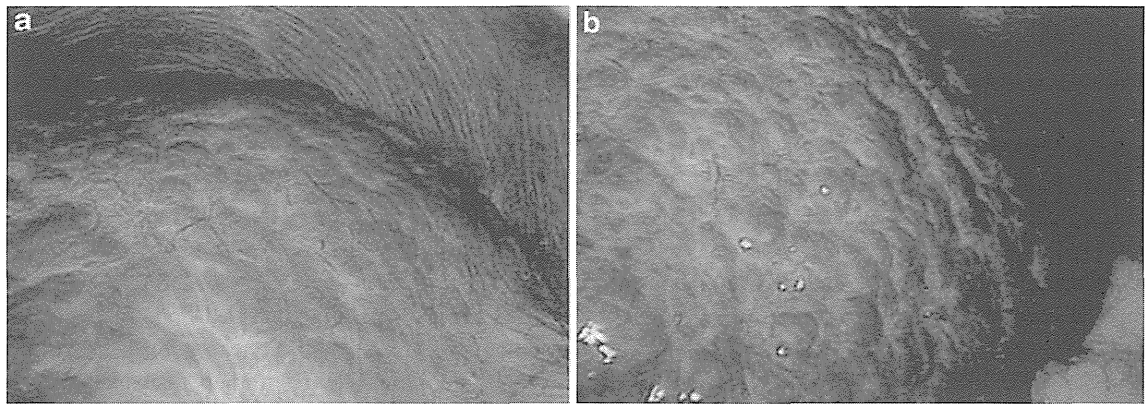

Fig. 3 Conventional laparoscopic image of the liver of the same patient in Fig. 1d. **a** View of the right lobe of the liver. The surface of the liver has diffuse large irregularities with nodular areas. **b** View of the left lobe of the liver. The surface of the liver has diffuse large irregularities with nodular areas

Table 2 Diagnostic features of three-dimensional magnetic resonance imaging and the APRI, FIB-4 index, and BARD scoring systems used to predict advanced liver fibrosis

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
3D-MRI (virtual MR-laparoscopy)	100	90	82	100
APRI	78	71	54	88
FIB-4 index	78	90	78	90
BARD score	89	81	67	94

