative predictors for sustained virological response (SVR) in hepatitis C virus (HCV) genotype 1 infected patients treated with pegylated interferon-α plus ribavirin. They are also known to affect serum levels of several cytokines including adipocytokines. But the association between these cytokines and treatment outcome has not been fully elucidated. We examined pretreatment serum levels of 14 cytokines among 190 patients who were treated with pegylated interferon-α-2b plus ribavirin for chronic HCV-1b infection with high viral load (≥5 log IU/mL) and analyzed their contribution to treatment response. Plasminogen activator inhibitor-1 (PAI-1), vascular endothelial growth factor, and 11 clinical factors showed significant association with SVR in univariate logistic regression analysis. Four significant factors in multivariate analysis; serum PAI-1 (odds ratio [OR] = 15.42), body mass index (OR = 4.56), rs8099917 (OR = 4.95) and fibrosis stage (OR = 5.18) were identified as independent predictors. We constructed a simple and minimally invasive prediction score for SVR based on the presence of these factors except for fibrosis stage. The accuracy of this score was 73%, and was confirmed using an independent validation cohort consisting of 31 patients (68%). The strongest correlation was between PAI-1 level and platelet count ($r = 0.38$, $P = 1.8 \times 10^{-7}$), and PAI-1 level was inversely correlated with fibrosis stage. Serum PAI-1 is a novel predictor for the response to combination therapy against chronic HCV-1b infection and may be associated with liver fibrosis.

Keywords: adipocytokine, genotype 1b, liver fibrosis, obesity, sustained virological response.

INTRODUCTION

Hepatitis C virus (HCV) is one of the major causes of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma in Japan as well as in most countries [1,2]. The current standard of care is pegylated interferon-α (PEG-IFN-α) and ribavirin (RBV) combination therapy [3]. In spite of recent progress in anti-HCV therapy, still only half of patients infected with genotype 1 show complete eradication. Therefore, it is important to identify reliable predictors for response to combination therapy.

To date, several viral and host factors responsible for sustained virological response (SVR) have been identified. Both HCV genotype and viral load [4] are strong predictors for SVR. The most difficult patients to treat are those having HCV genotype 1 and high viral load, both of which are common in Japan as well as other Eastern countries. Within genotype 1b, amino acid substitutions at positions 70 and 91 of the HCV core protein and multiple substitutions in the interferon sensitivity determining region of the NS5A protein

Abbreviations: AUC, area under the curve; BMI, body mass index; HCV, hepatitis C virus; NPV, negative predictive value; OR, odds ratio; PEG-IFN, pegylated interferon; PPV, positive predictive value; RBV, ribavirin; ROC, receiver operating characteristic; RT-PCR, reverse-transcription polymerase chain reaction; RVR, rapid virological response; SNP, single nucleotide polymorphism; SVR, sustained virological response

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have also been reported to affect treatment outcome, especially among Japanese patients [5–7]. Host factors responsible for SVR include age [8], gender, degree of hepatic fibrosis [9] and *IL28B* gene variants [10–12]. In addition, obesity and insulin resistance have been reported as independent negative predictors for SVR following combination therapy in chronic hepatitis C patients [13]. On the other hand, it has also been reported that adipocytokines, adipose tissue-derived cytokines, may play an important role in the development of obesity-related insulin resistance [14,15] and that many cytokines including adipocytokines are elevated or decreased in chronic hepatitis C patients. Therefore, it is reasonable to predict that adipocytokines may be associated with outcome of combination therapy, but such associations have yet to be fully investigated. In this study, we aim to elucidate the associations among several cytokines, other clinical data and treatment outcome of combination therapy.

MATERIALS AND METHODS

Study subjects

For the study cohort, data from 190 consecutive patients who were treated between 2005 and 2007 with PEG-IFN-α-2b plus RBV combination therapy for chronic hepatitis C genotype 1b infection with high viral load (≥5 log IU/mL) were collected from Hiroshima University Hospital. Data from 31 patients treated between 2007 and 2008 were also collected as an independent validation cohort using the same criteria. Study subjects tested positive for HCV RNA over a span of more than 6 months and were negative for hepatitis B virus and HIV and showed no evidence for other liver diseases. Patients received weekly injections of PEG-IFN-α-2b at 1.5 μg/kg body weight for 48 weeks, and RBV was administered orally. The amount of RBV was adjusted based on body weight (600 mg for <60 kg, 800 mg for 60–80 kg, and 1000 mg for >80 kg). Patients who received less than 80% of the prescribed dose of both drugs were excluded. Treatment success was evaluated based on SVR, defined as undetectable HCV RNA levels 24 weeks after cessation of treatment. We also defined rapid virological response (RVR) as undetectable HCV RNA at week 4 of treatment. Histopathological diagnosis was made according to the criteria of Desmet *et al.* [16]. All subjects provided written informed consent to participate in the study according to the process approved by the ethical committee of Hiroshima University and RIKEN and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

HCV RNA levels

HCV RNA levels were measured by reverse-transcription polymerase chain reaction (RT-PCR) using the original Amplicor method, the high range method, or the TaqMan RT-PCR test. The measurement ranges of these assays were 0.5–850 kIU/mL, 5–5000 kIU/mL, and 1.2–7.8 log IU, respectively. Samples exceeding the measurement range were diluted with PBS and reanalyzed. All values are reported as log IU/mL.

