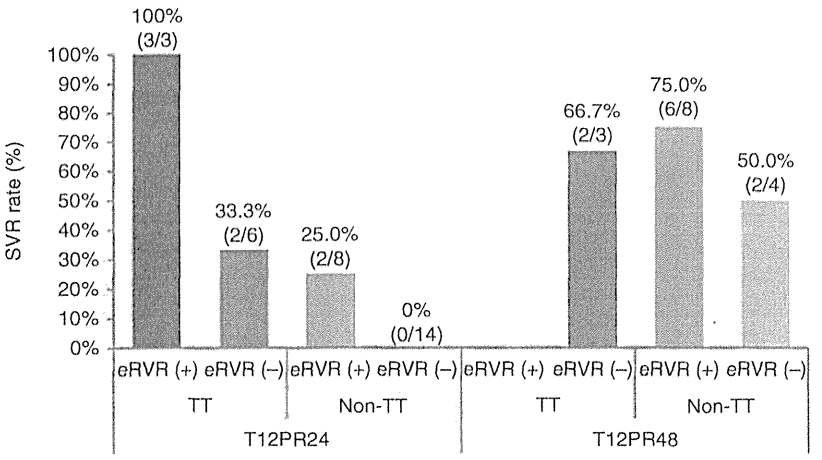

Figure 4 SVR rate in null responders stratified by eRVR according to treatment regimen and *IL28B* genotype. eRVR, extended rapid virological response; IL, interleukin; SVR, sustained virological response; T12PR24, 12 weeks of triple combination therapy followed by 12 weeks of peginterferon and ribavirin combination; T12PR48, 12 weeks of triple combination therapy followed by 36 weeks of peginterferon and ribavirin combination therapy.

achieved and did not achieve eRVR were treated with T12PR24 and T12PR48, respectively.[15] These results suggest that approximately 70% of partial responders may achieve SVR using response-guided therapy, but the SVR rate was extremely low in null responders treated with T12PR24. Although the study of Muir *et al.* was not a randomized controlled trial study, their results indicated that T12PR48 may improve the SVR rate in null responders to a greater extent than T12PR24.[15] The present study revealed the SVR rates of partial and null responders treated with T12PR24 were 70.0% and 22.6%, respectively. The lower SVR rate in null responders than partial responders is concordant with the results of the previous study on T12PR24.[15] To our knowledge, besides our previous reports,[22–24] only two studies performed in Japan have analyzed non-responders to PR classified as partial and null responders.[16,25] Akuta *et al.* reported that the SVR rates in partial and null responders treated with T12PR24 in clinical trials were 50% (4/8) and 0% (0/7), respectively.[16] Meanwhile, Ogawa *et al.* recently reported that the SVR rates in partial and null responders for CHC patients with advanced fibrosis (METAVIR score F3–4) treated with T12PR24 in clinical practice were 50% (9/18) and 16.7% (2/12), respectively.[25] Similarly, the SVR rate was lower in null responders than partial responders in the present study, when treated with T12PR24.

Genetic variations near the *IL28B* gene (rs8099917 and rs12979860) are strongly associated with treatment outcome of PR.[32–34] In addition, these genetic variations are also strong predictors of SVR with the T12PR24 regimen when including both treatment-naïve and treatment-experienced patients.[14,20–25,35] However, Pol *et al.* reported the *IL28B* genotype (rs12979860) has a limited and non-significant impact on SVR in treatment-experienced patients treated with T12PR48 regardless of relapsers, partial responders or null responders to previous PR.[19] In the present study, the SVR rate of partial responders with the TT genotype treated with T12PR24 was very high at approximately 90%, whereas that in patients with the non-TT genotype was significantly lower. Similarly, among null responders, the SVR rate was significantly lower in those with the non-TT genotype than those with the TT genotype. Ogawa *et al.* reported a similar trend that among previous partial and null responders treated with T12PR24, the SVR rate was lower in patients with the non-TT genotype than those with the TT genotype.[25] In contrast, the SVR rate did not differ significantly between either partial or null responders with the *IL28B* TT or non-TT genotype treated with T12PR48 in our study, although there were too few patients to make a meaningful comparison. Thus, the present and previous[25] results possibly indicated that *IL28B* genotype still affects SVR with T12PR24 but the impact of *IL28B* genotype is attenuated with T12PR48. Thus, the present results suggested that *IL28B* genotype is a strong pretreatment predictor of SVR in non-responders to previous PR treated with either T12PR24 or T12PR48. Nevertheless, the SVR rate was lower in null responders than partial responders treated with T12PR24 or T12PR48 even in patients had the same genotype. Therefore, the present results suggested that response to previous PR is a better predictor of SVR than *IL28B* genotype like the previous study.[19] However, in actual clinical practice, it may be impossible to differentiate between previous null and partial responders

in some cases because of the absence of relevant data from medical records. Therefore, in such treatment-experienced patients, *IL28B* genotyping may have clinical utility by serving as a pretreatment marker of interferon responsiveness and treatment duration to guide physicians.

This study also demonstrated that eRVR is a useful on-treatment predictive factor associated with SVR. In particular, partial responders with the *IL28B* TT genotype who achieved eRVR showed an extremely high SVR rate, and all three null responders with the TT genotype who achieved eRVR showed SVR with T12PR24. However, the SVR rate was very low in partial responders with the non-TT genotype who failed to achieve eRVR; no null responders with a non-TT genotype who failed to achieve eRVR showed SVR with T12PR24. This study revealed T12PR48 is a useful strategy for null responders and patients with an unfavorable *IL28B* non-TT genotype. Partial responders with the non-TT genotype had a very high relapse rate except for patients who achieved SVR (11/13, 84.6%; data not shown) with T12PR24. Therefore, partial responders with the TT genotype should be treated with T12PR24 regardless of achievement of eRVR. Meanwhile, partial responders with the non-TT genotype should be treated with T12PR48 regardless of achievement of eRVR in order to increase the SVR rate by decreasing the relapse rate. Furthermore, excluding VBT and non-response, null responders should be treated with T12PR48 irrespective of *IL28B* genotype and achievement of eRVR. However, all three null responders with the TT genotype who achieved eRVR showed SVR. Therefore, these patients might be treated with T12PR24. This study may provide useful information for treatment selection on the basis of previous treatment response, *IL28B* genotype and eRVR.

This study has some limitations that should be mentioned. First, there were too few patients to conclusively determine the factors contributing to SVR; in particular, only 22 patients were treated with T12PR48. Second, this study was not a randomized controlled trial. Accordingly, a randomized controlled trial randomizing partial and null responders to receive T12PR24 or T12PR48 should be conducted to corroborate the present findings.

The second-generation DAA simeprevir, a once-daily oral NS3/4A protease inhibitor, was approved in Japan and has been marketed since December 2013, ahead of the rest of the world. Furthermore, the guideline for CHC with HCV genotype 1 by the Japan Society of Hepatology was recently updated.[36] Zeuzem *et al.* recently studied simeprevir-based triple therapy for treatment-experienced patients. The SVR rates of partial and null responders with HCV genotype 1b who received simeprevir (100 mg once daily) for 12 weeks plus PR for 48 weeks were 68% and 56%, respectively.[37] Therefore, prospective studies are required to confirm whether a 48-week regimen of simeprevir-based therapy improves the SVR rate as well as T12PR48 in clinical practice.

In conclusion, this multicenter study demonstrated that the T12PR48 regimen improves the SVR rate in Japanese genotype 1b CHC patients who were previous non-responders to PR. T12PR48 improved the SVR rate to a greater extent than T12PR24, especially in null responders, those with the *IL28B* non-TT genotype, and patients with the unfavorable non-TT genotype. Further large-scale prospective studies including a 48-week simeprevir-based triple combination therapy are required to confirm the present findings, individualize treatment and optimize therapeutics.

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Original Article

Serum 25-hydroxyvitamin D_3 levels affect treatment outcome in pegylated interferon/ribavirin combination therapy for compensated cirrhotic patients with hepatitis C virus genotype 1b and high viral load

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Aim: Much is unknown about the effect of 25-hydroxyvitamin D_3 levels on the outcome of pegylated interferon/ribavirin (PEG IFN/RBV) therapy for hepatitis C virus-related cirrhosis. The purpose of the present study was to analyze and elucidate factors, including 25-hydroxyvitamin D_3, that contribute to a sustained virological response (SVR) in patients with cirrhosis.

Methods: We analyzed whether 25-hydroxyvitamin D_3 contributes to the response to PEG IFN/RBV therapy among 134 cirrhotic patients.

