log-rank tests. The time frame for HCC incidence was defined as the time from the end of antiviral treatment to the diagnosis of HCC. A p value less than 0.05 was regarded as statistically significant in all analyses.

Results

Patient characteristics

The baseline characteristics of the 1013 studied patients at the start of antiviral treatment, as classified by the existence of cirrhosis and treatment outcome, are shown in Table 1. HCV genotype 1 was detected in 710 patients and genotype 2 in 303. Of all patients, 151 (14.9%) discontinued antiviral treatment because of adverse effects or other reasons (e.g., poor virological response, economic reasons, or dropout). The discontinuation rate of patients with HCV genotype 1 (129 of 710, 18.2%) was significantly higher than that of those with HCV genotype 2 (22 of 303, 7.3%) (p <0.001). Of the studied patients, 557 achieved SVR (55.0%), 304, including 20 with breakthrough, were TVR (30.0%), and 152 (15.0%) were NVR. The SVR rate of patients infected with HCV genotype 1 was 43.9% (312 of 710), significantly lower than the 80.9% (245 of 303) found for patients with genotype 2 (p <0.001).

In the non-cirrhosis group (n = 863), the three treatment outcome groups differed significantly for age, sex, HCV genotype, and laboratory values associated with liver and metabolic disease (e.g., ALT, platelet count, AFP and HbA1c). The SVR group was more likely to be infected with HCV genotype 2 and to have mild liver fibrosis, but less likely to have laboratory values associated with advanced liver and metabolic disease (e.g., low platelet count, or high AFP and HbA1c level) than the TVR and NVR groups. Independent comparisons of SVR and TVR patients extracted age (p <0.001), sex distribution (p = 0.01), ALT level (p = 0.01), platelet count (p <0.001) and HCV genotype (p <0.001). Likewise, independent comparisons of TVR and NVR patients extracted only AFP level (p = 0.01).

Liver cirrhosis was diagnosed according to clinical (n = 77) and histological (n = 73) findings. In the cirrhosis group (n = 150), however, no significant differences, except for ALT and HCV genotype, were found among the clinical and biochemical parameters of the three treatment outcome groups.

SVR and TVR patients had fewer deaths from any cause (four [0.7%] and four [1.3%], respectively) in comparison to NVR patients (six [3.9%]). Similarly, the frequency of SVR and TVR patients who developed ascites and encephalopathy, symptoms of hepatic decompensation, was lower than that of NVR patients (ascites: two [0.4%], six [2.0%] and eight [5.3%], and encephalopathy: two [0.4%], two [0.7%] and five [3.3%] patients with SVR, TVR and NVR, respectively). None of the patients underwent liver transplantation during the observation period.

Risk of HCC classified by treatment outcome

Of 1013 patients who were followed for a median of 3.6 (range 0.3–7.0) years, 47 (4.6%) developed HCC during the observation period. The baseline characteristics of these patients classified by the development of HCC are shown in Table 2. By univariate analysis, the development of HCC was associated with older age, male sex, higher ALT level, lower serum albumin, lower platelet count, higher AFP level, cirrhosis, and NVR. No significant difference in the duration of HCV RNA negativity was found between the HCC (median [first-third quartiles]: 30.0 [24.0–48.5] weeks) and non-HCC group (41.0 [27.0–48.0] weeks) (p = 0.36) in patients with TVR.

Multivariable logistic regression analysis of possible predictors of HCC development is shown in Table 3. We examined eight factors (age [<60 *vs.* ⩾60 years], sex [men *vs.* women], ALT [<40 *vs.* ⩾40 IU/L], platelet count [<150 *vs.* ⩾150 × 10^9/L], AFP [<10 *vs.* ⩾10 ng/ml], serum albumin [<40 *vs.* ⩾40 g/L], liver pathophysiology [non-cirrhosis *vs.* cirrhosis] and treatment outcome [SVR *vs.* TVR *vs.* NVR]). Significant independent pretreatment predictors of HCC were age 60 years and over (HR 2.81; 95%CI 1.39–5.69; p = 0.004), male sex (HR 2.98; 95%CI 1.46–6.05; p = 0.003), low platelet count (<150 × 10^9/L) (HR 4.04; 95%CI 1.57–10.44; p = 0.004), higher AFP level (⩾10 ng/ml) (HR 2.50; 95%CI 1.09–5.78; p = 0.03), cirrhosis (HR 3.22; 95%CI 1.28–8.13; p = 0.01), and NVR (HR 3.72; 95%CI 1.69–8.18; p = 0.001). Baseline ALT level, serum albumin level, and TVR were not associated with the development of HCC.

Table 1. Pretreatment characteristics of 1013 patients with chronic hepatitis C classified by the existence of cirrhosis and treatment outcome.

Characteristic	Non-cirrhosis n = 863				Cirrhosis n = 150			
	SVR n = 504	TVR n = 255	NVR n = 104	p value*	SVR n = 53	TVR n = 49	NVR n = 48	p value*
Age (yr)	54 (46-63)	61 (55-67)	61 (53-67)	<0.001	61 (57-67)	63 (53-68)	60 (54-68)	0.94
Male, n (%)	263 (52.2)	109 (42.7)	52 (50.0)	0.05	30 (56.6)	19 (38.8)	25 (52.1)	0.18
Body mass index (kg/m²)	22.9 (20.8-25.2)	23.3 (21.3-25.7)	23.1 (21.2-25.1)	0.12	23.0 (20.4-25.6)	23.7 (21.9-26.7)	24.6 (22.8-26.9)	0.07
ALT (IU/L)	52 (34-91)	47 (33-78)	51 (31-80)	0.02	88 (69-127)	65 (53-107)	66 (48-102)	0.01
Albumin (g/L)	42 (40-44)	42 (39-44)	42 (39-44)	0.26	37 (35-39)	37 (35-40)	37 (33-39)	0.87
Platelet count (x10⁹/L)	177 (144-212)	158 (129-194)	159 (130-197)	<0.001	103 (89-116)	97 (84-111)	99 (84-118)	0.26
Hemoglobin (g/L)	137 (129-148)	136 (128-147)	138 (127-149)	0.49	130 (122-140)	133 (123-142)	137 (126-147)	0.37
Ferritin (ng/ml)	156 (75-280)	174 (92-316)	213 (116-361)	0.16	200 (127-317)	202 (134-327)	250 (170-452)	0.05
α-fetoprotein (ng/ml)	4.1 (2.9-6.0)	4.8 (2.9-7.8)	5.9 (3.4-8.9)	<0.001	14.0 (9.2-36.0)	14.1 (9.3-31.3)	30.2 (15.4-42.9)	0.24
Hemoglobin A1c (%)	5.8 (5.7-6.3)	5.9 (5.7-6.4)	6.0 (5.7-6.7)	0.005	5.8 (5.4-6.4)	5.6 (5.3-6.4)	6.0 (5.4-6.6)	0.73
HCV genotype (1/2), n (%)	288/216 (57.1/42.9)	220/35 (86.3/13.7)	92/12 (88.5/11.5)	<0.001	24/29 (45.3/54.7)	43/6 (87.8/12.2)	43/5 (89.6/10.4)	<0.001

Data are expressed as number (%) or median (first-third quartiles).
SVR, sustained virological response; TVR, transient virological response; NVR, non-virological response; HCV, hepatitis C virus; ALT, alanine aminotransferase.
*Comparison among the three groups.

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Table 2. Risk factors for the development of HCC by chronic hepatitis C patients treated with PegIFNα2b and RBV.

Characteristic	All patients n = 1013	HCC n = 47	non-HCC n = 966	*p* value*
Age (yr)	58 (50-65)	67 (58-71)	58 (49-65)	<0.001
Male, n (%)	498 (49.2)	32 (68.1)	466 (48.2)	0.007
Body mass index (kg/m²)	23.0 (21.1-25.2)	23.6 (21.6-25.7)	23.0 (21.1-25.2)	0.15
ALT (IU/L)	54 (35-89)	74 (46-100)	54 (34-89)	0.008
Albumin (g/L)	41 (39-44)	40 (37-42)	44 (41-46)	0.002
Platelet count (x10⁹/L)	159 (120-199)	110 (88-132)	161 (123-201)	<0.001
Hemoglobin (g/L)	136 (127-147)	136 (128-149)	136 (127-147)	0.89
Ferritin (ng/ml)	165 (84-376)	187 (80-462)	167 (80-306)	0.68
α-fetoprotein (ng/ml)	4.9 (3.0-9.3)	11.7 (6.8-32.7)	4.8 (3.0-8.7)	<0.001
Hemoglobin A1c (%)	5.5 (5.3-5.9)	5.8 (5.4-6.3)	5.5 (5.3-5.9)	0.96
HCV genotype (1/2), n (%)	710/303 (70.1/29.9)	38/9 (80.9/19.1)	672/294 (69.6/30.4)	0.09
Non-cirrhosis/cirrhosis, n	863/150 (85.2/14.8)	19/28 (40.4/59.6)	844/122 (87.4/12.6)	<0.001
Treatment duration (wk)	47 (24-48)	43 (23-48)	47 (24-48)	0.58
Virological response (SVR/TVR/NVR), n (%)	557/304/152 (55.0/30.0/15.0)	13/13/21 (27.7/27.7/44.7)	544/291/131 (56.3/30.1/13.6)	<0.001

Data are expressed as number (%) or median (first-third quartiles).
All demographic and clinical data are those at the start of antiviral treatment.
HCV, hepatitis C virus; HCC, hepatocellular carcinoma; SVR, sustained virological response; TVR, transient virological response; NVR, non-virological response; ALT, alanine aminotransferase.
*Comparison between HCC and non-HCC.

Overall cumulative incidence of HCC classified by treatment outcome

The 5-year cumulative incidence rates of HCC of the SVR (3.1%) and TVR groups (5.8%) were significantly lower than those of the NVR group (18.8%) (both $p < 0.001$), and the rate of the SVR group was lower, but not significantly, than that of the TVR group ($p = 0.21$).

Cumulative incidence of HCC classified by treatment outcome in the non-cirrhosis group

The Kaplan–Meier curves for the incidence of HCC classified by treatment outcome in the non-cirrhosis group are shown in Fig. 1A ($p = 0.009$ by log-rank test). The 5-year cumulative incidence rates of HCC in the SVR (1.7%) and TVR groups (3.2%) were significantly lower than those of the NVR group (7.6%) ($p = 0.003$ and $p = 0.03$, respectively), and the rate of the SVR group was lower, but not significantly, than that of the TVR group ($p = 0.47$).

