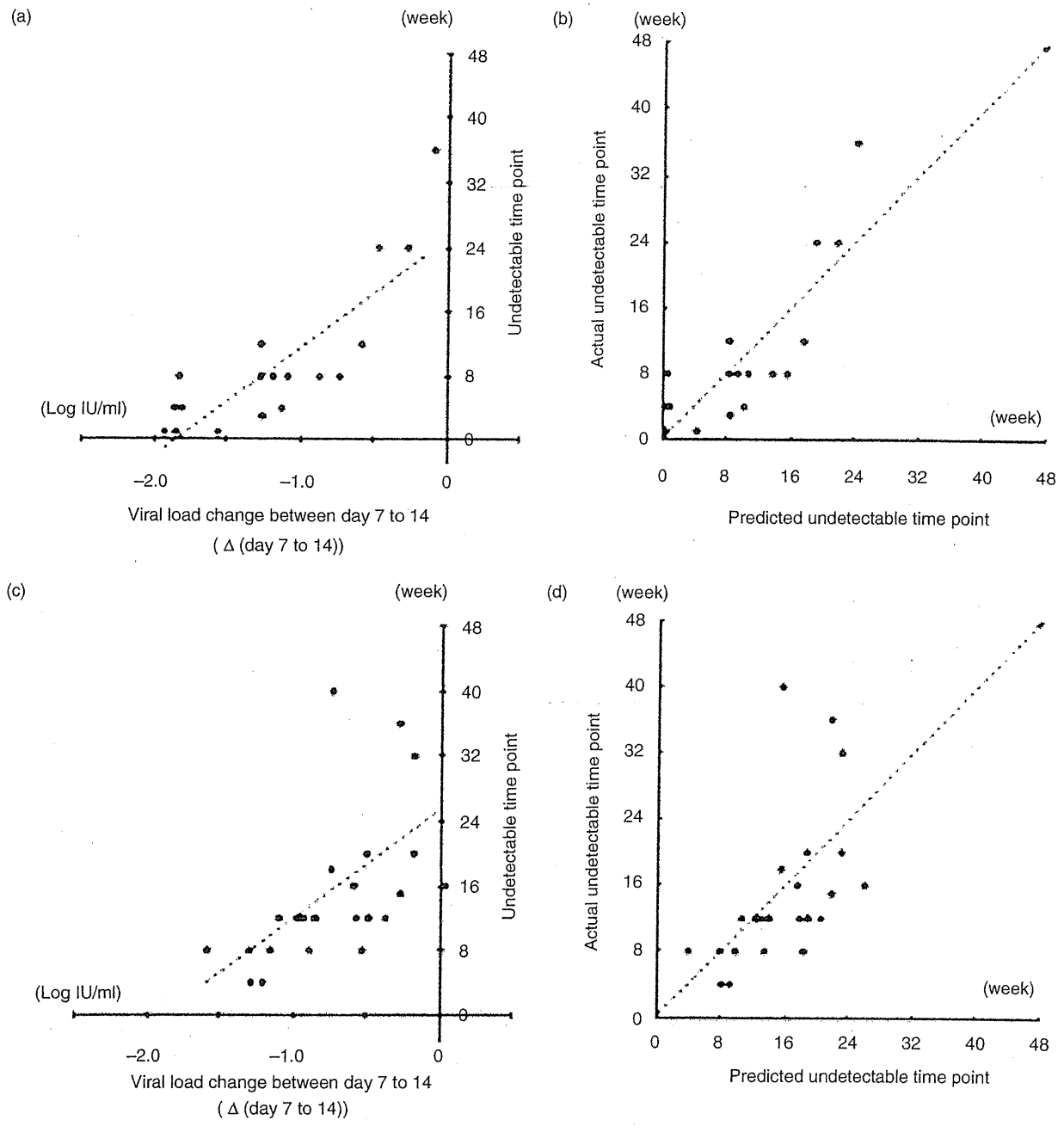

Figure 2 Correlation between the undetectable time point and the decrease in viral load from day 7 to 14 (a,b) and correlation between the actual and predicted undetectable time points (c,d). (a,c) Results of analyses for the model preparation group; and (b,d) analyses for the model validation group. Black circles, actual cases; dotted line, (a,c) estimate obtained from the prediction formula; (b,d) equal values of actual and predicted undetectable time points.

undetectable time point are plotted in Figure 2(a), which shows a very strong and a significant correlation ($r^2 = 0.67$, $P < 0.0005$).

The validity of the prediction formula was investigated in the validation group. Analysis was possible in 32 patients, as the other patients were excluded from the analysis due to the following reasons: therapy was discontinued before viral clearance in eight patients, PEG-IFN dosage was reduced before viral clearance in nine patients and viral clearance was achieved before day 14 in two patients. There were six cases of NVR, and incomplete blood collections from 13 patients on day 7 and/or 14. A strong and a significant correlation was demonstrated between the undetectable time points that were predicted using this formula and the actual undetectable time points (Fig. 2c, $r = 0.53$, $P = 0.005$).

Although only one case was predicted to achieve a rapid virological response (undetectable viral load at week 4)[13] in the model validation group, the actual undetectable time point of this patient was week 8 (Fig. 2d). In contrast, all nine cases who were predicted to achieve a complete early virological response (undetected viral load until week 12),[13] the actual undetectable time points of these patients were within week 12. Because the prediction formula was derived by the least squares method, half of the patients, who were predicted not to achieve the complete early virological response, actually achieved it.

DISCUSSION

NUMEROUS STUDIES HAVE documented that the undetectable time point is related to therapeutic responses, and its usefulness in predicting therapeutic efficacy is clear.[9–13] In the present study, we were able to derive a formula for predicting the undetectable time point for patients with HCV genotype 1b and high serum viral loads during PEG-IFN-α-2b/ribavirin combination therapy. Though the various parameters for the HCV dynamics were investigated, the change in viral load from day 7 to 14 was the only parameter that was useful for predicting the undetectable time point.

The standard length of PEG-IFN/ribavirin combination therapy is 48 weeks for patients with HCV genotype 1b and high serum viral loads; however, a 72-week administration is recommended to improve therapeutic response.[3,13,18] Therefore, when undetectable time points are predicted as from weeks 13–24 by our formula, the SVR rates could be improved by continuing the IFN therapy for longer periods. By prediction of the undetectable time point early during the treatment using our formula, the physician can make early modification and optimization of currently ongoing therapy.

Another important issue of PEG-IFN/ribavirin treatment is adherence to treatment. Because dose reductions may delay the time until serum viral clearance, patients in whom the dosage of IFN and ribavirin was reduced during therapy were excluded in the present study. However, there are many patients in whom the dosage of drugs has to be reduced during therapy for a wide variety of clinical reasons. If reducing dosage before the predicted undetectable time point, administration of IFN for longer periods should be considered.

In conclusion, we created a formula for predicting the undetectable time point in patients treated with PEG-IFN-α-2b/ribavirin combination therapy. Viral eradication is the ultimate objective of IFN-based therapy, but many patients failed to achieve viral eradication for some reason. Because our prediction formula for the undetectable time point was made with a small population, it is necessary to correct it by further analysis with a larger population. However, an early viral response reflects efficacy of the therapy, and the estimation of an undetectable time point by our formula would be useful for determining the optimal duration of treatment in the early period of the therapy for each chronic hepatitis C patient.

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Hepatology Research 2011; 41: 955–964 doi: 10.1111/j.1872-034X.2011.00847.x

Original Article

Cancer preventive effect of pegylated interferon α-2b plus ribavirin in a real-life clinical setting in Japan: PERFECT interim analysis

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Aim: This study was conducted to clarify the incidence of hepatocellular carcinoma (HCC) and the factors contributing to its occurrence by following chronic hepatitis C patients who received pegylated interferon (PEG-IFN) α-2b plus ribavirin (RBV) combination therapy.

Methods: Patients who received PEG-IFN α-2b and RBV combination therapy with no history of HCC or HCC within 3 months after the start of treatment were observed for the onset of HCC at 67 centers.

Results: Sustained virological response (SVR) was observed in 999 (53.5%) of 1865 patients eligible for analysis. During the observation period (median duration: 4 years and 3 months), HCC developed in 59 patients (3.1%). A significant difference was observed in the 5-year cumulative incidence of HCC between SVR and non-SVR patients (1.1% vs. 7.1%). Factors contributing to HCC selected in multivariate analysis were therapeutic efficacy, sex, age, alanine aminotransferase (ALT) level at 24 weeks after the end of treatment, and platelet count. Non-SVR patients with ALT improvement after the end of treatment had a significantly lower 5-year cumulative incidence of HCC than those without (3.4% vs. 11.0%). HCC developed in 10 patients who achieved SVR, and multivariate analysis indicated that ALT level at 24 weeks after the end of treatment was the only significant factor contributing to HCC.

