(n=6) *IL28B* genotypes on days 1, 3, 7 and 14 (−1.2 vs −1.3, −1.4 vs −1.4, −1.8 vs −1.7, and −2.3 vs −1.9 log copies/ml) (figure 4A). Moreover, we prepared two additional serum samples from the other HCV-1b patients (serum B and C)[21] to confirm the influence of *IL28B* genotype in early viral kinetics during IFN treatment. After establishing persistent infection with new HCV-1b strains in all chimeric mice, they were also administered four times injections of the bolus dose of peg-IFN-α2a for 2 weeks (figure 4B,C). In a similar fashion, no significant difference in HCV-RNA reduction in chimeric mice sera was observed between favourable and unfavourable *IL28B* genotypes.

Expression levels of ISG in chimeric mice livers

Because chimeric mice have the characteristic of severe combined immunodeficiency, the viral kinetics in chimeric mice sera during IFN treatment could be contributed by the innate immune response of HCV-infected human hepatocytes. Therefore, ISG expression levels in mice livers transplanted with human hepatocytes were compared between favourable and unfavourable *IL28B* genotypes (figure 5).

As shown in figure 5A, ISG expression levels in mice livers were measured at 8 h and 24 h after IFN treatment. The levels of representative antiviral ISG (eg, myxovirus resistance protein A, oligoadenylate synthetase 1, RNA-dependent protein kinase) and other ISG for promoting antiviral signalling (eg, Toll-like receptor 3, retinoic acid-inducible gene 1) were significantly induced at least 8 h after treatment, and prolonged at 24 h. No significant difference in ISG expression levels in HCV-infected livers was observed between favourable and unfavourable *IL28B* genotypes. The other inoculum for persistent infection of HCV-1b also demonstrated no significant difference in ISG

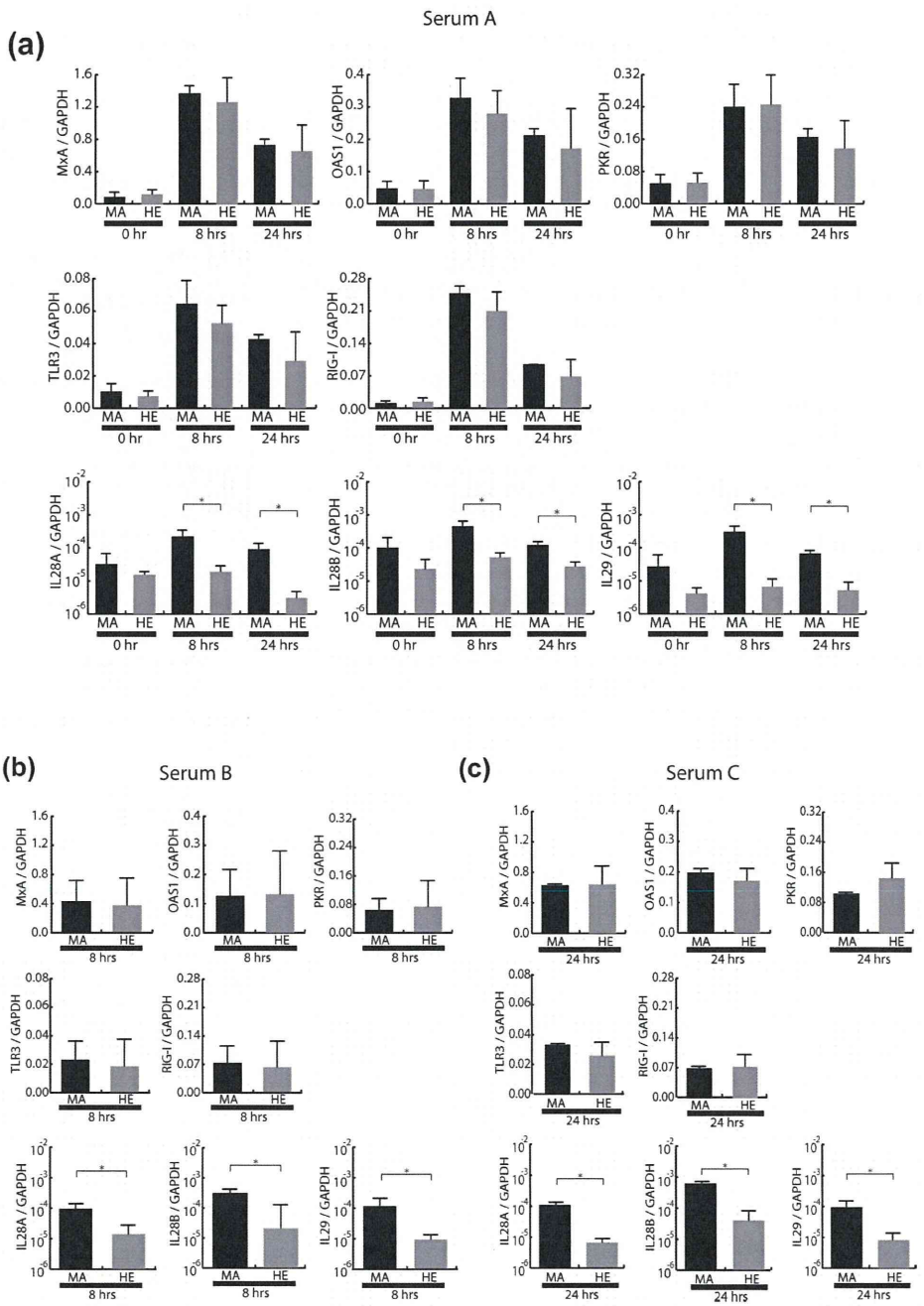

Figure 5 Intrahepatic interferon (IFN)-stimulated gene (ISG) expression levels in the pegylated interferon α (peg-IFN-α)-treated chimeric mice having human hepatocytes containing homozygous favourable allele (rs8099917 TT; MA) and heterozygous unfavourable allele (rs8099917 TG; HE) were measured and expressed relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) messenger RNA. Data are represented as mean+SD. (A) Time kinetics of ISG after administration of the peg-IFN-α in serum A-infected chimeric mice (n=3, each genotype). Comparison of ISG expression levels at (B) 8 h in serum B-infected mice and (C) 24 h in serum C-infected mice after administering peg-IFN-α (n=3, each genotype). Predesigned real-time PCR assay of IL28B transcript purchased from Applied Biosystems can be cross-reactive to IL28A transcript. *p<0.05. MxA, myxovirus resistance protein A; OAS1, oligoadenylate synthetase 1; PKR, RNA-dependent protein kinase; RIG-1, retinoic acid-inducible gene 1; TLR3, Toll-like receptor 3.

expression levels between favourable and unfavourable *IL28B* genotypes (figure 5B,C). Interestingly, IFN-λ expression levels by treatment of peg-IFN-α were significantly induced in HCV-infected human hepatocytes harbouring the favourable *IL28B* genotype (figure 5 A–C).

DISCUSSION

Several recent studies have demonstrated a marked association between the chronic hepatitis C treatment response[6–9] and SNP (rs8099917, rs8103142 and rs12979860) near or within the region of the *IL28B* gene, which affected the viral dynamics during peg-IFN-α plus ribavirin therapy in Caucasian, African American and Hispanic individuals.[13]

It has been reported that when patients with chronic hepatitis C are treated by IFN-α or peg-IFN-α plus ribavirin, HCV-RNA generally declines after a 7–10 h delay.[25] The typical decline is biphasic and consists of a rapid first phase lasting for approximately 1–2 days during which HCV-RNA may fall 1–2 logs in patients infected with genotype 1, and subsequently a slower second phase of HCV-RNA decline.[26] The viral kinetics had a predictive value in evaluating antiviral efficacy.[14] In this study, biphasic decline of the HCV-RNA level during peg-IFN-α treatment was observed in both patients and chimeric mice infected with HCV genotype 1; however, in the first and second phases of viral kinetics, a difference between *IL28B* genotypes was observed only in HCV-infected patients; a more rapid decline in serum HCV-RNA levels after administering peg-IFN-α plus ribavirin was confirmed in patients with the TT genotype of rs8099917 compared to those with the TG/GG genotype.

On the other hand, in-vivo data using the chimeric mouse model showed no significant difference in the reduction of HCV-RNA titers in mouse serum among four different lots of human hepatocytes containing *IL28B* favourable (rs8099917 TT) or unfavourable (rs8099917 TG) genotypes, which was confirmed by the inoculation of two additional HCV strains. These results indicated that variants of the *IL28B* gene in donor hepatocytes had no influence on the response to peg-IFN-α under immunosuppressive conditions, suggesting that the immune response according to *IL28B* genetic variants could contribute to the first and second phases of HCV-RNA decline and might be critical for HCV clearance by peg-IFN-α-based therapy.