Cumulative incidence of HCC classified by treatment outcome in the cirrhosis group

The Kaplan–Meier curves for the incidence of HCC classified by treatment outcome in the cirrhosis group are shown in Fig. 1B ($p = 0.03$ by log-rank test). The 5-year cumulative incidence rates of HCC in the SVR (18.9%) and TVR groups (20.8%) were significantly lower than those of the NVR group (39.4%) ($p = 0.03$ and $p = 0.04$, respectively), and the rate of the SVR group was lower, but not significantly, than that of the TVR group ($p = 0.94$).

Adjusted rates of HCC incidence classified by treatment outcome of non-cirrhotic patients under 60 years of age

The Kaplan–Meyer curves of the estimation of the incidence of HCC by non-cirrhosis patients under 60 years of age, classified by treatment outcome, are shown in Fig. 2A ($p = 0.51$ by log-rank test). The 5-year cumulative incidence rates of HCC in the SVR (0.9%) and TVR groups (1.7%) were lower, but not significantly, than those of the NVR group (2.6%) ($p = 0.25$ and $p = 0.45$, respectively).

Table 3. Multivariate logistic regression analysis of possible predictors of HCC development.

Parameter	Hazard ratio	95% CI	*p* value
Age			
<60 yr	1		
≥60 yr	2.81	1.39-5.69	0.004
Sex			
Female	1		
Male	2.98	1.46-6.05	0.003
Platelet count			
≥150 x 10⁹/L	1		
<150 x 10⁹/L	4.04	1.57-10.44	0.004
α-fetoprotein			
<10 ng/ml	1		
≥10 ng/ml	2.50	1.09-5.78	0.03
Liver pathophysiology			
Non-cirrhosis	1		
Cirrhosis	3.22	1.28-8.13	0.01
Treatment outcome			
SVR	1		
TVR	1.50	0.65-3.44	0.34
NVR	3.72	1.69-8.18	0.001

HCC, hepatocellular carcinoma; SVR, sustained virological response; TVR, transient virological response; NVR, non-virological response.

Adjusted rates of HCC incidence classified by treatment outcome of non-cirrhotic patients aged 60 years and over

The Kaplan–Meyer curves of the estimation of the incidence of HCC in non-cirrhosis patients, aged 60 years and over classified by treatment outcome, are shown in Fig. 2B ($p = 0.05$ by log-rank test). The 5-year cumulative incidence rates of HCC in the SVR (3.5%) and TVR groups (4.2%) were significantly lower than those

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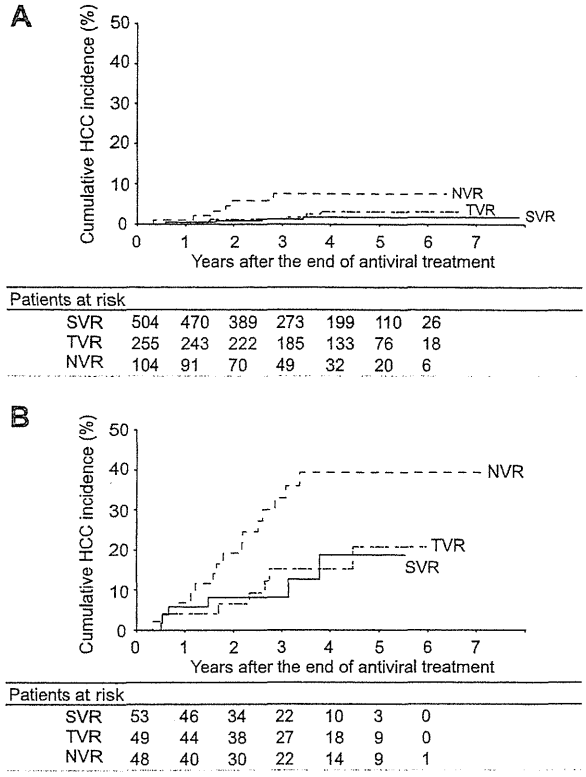

Fig. 1. Cumulative incidence of HCC after PegIFNα2b and RBV treatment stratified by treatment outcome (SVR: continuous line, TVR: long dashed-dotted line, NVR: dashed line). (A) Non-cirrhosis group (overall: $p = 0.009$; SVR *vs.* TVR: $p = 0.47$; SVR *vs.* NVR: $p = 0.003$; and TVR *vs.* NVR: $p = 0.03$ by log-rank test). (B) Cirrhosis group (overall: $p = 0.03$; SVR *vs.* TVR: $p = 0.94$; SVR *vs.* NVR: $p = 0.03$; and TVR *vs.* NVR: $p = 0.04$ by log-rank test).

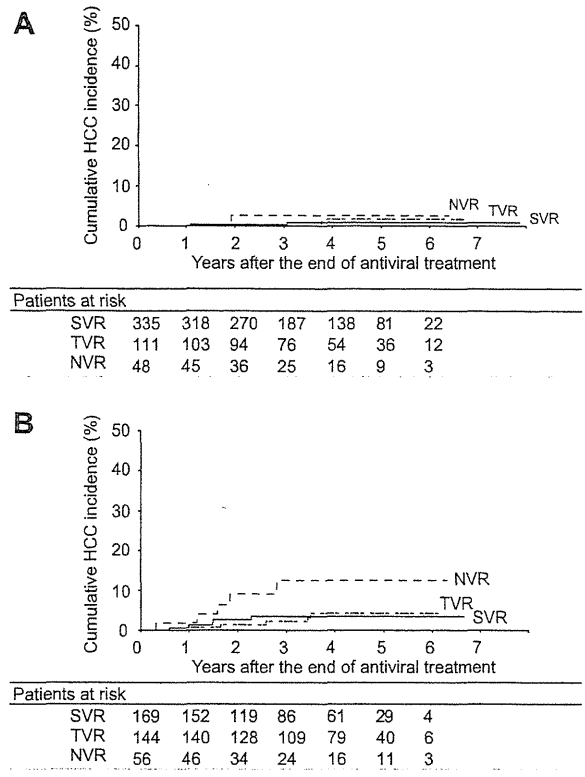

Fig. 2. Cumulative incidence of HCC after PegIFNα2b and RBV treatment stratified by treatment outcome of the non-cirrhosis group (SVR: continuous line, TVR: long dashed-dotted line, NVR: dashed line). (A) Under 60 years of age (overall: $p = 0.51$; SVR *vs.* TVR: $p = 0.94$; SVR *vs.* NVR: $p = 0.25$; and TVR *vs.* NVR: $p = 0.45$ by log-rank test). (B) Aged 60 years and over (overall: $p = 0.05$; SVR *vs.* TVR: $p = 0.96$; SVR *vs.* NVR: $p = 0.04$; and TVR *vs.* NVR: $p = 0.03$ by log-rank test).

of the NVR group (12.4%) ($p = 0.04$ and $p = 0.03$, respectively), and the rate of the SVR group was slightly lower, but not significantly, than that of the TVR group ($p = 0.96$).

The development of HCC by SVR patients

Thirteen patients who achieved SVR (2.3%) (6 non-cirrhotic and 7 cirrhotic patients) developed HCC during the follow-up period. Their individual pretreatment characteristics are shown in Table 4. Of these patients, 3 (patients 1–3) under 55 years of age had liver cirrhosis and the period from the end of antiviral treatment to the diagnosis of HCC was over 3 years. Of the remaining 10 patients (patients 4–13) aged 55 years and over, 6 did not have cirrhosis and the period from the end of antiviral treatment to the diagnosis of HCC was under 2.5 years.

Discussion

We here report the results of a prospective, long-term follow-up study done to evaluate the effect of treatment outcome on the development of HCC in a large cohort of Japanese patients with chronic hepatitis C, who were treated with PegIFNα2b and RBV. We found that those patients who achieved SVR or TVR had a lower risk of developing HCC within 5 years after the end of PegIFNα2b and RBV treatment when compared with NVR, in both cirrhosis and non-cirrhosis groups. Although SVR patients have been reported to have little risk of HCC incidence, a small number of our patients who achieved SVR did develop HCC, showing the necessity of a continued screening of patients with SVR.

Previously, the likelihood of HCC development by PegIFNα- and RBV-treated patients was difficult to determine because of the paucity of adequate long-term prospective studies. Based on the results of this prospective study, sex, age, platelet count, AFP level, and treatment outcome are significant, independent factors for the development of HCC. In addition to our present data, the incidence rate of HCC has been shown to be significantly lower for patients with TT genotype at rs8099917 and CC genotype at rs12979860 near the *IL28B* gene, which are associated with good response to antiviral treatment (data not shown). Of particular interest, the adjusted cumulative incidence of HCC was not significantly different between SVR and TVR for the 5 years after the end of treatment. Two randomized studies of maintenance therapy with low-dose PegIFNα to prevent hepatic decompensation and HCC have been recently reported [25,26]. However, maintenance therapy did not prevent HCC in presence of HCV viremia for at least 5 years, regardless of the degree of viral suppression. Our results showed that complete HCV sup-

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Table 4. Individual characteristics of SVR patients who developed HCC.

Patient number	Age (yr)	Sex	Liver pathophysiology	Time to HCC* (yr)	HCV genotype	ALT (IU/L)	Albumin (g/L)	Platelet count (x10⁹/L)	AFP (ng/ml)	HbA1c (%)
1	47	F	Cirrhosis	3.1	1	44	40	134	3.3	7.1
2	53	M	Cirrhosis	3.1	2	105	42	68	31.0	6.1
3	54	M	Cirrhosis	3.8	1	86	36	88	13.9	5.9
4	59	M	Non-cirrhosis	1.1	2	227	44	131	4.4	6.6
5	63	F	Cirrhosis	1.5	2	81	33	130	16.3	5.3
6	64	F	Non-cirrhosis	1.5	2	72	38	120	6.6	6.8
7	64	M	Non-cirrhosis	1.5	1	29	46	124	20.7	5.1
8	66	F	Cirrhosis	0.7	2	169	42	105	106.0	6.4
9	66	M	Non-cirrhosis	0.6	1	36	35	147	6.2	5.5
10	71	M	Cirrhosis	0.6	2	80	32	106	10.6	5.5
11	71	M	Non-cirrhosis	1.0	1	47	42	108	4.3	5.7
12	74	M	Non-cirrhosis	2.3	1	47	43	143	12.9	6.9
13	77	M	Cirrhosis	0.5	1	73	30	124	11.6	5.4

All data are those at the start of antiviral treatment.
SVR, sustained virological response; HCC, hepatocellular carcinoma; F, female; M, male; HCV, hepatitis C virus; ALT, alanine aminotransferase; AFP, α-fetoprotein; HbA1c, hemoglobin A1c.
*The time frame for HCC incidence starts from the end of antiviral treatment.

pression during antiviral treatment played an important role in preventing the development of HCC.

