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Original Article

Factors responsible for the discrepancy between *IL28B* polymorphism prediction and the viral response to peginterferon plus ribavirin therapy in Japanese chronic hepatitis C patients

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Aim: IL28B polymorphisms serve to predict response to pegylated interferon plus ribavirin therapy (PEG IFN/RBV) in Japanese patients with chronic hepatitis C (CHC) very reliably. However, the prediction by the *IL28B* polymorphism contradicted the virological response to PEG IFN/RBV in some patients. Here, we aimed to investigate the factors responsible for the discrepancy between the *IL28B* polymorphism prediction and virological responses.

Methods: CHC patients with genotype 1b and high viral load were enrolled in this study. In a case–control study, clinical and virological factors were analyzed for 130 patients with rs8099917 TT genotype and 96 patients with rs8099917 TG or GG genotype who were matched according to sex, age, hemoglobin level and platelet count.

Results: Higher low-density lipoprotein (LDL) cholesterol, lower γ-glutamyltransferase and the percentage of wild-type phenotype at amino acids 70 and 91 were significantly associated with the rs8099917 TT genotype. Multivariate analysis showed that rs8099917 TG or GG genotype, older age and lower LDL cholesterol were independently associated with the non-virological responder (NVR) phenotype. In patients with rs8099917 TT genotype (predicted as virological responder [VR]), multivariate analysis showed that older age was independently associated with NVR. In patients with rs8099917 TG or GG genotype (predicted as NVR), multivariate analysis showed that younger age was independently associated with VR.

Conclusion: Patient age gave rise to the discrepancy between the prediction by *IL28B* polymorphism and the virological responses, suggesting that patients should be treated at a younger age.

Key words: aging, genotype, IL28B, low-density lipoprotein cholesterol, single nucleotide polymorphism

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a global health problem with worldwide estimates of 120–130 million carriers.[1] Chronic HCV infection, the leading cause of liver transplantation, can lead to progressive liver disease, resulting in cirrhosis and complications, including decompensated liver disease and hepatocellular carcinoma.[2] The current standard-of-care treatment for suitable patients with chronic HCV infection consists of pegylated interferon-α-2a or -2b (PEG IFN) given by injection in combination with oral ribavirin (RBV) for 24 or 48 weeks, depending on HCV genotype. Large-scale treatment in the USA and Europe showed that 42–52% of patients with HCV genotype 1

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achieved a sustained virological response (SVR),[3–5] and studies conducted in Japan produced similar results. This treatment is associated with well-known side-effects (e.g. influenza-like syndrome, hematological abnormalities and neuropsychiatric events) resulting in reduced compliance and fewer patients completing treatment.[6] It is important to predict an individual's response before treatment with PEG IFN/RBV to avoid side-effects, as well as to reduce the treatment cost. The HCV genotype, in particular, is used to predict the response: patients with the HCV genotype 2/3 have a relatively high rate of SVR (70–80%) with 24 weeks of treatment, whereas those infected with genotype 1 have a much lower rate of SVR, despite 48 weeks of treatment.[5]

Our recent genome-wide association studies (GWAS) revealed that several highly correlated common single nucleotide polymorphisms (SNP) in the region of the interleukin-28B (*IL28B*) gene on chromosome 19, coding for interferon (IFN)-λ3, are implicated in the non-virological responder (NVR) to PEG IFN/RBV phenotype among patients infected by HCV genotype 1.[7] The association between response to PEG IFN/RBV and SNP associated with *IL28B* was concurrently reported by two other groups who also employed GWAS.[8,9] The *IL28B* polymorphism was highly predictive of the response to PEG IFN/RBV therapy in Japanese chronic hepatitis C (CHC) patients.[10–12] However, this was not always the case. Therefore, we attempted to determine why the *IL28B* polymorphism did not predict the response of all patients. The nature of the functional link between the *IL28B* polymorphism and HCV clearance is unknown, and this must be defined to understand how the *IL28B* polymorphism correlates with HCV clearance. Therefore, we also investigated the association between the *IL28B* polymorphism and clinical characteristics of CHC patients.

METHODS

Patients

A TOTAL OF 696 CHC patients with genotype 1b and high viral load were recruited from the National Center for Global Health and Medicine, Hokkaido University Hospital, Tokyo Medical and Dental University Hospital, Yamanashi University Hospital, Tonami General Hospital, and Shin-Kokura Hospital in Japan. In a case–control study, sex, age, hemoglobin level and platelet count were matched between patients with the rs8099917 TT genotype ($n = 130$) and patients with rs8099917 TG or GG genotypes ($n = 96$) to eliminate background biases.

Each patient was treated with PEG IFN-α-2b (1.5 μg/kg s.c. weekly) or PEG IFN-α-2a (180 μg/body s.c. weekly) plus RBV (600–1000 mg daily, depending on bodyweight). Because a reduction in the dose of PEG IFN/RBV can contribute to a lower SVR rate,[13] only patients with an adherence of more than 80% dose for both drugs during the first 12 weeks were included in this study. Those positive for hepatitis B surface antigen and/or anti-HIV were excluded from this study.

Non-virological response was defined as less than a 2 log-unit decline in the serum level of HCV RNA from the pretreatment baseline value within the first 12 weeks and detectable viremia 24 weeks after treatment. Virological response (VR) was defined as attaining SVR or transient virological response (TVR) in this study; SVR was defined as undetectable HCV RNA in serum 6 months after treatment, whereas TVR was defined as a reappearance of HCV RNA in serum after the treatment was discontinued for a patient who had undetectable HCV RNA during the therapy or on completion of the therapy. At the time of enrollment, written informed consent was obtained for the collection and storage of serum and peripheral blood. This study was conducted in accordance with provisions of the Declaration of Helsinki.

Clinical and laboratory data

The sex, age, hemoglobin (Hb) and platelet counts were matched between study groups. Other parameters determined were as follows: alkaline phosphatase (ALP), alanine transaminase (ALT), total cholesterol, fasting blood sugar (FBS), low-density lipoprotein (LDL) cholesterol, γ-glutamyl transpeptidase (γ-GTP), α-fetoprotein (AFP), HCV RNA level and the rs8099917 polymorphism near *IL28B*.

DNA extraction

Genomic DNA was extracted from the buffy coat fraction of patients' whole blood using a GENOMIX kit (Talent SRL; Trieste, Italy).

IL28B genotyping

We have reported that the rs8099917 polymorphism is the best predictor for the response of Japanese CHC patients to PEG IFN/RBV therapy than other SNP near *IL28B*.[14] Therefore, the rs8099917 polymorphism was genotyped using the InvaderPlus assay (Third Wave Japan, Tokyo, Japan), which combines polymerase

chain reaction (PCR) and the invader reaction.[15,16] The InvaderPlus assay was performed using the LightCycler LC480 (Roche Applied Science, Mannheim, Germany).

Detection of amino acid substitutions in core and NS5A regions of HCV-1b

In the present study, substitutions of amino acid residues 70 (s-aa 70) and 91 (s-aa 91), and the presence of the IFN sensitivity-determining region (ISDR) were determined by direct nucleotide sequencing. HCV RNA was extracted from serum samples at the start of patients' therapy and reverse transcribed with a random primer and SuperScript III reverse transcriptase (Life Technologies, Carlsbad, CA, USA). Nucleic acids were amplified by PCR as described.[17]

Statistical analysis

Quantitative variables were expressed as the mean ± standard error (SE) unless otherwise specified. Categorical variables were compared using a χ^2-test or Fisher's exact test, as appropriate, and continuous variables were compared using the Mann–Whitney *U*-test. $P < 0.05$ was considered statistically significant. Multivariate analysis was performed using a stepwise logistic regression model. We performed statistical analyses using STATA ver. 11.0 (StataCorp, College Station, TX, USA).

RESULTS

Patient characteristics and *IL28B* genotype in a matched case–control study

TABLE 1 SHOWS PATIENT characteristics according to *IL28B* genotype. In a matched case–control study, sex, age, Hb levels and platelet counts were matched between 130 patients with rs8099917 TT genotype and 96 patients with rs8099917 TG or GG genotype. Lower γ-GTP ($P = 0.013$) and higher LDL cholesterol levels ($P < 0.001$) were significantly associated with the TT genotype of rs8099917. The percentages of wild type of s-aa 70 and s-aa 91 of patients with the rs8099917 TT genotype were significantly higher than those of patients with rs8099917 TG or GG genotype (s-aa 70: TT vs TG + GG, 68% vs 37% [$P < 0.001$]; s-aa 91: TT vs TG + GG, 68% vs 51% [$P = 0.017$]).