NPV negative predictive value, *PPV* positive predictive value

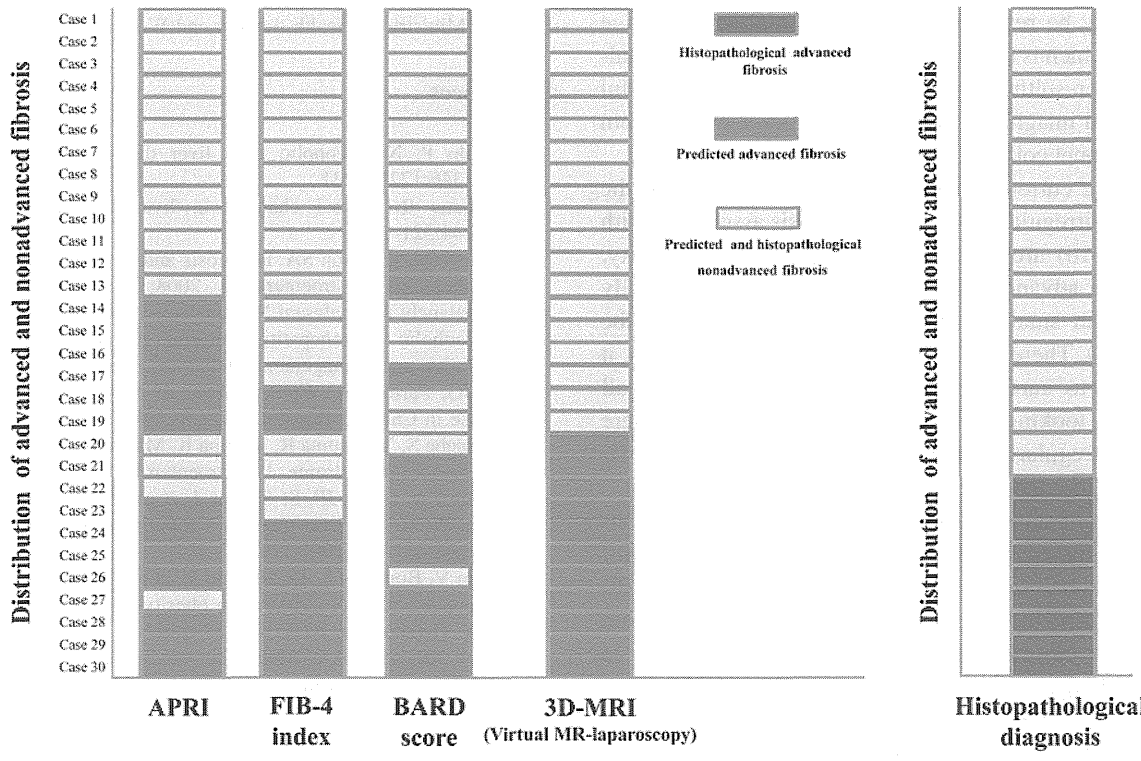

Fig. 4 Distribution of patients predicted to have advanced fibrosis by 3D-MRI and the APRI, FIB-4 index, and BARD scoring systems, along with the distribution of patients diagnosed with advanced fibrosis by histopathological evaluation

advanced fibrosis (82 and 100 %, respectively) and a sensitivity and specificity of 100 and 90 %, respectively.

We also evaluated the APRI, FIB-4 index, and BARD scoring systems, which are easy to calculate using three or four parameters that are routinely measured in outpatient medical practice. All these systems were found to have high predictive values for advanced fibrosis of the liver in this cohort. Figure 4 suggests that the combined use of 3D-MRI and a scoring system may be more advantageous for routine medical care than a single evaluation system. For example, it may be assumed that a double positive for advanced fibrosis provided by 3D-MRI and any one of the scoring systems would lead to a PPV increase from 82 to 90 %, while the NPV would remain 100 %.

However, there are some technical problems with 3D-MRI that still need to be resolved. Inadequate breath holding during the hepatobiliary phase leads to distorted hepatobiliary phase images and inaccurate findings. The 1.5-T MR-imaging system (Avanto) used for the patients in the present study required a 25-s breath hold; therefore, a patient with pulmonary emphysema might not be able to undergo this procedure. There is also the problem of motion artifact; the patients were all found to have linear surface irregularities where the superior border of the liver is near the inferior border of the heart. At present, the heart-beat-motion artifact is difficult to remove. Therefore, evaluation of liver surface irregularities seen on 3D-MRI should take into consideration the effect of the heart on the area of the liver below it. Both these problems may be resolved by increased high-speed image acquisition, which is based on the improvement of the signal-to-noise ratio resulting from the introduction of a powerful magnetic-field imaging system such as a 3.0-T MRI system and multichannel coil.

Despite the current technical problems of 3D-MRI, the large surface irregularities of liver cirrhosis associated with NASH (Fig. 1d) are easy to observe with this modality. However, the advanced fibrosis of NASH stage 3 usually manifests with small irregularities of the surface of the liver (Fig. 1c). Therefore, in patients with NASH stage 3, it is important to carefully examine the images for small irregularities, looking closely at the edge of the liver where the surface irregularities are most clearly depicted by 3D-MRI and clearly seen during conventional laparoscopy.

In the present study has some limitations. This was a retrospective cohort trial evaluating a small number of patients. There were a small number of patients because of the enrollment requirement that patients had to undergo 3D-MRI within 1 year before biopsy and histopathological evaluation. In addition, with regard to the liver biopsy specimens, there were significant differences in the length and number of portal areas of the specimens, and these differences may have led to underestimation of the extent of liver fibrosis in patients with nonadvanced fibrosis. Although in the present study there were no discrepancies among the three experienced hepatologists regarding the diagnosis of advanced fibrosis, 3D-MRI has qualitative and subjective features that might be affected by the different clinical experiences of physicians assessing the images derived from 3D-MRI. Because there were no methods for quantitative assessment of the surface irregularities of the liver seen on 3D-MRI, it was impossible to compare 3D-MRI with the other scoring systems by means of receiver-operating characteristic curve analysis. As stated above, the number of patients in the present study is too small, and some limitations have to be solved. In the near future, further additional large studies that include quantitative evaluation of the surface of the liver are needed.