Results: SVR was achieved in 43 patients. The median 25-hydroxyvitamin D_3 level was 20 ng/mL. Univariate analysis showed that the following factors contributed to SVR: low-density lipoprotein cholesterol, albumin, 25-hydroxyvitamin D_3, core a.a.70 (a.a.70) substitutions, the number of mutations at the interferon sensitivity-determining region and IL28B genotype. Multivariate analysis identified IL28B genotype and 25-hydroxyvitamin D_3 as independent factors contributing to SVR. Subsequently, SVR rate was examined by using 25-hydroxyvitamin D_3 and other important factors. The SVR rate was 51.8% in patients with core a.a.70 wild and ≥15 ng/mL of 25-hydroxyvitamin D_3, whereas the SVR rate was 7.1% in patients with core a.a.70 wild and <15 ng/mL of 25-hydroxyvitamin D_3. The SVR rate was 56.9% in patients with IL28B major genotype and ≥15 ng/mL of 25-hydroxyvitamin D_3. Surprisingly, the SVR rate was 0% in patients with IL28B minor genotype and <15 ng/mL of 25-hydroxyvitamin D_3.

Conclusion: IL28B genotype and 25-hydroxyvitamin D_3 were identified as independent factors contributing to SVR. Stratified analyses according to core a.a.70 substitution and IL28B genotype suggested that 25-hydroxyvitamin D_3 influences the outcome of PEG IFN/RBV therapy for cirrhosis.

Key words: cirrhosis, hepatitis C virus, 25-hydroxyvitamin D_3, pegylated interferon, ribavirin, sustained virological response

INTRODUCTION

WORLDWIDE, AN ESTIMATED 1.7 million people have chronic hepatitis C virus (HCV) infection.[1] If untreated, chronic hepatitis will progress to cirrhosis in many of these patients, some of whom will develop hepatocellular carcinoma.[2–5] Interferon (IFN) therapy for chronic hepatitis C inhibits progression to cirrhosis by achieving a sustained virological response (SVR).[6] The occurrence of hepatocellular carcinoma and liver disease-related death can be inhibited and decreased even after cirrhosis has already developed, by administrating IFN-based treatment and achieving SVR.[7–15]

Striking advances have been made in antiviral therapy for chronic hepatitis C. SVR is obtained in 40–50% of patients who receive pegylated (PEG) IFN/ribavirin (RBV) combination therapy for genotype 1 chronic hepatitis C, which is resistant to treatment.[16–18] Protease

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inhibitors, such as telaprevir and boceprevir, have also become available for use, and SVR is obtained in more than 70% of patients when protease inhibitors are combined with PEG IFN/RBV therapy.[19–21]

Nevertheless, the outcome of antiviral therapy for cirrhotic patients remains inadequate.[7,22,23] While PEG IFN/RBV in combination with a protease inhibitor has an outstanding antiviral effect on the infection, the combination therapy may possibly produce serious adverse events, specifically in patients with advanced fibrosis or cirrhosis.[24] Patients with chronic hepatitis including cirrhosis have a higher average age (~60 years old) in Japan as compared with patients in Western countries. Therefore, adherence to the treatment may be inadequate or treatment may be prematurely ceased in Japanese patients because of the decreased tolerability and increased incidence of more serious adverse events.[22,25]

Several factors that contribute to SVR to PEG IFN/RBV therapy in cirrhotic patients have been identified.[26–28] In recent years, a relationship between chronic hepatitis C and serum vitamin D levels has been suggested.[29–33] Vitamin D is produced in the skin or ingested from food. It exists in various forms in the body, including $1.25(OH)_2D_3$, which is an active form metabolized in the kidneys, and 25-hydroxyvitamin D_3, which is metabolized in the liver and determines the level of vitamin D in the body.[34] In chronic hepatitis C, forms of vitamin D, such as $1.25(OH)_2D_3$ and 25-hydroxyvitamin D_3, were reported to have important roles in host immune activation and inhibition of HCV replication.[35,36] The levels of serum 25-hydroxyvitamin D_3 were lower in chronic hepatitis C patients than in healthy patients and decreased with the progression of hepatic fibrosis.[29] The mechanism by which low serum 25-hydroxyvitamin D_3 levels in cirrhotic patients influence the disease condition and treatment response is unknown. Racial differences in serum 25-hydroxyvitamin D_3 levels have also been identified, but most studies have been performed in Caucasians.[30,32]

In the present study, we analyzed whether serum 25-hydroxyvitamin D_3 levels contribute to the response to PEG IFN/RBV therapy among genotype 1b, high viral load patients with compensated cirrhosis in Japan.

METHODS

Study design

THE INCLUSION CRITERIA were as follows: patient age between 20 and 85 years, high viral load (>5.0 log IU/mL) by quantitative analysis of HCV RNA with real-time polymerase chain reaction (PCR), infection with HCV genotype 1b, white blood cell count of more than 1500/mm^3, neutrophil count of more than 500/mm^3, platelet count of more than 50 000/mm^3 and hemoglobin level of more than 8.5 g/dL. Patients were also required to have a liver biopsy specimen indicative of cirrhosis (F4 based on the METAVIR classification system).[37] The exclusion criteria were as follows: other liver diseases, including autoimmune hepatitis, primary biliary cirrhosis and alcoholic disease; decompensated liver cirrhosis, such as poorly controlled ascites, hepatic encephalopathy or jaundice; liver failure; severe renal disorder; abnormal thyroid function; poorly controlled diabetes; poorly controlled hypertension; medication with Chinese herbal medicine; medical history of interstitial pneumonia; severe depression; and allergy to IFN, RBV and biological preparations such as vaccines.

There were 134 patients who visited Nippon Medical School Chiba Hokusoh Hospital and Shinmatsudo Central General Hospital between January 2006 and December 2011, and who met the inclusion criteria and agreed to receive PEG IFN/RBV therapy. The study protocol followed the ethical guidelines established in accordance with the 2004 Declaration of Helsinki and was approved by the ethics committee of Nippon Medical School Chiba Hokusoh Hospital and Shinmatsudo Central General Hospital. All patients provided written informed consent.

Treatment and definition of virological response

Patients received an s.c. injection of PEG IFN-α-2b (PegIntron; MSD, Tokyo, Japan) at a dose of 1.5 μg/kg per week and p.o. administration of RBV (Rebetol; MSD). The dose of RBV was determined on the basis of bodyweight (600, 800 and 1000 mg/day for <60, 60–80 and >80 kg, respectively) according to the manufacturer's instructions in Japan. The doses were reduced appropriately when a critical adverse event occurred during the treatment course, such as anemia. The treatment period was 48 weeks if the viral load was undetectable 12 weeks after the treatment initiation. However, the treatment course was prolonged to 72 weeks if the viral load became undetectable at 13 weeks or later. Patients who failed to achieve HCV RNA negativity by the end of treatment were considered to have a non-virological response (NVR). Patients in whom treatment was discontinued because of adverse effects or lack of effect were also considered to have an NVR. The patients were followed for 24 weeks after the completion of treatment. SVR was defined as virus-undetectable

status 24 weeks after treatment completion. Patients who exhibited end-of-treatment response but had detectable levels of virus 24 weeks after the completion of treatment were considered to have a relapse.

Laboratory tests

Peripheral blood examination and liver function tests were performed weekly until 8 weeks after treatment initiation and then monthly until 24 weeks after the completion of treatment. For biochemical tests before treatment initiation, data was obtained in the fasting state. Serum levels of 25-hydroxyvitamin D_3 were measured as that of vitamin D, because 25-hydroxyvitamin D_3 is stable in the blood circulation and comprises the major portion of vitamin D in the body. Serum 25-hydroxyvitamin D_3 levels were measured by double-antibody radioimmunoassay (RIA2 antibody assay) at a commercial laboratory (SRL Laboratory, Tokyo, Japan). HCV RNA levels were measured by real-time PCR (COBAS AmpliPrep; Roche Diagnostics, Tokyo, Japan). Gene mutations in the core and NS5A regions of the HCV genome were determined by the direct sequencing method. Core amino acid (a.a.)70 was defined as wild type (arginine) or mutant type (glutamine or histidine). Genomic DNA was extracted from whole blood by using a DNA Isolation kit on a MagNA Pure LC instrument (Roche Diagnostics, Basel, Switzerland). Single nucleotide polymorphisms (SNP) at rs8099917, which is located in the locus adjacent to the interleukin 28B (IL28B) gene on chromosome 19, were determined by real-time PCR with TaqMan SNP Genotyping Assays on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The rs8099917 genotypes were classified into two categories: T/T (major genotype) and non-T/T (minor genotype: T/G or G/G).

Statistical analysis

Fisher's exact test was performed to compare the SVR rates according to IL28B genotype, core a.a.70 substitutions and serum 25-hydroxyvitamin D_3 levels. Logistic regression analysis for univariate comparison was performed to investigate whether each factor influenced SVR and decreased serum 25-hydroxyvitamin D_3 levels. Multiple logistic regression analyses were performed to identify significant independent factors that contributed to SVR and decreased serum 25-hydroxyvitamin D_3 levels. A receiver-operator curve (ROC) was generated to analyze the concentration of serum 25-hydroxyvitamin D_3 levels to most reasonably predict SVR. All statistical analyses were performed with IBM SPSS version 17.0 (IBM Japan, Tokyo, Japan). The level of statistical significance was set at $P < 0.05$.