Factors associated with NVR in total patients

Table 2 shows the factors associated with NVR by univariate and multivariate analyses. Univariate analysis showed that older age ($P = 0.002$), lower platelet counts ($P = 0.01$), higher γ-GTP ($P = 0.013$), lower total cholesterol ($P = 0.017$), lower LDL cholesterol ($P < 0.001$) levels and higher AFP levels ($P = 0.019$) were significantly associated with NVR. The percentage of TG or GG genotype of rs8099917 of patients with NVR was

Table 1 Univariate analysis of *IL28B* TT and TG + GG genotypes

Variable	TT genotype ($n = 130$)	TG + GG genotype ($n = 96$)	*P*-value
Sex (% male)	61 (47)	46 (48)	Matched
Age (years), mean (SE)	57.2 (0.8)	57.5 (0.9)	Matched
Hemoglobin (g/dL), mean (SE)	14.3 (0.3)	13.9 (0.2)	Matched
Platelet count (/μL), mean (SE)	16.2 (0.5)	16.0 (0.5)	Matched
ALT (IU/L), mean (SE)	79.4 (5.4)	80.5 (7.8)	0.281
ALP (IU/L), mean (SE)	273.8 (11.7)	283.9 (11.8)	0.313
γ-GTP (IU/L), mean (SE)	63.4 (6.0)	76.0 (6.4)	0.013
Total cholesterol (mg/dL), mean (SE)	177.5 (3.3)	172.3 (3.2)	0.345
LDL cholesterol (mg/dL), mean (SE)	99.0 (2.6)	83.5 (2.8)	<0.001
Fasting blood sugar (mg/dL), mean (SE)	114.1 (4.1)	104.4 (1.9)	0.97
AFP (ng/dL), mean (SE)	9.8 (1.1)	11.5 (1.6)	0.190
HCV RNA (log IU), mean (SE)	6.2 (0.1)	6.1 (0.1)	0.186
s-aa 70 wild type (%)	70/103 (68)	30/81 (37)	<0.001
s-aa 91 wild type (%)	70/103 (68)	41/81 (51)	0.017
ISDR mutation 0–1 point (%)	82/100 (82)	70/81 (86)	0.42

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; SE, standard error.

Table 2 Univariate and multivariate analyses of patients with chronic hepatitis C treated with PEG IFN/RBV with respect to VR and NVR

Variable	Univariate analysis			Multivariate analysis	
	VR ($n = 128$)	NVR ($n = 98$)	*P*-value	OR (95% CI)	*P*-value
Sex (% male)	65 (51)	42 (43)	0.237		
Age (years), mean (SE)	55.6 (0.8)	59.6 (0.9)	0.002	1.075 (1.012–1.143)	0.02
rs8099917 (TG or GG genotype) (%)	23/128 (18)	73/98 (74)	<0.001	25.460 (7.436–87.169)	<0.001
Hemoglobin (g/dL), mean (SE)	14.4 (0.3)	13.7 (0.2)	0.053		
Platelet count (/μL), mean (SE)	16.9 (0.5)	15.0 (0.5)	0.01		
ALT (IU/L), mean (SE)	83.9 (6.4)	74.5 (6.2)	0.116		
ALP (IU/L), mean (SE)	274.1 (12.3)	282.9 (11.2)	0.169		
γ-GTP (IU/L), mean (SE)	65.9 (6.4)	72.6 (5.6)	0.013		
Total cholesterol (mg/dL), mean (SE)	180.3 (3.1)	168.4 (3.5)	0.017		
LDL cholesterol (mg/dL), mean (SE)	100.5 (2.7)	83.5 (2.8)	<0.001	0.978 (0.956–0.999)	0.046
Fasting blood sugar (mg/dL), mean (SE)	106.6 (2.9)	114.8 (4.4)	0.058		
AFP (ng/dL), mean (SE)	9.6 (1.1)	12.0 (1.6)	0.021		
HCV RNA (Log IU), mean (SE)	6.2 (0.1)	6.2 (0.1)	0.876		
s-aa 70 wild type (%)	67/102 (66)	33/82 (54)	0.001		
s-aa 91 wild type (%)	67/102 (66)	44/82 (54)	0.097		
ISDR mutation 0–1 point (%)	79/96 (82)	73/85 (86)	0.511		

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; NVR, non-virological response; OR, odds ratio; PEG IFN, peginterferon; SE, standard error; RBV, ribavirin; VR, virological response.

significantly higher than that of patients with VR (VR vs NVR: 23/128 [18%] vs 73/98 [74%], $P < 0.001$). The percentage of wild-type s-aa 70 in patients with NVR was significantly lower than that in patients with VR [VR vs NVR: 67/102 [66%] vs 33/82 [54%], $P = 0.001$]. Multivariate analysis showed that older age (odds ratio [OR] = 1.075; 95% confidence interval [CI] = 1.012–1.14; $P = 0.02$), TG or GG genotype of rs8099917 (OR = 25.460; 95% CI = 7.436–87.169; $P < 0.001$) and lower LDL cholesterol levels (OR = 0.978; 95% CI = 0.956–0.999; $P = 0.046$) were independently associated with NVR.

VR to treatment depending on *IL28B* genotype

In the patients with the rs8099917 TT genotype, the rates of SVR, TVR and NVR were 62%, 19% and 19%, respectively. Therefore, 19% patients were NVR, even though rs8099917 represents the TT genotype (predicted as VR). In contrast, in the patients with rs8099917 TG or GG, the rates of SVR, TVR and NVR were 14%, 10% and 76%, respectively. Therefore, 24% patients were VR, even though rs8099917 was TG or GG genotype (predicted as NVR) (Fig. 1).

Factors associated with NVR in patients with the rs8099917 TT genotype

Table 3 shows the factors associated with NVR in patients with the rs8099917 TT genotype (predicted as VR) by univariate and multivariate analyses. Univariate analysis showed that female sex ($P = 0.003$), older age

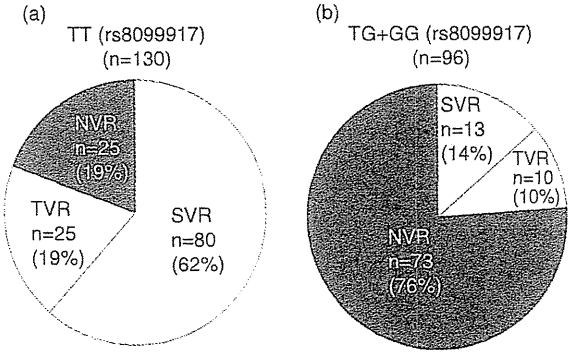

Figure 1 Virological responses to pegylated interferon and ribavirin therapy were shown in patients with rs8099917 TT (a) and TG + GG (b). NVR, non-virological response; SVR, sustained virological response; TVR, transient virological response.

Table 3 Variables associated with NVR by univariate and multivariate analyses in patients with rs8099917 TT genotype

Variable	Univariate analysis			Multivariate analysis	
	VR ($n = 105$)	NVR ($n = 25$)	P-value	OR (95% CI)	P-value
Sex (% male)	56 (53)	5 (20)	0.003		
Age (years), mean (SE)	56.1 (0.8)	61.7 (1.6)	0.001	1.142 (1.026–1.271)	0.015
Hemoglobin (g/dL), mean (SE)	14.6 (0.4)	13.1 (0.3)	0.005		
Platelet count (/μL), mean (SE)	16.7 (0.6)	13.8 (1.0)	0.019		
ALT (IU/L), mean (SE)	83.6 (6.3)	61.0 (7.9)	0.053		
ALP (IU/L), mean (SE)	270.6 (13.6)	285.9 (22.3)	0.206		
γ-GTP (IU/L), mean (SE)	66.9 (7.1)	49.2 (7.4)	0.473		
Total cholesterol (mg/dL), mean (SE)	180.2 (3.6)	165.0 (7.6)	0.072		
LDL cholesterol (mg/dL), mean (SE)	101.2 (2.9)	88.5 (5.2)	0.067		
Fasting blood sugar (mg/dL), mean (SE)	108.4 (3.5)	140.0 (15.5)	0.127		
AFP (ng/dL), mean (SE)	9.4 (1.2)	12.2 (3.6)	0.245		
HCV RNA (log IU), mean (SE)	6.2 (0.1)	6.2 (0.1)	0.948		
s-aa 70 wild type (%)	57/83 (66)	13/20 (75)	0.752		
s-aa 91 wild type (%)	55/83 (66)	15/20 (75)	0.452		
ISDR mutation 0–1 point (%)	64/79 (81)	18/21 (86)	0.618		

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; NVR, non-virological response; OR, odds ratio; SE, standard error; VR, virological response.