A recent prospective study that included Caucasian, Hispanic, and Black patients treated with PegIFNα2a and RBV reported that the adjusted mortality from any cause or liver transplantation, or of any liver-related outcome, was significantly lower in TVR patients than in NVR patients [13]. Similarly, the risk of decompensated liver disease, HCC and liver-related death was also lower in TVR patients than in NVR patients, although these differences did not reach statistical significance [13]. Therefore, the significantly low incidence rate of HCC, for the patients of this study with TVR in comparison with NVR, is an original finding, but the trend was true for cirrhotic patients of all ages and for non-cirrhotic patients aged 60 years and over. One possible explanation for this difference may be related to the rising incidence of HCC for NVR patients aged 60 years and over. Our results indicate that the duration of clinical benefit may outlast the period of actual viral suppression in the 5 years after treatment, however, it remains unclear how older age would explain why TVR resulted in a lower incidence of HCC that matched the incidence in SVR. Therefore, it will be necessary to investigate the development of HCC in SVR and TVR patients beyond five years.

Recently, a number of direct-acting antivirals (DAAs) have been designed and developed. Among them, telaprevir and boceprevir, non-structural 3/4A protease inhibitors, have shown promising results in various clinical trials and have led to an increased SVR rate when given in combination with PegIFNα and RBV, as compared with PegIFNα and RBV alone [27,28]. Furthermore, several IFN-free clinical trials, using regimens that combine several potent DAAs, are ongoing. As a result of advances in antiviral treatment, almost all patients can experience complete HCV suppression during treatment. We showed that TVR patients had a lower incidence rate of HCC than did NVR patients. It will be necessary to study the impact of virological response on the development of HCC by patients who undergo DAAs with and without IFN antiviral treatment.

Findings on the effect of SVR on liver-related preferable clinical outcomes have been reported in many previous reports [13,29–31], however, the analysis of the effect of SVR on the development of HCC is statistically difficult, because the number of events is too small to draw meaningful conclusions. In fact, there were only 13 patients with SVR who developed HCC during the observation period, reducing the validity of the analysis. Additional prospective studies that include a larger number of patients with SVR will be necessary to evaluate the relationship between SVR and the development of HCC.

Risk factors for HCV-related HCC have been reported previously, such as older age, male sex, obesity, diabetes mellitus, alcohol consumption, HCV genotype 1b, insulin resistance, complicated hepatic steatosis, and co-infection with hepatitis B virus or HIV [32,33]. Unfortunately, this study lacks data on insulin resistance and hepatic steatosis. Homeostasis Model Assessment of Insulin Resistance value is also related to a profound effect on PegIFNα2b and RBV treatment outcome [34], thus, there may be a significant difference in HbA1c level between the SVR, TVR and NVR non-cirrhotic groups, indicating differences in glucose metabolism. Moreover, it is known that hepatic steatosis occurs in about 40% of the chronic hepatitis C patients, when all common factors of fatty liver, such as alcohol abuse, obesity, and diabetes, have been excluded [35]. Therefore, it remains unclear whether or not there is a significant bias due to different rates of patients with insulin resistance or hepatic steatosis. Another limitation is the generalizability of the extremely high cumulative incidence rate of HCC, especially for cirrhotic NVR patients. The reasons for this exceedingly high rate are not well understood, although it may be explained by the increasing number of aging chronic hepatitis C patients in Japan, earlier than other countries [14]. Our results, therefore, may not be generalized to other ethnic groups that do not have such high rates of HCC.

In summary, this prospective study demonstrated that SVR and TVR patients had a significantly lower rate than NVR patients of HCC incidence within five years after the end of treatment, both for patients with and without cirrhosis. Because the risk of developing HCC remains present even after HCV eradication, long-term screening of patients with SVR is important.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Acknowledgments

We are grateful to Drs. Masayuki Murata, Mosaburo Kainuma, Kyoko Okada, Kazuhiro Toyoda, Haru Mukae, Kunimitsu Eiraku, Hiroaki Ikezaki, Takeshi Ihara, Takeo Hayashi, Satoshi Hiramine, Fujiko Mitsumoto, and Koji Takayama from the Department of General Internal Medicine, Kyushu University Hospital for their assistance with data collection for this study. We also thank Takeru Abe from the Medical Information Center, Kyushu University Hospital for statistical analysis support.

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Please cite this article in press as: Ogawa E et al. Efficacy of pegylated interferon alpha-2b and ribavirin treatment on the risk of hepatocellular carcinoma in patients with chronic hepatitis C: A prospective, multicenter study. J Hepatol (2012), http://dx.doi.org/10.1016/j.jhep.2012.10.017

J Infect Chemother
DOI 10.1007/s10156-012-0452-1

ORIGINAL ARTICLE

Estimation of two real-time RT-PCR assays for quantitation of hepatitis C virus RNA during PEG-IFN plus ribavirin therapy by HCV genotypes and IL28B genotype

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Received: 5 January 2012 / Accepted: 20 June 2012

Abstract Hepatitis C virus (HCV) RNA values measured with two real-time PCR methods (Cobas Ampliprep/Cobas TaqMan, CAP/CTM, and the Abbott real-time PCR test, ART) vary among patients with genotype 1. We investigated HCV RNA values measured by two real-time PCR assays during pegylated interferon plus ribavirin (PEG-IFN/RBV) therapy. We evaluated 185 cases of chronic hepatitis C patients, among which 97 patients received the PEG-IFN/RBV therapy. HCV RNA values of CAP/CTM for genotype 1 were significantly higher than those of ART ($p < 0.05$) The difference in HCV RNA values (CAP/CTM minus ART) of genotype 1 was significantly higher than those in genotype 2 ($p < 0.0001$). The positive rate (>0) of the difference of HCV RNA values in genotype 1 was 100 % (55/55), which was significantly higher than the 78.6 % (33/42) of genotype 2 ($p < 0.001$). There was no difference between TT and TG/GG genotype groups in terms of difference of HCV RNA values (CAP/CTM minus ART). After PEG-IFN/RBV therapy was administered, reduction of HCV measurements was observed from day 1 for both assays regardless of genotype. The HCV value of CAP/CTM during PEG-IFN/RBV therapy was consistently higher than the value of ART, although the difference in these two values gradually became smaller during the course of therapy, and eventually no significant difference was observed near the detection level. No correlation was observed between the sustained virological response (SVR) rate and the difference between the CAP/CTM HCV values and the ART HCV value before treatment.

Keywords Abbott real-time PCR test · Cobas Ampliprep/Cobas TaqMan · Hepatitis C virus · Genotype · PEG-IFN plus ribavirin therapy · Real-time RT-PCR assay

Introduction

Approximately 80 % of patients infected by hepatitis C virus (HCV) develop chronic hepatitis [1, 2]. Currently, there are more than 100 million HCV carriers worldwide. Chronic hepatitis C could gradually progress to cirrhosis and liver cancer. The first treatment option for chronic hepatitis C is the pegylated interferon plus ribavirin (PEG-IFN/RBV) combination therapy [3, 4]. Several virological predictive factors for sustained virological response (SVR) of PEG-IFN/RBV combination therapy are HCV genotype, baseline viral loads, and early virological response [5–7]. The SVR rate of PEG-IFN/RBV therapy is approximately 50 % for genotype 1 and 80 % for genotype 2. HCV RNA monitoring early in PEG-IFN/RBV therapy is an important predictive factor for SVR for either genotype 1 or genotype 2 [8, 9]. Detection of HCV RNA during PEG-IFN/RBV therapy is important in determining the length of IFN treatment [10]. Currently, Cobas Ampliprep/Cobas TaqMan (CAP/CTM) and Abbott real-time PCR test (ART) are used for HCV RNA measurement. The HCV RNA value in genotype 1 measured by CAP/CTM assay was significantly higher than values by ART assay [11]. The HCV RNA

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Published online: 21 July 2012

value in genotype 1 was measured by two real-time polymerase chain reaction (PCR) methods in this study to investigate whether there is a significant difference in HCV RNA values during PEG-IFN/RBV therapy.

Materials and methods

Of patients with chronic hepatitis C who visited Shin-Kokura hospital from April 2009 to December 2010, 185 were enrolled in this study. Of these 185 patients, 92 subjects were male and 93 were female, 96 subjects were 60 years old or older, and 89 were younger than 60 years old. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki and was approved by the Institutional Review Board. Each patient gave informed consent before participating in this trial. Of the 185 subjects in the study, 97 patients received the PEG-IFNa-2b plus ribavirin combination therapy: 55 patients had genotype 1, and 42 patients had genotype 2. PEG-IFNa-2b (PEG-Intron; MSD, Tokyo, Japan) was injected subcutaneously at a median dose 1.5 μg/kg (range, 1.3–1.5 μg/kg) once a week. Ribavirin (Rebetol; MSD, Tokyo, Japan) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose, 600–1,000 mg). Patients were considered to have an SVR if HCV RNA remained undetectable at 24 weeks after the completion of treatment. The SVR rate was evaluated separately in patients with genotype 1 and genotype 2. Fifty-two of 55 cases of genotype 1 that received PEG-IFN/RBV were evaluated because the treatment was discontinued in 3 patients. Forty-one of 42 cases of genotype 2 were evaluated; treatment was discontinued in 1 patient.

Two real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assays, CAP/CTM (Roche Molecular Systems, Pleasanton, CA, USA) and Abbott real-time HCV test (ART; Abbott Molecular, Abbott Park, IL, USA) were used for the quantitative measurement of HCV RNA concentrations before PEG-IFN/RBV treatment and at day 1, week 1, and week 2 during PEG-IFN/RBV treatment.