However, we believe that the impact of the present study on the routine clinical care of patients with NAFLD, especially NASH patients, will be enormous. We also think that the progression of many high-risk patients to advanced liver disease, including decompensated liver cirrhosis and hepatocellular carcinoma, will be prevented by early detection of advanced fibrosis using 3D-MRI.

In conclusion, the diagnostic features of 3D-MRI for predicting advanced fibrosis associated with NASH were superior to those of other previously reported diagnostic methods.

Conflict of interest The authors state that they have no conflicts of interest regarding the content of the article.

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Prediction of Treatment Efficacy and Telaprevir-Resistant Variants after Triple Therapy in Patients Infected with Hepatitis C Virus Genotype 1

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It is often difficult to predict the response to telaprevir-pegylated interferon (PEG-IFN)-ribavirin triple therapy and the appearance of telaprevir-resistant variants. The present study determined the predictive factors of a sustained virological response (SVR) to 12- or 24-week triple therapy (T12PR12 or T12PR24, respectively) in 194 Japanese patients infected with hepatitis C virus genotype 1b (HCV-1b). The study also evaluated whether ultradeep sequencing technology can predict at baseline the emergence of resistant variants after the start of therapy. Analysis of the data of the entire group indicated that an SVR was achieved in 78% of the patients. Multivariate analysis identified *IL28B* rs8099917 (genotype TT), the substitution of amino acid (aa) 70 (Arg70), response to prior treatment (naive or relapse), PEG-IFN dose (≥1.3 μg/kg of body weight), and treatment regimen (T12PR24) as significant determinants of SVR. Among patients of the T12PR24 group, 92% with genotype TT achieved an SVR, irrespective of a substitution at aa 70. In patients with the non-TT genotype, an SVR was achieved in 76% of those with Arg70, while only 14% of patients with the non-TT genotype, Gln70(His70), and nonresponse to ribavirin combination therapy achieved an SVR. Ultradeep sequencing was conducted for 17 patients who did not achieve an SVR to determine the emergence of resistant variants during therapy. *De novo* resistant variants were detected in 16 of 17 patients (94%), regardless of the variant frequencies detected at baseline. In conclusion, the results indicate that the response to triple therapy can be predicted by the combination of host, viral, and treatment factors and that it is difficult to predict at baseline the telaprevir-resistant variants that emerge during triple therapy, even with the use of ultradeep sequencing.

New strategies have been introduced recently for the treatment of chronic hepatitis C virus (HCV) infection based on the inhibition of protease in the nonstructural NS3/NS4 proteins of the HCV polyprotein. Of the new agents currently available, telaprevir (VX-950) is used for the treatment of chronic HCV infection (1). Three studies (PROVE1, PROVE2, and a Japanese study) showed that a 24-week regimen of triple therapy (consisting of telaprevir, pegylated interferon [PEG-IFN], and ribavirin) for 12 weeks followed by dual therapy (PEG-IFN and ribavirin) for 12 weeks (also called the T12PR24 regimen) achieved sustained virological response (SVR) (defined as negative HCV RNA lasting >24 weeks after withdrawal of treatment) rates of 61%, 69%, and 73%, respectively, for the three studies, in patients infected with HCV genotype 1 (HCV-1) (2–4). However, a recent study (PROVE3) showed lower SVR rates following the T12PR24 regimen (39%) in HCV-1-infected nonresponders to previous PEG-IFN-ribavirin therapy, and who did not achieve HCV RNA negativity during or at the end of the initial triple therapy (5).

Telaprevir-based therapy is reported to induce resistant variants in HCV (6, 7). Recent reports have described the advantages of ultradeep sequencing technology, including faster processing and large-scale sequencing, in addition to providing a better understanding of the dynamics of variants in HCV quasispecies (8–11). However, it is not clear at this stage whether such technology is useful for the prediction of the emergence of telaprevir-resistant variants during or after the administration of triple therapy.