($P = 0.001$), lower Hb levels ($P = 0.005$) and lower platelet counts ($P = 0.019$) were significantly associated with NVR in patients with the rs8099917 TT genotype. Multivariate analysis showed that only older age was independently associated with NVR in patients with the rs8099917 TT genotype (predicted as VR) (OR = 1.142; 95% CI = 1.026–1.27; $P = 0.015$).

Factors associated with VR in patients with the rs8099917 TG or GG genotypes

Table 4 shows the factors associated with VR in patients with the rs8099917 TG or GG genotypes (predicted as NVR) by univariate and multivariate analyses. Younger age ($P = 0.005$), lower γ-GTP ($P = 0.009$) and higher LDL cholesterol levels ($P = 0.032$) were significantly associated with VR by univariate analysis. Multivariate analysis showed that only younger age was independently associated with VR in patients with the rs8099917 TG or GG genotype (predicted as NVR) (OR = 0.926; 95% CI = 0.867–0.990; $P = 0.023$).

Rate of VR depending on the rs8099917 genotype of each age group

We divided patients into four age groups and compared VR rates by the differences in rs8099917 genotype for each group. The rate of VR decreased gradually in the older age groups independent of genotype. In the less than 49 years age group, the rate of VR in patients with the rs8099917 TT genotype was significantly higher than that in patients with the rs8099917 TG + GG genotypes ($P = 0.0002$). Further, in the 50–59 and 60–69 years age groups, the rates of VR in patients with the rs8099917 TT genotype were significantly higher than those in patients with the rs8099917 TG + GG genotypes ($P < 0.0001$, respectively). In the group that included subjects aged older than 69 years, only 50% of patients achieved VR even in those with the rs8099917 TT genotype (predicted as VR). In contrast, 47.6% of patients achieved VR, including those with the rs8099917 TG or GG genotypes (predicted as NVR) in the less than 49 years group (Fig. 2).

DISCUSSION

SINGLE NUCLEOTIDE POLYMORPHISM array analysis employing GWAS technology conducted by our laboratory and others revealed the relationships between SNP associated with the *IL28B* locus or present within the coding sequences for IFN-λ3, or the response to PEG IFN/RBV therapy for CHC.[7–9] Subsequent studies have confirmed that the response to PEG IFN/RBV therapy correlates with the SNP associated with *IL28B*[18,19] and indicates their value for predicting the response to PEG IFN/RBV therapy. Unfortunately, these predictions do not hold for some patients. In an attempt to understand the reasons for this, in the present study,

Table 4 Variables associated with VR by univariate and multivariate analyses in patients with rs8099917 TG or GG genotypes

Variable	Univariate analysis			Multivariate analysis	
	VR ($n = 23$)	NVR ($n = 73$)	*P*-value	OR (95% CI)	*P*-value
Sex (% male)	9 (40%)	37 (51%)	0.333		
Age (years), mean (SE)	53.2 (1.7)	58.8 (1.1)	0.005	0.926 (0.867–0.990)	0.023
Hemoglobin (g/dL), mean (SE)	13.6 (0.3)	13.9 (0.2)	0.44		
Platelet count (/μL), mean (SE)	17.6 (1.1)	15.5 (0.6)	0.059		
ALT (IU/L), mean (SE)	85.5 (21.6)	78.9 (7.8)	0.767		
ALP (IU/L), mean (SE)	291.9 (28.6)	281.8 (13.0)	0.921		
γ-GTP (IU/L), mean (SE)	62.2 (15.1)	80.4 (6.9)	0.009		
Total cholesterol (mg/dL), mean (SE)	180.5 (6.2)	169.5 (3.7)	0.17		
LDL cholesterol (mg/dL), mean (SE)	97.6 (6.9)	81.9 (3.6)	0.032		
Fasting blood sugar (mg/dL), mean (SE)	98.1 (2.8)	106.3 (2.3)	0.084		
AFP (ng/dL), mean (SE)	10.3 (3.4)	11.9 (1.8)	0.123		
HCV RNA (log IU), mean (SE)	5.9 (0.1)	6.2 (0.1)	0.087		
s-aa 70 wild type (%)	10/19 (53)	20/62 (32)	0.108		
s-aa 91 wild type (%)	12/19 (63)	29/62 (47)	0.211		
ISDR mutation 0–1 point (%)	15/17 (88)	55/64 (86)	0.806		

AFP, α-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; LDL, low-density lipoprotein; NVR, non-virological response; OR, odds ratio; SE, standard error; VR, virological response.

we recruited a new set of patients for further analysis. Here, we confirmed that *IL28B* polymorphism was the most significant predictive factor for NVR with respect to PEG IFN/RBV treatment. Moreover, 19% of patients exhibiting the rs8099917 TT genotype were NVR, although they were predicted as VR. Twenty-four percent of patients with the rs8099917 TG or GG genotypes were VR, although they were predicted as NVR. We were able to determine by multivariate analysis that age was the most likely factor responsible for the discordance

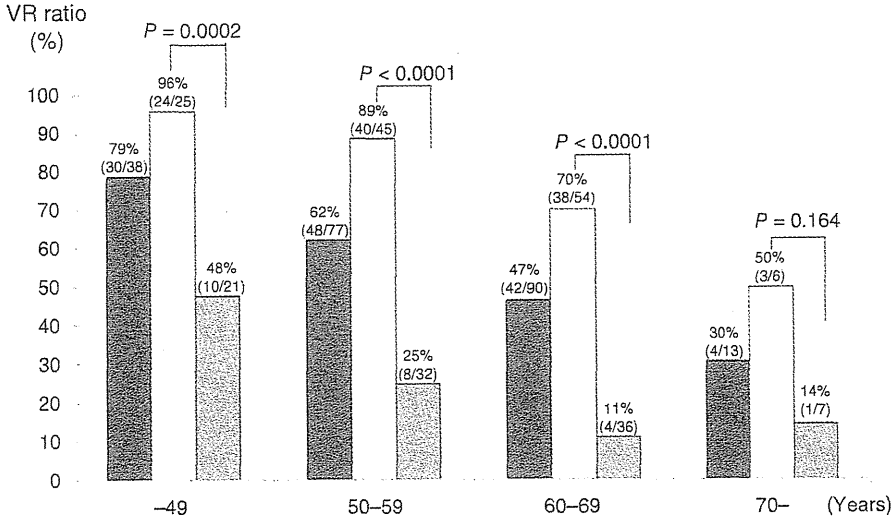

Figure 2 Virological responses (VR) to pegylated interferon and ribavirin therapy were compared between the patients with rs8099917 TT and TG + GG in each generation group. (■) Total patients, (□) TT genotype (rs8099917), (▨) TG + GG genotype (rs8099917).

between *IL28B* genotype and patients' response to viral infection.

How does age influence the VR to PEG IFN/RBV therapy? First, the lower rate of VR to PEG IFN/RBV therapy in patients with CHC was attributed to lower compliance with the IFN or RBV dose.[20,21] Because lower compliance with PEG IFN or RBV therapy was expected to be associated with a lower rate of VR in older patients, we recruited patients who were administrated over 80% of the prescribed dose of IFN/RBV. Therefore, lower compliance can be discounted as a reason for reduced response. Second, a more advanced stage of fibrosis might have been present in the older group. Platelet counts in patients with NVR were significantly lower than those in patients with VR, and lower platelet counts may be associated with advanced fibrosis.[22] Moreover, advanced fibrosis is associated with lower rates of SVR to IFN-based therapy.[23] Third, epigenetic factors such as DNA methylation induced by aging may be involved in the reduced efficacy of PEG IFN/RBV treatment in older patients. DNA methylation near gene promoters is known to turn off transcription or reduce it considerably,[24] and advanced age is strongly associated with the increased DNA methylation.[25] Therefore, DNA methylation may be increased near or in the *IL28B* promoter as a function of age resulting in suppression of *IL28B* transcription.

Lower LDL cholesterol levels were significantly associated with NVR in patients with CHC. Moreover, LDL cholesterol levels in patients with the rs8099917 TT genotype were significantly higher than those in patients with the TG + GG genotypes. The association between LDL cholesterol and *IL28B* polymorphism as well as the VR to PEG IFN/RBV has been reported.[26] Higher pretreatment levels of LDL cholesterol have been shown to predict increased response to standard PEG IFN/RBV treatment for patients with CHC.[27,28] Although the mechanisms responsible for the association between LDL cholesterol levels and the VR to PEG IFN/RBV are unknown, the *IL28B*-rs8099917 TT responder genotype, which may correlate with an increased likelihood of treatment response and higher LDL cholesterol levels, is associated with either lower IFN-λ3 activity or reduced expression of genes regulated by IFN-mediated signaling pathways.