RESULTS

Contributing factors to achieving SVR

PATIENT CHARACTERISTICS ARE shown in Table 1. The patient group consisted of 73 men and 61 women. The median patient age was 63 years (range, 41–82). Among the 134 patients, 43 (32.1%) achieved SVR, 39 (29.1%) relapsed into viremia and 52 (38.8%) showed NVR (38.8%).

Univariate analysis identified the following factors contributing to SVR: low-density lipoprotein ($P = 0.010$, odds ratio [OR] = 1.026, 95% confidence interval [CI] = 1.006–1.045), albumin level ($P = 0.006$, OR = 4.259, 95% CI = 1.521–11.928), 25-hydroxyvitamin D_3

Table 1 Baseline clinical and demographic characteristics of 134 patients with compensated cirrhosis

Factor	$n = 134$
Age (years), median (range)	63 (41–82)
Sex (men/women)	73/61
History of interferon therapy (naïve/relapse/non-response)	107/20/7
White blood cell count (/μL)	4355 (1800–10 400)
Hemoglobin (g/dL)	13.1 (9.7–17.2)
Platelet count (/mm^3) ×10^3	113 (53–373)
AST (IU/L)	71 (18–398)
ALT (IU/L)	64 (16–362)
γ-GT (IU/L)	57 (17–387)
Albumin (g/dL)	3.5 (2.5–5.0)
Total bilirubin (mg/dL)	0.8 (0.2–2.7)
LDL-C (mg/dL)	82 (34–137)
Triglycerides (mg/dL)	98 (34–235)
Cholinesterase (IU/mL)	208 (46–415)
25-hydroxyvitamin D_3 (ng/mL)	20 (7–45)
Alpha-fetoprotein (ng/mL)	12.1 (2.0–754.7)
Prothrombin time (%)	86.8 (36.5–141)
HCV RNA (log IU/mL)	6.5 (5.0–7.4)
ISDR (0–1/≥2)	99/29
Core a.a.70 (wild type/mutant type)	70/60
Core a.a.91 (wild type/mutant type)	77/53
IL28B (rs8099917) (TT/nonTT)	75/58

Categorical variables are given as number. Continuous variables are given as median (range).
γ-GT, γ-glutamyltransferase; a.a., amino acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; HCV, hepatitis C virus; IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; LDL-C, low-density lipoprotein cholesterol.

Table 2 Univariate logistic regression analyses of baseline factors associated with sustained virological response in patients with cirrhosis

		Univariate		
Factors	Category	OR	95% CI	*P*
LDL-C (mg/dL)	By 10 up	1.026	1.006–1.045	0.010
Albumin (mg/dL)	By 0.1	4.259	1.521–11.928	0.006
25-Hydroxyvitamin D3 (ng/mL)	By 1 up	1.064	1.001–1.132	0.049
ISDR mutation	Mutant type	2.750	1.143–6.620	0.024
Core a.a.70 substitution	Wild type	4.500	1.856–10.910	0.001
IL28B genotype	TT	8.041	3.067–21.081	0.00002

a.a., amino acid; CI, confidence interval; IL28B, interleukin 28B; ISDR, interferon sensitivity determining region; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio.

level ($P = 0.049$, OR = 1.064, 95% CI = 1.001–1.132), the number of mutations at the interferon sensitivity-determining region in NS5A ($P = 0.024$, OR = 2.750, 95% CI = 1.143–6.620), core a.a.70 wild type ($P = 0.001$, OR = 4.500, 95% CI = 1.856–10.910) and IL28B major genotype ($P = 0.00002$, OR = 8.041, 95% CI = 3.067–21.081) (Table 2).

Multivariate analysis identified 25-hydroxyvitamin D_3 level ($P = 0.048$, OR = 1.087, 95% CI = 1.001–1.181) and IL28B major genotype ($P = 0.001$, OR = 8.565, 95% CI = 2.491–29.450) as independent factors contributing to SVR (Table 3).

Relationship between serum 25-hydroxyvitamin D_3 levels and SVR rates

Serum 25-hydroxyvitamin D_3 levels were divided into three groups in accordance with previous reports: deficient (≤20 ng/mL), insufficient (21–29 ng/mL) and sufficient (≥30 ng/mL).[34] The deficient group contained 74 patients (55.2%), the insufficient group contained 50 patients (37.3%) and the sufficient group contained 10 patients (7.5%) (Fig. 1). The SVR rates were 29.7% (22/74) in the deficient group, 38.0% (19/50) in the insufficient group and 20% (2/10) in the sufficient group.

When the cut-off value for serum 25-hydroxyvitamin D_3 levels was 15 ng/mL (sensitivity = 0.91, specificity = 0.33, area under the curve = 0.564), the SVR rates were 11.8% (4/34) in patients with less than 15 ng/mL of serum 25-hydroxyvitamin D_3 and 39.0% (39/100) in patients with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 ($P = 0.006$; Fig. 2).

Relationship between serum 25-hydroxyvitamin D_3 levels and core a.a.70 substitutions and SVR rates

Among patients with core a.a.70 wild type, the SVR rates were 51.8% (29/56) in those with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 and 7.1% (1/14) in those with less than 15 ng/mL of serum 25-hydroxyvitamin D_3 ($P = 0.002$; Fig. 3). Among patients with core a.a.70 mutant type, the SVR rates were 19.5% (8/41) in those with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 and 10.5% (2/19) in those with less than 15 ng/mL of

Table 3 Multivariate logistic regression analysis of baseline factors associated with sustained virological response in patients with cirrhosis

		Multivariate		
Factors	Category	OR	95% CI	*P*
25-Hydroxyvitamin D3 (ng/mL)	By 1 up	1.087	1.001–1.181	0.048
ISDR mutation	Mutant type	2.761	0.920–8.290	0.070
Core a.a.70 substitution	Wild type	3.052	0.994–9.374	0.051
IL28B genotype	TT	8.565	2.491–29.450	0.001

Multivariate analysis was performed with four selected factors significantly associated with sustained virological response by univariate analysis.
a.a., amino acid; CI, confidence interval; IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; OR, odds ratio.

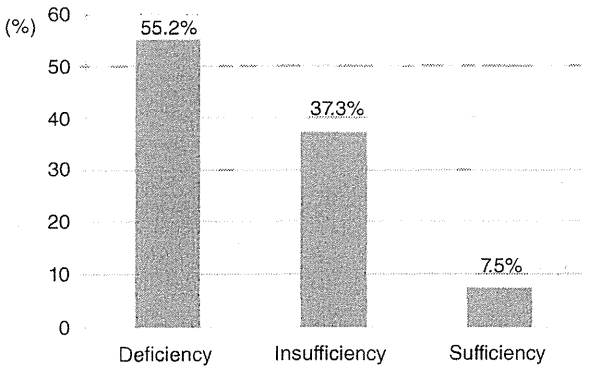

Figure 1 Categories of serum 25-hydroxyvitamin D_3 levels in the cirrhotic patient cohort. Serum 25-hydroxyvitamin D_3 levels were divided into three groups: deficient (≤20 ng/mL), insufficient (21–29 ng/mL) and sufficient (≥30 ng/mL).

serum 25-hydroxyvitamin D_3 ($P = 0.480$; Fig. 3). From the viewpoint of serum 25(OH)D_3 levels, a significant difference in the SVR rate was noted among patients with core a.a.70 wild type but not among patients with core a.a.70 mutant type.

Relationship between serum 25-hydroxyvitamin D_3 levels and IL28B genotype and SVR rates

Among patients with IL28B major genotype TT, the SVR rates were 56.9% (33/58) in those with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 and 23.5% (4/17) in those with less than 15 ng/mL of serum 25-hydroxyvitamin D_3 ($P = 0.026$; Fig. 4). Among patients with IL28B minor genotype non-TT, the SVR rates were 14.6% (6/41) in those with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 and 0% (0/17) in those with less than 15 ng/mL of serum 25-hydroxyvitamin D_3 ($P = 0.166$; Fig. 4). From the viewpoint of serum 25(OH)D_3 levels, a significant difference in the SVR rate was noted among IL28B TT patients,

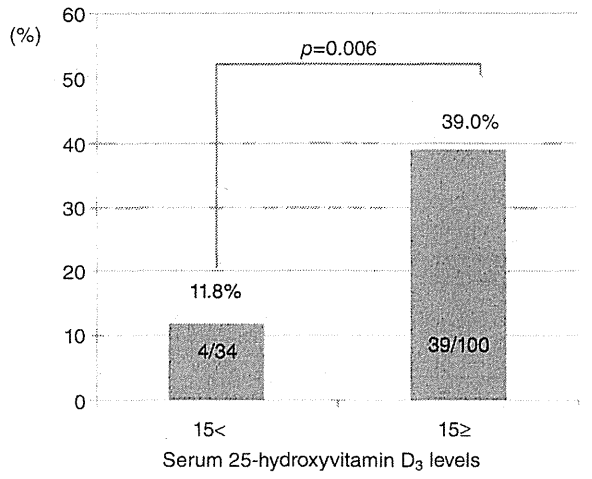

Figure 2 Comparison of sustained virological response (SVR) rates according to serum 25-hydroxyvitamin D_3 levels. The cut-off values of serum 25-hydroxyvitamin D_3 levels that predict SVR were determined from the receiver–operator curve analysis and divided into two groups: <15 ng/mL and ≥15 ng/mL.