Conclusion: Several known risk factors for HCC contributed to HCC in patients who received PEG-IFN α-2b and RBV combination therapy, and ALT abnormality after the end of treatment contributes to the onset of HCC in both non-SVR and SVR patients.

Key words: alanine aminotransferase, chronic hepatitis C virus, hepatocellular carcinoma, pegylated inteferon, ribavirin

Abbreviations: AFP, alpha fetoprotein; ALT, alanine aminotransferase; BR, biochemical response; CHC, chronic hepatitis C; HCC, hepatocellular carcinoma; IFN, interferon; LVR, late virological response; NR, no response; NVR, non-virological response; PEG-IFN, pegylated interferon; ; RBV, ribavirin; SVR, sustained virological response; TR, transient response.

INTRODUCTION

THE INCREASE IN the incidence of hepatocellular carcinoma (HCC) in Japan peaked in 2004 and is now in a declining trend.[1] The HCC mortality rate, however, is still particularly high among developed countries,[2] and even now nearly 35 000 people die

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Received 17 February 2011; revision 18 May 2011; accepted 23 May 2011.

annually from HCC. In Japan, about 70% of patients diagnosed with HCC are positive for hepatitis C virus antibody.[3] The hepatitis C virus infection rate[2] and incidence of HCC both increase with the age of the patient,[4] and curing chronic hepatitis C (CHC) to reduce HCC and deaths due to HCC is a pressing issue.

With the discovery of interferon (IFN), CHC became a curable disease, and with the addition of ribavirin (RBV), therapeutic outcomes have improved dramatically. Currently, about 50%[5–8] of patients with HCV genotype 1b and high virus load and more than 80%[9] of genotype 2 patients achieve sustained virologic response (SVR), and the SVR rate is reported to improve further with long-term treatment[10,11] and with combination therapy plus a statin.[12]

The efficacy achieved with these IFN therapies is also reported to lead to the inhibition of the onset of HCC and deaths due to HCC[13–19], but only a few reports are available of long-term observation of patients receiving PEG-IFN α plus RBV combination therapy.

We therefore examined the HCC preventive effect of combination therapy in 1865 patients who received PEG-IFN α-2b and RBV.

METHODS

Patients and treatment

PERFECT (THE PEG-IFN and Ribavirin, Find Evidence of Chronic Hepatitis C Therapy in Tokyo) Study Group, consisting of 67 centers in Tokyo and Yamanashi Prefecture, conducted a retrospective study to investigate the efficacy and safety of PEG-IFN α-2b plus RBV in CHC patients in a real-life clinical setting. The participating centers, targeted patients, and the treatment method have already been reported[10] and are summarized below.

Patients seen from December 2004 who completed PEG-IFN α-2b plus RBV combination therapy by September 2007 were registered regardless of genotype, history of IFN treatment, or alanine aminotransferase (ALT) levels. Excluded from this study were pregnant or possibly pregnant and lactating women, and patients with severe heart disease, chronic kidney failure or creatinine clearance of ≤50 mL/min, current or history of severe psychiatric disorder, and autoimmune hepatitis. Doses of PEG-IFN α-2b and RBV and dose adjustment followed the Japanese package insert. The duration of treatment was 48 weeks, the standard of care for patients with genotype 1 and high virus load. In patients with late viral response (LVR) who did not achieve viral negativity by week 12, treatment could be extended up to 72 weeks. Patients other than those with genotype 1 and high virus load were treated for 24 weeks.

Included in this analysis were the patients registered in the PERFECT Study who had no history of HCC and for whom SVR/non-SVR status could be confirmed. The patients who developed HCC within 3 months of the start of treatment were excluded from analysis to rule out the possibility of inclusion of patients with HCC already present at the start of treatment.

The start of the follow-up period was defined as the first day of PEG-IFN α-2b and RBV treatment. The patients were monitored for the onset of HCC by routine follow-up methods practiced by each center. The diagnosis of HCC was based on the presence of typical hypervascular characteristics on angiography in addition to the findings on computed tomography and ultrasonography. Microscopic examination of fine-needle biopsy specimens was performed in patients whose angiograms did not demonstrate a typical image of HCC.

This multicenter study was approved by the institutional review board of each participating center. The study protocol was carried out according to the ethical guidelines of the 1975 Declaration of Helsinki, and informed consent was obtained from each patient.

Statistical analysis

All statistical analyses were performed using SAS, version 9.13 (SAS Institute, Cary, NC, USA). Intergroup comparison of background variables was performed by Fisher's exact test and Mann–Whitney *U*-test.

The cumulative incidence of HCC was calculated by the Kaplan–Meier method, and intergroup comparison was conducted using the log-rank test. The determination of the factors contributing to HCC was conducted by Cox proportional hazards regression model using a stepwise procedure, incorporating the factors exhibiting $P < 0.2$ by the log-rank test and excluding factors with more than 30% of values missing. The determination of factors associated with biochemical response (BR) was conducted by a stepwise procedure using the results of logistic univariate analysis ($P < 0.2$) in logistic multivariate analysis.

All tests were two-sided, with a significance level set at $P < 0.05$.

RESULTS

Study population

A TOTAL OF 1865 subjects, consisting of 999 SVR patients (SVR rate 53.5%) and 866 non-SVR patients, were eligible for analysis. Of the non-SVR patients, 441 had transient response (TR) defined as viral negativity achieved during treatment (relapse: 408, virus breakthrough: 33), 400 patients had non-virological response (NVR) defined as viral negativity not being achieved, and the change in viral load during treatment was not known for 25 patients.

The duration of observation ranged from 3 months to 5 years and 8 months, with a median of 4 years and 3 months.

During the observation period, HCC developed in 59 patients (3.1%). Between patients who developed HCC and those who did not, significant differences in background factors were detected in age ($P < 0.0001$), hepatic fibrosis ($P = 0.0002$), virological efficacy ($P < 0.0001$), ALT levels ($P = 0.0089$), ALT level at 24 weeks after the end of treatment (≤40 vs. >40 IU/L) ($P < 0.0001$), platelet count ($P = 0.0001$), serum albumin ($P = 0.0062$), and alpha fetoprotein (AFP) ($P < 0.0001$) (Table 1).

Virological efficacy and incidence of HCC

The 5-year cumulative incidence of HCC by the Kaplan–Meier method was 1.1% in SVR patients and 7.1% in non-SVR patients, a difference that was significant ($P < 0.001$) (Fig. 1). No significant difference was observed in the incidence of HCC between TR and NVR patients among non-SVR patients, but the difference between TR and SVR patients was significant ($P < 0.0001$) (Fig. 2). This trend was also observed regardless of gender, with no significant difference in the incidence of HCC observed between TR and NVR in either male or female patients and a significant difference observed between TR and SVR in both male patients ($P = 0.0007$) and female ($P = 0.0065$) patients.

Factors contributing to HCC

The factors contributing to HCC selected in the multivariate analysis were therapeutic efficacy (SVR vs. NVR), sex, age (<60 vs. ≥60 years), ALT level at 24 weeks after the end of treatment (≤40 vs. >40 IU/L), and platelet count (<10 vs. ≥10 × 10 000/mm³) (Table 2).