Two recent studies indeed revealed an association between the *IL28B* genotype and the expression level of hepatic ISG in human studies.[27 28] Quiescent hepatic ISG before treatment among patients with the *IL28B* favourable genotype have been associated with sensitivity to exogenous IFN treatment and viral eradication; however, it is difficult to establish whether the hepatic ISG expression level contributes to viral clearance independently or appears as a direct consequence of the *IL28B* genotype. Another recent study addressed this question and the results suggested that there is no absolute correlation with the *IL28B* genotype and hepatic expression of ISG.[29] Our results on the hepatic ISG expression level in immunodeficient chimeric mice also suggested that no significant difference in ISG expression levels was observed between favourable and unfavourable *IL28B* genotypes. However, these results were not consistent with a previous report using chimeric mice that the favourable *IL28B* genotype was associated with an early reduction in HCV-RNA by ISG induction.[30] The reasons for the discrepancy might depend on the dose and type of IFN treatment, as well as the time point when ISG expression was examined in the liver. In addition, although IFN-λ transcript levels measured in peripheral blood mononuclear cells or liver revealed inconsistent results in the context of an association with the *IL28B* genotype,[7 8] our preliminary assay on the IL28A, IL28B and IL29 transcripts in the liver first indicated that the induction of IFN-λ on peg-IFN-α administration could be associated with the *IL28B* genotype. Therefore, the induction of IFN-λ followed by immune response might contribute to different viral kinetics and treatment outcomes in HCV-infected patients, because no difference was found in chimeric mice without immune response.

It has also been reported that the mechanism of the association of genetic variations in the *IL28B* gene and spontaneous clearance of HCV may be related to the host innate immune response.[11] Interestingly, participants with seroconversion illness with jaundice were more frequently rs8099917 homozygous favourable allele (TT) than other genotypes (32% vs 5%, p=0.047). This suggests that a stronger immune response during the acute phase of HCV infection among patients with the *IL28B* favourable genotype would induce more frequent spontaneous clearance of HCV.

Taking into account both the above results in acute HCV infection and our results conducted on chimeric mice that have the characteristic of immunodeficiency, it is suggested that the response to peg-IFN-α associated with the variation in *IL28B* alleles in chronic hepatitis C patients would be composed of the intact immune system.

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Contributors YT and MM conceived the study. TW and FS and YT conducted the study equally. TW and FS coordinated the analysis and manuscript preparation. All the authors had input into the study design, patient recruitment and management or mouse management and critical revision of the manuscript for intellectual content. TW, FS and YT contributed equally.

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Patient consent Obtained.

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Hepatitis C virus kinetics by administration of pegylated interferon- α in human and chimeric mice carrying human hepatocytes with variants of the *IL28B* gene

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Clinical Study

Baseline Serum Cholesterol Is Associated with a Response to Pegylated Interferon Alfa-2b and Ribavirin Therapy for Chronic Hepatitis C Genotype 2

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Background. HCV infection is associated with lipid disorders because this virus utilizes the host lipid metabolism to sustain its life cycle. Several studies have indicated that higher concentrations of serum cholesterol and LDL before treatment are important predictors of higher rates of sustained virological response (SVR). However, most of these studies involved patients infected with HCV genotype 1. Thus, we performed a multi-institutional clinical study to evaluate the impact of lipid profiles on SVR rates in patients with HCV genotype 2. *Methods.* A total of 100 chronic hepatitis C patients with HCV genotype 2 who received peg-IFN alfa-2b and ribavirin therapy were consecutively enrolled. The significance of age, sex, BMI, AST level, ALT level, WBC, hemoglobin, platelet count, gamma-glutamyltransferase, total cholesterol level (TC), LDL level, HCV RNA, and histological evaluation was examined for SVR using logistic regression analysis. *Results.* The 100 patients infected with HCV genotype 2 were divided into 2 groups, an SVR group and a non-SVR group. Characteristics of each group were subsequently compared. There was no significant difference in the level of HCV RNA, BMI, platelet, TG, or stage of fibrosis between the groups. However, there were significant differences in the levels of TC and LDL-C. In multivariate logistic regression analysis using baseline characteristics, high TC level was an independent and significant risk factor (relative risk 18.59, $P = 0.015$) for SVR. *Conclusion.* Baseline serum total cholesterol levels should be considered when assessing the likelihood of sustained treatment response following the course of peg-IFN and ribavirin therapy in patients with chronic HCV genotype 2 infection.

1. Introduction

Hepatitis C virus (HCV) causes acute and chronic hepatitis as well as liver cirrhosis and hepatocellular carcinoma [1]. A single-stranded RNA genome encodes 1 large open reading frame that is processed into at least 10 proteins by host and viral enzymes [2]. Some viral proteins are known to affect the outcome of pegylated interferon (PEG-IFN) and ribavirin combination therapy, which is the current standard for treating chronic hepatitis [3, 4].

HCV infection is associated with lipid disorders because this virus utilizes the host lipid metabolism to sustain its life cycle [5, 6]. Accordingly, understanding lipid metabolism in HCV infection is necessary for developing new strategies for complete eradication of this virus. Characteristic lipid disorders observed in chronic hepatitis C patients include steatosis and hypocholesterolemia, which are primarily caused by abnormal triglyceride (TG) and cholesterol metabolism, respectively [7]. The metabolic pathways of these 2 lipids are closely related to each other.

Several studies have indicated that higher concentrations of serum cholesterol and LDL before treatment are important predictors of high rates of sustained virological response (SVR) [8–10]. However, most of these studies involved patients who were infected with HCV genotype 1. Prognostic factors are likely to differ considerably between genotypes 1 and 2. For example, two studies have shown that total PEG-IFN and ribavirin doses are independent predictive factors of an SVR to the HCV genotype 1, whereas another found that dosages of PEG-IFN and ribavirin on SVR are not related to the genotype 2 [11, 12]. Total dosages of PEG-IFN and ribavirin may similarly influence the SVR to genotypes 1 and 2. Identifying factors involved in the responses of patients infected with HCV genotype 2 to PEG-IFN and ribavirin is important when considering treatment strategies. Fewer patients are infected with HCV genotype 2 than genotype 1. Thus, we performed a multi-institutional clinical study to evaluate the impact of lipid profiles on SVR rates in patients with HCV genotype 2.

2. Patients and Methods

2.1. Patients. A total of 685 patients with chronic hepatitis C diagnosed between 2004 and 2008 in the Nagasaki Association for the Study of Liver Disease (NASLD) were recruited for this study. All patients were included if they were positive for HCV antibodies and serum HCV RNA. One hundred patients with HCV genotype 2 who received pegylated interferon alfa-2b (PEG-INF) and ribavirin therapy were consecutively enrolled. Exclusion criteria were as follows: (1) positive for serum hepatitis B virus surface antigen, (2) abnormal thyroid and kidney functions, (3) decompensated liver disease, (4) presence of human immunodeficiency virus type I infection, and (5) ever received specific antiviral therapy prior to referral.

2.2. Study Protocol. This study is retrospective study. Response to antiviral treatment was assessed in patients based on HCV viremia and aminotransferase levels. Patients treated with a combination of PEG-IFN alfa-2b (product by MSD) and ribavirin received 1.0–1.5 μg/kg and 600–800 mg daily of each drug, respectively. SVR was defined as both normal aminotransferase levels and undetectable serum HCV RNA 24 weeks after the end of antiviral therapy. The remaining patients were considered nonvirus responders (non-SVR).

Fasting serum samples were obtained in the early morning for biochemical analysis. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters (kg/m^2). Liver biopsy specimens were fixed in 10% formalin, embedded in paraffin, cut to a thickness of 4 μm, and stained with hematoxylin-eosin and Azan. All liver tissue specimens were evaluated by one pathologist who was unaware of patient clinical conditions. Liver histology was evaluated according to the degree of fibrosis and necroinflammatory activity [13]. The extent of fibrosis (staging) was classified as follows: F1 (periportal expansion), F2 (portoportal septa), F3 (portocentral linkage or bridging fibrosis), and F4 (cirrhosis). Necroinflammatory activity (grading) was classified as follows: A1 (mild), A2 (moderate), and A3 (severe). In order to define the cutoff parameter for total cholesterol level (TC), LDL, and TG for the SVR of PEG-IFN alfa-2b and ribavirin in HCV patients, we used the ROC curve. The area under the curve was 62% (CI 95%: 51%–75%), 72% (CI 95%: 59%–86%), and 61% (CI 95%: 46%–76%), respectively. The ideal cutoff point for the TC, LDL, and TG was calculated to be 177 with sensitivity equal to 58% and specificity equal to 77%, 98 with sensitivity equal to 57% and specificity equal to 77%, and 88 with sensitivity equal to 56% and specificity equal to 67%, respectively.

The protocol was approved by the Ethical Committee of the Nagasaki University School of Medicine.

2.3. Statistical Analysis. Descriptive summaries of study groups are reported as the median (range) and number (%). Data were analyzed using the Mann-Whitney U test for continuous ordinal data, and the chi-square test with Yates' correction and Fisher's exact test were performed for intergroup comparisons to determine the association between 2 qualitative variables. P-values <0.05 were considered statistically significant. Variables achieving statistical significance according to univariate analysis were subsequently included in the multivariate analysis using a logistic regression model and were described as relative risk (RR) with 95% confidence intervals (CI). Coefficients were calculated from the linear discriminating function of the variables. Data analysis was performed using SPSS version 16.0 for Windows.