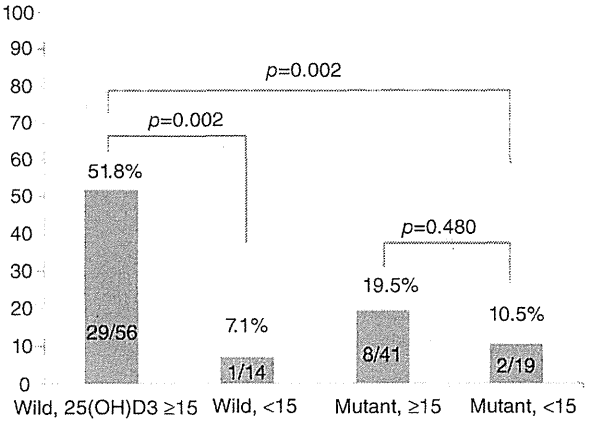

Figure 3 Comparison of sustained virological response (SVR) rates according to core amino acid (a.a.)70 substitutions and serum 25-hydroxyvitamin D_3 levels. Core a.a.70 substitutions were classified into two categories: wild type and mutant type.

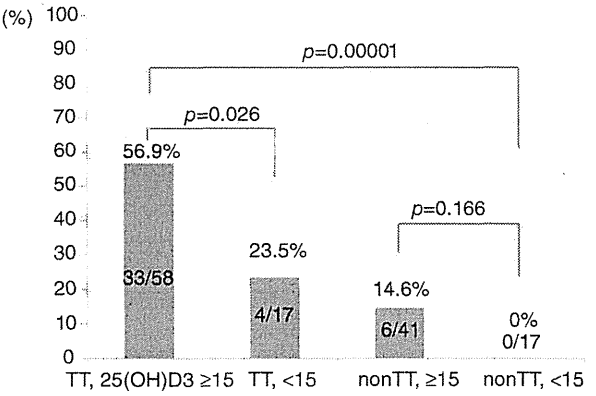

Figure 4 Comparison of sustained virological response (SVR) rates according to IL28B genotype (rs8099917) and serum 25-hydroxyvitamin D_3 levels. The IL28B (rs8099917) genotypes were classified into two categories: TT (major genotype) and non-TT (minor genotype: TG or GG).

Table 4 Univariate and multivariate logistic regression analyses of factors associated with decrease in serum 25-hydroxyvitamin D_3 levels in the 134 cirrhotic patients

		Univariate			Multivariate		
Factors	Category	OR	95% CI	*P*	OR	95% CI	*P*
Sex	Female	2.423	1.090–5.388	0.030			
Platelet count ($/mm^3$) $\times 10^3$	By 10 down	1.132	1.014–1.264	0.028			
Total bilirubin (mg/dL)	By 0.1 up	3.571	1.397–9.091	0.008			
Albumin (mg/dL)	By 0.1 down	7.781	2.582–23.443	0.0003	5.869	1.152–29.885	0.033
Cholinesterase (IU/mL)	By 10 down	1.001	1.001–1.013	0.020			
Creatinine (mg/dL)	By 0.1 up	12.560	1.167–134.958	0.037			
Prothrombin time (%)	By 10 down	1.031	1.002–1.062	0.036			

Multivariate analysis was performed with factors significantly associated with decreased 25-hydroxyvitamin D_3 levels by univariate analysis.
CI, confidence interval; OR, odds ratio.

whereas it was not observed among non-TT patients. In particular, the SVR rate of IL28B TT patients with 15 ng/mL or more of serum 25-hydroxyvitamin D_3 was significantly higher as compared to other patient subgroups (Fig. 4). By contrast, non-TT patients with less than 15 ng/mL of serum 25-hydroxyvitamin D_3 responded very poorly to the treatment.

Factors associated with low serum 25-hydroxyvitamin D_3 levels

The factors that influenced serum 25-hydroxyvitamin D_3 levels in cirrhotic patients were also investigated. Univariate analysis showed that the following factors were significantly associated with decreased levels of 25-hydroxyvitamin D_3: female sex ($P = 0.030$, OR = 2.423, 95% CI = 1.090–5.388), low platelet count ($P = 0.028$, OR = 1.132, 95% CI = 1.014–1.264), high total bilirubin level ($P = 0.008$, OR = 3.571, 95% CI = 1.397–9.091), low albumin level ($P = 0.0003$, OR = 7.781, 95% CI = 2.582–23.443), low cholinesterase level ($P = 0.020$, OR = 1.001, 95% CI = 1.001–1.013), high serum creatinine level ($P = 0.037$, OR = 12.560, 95% CI = 1.167–134.958) and low prothrombin time ($P = 0.036$, OR = 1.031, 95% CI = 1.002–1.062) (Table 4). Multivariate analysis identified albumin level ($P = 0.033$, OR = 5.869, 95% CI = 1.152–29.885) as the only independent factor associated with decreased levels of 25-hydroxyvitamin D_3 (Table 4).

DISCUSSION

THE CONTRIBUTION OF serum 25-hydroxyvitamin D_3 levels to the response to antiviral treatment or the association with liver fibrosis has been previously reported for patients with chronic hepatitis C infection.[30–33,38] However, these previous studies included exclusively Caucasian patients with various stages of liver fibrosis and different HCV genotypes. The present study was the first report to investigate serum 25-hydroxyvitamin D_3 status in HCV genotype 1b-mono-infected Asian patients with compensated cirrhosis and to clarify the influence of vitamin D on the outcome of PEG IFN/RBV combination therapy.

The association between the serum 25-hydroxyvitamin D_3 levels and SVR rates to PEG IFN/RBV combination therapy has been previously examined. In some studies, patients with higher serum 25-hydroxyvitamin D_3 levels were more likely to achieve SVR;[30,32] however, in another study, patients who had achieved SVR had lower serum 25-hydroxyvitamin D_3 levels.[38] The present study showed that it was difficult for cirrhotic patients with low serum 25-hydroxyvitamin D_3 levels to achieve SVR.

Advanced fibrosis or cirrhosis has a negative impact on the treatment outcome in PEG IFN/RBV therapy and even in protease inhibitor-based combination therapy.[7,20,22,23] Indeed, the SVR rate was only 32.1% in this cirrhotic patient cohort. The refractory reason is that cirrhotic patients are commonly characterized by cytopenia resulting from hypersplenism and/or impaired thrombopoiesis. Cytopenia results in reduced adherence to the treatment and/or premature treatment cessation. The mean doses of PEG IFN-α-2b and RBV in this study were 1.42 μg/kg per week (range, 0.74–1.67) and 9.05 mg/kg per day (range, 1.1–14.0), respectively, which were less than the generally recommended doses of 1.5 μg/kg per week and 13 mg/kg per day, respectively.[39] Another possible reason is that patients with

HCV-related cirrhosis are commonly elderly and thus less tolerant of the treatment, resulting in an inadequate treatment regimen.[40] Additionally, low vitamin D activity in cirrhotic patients may negatively influence the efficacy of treatment. Vitamin D supplementation may improve the poor response to treatment in cirrhotic patients.

Multivariable regression analysis in the present study identified IL28B genotype and 25-hydroxyvitamin D_3 levels as significant independent factors associated with SVR. Despite the presence of cirrhosis, IL28B major genotype patients had the SVR rate of 49.3%. In contrast, the SVR rate was very low (11.1%) in patients with IL28B minor genotype. Moreover, the present study found that serum vitamin D levels were also important for cirrhotic patients to achieve SVR.