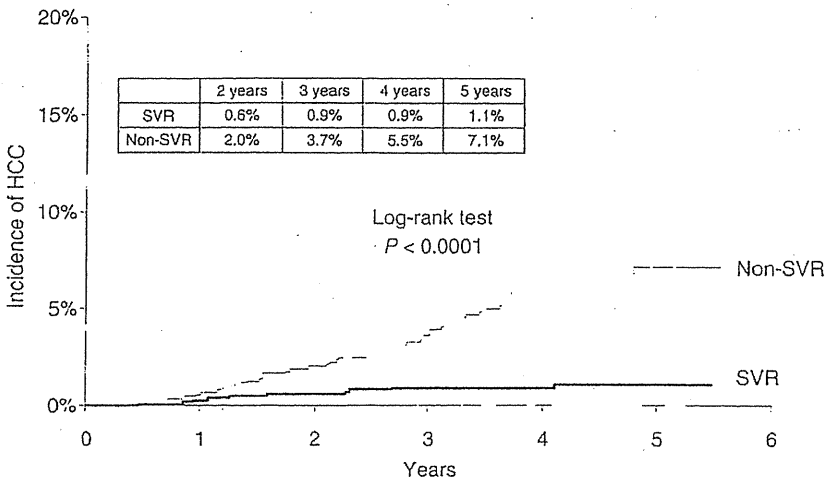
Biochemical response and incidence of HCC in non-SVR patients

Since ALT levels at 24 weeks after the end of treatment was selected as one factor contributing to HCC, the changes in ALT levels and onset of HCC were examined in 514 non-SVR patients with a pretreatment ALT level of more than 40 IU/L whose ALT level at 24 weeks after the end of treatment was obtained. Of these 514

Table 1 Patient background by onset of hepatocellular carcinoma (HCC) (1865 patients)

Factor	With onset of HCC ($n = 59$)	Without onset of HCC ($n = 1806$)	*P*-value
Gender (male/female)	40/19	1014/792	0.0832
Age	62 (44–74)	56 (17–77)	<0.0001
Diabetes (yes/no/unknown)	6/33/20	100/1040/666	0.1539
Hypertension (yes/no/unknown)	4/6/49	116/569/1121	0.0763
Alcohol abuse (yes/no/unknown)	11/16/32	195/493/1118	0.1930
Fibrosis (0/1/2/3/4/unknown)	0/12/13/15/4/15	57/573/355/205/56/560	0.0002
Genotype (1/2/3/unknown)	52/5/0/2	1421/365/2/18	0.0876
Effect of IFN (SVR/non-SVR)	10/49	989/817	<0.0001
Body mass index (kg/m²)	22.6 (14.2–34.0)	22.9 (14.9–41.2)	0.8546
ALT (IU/L)	79 (24–343)	60 (8–984)	0.0089
ALT at 24 weeks after end of treatment (IU/L) (≤40/>40/unknown)	16/30/13	1105/352/349	<0.0001
Platelet count (×10 000/mm³)	13.3 (4.3–22.2)	16.3 (3.6–213.3)	0.0001
Serum albumin (g/dL)	3.9 (2.9–4.7)	4.1 (2.8–5.9)	0.0062
AFP (ng/mL)	13 (2.2–327.9)	5 (0–875)	<0.0001

Median (minimum – maximum).
AFP, alpha fetoprotein; ALT, alanine aminotransferase; IFN, interferon; SVR, sustained virological response.



	2 years	3 years	4 years	5 years
SVR	0.6%	0.9%	0.9%	1.1%
Non-SVR	2.0%	3.7%	5.5%	7.1%

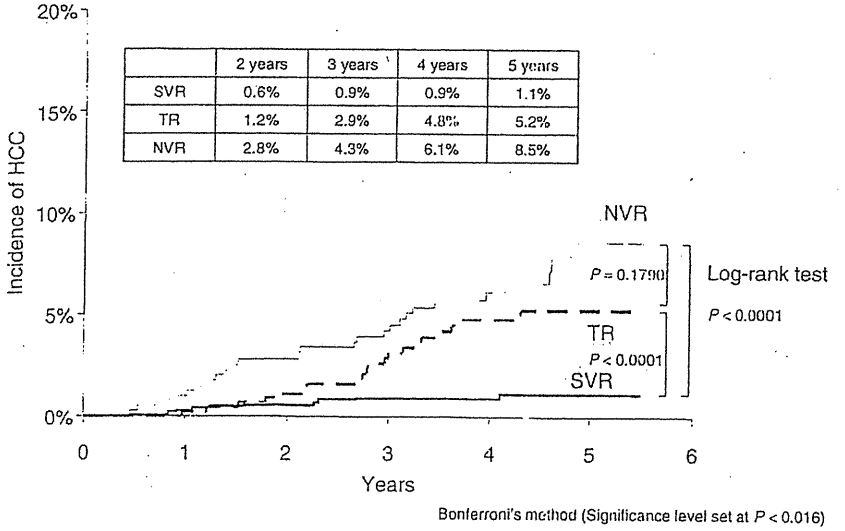
Figure 1 Onset of hepatocellular carcinoma (HCC) by therapeutic efficacy (1865 patients) (sustained virological response [SVR] vs. non-virological response [NVR]). The cumulative incidence of HCC was calculated by the Kaplan–Meier method. The difference between SVR and non-SVR was examined using the log-rank test.

patients, ALT level at 24 weeks after the end of treatment was reduced to less or equal to 40 IU/L (biochemical response: BR) in 234 patients, and the remaining 280 patients had values of more than 40 IU/L (non-BR). There were significant differences between BR and non-BR patients in the background factors of pretreatment ALT level, age, hepatic fibrosis, platelet count, AFP, and treatment duration. Selected as the factors contributing to BR in non-SVR patients in the multivariate analysis were TR, long treatment duration, and high platelet count before the start of treatment (Table 3).

The 5-year cumulative incidence of HCC was 3.4% in BR patients and 11.0% in non-BR patients, and the difference in incidence was significant ($P = 0.0012$) (Fig. 3). The 5-year cumulative incidence of HCC in male patients was 3.6% in BR patients and 13.9% in non-BR patients, and the difference was significant ($P = 0.0012$). In female patients, however, it was 3.5% in BR patients and 7.6% in non-BR patients, and although the incidence of HCC was lower in BR patients, the difference was not significant ($P = 0.0706$).

Incidence of HCC in patients with normal pretreatment ALT levels

When the incidence of HCC was compared between SVR (288) and non-SVR (214) patients among 502 patients with pretreatment ALT levels less or equal to 40 IU/L, the 5-year cumulative incidence of HCC was 0% in SVR patients and 4.8% in non-SVR patients, indicating a significant difference ($P = 0.0005$) between the groups



	2 years	3 years	4 years	5 years
SVR	0.6%	0.9%	0.9%	1.1%
TR	1.2%	2.9%	4.8%	5.2%
NVR	2.8%	4.3%	6.1%	8.5%

Figure 2 Onset of hepatocellular carcinoma (HCC) by therapeutic efficacy (sustained virological response [SVR] vs. transient response [TR] vs. non-virological response [NVR]). The cumulative incidence of HCC was calculated by the Kaplan–Meier method. The difference between each group was examined using the log-rank test (Bonferroni's Method, significance level set at $P < 0.016$).

Table 2 Factors contributing to hepatocellular carcinoma (all patients) Cox regression analysis (multivariate)

		Hazard ratio	95% confidence interval	*P*-value
Therapeutic efficacy	SVR	1		
	TR	2.055	0.709–5.955	0.1845
	NVR	2.985	1.036–8.601	0.0428
Sex	Male	1		
	Female	0.486	0.243–0.969	0.0405
Age	<60	1		
	≥60	2.005	1.035–3.883	0.0391
ALT at 24 weeks after end of treatment (IU/L)	≤40	1		
	>40	3.940	1.754–8.850	0.0009
Platelet count (×10 000/mm^3)	<10	1		
	≥10	0363	0.169–0.779	0.0093
Serum albumin (g/dL)	<4	1		
	≥4	0.594	0.310–1.140	0.1175

Factors examined: Of the 15 factors exhibiting $P < 0.2$ by log-rank test (therapeutic efficacy [1: SVR, 2: TR, 3: NVR], genotype [1: 1, 2: 2 or 3], sex [1: male, 2: female], age [1: <60, 2: ≥60], pre ALT [1: ≤40, 2: >40], +24 w ALT [1: ≤40, 2: >40], pre PLT [1: <10, 2: ≥10], pre ALB [1 <4, 2: ≥4], pre AFP [1: <20, 2: ≥20], grade [1: A0–1, 2: A2–3], stage [1: F0–1, 2: F2–4], hypertension [1: absent, 2: present], diabetes [1: absent, 2: present], heavy drinking [1: absent, 2: present], and treatment duration [1: ≤48 W, 2: >48 W]), nine factors were examined. Excluded were factors for which approximately 30% of values were missing (AFP, grade, stage, diabetes, hypertension, and heavy drinking).
AFP, alpha fetoprotein; ALB, albumin; ALT, alanine aminotransferase; NVR, non-virological response; PLT, platelet count; SVR, sustained virological response; TR, transient response.

(Fig. 4). This tendency is also observed with the 280 patients having pretreatment ALT levels of less or equal to 30 IU/L.