3. Results

3.1. Patient Clinical Features. Baseline characteristics of the 100 patients infected with HCV genotype 2 are shown in Table 1. There were 54 male (54%) and 46 female (46%) patients, with a median age of 57 years.

The 100 patients infected with HCV genotype 2 were then divided into 2 groups, an SVR group (74 patients) and Non-SVR group (26 patients). Characteristics of each group were subsequently compared (Table 2). There was no

Table 1: Characteristics of 100 studied patients with HCV genotype 2.

All	100	
Age	57.0	(24–76)
Sex (%)		
Male	54	(54)
Female	46	(46)
Height (cm)	162	(138–186)
Weight (kg)	58	(37–87)
BMI (kg/m^2)	22.7	(18.4–30.8)
Clinical finding (%)		
Chronic hepatitis	93	(93)
Cirrhosis	7	(7)
WBC (/μL)	5100	(2100–9730)
Hemoglobin (g/dL)	14.0	(10–16)
Platelet (10^4/μL)	20.4	(6.9–26.5)
AST (IU/L)	42	(17–157)
ALT (IU/L)	52	(11–280)
TC (mg/dL)	177	(106–269)
<177 mg/dL (%)	50	(50)
≥177 mg/dL (%)	50	(50)
TG (mg/dL)	88	(56–262)
<88 mg/dL (%)	50	(50)
≥88 mg/dL (%)	50	(50)
LDL-C (mg/dL)	98	(30–167)
<98 mg/dL (%)	50	(50)
≥98 mg/dL (%)	50	(50)
HCV RNA (KIU/mL)	1000	(20–40900)
Distribution of stage of fibrosis (%)		
0-1	43	(43)
2	17	(17)
3	11	(11)
4	4	(4)
Unknown	25	(25)
Distribution of grade of inflammation (%)		
0-1	39	(39)
2	34	(34)
3	2	(2)
Unknown	25	(25)
Treatment period (week) (%)		
<24	10	(10)
24	83	(83)
25–48	5	(5)
>48	2	(2)
Therapeutic efficacy (%)		
SVR	74	(74)
Non-SVR	26	(26)

Data are median (range) or frequency (%).

significant difference in the level of HCV RNA, BMI, platelet, TG, or stage of fibrosis between the groups. However, there were significant differences in the level of TC and LDL-C.

3.2. Univariate and Multivariate Analysis of Factors Associated with SVR to Pegylated Interferon Alfa-2b and Ribavirin Therapy. Univariate and multivariate analysis in 100 patients infected with HCV genotype 2 was performed to identify independent factors relevant to an SVR (Table 3). In univariate analysis, the following 2 factors significantly influenced the SVR: TC (≥177 mg/dL; relative risk, 3.77; 95% confidence interval (95% CI), 1.41–10.05; $P = 0.008$) and LDL-C (≥98 mg/dL; relative risk, 4.91; 95% CI, 1.19–20.23; $P = 0.028$). However, in multivariate analysis, TC was the only independent factor for SVR (relative risk, 18.59; 95% CI, 1.78–193.65; $P = 0.015$).

3.3. Association of SVR Rate to Combination Therapy and TC Level. The 100 patients infected with HCV genotype 2 were then divided into 2 groups, a high serum TC level group (≥177 mg/dL) and a low serum TC level group (<177 mg/dL). Characteristics of each group were subsequently compared (Table 4). There was no significant difference in age, the level of ALT, WBC, hemoglobin, platelet, TG, stage of fibrosis or grade of inflammation between the groups. However, there were significant differences in sex, BMI, the level of AST, TG, LDL-C, and HCV RNA.

We examined the differences in the 4 indices related to SVR rate between high serum TC level and low serum TC level in HCV genotype 2 patients (Figure 1). The SVR rate in low serum TC level patients was 62% (31 of 50), whereas 86% of patients (43 of 50) had serum high TC levels. The significantly higher SVR rate of serum high TC level than low serum TC levels was observed in 100 patients infected with HCV genotype 2.

4. Discussion

In this retrospective study, we showed a significant association of treatment response with baseline characteristics of patients infected with HCV genotype 2, including HCV viral load, BMI, and serum cholesterol level. Several baseline predictors for SVR have been identified in earlier studies [14–17]. Notably, among pretreatment features in the present study, serum TC levels appeared to discriminate responders from nonresponders independently of different treatment schedules. The response rate to standard treatment for patients with HCV genotype 2 using a combination of PEG-IFN and ribavirin is approximately 80% and remains a major concern in patient care. Our findings confirm serum high TC level as a good predictor of SVR in genotype 2. In patients with genotype 2, the SVR rate in patients with low serum TC levels was 62%, whereas 86% had high serum TC levels. Serum cholesterol as a predictor of SVR in patients with chronic hepatitis C is in accordance with the results of previous studies [8–10, 18–20]. However, our study design included only patients with HCV genotype 2.

A cutoff value of total cholesterol of 177 mg/dL in this study represented the best value in terms of sensitivity and specificity for SVR. Our cutoff total cholesterol level was lower than other previous studies [8–10, 18–20]. However, American Diabetes Association guidelines suggest that a goal should be a total cholesterol of <160 mg/dL in patient with

Table 2: Factors associated with response to peginterferon alfa-2b and ribavirin therapy.

	SVR	(Range or %)	Non-SVR	(Range or %)	*P* value
Total	74		26		
Age (y.o.)	57	(24–72)	57	(31–78)	NS
Sex (%)					
Male	33	(45)	13	(50)	
Female	41	(55)	13	(50)	NS
BMI (kg/m^2)	23.1	(15.4–30.9)	21.0	(18.4–26.0)	NS
WBC (/μL)	5100	(2100–9730)	5145	(3000–8300)	NS
Hemoglobin (g/dL)	14.1	(10–16)	14.0	(10–16)	NS
Platelet ($10^4/\mu$L)	21.7	(6.9–26.5)	11.5	(7.3–21.1)	NS
AST (IU/L)	39	(17–377)	44	(17–140)	NS
ALT (IU/L)	51	(11–751)	53	(14–169)	NS
TC (mg/dL)	183	(106–269)	163	(127–248)	NS
<177 mg/dL (%)	31	(42)	19	(73)	
≥177 mg/dL (%)	43	(58)	7	(27)	0.005
TG (mg/dL)	98	(56–262)	83	(74–176)	NS
<88 mg/dL (%)	33	(44)	17	(67)	
≥88 mg/dL (%)	41	(56)	9	(33)	NS
LDL-C (mg/dL)	109	(30–167)	88	(64–117)	0.015
<98 mg/dL (%)	30	(40)	20	(77)	
≥98 mg/dL (%)	44	(60)	6	(23)	0.020
HCV RNA (KIU/mL)	1000	(20–40900)	1850	(37–24200)	NS
Distribution of stage of fibrosis (%)					
1	31	(42)	12	(46)	
2	14	(19)	3	(12)	
3	6	(8)	5	(19)	
4	3	(4)	1	(4)	
Unknown	20	(27)	5	(19)	NS
Distribution of grade of inflammation (%)					
1	27	(36)	12	(46)	
2	25	(34)	9	(35)	
3	2	(3)	0	(0)	
Unknown	20	(27)	5	(19)	NS

Data are median (range) or frequency (%).

type 2 diabetes who is at low risk [21]. Furthermore, Miller et al. reported that American type 2 diabetic patients had an average cholesterol level of 179 mg/dL [22].

The reason for SVR improvement in patients with elevated serum cholesterol levels is unknown. In patients with chronic hepatitis B and hepatitis C, serum lipid levels have been reported to be correlated with specific cytokines that may have antiviral activity, including tumor necrosis factor-alpha and interleukin-6 [23]. This hyperlipidemia-induced increase in cytokine levels may have a favorable and potentially additive effect on antiviral treatment in patients with chronic hepatitis C. Another proposed mechanism may be related to a possible regulatory effect of cholesterol in HCV binding to cell surface receptors, which in turn may be relevant to viral clearance [24]. The LDL receptor, a membrane glycoprotein, has been shown to be involved in HCV entry into hepatocytes, and data suggest that HCV RNA levels correlate with LDL receptor expression [25, 26]. Elevated serum concentrations of LDL may decrease the number of LDL receptors located on hepatocytes.

Recent studies have shown that single nucleotide polymorphisms located in the gene region encoding interleukin 28b (IL28B) are strongly associated with the response to PEG-IFN and ribavirin therapy [17, 27, 28]. Total cholesterol, LDL cholesterol, and ApoB concentrations are significantly higher in chronic hepatitis C patients carrying a second IL28B major allele (CC in rs 12979860) compared with those possessing minor alleles (CT or TT) [29]. Therefore, the association between serum LDL cholesterol concentration and SVR may be reflected by the underlying link

Table 3: Univariate and multivariate analysis of the factors associated with SVR to peginterferon alfa-2b and ribavirin therapy.