Serum 25-hydroxyvitamin D_3 levels are often divided into categories based on several proposed criteria. According to the criteria proposed by Holick *et al.*,[34] 92.5% of the cirrhotic patients in the present study were either deficient or insufficient in serum 25-hydroxyvitamin D_3 concentration. According to previous reports, the serum 25-hydroxyvitamin D_3 levels of 46% of chronic hepatitis C patients were less than 20 ng/mL (deficient)[30,32] and those of healthy individuals were an average of 28.4 ng/mL (range, 9.5–54.8) with only 14.4% of them having deficient levels.[32] In the present study, 55.2% of the cirrhotic patients had less than 20 ng/mL of serum 25-hydroxyvitamin D_3. In patients with compensated cirrhosis, it is unclear which factors influence low serum 25-hydroxyvitamin D_3 levels. The present study identified several factors that were associated with decreased serum 25-hydroxyvitamin D_3 levels, especially as multivariate analysis identified serum albumin level as the only independent factor associated with decreased serum 25-hydroxyvitamin D_3 levels. Serum albumin level reduction may reflect the severity of liver fibrosis. Previously reported causes of vitamin D deficiency are aging, obesity, liver failure, nephrotic syndrome, chronic kidney disease and lack of sunlight.[34]

In a comparison of the SVR rate with serum 25-hydroxyvitamin D_3 levels of 15 ng/mL as the cut-off value obtained from the ROC, the SVR rate was lower in cirrhosis patients with less than 15 ng/mL of serum 25-hydroxyvitamin D_3. Based on these findings, it may be possible to designate 15 ng/mL as the cut-off value for deficient/insufficient serum 25-hydroxyvitamin D_3 levels in cirrhotic patients who receive PEG IFN/RBV combination therapy. Surprisingly, the SVR rate in patients with IL28B minor genotype and less than 5 ng/mL of serum 25-hydroxyvitamin D_3 was extremely poor (0%, 0/17). Improving the treatment outcomes of cirrhotic patients with such decreased serum 25-hydroxyvitamin D_3 levels will be crucial. These findings suggest that the SVR rate may be predicted by using the combination of serum 25-hydroxyvitamin D_3 levels and IL28B genotype,[41–43] which is currently the most influential factor in the therapeutic outcome of IFN-based treatment of chronic hepatitis C.

The addition of vitamin D to PEG IFN/RBV combination therapy was reported to improve the treatment outcome for chronic hepatitis C patients.[44] *In vitro*, vitamin D appears to play an important role in immune activation such as enhancement of the antigen-presenting capacity of dendritic cells and the cytotoxic activity of natural killer cells.[45,46] Moreover, it was reported that $1.25(OH)_2D_3$ and 25-hydroxyvitamin D_3 demonstrated a direct anti-HCV effect.[35,36] However, there are no reports of vitamin D administration to patients with compensated cirrhosis. The results of the present study encourage us to investigate the efficacy and roles of vitamin D in HCV-related cirrhosis. It is possible that supplementary vitamin D may improve treatment efficacy, especially in patients with compensated cirrhosis who have low 25-hydroxyvitamin D_3 levels.

As shown in the present investigation, it is thought that treatment should be aggressively introduced in patients with core a.a.70 wild type and/or IL28B major genotype with 15 ng/mL or more of serum 25-hydroxyvitamin D_3. On the other hand, more effective drug therapy is needed for patients in whom the response is poor, such as patients with core a.a.70 mutant type and/or IL28B minor genotype with very low serum 25-hydroxyvitamin D_3 levels.

The limitations of the present study include its small sample size and the fact that the serum 25-hydroxyvitamin D_3 levels were measured in various seasons. The serum 25-hydroxyvitamin D_3 levels are higher in summer and autumn than in winter and spring.[38] Additionally, although genes related to vitamin D levels, CYP2R1 (rs1993116, rs10741657), GC (rs2282679) and DHCR7 (rs7944926, rs12785878), have been reported,[47,48] we did not investigate these factors. Also, although an association between the serum vitamin D levels and insulin resistance has been reported,[34] it was not evaluated as a background factor in our study.

In conclusion, serum 25-hydroxyvitamin D_3 levels were decreased overall in patients with compensated cirrhosis. 25-Hydroxyvitamin D_3 levels were identified

as independent factors contributing to SVR. Stratified analyses according to core a.a.70 substitutions and IL28B genotype suggested that serum 25-hydroxyvitamin D_3 levels critically influence the outcome of PEG IFN/RBV combination therapy for HCV genotype 1b patients with compensated cirrhosis.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Baseline factors and very early viral response (week 1) for predicting sustained virological response in telaprevir-based triple combination therapy for Japanese genotype 1b chronic hepatitis C patients: a multicenter study

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Abstract
Background Genetic polymorphisms near *Interleukin 28B* (*IL28B*) (rs8099917) and a rapid virological response (RVR) have been reported as predictors for a sustained virological response (SVR) to telaprevir (TVR)-based triple combination therapy. However, the association between SVR and viral kinetics earlier than week 4 after initiation of therapy remains unclear. Thus, we evaluated the SVR prediction ability of baseline factors and reduced hepatitis C virus (HCV) RNA levels at week 1 after the initiation of TVR-based therapy in Japanese genotype-1b chronic hepatitis C (CHC) patients.

Methods A total of 156 Japanese CHC patients received a 24-week regimen of TVR-based therapy. Baseline factors and reduction in HCV RNA levels at weeks 1 and 4 after the initiation of therapy were analyzed for SVR prediction.
Results Multiple logistic regression analysis for SVR in TVR-based therapy identified the *IL28B* TT genotype, a reduction of $\geq$4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1, RVR, and treatment-naïve/relapse. Whereas the SVR rate was higher than 90 % regardless of the reduction in HCV RNA levels at week 1 in patients with the TT genotype, a reduction of $\geq$4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1 was the strongest predictor of SVR in patients with the non-TT genotype, as determined by multiple logistic regression analysis ($P = 0.0043$).
Conclusions The *IL28B* TT genotype is the most important baseline factor for predicting SVR, and a $\geq$4.7 $\log_{10}$IU/mL reduction in HCV RNA at week 1 is a useful very early on-treatment predictor of SVR, especially in the non-TT genotype.

Keywords Chronic hepatitis C · Reduction in HCV RNA at week 1 · Telaprevir · *IL28B*

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Introduction

In 2011, the first-generation direct-acting antiviral agents telaprevir (TVR) and boceprevir (BOC) were approved for treatment of chronic hepatitis C (CHC) patients with hepatitis C virus (HCV) genotype 1 in the United States (US), Canada, and the European Union (EU). Triple combination therapy with TVR or BOC, PEG-interferon (PEG-IFN), and ribavirin (RBV) is the current standard of care for genotype 1 CHC patients [1]. In Japan, TVR, which is a nonstructural (NS) 3/4A serine protease inhibitor, was

approved in September 2011 and has been marketed since November 2011. In treatment-naïve genotype 1 CHC patients, TVR-based triple combination therapy for a shortened period was reported to remarkably improve the rate of sustained virological response (SVR) compared with PEG-IFN and RBV alone [2–4]. In treatment-experienced patients, the effect of TVR-based triple combination therapy reportedly depends on the response to PEG-IFN and RBV combination therapy [5–16].

Pivotal genome-wide association studies have found that genetic variations near the interleukin 28B (*IL28B*) gene (rs8099917 and rs12979860) are strongly associated with the treatment outcome of PEG-IFN and RBV combination therapy [17–19]. We previously confirmed that the *IL28B* single-nucleotide polymorphism (SNP) genotype was the strongest factor contributing to SVR in PEG-IFN and RBV combination therapy [20–23]. These genetic variations appear to be strong predictors of SVR to a 24-week regimen of TVR-based triple therapy, as well as PEG-IFN and ribavirin combination therapy [7, 11, 14–16, 24].

Two guidelines for treatment of genotype 1 CHC patients, which were based on the results of the clinical trials of a 24-week regimen of TVR-based triple therapy for Japanese patients [4, 10], provided recommendations for patient selection for TVR-based therapy [25]. Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis published by the Ministry of Health, Labour and Welfare of Japan, and Japan Society of Hepatology guidelines provided recommendations of a 24-week regimen of TVR-based triple therapy for Japanese genotype 1 CHC patients. These recommendations are based on baseline factors, including patient's age, sex, *IL28B* genotype, core amino acid substitution at position 70, previous treatment history and response, stage of fibrosis, viral load, and baseline hemoglobin level [25, 26].

In addition to the baseline predictive factors, changes in HCV RNA levels after the start of therapy are predictive for treatment outcomes. A rapid virological response (RVR), defined as undetectable serum HCV RNA at week 4 after the start of therapy, and an extended rapid virological response, defined as undetectable serum HCV RNA at both weeks 4 and 12, were also reported as significant predictors of TVR-based treatment outcome [7, 11, 15, 16, 27]. However, the association between SVR and viral kinetics earlier than 4 weeks after initiating TVR-based triple combination therapy remains unclear. RVR was achieved in only approximately 3–11 % of cases receiving PEG-IFN and RBV combination therapy [2, 3, 6, 27, 28]. In contrast, RVR was achieved in approximately 61–84 % of cases receiving TVR-based triple combination therapy [2–6, 8, 10, 11, 15, 16, 27, 29]. It is therefore important to determine whether viral kinetics earlier than week 4 after the start of therapy is predictive for SVR in TVR-based triple combination therapy?