Onset of HCC in SVR patients

Hepatocellular carcinoma developed in 10 patients who achieved SVR. Multivariate analysis indicated that in SVR patients, the ALT level at 24 weeks after the end of treatment was the only significant factor contributing to HCC ($P = 0.0007$) (Table 4). In SVR patients with an ALT level of more than 40 IU/L at 24 weeks after the end of treatment, the 5-year cumulative incidence of HCC was 5.6% while the incidence in patients with an ALT level of less or equal to 40 IU/L was 0.7%, indicating a significant difference ($P = 0.0004$) between the groups (Fig. 5).

DISCUSSION

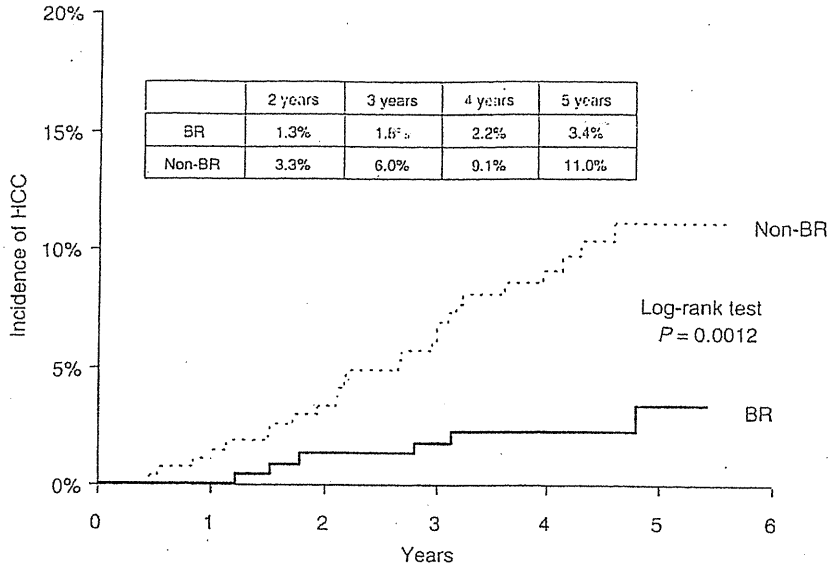
THIS STUDY INDICATED that the risk factors for HCC after PEG-IFN α-2b plus RBV combination therapy are NVR, male sex, older age, low platelet count, and an ALT level of more than 40 IU/L at 24 weeks after the end of treatment.

Kurokawa *et al.*[16] tracked 403 patients receiving PEG-IFN α-2b plus RBV combination therapy for a median

Table 3 Factors contributing to biochemical response in non-sustained virological response patients Logistic regression analysis (multivariate)

		Odds ratio	95% confidence interval	*P*-value
Virological response	NVR	1	1.480–3.203	0.0001
	TR	2.177		
Treatment duration	per week	1	1.000–1.022	0.0424
		1.011		
Platelet count	per 10 000/mm^3	1	1.018–1.099	0.0043
		1.058		

Factors examined were those exhibiting $P < 0.2$ by log-rank test: Genotype, virological response (TR/NVR), treatment duration, pre platelet count, diabetes, stage, and alanine aminotransferase (ALT).

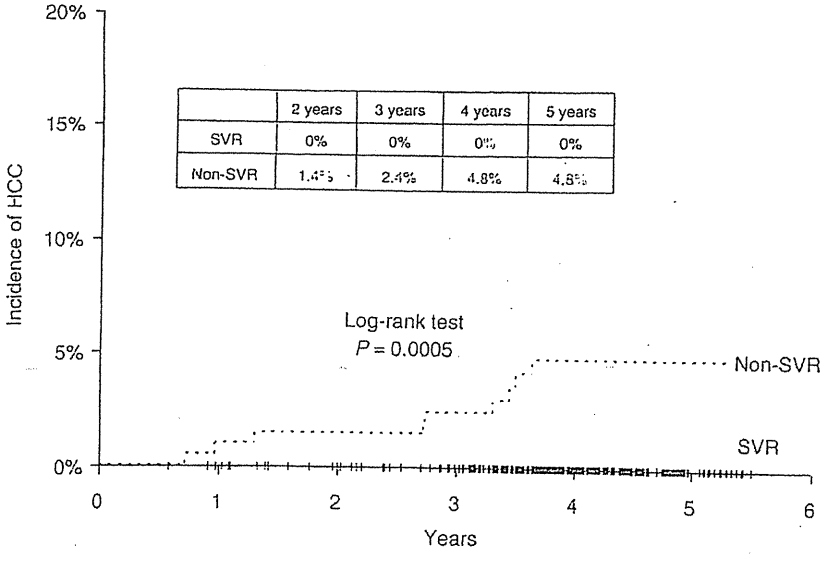


	2 years	3 years	4 years	5 years
BR	1.3%	1.5%	2.2%	3.4%
Non-BR	3.3%	6.0%	9.1%	11.0%

Figure 3 Alanine aminotransferase (ALT) normalization and hepatocellular carcinoma (HCC) in non-virological response [NVR] patients. The cumulative incidence of HCC was calculated by the Kaplan–Meier method. Log-rank test was used to study the difference between biochemical response (BR) and non-BR.

duration of 36.5 months and reported that in multivariate analysis, virological efficacy (SVR vs. non-SVR), age, and hepatic fibrosis were selected as the factors contributing to HCC. Arase *et al.*[15] tracked 500 patients 60 years of age and older receiving IFN alone or in combination with RBV for an average duration of 7.4 years and also reported that the factors contributing to HCC are virological efficacy (SVR vs. non-SVR), age, and hepatic fibrosis. In our study, hepatic fibrosis was not tested with multivariate analysis because more than 30% of values were missing, but it was selected as a significant factor in the univariate analysis. Platelet count was selected in multivariate analysis, and the results in our study are therefore considered to be generally consistent with these reports.

The results of the present study indicated no significant difference between TR and NVR in non-SVR in stratified cumulative incidence of HCC, and although there was a significant difference between SVR and both TR and NVR, TR was not significant against SVR in multivariate analysis, and NVR was the only significant factor. Kurokawa *et al.*[16] reported the same results by



	2 years	3 years	4 years	5 years
SVR	0%	0%	0%	0%
Non-SVR	1.4%	2.4%	4.8%	4.8%

Figure 4 Therapeutic efficacy and hepatocellular carcinoma (HCC) in patients with pretreatment alanine aminotransferase (ALT) of ≤40. The cumulative incidence of HCC was calculated by the Kaplan–Meier method. Log-rank test was used to study the difference between sustained virological response (SVR) and non-virological response (NVR).

Table 4 Factors contributing to hepatocellular carcinoma (sustained virological response [SVR] patients) Cox regression analysis (multivariate)

		Hazard ratio	95% confidence interval	*P*-value
ALT at 24 weeks after end of treatment (IU/L)	≤40	1		
	>40	16.054	3.235–79.681	$P = 0.0007$
Serum albumin (g/dL)	<4	1		
	≥4	0.196	0.036–1.073	$P = 0.0603$

Factors examined: Of the 10 factors exhibiting $P < 0.2$ by log-rank test (Genotype [1: 1, 2: 2 or 3], age [1: <60, 2: ≥60], pre ALT [1: ≤40, 2: >40], +24 w ALT [1: ≤40, 2: >40], pre PLT [1: <10, 2: ≥10], pre ALB [1: <4, 2: ≥4], pre AFP [1: <20, 2: ≥20], grade [1: A0–1, 2: A2–3], stage [1: F0–1, 2: F2–4], and diabetes [1: absent, 2: present]), 5 factors were examined. Excluded were pre ALT, with which HCC did not occur in the ≤40 group, and AFP, grade, stage, and diabetes, the factors for which approximately 30% of values were missing.
ALB, albumin; ALT, alanine aminotransferase; PLT, platelet count;

comparing cumulative incidences of HCC among SVR, TR and NVR (the results of multivariate analysis are not known). On the other hand, Morgan *et al.*,[19] in their follow-up study of the HALT-C Trial, reported that there was no difference between TR and NVR in the incidence of HCC or death related to hepatic disease/liver transplantation, but when all hepatic-related outcomes were examined, a significantly superior inhibition was observed with TR compared to NVR. Our results also demonstrate that although the difference is not significant, the cumulative incidence of HCC is lower in TR patients than in NVR patients, especially in male patients (5-year cumulative incidence of HCC: 6.0% vs. 10.7%). It is therefore necessary to continue to observe this for an extended number of years.