In conclusion, our studies provide compelling evidence that patient age is most likely responsible for incorrect predictions of VR to PEG IFN/RBV therapy in Japanese CHC patients based on *IL28B* genotypes. Our findings indicated that patients should be treated as soon as they are diagnosed. It will be important to investigate the role of the epigenetic factors associated with *IL28B* expression to develop more effective PEG IFN/RBV-based therapies for patients with CHC.

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HEPATOLOGY

Evaluation of the adverse effect of premature discontinuation of pegylated interferon α-2b and ribavirin treatment for chronic hepatitis C virus infection: Results from Kyushu University Liver Disease Study

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Conflict of interest
None declared.

Abstract

Background and Aims: Pegylated interferon (PEG-IFN) α-2b and ribavirin (RBV) treatment of chronic hepatitis C virus (HCV) infection is associated with a substantially elevated risk of discontinuation. The aim of this study is to evaluate the reason for premature discontinuation during PEG-IFN α-2b and RBV treatment due to adverse effects in patients with chronic HCV infection.

Methods: A total of 2871 Japanese patients who had chronic HCV infection treated with PEG-IFN α-2b and RBV were screened. We prospectively investigated the reasons for premature discontinuation of treatment classified by sex and age, and analyzed the timing of discontinuation.

Results: Of the 2871 patients, 250 (8.7%) discontinued treatment because of adverse effects. The main reasons for premature discontinuation were neurovegetative symptoms ($n = 77$, 30.8%), depression-related syndrome ($n = 46$, 18.4%), hematologic effects ($n = 41$, 16.4%) and dermatologic effects ($n = 27$, 10.8%). The rate of discontinuation of treatment for patients aged ≥ 65 years was significantly higher than for patients aged < 65 years, for both men ($P < 0.0001$) and women ($P = 0.0121$). Moreover, the frequency of discontinuation due to neurovegetative symptoms, depression-related syndrome, and hematologic effects for men aged ≥ 65 years was significantly higher than for those aged < 65 years ($P = 0.0001$, $P = 0.0016$, and $P = 0.0170$, respectively), but not for women.

Conclusion: Premature discontinuation due to the adverse effects of PEG-IFN α-2b and RBV treatment by patients with chronic HCV infection is mainly due to neuropsychiatric symptoms and is more common for older than for younger patients.

Introduction

Hepatitis C virus (HCV) infection is the main cause of chronic liver disease and is reported to have infected approximately 170 million people worldwide. Most patients with chronic HCV infection may achieve normal life expectancy, but about 30% develop the life-threatening complications of end-stage liver disease, including cirrhosis and hepatocellular carcinoma.[1,2] Antiviral treatment with interferon (IFN) for chronic HCV infection can induce viral clearance and biochemical and histological improvement.[3–5]

Although antiviral treatment has steadily improved over the last decades, the rates of sustained virological response (SVR) were only 40–50% for patients treated with pegylated IFN (PEG-IFN) α and ribavirin (RBV) for chronic hepatitis infected with HCV genotype 1.[6,7] SVR depends mainly on factors such as viral (HCV genotype, the HCV core 70[8] and NS5A interferon sensitivity-determining regions[9]), host factors (polymorphisms in the

interleukin 28B region[8,9]), and the early viral kinetics of antiviral treatment.[10,11] One of the reasons for the low SVR rates is the high frequency of adverse events related to PEG-IFN α and RBV treatment;[12] therefore, the adherence to antiviral treatment would have a favorable effect on the SVR rate. In fact, patients who discontinued PEG-IFN α and RBV treatment prematurely for whatever reason had an SVR rate of 12% compared with 65% of those who continued treatment despite dose reduction.[13]

Laboratory abnormalities, such as neutropenia, anemia and thrombocytopenia, are the most frequent side-effects of PEG-IFN α and RBV treatment. Dose reductions of either PEG-IFN α and/or RBV for mainly laboratory abnormalities were required by 32–42% of patients in one study; however, the rate of premature discontinuation due to laboratory abnormalities was only 2–3%.[3,7] Although the most common adverse events of PEG-IFN α and RBV treatment were fatigue (64%), headache (62%) and injection-site reaction (58%) in another study,[14] there have been few large-scale reports of the correlation between adverse effects and the premature discontinuation of PEG-IFN α and RBV treatment.

The aim of this large-scale, prospective study was to assess the reasons for and frequency of premature discontinuation of PEG-IFN α-2b and RBV treatment of Japanese patients with chronic HCV infection due to adverse effects.

Methods

Patients. This prospective study was of 2871 Japanese patients with chronic HCV infection aged 18 years or older treated with PEG-IFN α-2b and RBV between December 2004 and February 2009. The number with HCV genotype 1 was 2018 (70.3%, median age: 59 years), of whom 1066 (52.8%) were men. The number with HCV genotype 2 was 853 (29.7%, median age: 54 years), of whom 430 (50.4%) were men. Exclusion criteria were as follows: (i) positivity for antibody to HIV or positivity for hepatitis B surface antigen; (ii) clinical or biochemical evidence of hepatic decompensation; (iii) excessive active alcohol consumption (> 60 g/day converted into ethanol) or drug abuse; (iv) suspected hepatocellular carcinoma at entry; (v) other forms of liver disease (e.g. autoimmune hepatitis, alcoholic liver disease, hemochromatosis); and (vi) treatment with antiviral or immunosuppressive agents prior to enrollment. A total of 2871 patients were recruited at Kyushu University Hospital and 32 affiliated hospitals in the northern Kyushu area of Japan.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of each participating hospital. Informed consent was obtained from all patients before enrollment.

Clinical and laboratory assessment. Clinical parameters included serum albumin, alanine aminotransferase (ALT), γ-glutamyl-transpeptidase, creatinine clearance (Ccr), total cholesterol, hemoglobin (Hb), complete platelet counts, HCV genotype and HCV RNA. All were measured by standard laboratory techniques at a commercial laboratory. Body mass index (BMI) was calculated as weight in kilograms/height in square meters.

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 assay (COBAS TaqMan HCV assay; Roche Diagnostics, Tokyo, Japan), with a lower limit of quantitation of 15 IU/mL and an outer limit of quantitation of 6.9×10^7 IU/mL (1.2–7.8 log IU/mL referred to $\log_{10}$ units/mL). HCV genotype determination was performed by means of sequence determination in the 5′-nonstructual (NS) region of the HCV genome followed by phylogenetic analysis, as previously described.[15]

Therapeutic protocol. All patients received a combination treatment of PEG-IFN α-2b (PEG-Intron; MSD, Tokyo, Japan) plus RBV (Rebetol; MSD). The length of treatment was 48 weeks for HCV genotype 1 and 24 weeks for HCV genotype 2. PEG-IFN α-2b was administered subcutaneously once weekly at a dose of 60–150 μg based on bodyweight (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 600–1000 mg based on bodyweight (600 mg for patients weighing < 60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing > 80 kg). The above dosages and duration are those approved by the Japanese Ministry of Health, Labor and Welfare.

In the event of a serious adverse effect developing during the course of treatment, we modified the dosage of PEG-IFN α-2b and RBV until the adverse event abated or decreased in severity. Patients were considered to have RBV-induced anemia if the Hb level decreased to < 100 g/L. In such cases, a reduction in the dose of RBV was required. Some patients also had PEG-IFN α-2b-induced psychological adverse effects or a decrease of white blood cell and platelet count. In such cases, a reduction in the dosage of PEG-IFN α-2b was required.

Discontinuation of PEG-IFN α-2b and RBV treatment. Both PEG-IFN α-2b and RBV were discontinued if the Hb level, white blood cell count, or platelet count fell below 85 g/L, 1×10^9 /L, or 25×10^9 /L, respectively. The treatment was discontinued if severe general fatigue, severe neuropsychiatric symptoms, uncontrolled thyroid disease, interstitial pneumonia, progressive IFN retinopathy, the onset of carcinoma, severe hematologic problems developed, continuation of treatment was judged not to be possible by the attending physician, or the patient desired discontinuation of treatment.

Virological response. COBAS TaqMan HCV assay was used to evaluate HCV viremia as a surrogate marker of virological outcome to 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 of patients who had undetectable HCV RNA at the end of treatment.