Abbott real-time HCV test

The Abbott real-time HCV test is based upon RT-PCR followed by real-time fluorescent detection of HCV RNA (RT-PCR assay). The assay has adopted the second international WHO standard for HCV RNA (code 96/798) for calibration. HCV RNA concentration is expressed in IU/ml. The ART assay has a lower limit of detection (LOD) of 12 IU/ml with a linear quantitation range of 12×10^7 IU/ml.

Cobas Ampliprep/Cobas TaqMan assay

The Cobas Ampliprep/Cobas TaqMan assay is based upon RT-PCR followed by real-time fluorescent detection of HCV RNA from 850 ml serum. CAP/CTM is standardized against the first WHO international standard for HCV RNA (code 96/798). HCV RNA concentration is reported in IU/ml. CAP/CTM assay has an LOD of 15 IU/ml with a linear quantitation range of 43–6.9×10^7 IU/ml.

We genotyped 115 patients for a single nucleotide polymorphism (SNP): rs8099917, an IL28B SNP previously reported to be associated with PEG-IFN/RBV therapy outcome. Samples were genotyped using the Illumina Human Hap 610-Quad Genotyping Bead Chip, with the Invader, or TaqMan assay, as described elsewhere [11–13].

Data analysis

Statistical analysis was performed using PASW Statistics, version 18 (SPSS) and R, version 2.11. Categorical data were analyzed using the chi-squared test and Fisher's exact tests, and continuous data were analyzed using the nonparametric Mann–Whitney U test. p values (two-tailed) <0.05 were considered statistically significant. Correlation coefficient (R) was assessed by the Spearman's correlation coefficient implemented in STATA software version 8.0 (Stata-Corp. LP, College Station, TX, USA).

Results

Figure 1 shows the correlation between the HCV RNA measurements obtained by the two real-time PCR assays: CAP/CTM versus ART in the study variables. A strong correlation was noted between the two real-time RT-PCR assays with an overall coefficient of correlation (R^2) of 0.8975 ($p < 0.0001$).

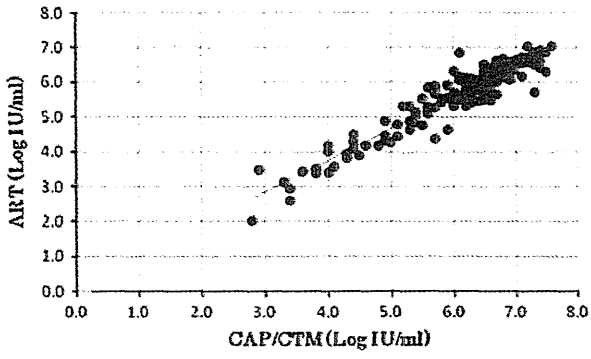

Fig. 1 Correlation between hepatitis C virus (HCV) RNA measurements obtained by two real-time RT-PCR assays: Cobas Ampliprep/Cobas TaqMan (*CAP/CTM*) versus Abbott real-time HCV test (*ART*)

Table 1 Correlation between hepatitis C (HCV) RNA measurements obtained by two real-time RT-PCR assays: CAP/CTM versus ART for study variables ($y = 0.9064x + 0.1176$, $R^2 = 0.8975$, $p < 0.0001$)

Study variables	*n*	CAP/CTM (log IU/ml)		ART (log IU/ml)		Correlation coefficient	
		Mean	SD	Mean	SD	R^2	*p* value
Gender							
Male	92	6.18	1.06	5.71	0.97	0.9009	<0.0001
Female	93	6.18	0.92	5.72	0.92	0.8972	<0.0001
Age (years)							
≥60	96	6.04	1.03	5.56	1.00	0.9142	<0.0001
<60	89	6.33	0.92	5.89	0.89	0.8709	<0.0001
Platelet counts (10^9/l)							
≥150	126	6.24	0.96	5.78	0.94	0.9106	<0.0001
<150	59	6.04	1.03	5.58	0.96	0.8705	<0.0001
IL28B							
TT	84	6.06	1.06	5.67	1.06	0.8826	<0.0001
TG/GG	31	5.91	1.18	5.45	1.07	0.9461	<0.0001
Genotype							
1	55	6.06	1.09	5.52	1.09	0.9647	<0.0001
2	42	5.94	1.21	5.65	1.15	0.9233	<0.0001

CAP/CTM Cobas Ampliprep/Cobas TaqMan, *ART* Abbott real-time HCV test, *IL28B* interleukin 28B

Table 1 shows the correlation between the HCV RNA measurements obtained by the two real-time RT-PCR assays, CAP/CTM versus ART, in the study variables. All the coefficient of correlation (R^2) values based on the variables, such as gender, age (≥60 or >60 years), and number of platelets (≤150 × 10^9/l or >150 × 10^9/l), were more than 0.8700 ($p < 0.0001$) and were strongly correlated with the HCV RNA values obtained by the two real-time RT-PCR assays. The coefficients of correlation (R^2) for IL28B genotype (TT, TG/GG) were 0.8826 ($p < 0.000$) and 0.9461 ($p < 0.0001$), respectively, and a strong correlation was observed also for the HCV RNA values obtained by the two real-time RT-PCR assays. Similarly, the coefficients of correlation (R^2) for the HCV genotypes (genotypes 1, 2) were 0.9647 ($p < 0.0001$) and 0.9233 ($p < 0.0001$), respectively, and a strong correlation was observed also for the HCV RNA values obtained by the two real-time PCR assays.

Table 2 shows HCV RNA concentrations of study variables as measured by the two real-time RT-PCR assays. HCV RNA values measured by CAP/CTM were significantly higher than those by ART for all variables, such as gender, age (≥60 or >60 years), and the number of platelets (≥150 × 10^9/l or >150 × 10^9/l) ($p < 0.05$). The HCV RNA values of the IL28B group with TT genotype measured by CAP/CTM were significantly higher than those by ART ($p < 0.05$); however, no difference was observed for the TG/GG genotypes. The difference of HCV RNA values (CAP/CTM minus ART) between the TT genotype and the TG/GG genotypes was not statistically significant (Fig. 2). The positive rates of the difference of HCV RNA values in the TT genotype and the TG/GG genotypes (CAP/CTM minus ART) were 90.5 and 90.3 %, respectively, and were not statistically significant. The difference of HCV RNA values in the TG/GG genotypes were not statistically significant, which can be explained by the small number of subjects enrolled in this study. The HCV RNA values in genotype 1 measured by CAP/CTM were significantly higher than those by ART ($p < 0.05$); however, the difference was not statistically significant in genotype 2. The difference of HCV RNA values (CAP/CTM minus ART) was significantly higher in genotype 1 than in genotype 2 ($p < 0.0001$) (Fig. 3). The positive rate (>0) of the difference of HCV RNA values (CAP/CTM minus ART) in genotype 1 was 100 % (55/55), significantly higher ($p < 0.001$) compared to the positive rate of 78.6 % (33/42) in genotype 2.

Table 3 shows HCV RNA concentrations of HCV genotypes 1 and 2 during PEG-IFN/RBV treatment as measured by the two real-time PCR assays. The HCV RNA values decreased during PEG-IFN/RBV therapy for both genotype 1 and genotype 2. The difference of HCV RNA values (CAP/CTM minus ART) in the genotype 1 group decreased gradually. The difference was not statistically significant at day 1 of treatment. The HCV RNA values of CAT/CTM in the genotype 1 group were higher than those of ART at day 1, week 1, and week 2; however, the difference was not statistically significant.

Table 4 shows the SVR rate in patients who received PEG-IFN/RBV therapy by differences of HCV RNA values between CAT/CTM and ART before PEG-IFN/RBV

Table 2 HCV RNA concentrations for study variables as measured by two real-time RT-PCR assays: CAP/CTM and ART

Study variables	*n*	CAP/CTM (log IU/ml)		ART (log IU/ml)		Average HCV RNA level (CAP/CTM−ART)		Quantitation difference		*p* value*
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Gender										
Male	92	6.18	1.06	5.71	0.97	5.95	1.00	0.47	0.33	0.0019
Female	93	6.18	0.92	5.72	0.92	5.95	0.91	0.45	0.30	0.0010
Age (years)										
≥60	96	6.04	1.03	5.56	1.00	5.80	1.00	0.48	0.30	0.0010
<60	89	6.33	0.92	5.89	0.89	6.11	0.87	0.44	0.33	0.0016
Platelet counts (10^9/l)										
≥150	126	6.24	0.96	5.78	0.94	6.01	0.94	0.46	0.29	0.0002
<150	59	6.04	1.03	5.58	0.96	5.81	0.98	0.47	0.37	0.0123
IL28B										
TT	84	6.06	1.06	5.67	1.06	5.86	1.04	0.40	0.34	0.0131
TG/GG	31	5.91	1.18	5.45	1.07	5.68	1.12	0.45	0.29	0.1199
Genotype										
1	55	6.06	1.09	5.52	1.09	5.00	1.09	0.54	0.21†	0.0161
2	42	5.94	1.21	5.65	1.15	5.79	1.17	0.28	0.34†	0.2734

CAP/CTM Cobas Ampliprep/Cobas TaqMan, *ART* Abbott real time HCV test, *IL28B* interleukin 28B

* CAP/CTM versus ART

† $p < 0.0001$, genotype 1 versus genotype 2

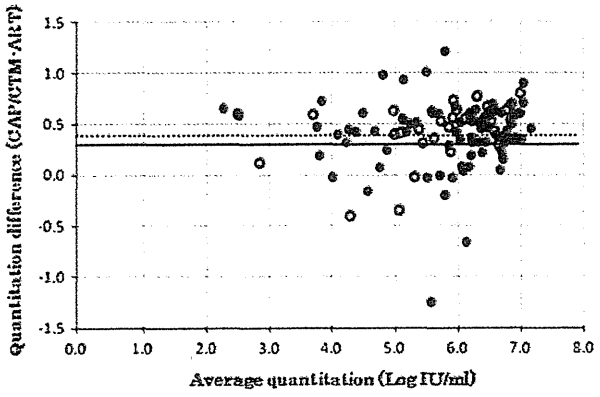

Fig. 2 Genotype-specific HCV RNA level difference in HCV RNA measurements by CAP/CTM versus those by ART test in samples with interleukin 28B (IL28B) genotypes; TT and TG/GG, before pegylated interferon plus ribavirin (PEG-IFN/RBV) treatment [*closed circles* genotype TT, *open circles* genotype TG/GG, *solid line* mean HCV RNA values of the difference (CAP/CTM minus ART) in TT, *dotted line* mean HCV RNA values of the difference (CAP/CTM minus ART) in TG/GG]