Our results study indicated that in non-SVR patients, whether or not ALT level is normalized after treatment is a greater contributing factor for the onset of HCC than virological response. Normalization of ALT has already been reported to contribute to the inhibition of the onset of HCC even under HCV-positive conditions,[13,20] and this was found to apply also to non-SVR patients receiving PEG-IFN α plus RBV combination therapy.

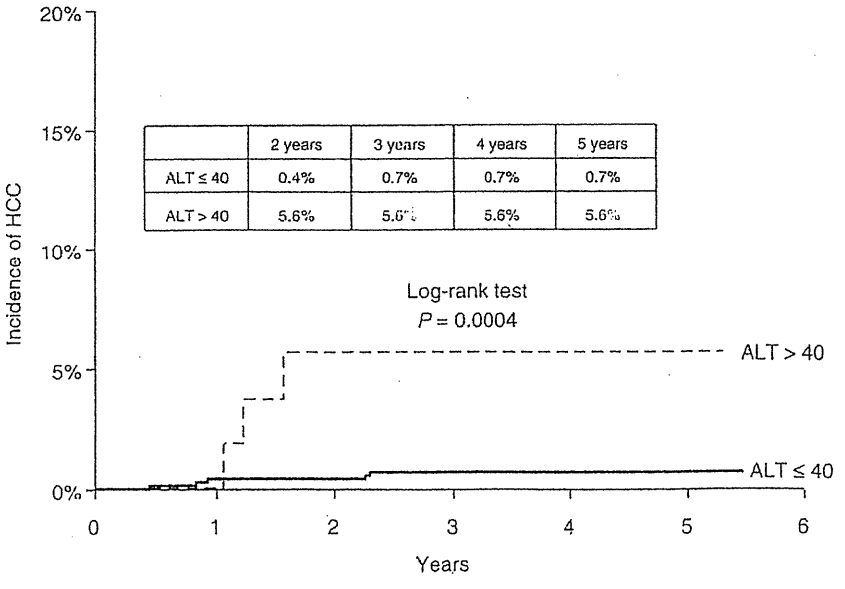


Figure 5 Alanine aminotransferase (ALT) levels at 24 weeks after end of treatment and hepatocellular carcinoma (HCC) in patients with sustained virological response (SVR). The cumulative incidence of HCC was calculated by the Kaplan–Meier method. Log-rank test was used to study the difference between SVR patients with an ALT level of more than 40 IU/L at 24 weeks after the end of treatment and those with an ALT level of less or equal to 40 IU/L.

Our investigation also indicated that abnormal ALT levels also contribute to the onset of HCC in SVR patients. In multivariate analysis, the only contributing factor to the development of HCC in SVR patients was ALT levels at 24 weeks after the end of treatment. However, the onset of HCC is also observed in patients who achieve ALT normalization after treatment, and it is therefore difficult to conclude that ALT is the only risk factor for the onset of HCC in SVR patients. The potential involvement of hepatic fibrosis as well as hepatic steatosis, which persists after viral clearance[21] and small amounts of virus remaining in the liver[22] have also been suggested as risk factors for the onset of HCC in SVR patients. Further detailed investigation is therefore necessary. Nevertheless, regardless of whether or not SVR is achieved, it is clear that abnormal ALT is a factor affecting the onset of HCC. Careful monitoring of changes in ALT and instituting measures to normalize ALT are therefore important regardless of whether or not SVR is achieved.

With the administration of PEG-IFN α plus RBV combination therapy tailored for individual patients and the addition of direct-acting antivirals to current combination therapy, the therapeutic outcomes for CHC will continue to further improve, and the number of patients who develop hepatic cirrhosis and HCC from hepatitis C can be expected to decrease in the future. HCC can occur even in patients achieving SVR, and even if SVR is not achieved, as long as the possibility to inhibit the onset of HCC remains, there will be a need for various treatment innovations to achieve the prevention of HCC, the ultimate goal of treatment of CHC.

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APPENDIX I

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Journal of Medical Virology 83:445–452 (2011)

Sequences in the Interferon Sensitivity-Determining Region and Core Region of Hepatitis C Virus Impact Pretreatment Prediction of Response to PEG-Interferon Plus Ribavirin: Data Mining Analysis

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The aim of the present study was to clarify the significance of viral factors for pretreatment prediction of sustained virological response to pegylated-interferon (PEG-IFN) plus ribavirin (RBV) therapy for chronic hepatitis C using data mining analysis. Substitutions in the IFN sensitivity-determining region (ISDR) and at position 70 of the HCV core region (Core70) were determined in 505 patients with genotype 1b chronic hepatitis C treated with PEG-IFN plus RBV. Data mining analysis was used to build a predictive model of sustained virological response in patients selected randomly (n = 304). The reproducibility of the model was validated in the remaining 201 patients. Substitutions in ISDR (odds ratio = 9.92, $P < 0.0001$) and Core70 (odds ratio = 1.92, $P = 0.01$) predicted sustained virological response independent of other covariates. The decision-tree model revealed that the rate of sustained virological response was highest (83%) in patients with two or more substitutions in ISDR. The overall rate of sustained virological response was 44% in patients with a low number of substitutions in ISDR (0–1) but was 83% in selected subgroups of younger patients (<60 years), wild-type sequence at Core70, and higher level of low-density lipoprotein cholesterol (LDL-C) (≥120 mg/dl). Reproducibility of the model was validated ($r^2 = 0.94$, $P < 0.001$). In conclusion, substitutions in ISDR and Core70 of HCV are significant predictors of response to PEG-IFN plus RBV therapy. A decision-tree model that includes these viral factors as predictors could identify patients with a high probability of sustained virological response. ***J. Med. Virol. 83:445–452, 2011.***

KEY WORDS: data mining; decision-tree model; ISDR; core region; PEG-interferon

INTRODUCTION

The combination of pegylated-interferon (PEG-IFN) plus ribavirin (RBV) is currently the most effective therapy for chronic hepatitis C, but the rate of sustained virological response after 48 weeks of therapy is about 50% in patients with HCV genotype 1b and a high HCV

Grant sponsor: Ministry of Health, Labor and Welfare, Japan.
The authors report no conflicts of interest.

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Accepted 26 October 2010
DOI 10.1002/jmv.22005
Published online in Wiley Online Library (wileyonlinelibrary.com).

RNA titer [Manns et al., 2001; Fried et al., 2002]. The most reliable means to predict sustained virological response is to monitor the viral response during the early weeks of treatment. The early virological response, defined as undetectable HCV RNA at week 12, is associated with a high rate of sustained virological response [Davis et al., 2003; Lee and Ferenci, 2008]. The rapid virological response, defined as undetectable HCV RNA at week 4 of therapy, is even more predictive of sustained virological response than the early virological response [Jensen et al., 2006; Yu et al., 2008; Izumi et al., 2010]. However, there is no established means that predicts the virological response before commencing treatment. Recent reports have revealed that single nucleotide polymorphisms located near the *IL28B* gene show a strong association with the response to PEG-IFN plus RBV therapy [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Kurosaki et al., 2010c]. These findings indicate that the host factor is an important determinant of the treatment response. On the other hand, the present study's authors have reported that a stretch of 40 amino acids in the NS5A region of HCV, designated as the interferon sensitivity-determining region (ISDR), has a close association with the virological response to interferon mono-therapy [Enomoto et al., 1995, 1996; Kurosaki et al., 1997]. More recently, amino acid substitutions at positions 70 and 91 of the core region have been reported to be associated with response to PEG-IFN plus RBV combination therapy [Akuta et al., 2005, 2007a]. The impact of these HCV substitutions on treatment response is yet to be validated.

Decision-tree analysis is a core component of data mining analysis that can be used to build predictive models [Breiman et al., 1980]. This method has been used to define prognostic factors in various diseases such as prostate cancer [Garzotto et al., 2005], diabetes [Miyaki et al., 2002], melanoma [Averbook et al., 2002; Leiter et al., 2004], colorectal carcinoma [Zlobec et al., 2005; Valera et al., 2007], and liver failure [Baquerizo et al., 2003]. The major advantage of decision-tree analysis over logistic regression analysis is that the results of analysis are easy to understand. The simple allocation of patients into subgroups by following the flowchart form could define the predicted possibility of outcome [LeBlanc and Crowley, 1995].