TVR-based triple combination therapy remarkably improves the SVR rate in CHC patients with the difficult-to-treat HCV genotype 1. However, some patients still fail to achieve SVR. Adverse events occurred more frequently and were more severe in patients treated with TVR-based therapy than in those treated with PEG-IFN and RBV alone [2–6]. Additionally, TVR-based therapy is expensive. In clinical practice, the determination of predictive factors of successful treatment outcome as early as possible is necessary for preventing unnecessary treatment in addition to physical and economic burden. Thus, in this prospective, multicenter study, we evaluated the clinical relevance of baseline predictors and the reduction in HCV RNA levels at week 1 after starting therapy for predicting SVR in a 24-week regimen of TVR-based triple combination therapy for genotype 1b CHC patients.

Methods

Patients, treatment, and definition of outcomes

Between December 2011 and September 2012, 156 Japanese genotype 1b monoinfected CHC patients were enrolled in this multicenter study at Shinmatsudo Central General Hospital, Kurume University School of Medicine, Kagawa Prefectural Central Hospital, Jikei University School of Medicine Kashiwa Hospital, and Ogaki Municipal Hospital. The inclusion criteria for the study included persistently positive sera for HCV RNA for > 6 months as determined using the quantitative real-time PCR method (COBAS AmpliPrep/COBAS TaqMan HCV test, Roche Diagnostics, Tokyo, Japan), HCV RNA $\geq$5.0 $\log_{10}$IU/mL in treatment-naïve patients, age of 18–75 years, and body weight >35 kg at the time of entry into the study. Exclusion criteria were: (1) decompensated cirrhosis; (2) positive for hepatitis B surface antigen or antibodies against human immunodeficiency virus; (3) previous or current development of hepatocellular carcinoma; (4) co-existence of other liver diseases, such as autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson disease, and alcoholic liver disease; (5) renal disease or creatinine clearance $\leq$ 50 mL/min at baseline; (6) hemoglobin level < 12 g/dL, white blood cell count < 2000/μL, neutrophil count < 1500/μL, and platelet count < 8.0 × 10^4/μL at baseline; (7) depression, schizophrenia or its history, or history of suicide attempts, (8) pregnancy in progress or planned for either partner during the study period. For 114 of 156 (73.1 %) patients, liver biopsy was conducted within 12 months of enrollment. The presence or absence of cirrhosis was established according to the Metavir score

Table 1 Patient profiles

Number of patients	156
Sex (male/female)	78/78
Age (years)	58.4 ± 10.3
Body weight (kg)	61.8 ± 12.8
Body mass index (kg/m^2)	23.7 ± 3.5
Absence or presence of cirrhosis (non-cirrhosis/cirrhosis)	120/36
Response to previous treatment (treatment-naïve/relapsers/partial responders/null responders)	78/50/14/14
rs8099917 (TT/TG/GG)	106/48/2
Core amino acid substitution 70 (wild-type/mutant-type)	97/59
ISDR of NS5A (wild-type/non-wild-type)	138/18
White blood cells (/μL)	4972 ± 1542
Hemoglobin (g/dL)	14.2 ± 1.4
Platelets (×10^4/μL)	17.1 ± 5.6
Aspartate aminotransferase I (U/L)	54 ± 36
Alanine aminotransferase I (U/L)	60 ± 50
Gamma-glutamyl-transpeptidase I (U/L)	59 ± 68
Albumin (g/dL)	4.2 ± 0.3
Total cholesterol (mg/dL)	173 ± 31
Low-density lipoprotein cholesterol (mg/dL)	103 ± 28
Alpha-fetoprotein (ng/mL)	10.9 ± 20.7
HCV RNA ($\log_{10}$IU/mL)	6.4 ± 0.9
Initial dose of PEG-IFN (μg/kg)	1.5 ± 0.2
Initial dose of ribavirin (mg/kg)	11.2 ± 1.6
Initial daily dose of telaprevir (1500/2250 mg)	84/72
Administration intervals of telaprevir (q8/q12 h)	96/60

Data are expressed as numbers or mean ± standard deviation

ISDR interferon sensitivity-determining region, *HCV* hepatitis C virus, *PEG-IFN* PEG-interferon

[30]. For the remaining 42 patients, the presence or absence of cirrhosis was evaluated using ultrasonography and/or computed tomography findings.

Patient profiles are shown in Table 1. In this study, all treatment-experienced patients were treated with PEG-IFN and ribavirin combination therapy. Patients in this study were categorized as relapsers (HCV RNA undetectable at the end of treatment and then positive in follow-up), partial responders (≥2 $\log_{10}$IU/mL reduction in HCV RNA at week 12 but never undetectable), or null responders (< 2 $\log_{10}$IU/mL reduction in HCV RNA at week 12). In this study, partial responders and null responders were analyzed as non-responders.

All patients were treated with PEG-IFN-α-2b, RBV, and TVR triple therapy. TVR (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered every 8 h after meals (q8 h) at 500 or 750 mg, or every 12 h after meals (q12 h) at 750 or 1125 mg. The initial daily dose of TVR (1500 or 2250 mg per day) and administration intervals (q8 or q12 h) were determined by each attending physician according to age, sex, body weight, and hemoglobin level. PEG-IFN-α-2b (PEG-Intron, MSD, Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 μg/kg per week. The RBV (Rebetol, MSD, Tokyo, Japan) dose was adjusted by body weight (600 mg for < 60 kg; 800 mg for ≥60 to <80 kg; and 1000 mg for ≥80 kg; in the case of hemoglobin < 13 g/dL at start of therapy, the RBV dose was reduced by 200 mg), based on the guidelines of the Ministry of Health, Labor and Welfare of Japan, and the drug was administered orally after breakfast and dinner. Triple therapy was given for 12 weeks, followed by an additional 12 weeks of PEG-IFN-α-2b and RBV combination therapy (T12PR24). Administration of each drug was appropriately reduced or withdrawn when a serious adverse event was suspected to be developing or if a serious adverse event occurred during the course of treatment. Regardless of adverse events, treatment was stopped for patients who had HCV RNA > 3 $\log_{10}$IU/mL at week 4 or detectable HCV RNA at week 12, or those showing a > 2 $\log_{10}$IU/mL increase in HCV RNA levels from the lowest level during therapy, because of the low likelihood of achieving SVR and the high risk of developing antiviral resistance.

Adherence to PEG-IFN was calculated based on the initial weekly dose, and that to RBV was calculated based on the initial daily dose. Adherence to TVR was defined as 100 % when 2250 mg was given each day for 12 weeks, which is the recommended daily dose.

The virological response was analyzed on an intent-to-treat basis. The successful endpoint of treatment was SVR for patients showing undetectable HCV RNA for 24 weeks after cessation of treatment. Patients were defined as relapse when HCV RNA levels became undetectable until the end of treatment, but became positive during the follow-up period. Patients were defined as at viral breakthrough when HCV RNA became undetectable during the treatment period, but then became positive again before the end of the treatment period. Patients were defined as non-response when HCV RNA was detectable throughout the treatment period. Furthermore, RVR was defined as undetectable HCV RNA at week 4 after starting treatment.

All patients provided written informed consent. This study protocol was prepared following ethics guidelines established in conformity with the 2008 Declaration of Helsinki, and was approved by the Ethics Committee of each participating institution.

Measurement of HCV RNA, and amino acid substitution in the core and NS5A regions of HCV genotype 1b

HCV genotype was determined by direct sequencing followed by phylogenic analysis of the NS5B region [31]. The

antiviral effects of the therapy on HCV were assessed by measuring serum HCV RNA levels. In this study, HCV RNA levels were evaluated at baseline; weeks 1, 4, 8, 12, 16, 20, and 24 during treatment; and once every 4 weeks after cessation of treatment. HCV RNA levels were determined using the COBAS AmpliPrep/CABAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). The linear dynamic range of the assay was 1.2–7.8 $\log_{10}$IU/mL, and undetectable samples were defined as negative.

Core amino acid substitution at position 70 was determined according to a previously described method [32, 33]. Core amino acid substitution at position 70 was defined as wild-type (arginine) or mutant-type (glutamine or histidine). Additionally, substitutions at amino acids 2290–2248 of the NS5A region [interferon-sensitivity determining region (ISDR)] were determined using a previously described method [34]. Amino acid substitutions in ISDR were defined as wild-type (0 or 1) or non-wild-type ($\geq$2).

Single-nucleotide polymorphism genotyping

Genomic DNA was extracted from whole blood using the MagNA Pure LC and a DNA Isolation Kit (Roche Diagnostics). The genetic polymorphism rs8099917, near the *IL28B* gene [17, 18], was genotyped by real-time detection PCR using the TaqMan SNP Genotyping Assays and the 7500Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The rs8099917 genotypes were classified into 2 categories, including TT (major genotype) and non-TT genotype (minor genotype: TG or GG).