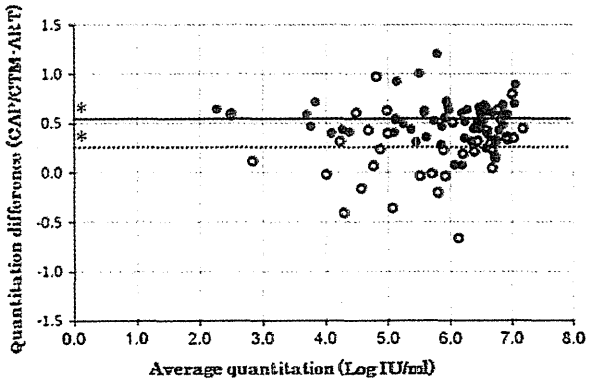

Fig. 3 Genotype-specific HCV RNA level difference in HCV RNA measurements by CAP/CTM versus those by ART test in samples with HCV subtypes 1 and 2 before PEG-IFN/RBV treatment [*closed circles* genotype 1, *open circles* genotype 2, *solid line* mean HCV RNA values of the difference (CAP/CTM minus ART) in HCV genotype 1, *dotted line* mean HCV RNA values of the difference (CAP/CTM minus ART) in HCV genotype 2]. *$p < 0.0001$, genotype 1 versus genotype 2

therapy. Group L comprises patients with a difference of HCV RNA values of 0.5 IU/ml or more (CAP/CTM minus ART), and group S comprises patients with a difference of HCV RNA values of less than 0.5 IU/ml (CAP/CTM minus ART). The SVR rate of genotype 1 (55.8 %) was significantly higher than that of genotype 2 (78.0 %, $p = 0.015$). For genotype 1, the SVR rate of IL28B genotype TT was significantly higher ($p = 0.016$) than that of genotype TG or GG. The SVR rates in group L and group S were not significantly different for IL28B genotypes TT, TG, or GG. The SVR rate of genotype TT was significantly higher than that of genotype TG or GG. The SVR rates in groups L and S were evaluated for the CAP/CTM HCV RNA values and ART before therapy, but no significant difference was

Table 3 HCV RNA concentrations for HCV genotypes 1 and 2 during PEG-IFN/RBV treatment as measured by two real-time RT-PCR assays: CAP/CTM and ART

Genotype	*n*	CAP/CTM (log IU/ml)		ART (log IU/ml)		Average HCV RNA level (log IU/ml)		Quantitation difference (CAP/CTM−ART)		*p* value*
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Genotype 1										
Before treatment	55	6.06	1.09	5.52	1.09	5.00	1.09	0.54	0.21	0.0161
Day 1	53	4.64	1.11	4.23	1.12	4.44	1.12	0.41	0.15	0.0662
Week 1	55	3.79	1.93	3.41	1.74	3.60	1.83	0.38	0.38	0.2739
Week 2	55	3.06	2.05	2.80	1.74	2.93	1.88	0.26	0.49	0.4966
Genotype 2										
Before treatment	42	5.94	1.21	5.65	1.15	5.79	1.17	0.28	0.34	0.2734
Day 1	39	4.55	1.19	4.49	1.08	4.52	1.13	0.06	0.27	0.8618
Week 1	41	3.39	1.51	3.21	1.48	3.30	1.47	0.18	0.45	0.6946
Week 2	42	1.98	1.30	1.89	1.06	1.96	1.14	0.09	0.29	0.8014

CAP/CTM Cobas Ampliprep/Cobas TaqMan, *ART* Abbott real time HCV test

* CAP/CTM versus ART

observed. The SVR rate of patients with less than 6.0 log IU/ml of the HCV RNA values measured by both CAP/CTM and assay was higher than that with 6.0 log IU/ml or more, but this difference was not significant. For genotype 2, the SVR rate of IL28B genotype TT was higher than that of genotype TG or GG, but this difference also was not significant. There was no difference in the SVR rates between less than 6.0 and 6.0 log IU/ml or more. The SVR rates in group L and group S were not significantly different. The SVR rate of patients with genotype 2 was high regardless of HCV RNA values and IL28B genotype.

Discussion

The study revealed that the HCV RNA values measured by CAP/CTM were higher than those by ART among the subjects with genotype 1; however, no difference was observed among the patients with genotype 2. The difference of HCV RNA values (CAP/CTM minus ART) in genotype 1 was significantly higher than those in genotype 2 ($p < 0.0001$). The positive rate (>0) of the difference of HCV RNA values (CAP/CTM minus ART) in genotype 1 was 100 %, which was significantly higher than the positive rate of 78.6 % in the genotype 2 group ($p < 0.001$). The differences of HCV RNA values (CAP/CTM minus ART) in the genotype 1 group were all positive (>0), and all the viral load measurements obtained from CAP/CTM were higher than those from ART (Fig. 3). Ohnishi et al. [11] reported that the difference of the WHO standard versions used for each assay calibration might be the reason for the findings. CAP/CTM adopted the First International HCV RNA WHO standard for the assay calibration, while ART adopted the Second International HCV RNA WHO standard. Direct comparison of the two assays for measuring the WHO standard revealed a consistently higher quantitation of the WHO standard by CAP/CTM than by ART. The HCV RNA values of CAT/CTM in genotype 2 were reported to be lower those of ART [14]. Some cases in this evaluation also had lower CAP/CTM HCV RNA values (Fig. 3). No consistency was observed in genotype 2; some CAT/CTM HCV RNA values were higher than ART and some were lower. Base substitution is thought to contribute to this inconsistency [14]; this could have resulted from the differences between the two PCR methods. Also, this is consistent with a previous study in which CAP/CTM values were relatively higher for genotype 1 and lower for genotype 2 [2].

The difference of the HCV RNA values between CAP/CTM and ART was investigated in this study based on viral kinetics from the early stage of PEG-IFN/RBV treatment. After administration of PEG-IFN/RBV treatment, reduction of HCV RNA measurements obtained from both the CAP/CTM assay and the ART assay was observed from day 1 regardless of genotype (1 or 2). The HCV RNA values of CAP/CTM were consistently higher than those of ART during PEG-IFN/RBV therapy. The difference between these two values eventually became smaller because of the effect of PEG-IFN/RBV therapy, and a significant difference was no longer observed.

The IL28B genotype is one of the predictors of PEG-IFN/RBV therapy outcome before administration of treatment [15, 16]. In this study, for the genotype 1 patients, the SVR rate of IL28B genotype TT was significantly higher than the SVR rates of genotype TG or GG.

Table 4 Sustained virological response (SVR) rate in patients who received PEG-IFN/RBV therapy by difference between CAT/CTM HCV value and ART value before PEG-IFN/RBV therapy

	Group S SVR/*n* (%)	Group L SVR/*n* (%)	*p* value*	Total SVR/*n* (%)
Genotype 1				
IL28B				
TT	15/19 (79)	10/19 (53)	0.087	25/38 (66)
TG or GG	1/5 (20)	2/9 (22)	0.481	3/14 (21)
p value	0.012	0.128		0.004
CAP/CTM				
<6.0 log IU/ml	6/8 (75)	5/7 (71)	0.875	11/15 (73)
≥6.0 log IU/ml	10/16 (63)	7/21 (33)	0.077	17/37 (46)
p value	0.540	0.077		0.072
ART				
<6.0 log IU/ml	8/10 (80)	8/14 (57)	0.241	16/24 (67)
≥6.0 log IU/ml	8/14 (57)	4/14 (29)	0.126	12/28 (43)
p value	0.241	0.126		0.085
Total	16/24 (67)	12/28 (43)	0.086	28/52 (54)†
Genotype 2				
IL28B				
TT	19/22 (86)	4/6 (67)	0.264	23/28 (82)
TG or GG	5/7 (71)	4/6 (67)	0.852	9/13 (69)
p value	0.362	1.000		0.112
CAP/CTM				
<6.0 log IU/ml	11/15 (73)	3/4 (75)	0.946	14/19 (74)
≥6.0 log IU/ml	12/14 (86)	6/8 (75)	0.531	18/22 (82)
p value	0.411	1.000		0.530
ART				
<6.0 log IU/ml	14/15 (93)	4/5 (80)	0.717	18/20 (90)
≥6.0 log IU/ml	10/14 (71)	4/7 (57)	0.305	14/21 (67)
p value	0.564	0.407		0.293
Total	24/29 (83)	8/12 (67)	0.257	32/41 (78)†

Group L, ≥0.5 log IU/ml (CAP/CTM−ART); group S, <0.5 log IU/ml (CAP/CTM−ART)

* Group S versus group L

† $p = 0.015$, genotype 1 versus genotype 2

The HCV RNA values obtained from the two real-time PCR assays were analyzed based on the IL28B genotypes in this study. The HCV RNA values in the TT genotype group measured by CAP/CTM were significantly higher than those by ART; however, there was no significant difference in the TG or GG genotype groups. IL28B genotypes TT, TG, or GG were evaluated by differences of HCV RNA values between the CAP/CTM and ART: only the genotype TT group had a higher SVR rate. No SVR rate difference depending on the difference of HCV RNA values between CAP/CTM and ART was observed for genotypes 1 and 2. Clinically, a higher SVR rate was observed in the genotype TT group. It is assumed that the HCV RNA values of CAP/CTM were significantly higher than those of ART in genotype 1 patients because 73 % were in the genotype TT group. Therefore, there is assumed to be no correlation between IL28B and the difference of HCV RNA values between CAP/CTM and ART.

The data were also analyzed based on gender, age, and the number of platelets. For all variables, HCV RNA values as measured by CAP/CTM were significantly higher than those by ART; however, there was no difference in the HCV RNA values measured by CAP/CTM and ART when the measurements were compared against each variable.

The difference in HCV RNA measurements is suggested to be the result of HCV genotype. The prevalence of genotype 1 is higher in Japanese; therefore, the difference was observed in the measurements obtained from both assays.

The details of primer design and the PCR protocol for the products of both manufacturers used for this evaluation

are not disclosed. The PCR protocol of CAP/CTM method has two steps whereas the ART method has a three-step protocol. For the CAP/CTM method, elongation and probe hybridization are conducted simultaneously in the low-temperature step, and the temperature is generally 50–60 °C . In the ART method, a single-stranded linear probe is used instead of a TaqMan Probe and it has three steps, although it is also a real-time PCR method. Also, probe hybridization takes place at a lower temperature than for the CAP/CTM method, which is thought to optimize the tolerance level for HCV detection.