		Univariate analysis		Multivariate analysis	
		P	RR (95% CI)	*P*	RR (95% CI)
Age	<57 years	0.646	1.24 (0.50–3.09)		
Sex	Female	0.634	0.80 (0.33–1.97)		
BMI	≥23 kg/m^2	0.221	1.86 (0.69–5.02)		
Underlying liver disease					
	CH	0.872	1.15 (0.21–6.32)		
WBC	≥5100 /μL	0.827	0.75 (0.37–2.22)		
Hb	≥14.0 g/dL	0.317	0.62 (0.25–1.58)		
Plt	≥20 × 10^4/μL	0.112	2.10 (0.84–5.24)		
AST	<40 IU/L	0.429	1.44 (0.58–3.55)		
ALT	<52 IU/L	0.649	1.23 (0.50–3.02)		
γ-GTP	<35 IU/L	0.525	0.75 (0.30–1.83)		
TC	≥177 mg/dL	0.008	3.77 (1.41–10.05)	0.015	18.59 (1.78–193.65)
TG	≥88 mg/dL	0.101	2.60 (0.83–8.13)		
LDL-C	≥98 mg/dL	0.028	4.91 (1.19–20.23)	0.800	1.25 (0.22–7.01)
Stage	F 3-4	0.419	0.60 (0.17–2.07)		
Grade	A 2-3	0.809	1.13 (0.41–3.18)		
HCV RNA					
	<1000 KIU/mL	0.310	1.65 (0.63–4.31)		

Relative risk (RR); 95% confidence interval (95% CI).

Table 4: Comparison between HCV patients with high and low serum TC.

TC	<177 mg/dL	(Range or %)	≥177 mg/dL	(Range or %)	*P* value
Total	50		50		
Age (y.o.)	57	(24–78)	57	(36–69)	NS
Sex (%)					
Male	34	(68)	20	(40)	
Female	16	(32)	30	(60)	0.005
BMI (kg/m^2)	21.5	(18.4–26.8)	23.5	(15.4–30.6)	0.027
WBC (/μL)	5100	(2100–9730)	5100	(3000–8300)	NS
Hemoglobin (g/dL)	14.2	(10–16)	13.9	(10–16)	NS
Platelet (10^4/μL)	17.6	(7.3–26.5)	21.7	(6.9–26.1)	NS
AST (IU/L)	48	(17–377)	33	(18–199)	0.033
ALT (IU/L)	67	(16–751)	40	(11–283)	NS
TG (mg/dL)	83	(46–203)	111	(43–262)	NS
<88 mg/dL (%)	32	(63)	19	(38)	
≥88 mg/dL (%)	18	(37)	31	(62)	0.045
LDL-C (mg/dL)	84	(43–118)	121	(30–167)	<0.001
<98 mg/dL (%)	40	(79)	9	(19)	
≥98 mg/dL (%)	10	(21)	41	(81)	<0.001
HCV RNA (KIU/mL)	1000	(20–24200)	2670	(20–40900)	0.029
Distribution of stage of fibrosis (%)					
1	18	(36)	25	(50)	
2	7	(14)	10	(20)	
3	8	(16)	3	(6)	
4	2	(4)	2	(4)	
Unknown	15	(30)	10	(20)	NS
Distribution of grade of inflammation (%)					
1	20	(40)	19	(38)	
2	14	(28)	20	(40)	
3	1	(2)	1	(2)	
Unknown	15	(30)	10	(20)	NS

Data are median (range) or frequency (%).

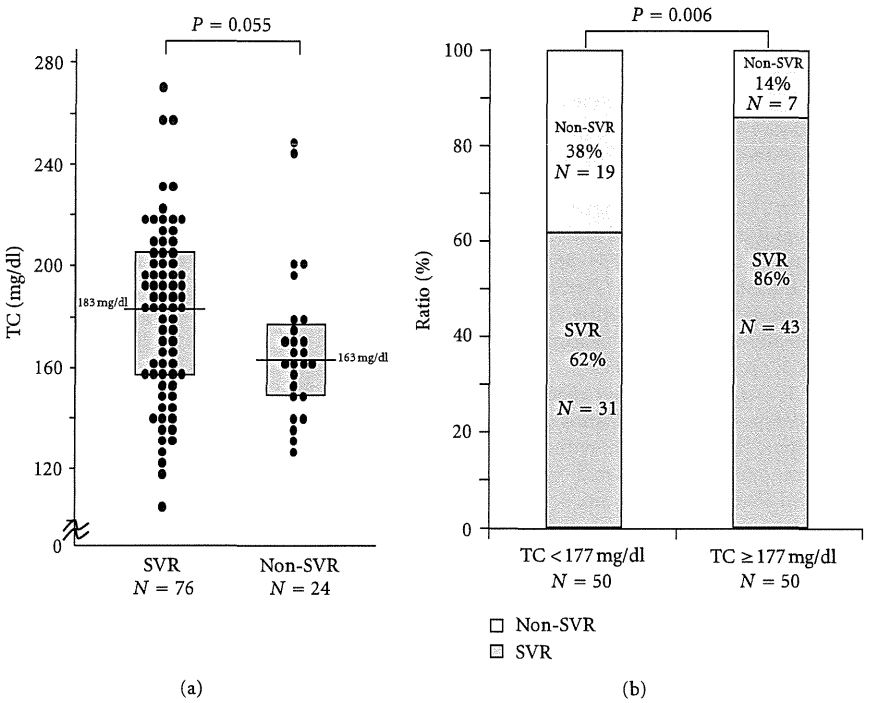

FIGURE 1: Comparison between SVR rate in patients with high serum TC levels (≥177 mg/dL) and patients with low serum TC levels (<177 mg/dL) in HCV genotype 2 patients.

between IL28B genotypes and LDL cholesterol concentrations. As discussed above, we cannot exclude the possibility that high cholesterol levels in patients with HCV only reflect the presence of the IL28B major allele. It may simply reflect the wild-type sequence at core amino acid 70 because substitution in the core protein correlated significantly with a low concentration of LDL cholesterol [30, 31]. However, we could not identify IL28-B and core amino acid 70 as predictive for our patients with HCV genotype 2 because our sample population was limited.

Petta and Craxì reported that age, sex, stage of fibrosis, and baseline viral load were important predictive factors SVR in patient with HCV genotype 2 [32]. However, our study was not significantly differente in these factors for SVR. Furthermore, there was no significant difference in the serum TC between SVR and non-SVR by the Mann-Whitney *U* test. The reason may be explained as follows: severe stage of fibrosis (F3-4) was recruited only 15%, and 25% was stage unknown in this study. HCV RNA in high TC group was significant higher than low TC group. Finally, this was the limitation small sample size and retrospective study. The discrepancies of the observation from different reports need further investigation.

In conclusion, our data suggest that baseline serum total cholesterol levels should be considered when assessing the likelihood of sustained treatment response following PEG-IFN and ribavirin therapy in patients with chronic HCV genotype 2 infection. However, this finding requires further analysis.

Conflict of Interests

The authors declare that they have no conflict of interests and financial support.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Anemia and thrombocytosis induced by ribavirin monotherapy in patients with chronic hepatitis C

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Abstract
Background An inosine triphosphatase (*ITPA*) single-nucleotide polymorphism (SNP) is associated with anemia induced by pegylated interferon and ribavirin (RBV) combination therapy in patients with chronic hepatitis C (CHC). However, there are very few reports on the hematological effects of RBV monotherapy. Here, hematological changes were monitored in patients with CHC who received RBV monotherapy, and the mechanism of these changes was investigated.
Methods Patients with CHC ($n = 30$) received RBV monotherapy for 4 weeks. The RBV dose was determined on the basis of body weight. Complete blood count, and serum erythropoietin (EPO) and thrombopoietin (TPO) levels were assessed. The associations between these parameters and the *ITPA* SNP (*rs1127354*) were analyzed.
Results Over the 4 weeks, the median hemoglobin level of all patients decreased significantly, from 13.6 (10.5–16.6) to 11.7 (9.4–14.9) g/dl ($P < 0.001$), and the platelet counts increased, from 14.0×10^4 ($8.9–37.4 \times 10^4$) to 15.8×10^4 ($10.2–40.6 \times 10^4$) /mm^3 ($P = 0.003$). At week 4, hemoglobin levels differed between patients with the *ITPA* CC genotype and those with the AA or AC genotypes [11.1 (9.4–13.5) vs. 12.9 (12.5–14.9) g/dl, $P = 0.001$]. The platelet change ratio (i.e., platelet count at week 4/platelet count at baseline) in the patients with developing anemia was correlated with the increase in the serum EPO level over 4 weeks ($r = 0.88$, $P = 0.002$), but not with the increase in the serum TPO level over 4 weeks.
Conclusions RBV monotherapy induced anemia and affected thrombocytosis in patients with CHC. Elevated endogenous EPO may stimulate platelet production.