SNP genotyping

Single nucleotide polymorphism (SNP) genotyping of rs8099917 was performed using the Invader assay as described previously [17].

Cytokine measurements

Fourteen cytokines, including interleukin (IL) 1β, IL4, IL6, IL10, IL12p70, IL17, IL28a, tumour necrosis factor-α (TNFα), vascular endothelial growth factor (VEGF), adiponectin, total plasminogen activator inhibitor-1 (PAI-1), resistin, leptin and monocyte chemotactic protein-1 (MCP-1) were measured using the multiplex bead array assay on the Luminex Complete System 200 (Luminex Co., Austin, TX, USA) [18,19], with MILLIPLEX™ MAP kits (Millipore, Billerica, MA, USA) and Bio-Plex Pro™ Cytokine Assay kit (Bio-Rad, Benicia, CA, USA) according to the manufacturers' instructions. High Sensitivity Human Cytokine (HSCYTO-60SK) was used for IL 1β, IL4, IL6, IL10, IL12p70, and TNFα, and Human Serum Adipokine Panel A (HADK1-61K-A) was used for adiponectin, total PAI-1, and resistin, Human Serum Adipokine Panel B (HADK2-61K-B) was used for leptin and MCP-1, and Human Cytokine/Chemokine Panel II (MPXHCYP2-62K) was used for IL28a. Bio-Plex Pro Human Cytokine Group I (M50-0KCAF0Y) was used for IL17 and VEGF. All serum samples obtained from patients were stored −80 °C. Less than 60% of the analyzed samples had values above the lower limit of detection for four cytokines (IL4, IL12p70, IL17 and IL28a), so we excluded them from further analysis.

Statistical analysis

All analyses were performed using the R statistical package (http://www.r-project.org). We assessed the normality of continuous data using the Kolmogorov–Smirnov test and found that only total cholesterol had a normal distribution (data not shown), so we used the non-parametric Mann–Whitney *U*-test for all continuous variables despite our relatively large cohort. Chi-squared tests were used for the analysis of categorical data. All statistical analyses were two sided, and $P < 0.05$ was considered significant. Odds ratios (ORs) and 95% confidence intervals were calculated for each factor. Univariate and multivariate logistic regression analyses were used to identify predictors for SVR. Cut-off points for continuous variables were determined by analysis of the receiver operating characteristic (ROC) curve [20] based on the minimum balanced error rate (BER). BER is the average of the proportion of incorrect classification in each class. Variables that achieved statistical significance in univariate

tests were entered into multivariate logistic regression analysis. Patients for whom data was unavailable for one or more of the selected variables were excluded from multivariate analysis. Multivariate logistic regression analysis was performed using stepwise backward elimination based on the AIC score. The chi-squared test was used to compare the accuracy of the prediction score in the study cohort with that of the validation cohort. Correlation coefficients (r) between PAI-1 level and other clinical data were evaluated using Spearman's rank correlation test.

RESULTS

Comparison of characteristics between patients with and without SVR

We compared the characteristics of patients with and without SVR. As shown in Table 1, younger age, higher leukocyte count, higher platelet count, lower gamma-GTP levels, lower fasting blood sugar levels and homozygosity for the rs8099917 major allele (TT) were significantly associated with SVR. Histological findings revealed that lower fibrosis stage and lower activity grade were also associated with SVR. Two cytokines, IL10 and PAI-1, were significantly higher in patients with SVR (P = 0.048 and 0.0034, respectively).

Predictive factors for SVR

In univariate analysis, the following 13 factors were significantly associated with SVR (Table 2): age (<54 years; P = 0.0000138), body mass index (BMI) (<22.2 kg/m^2; P = 0.0135), leukocyte count (≥4440/mm^3; P = 0.00449), platelet count (≥18.1 × 10^4/mm^3; P = 0.000206), alanine aminotransferase (≥68 IU/L; P = 0.00824), gamma-GTP (<38 IU/L; P = 0.00166), triglycerides (≥75 mg/dL; P = 0.0491), fasting blood sugar (<114 mg/dL; P = 0.00379), rs8099917 genotype (TT; P = 0.00068), VEGF (≥79.26 pg/mL; P = 0.00927), PAI-1 (≥25 117.24 pg/mL; P = 0.000157), fibrosis stage (<3; P = 0.0122), and activity grade (<2; P = 0.0364). All nine factors for which the area