The newly developed ART features nucleic acid extraction using m2000 system, automated real-time PCR analysis, and high processing capacity. The assay results correlate well with the CAP/CTM assay, which suggests the wide application of the platform in clinical settings in the future. Additionally, the sample volume is 0.5 or 0.2 ml, which is highly practical for pediatric patients or when only a limited amount of patient sample is available. Also, some research has suggested that the genotype reactivity of ART is superior [17, 18].

In this study, the SVR rate was higher in genotype 2 than in genotype 1. For genotype 1, the SVR rate in IL28B genotype TT was higher than that in genotype TG or GG. For genotype 2, there was no difference of SVR rate between genotype TT and genotype TG or GG. These results were similar to the results of a previous study.

In summary, the HCV RNA values in genotype 1 obtained from the CAP/CTM assay were significantly higher compared to the values obtained from ART; however, no difference was observed in genotype 2. The HCV RNA values decreased during PEG-IFN/RBV therapy regardless of genotype. The HCV RNA value for CAP/CTM during PEG-IFN/RBV therapy was consistently higher than that for ART. However, the difference in these two values gradually became less during the course of therapy, and eventually no significant difference was observed near the detection level. No correlation was observed between the SVR rate and the difference between the CAP/CTM HCV values and the ART HCV value before treatment. Both CAP/CTM assay and ART assay were useful for PEG-IFN/RBV therapy. In this study, it was not clear which of the two HCV RNA assays was useful regarding the effects of IFN therapy. More detailed study is necessary.

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J Infect Chemother (2012) 18:689–697
DOI 10.1007/s10156-012-0396-5

ORIGINAL ARTICLE

An inadequate dose of ribavirin is related to virological relapse by chronic hepatitis C patients treated with pegylated interferon alpha-2b and ribavirin

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Received: 20 September 2011 / Accepted: 14 February 2012 / Published online: 27 March 2012

Abstract The aim of this large-scale analysis was to assess the effect of 48-week pegylated interferon (PEG-IFN) α-2b and ribavirin (RBV) therapy on virological relapse by patients infected with hepatitis C virus (HCV) genotype 1. The relationship between virological relapse and the dose of PEG-IFNα-2b and RBV was investigated in 619 patients who had once cleared HCV RNA during PEG-IFNα-2b and RBV treatment for 48 weeks. The overall virological relapse rate was 34.1% (211 of 619). The relapse rate was 59.5% (22 of 37) for patients who received <6 mg/kg/day of RBV, even if a sufficient dose of PEG-IFNα-2b (≥1.5 μg/kg/day) was received. In contrast, the relapse rate was 28.1% (16 of 57) for patients who received ≥12 mg/kg/day of RBV, irrespective of the PEG-IFNα-2b dose. The relapse rates were significantly increased with the reduction of the RBV dose for both PEG-IFNα-2b doses of ≥1.2 and <1.2 μg/kg/week ($P < 0.0001$ and $P = 0.0006$, respectively). Moreover, the relapse rate was 41.2% (35 of 85) for patients with an early virological response (EVR) who received <6 mg/kg/day of RBV. The relapse rates were significantly increased with the reduction of the RBV dose in both those patients with an EVR and those with a late virological response ($P = 0.0006$ and $P = 0.0088$, respectively). To summarize, for HCV genotype 1 patients treated with PEG-IFNα-2b and RBV, the virological relapse of HCV was RBV dose-dependent, irrespective of the dose of PEG-IFNα or the effect of early viral kinetics.

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Keywords Hepatitis C virus · Relapse · Pegylated interferon · Ribavirin

Introduction

Chronic hepatitis C virus (HCV) infection is a major cause of viral hepatitis. An estimated 170 million people worldwide are infected with persistent HCV, and cirrhosis and complications of endstage liver disease will develop in many of these people [1]. Previous studies have shown that interferon (IFN) is among the most frequently used agents against HCV infection and is effective for eradicating HCV [2, 3] and that it significantly reduces the progression of liver fibrosis and the risk of hepatocellular carcinoma [4, 5]. Currently, a combination of pegylated interferon alpha (PEG-IFNα) and ribavirin (RBV) given for 48 weeks is the standard of care for chronic HCV infection [6, 7]. Predictive factors for achieving a sustained virological response (SVR) have been reported, including pretreatment demographics (age, sex) and clinical [alanine aminotransferase (ALT) level, liver histology], viral (HCV genotype, viral load, amino acid substitutions in the HCV) and treatment (RBV concentration) parameters [2–4, 8, 9].

However, because such a high percentage of patients who complete PEG-IFNα and RBV treatment relapse, defined as having undetectable HCV RNA at the end of treatment but detectable HCV RNA during the follow-up period, it is important to accurately determine the factors involved in relapse. Previous reports have suggested that early viral kinetics, HCV genotype, host (HIV coinfection) and drug (PEG-IFNα and RBV dosing) factors influence the relapse rate [10–12]. From the perspective of drug factors, Shiffman et al. [13], in a study of HCV genotype 1 patients, reported that the relapse rate was lower among patients receiving high-dose (1,000–1,600 mg/day or 15.2 mg/kg/day) compared with standard dose (800–1,400 mg/day or 13.3 mg/kg/day) RBV. Similarly, Fried et al. [14], in a study of HCV genotype 1 patients with high baseline HCV RNA and high body mass index (BMI), reported that a lower relapse rate was attained for patients receiving high doses of PEG-IFNα-2a (270 μg/week) and high doses of RBV (1,600 mg/day) than that attained for patients treated with a standard dosing regimen. These findings indicate that the therapeutic dose, especially that of RBV, plays an important role in reducing relapse in the treatment of chronic HCV infection. However, no medical consensus has been reached regarding evaluation of the treatment dose related to virological relapse (i.e., detectable HCV RNA after HCV RNA has been cleared).

The aim of this large-scale treatment analysis was to assess, in HCV genotype 1 patients treated with PEG-IFNα-2b and RBV for 48 weeks, whether or not the PEG-IFNα and RBV doses had an effect on virological relapse after the clearance of HCV RNA.

Patients and methods

Patients

This prospective study was of 2,871 Japanese patients with chronic HCV infection aged 18 years or older treated with PEG-IFNα-2b and RBV between December 2004 and February 2009. Of the 2,871 patients screened, 1,712 were excluded from the study because of having HCV genotypes other than genotype 1, lack of treatment response evaluation, unclear HCV genotype and viral load, history of hepatocellular carcinoma, or prolonged duration of treatment (more than 48 weeks). Of the remaining 1,159 patients who met the enrollment criteria, 402 were excluded because they did not complete the standard treatment (48 weeks), because of side effects, ineffective virological response, or for economic reasons. To ensure that we were able to accurately evaluate virological relapse, we also excluded patients with a non-viral response (NVR) ($n = 138$). Finally, we investigated the correlation between virological relapse and demographic factors, clinical parameters, and the doses of PEG-IFNα-2b and RBV in 619 patients who had once cleared HCV RNA with PEG-IFNα-2b and RBV treatment. Of these 619 patients, 414 (66.9%) had been treated previously with IFN before being enrolled in this prospective study.

All 619 patients satisfied the following exclusion criteria and were recruited at Kyushu University Hospital and 32 affiliated hospitals in the northern Kyushu area of Japan : (1) positivity for antibody to human immunodeficiency virus (HIV) or positivity for hepatitis B surface antigen; (2) clinical or biochemical evidence of hepatic decompensation; (3) excessive active alcohol consumption (>60 g/day converted into ethanol) or drug abuse; (4) suspected hepatocellular carcinoma; (5) other forms of liver disease; or (6) treatment with antiviral or immunosuppressive agents prior to enrollment.

Informed consent was obtained from all patients before enrollment. The study was approved by the institutional Ethics Committees of the hospitals involved and was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Clinical and laboratory assessments

Clinical parameters assessed included serum albumin, ALT, γ-glutamyl transpeptidase (γGTP), creatinine clearance, hemoglobin, platelet count, plasma glucose, HCV genotype, and HCV RNA. All these parameters were

measured by standard laboratory techniques at a commercial laboratory. Body mass index (BMI) was calculated as weight in kilograms/height in square meters. Insulin resistance was calculated by means of the homeostasis model assessment-insulin resistance (HOMA-IR) method [15].

Therapeutic protocol, dose reduction, and discontinuation of treatment

All patients were treated with PEG-IFNα-2b (PEG-Intron; MSD, Tokyo, Japan) plus RBV (Rebetol; MSD). The duration of treatment for these HCV genotype 1 patients was 48 weeks. PEG-IFNα-2b was given subcutaneously once weekly at a dose of 60–150 μg based on body weight (60 μg for patients weighing 35–45 kg, 80 μg for those weighing 46–60 kg, 100 μg for those weighing 61–75 kg, 120 μg for those weighing 76–90 kg, and 150 μg for those weighing 91–120 kg). RBV was given orally at a daily dose of 600–1,000 mg based on body weight (600 mg for patients weighing <60 kg, 800 mg for those weighing 60–80 kg, and 1,000 mg for those weighing >80 kg).

Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to <100 g/L. In such cases, a reduction in the dose of RBV was required. Some patients also had PEG-IFNα-2b-induced psychological adverse effects or a decrease in the white blood cell and platelet counts. In such cases, a reduction in the dose of PEG-IFNα-2b was required. Both PEG-IFNα-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 80 g/L, 1×10^9/L, or 25×10^9/L, respectively. The treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic problems developed; continuation of treatment was judged not to be possible by the attending physician; or the patient desired discontinuation of treatment.

Assessment of drug exposure

The doses of PEG-IFNα-2b and RBV were calculated individually as averages on the basis of body weight at baseline by reviewing the patients' medical records: the PEG-IFNα-2b dose was expressed as μg/kg/week and RBV dose was expressed as mg/kg/day.

Determination of HCV-RNA level and HCV genotype

Clinical virological follow up was performed by HCV viremia detection using a real-time reverse transcriptase polymerase chain reaction (PCR) assay (COBAS TaqMan HCV assay; Roche Diagnostics, Tokyo, Japan), with a lower limit of quantitation of 15 IU/mL and an upper limit of quantitation of 6.9×10^7 IU/mL (1.2–7.8 log IU/mL referred to as $\log_{10}$ units/mL). HCV genotype was determined by means of sequence determination in the 5′-nonstructural region of the HCV genome followed by phylogenetic analysis, as previously described [2].