Definition of neuropsychiatric symptoms and the assessment of psychiatric problems. Neuropsychiatric symptoms included two distinct dimensions; a neurovegetative symptom and a depression-related syndrome.[16] The neurovegetative symptoms were reduced energy, anorexia, and psychomotor retardation, while depression-related syndromes were worsening

mood, anxiety, suicidal ideation, and aggressive behavior towards others. All patients were seen by hepatologists and psychiatrists at least weekly but as often as necessary during the first 8 weeks and then once a month. Mental status was continuously, carefully monitored. Major depressive episodes during PEG-IFN α-2b and RBV treatment were diagnosed by clinical assessment according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition.[17]

Statistical analysis. Statistical analysis was performed using SAS ver. 9.2 (SAS Institute, Cary, NC, USA). Quantitative variables were expressed as median and interquartile range. The paired *t*-test, unpaired *t*-test, Mann–Whitney *U*-test, χ^2-test or the Kruskal–Wallis test were used where appropriate. A *P*-value less than 0.05 was regarded as statistically significant.

Results

Rates of premature discontinuation of PEG-IFN α-2b and RBV treatment (Table 1). Of the 2871 patients screened, 551 (19.2%) had PEG-IFN α-2b and RBV treatment discontinued during the treatment period. The discontinuation rate of patients with HCV genotype 1 (209 of 2010, 10.4%) due to adverse effects was more than double that of those with HCV genotype 2 (41 of 861, 4.8%) ($P < 0.0001$), possibly due to the difference of the treatment period. Of the 551, 250 (45.4%) had treatment discontinued because of adverse effects and 301 (54.6%) discontinued for other reasons (e.g. non-virological response, the onset of carcinoma, economic reasons, or dropout).

The rate of premature discontinuation of treatment due to adverse effects was significantly higher for patients aged ≥ 65 years than for those aged < 65 years, both for men (14.4% *vs* 7.3%, $P < 0.0001$) and women (11.2% *vs* 7.0%, $P = 0.0121$). Although the rates of premature discontinuation of treatment were not significantly different for patients aged ≥ 65 years and those < 65 years with HCV genotype 2, for both men and women, the discontinuation rates of the group of patients aged ≥ 65 years with HCV genotype 1 was significantly higher than that of the group aged < 65 years, both for men (16.9% *vs* 8.3%, $P < 0.0001$) and women (13.6% *vs* 8.4%, $P = 0.0143$).

Demographic characteristics of the studied 250 patients who discontinued PEG-IFN α-2b and RBV treatment prematurely (Table 2). Demographic characteristics of the 209 patients with HCV genotype 1 and the 41 with HCV genotype 2 who discontinued the combined treatment are compared in Table 2. They all discontinued PEG-IFN α-2b and RBV treatment prematurely because of adverse effects during the treatment period. The age was significantly higher ($P = 0.0002$) and Ccr and platelet count were lower ($P = 0.0003$ and $P = 0.0139$, respectively) for patients with HCV genotype 1 than for those with genotype 2. This was due to the age difference between HCV genotype 1 (median 59 years) and 2 (median 54 years) ($P < 0.0001$) patients at entry to the study.

Breakdown of the reasons for premature discontinuation of PEG-IFN α-2b and RBV treatment (Table 3). The reasons for premature discontinuation of PEG-IFN α-2b and RBV treatment included neurovegetative symptoms ($n = 77$, 30.8%), depression-related syndrome ($n = 46$, 18.4%), hematologic effects ($n = 41$, 16.4%), dermatologic effects ($n = 27$, 10.8%), thyroid disease ($n = 10$, 4.0%), pulmonary disease (including interstitial pneumonia and tuberculosis) ($n = 10$, 4.0%), IFN-induced retinopathy ($n = 6$, 2.4%), autoimmune disease ($n = 4$, 1.6%), elevation of ALT (over 10 times the upper limit of the normal range) ($n = 2$, 0.8%), cerebral vascular disease ($n = 2$, 0.8%), diabetes mellitus type 1 ($n = 2$, 0.8%) and others ($n = 23$, 9.2%).

For HCV genotype 1 ($n = 209$), the reasons were neurovegetative symptoms ($n = 69$, 33.0%), depression-related syndrome ($n = 38$, 18.2%), hematologic effects ($n = 33$, 15.8%), dermatologic effects ($n = 20$, 9.6%), thyroid disease ($n = 8$, 3.8%), pulmonary disease (including interstitial pneumonia and tuberculosis) ($n = 8$, 3.8%), IFN-induced retinopathy ($n = 5$, 2.4%), autoimmune disease ($n = 3$, 1.2%), cerebral vascular disease ($n = 2$, 1.0%), diabetes mellitus type 1 ($n = 2$, 1.0%), elevation of ALT ($n = 1$, 0.5%) and others ($n = 20$, 9.6%). Similarly, for HCV genotype 2 ($n = 41$), the reasons were neurovegetative symptoms ($n = 8$, 19.5%), depression-related syndrome ($n = 8$, 19.5%), hematologic effects ($n = 8$, 19.5%), dermatologic effects ($n = 7$, 17.1%), thyroid disease ($n = 2$, 4.9%), interstitial pneumonia

Table 1 Rates of premature discontinuation due to AE of PEG-IFN α-2b and RBV treatment

	Overall			HCV Genotype 1			HCV Genotype 2		
	Total	Discontinuation due to AE	*P*-value*	Total	Discontinuation due to AE	*P*-value*	Total	Discontinuation due to AE	*P*-value*
All patients	2871	250 (8.7)		2010	209 (10.4)		861	41 (4.8)	
Men									
< 65 years	1104	81 (7.3)	< 0.0001	756	63 (8.3)	< 0.0001	348	18 (5.2)	0.7946
≥ 65 years	397	57 (14.4)		308	52 (16.9)		89	5 (5.6)	
Women									
< 65 years	995	70 (7.0)	0.0121	667	56 (8.4)	0.0143	328	14 (4.3)	> 0.9999
≥ 65 years	375	42 (11.2)		279	38 (13.6)		96	4 (4.2)	

Data is shown as *n* (%).

*Comparison of patients aged < 65 years and ≥ 65 years.

AE, adverse effects; HCV, hepatitis C virus; PEG-IFN, pegylated interferon; RBV, ribavirin.

Table 2 Baseline characteristics of 250 patients with chronic HCV infection who discontinued PEG-IFN α-2b and RBV treatment prematurely due to adverse effects

Characteristics	Total	HCV		*P*-value*
		Genotype 1	Genotype 2	
	n = 250	*n* = 209	*n* = 41	
Men, *n* (%)	138 (55.2)	116 (55.6)	22 (53.7)	0.2428
Age (years)	62 [16]	63 [15]	56 [25]	0.0002
Body mass index (kg/m²)	23.2 [4.0]	23.1 [3.8]	23.2 [5.3]	0.2428
Creatinine clearance (mL/min)	86 [34]	84 [31]	104 [45]	0.0003
Albumin (g/L)	40 [6]	40 [6]	41 [8]	0.3663
ALT (IU/L)	57.0 [60.0]	61.0 [56.5]	48.5 [79.7]	0.3354
γ-GT (IU/L)	48.0 [56.3]	48.0 [57.0]	45.0 [54.0]	0.4948
Total cholesterol (mg/dL)	166 [41]	162 [41]	175 [35]	0.4322
White blood cells (10^9/L)	47 [18]	47 [18]	50 [26]	0.4145
Hemoglobin (g/L)	137 [21]	136 [21]	141 [24]	0.1333
Platelets (10^9/L)	144 [81]	141 [70]	187 [94]	0.0139
HCV RNA (log IU/mL)	6.5 [1.0]	6.5 [0.9]	5.9 [1.6]	0.1507

Data is shown median [interquartile range] or *n* (%).
*Compared with HCV genotype 1 and 2.
ALT, alanine aminotransferase; γ-GT, γ-glutamyl-transpeptidase; HCV, hepatitis C virus; PEG-IFN, pegylated interferon; RBV, ribavirin.

Table 3 Breakdown of reasons for premature discontinuation due to adverse effects of PEG-IFN α-2b and RBV treatment

	Genotype 1 (*n* = 209)	Genotype 2 (*n* = 41)	Total (*n* = 250)
Neurovegetative symptom, *n*	69	8	77
Depression-related syndrome, *n*	38	8	46
Hematologic effect, *n*	33	8	41
Anemia	18	3	21
Thrombocytopenia	8	3	11
Neutropenia	7	2	9
Dermatologic effect, *n*	20	7	27
Thyroid disease, *n*	8	2	10
Hyperthyroidism	7	1	8
Hypothyroidism	1	1	2
Pulmonary disease, *n*	8	2	10
Interstitial pneumonia	6	2	8
Pulmonary tuberculosis	2	0	2
Retinopathy, *n*	5	1	6
Autoimmune disease, *n*	3	1	4
Rheumatoid arthritis	2	1	3
Myasthenia gravis	1	0	1
Elevation of ALT†, *n*	1	1	2
Cerebrovascular disease, *n*	2	0	2
Diabetes mellitus type 1, *n*	2	0	2
Others, *n*	20	3	23

†Over 10 times the upper limit of the normal range.
ALT, alanine aminotransferase; PEG-IFN, pegylated interferon; RBV, ribavirin.