Keywords Ribavirin · Anemia · Erythropoietin · Thrombocytosis · *ITPA* SNP

Abbreviations

ITPA	Inosine triphosphatase
SNP	Single-nucleotide polymorphism
PEG-IFN	Pegylated interferon
RBV	Ribavirin
CHC	Chronic hepatitis C
EPO	Erythropoietin
TPO	Thrombopoietin
HCV	Hepatitis C virus
GWASs	Genome-wide association studies
IL28B	Interleukin 28B
DDRGK1	DDRGK domain-containing protein 1

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Introduction

Hepatitis C virus (HCV) infection currently affects an estimated 160 million individuals, or 2.35 % of the world population [1]. Of the patients with a primary HCV infection, 70–80 % develop chronic infection and are consequently at significant risk for progressive liver fibrosis, which can lead to liver cirrhosis (LC) and/or hepatocellular carcinoma (HCC) [2, 3].

Current antiviral treatment for chronic hepatitis C (CHC) patients is pegylated interferon alfa (PEG-IFN) and ribavirin (RBV) combination therapy. However, despite advances in the treatment of CHC, the sustained viral response (SVR) rate of patients infected with HCV genotype 1 and with a high viral load is <50 %; these patients have the most difficulty achieving SVR [4, 5].

In the 1970s, RBV, a guanosine analog, was demonstrated to have antiviral activity against a broad spectrum of DNA and RNA viruses in tissue culture cells [6]. RBV monotherapy has transient antiviral effects in patients with HCV, but the treatment response improves markedly when RBV is combined with IFN [4].

Drug tolerance is an important factor associated with the treatment response. Side effects induced by PEG-IFN/RBV combination therapy lead to dose reduction and sometimes to discontinuation of the combination therapy. Treatment-induced anemia is a common cause of RBV dose reduction. Reportedly, patients receiving less than 60 % of the planned RBV dose have a lower response rate and a higher relapse rate than patients receiving a higher dose [7, 8].

In recent years, genome-wide association studies (GWASs) have demonstrated a marked association between particular single-nucleotide polymorphisms (SNPs) near the interleukin 28B (*IL28B*) gene and treatment outcome with PEG-IFN/RBV combination therapy in patients with CHC [9].

In addition, some studies indicate that inosine triphosphatase (*ITPA*) SNPs are associated with anemia induced by PEG-IFN/RBV combination therapy [10, 11].

Tanaka et al. [12] reported that the *ITPA rs1127354* genotype was associated with the outcome of PEG-IFN/RBV combination therapy in a Japanese population, and Ochi et al. [11] reported a marginally significant association between the *ITPA* SNP and treatment outcomes of combination therapy, based on univariate analysis. Taken together, these findings indicate that there is a correlation between the *ITPA* SNP and the outcome of combination therapy in a Japanese population. Furthermore, it was surmised that the *ITPA* SNP may be associated with some treatment outcomes because this SNP affected RBV dose reduction and may have contributed to treatment failures.

Tanaka et al. [12] have demonstrated that *DDRGK1* (DDRGK domain-containing protein 1) SNPs are also associated with treatment-induced anemia and treatment-induced thrombocytopenia associated with PEG-IFN/RBV combination therapy.

IFN/RBV combination therapy leads to thrombocytopenia primarily because of the administration of IFN. However, in most studies of hematological changes associated with CHC treatments, patients received IFN/RBV or PEG-IFN/RBV combination therapy. Therefore, these studies did not address the hematological effects of RBV monotherapy.

Here, we assessed hematological changes in patients with CHC who received RBV monotherapy, and we studied factors associated with these changes, including *ITPA* SNPs and hematopoietic hormones.

Patients and methods

Patients and treatment protocol

Patients ($n = 30$; 14 males and 16 females; median age 56 years; age range 31–71) with chronic HCV infection who received RBV monotherapy at our hospital between April 2002 and March 2004 were enrolled in this study; the RBV monotherapy was administered for 4 weeks. All patients received IFN alfa-2b/RBV combination therapy after the RBV monotherapy.

The characteristics of the patients are shown in Table 1. The initial diagnosis was made using a second-generation enzyme-linked immunosorbent assay (ELISA) for antibodies against HCV and confirmed by quantitative reverse transcriptase (RT)-polymerase chain reaction (PCR) amplification of HCV from serum samples.

Patients who were positive for hepatitis B surface antigen or HIV antibodies were excluded from the study. The dose of RBV (Rebetol™; MSD, Tokyo, Japan) was determined based on body weight: the daily dose was 600 mg for patients <60 kg, 800 mg for those between 60 and 80 kg, and 1000 mg for those ≥80 kg. Complete blood counts were assessed at weeks 0, 1, 2, 3, and 4. The daily RBV dose was reduced by 200 mg if hemoglobin was <10 g/dl or if there was a 2 g/dl decline from the week-0 baseline; additionally, RBV treatment was withheld if the hemoglobin level was <8.5 g/dl. Serum samples were collected at weeks 0, 1, 2, 3, and 4 of RBV monotherapy and stored at −30 °C.

This protocol was approved by the Ethics Committee of Hokkaido University Hospital (Sapporo, Japan) and written informed consent was obtained from all patients before starting the trial.

Of the 30 patients who were enrolled in the study, 26 received all the planned dose of RBV. Owing to anemia, three patients received 70 % of the planned RBV dose, and

Table 1 Characteristics of the patients enrolled in this study

Characteristic	No. of patients or median	Range
Gender (male/female)	14/16	
Age (years)	56	31–71
BMI (kg/m^2)	24.5	19.4–32.0
rs8099917 (TT/TG or GG)	25/5	
rs1127354 (AA/AC or CC)	7/23	
rs11697186 (TT/TA or AA)	7/23	
WBC (/mm^3)	4500	3100–7700
Hemoglobin (g/dl)	13.6	10.5–16.6
Hematocrit (%)	40.8	32.0–48.6
Platelets ($\times 10^4$/mm^3)	14.0	8.9–37.4
AST (IU/l)	55	17–228
ALT (IU/l)	81	14–397
γ-GT (IU/l)	43	11–219
LDH (IU/l)	339	135–594
Albumin (g/dl)	4.1	2.6–5.0
T-bilirubin (mg/dl)	0.8	0.5–1.4
Creatinine (mg/dl)	0.7	0.4–1.1
HCV-RNA ($\log_{10}$ IU/ml)	6.0	3.7–6.6
Fibrosis (0/1/2/3/4)	3/6/11/9/1	
Activity (0/1/2/3)	1/9/20/0	

The data shown are medians and ranges unless otherwise specified

BMI body mass index, *WBC* white blood cell, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GT* gamma-glutamyl transpeptidase, *HCV* hepatitis C virus, *LDH* lactate dehydrogenase

one patient received just 53 %. No patients required a blood transfusion or administration of recombinant human erythropoietin (rhEPO).

SNP genotyping

To determine the *IL28B*, *ITPA*, and *DDRGK1* genotypes at select SNPs, genomic DNA was extracted from 200 μl of whole blood, using the QIAamp DNA Blood Mini Kit (QIAGEN Sciences, Germantown, MD, USA). SNP genotypes were determined using the real-time PCR method (TaqMan™ SNP Genotyping Assay; Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Genotypes at three SNPs—*rs8099917*, *IL28B* (Assay ID: C_11710096_10); *rs1127354*, *ITPA* (Assay ID: C_27465000_10); and *rs11697186*, *DDRGK1* (Assay ID: C_11815649_20)—were determined. The genotype of *DDRGK1* could be determined by this method in all patients, but the genotypes of *ITPA* and *IL28B* could not be determined by this method in some patients. Therefore, when the genotype of a patient could not be determined by this method, the genotype was determined using standard PCR (ExTaq Hot Start version; Takara Bio, Otsu, Japan) and direct sequencing (BigDye Terminator; Applied Biosystems). A 2-μl sample of the genomic DNA extracted from a whole blood sample was amplified over 40 cycles of PCR. The PCR thermal profile comprised an initial denaturation at 95 °C for 10 min and 40 cycles of amplification (denaturation at 95 °C for 60 s, annealing at 55 °C for 60 s, and extension at 72 °C for 60 s). The forward primer for *rs8099917* was TTTGTCACTGTTCCTCCTTTTG and the reverse primer was TGCTGGGCCCTAACTGATAC. The forward primer for *rs1127354* was ATGAGAAAGG CGGATGACAG and the reverse primer was CGGCACT TATCAGGGAAACA.

Measurement of serum EPO and thrombopoietin (TPO) levels

Serum levels of EPO were measured using an ELISA (EPO ELISA; Roche, Mannheim, Germany) in stored blood samples taken from patients at weeks 0, 1, 2, 3, and 4. Serum TPO levels were measured using an ELISA (Quantikine™ Human TPO; R&D Systems, Minneapolis, MN, USA) in patient blood samples taken at 0, 2, and 4 weeks. Both assays were performed according to the manufacturers' instructions.