Table 1 Comparison of characteristics between patients with and without SVR

	All patients (n = 190)	SVR (n = 74)	Non-SVR (n = 116)	P value
Age (years)†	62 (54–68)	58 (48–66)	64 (57–68)	0.00084*
Gender (M/F)‡	99/91	41/33	58/58	0.47
BMI (kg/m^2)†	22.5 (20.7–25.3)	22.0 (20.6–25.2)	23.1 (20.9–25.2)	0.17
WBC (/mm^3)†	4810 (3960–5940)	5100 (4490–6330)	4585 (3600–5710)	0.014*
Hb (g/dL)†	13.7 (12.6–14.6)	14.0 (13.1–14.7)	13.5 (12.5–14.5)	0.25
Platelet count (×10^4/mm^3)†	14.5 (10.4–18.8)	16.4 (12.5–20.6)	12.7 (9.9–17.4)	0.00035*
ALT (IU/L)†	50 (34–71)	54 (32–84)	50 (37–65)	0.56
Gamma-GT (IU/L)†	39 (25–69)	31 (23–56)	46 (26–77)	0.023*
Total cholesterol (mg/dL)†	170 (150–191)	178 (153–200)	167 (146–185)	0.25
Triglyceride (mg/dL)†	95 (71–150)	99 (77–181)	92 (69–140)	0.20
Fasting blood sugar (mg/dL)†	106 (88–127)	95 (85–112)	110 (92–133)	0.0047*
HbA1c (%)†	5.2 (4.9–5.6)	5.2 (4.9–5.5)	5.2 (4.9–5.6)	0.60
rs8099917 (TT/TG or GG)‡	137/53	64/10	73/43	0.00042*
IL1β (pg/mL)†	0.22 (<LDD–1.15)	0.19 (<LDD–1.13)	0.23 (<LDD–1.10)	0.54
IL6 (pg/mL)†	3.63 (1.79–8.05)	3.29 (1.79–7.77)	3.75 (1.81–8.38)	0.41
IL10 (pg/mL)†	16.58 (10.30–24.67)	18.27 (11.28–28.70)	15.55 (9.73–22.30)	0.048*
TNFα (pg/mL)†	8.96 (5.88–17.93)	8.71 (5.64–17.90)	9.04 (6.25–17.98)	0.87
VEGF (pg/mL)†	104.66 (69.21–156.72)	108.81 (79.38–198.81)	96.46 (65.30–149.49)	0.098
Adiponectin (ng/mL)†	10 841 (7346–16 141)	10 697 (7313–16 066)	11 112 (7346–16 047)	0.73
PAI-1 (pg/mL)†	18 651 (13 830–22 667)	20 302 (15 564–26 746)	17 639 (13 122–21 278)	0.0034*
Resistin (pg/mL)†	14 254 (5058–25 267)	15 025 (6095–27 098)	12 844 (4605–23 251)	0.19
Leptin (pg/mL)†	5026 (2100–9488)	5161 (2100–9670)	4793 (2126–9263)	0.89
MCP-1 (pg/mL)†	284.8 (226.7–373.3)	278.2 (216.7–364.5)	291.9 (231.1–375.4)	0.56
Fibrosis stage (0–1/2/3/4/ND)†	59/55/23/18/35	27/24/8/1/14	32/31/15/17/21	0.015*
Activity grade (0–1/2/3/ND)†	46/85/21/38	24/32/4/14	22/53/17/24	0.0099*

<LDD, less than the lower limit of detection; ND, not determined; BMI, body mass index; WBC, leukocytes; Hb, haemoglobin; ALT, alanine aminotransferase; Gammma-GT, gammma-glutamyl transpeptidase; IL, interleukin; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor; PAI, plasminogen activator inhibitor; MCP, monocyte chemotactic protein.
For categorical data, the number of patients in each category is shown. For continuous data, the median and 25–75th percentile are displayed. †Mann–Whitney U-test. ‡Chi-square test. *Siginificant at the 0.05 level.