Decision-tree analysis was used for the prediction of early virological response (undetectable HCV RNA within 12 weeks of therapy) to PEG-IFN and RBV combination therapy in chronic hepatitis C [Kurosaki et al., 2010a], and more recently for the pretreatment prediction of sustained virological response [Kurosaki et al., 2010b]. In the latter model, simple and noninvasive standard tests were used as parameters; specialized tests such as viral mutations and host genetics, or invasive tests such as liver histology, were not included because the aim of that model was for use in general medical practice, especially in some countries or areas where resources are limited. Thus, the impact of viral mutations or liver histology was not considered in that model.

The present study examined whether including viral substitutions in ISDR and the core region of HCV in the decision-tree model could improve its predictive accuracy over the previous model to identify chronic hepatitis C patients who are likely to respond to PEG-IFN plus RBV therapy.

MATERIALS AND METHODS

Patients

This multicenter retrospective cohort study included 505 chronic hepatitis C patients who were treated with PEG-IFN alpha-2b and RBV at Musashino Red Cross Hospital, Toranomon Hospital, Tokyo Medical and Dental University, Osaka University, Nagoya City University Graduate School of Medical Sciences, Yamanashi University, Osaka City University, and their related hospitals. The inclusion criteria were: (1) genotype 1b, (2) HCV RNA titer higher than 100 kIU/ml by quantitative PCR (Cobas Amplicor HCV Monitor v 2.0, Roche Diagnostic Systems, Pleasanton, CA), (3) no co-infection with hepatitis B virus or human immunodeficiency virus, (4) no other causes of liver disease, (5) patients having undergone liver biopsy prior to IFN treatment, (6) number of substitutions in ISDR having been determined, (7) substitutions in the amino acid positions 70 and 91 of the core region having been determined, and (8) completion of at least 12 weeks of therapy. Patients were treated with PEG-IFN alpha-2b (1.5 μg/kg) weekly plus RBV. The daily dose of RBV was adjusted by weight: 600 mg for <60 kg, 800 mg for 60–80 kg, and 1,000 mg for >80 kg. For the analysis, patients were assigned randomly to either the model building (304 patients) or validation (201 patients) groups. There were no significant differences in the clinical backgrounds between these two groups (Table I). Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committees of all concerned hospitals.

Laboratory Tests

Hematological tests, blood chemistry, and HCV RNA titer were analyzed before therapy and at least once every month during therapy. Sequences of ISDR and the core region of HCV were determined by direct sequencing after amplification by reverse transcription and polymerase chain reaction as reported previously. At position 70 of the core region (Core70), arginine was defined as the wild type, and glutamine or histidine was defined as the mutant type. At position 91 of the core region, leucine was defined as the wild type and methionine was defined as the mutant type, as described previously [Akuta et al., 2005]. Fibrosis and activity were scored according to the METAVIR scoring system [Bedossa and Poynard, 1996]. Fibrosis was staged on a scale of 0–4: F0 (no fibrosis), F1 (mild fibrosis), F2 (moderate fibrosis), F3 (severe fibrosis), and F4 (cirrhosis). Activity of necroinflammation was graded on a scale of

TABLE I. Comparison of Pretreatment Factors Between Model Building and Validation Patients

	Model (n = 304)	Validation (n = 201)	*P*-value
Age (years)	55.6 (9.4)	56.0 (12.2)	0.80
Male (%)	53 (%)	55 (%)	0.13
Body mass index (kg/m^2)	23.1 (3.1)	23.1 (4.0)	0.99
Albumin (g/dl)	4.0 (0.3)	4.0 (0.3)	0.47
Creatinine (mg/dl)	0.72 (0.15)	0.72 (0.14)	0.62
AST (IU/L)	63.3 (45.6)	58.9 (46.4)	0.91
ALT (IU/L)	78.7 (58.6)	74.5 (67.5)	0.68
GGT (IU/L)	53.2 (49.1)	57.4 (63.5)	0.43
Total cholesterol (mg/dl)	170.9 (32.6)	169.4 (34.1)	0.33
Triglyceride (mg/dl)	107.0 (44.7)	105.7 (48.0)	0.90
LDL-C (mg/dl)	95.5 (28.0)	96.4 (28.8)	0.34
White blood cell count (/μl)	4,902 (1,489)	4,906 (1,319)	0.86
Hemoglobin (g/dl)	14.1 (1.3)	14.3 (1.4)	0.09
Platelets (10^9/L)	164 (56)	172 (55)	0.68
HCVRNA (10^3 IU/ml)	1,859 (1,468)	2,021 (1,393)	0.09
ISDR mutations: ≥2 (%)	15 (%)	20 (%)	0.11
Core70: mutant (%)	36 (%)	29 (%)	0.22
Core91: mutant (%)	40 (%)	36 (%)	0.20
Fibrosis: F2–4 (%)	49 (%)	48 (%)	0.36
Activity: A2–3 (%)	42 (%)	34 (%)	0.10

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; LDL-C, low-density-lipoprotein-cholesterol; ISDR, interferon sensitivity-determining region.
Data expressed as mean (SD).

0–3: A0 (no activity), A1 (mild activity), A2 (moderate activity), and A3 (severe activity). Sustained virological response was defined as undetectable HCV RNA by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor, Roche Diagnostic Systems) at week 24 after the completion of therapy.

Statistical Analysis

A database of pretreatment variables included hematological tests (hemoglobin level, white blood cell count, and platelet count), blood chemistry tests (serum levels of creatinine, albumin, aspartate aminotransferase, alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C)), viral factors (HCV RNA titer, number of substitutions in ISDR, substitutions in the amino acid positions 70 and 91 of the core region), histological findings (stage of fibrosis and grade of activity) and patient characteristics (age, sex, and body mass index). Based on this database, decision-tree analysis was used to define a predictive model for sustained virological response.

Student's *t*-test was used for the univariable comparison of quantitative variables and Fisher's exact test was used for the comparison of qualitative variables. For the multivariable analysis for factors associated with sustained virological response, logistic regression models with backward selection were used to identify independent predictors of sustained virological response. Variables that showed significant association with sustained virological response by univariable analysis were included in the multivariable analysis. IBM-SPSS software v.15.0 (SPSS, Inc., Chicago, IL) was used for these analyses. For the decision-tree analysis [Segal and Bloch, 1989], the data mining software IBM SPSS Modeler 13 (IBM SPSS, Inc.) was used, as reported previously [Kurosaki et al., 2010a,b]. In brief, the software searched for the optimal split variables to build a decision-tree structure. The entire study population was first evaluated to determine the variables and cut-off points for the most significant division into two subgroups having different probabilities of sustained virological response. Thereafter, analysis was repeated on all subgroups in the same way until either no additional significant variable was detected or the sample size was below 20.

RESULTS

Generation of the Decision-Tree Model

The decision-tree analysis selected five predictive variables to produce six subgroups of patients (Fig. 1). The number of substitutions in ISDR was selected as the best predictor of sustained virological response. The possibility of achieving sustained virological response was 83% for patients with two or more substitutions in ISDR compared with 44% for patients with a single or no substitution. Among patients with a single or no substitution in ISDR, age, with an optimal cut-off of 60 years, was selected as the variable of second split. Patients younger than 60 had the higher probability of sustained virological response (55%) compared with those older than 60 years (31%). Among younger patients, amino acid substitution at Core70 was selected as the third variable of split—wild-type sequence being the predictor of favorable response compared with the mutant type (65% vs. 36%). Among patients with wild-type Core70, the level of serum LDL-C was selected as the fourth variable of split, with an optimal cutoff of