(*n* = 2, 4.9%), IFN-induced retinopathy (*n* = 1, 2.4%), autoimmune disease (*n* = 1, 2.4%), elevation of ALT (*n* = 1, 2.4%), and others (*n* = 3, 7.3%).

About half of the premature discontinuations by both genotype 1 and 2 patients were for neuropsychiatric symptoms (123 of 250, 49.2%).

Timing of discontinuation of PEG-IFN α-2b and RBV treatment classified by the type of adverse effect (Fig. 1a,b). In the case of premature discontinuation due to neuropsychiatric symptoms, including neurovegetative symptoms and depression-related syndrome, 66 of 107 patients (61.7%) with HCV genotype 1 discontinued between 5 and 24 weeks after the start of treatment. For premature discontinuation due to hematologic effects, 32 of 33 patients (97.0%) with HCV genotype 1 discontinued within the first 36 weeks of treatment. For premature discontinuation due to dermatologic effects, 11 of 20 patients (55.0%) with HCV genotype 1 discontinued within the first 12 weeks of treatment. However, analyses of HCV genotype 2 found no significant differences in the timing of discontinuation of treatment among the tested adverse effects.

Breakdown of the reasons for premature discontinuation classified by sex, age, and adverse effects (Table 4). Of the 250 patients who prematurely discontinued treatment, 138 were men and 112 women ($P = 0.3336$). The most common reason for premature discontinuation was neuropsychiatric symptoms (neurovegetative symptoms and depression-related syndrome), both for men (64 of 138, 46.3%) and women (59 of 112, 52.7%). No significant differences in premature discontinuation due to neurovegetative symptoms, depression-related syndrome, or dermatologic effects were found in the sex-based analysis, except for a difference in premature discontinuation due to hematologic effects (men 2.1% *vs* women 0.7%, $P = 0.0026$).

In analyses of neurovegetative symptoms, depression-related syndrome, and hematologic effects classified by sex, age, and adverse effects, the rate of discontinuation of treatment for male patients aged ≥ 65 years was significantly higher than for male patients aged < 65 years ($P = 0.0001$, $P = 0.0016$ and $P = 0.0170$, respectively); however, no such difference was found for women.

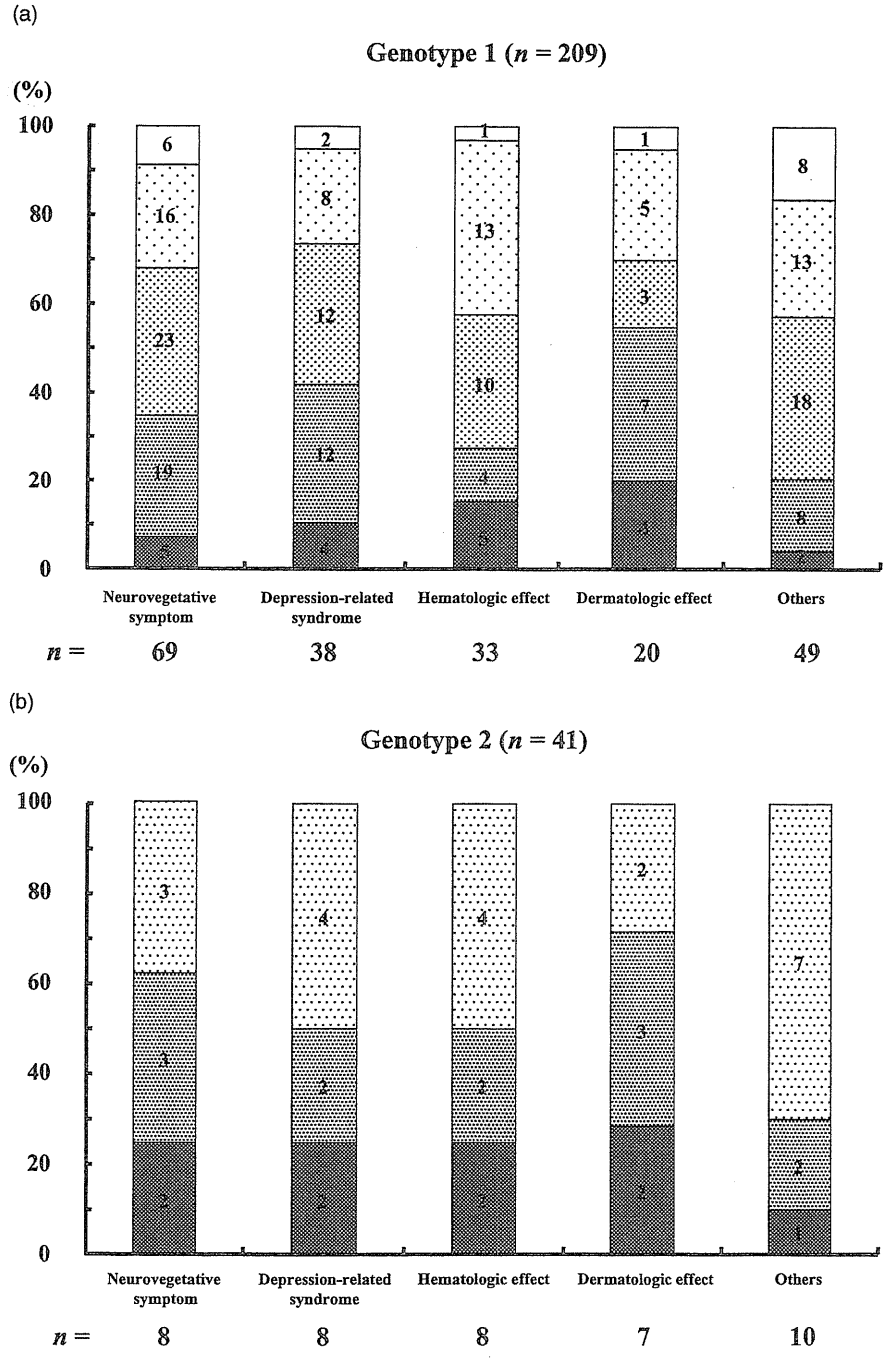

Figure 1 The timing of discontinuation of pegylated interferon α-2b and ribavirin treatment classified by the type of adverse effect. (a) Hepatitis C virus (HCV) genotype 1. ▩, 1–4 weeks; ▨, 5–12 weeks; ⊡, 13–24 weeks; □, 25–36 weeks; □, 37–47 weeks. (b) Genotype 2. ▩, 1–4 weeks; ▨, 5–12 weeks; ⊡, 13–24 weeks.

Similarly, in analysis of the dermatologic effect classified by sex, age, and adverse effect, the rate of discontinuation of treatment for female patients aged ≥ 65 years was significantly higher than for female patients aged < 65 years ($P = 0.0050$); however, no such difference was found for men.

Discussion

This large-scale, prospective study documented the reasons for premature discontinuation of PEG-IFN α-2b and RBV treatment by Japanese patients with chronic HCV infection, including patients aged ≥ 65 years. Although some reports have described the adverse effects of PEG-IFN α-2b and RBV treatment,[13,14] Japanese patients with chronic HCV infection who are candidates for antiviral treatment are currently 10–15 years older than the patients reported in Western countries,[18] therefore, it is necessary to investigate the adverse effects of PEG-IFN α-2b and RBV treatment on Japanese patients.

The results of the present study suggested that premature discontinuation due to adverse effects during PEG-IFN α-2b and

Table 4 Breakdown of the reasons for premature discontinuation classified by sex, age, and adverse effects

	Overall	Neurovegetative symptom	*P*-value	Depression-related syndrome	*P*-value	Hematologic effect	*P*-value	Dermatologic effect	*P*-value
Men	1501	44 (2.9)	0.3866	20 (1.3)	0.2282	31 (2.1)	0.0026	15 (1.0)	0.7322
Women	1370	33 (2.4)		26 (1.9)		10 (0.7)		12 (0.9)	
Men									
< 65 years	1104	23 (1.1)	0.0001	10 (0.9)	0.0016	17 (1.5)	0.0170	10 (0.9)	0.5596
≥ 65 years	397	21 (5.3)		10 (2.5)		14 (3.5)		5 (1.3)	
Women									
< 65 years	995	21 (2.1)	0.2409	17 (1.7)	0.4030	7 (0.7)	> 0.9999	4 (0.4)	0.0050
≥ 65 years	375	12 (3.2)		9 (2.4)		3 (0.8)		8 (2.1)	

Data is shown as *n* (%).