Statistical analysis

Continuous variables are expressed as the mean and standard deviation. Categorical data were analyzed using the Chi-squared test and Fisher's exact test, while continuous data were analyzed using the non-parametric Mann–Whitney *U* test. Univariate and multiple logistic regression analyses were used to identify factors that significantly contributed to SVR. The odds ratios (OR) and 95 % confidence intervals (95 % CI) were also calculated. All *P* values for statistical tests were 2-tailed, and values of < 0.05 were considered statistically significant. Variables that achieved statistical significance ($P < 0.05$) according to univariate analysis were entered into multiple logistic regression analyses to identify significant independent predictive factors of SVR.

Receiver-operating characteristics (ROC) analyses were performed to determine cut-off values for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for predicting SVR. Statistical analysis was performed using SPSS version 17.0 (IBM-SPSS, Chicago, IL, USA).

Results

Characteristics of patients and treatment outcome

Table 1 summarizes the characteristics of the patients. In total, 78 patients (50.0 %) were treatment-naïve, and 78 patients (50.0 %) were treatment-experienced with PEG-IFN and RBV. The *IL28B* TT genotype was present in 67.9 % (106 of 156) of the patients. The proportion of patients with cirrhosis was 23.1 % (36 of 156). In total, 72 patients (46.1 %) were treated with TVR at 2250 mg/day, and 84 patients (53.9 %) were treated with TVR at 1500 mg/day. In terms of dosing schedule, 96 patients (61.5 %) were treated q8 h, and 60 patients (38.5 %) were treated q12 h.

Regarding treatment outcomes, 125 patients (80.1 %) achieved SVR; 14 patients (9.0 %) relapsed. 12 patients (7.7 %) showed viral breakthrough, and the remaining five patients (3.2 %) showed non-response. For the *IL28B* SNP genotypes, among the 106 patients with the TT genotype, 102 (96.2 %) achieved an SVR, and one (0.9 %) relapsed; two (1.9 %) showed viral breakthrough, and one (0.9 %) showed non-response Among the 50 patients with the non-TT genotype, 23 (46.0 %) achieved an SVR; 13 (26.0 %) relapsed. Ten (20.0 %) showed viral breakthrough, and four (8.0 %) showed non-response. Thus, the SVR rate was significantly higher in patients with the TT genotype than in those with the non-TT genotype [102 of 106 patients (96.2 %) vs. 23 of 50 (46.0 %), $P < 0.0001$] (Fig. 1). According to previous treatment response, among the 78 treatment-naïve patients, 66 (84.6 %) achieved an SVR; five (6.4 %) relapsed. Five (6.4 %) showed viral breakthrough, and two (2.6 %) showed non-response. Among the 50 relapsers, 48 (96.0 %) achieved an SVR; one (2.0 %) relapsed, and one (2.0 %) showed viral breakthrough. Among the 14 partial responders, eight (57.1 %)

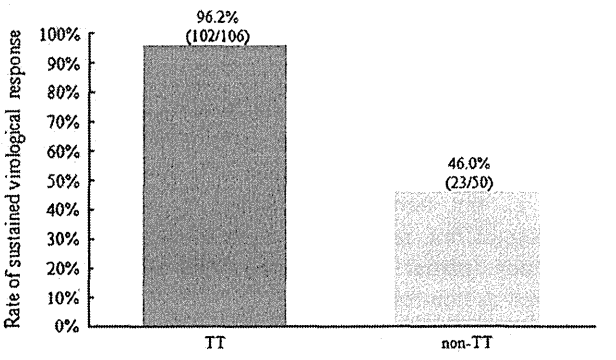

Fig. 1 Rate of sustained virological response according to the *IL28B* (rs8099917) genotype. The rate of sustained virological response was significantly higher in patients with the TT genotype than in those with the non-TT genotype ($P < 0.0001$)

achieved an SVR; four (28.6 %) relapsed, and two (14.3 %) showed viral breakthrough. Among the 14 null responders, three (21.4 %) achieved an SVR; four (28.6 %) relapsed. Four (28.6 %) showed viral breakthrough, and three (21.4 %) showed non-response. The SVR rate was significantly different across the four categories of previous treatment response ($P < 0.0001$). In particular, the SVR rate was significantly lower in non-responders than in treatment-naïve patients or relapsers [114 of 128 patients (89.1 %) vs. 11 of 28 patients (39.3 %), $P < 0.0001$].

Six patients stopped triple therapy before 12 weeks. The reasons were loss of appetite in three patients, severe anemia in one patient, systemic skin flare in one patient, and viral breakthrough in one patient. Among the six patients, five (83.3 %) with the IL28B TT genotype achieved an SVR, and one (16.7 %) with the non-TT genotype who showed viral breakthrough did not achieve an SVR.

Association between reduced serum HCV RNA levels at week 1 after starting therapy and SVR

ROC curve analysis was performed in 156 patients, to evaluate the association between reduced serum HCV RNA levels at week 1 after starting therapy and SVR. The area under the ROC curve was 0.754, and the best cut-off value was calculated as 4.7 $\log_{10}$IU/mL (Fig. 2). The SVR rate was significantly higher in patients with a reduction of $\geq$4.7 $\log_{10}$IU/mL at week 1 than in those with a reduction of <4.7 $\log_{10}$IU/mL [65 of 68 patients (95.6 %) with $\geq$4.7 $\log_{10}$IU/mL vs. 60 of 88 patients (68.2 %) with <4.7 $\log_{10}$IU/mL, $P < 0.0001$]. All four patients with the TT genotype who failed to show an SVR had a reduction of

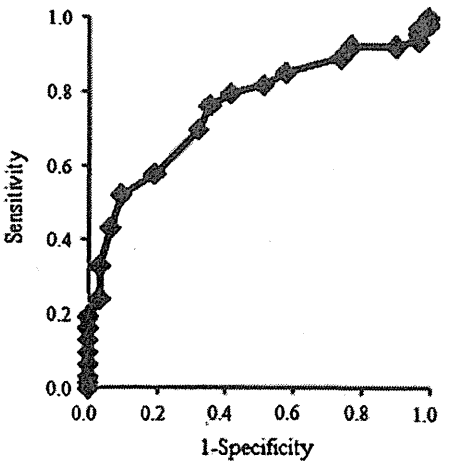

Fig. 2 Receiver operating characteristic (ROC) analysis for prediction of a sustained virological response according to the reduction in serum HCV RNA levels at week 1 after the start of therapy. The area under the ROC curve was 0.754

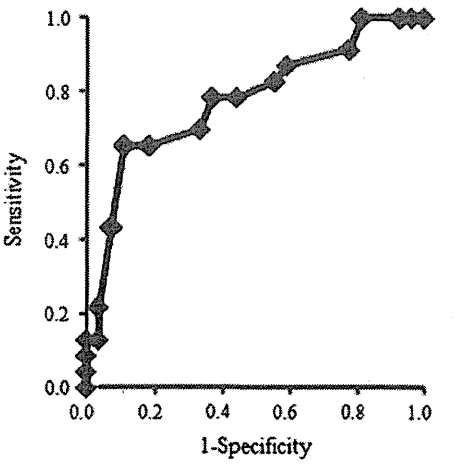

Fig. 3 Receiver operating characteristics (ROC) analysis for prediction of a sustained virological response in the *IL28B* (rs8099917) non-TT genotype according to the reduction in serum HCV RNA levels at week 1 after the start of therapy. The area under the ROC curve was 0.777

<4.7 $\log_{10}$IU/mL at week 1 (4.1 $\log_{10}$IU/mL in treatment-naïve patient, 3.8 $\log_{10}$IU/mL in partial responder, 3.7 $\log_{10}$IU/mL in null responder, and 4.6 $\log_{10}$IU/mL in null responder, respectively).

Patients with the *IL28B* TT genotype presented an extremely high SVR rate. Therefore, the ROC analysis focused on 50 patients with the *IL28B* non-TT genotype. The area under the ROC curve was 0.777, and the best cut-off value was calculated as 4.7 $\log_{10}$IU/mL, which was similar to the value calculated for all patients (Fig. 3). The SVR rate was significantly higher in patients with a reduction of $\geq$4.7 $\log_{10}$IU/mL at week 1 than in those with a reduction of <4.7 $\log_{10}$IU/mL [15 of 18 patients (83.3 %) with a reduction of $\geq$4.7 $\log_{10}$IU/mL vs. 8 of 32 patients (25.0 %) with a reduction of <4.7 $\log_{10}$IU/mL, $P = 0.0001$].