Virological response

The above COBAS TaqMan HCV assay was used to evaluate HCV viremia as a surrogate marker of the virological outcome of treatment. SVR was defined as serum HCV-RNA undetectable at 24 weeks after the end of treatment, and virological HCV relapse was defined as detectable HCV RNA during the 24-week post-treatment period in patients who had undetectable HCV RNA at the end of treatment.

Treatment response was defined as follows: early virological response (EVR), HCV RNA undetectable at week 12; late virological response (LVR), HCV RNA undetectable between week 12 and week 48; and non-viral response (NVR), HCV RNA detectable at the end of treatment.

Liver histology and quantitative variables

Liver biopsy was conducted under ultrasound guidance by experienced hepatologists. For each specimen, the stage of fibrosis and the grade of histological activity were established according to the METAVIR scoring [16]. Fibrosis was staged on a 0–4 scale as follows: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis. The grading of histological activity, including the intensity of necroinflammation, was scored as follows: A0 = no histological activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity.

Statistical analysis

Statistical analysis was performed using SAS ver. 9.2 (Statistical Analysis System, SAS Institute, Tokyo, Japan). Quantitative variables were expressed as medians and categorical variables were reported as frequencies and percentages. The paired *t*-test, unpaired *t*-test, Mann–Whitney *U*-test, or χ^2 test was used for the analysis. The significance of trends in values was determined with the Cochran–Armitage trend test. Logistic regression analysis was used to investigate the association of virological response with PEG-IFNα-2b plus RBV treatment, treatment dose, demographic factors, and clinical data. Area under the receiver operating characteristic curve (AUROC) analysis was performed to evaluate the relationship between the RBV dose and virological relapse. The cutoff

values were selected from the receiver operating characteristic (ROC) curve to maximize the total sensitivity and specificity. A two-tailed P value of less than 0.05 was regarded as statistically significant.

Results

Univariate analysis of factors for virological relapse

The overall relapse rate in the present study was 34.1% (211 of 619 patients). The results of univariate analysis of factors for relapse in the patients with virological response are shown in Table 1. The demographic factors of sex (women) and age (65 or over) and the clinical parameters of low creatinine clearance, low albumin, high ALT, low hemoglobin, low platelet count, high HOMA-IR, and liver histology that had progressed were significantly associated with virological relapse. In the multivariate logistic regression analysis, as shown in Table 2, a low total dose of PEG-IFNα-2b and RBV, late timing of HCV RNA negativity, and low average RBV dose were significantly associated with virological relapse. However, the average PEG-IFNα-2b dose and the initial dose of PEG-IFNα-2b or RBV were not associated with relapse.

Relapse rate according to PEG-IFNα-2b and RBV doses (Table 3)

We analyzed the relapse rate according to the degree of exposure to PEG-IFNα-2b and RBV. Table 3 shows the relapse rates according to the PEG-IFNα-2b and RBV doses given over the full treatment period (48 weeks). As a whole, the relapse rate revealed an elevation according to the reduction of RBV dose. The relapse rate for patients who received <6 mg/kg/day of RBV (59.5%, 22 of 37) was significantly higher than the rate for patients in the other RBV dose categories, even if a sufficient dose of PEG-IFNα-2b (≥1.5 μg/kg/day) was received. In contrast, the relapse rate for patients who received ≥12 mg/kg/day of RBV (28.1%, 16 of 57) was significantly lower than the rate for patients in the other RBV dose categories, irrespective of the PEG-IFNα-2b dose.

Multivariate analysis of virological relapse by background and treatment factors (Table 4)

Multivariate analysis was performed to select the background and treatment factors related to virological relapse. For the items affecting virological relapse shown in Table 4, the significant background factors for virological relapse were age (odds ratio 1.68, 95% confidence interval [CI] 1.36–2.08, $P < 0.0001$), ALT (odds ratio 0.93, 95% CI 0.88–0.97, $P = 0.0019$), HOMA-IR (odds ratio 1.24, 95% CI 1.10–1.40, $P = 0.0004$), platelet count (odds ratio 0.95, 95% CI 0.91–0.99, $P = 0.0102$), and liver fibrosis (odds ratio 2.17, 95% CI 1.08–4.34, $P = 0.0290$). Adding the treatment parameters to the analyses, age (odds ratio 1.48, 95% CI 1.20–1.84, $P = 0.0003$), HOMA-IR (odds ratio 1.22, 95% CI 1.08–1.38, $P = 0.0012$), RBV dose (odds ratio 0.90, 95% CI 0.84–0.96, $P = 0.0009$), and LVR (odds ratio 5.75, 95% CI 3.73–8.86, $P < 0.0001$) were found to be independent factors associated with a virological relapse. It is particularly worth noting that the PEG-IFNα-2b dose was not associated with relapse in this multivariate analysis.

Table 1 Baseline characteristics of 619 studied patients with chronic HCV infection

Characteristics	SVR ($n = 408$)	Relapse ($n = 211$)	P value
Men [no. (%)]	224 (54.9)	88 (41.7)	0.0022
Age (years)	55.0 [18–75]	60.0 [26–79]	<0.0001
Body mass index (kg/m^2)	23.1 [14.9–38.0]	23.1 [16.9–37.2]	0.4751
Prior IFN treatment [no. (%)]	277 (67.9)	137 (65.0)	0.3649
Creatinine clearance (L/h)	12.6 [5.1–37.1]	10.6 [4.4–28.6]	0.0002
Albumin (g/dL)	4.2 [3.0–5.1]	4.1 [3.1–4.9]	0.0120
Alanine aminotransferase (IU/L)	61 [14–590]	52 [12–295]	0.0126
γ-Glutamyl-transpeptidase (IU/L)	33 [10–380]	37 [7–255]	0.1225
Hemoglobin (g/L)	139 [96–184]	136 [107–178]	0.0274
Platelet count (10^9/L)	171 [80–343]	154 [61–329]	0.0004
HOMA-IR	1.7 [0.4–33.7]	3.0 [0.4–17.7]	<0.0001
Serum HCV RNA level (log IU/mL)	6.5 [5.0–8.1]	6.6 [5.1–7.6]	0.0888
Liver histology			
Fibrosis: 0–2/3–4	149/101	56/84	0.0002
Activity: 0–1/2–3	102/146	39/98	0.0151

Data are shown as numbers (%) or medians [ranges]

HCV hepatitis C virus, *SVR* sustained virological response, *HOMA-IR* homeostasis model assessment-insulin resistance, *IFN* interferon

Table 2 Treatment doses and virological response of 619 studied patients

Characteristics	SVR ($n = 408$)	Relapse ($n = 211$)	P value
Initial PEG-IFN dose (μg/kg/week)	1.46 [0.54–2.19]	1.45 [0.70–1.98]	0.4063
Initial RBV dose (mg/kg/day)	10.8 [3.4–16.9]	10.6 [3.3–18.0]	0.1065
Average PEG-IFN dose (μg/kg/week)	1.42 [0.54–2.45]	1.37 [0.52–1.94]	0.1506
Average RBV dose (mg/kg/day)	10.0 [3.3–16.6]	9.9 [3.4–13.6]	<0.0001
Assigned total cumulative PEG-IFN dose ≥80% and RBV dose ≥60% [no. (%)]	269 (65.9)	93 (44.1)	<0.0001
Virological response EVR/LVR	363/45	114/97	<0.0001

Data are shown as numbers (%) or medians [ranges]

SVR sustained virological response, *PEG-IFN* pegylated interferon, *RBV* ribavirin, *EVR* early virological response, *LVR* late virological response

Table 3 Relapse rates according to the PEG-IFNα-2b and RBV doses given over the full treatment period (48 weeks)

RBV (mg/kg/day) PEG-IFNα-2b (μg/kg/week)	≥12	10 to <12	8 to <10	6 to <8	<6	Total
≥1.5	19.6% (9/46)	31.8% (14/44)	22.7% (10/44)	35.3% (12/34)	59.5% (22/37)	32.7% (67/205)
1.2 to <1.5	26.3% (5/19)	27.9% (29/104)	30.6% (19/62)	28.9% (13/45)	50.8% (30/59)	33.2% (96/289)
0.9 to <1.2	25.0% (1/4)	17.6% (3/17)	27.8% (5/13)	22.2% (4/18)	58.1% (18/31)	35.2% (31/88)
<0.9	25.0% (1/4)	50.0% (3/6)	16.7% (1/6)	42.9% (3/7)	87.5% (7/8)	48.4% (15/31)
Total	28.1% (16/57)	28.7% (49/171)	26.9% (35/130)	30.8% (32/104)	57.0% (77/135)	34.1% (211/619)

PEG-IFN pegylated interferon, *RBV* ribavirin

ROC curve analysis of association of RBV dose and virological relapse

ROC curve analysis was performed to determine the optimal threshold value of RBV dose for predicting virological relapse. The relevant AUROC was 0.71 and the cutoff value for RBV dose was 6.3 mg/kg/day (sensitivity 85.0%, specificity 44.0%, positive predictive value 74.0%, negative predictive value 62.0%).

Relapse rates by PEG-IFNα-2b dose, sex, age, and the timing of HCV RNA negativity according to the RBV dose (Table 5)

We analyzed the association between the relapse rates and the dose of RBV received with the PEG-IFNα-2b dose divided into ≥1.2 μg/kg/week and <1.2 μg/kg/week. Relapse rates were significantly increased with the reduction of the RBV dose for both PEG-IFNα-2b ≥1.2 and <1.2 μg/kg/week ($P < 0.0001$ and $P = 0.0006$, respectively, by Cochran–Armitage trend test). There was no significant difference between PEG-IFNα-2b ≥1.2 and <1.2 μg/kg/week in any RBV dose category.

The association between relapse rates and the RBV dose received was analyzed by sex. The relapse rates were significantly increased with the reduction of the RBV dose for both men and women ($P < 0.0001$ and $P = 0.0084$, respectively, by Cochran–Armitage trend test). Relapse rates in women were significantly higher than those in men in the group given an average RBV dose of more than 10 mg/kg/day.