RBV treatment mainly depended on neuropsychiatric symptoms. The reasons for depressive mood changes during IFN treatment are still not well understood. Historical or current pretreatment psychiatric disorders have not been associated with the ability to tolerate IFN treatment. The alteration of the physiological stress response in the hypothalamic-pituitary-adrenal axis, the activation of inflammatory cytokines (IL-2, IL-6, IL-10, dipeptidyl-peptidase 4), abnormal 5-hydroxytryptamine (serotonin) metabolism,[19] and to some extent personality traits, may all play a major role in the development of depression during the IFN treatment. According to a recent study, a polymorphism (rs9657182) in the promoter region of the gene encoding indoleamine-2,3-dioxygenase is associated with moderate or severe IFN-α-induced depressive symptoms in Caucasians.[20]

Baseline depression significantly predicted the severity of depressive symptoms during PEG-IFN α-2b and RBV treatment; moreover, Leutscher *et al.*[21] reported that the emergence of therapy-induced major depression was associated with a reduced likelihood of achieving SVR. Hence, it is imperative to assess the presence or absence of underlying depression and other psychiatric diseases prior to antiviral treatment. Although our data did not show the frequency of psychiatric disease before the antiviral treatment, chronic HCV infection has been associated with higher rates of depression. Lee *et al.*[22] reported evidence of depressive symptoms in about 24% of patients with untreated HCV infection, with two-thirds eventually requiring antidepressant treatment.

Previous studies reported that antidepressive treatment may optimize the outcome of HCV treatment, in particular by reducing the risk of early premature discontinuation.[23,24] However, in contrast to this view, some studies reported that rates of IFN treatment completion did not significantly differ between categories of antidepressant use.[25,26] There is a general consensus on antidepressant medications that serotonin reuptake inhibitors (SSRI) are an effective choice for treating depression associated with IFN treatment with chronic hepatitis C. Although SSRI appear to be relatively safe and help maintain quality of life, their use did not significantly improve the likelihood of an SVR.[26]

In the present study, neuropsychiatric symptoms were the main reason for premature discontinuation, for patients both under and over 65 years, and they mainly occurred during the first 24 weeks of treatment. In previous studies, several demographic factors, including age, sex, ethnicity, and education level, had a potential impact on the occurrence of depression;[27] however, there is no consensus as to whether any of these factors have an impact on the development of depression in patients treated with IFN.[19] In our study, there was no significant sex-based difference in the frequency occurrence of neuropsychiatric symptoms. However, the frequency of occurrence of neuropsychiatric symptoms for male patients aged ≥ 65 years was significantly higher than for male patients aged < 65 years. As mentioned above, we need to monitor neuropsychiatric symptoms carefully using diagnostic criteria similar to those used for other adverse effects: hematologic effects, thyroid function, interstitial pneumonia and ophthalmologic complications.

The second most common reason for premature discontinuation of PEG-IFN α-2b and RBV treatment was hematologic effects. However, the rate of discontinuation because of a hematologic effect was only 1.4% (41 of 2871). IFN mainly affects white blood cell and platelet counts, in contrast to RBV, which can bring about hemolytic anemia. Even though women had significantly lower pretreatment Hb levels in this study, more men than women prematurely discontinued PEG-IFN α-2b and RBV treatment due to hematologic effects, mainly hemolytic anemia. Women more frequently than men had to reduce the RBV dosage at an early stage because of a lower pretreatment Hb level. Serum RBV concentration was significantly correlated with Hb decline after 12 weeks from the start of treatment;[28] thus, the reason for discontinuation due to hemolytic anemia of men might be the high RBV concentration.

In a recent genome-wide association analysis,[29] the inosine triphosphatase (ITPA)-AA/CA genotype was associated with a higher degree of reduction in platelet count at week 4, as well as protection against the reduction of Hb, whereas the ITPA-CC genotype had significantly less reduction in platelet count when compared with the ITPA-AA/CA genotype instead of a higher degree of reduction in Hb during the first 12 weeks of PEG-IFN α and RBV treatment. Based on the results of our study, the rate of discontinuation due to a hematologic effect during the first 12 weeks of treatment was 31.7% (13 of 41), indicating that ITPA gene analysis may be useful for tailoring the PEG-IFN and/or RBV dose to minimize hematologic abnormalities.

Another IFN-induced hematologic adverse effect is neutropenia. The incidence of neutropenia is a frequent indication for PEG-IFN α dose reduction or discontinuation. Rapid decreases in neutrophil counts may be seen within the first 2 weeks of treatment, but they usually stabilize over the next 4–6 weeks as steady state concentrations of PEG-IFN α are achieved. Although the use of granulocyte colony-stimulating factor (GCSF) can raise the neutrophil count during IFN treatment, there is no significant

correlation between IFN-induced neutropenia and the incidence of infection.[30] Thus, future studies will be required to determine the usefulness of GCSF for patients with neutropenia.

The third most common reason for the premature discontinuation of PEG-IFN α and RBV treatment was dermatologic effects. The frequency was very low (27 of 2871, 0.9%) and 16 of 27 (59.3%) of these patients discontinued during the first 12 weeks of treatment. Moreover, the rate of discontinuation of treatment for female patients aged ≥ 65 years was significantly higher than for female patients aged < 65 years ($P = 0.0050$). Most of them discontinued treatment due to severe rash or pruritus resistant to anti-histamine and/or steroid ointments.

Dermatologic adverse effects associated with PEG-IFN α were mainly reactions at the site of IFN injection. Infection and skin necrosis at the site of IFN injection is rare, thus, these symptoms do not necessarily warrant termination of antiviral treatment. Dermatologic adverse effects associated with RBV were generalized pruritus, skin xerosis, and eczema, which are mainly localized to the extremities. Transient rashes do not require RBV treatment interruption and we treat rashes with topical corticosteroids.

In the near future, the addition of telaprevir, an NS3/4A HCV protease inhibitor, to PEG-IFN α and RBV is expected to become the first choice for treating HCV genotype 1. Although a significant improvement in SVR has been shown for patients treated with the triple therapy, severe rash occurred in approximately 5% of patients.[31] Zeuzem *et al.*[31] reported that the most common reason for premature discontinuation was dermatologic effects, thus, physicians would need close cooperation with dermatologists for the care of patients with chronic HCV infection.

The remaining reasons for the premature discontinuation of PEG-IFN α-2b and RBV treatment were various clinical conditions (e.g. inadequately controlled hyperthyroidism, interstitial pneumonia, worsened retinopathy and autoimmune disease). Decisions about premature discontinuation of antiviral treatment must be individualized.

In conclusion, the premature discontinuation of PEG-IFN α-2b and RBV treatment by patients with chronic HCV infection is mainly because of neuropsychiatric symptoms, irrespective of sex and age. The management and careful monitoring of neuropsychiatric symptoms using well-established diagnostic criteria are needed during PEG-IFN α-2b and RBV treatment, especially in the first 24 weeks.

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Original Article

Pegylated interferon α-2b plus ribavirin for Japanese chronic hepatitis C patients with normal alanine aminotransferase

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Aim: To investigate the efficacy and safety of a pegylated interferon (PEG-IFN) α-2b plus ribavirin (RBV) combination treatment for patients with chronic hepatitis C virus (HCV) infection who have persistently normal alanine aminotransferase (NALT).

Methods: This multicenter study included 989 patients with HCV genotype 1 (114 with NALT and 875 with elevated ALT) who received weight-based doses of PEG-IFN α-2b plus RBV for 48 weeks. We compared the sustained viral response (SVR) rates of patients with NALT and elevated ALT who received at least 80% or more of the target dosage of PEG-IFN α-2b and 60% or more of the target RBV (minimum acceptable dosage).

Results: No significant difference was found in the overall SVR rate between the NALT (42.1%) and elevated ALT groups (37.3%). No significant difference in the SVR rates was found between NALT (63.3%) and elevated ALT group (61.6%) patients who received minimum acceptable dosage. Multivariate analysis showed that age (<65 years old) and total cholesterol (≧220 mg/dL) were significantly independent positive factors associated with an SVR in the NALT group. Twenty-four weeks after treatment, an ALT increase above the normal range was observed for 34.0% (18 of 53) of the non-responsive group of NALT patients.

Conclusions: The efficacy and safety of PEG-IFN α-2b plus RBV combination therapy for patients with chronic HCV infection are similar for patients with NALT and those with elevated ALT levels. These results indicate that patients with NALT should be considered for treatment with PEG-IFN α-2b plus RBV.