Predictive factors associated with SVR

According to the univariate analysis, the following factors were associated with SVR: treatment-naïve patients or relapsers ($P < 0.0001$); *IL28B* TT genotype ($P < 0.0001$); higher white blood cell count ($P = 0.0098$), platelet count ($P = 0.0299$), total cholesterol level ($P = 0.0467$), and low-density lipoprotein cholesterol level ($P = 0.0080$); lower gamma glutamyl transpeptidase level ($P = 0.0014$) and alpha-fetoprotein level ($P = 0.0175$); core amino acid substitution at position 70 of the wild-type ($P = 0.0010$); achievement of RVR ($P < 0.0001$); and reduction of $\geq$4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1 ($P = 0.0003$). Multiple logistic regression analysis identified the following four independent factors: *IL28B* TT

Table 2 Factors associated with sustained virological response

Variable	Simple			Multiple		
	OR	95 % CI	*P* value	OR	95 % CI	*P* value
Host-related factor						
Age (year)	1.00	0.96–1.04	0.9488			
Sex male vs. female	1.23	0.56–2.72	0.6019			
Body weight (kg)	0.99	0.97–1.02	0.7195			
Body mass index (kg/m^2)	0.97	0.87–1.08	0.5494			
Cirrhosis absence vs. presence	2.20	0.93–5.18	0.0711			
Treatment-naïve or relapsers vs. non-responders	12.58	4.92–32.21	< 0.0001	5.58	1.28–24.38	0.0224
rs8099917 TT vs. non-TT	29.93	9.54–93.92	< 0.0001	73.65	11.28–480.93	< 0.0001
White blood cells (/μL)	1.00	1.00–1.00	0.0098			
Hemoglobin (g/dL)	1.16	0.87–1.55	0.3196			
Platelets ($\times 10^4$/μL)	1.09	1.01–1.18	0.0299			
Aspartate aminotransferase I(U/L)	0.99	0.98–1.00	0.1034			
Alanine aminotransferase I(U/L)	1.00	0.99–1.00	0.3574			
Gamma-glutamyl-transpeptidase I(U/L)	0.99	0.99–1.00	0.0014			
Albumin (g/dL)	3.14	0.65–15.22	0.1548			
Total cholesterol (mg/dL)	1.01	1.00–1.03	0.0467			
Low-density lipoprotein-cholesterol (mg/dL)	1.03	1.01–1.05	0.0080			
Alpha-fetoprotein (ng/mL)	0.97	0.95–1.00	0.0175			
Virus-related factor						
HCV RNA ($\log_{10}$IU/mL)	1.01	0.64–1.60	0.9695			
Core amino acid substitution 70 wild-type vs. mutant-type	4.01	1.75–9.17	0.0010			
ISDR of NS5A non-wild-type vs. wild type	2.13	0.46–9.79	0.3319			
Treatment-response factor						
Rapid virological response + vs. −	9.43	3.89–22.87	< 0.0001	12.59	2.33–69.97	0.0032
Reduction in HCV RNA level at week 1 ≥4.7 $\log_{10}$/mL vs. <4.7 $\log_{10}$IU/mL	10.11	2.92–34.99	0.0003	18.99	2.74–131.63	0.0029
Treatment-related factor						
Administration intervals of telaprevir q8 vs. q12 h	1.20	0.54–2.67	0.6572			
Initial daily dose of telaprevir 2250 vs. 1500 mg	1.46	0.65–3.26	0.3545			
Duration of therapy (weeks)	0.66	0.92–1.13	1.0226			
Adherence of PEG-IFN (%)	1.00	0.98–1.01	0.5762			
Adherence of ribavirin (%)	1.00	1.00–1.00	0.8539			
Adherence of telaprevir (%)	1.01	0.99–1.03	0.4877			

HCV hepatitis C virus, *ISDR* interferon sensitivity-determining region, *Peg-IFN* PEG-interferon

genotype ($P < 0.0001$, OR = 73.65, 95 % CI = 11.28–480.93), reduction of ≥4.7 $\log_{10}$IU/mL in HCV RNA at week 1 ($P = 0.0029$, OR = 18.99, 95 % CI = 2.74–131.63), achievement of RVR ($P = 0.0032$, OR = 12.59, 95 % CI = 2.33–69.97), and treatment-naïve patients or relapsers ($P = 0.0224$, OR = 5.58, 95 % CI = 1.28–24.38) (Table 2).

When analyses focused on patients with the *IL28B* non-TT genotype alone, previous relapsers ($P = 0.0020$), higher white blood cell count ($P = 0.0255$) and platelet count ($P = 0.0161$), lower body mass index ($P = 0.0400$), aspartate aminotransferase level ($P = 0.0303$), alpha-fetoprotein level ($P = 0.0304$), achievement of RVR ($P = 0.0011$), and reduction of ≥4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1 ($P = 0.0003$) were identified as factors associated with SVR by univariate analysis. The multiple logistic regression analysis identified the following three independent factors: a reduction of ≥4.7 $\log_{10}$IU/mL in HCV RNA at week 1 ($P = 0.0043$, OR = 29.35, 95 % CI = 2.88–299.22), achievement of RVR

Table 3 Factors associated with sustained virological response in patients with the *IL28B* non-TT genotype

Variable	Simple			Multiple		
	OR	95 % CI	*P* value	OR	95 % CI	*P* value
Host-related factor						
Age (year)	0.99	0.94–1.05	0.7963			
Sex male vs. female	1.56	0.50–4.83	0.4421			
Body weight (kg)	0.97	0.93–1.02	0.2324			
Body mass index (kg/m^2)	0.82	0.69–0.99	0.0400			
Cirrhosis absence vs. presence	1.80	0.50–6.43	0.3657			
Relapsers vs. treatment-naïve or non-responders	13.64	2.60–71.46	0.0020	9.18	1.04–81.16	0.0461
White blood cells (/μL)	1.00	1.00–1.00	0.0255			
Hemoglobin (g/dL)	1.26	0.87–1.82	0.2145			
Platelets ($\times 10^4$/μL)	1.14	1.02–1.26	0.0161			
Aspartate aminotransferase I(U/L)	0.97	0.95–1.00	0.0303			
Alanine aminotransferase I(U/L)	0.98	0.96–1.00	0.0564			
Gamma-glutamyl-transpeptidase I(U/L)	0.99	0.99–1.00	0.1852			
Albumin (g/dL)	2.30	0.42–12.72	0.3380			
Total cholesterol (mg/dL)	1.00	0.98–1.02	0.9274			
Low-density lipoprotein cholesterol (mg/dL)	1.01	0.99–1.03	0.3557			
Alpha-fetoprotein (ng/mL)	0.90	0.82–0.99	0.0304			
Virus-related factor						
HCV RNA ($\log_{10}$IU/mL)	0.67	0.28–1.59	0.3590			
Core amino acid substitution 70 wild-type vs. mutant-type	1.56	0.50–4.83	0.4421			
ISDR of NS5A non-wild-type vs. wild type	1.87	0.29–12.33	0.5130			
Treatment-response factor						
Rapid virological response + vs. −	15.27	2.96–78.81	0.0011	17.96	1.73–186.57	0.0156
Reduction in HCV RNA level at week 1 ≥4.7 $\log_{10}$/mL vs. <4.7 $\log_{10}$IU/mL	15.00	3.43–65.59	0.0003	29.35	2.88–299.22	0.0043
Treatment-related factor						
Administration intervals of telaprevir q8 vs. q12 h	0.60	0.19–1.84	0.3698			
Initial daily dose of telaprevir 2250 vs. 1500 mg	0.71	0.21–2.41	0.5781			
Duration of therapy (weeks)	1.16	0.93–1.44	0.1973			
Adherence of PEG-IFN (%)	1.04	0.99–1.08	0.1084			
Adherence of ribavirin (%)	1.01	0.98–1.04	0.4767			
Adherence of telaprevir (%)	0.99	0.96–1.03	0.6851			

HCV hepatitis C virus, *ISDR* interferon sensitivity-determining region, *Peg-IFN* PEG-interferon

($P = 0.0156$, OR = 17.96, 95 % CI = 1.73–186.57), and previous relapsers ($P = 0.0461$, OR = 9.18, 95 % CI = 1.04–81.16) (Table 3).

Combination of the *IL28B* genotype and reduction in HCV RNA levels at week 1 after the start of therapy to identify patients with a high likelihood of SVR

Figure 4 shows the schematic representation of the process used to identify patients with a high likelihood to achieve SVR by combining the two factors most strongly associated with SVR. Patients with the *IL28B* TT genotype presented a high SVR rate [102 of 106 patients (96.2 %)], regardless of the reduction in HCV RNA levels at week 1 after the start of therapy. In contrast, patients with the non-TT genotype showed a high SVR rate [15 of 18 patients (83.3 %)] if they presented a reduction of ≥4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1 after the start of therapy. In contrast, the SVR rate was significantly lower [8 of 32 patients [25.0 %)] when patients did not present a reduction of ≥4.7 $\log_{10}$IU/mL at week 1 ($P = 0.0001$). In patients with the *IL28B* non-TT genotype and a reduction