Pathological findings

Baseline liver biopsies were performed on all patients prior to the treatment, to determine METAVIR activity and fibrosis score. The METAVIR scoring system grades fibrosis on a 5-point scale (F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous bridging septa without cirrhosis; F4, cirrhosis) and grades activity on a 4-point scale (A0, no activity; A1, mild activity; A2, moderate activity; A3, severe activity).

Measurement of serum ribavirin concentration

Serum concentrations of RBV after 4 weeks of monotherapy were measured using high-performance liquid chromatography (HPLC) as described previously [13].

Statistical analyses

All results are presented as medians and ranges. Statistical tests were performed based on Friedman's test to assess the change in a parameter over time, the Mann–Whitney test and Chi-square test to assess differences between groups, and the Spearman test to assess the correlation between hematological changes and hematopoietic hormones. The degree of platelet increase was measured using the platelet change ratio, specifically the platelet count at week 4/platelet count at week 0.

P values of <0.05 were considered significant. All statistical analyses were performed using PASW statistics 18 software (IBM, Armonk, NY, USA).

Results

Changes in hemoglobin, platelet count, serum alanine aminotransferase (ALT), and HCV RNA level during RBV monotherapy

Changes in values during RBV monotherapy are shown in Table 2. During 4 weeks of RBV monotherapy, the median hemoglobin level of the patients decreased significantly, from 13.6 (10.5–16.6) to 11.7 (9.4–14.9) g/dl ($P < 0.001$). The median platelet count increased significantly, from 14.0×10^4 ($8.9–37.4 \times 10^4$) to 15.8×10^4 ($10.2–40.6 \times 10^4$) /mm^3 ($P = 0.003$). The median mean corpuscular volume (MCV) increased from 98.3 (88.3–104.1) to 99.6 (89.9–105.3) fl ($P = 0.009$), and the median reticulocyte count increased from 9.2 (6.1–40.2) to 29.5 (9.0–80.2) ‰ ($P = 0.002$). There were no significant differences between baseline and week 4 in WBC, neutrophil counts, or lymphocyte counts. The median ALT level decreased significantly, from 81 (14–397) IU/l at baseline to 50 (12–312) IU/l at week 4 ($P = 0.007$), and the level of HCV RNA decreased significantly, from 6.0 (3.7–6.6) at baseline to 5.6 (3.3–6.5) $\log_{10}$ IU/ml at week 4 ($P = 0.045$). Serum EPO increased significantly during 4 weeks of RBV monotherapy, whereas serum TPO did not change significantly.

Association between *ITPA* SNP and hematological changes and hematopoietic hormones during RBV monotherapy

The 30 enrolled patients were divided into two groups based on *ITPA* genotype. Based on this grouping, baseline TPO level was significantly associated with the *ITPA* genotype, but other parameters, including gender, age, and renal function, were not (Table 3). Although the difference was not statistically significant, during the first 2 weeks of RBV monotherapy, hemoglobin levels in patients with the *ITPA* CC genotype tended to be lower than levels in those with the *ITPA* AA or AC genotypes [12.2 (9.8–15.9) vs. 13.2 (12.4–15.1) g/dl, $P = 0.07$]. After 4 weeks of RBV monotherapy, there was a significant difference in hemoglobin levels between the patients with the *ITPA* CC genotype and those with the AA or AC genotypes [11.1 (9.4–13.5) vs. 12.9 (12.5–14.9) g/dl, respectively, $P = 0.001$] (Fig. 1). Reticulocyte counts in patients with the *ITPA* CC genotype increased from 9.7 (6.1–40.4) to 31.0 (15.8–70.0) ‰ ($P = 0.001$) over the 4 weeks, while reticulocyte counts did not change significantly in the group of patients with the *ITPA* AA or AC genotypes [baseline, 8.8 (8.0–16.9) ‰; 4 weeks, 11.3 (9.0–20.5) ‰, not significant (NS)]. Serum concentrations of RBV were not different between the patients with the *ITPA* CC genotype and those with the AA or AC genotypes. The *DDRGK1* SNP was also analyzed. Because the *DDRGK1* TT or TA genotypes showed linkage with the *ITPA* AA or AC genotypes in all patients enrolled in the present

Table 2 Hematological changes and changes of ALT and HCV-RNA levels over a 4-week course of RBV monotherapy

	Week 0	Week 2	Week 4	P value
WBC (/mm^3)	4500 (3100–7700)	4800 (3800–8700)	4400 (2900–7500)	NS
Neutrophils (/mm^3)	2162 (1473–4068)	2355 (1867–4219)	2501 (1334–4219)	NS
Lymphocytes (/mm^3)	1659 (707–3796)	1678 (1092–2642)	1548 (616–2688)	NS
Hemoglobin (g/dl)	13.6 (10.5–16.6)	12.3 (9.8–15.9)	11.7 (9.4–14.9)	<0.001
MCV (fl)	98.3 (88.3–104.1)	97.2 (90.2–106.1)	99.6 (89.9–105.3)	0.009
Reticulocytes (‰)	9.2 (6.1–40.4)	23.3 (7.0–54.1)	29.5 (9.0–80.2)	0.002
Platelets ($\times 10^4$/mm^3)	14.0 (8.9–37.4)	15.3 (9.2–32.8)	15.8 (10.2–40.6)	0.003
ALT (IU/l)	81 (14–397)	58 (17–254)	50 (12–312)	0.007
HCV-RNA ($\log_{10}$ IU/ml)	6.0 (3.7–6.6)	5.9 (4.0–6.7)	5.6 (3.3–6.5)	0.045
EPO (pg/ml)	2.9 (0–35.8)	11.9 (0–114.8)	16.8 (0–184.2)	<0.001
TPO (fmol/ml)	1.84 (0.94–2.50)	1.95 (0.66–2.57)	1.93 (0.82–2.51)	NS
Serum RBV concentration (ng/ml)	–	1868 (1087–4656)	2266 (1157–4366)	0.004

The significance of the changes in each parameter was analyzed using Friedman's test

WBC white blood cell, *MCV* mean corpuscular volume, *ALT* alanine aminotransferase, *EPO* erythropoietin, *TPO* thrombopoietin, *NS* not significant, *RBV* ribavirin

Table 3 Characteristics of the patients grouped according to inosine triphosphatase (*ITPA*) SNP genotype

	ITPA (*rs1127354*)		
	CC allele ($n = 23$)	AA or AC allele ($n = 7$)	*P* value
Age (years)	56 (32–67)	60 (31–71)	NS
Gender (M/F)	10/13	4/3	NS
BMI (kg/m^2)	25.2 (19.4–32.0)	23.5 (20.5–27.6)	NS
rs8099917 (TT/non-TT)	18/5	7/0	NS
WBC (/mm^3)	4550 (3400–7500)	4500 (3100–7000)	NS
Hemoglobin (g/dl)	13.5 (10.5–16.6)	13.7 (11.8–15.6)	NS
Platelets ($\times 10^4$/mm^3)	13.2 (8.9–26.9)	15.5 (12.3–37.4)	NS
T-bilirubin (mg/dl)	0.8 (0.5–1.4)	0.8 (0.5–1.1)	NS
Albumin (g/dl)	4.0 (2.6–5.0)	4.0 (3.9–4.6)	NS
ALT (IU/l)	80 (14–176)	88 (18–397)	NS
γ-GT (IU/l)	46 (11–156)	36 (23–219)	NS
Creatinine (mg/dl)	0.7 (0.4–1.1)	0.6 (0.5–1.0)	NS
HCV-RNA ($\log_{10}$ IU/ml)	6.0 (4.5–6.6)	6.3 (3.7–6.6)	NS
EPO (pg/ml)	3.4 (0–35.8)	2.4 (0–12.2)	NS
TPO (fmol/ml)	1.75 (0.94–2.50)	2.03 (1.94–2.33)	0.038
Fibrosis (0–1/2–4)	6/17	7/0	NS
RBV concentration at 2 weeks (ng/ml)	1960 (1246–4656)	1395 (1087–2286)	NS
RBV concentration at 4 weeks (ng/ml)	2256 (1157–4366)	2551 (1349–3304)	NS

P values were calculated using the Mann–Whitney test

BMI body mass index, *WBC* white blood cell, *ALT* alanine aminotransferase, *γ-GT* gamma-glutamyl transpeptidase, *EPO* erythropoietin, *TPO* thrombopoietin, *NS* not significant, *RBV* ribavirin, *SNP* single-nucleotide polymorphism

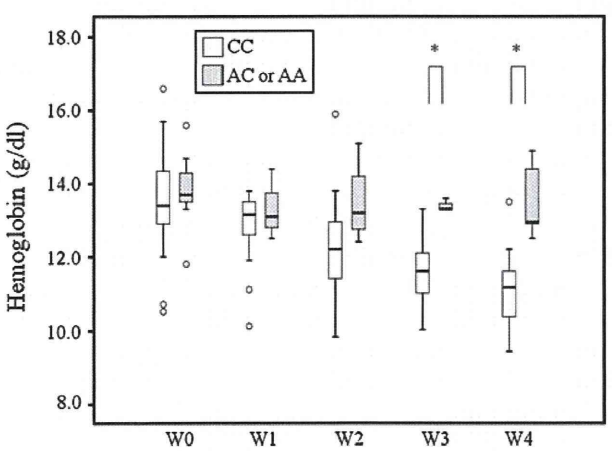

Fig. 1 Changes in hemoglobin according to inosine triphosphatase (*ITPA*) single-nucleotide polymorphism (SNP) genotype. *Box plots* display the minimum, the first quartile, the median, the third quartile, and the maximum values for hemoglobin in patients divided into two groups based on *ITPA* SNP genotype. *P* values were calculated using the Mann–Whitney test. The *white boxes* represent the patients with the CC genotype, and the *gray boxes* represent patients with the AC or AA genotype. $*P < 0.05$

study, the association between *DDRGK1* SNP and changes in platelet counts were not further examined.