The association between relapse rates and the RBV dose received was analyzed by age. The relapse rates were also significantly increased with the reduction of the RBV dose for patients aged 64 or less as well as for those aged 65 or more ($P < 0.0001$ and $P = 0.0037$, respectively, by Cochran–Armitage trend test). The relapse rates of patients aged 65 or more were significantly higher than those of patients aged 64 or less in the group given an average RBV dose of less than 6 mg/kg/day ($P = 0.0069$).

We analyzed the association between relapse rates and the prescribed RBV dose by virological response. The relapse rates were significantly increased with the reduction of the RBV dose both for patients with an EVR and those with an LVR ($P = 0.0006$ and $P = 0.0088$, respectively, by Cochran–Armitage trend test). Relapse rates of patients with an LVR were significantly higher than those of patients with an EVR in the group given an average RBV dose of less than 12 mg/kg/day.

Discussion

Virological HCV relapse is an exceedingly important clinical outcome in the treatment of chronic HCV infection. HCV genotype is a factor well known to affect

Table 4 Multivariate logistic regression analysis of virological relapse by background and treatment factors

Characteristics	Background factors		Background and treatment factors	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Age per 10 years	1.68 (1.36–2.08)	<0.0001	1.48 (1.20–1.84)	0.0003
ALT per 10 IU/L	0.93 (0.88–0.97)	0.0019		
HOMA-IR per 1	1.24 (1.10–1.40)	0.0004	1.22 (1.08–1.38)	0.0012
Platelet count per 1 × 10^9/L	0.95 (0.91–0.99)	0.0102		
Fibrosis 3–4 versus 0–2	2.17 (1.08–4.34)	0.0290		
RBV dose per 1 mg/kg/day			0.90 (0.84–0.96)	0.0009
LVR versus EVR			5.75 (3.73–8.86)	<0.0001

ALT alanine aminotransferase, *HOMA-IR* homeostasis model assessment-insulin resistance, *RBV* ribavirin, *LVR* late virological response, *EVR* early virological response, *CI* confidence interval

Table 5 Relapse rates by PEG-IFNα-2b dose, sex, age, and the timing of HCV RNA negativity during treatment according to RBV dose

RBV average dose (mg/kg/day)	RBV ≥12	RBV 10 to <12	RBV 8 to <10	RBV 6 to <8	RBV <6	Cochran–Armitage trend test
PEG-IFNα-2b						
≥1.2 μg/kg/week	21.2 (14/66)	28.7 (43/150)	27.4 (29/106)	32.1 (26/81)	54.2 (52/96)	$P < 0.0001$
<1.2 μg/kg/week	25.0 (2/8)	26.1 (6/23)	25.0 (6/24)	28.0 (7/25)	64.1 (25/39)	$P = 0.0006$
Men	11.1 (4/36)	21.0 (21/100)	24.2 (15/62)	30.6 (15/49)	53.2 (33/62)	$P < 0.0001$
Women	32.4 (12/37)	39.4 (28/71)	29.4 (20/68)	30.9 (17/55)	60.3 (44/73)	$P = 0.0084$
Age						
<65 years	19.4 (12/62)	26.1 (37/142)	25.0 (27/108)	30.1 (22/73)	50.0 (49/98)	$P < 0.0001$
≥65 years	36.4 (4/11)	41.4 (12/29)	36.4 (8/22)	32.3 (10/31)	75.7 (28/37)	$P = 0.0037$
EVR	20.0 (13/65)	16.8 (22/131)	22.2 (24/108)	22.9 (19/83)	41.2 (35/85)	$P = 0.0006$
LVR	37.5 (3/8)	67.5 (27/40)	50.0 (11/22)	61.9 (13/21)	84.0 (42/50)	$P = 0.0088$

Data is shown as the percentile

PEG-IFN pegylated interferon, *HCV* hepatitis C virus, *RBV* ribavirin, *EVR* early virological response, *LVR* late virological response

virological relapse, with HCV genotype 1 patients having a higher relapse rate than HCV genotype 2 or 3 patients (23–30% of HCV genotype 1 patients will experience relapse [11, 17]); however, strategies for the management of relapse are poorly understood. Thus, it is important to research and promote treatment regimens that will insure that patients with the potential to relapse achieve an SVR; such regimens would also improve the cost-effectiveness of treatment and improve the quality of life of patients with chronic HCV infection who are treated with PEG-IFNα and RBV.

The treatment doses used in the present study were strongly associated with virological HCV relapse, with the RBV dose being a more important factor than the PEG-IFNα-2b dose. Our multivariate analyses revealed that while RBV had a dose-dependent correlation with the relapse rate, PEG-IFNα-2b did not. Reducing the dose of RBV to <6 mg/kg/day resulted in an increase in the relapse rate to 57.0% (mean relapse rate was 34.1%) regardless of the PEG-IFNα-2b dose. Shiffman et al. [13] reported that combination treatment of epoetin alpha with PEG-IFNα plus RBV to maintain the hemoglobin level did not enhance SVR or reduce the relapse rate in HCV genotype 1 patients. Thus, maintaining as high an RBV dose as possible throughout the full treatment period may suppress relapse by HCV genotype 1 patients.

Recently, a genome-wide association study identified that polymorphisms of the inosine triphosphatase (ITPA) gene on chromosome 20 influenced RBV-induced anemia [18]. Patients with the ITPA CC genotype at rs1127354 are more likely to develop anemia than those with ITPA CA/AA genotypes during PEG-IFNα and RBV combination treatment for chronic hepatitis C. In fact, Thompson et al. [19] reported that the ITPase deficiency variable (ITPA CA/AA) was associated with a lower rate of anemia-related RBV dose reduction during PEG-IFNα and RBV treatment. Therefore, ITPA genotyping may help guide clinical decisions in considering antiviral treatment.

Although RBV may play an important role in eradicating HCV, RBV does not have a direct antiviral action

against HCV. Immunomodulation exerted by RBV, which acts on CD4 T cells by promoting T-cell differentiation toward the T-helper 1 phenotype, is associated with the clearance of HCV [20], and the proportion of quasi-species defective viruses created due to mutation of the nonstructural 5A and 5B proteins of the HCV genome makes the virus unable to infect new cells [21, 22]. RBV may act synergistically with IFN by upregulating host antiviral proteins or by enhancing IFN signaling [23]. Shiffman et al. [24] suggested that reducing the mean dose of RBV during the first 20 weeks of treatment had little impact on relapse for patients with HCV genotype 1 and they noted that the magnitude of the decline in HCV RNA induced by RBV in the early stages was significantly smaller than that induced by IFN. In the early stages, IFN can induce a decline of several orders of magnitude, while RBV induces a less than 0.5 log decline in HCV RNA. Therefore, the virological effect induced by RBV pharmacokinetics may occur in the later stages.

We confirmed that LVR was a predictive factor for virological relapse, similar to the RBV dose. Based on the results of our study, 37.5% of the patients with an LVR relapsed even when given an average RBV dose of ≥12 mg/kg/day. Because it is not necessarily the case that an adequate RBV dose reduces the relapse rate of patients with an LVR, extending the duration of treatment from 48 to 72 weeks must be considered. Relapse rates in HCV genotype 1 slow responders were significantly lower for patients treated with PEG-IFNα-2a and RBV (800 mg/day) for 72 weeks compared with 48 weeks' treatment (40 vs. 64%; $P = 0.021$) [25]. We showed in the present study that the relapse rates of patients with an LVR were significantly higher than those of patients with an EVR for patients treated with an average RBV dose of less than 12 mg/kg/day. However, even for patients with an EVR, the relapse rate increased with a reduction of the RBV dose. Hiramatsu et al. [26] reported a relapse rate of only 4% in HCV genotype 1 patients with an EVR given ≥12 mg/kg/day of RBV, and a stepwise reduction of the RBV dose was associated with a stepwise increase in the relapse rate. Therefore, the RBV dose cannot be easily reduced for patients treated with PEG-IFN plus RBV, even if an adequate dose of PEG-IFNα-2b is given, particularly for patients with an LVR.

Treatment of chronic HCV infection will include the addition of direct-acting antivirals with protease inhibitors to PEG-IFNα-2b and RBV regimens. According to some clinical trials, telaprevir, which is an NS3/4A protease inhibitor, has shown promising results, when combined with PEG-IFNα-2b and RBV, in patients infected with HCV genotype 1 [27–29]. However, in telaprevir-based regimens, the addition of RBV increased the SVR rates by preventing relapse and the emergence of telaprevir resistance [29]; therefore, RBV remains a key drug to treat HCV infection.

Host factors, including age, sex, ethnicity, liver histology, and obesity, have been reported to be associated with the outcome of PEG-IFNα and RBV treatment [30–33]. In women and elderly patients who received an adequate dose of RBV, the relapse rates were higher than those in men and younger patients. The mechanisms underlying this are unknown, but indirect evidence (e.g., cellular impairment or impairment of humoral or innate immunity in elderly persons, or a lack of estrogen in elderly women) suggests that chronic infection is associated with phenomena that protect HCV from the antiviral action of IFN and RBV [3, 34, 35].

As mentioned above, the most important result of the present study was that, in patients treated with PEG-IFNα-2b and RBV, relapse after the clearance of HCV RNA was associated with the RBV dose but not with the PEG-IFNα-2b dose. Recently, genetic studies have identified several single nucleotide polymorphisms in and near the interleukin-28B gene region, encoding IFN-λ3, that are correlated with HCV clearance [36–38]. IFN-λ3 upregulates IFN-stimulated genes and affects the adaptive immune response. Fortunately, East Asian populations, including Japanese, have the highest frequencies of the alleles associated with HCV clearance [37], so the PEG-IFNα dose probably did not influence HCV virological relapse to a great degree in our patients.

In conclusion, in HCV genotype 1 patients treated with PEG-IFNα-2b and RBV, the HCV virological relapse rate was dose-dependently correlated with RBV, irrespective of age, sex, the effect of early viral kinetics, or the dose of PEG-IFNα-2b. Careful consideration must be given to decisions on the tapering of the RBV dose, even if an EVR is achieved.

Acknowledgments We greatly thank Drs. Masayuki Murata, Mosaburo Kainuma, Kyoko Okada, Hiroaki Taniai, Kazuhiro Toyoda, Haru Mukae, Tsunehisa Koga, Hiroaki Ikezaki, Takeshi Ihara, and Takeo Hayashi of our department for their clinical and research assistance with the present study.

Conflict of interest None declared.

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