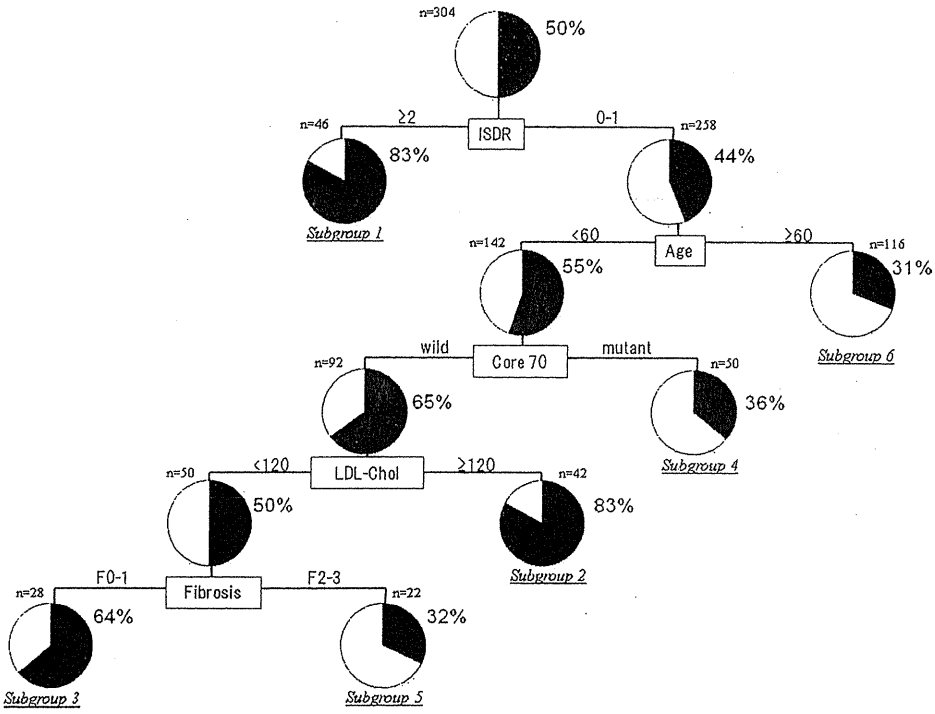

Fig. 1. Decision-tree model. Boxes indicate the factors used for splitting and the cutoff value for the split. Pie charts indicate the rate of sustained virological response for each group of patients after splitting. Terminal subgroups of patients discriminated by the analysis are numbered from 1 to 7. The rate of sustained virological response was >80% in subgroups 1 and 2, 64% in subgroup 3, and 31–36% in subgroups 4, 5, and 6. LDL-C represents low-density lipoprotein cholesterol and Core70 represents amino acid substitution at position 70 of the core region.

120 mg/dl. Patients with higher LDL-C level had the higher probability of sustained virological response (83% vs. 50%). The stage of fibrosis was selected as the final variable of split, with significant fibrosis (F2–4) being the predictor of lower sustained virological response probability (64% vs. 32%).

Among the six subgroups derived by this decision tree, the subgroup of patients with two or more substitutions in ISDR (subgroup 1) or with a single or no substitution in ISDR but younger than 60 years of age, having the wild-type Core70 and high serum level of LDL-C (≥120 mg/dl) (subgroup 2) showed the highest probability of sustained virological response (83%).

Validation of the Decision-Tree Model

The decision-tree model was validated using a validation dataset of 201 cases that were not included the model-building dataset. Each patient in the validation set was allocated to subgroups 1–6 using the flowchart form of the decision tree. The rates of sustained virological response were 75% for subgroup 1, 73% for subgroup 2, 65% for subgroup 3, 41% for subgroup 4, 46% for subgroup 5, and 33% for subgroup 6. The rates of sustained virological response for each subgroup of patients were correlated closely between the model building dataset and the validation dataset ($r^2 = 0.94$) (Fig. 2).

The six subgroups were reconstructed into three groups according to their rate of sustained virological response: the high-probability group consisted of subgroups 1 and 2, the intermediate-probability group consisted of subgroup 3, and the low-probability group consisted of subgroups 4, 5, and 6. The rate of sustained virological response in the high-probability group was high on a consistent basis: 83% for model-building patients and 74% for validation patients. The rate of sustained virological response in the intermediate-probability group was 64% for model building patients and 65% for internal validation patients. The rate of sustained virological response in the low-probability group was low on a consistent basis: 32% for model-building patients and 36% for internal validation patients (Fig. 3). Thirty percent of the patients were classified into the high-probability group and 10% of the patients were classified into intermediate-probability group, which means that about 40% of patients with higher than average probability of achieving sustained virological response were identified.

Effect of Dose Reductions of PEG-IFN and RBV

The possible effect of drug reductions was analyzed in the three groups of patients divided by decision tree (low-, intermediate-, and high-probability groups)

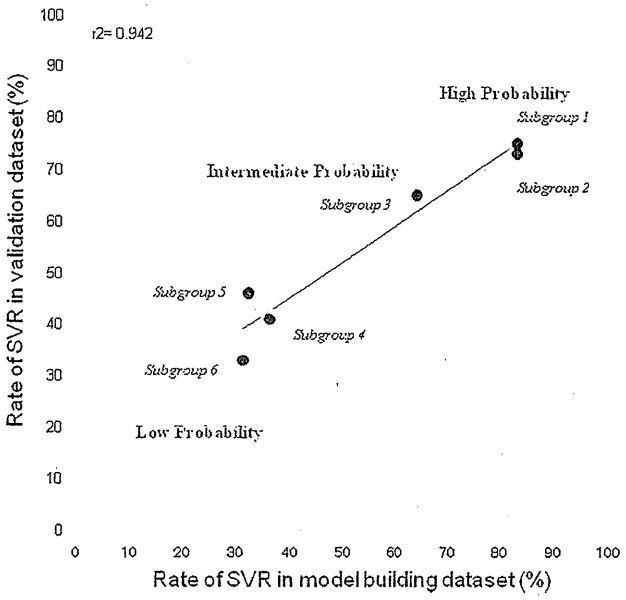

Fig. 2. Validation of the decision-tree analysis: Subgroup-stratified comparison of the rate of sustained virological response. Each patient in the validation set was allocated to subgroups 1–6 by following the flowchart form of the decision tree, and the rates of sustained virological response were then calculated and plotted for each subgroup. The *x*-axis represents the rate of sustained virological response in the model-building datasets and the *y*-axis represents the rate of sustained virological response in the validation datasets. The rates of achieving sustained virological response in each subgroup of patients correlated closely between the model-building dataset and the validation dataset (correlation coefficient: $r^2 = 0.94$).

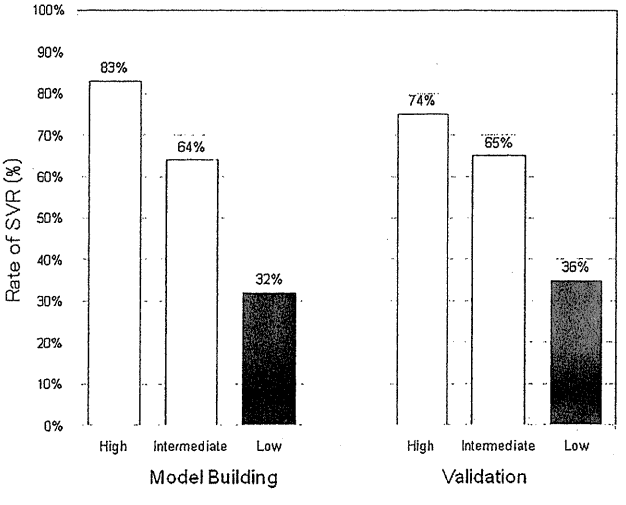

Fig. 3. Comparison of sustained virological response rates between groups divided by the decision tree. The rate of sustained virological response was compared between three groups of patients as divided by the decision-tree analysis. Black, gray, and white boxes indicate the low-probability group (subgroup 4, 5, and 6), intermediate-probability group (subgroup 3), and high-probability group (subgroup 1 and 2), respectively. The rate of sustained virological response showed significant difference between the three groups.

(Fig. 4). Patients were stratified according to the cumulative drug exposure with PEG-IFN and RBV: the good adherence group consisted of patients who took ≥80% planned doses of both PEG-IFN and RBV; the poor adherence group consisted of patients who took <80% of planned doses of both PEG-IFN and RBV. Even after adjustment for drug adherence, the three groups of patients divided by decision-tree analysis still had low, intermediate, and high probability of achieving sustained virological response, respectively, indicating that this model predicts sustained virological response independent of drug exposure.

Multivariable Logistic Regression Analysis

Age, sex, serum levels of creatinine, ALT, GGT, LDL-C, hemoglobin, platelet count, HCV RNA titer, ISDR substitution, substitution at Core70, substitution at Core91, histological stage of fibrosis, and grade of activity were found to be associated with sustained virological response by standard univariable analysis. Multivariable analysis including these factors showed that age, sex, LDL-C levels, GGT levels, platelet count, ISDR substitution, and substitution at Core70 showed independent associations with sustained virological response (Table II). Substitution in ISDR had the highest odds ratio, at 9.92. Fibrosis, which was selected as a significant predictor of response in the decision-tree analysis, was not found to be an independent predictor of response in standard multivariable analysis, indicating that the decision-tree analysis could identify significant predictors that would apply specifically to selected patients.