Key words: hepatitis C virus, normal alanine aminotransferase, pegylated interferon, ribavirin

INTRODUCTION

IT IS WELL documented that hepatocellular carcinoma (HCC) caused by HCV infection develops at a high rate in patients with advanced chronic hepatitis (CH) and liver cirrhosis.[1] Interferon (IFN) therapy for chronic hepatitis C is useful for eliminating hepatitis C virus (HCV)[2,3] and for reducing the progression of hepatic fibrosis,[4] and consequently the development of HCC.[5] Alanine aminotransferase (ALT) values are persistently normal for 20–40% of HCV patients,[6–9] with these patients generally having milder disease and a relatively favorable prognosis, and thus they have in the past been excluded from antiviral treatment.[10,11] However, the current American Association for the Study of Liver Disease (AASLD) practice guidelines recommend that

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the decision to treat HCV-infected patients with persistently normal ALT (NALT) should be individualized based on the severity of liver disease by liver biopsy, the potential for serious side effects, the likelihood of response, and the presence of comorbid conditions.[12] Because, several studies conducted over the past several years have shown that the liver histology of patients with NALT levels shows advanced fibrosis, and in some reports, 5–30% of these patients were found to have marked fibrosis or even cirrhosis (1.3%).[13–15] Further, previous studies reported that the efficacy and safety of pegylated interferon (PEG-IFN) α-2a and ribavirin (RBV) combination treatment for NALT patients with chronic hepatitis C were comparable or even higher than was found for patients with elevated ALT levels.[16–18]

Most patients in previous studies were from western countries and were aged in their 40s on average. The influence of aging of the patient population has not been adequately studied. In Japan, patients with chronic hepatitis C currently under treatment with IFN are 10 to 15 years older than corresponding patients in the United States and other western countries, where patients treated with antiviral therapy tend to average 45 years of age.[19,20] Moreover, a racial analysis reported that being Asian (non-south) is a strong independent predictor of sustained virological response to antiviral therapy.[21] However, there is no Asian data concerning the response and safety of this combination therapy from large scale trials of NALT patients with chronic HCV infection. The present prospective study was done to analyze the efficacy and safety of a combination treatment of PEG-IFN α-2b plus RBV for Japanese NALT patients with HCV genotype 1.

METHODS

Patients

A MULTICENTER STUDY of the efficacy and safety of antiviral treatments for Japanese chronic liver disease patients, the Kyushu University Liver Disease Study (KULDS), was launched in 2003.[22,23] For the present study, combination PEG-IFN α-2b and RBV treatment was done from December 2004 to September 2008, and chronic hepatitis C patients were enrolled with exclusion criteria that included: (i) clinical or biochemical evidence of hepatic decompensation, advanced cirrhosis identified by bleeding-risky esophageal varices, history of gastrointestinal bleeding, ascites, encephalopathy, or hepatocellular carcinoma; (ii) hemoglobin level <11.5 g/dL, white blood cell count $<3 \times 10^9$/L, and platelet count $<50 \times 10^9$/L; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen positive or HIV positive); (iv) excessive active alcohol consumption >60 g/day or drug abuse; (v) severe psychiatric disease; or (vi) antiviral or corticosteroid treatment within 12 months prior to enrollment. Patients who fulfilled the above criteria were recruited at Kyushu University Hospital and 40 affiliated hospitals in the northern Kyushu area of Japan. We have treated 2270 Japanese patients aged 18 years or older with PEG-IFN α-2b plus RBV. Of the 2270 patients, 989 were HCV genotype 2, and the remaining 292 patients were currently undergoing combination treatment or we were not yet able to judge the effect of combination treatment. The 989 HCV genotype 1 patients were enrolled for analysis in the present study. All who were positive for both antibody to HCV and HCV RNA for over 6 months were enrolled in the KULDS study. Within 3 months before the start of the treatment and every 3 months during the treatment period, each patient was tested for α-fetoprotein (AFP) and had abdominal ultrasonographic examination. If an abnormal AFP level of 40 ng/mL and/or an appearance of focal lesions on ultrasonographic examination was found at any testing, further testing for HCC was done, which included dynamic computed tomography, angiography, and/or tumor biopsy. In this study, NALT was defined as ALT persistently below 30 IU/L in at least three measurements within the past 6 months, and we defined an ALT-flare up as an ALT level ≧30 IU/L at the 24-week follow-up after the end of treatment. Of the enrolled patients, 114 were assigned to a NALT group (group A) and the remaining 875 to an elevated ALT group (group B) (Table 1). The number of the women and platelet count were significantly higher in group A than in group B. Furthermore, in group A, body mass index, γ-glutamyltranspeptidase and hemoglobin were significantly lower than for group B ($P < 0.001$), and the total cholesterol level was significantly lower in group B than group A ($P < 0.001$).

Informed consent was obtained from all patients before enrollment. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization of Guidelines for Good Clinical Practice.

Liver histology

Liver biopsy was done for 63 (55.3%) of the group A and 518 (59.2%) of the group B patients: The other patients refused biopsy. Fibrosis was staged on a 0–4

Table 1 Characteristics of 989 chronic hepatitis C virus (HCV) infected patients treated with a combination of pegylated interferon IFN) α-2b plus RBV

	Group A (ALT < 30 IU/L) (n = 114)	Group B (ALT ≧ 30 IU/L) (n = 875)	P-value
Men/Women	37/77	502/373	<0.001
Age (years)	57.4 ± 11.9	58.0 ± 10.1	0.607
Body mass index (kg/m^2)	22.5 ± 2.9	23.6 ± 3.2	<0.001
Prior non-pegylated IFN monotherapy n (%)	26 (22.8)	235 (26.9)	0.350
Prior combined non-pegylated IFN plus RBV treatment n (%)	6 (5.3)	77 (8.8)	0.200
Alanine aminotransferase (IU/L)	22.9 ± 4.4	82.9 ± 56.3	<0.001
γ-glutamyltranspeptidase (IU/L)	31.6 ± 24.8	64.3 ± 57.1	<0.001
Albumin (g/dL)	4.2 ± 0.3	4.1 ± 0.4	0.015
White blood cell (×10^9/L)	5.1 ± 1.6	5.0 ± 1.4	0.629
Hemoglobin (g/dL)	13.4 ± 1.3	13.9 ± 1.4	<0.001
Platelet count (×10^9/L)	188 ± 5.5	157 ± 5.2	<0.001
Creatinine (mg /dL)	0.7 ± 0.2	0.8 ± 0.9	0.284
Creatinine clearance (mL/min)	93.9 ± 32.6	97.6 ± 28.6	0.168
Total cholesterol (mg/dL)	182.6 ± 31.7	167.6 ± 30.5	<0.001
Tryglyceride (mg/dL)	102.6 ± 429	105.8 ± 52.7	0.638
HDL-C (mg/dL)	54.4 ± 15.7	50.1 ± 14.4	0.058
LDL-C (mg/dL)	100.2 ± 26.5	95.6 ± 25.9	0.233
Fasting plasma glucose (mg/dL)	95.8 ± 15.2	99.8 ± 21.9	0.075
HbA1c (%)	5.2 ± 0.5	5.4 ± 0.8	0.100
HOMA-IR	2.4 ± 1.8	2.7 ± 1.8	0.158
Serum HCV RNA level (logIU/mL)	6.5 ± 0.6	6.5 ± 0.6	0.332
Histological fibrosis F0/F1/F2/F3/F4	10/31/14/5/3	31/166/165/97/59	0.008

Data are shown as the mean ±standard deviation Group A; ALT<30 IU/L, Group B; ALT ≧ 30 IU/L.
ALT, alanine aminotransferase; HDL-C, high density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance (plasma fasting glucose (mg/dL) × IRI(ng/mL) ÷ 405); LDL-C, Low density lipoprotein-cholesterol; RBV, ribavirin.

scale as follows: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis. Liver fibrosis was more advanced in group B than group A (P = 0.008).

Treatment regimen

All patients were treated with a weight-based, 1.5 μg/kg weekly dosage of subcutaneous PEG-IFN α-2b (PegIntron, Schering-Plough, Osaka, Japan), in combination with RBV (Rebetol, Schering-Plough), which was given orally at a daily dose of 600–1000 mg based on body weight (600 mg for patients weighing less than 60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing 80 kg or more). The length of treatment was 48 weeks, and the above duration and dosage are those approved by the Japanese Ministry of Health, Labor and Welfare. Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to less than 10.0 g/dL. In such cases, a reduction in the dosage of RBV was required. Some patients also had PEG-IFN α-2b-induced psychological adverse effects or a decrease of white blood cell and platelet count. In such cases, a reduction in the dose of PEG-IFN α-2b was required. Both PEG-IFN α-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 8.5 g/dL, 1×10^9/L, and 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.

Determination of baseline HCV RNA level and HCV genotype

The pretreatment, baseline, serum HCV RNA level was measured by COBAS TaqMan HCV assay (TaqMan)