The median serum EPO level in patients with the *ITPA* CC genotype increased significantly, from 3.4 (0.0–35.8) to 26.1 (3.1–154.2) pg/ml ($P = 0.005$), over the 4 weeks. In contrast, serum EPO levels in patients with the *ITPA* AA or AC genotypes did not change significantly [2.4 (0.0–12.2) pg/ml at baseline and 4.7 (0.0–17.3) pg/ml at week 4, NS] (Fig. 2). There were no significant differences in WBC, neutrophil, lymphocyte, or platelet counts (Fig. 3) or TPO levels between the patients with the *ITPA* CC genotype and those with the AA or AC genotypes.

Correlation between hemoglobin levels, platelet counts, and EPO levels

There was a significant negative correlation between hemoglobin levels at week 2 and the increase in serum EPO over those 2 weeks ($r = -0.758$, $P = 0.003$) and between hemoglobin levels at week 4 and the increase in serum EPO over those 4 weeks ($r = -0.622$, $P = 0.004$) (Fig. 4).

Next, the association between EPO and the degree of platelet increase as measured by the platelet change ratio (i.e., platelet count at week 4/platelet count at baseline) was analyzed. Although not statistically significant, the platelet change ratio for 4 weeks tended to be correlated with the increase of EPO for 4 weeks ($r = 0.426$, $P = 0.056$). There was no significant correlation between the platelet change ratio and serum TPO over the 4 weeks. Similarly,

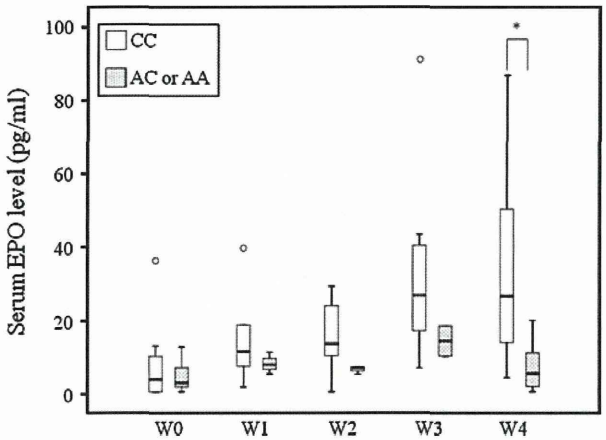

Fig. 2 Changes in serum erythropoietin (*EPO*) according to *ITPA* SNP genotype. *Box plots* display the minimum, the first quartile, the median, the third quartile, and the maximum values for serum EPO in patients divided into two groups based on the *ITPA* SNP genotype. *P* values were calculated using the Mann–Whitney test. The *white boxes* represent patients with the CC genotype, and the *gray boxes* represent patients with the AC or AA genotype. $*P < 0.05$

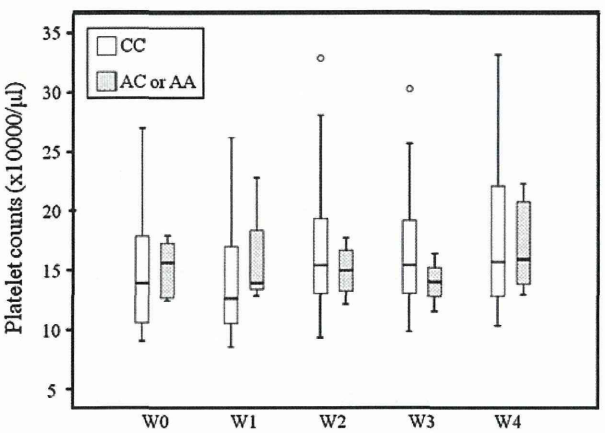

Fig. 3 Changes in platelet counts according to *ITPA* SNP genotype. *Box plots* display the minimum, the first quartile, the median, the third quartile, and the maximum value for platelet counts in patients divided into two groups based on *ITPA* SNP genotype. The *white boxes* represent patients with the CC genotype, and the *gray boxes* represent patients with the AC or AA genotype

there was no significant correlation between hemoglobin levels and the platelet change ratio, or between the increase in serum EPO and the increase in serum TPO (Fig. 5).

Association between serum EPO and platelet counts according to anemia

Because there was a correlation between serum EPO and the platelet count, it was expected that platelet counts would not increase in patients who had not developed anemia.

Therefore, the correlation between serum EPO and platelet count was determined in patients with and without anemia. Here, anemia was defined as a decrease in hemoglobin of >2 g/dl or a hemoglobin level of <10 g/dl. All patients with anemia ($n = 15$) had the *ITPA* CC genotype, while the group of patients who did not develop anemia ($n = 15$) included 8 patients with the CC allele and 7 patients with the AA or AC genotype. Among the group of patients with anemia, platelet counts increased significantly from baseline over the 4 weeks ($P = 0.001$). However, there was no significant increase in platelet counts among the patients who did not develop anemia. There was a significant correlation between serum EPO and the platelet change ratio from baseline to week 4 in the anemia group ($r = 0.88$, $P = 0.002$), but there was no such correlation in the non-anemia group ($r = 0.39$, $P = 0.27$) (Fig. 6).

Factors associated with increase in platelet count

The patients were divided into two groups based on the degree of platelet increase as measured by the platelet change ratio (i.e., platelet count at week 4/platelet count at baseline); specifically, patients with a platelet change ratio greater than or equal to the median of 1.05 were placed in one group, and those with a ratio below the median were placed in the other group (Table 4). The factors that contributed to a platelet increase were examined. The group with a ratio of ≥1.05 tended to be younger than the other group ($P = 0.062$) in univariate analyses. A multivariate analysis could not be performed because of the small number of patients enrolled in this study.

Furthermore, factors that contributed to the platelet increase were examined in the patients without anemia (Table 5). The patients who did not have anemia and had a ratio of platelet increase of ≥1.05 were significantly younger [age 48 years (range 31–56) vs. 61 years (range 54–71), $P < 0.001$] and tended to have higher platelet counts at baseline [17.1×10^4 ($9.1–37.1 \times 10^4$) vs. 12.4×10^4 ($8.9–15.5 \times 10^4$) /mm^3] than those who had a platelet ratio of <1.05.

Discussion

Although RBV has antiviral activity against a broad spectrum of DNA and RNA viruses, RBV itself has only transient effects on HCV. In spite of the minimal antiviral effect of RBV on HCV, some studies show that IFN alpha and RBV combination therapy has significantly better treatment outcomes than IFN monotherapy [6, 14]. Furthermore, in recent years, direct-acting antiviral agents (DAAs), such as telaprevir, were shown to have a strong antiviral effect on HCV. However, in clinical trials of IFN

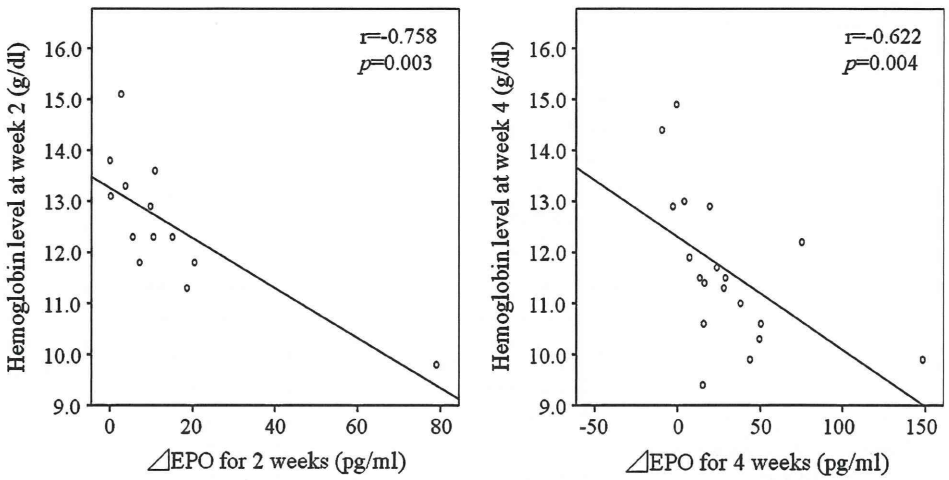

Fig. 4 Correlation between hemoglobin levels and increases in serum EPO. Correlation coefficients and *P* values were calculated using Spearman's rank correlation coefficient test