DISCUSSION

The present study revealed that viral factors such as substitutions in ISDR and Core70 are significant and independent predictors of sustained virological response to PEG-IFN plus RBV in chronic hepatitis C. In a decision-tree model for the pretreatment prediction of sustained virological response, the number of substitutions in ISDR was the best predictor of sustained virological response, followed by younger age, wild-type sequence at Core70, higher level of LDL-C, and absent fibrosis. This decision-tree model could identify patients with high probability of sustained virological response (83%) among difficult-to-treat genotype 1b chronic hepatitis C patients. Using this model, rapid estimates of the response before treatment can be made by allocating patients to specific subgroups with a defined rate of response simply by following the flowchart form. Because more potent therapy, such as a combination of protease inhibitor, PEG-IFN, and RBV, is under clinical trial and may become available in the near future [Hezode et al., 2009; McHutchison et al., 2009], pretreatment prediction of the likelihood of sustained virological response may be useful for both patients and physicians to support clinical decisions whether to start current standard therapy or to wait for emerging new therapies.

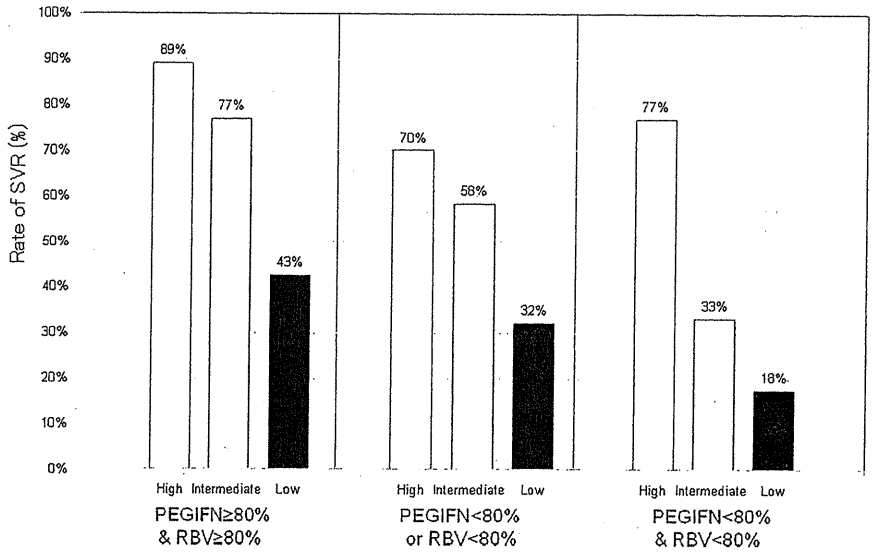

Fig. 4. Comparison of the rate of sustained virological response between the decision-tree groups stratified by drug adherence. The three groups of patients divided by the decision tree (black, gray, and white boxes indicating the low-, intermediate-, and high-probability groups, respectively) were further stratified according to cumulative drug exposure to PEG-IFN and RBV.

Two or more substitutions in ISDR had a strong impact on sustained virological response, because this factor was selected as a top variable in decision-tree analysis and had the highest odds ratio in multivariable analysis. Moreover, even among patients with unfavorable ISDR (0 or 1 mutation), younger patients (<60 years) with the wild-type sequence at Core70 and high level of LDL-C (≥120 mg/dl) had a high rate of sustained virological response. The sustained virological response rate of these two subgroups of patients was 83% in the model-building patients and 75% in the validation patients. Thus, patients with high possibility of sustained virological response could be extracted by the combined analysis of ISDR and Core70. These patients may be the best-suited candidates for treatment with the current combination therapy. Conversely, the following patients with 0–1 mutation in ISDR had a low probability of sustained virological response (32–35%): (1) older (>60 years); or (2) younger (<60 years) patients but having mutant-type sequence at Core70; or (3) younger (<60 years) patients having a wild-type sequence at Core70, but having a low level of LDL-C (<120 mg/dl) and advanced fibrosis. These patients may be advised to wait for a more effective therapy. Decision may be made on a case-by-case basis, taking into account the potential risk of disease progression while waiting.

In a previous decision-tree model using simple and noninvasive standard tests that are available readily worldwide [Kurosaki et al., 2010b], the rate of sustained virological response was at most 65–76% among those in the high-probability group. That model focused on use by general physicians in routine general practice, especially where specialized resources, such as liver biopsy or determination of viral sequences, are not available. In that model, younger age, male sex, higher platelet counts, lower alpha-fetoprotein (AFP) levels, and lower GGT levels were identified as favorable predictive parameters. Higher AFP levels and lower platelet counts that are hallmarks of advanced fibrosis [Shiratori and Omata, 2000; Akuta et al., 2007b] were associated with low probability of sustained virological response in that model. On the other hand, the present analysis aimed to clarify the significance of viral factors for pretreatment prediction of sustained virological response, and to build an advanced model that may be used by specialist physicians engaged in the

TABLE II. Multivariable Logistic Regression Analysis for Factors Associated With SVR

Parameter		Odds	95% CI	*P*-value
Age (years)	<60 vs. ≥60	2.28	1.31–3.94	0.003
Sex	Male vs. female	3.36	1.87–5.99	<0.0001
GGT (IU/L)	<40 vs. ≥40	2.65	1.45–4.85	0.002
LDL-C (mg/dl)	≥120 vs. <120	1.79	0.91–3.53	0.094
Platelets (109/L)	≥120 vs. <120	2.69	1.22–5.90	0.014
ISDR mutations	≥2 vs. 0–1	9.92	3.71–26.54	<0.0001
Core70	Wild vs. mutant	1.92	1.07–3.47	0.030

GGT, gamma-glutamyltransferase; LDL-C, low-density-lipoprotein-cholesterol; ISDR, interferon sensitivity-determining region.

treatment of hepatitis. In the present model, stage of fibrosis was selected as a predictive factor, but at lower level of significance than HCV mutations. The predicted rate of sustained virological response in the high-probability group of the present model is higher than that in the previous model (75–83% vs. 65–76%). These results indicate that substitutions in ISDR and Core70 were important pretreatment predictors of sustained virological response. Determination of these viral factors is not available readily in clinical practice, but is of value for improving the accuracy of pretreatment prediction of sustained virological response.

Substitutions in ISDR and Core70 have been reported previously to be associated with efficacy of IFN therapy. The association between the number of substitutions in ISDR and response to therapy was demonstrated originally in patients treated with IFN mono-therapy [Enomoto et al., 1995, 1996; Kurosaki et al., 1997], but recent studies have reported a positive correlation with PEG-IFN and RBV combination therapy as well [Munoz de Rueda et al., 2008; Shirakawa et al., 2008; Ikeda et al., 2009]. Another important viral factor relevant to treatment response is amino acid substitution in Core70. The sequence of this amino acid was reported originally to be associated with nonresponse to therapy [Akuta et al., 2005], but subsequent studies confirmed the positive correlation of a wild-type Core70 with sustained virological response [Akuta et al., 2009]. The multiple logistic regression analysis showed that ISDR and Core70 were independent factors associated with sustained virological response along with host factors. How these important viral factors and other host factors can be combined to predict response to PEG-IFN plus RBV is an important clinical question. Decision-tree modeling can make the response probability apparent by combining all these factors. Some factors that may be associated with treatment outcome, such as levels of ferritin or homocysteine, were not included. This may be a potential limitation of the present study.

It is of interest that a recent study by Li et al. [2010] has shown that a high serum level of LDL-C is linked to the *IL28B* major allele (CC in rs12979860). In that study, a high serum level of LDL-C was associated with sustained virological response, but it was no longer significant when analyzed together with the *IL28B* genotype in multivariate analysis. Thus, the association between treatment response and LDL cholesterol levels in the present study may reflect the underlining link of LDL cholesterol levels to the *IL28B* genotype. Recent reports indicate that the *IL28B* genotype and HCV substitutions are correlated closely [Akuta et al., 2010; Kurosaki et al., 2010c]. Still, Core70 [Akuta et al., 2010] or ISDR [Kurosaki et al., 2010c] were predictors of response to therapy independent of *IL28B* genotype. Future study is needed to elucidate the possible mechanisms underlying the association between HCV sequences and host genetic factors, and also the role of host and viral factors for the prediction of treatment response.

In conclusion, a data mining analysis emphasized the impact of substitutions in ISDR and Core70 on pretreatment prediction of sustained virological response to PEG-IFN plus RBV therapy. A decision-tree model that includes substitutions in ISDR and Core70 of HCV could identify patients with high probability of sustained virological response, and could thereby improve the predictive accuracy over predictions that are based on standard tests.

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