Table 2 Significant predictive factors for SVR using univariate and multivariate logistic regression analysis

		Univariate				Multivariate			
Variable[†]	ROC_AUC	*n*	OR	95% CI	*P* value	*n*	OR	95% CI	*P* value
Age <54 years	0.64	190	4.86	2.39–9.90	0.0000138[*]				
Gender: male	0.53	190	1.25	0.70–2.24	0.467				
BMI <22.2 kg/m^2	0.56	190	2.12	1.17–3.82	0.0135[*]	100	4.56	1.34–15.53	0.0154[*]
WBC ≥4440/mm^3	0.61	189	2.59	1.35–4.98	0.00449[*]				
Hb ≥14.0 g/dL	0.55	190	1.61	0.90–2.90	0.114				
Platelet count ≥18.1 × 10^4/mm^3	0.65	190	3.44	1.80–6.61	0.000206[*]	100	2.87	0.84–9.85	0.0955
ALT ≥68 IU/L	0.53	190	2.37	1.25–4.47	0.00824[*]				
Gamma-GT <38 IU/L	0.60	189	2.63	1.44–4.81	0.00166[*]				
Total cholesterol ≥178 mg/dL	0.55	171	1.79	0.96–3.34	0.0708				
Triglyceride ≥75 mg/dL	0.57	138	2.23	1.01–4.93	0.0491[*]	100	3.37	0.93–12.23	0.0648
Fasting blood sugar <114 mg/dL	0.64	143	3.10	1.45–6.67	0.00379[*]				
HbA1c <6.4%	0.53	146	2.96	0.81–10.8	0.102				
rs8099917: TT	0.62	190	3.77	1.76–8.11	0.00068[*]	100	4.95	1.13–21.85	0.0349[*]
IL1β <1.74 pg/mL	0.53	190	1.58	0.73–3.43	0.254				
IL6 <13.66 pg/mL	0.54	190	2.51	0.96–6.54	0.0606				
IL10 ≥12.84 pg/mL	0.59	190	1.86	1.00–3.45	0.0504				
TNFα <6.03 pg/mL	0.51	190	1.49	0.78–2.86	0.235				
VEGF ≥79.26 pg/mL	0.57	190	2.36	1.24–4.50	0.00927[*]	100	2.70	0.71–10.23	0.146
Adiponectin <11 076.65 ng/mL	0.51	190	1.46	0.81–2.62	0.21				
PAI-1 ≥25 117.24 pg/mL	0.63	190	4.59	2.09–10.09	0.000157[*]	100	15.42	3.00–79.18	0.00105[*]
Resistin ≥21 580.23 pg/mL	0.56	190	1.75	0.94–3.27	0.0819				
Leptin ≥4418.96 pg/mL	0.51	190	1.49	0.82–2.69	0.195				
MCP-1 <186.81 pg/mL	0.52	190	2.01	0.94–4.33	0.0746				
Fibrosis stage <3	0.61	155	2.88	1.26–6.58	0.0122[*]	100	5.18	1.12–24.11	0.0362[*]
Activity grade <2	0.61	152	2.13	1.05–4.30	0.0364[*]				

ROC_AUC, area under the receiver operating characteristic curve; OR, odds ratio; CI, confidence interval.
Variables with a *P* value <0.05 were included in the multivariate model. Variables were selected using stepwise backward selection. [†]Continuous variables were split into two categories by analysis of the ROC curve. The favourable category for SVR is shown for each variable. [*]Significant.

under the ROC curve (ROC_AUC) was ≥0.60 were significant in univariate logistic regression analysis. Four out of these 13 factors were independently associated with SVR under multivariate analysis: PAI-1 (*P* = 0.00105, OR = 15.42), BMI (*P* = 0.0154, OR = 4.56), rs8099917 (*P* = 0.0349, OR = 4.95), and fibrosis stage (*P* = 0.0362, OR = 5.18). Finally, pretreatment serum PAI-1 level, which has not been reported previously, was revealed as the most significant factor for SVR in this study cohort (Table 2).

Prediction score for SVR in the study and validation cohorts

We tried to construct a simple and minimally invasive prediction score for SVR based on these independent predictors except for fibrosis stage. Liver biopsy has long remained the gold standard for staging of fibrosis, but it is an invasive test with potential for serious, albeit rare, complications [21]. Hence, we excluded fibrosis stage from the score. In this scoring method, 1 point each was assigned for PAI-1 (≥25 117.24 pg/mL), BMI (<22.2 kg/m^2), and rs8099917 (TT). Points were summed in each patient, and the combined point value was assigned as the prediction score of the patient. As shown in Table 3, the proportion of patients assigned 1 point was higher in the SVR group than the non-SVR group for each of the three factors. Higher combined scores predicted higher SVR rates, i.e., patients who scored 2 or 3 had a higher SVR rate (63.1%; Fig. 1). In contrast, patients who scored 0 or 1 had a lower SVR rate (19.8%). Based on this threshold, the accuracy of the prediction score for SVR versus non-SVR was 73% (138/190) in this study cohort with ROC_AUC of 0.74. Specificity, sensitivity, and positive predictive value (PPV) and negative predictive value (NPV) for SVR were 73%, 72%, 63% and 80%, respectively.

We next evaluated the accuracy of the prediction score using an independent validation cohort consisting of 31 patients (SVR, 9; non-SVR, 22) and reobserved the same