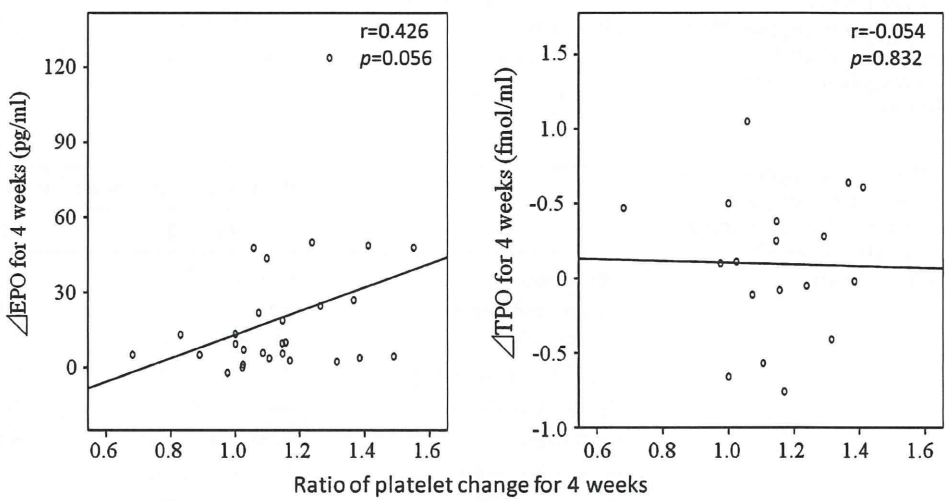

Fig. 5 Correlations between platelet counts and hematopoietic hormones. Correlation coefficients and *P* values were calculated using Spearman's rank correlation coefficient test. *TPO* thrombopoietin

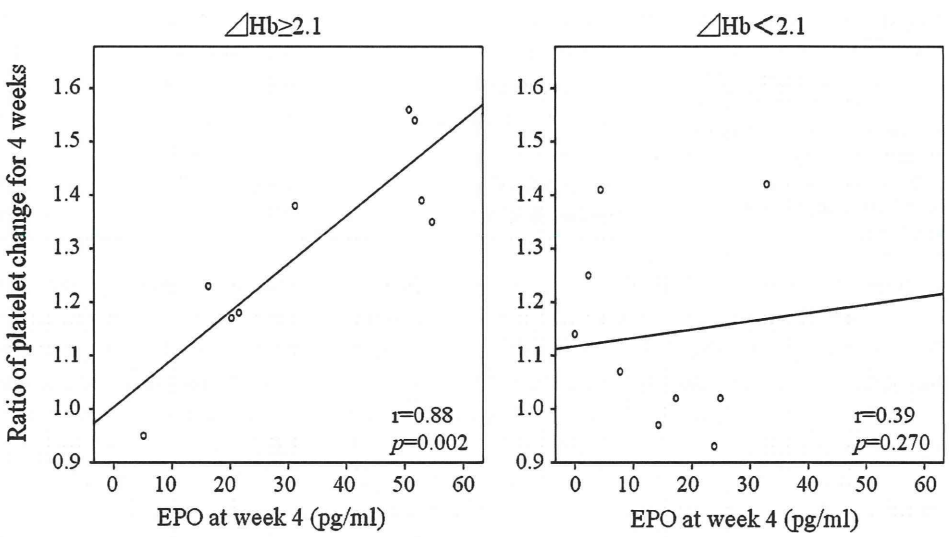

Fig. 6 Correlation between the platelet change ratio and EPO based on the presence/absence of treatment-induced anemia. The platelet change ratio was defined as the platelet count at week 4/platelet count at baseline. Correlation coefficients and *P* values were calculated using Spearman's rank correlation coefficient test. *Hb* hemoglobin

Table 4 Associations between hematological parameters and platelet counts

	Ratio of platelet increase for 4 weeks		
	<1.05 (n = 11)	≥1.05 (n = 19)	P value
Age (years)	61 (41–71)	55 (31–67)	0.062
Gender (M/F)	6/5	8/11	NS
BMI (kg/m^2)	25.2 (19.4–28.1)	24.0 (19.8–32.0)	NS
rs8099917 (TT/non-TT)	10/1	13/4	NS
rs1127354 (CC/non-CC)	7/4	16/3	NS
WBC (/mm^3)	4750 (3800–7400)	4500 (3100–7700)	NS
Hemoglobin (g/dl)	14.1 (10.5–16.6)	13.5 (11.8–15.7)	NS
Platelets (×10^4/mm^3)	13.5 (10.0–26.9)	13.8 (8.9–37.4)	NS
T-bilirubin (mg/dl)	0.7 (0.5–1.4)	0.8 (0.5–1.2)	NS
Albumin (g/dl)	4.0 (2.6–4.6)	4.1 (3.4–5.0)	NS
ALT (IU/l)	49 (18–397)	93 (14–176)	NS
γ-GT (IU/l)	43 (15–219)	48 (11–156)	NS
Creatinine (mg/dl)	0.7 (0.5–1.1)	0.7 (0.4–0.9)	NS
HCV-RNA ($\log_{10}$ IU/ml)	6.2 (3.7–6.6)	6.0 (4.5–6.6)	NS
EPO (pg/ml)	2.0 (0.0–12.2)	2.9 (0.0–35.8)	NS
TPO (fmol/ml)	1.96 (1.41–2.33)	1.75 (0.94–2.5)	NS
Fibrosis (0–1/2–4)	4/7	5/14	NS

P values were calculated using the Mann–Whitney test

BMI body mass index, *WBC* white blood cell, *ALT* alanine aminotransferase, *γ-GT* gamma-glutamyl transpeptidase, *EPO* erythropoietin, *TPO* thrombopoietin, *NS* not significant

Table 5 Associations between increases in hematological parameters and platelet counts in patients without RBV-induced anemia

	Ratio of platelet increase for 4 weeks		
	<1.05 (n = 8)	≥1.05 (n = 6)	P value
Age (years)	61 (54–71)	48 (31–56)	<0.01
Gender (male/female)	3/5	4/2	NS
BMI (kg/m^2)	23.5 (19.4–27.6)	23.0 (19.8–25.8)	NS
rs8099917 (TT/non-TT)	8/0	5/1	NS
rs1127354 (CC/non-CC)	4/4	3/3	NS
WBC (/mm^3)	4400 (3500–7400)	5000 (3100–7700)	NS
Hemoglobin (g/dl)	13.2 (10.5–15.6)	13.6 (11.8–14.1)	NS
Platelets (× 10^4/mm^3)	12.4 (8.9–15.5)	17.1 (9.1–37.4)	0.052
T-bilirubin (mg/dl)	0.7 (0.5–1.1)	0.8 (0.5–1.1)	NS
Albumin (g/dl)	4.0 (2.6–4.6)	4.0 (3.9–4.6)	NS
ALT (IU/l)	52.5 (18–219)	107 (30–119)	NS
γ-GT (IU/l)	47.5 (21–219)	43 (19–96)	NS
Creatinine (mg/dl)	0.65 (0.40–1.00)	0.70 (0.50–1.90)	NS
HCV-RNA ($\log_{10}$ IU/ml)	6.0 (3.7–6.6)	5.9 (5.4–6.6)	NS
EPO (pg/ml)	6.6 (0.0–35.8)	1.94 (0.0–8.3)	NS
TPO (fmol/ml)	2.1 (1.15–2.33)	1.85 (0.94–2.09)	NS
Fibrosis (0–1/2–4)	2/6	1/5	NS

P values were calculated by Mann–Whitney test

BMI body mass index, *WBC* white blood cell, *ALT* alanine aminotransferase, *γ-GT* gamma-glutamyl transpeptidase, *EPO* erythropoietin, *TPO* thrombopoietin, *NS* not significant

and telaprevir with or without RBV, response rates were lower when the treatment regimen did not include RBV. This finding indicates that RBV is a key drug in treatments that achieve SVR for patients with CHC [15].

It is well known that RBV induces anemia, but few reports have shown that RBV monotherapy induced anemia. In 1984, Canonico et al. [16] reported that RBV administration to rhesus monkeys led to anemia, increased platelet counts, and increased megakaryocytes in the bone marrow, indicating that RBV influences bone marrow function. Bone marrow aspiration was not performed in the present study, but our findings confirmed that RBV monotherapy can lead to anemia and increases in platelet counts. Decreases in hemoglobin and increases in serum EPO were evident just 1 week after the start of RBV monotherapy. Increases in platelet counts were evident 2 weeks after the start of RBV monotherapy. However, RBV did not affect serum TPO levels. The patients who did