Based on the above background, there is a need to determine the predictive factors of non-SVR to triple therapy with telaprevir-PEG-IFN-ribavirin before the use of this treatment in order to avoid the appearance of telaprevir-resistant variants. The aim of this study was to determine the predictive factors of SVR to triple therapy and the emergence of telaprevir-resistant variants during such therapy (using ultradeep sequencing technology) in patients infected with HCV genotype 1b.

MATERIALS AND METHODS

Study population. From May 2008 through April 2013, 332 consecutive patients infected with HCV were selected for triple therapy with telaprevir (MP-424 or Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan), PEG-IFN-α-2b (PEG-Intron; MSD, Tokyo, Japan), and ribavirin (Rebetol; MSD, Tokyo) at the Department of Hepatology, Toranomon Hospital (located in metropolitan Tokyo). Subsequently, 194 of these patients received the triple therapy based on the following inclusion criteria: (i) diagnosis of chronic hepatitis C, (ii) HCV genotype 1b confirmed by sequence analysis, (iii) HCV RNA levels of ≥5.0 log IU/ml determined by the cobas TaqMan HCV test (Roche Diagnostics, Tokyo), (iv) age at study entry of 20 to 65 years, (v) follow-up duration of ≥24 weeks after the completion of triple therapy, (vi) no history of treatment with NS3/NS4A protease inhibitors, (vii) lack of decompensated liver cirrhosis and hepatocellular carcinoma (HCC), (viii) negativity for hepatitis B surface anti-

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TABLE 1 Profile and laboratory data at commencement of telaprevir, pegylated interferon, and ribavirin triple therapy in patients infected with HCV genotype 1b

Study or patient characteristics	Data
Demographic data	
No. of patients	194
Sex (no.)	
Male	117
Female	77
Age (median [range]) (yr)	56 (21–65)
Body mass index (median [range]) (kg/m^2)	22.7 (16.0–36.7)
Blood plasma levels (median [range])	
Viremia (log IU/ml)	6.7 (5.0–7.8)
Aspartate aminotransferase (IU/liter)	36 (15–118)
Alanine aminotransferase (IU/liter)	41 (12–175)
Albumin (g/dl)	3.9 (2.9–4.6)
Total bilirubin (mg/dl)	0.8 (0.2–2.0)
Gamma-glutamyl transpeptidase (IU/liter)	34 (3–240)
Creatinine (g/dl)	0.7 (0.4–1.1)
Leukocyte count (cells/mm^3)	4,800 (2,000–8,400)
Hemoglobin (g/dl)	14.4 (12.1–17.4)
Platelet count ($\times 10^4$/mm^3)	17.5 (8.9–33.8)
Alpha-fetoprotein (μg/liter)	4 (2–104)
Total cholesterol (mg/dl)	174 (112–301)
High-density lipoprotein cholesterol (mg/dl)	48 (20–117)
Low-density lipoprotein cholesterol (mg/dl)	97 (41–216)
Triglycerides (mg/dl)	97 (36–336)
Uric acid (mg/dl)	5.7 (2.0–8.6)
Fasting plasma glucose (mg/dl)	93 (64–169)
Treatment dose	
PEG-IFN-α-2b (median [range]) (μg/kg)	1.5 (0.9–1.7)
Ribavirin (median [range]) (mg/kg)	11.0 (4.3–15.8)
Telaprevir (median [range]) (mg/kg)	31.8 (14.5–59.2)
Telaprevir (no.)	
1,500 mg/day	74
2,250 mg/day	120
Treatment regimen (no.)	
T12PR12 group	20
T12PR24 group	174
Response to prior treatment (no.)	
Treatment naive/relapse to prior treatment	71
Relapse after prior treatment	78
No response to prior treatment	44
IFN monotherapy	10
IFN-ribavirin dual therapy	34
Unknown	1
Amino acid substitutions in HCV genotype 1b (no.)	
Core aa 70	
Arginine	128
Glutamine (histidine)	65
ND[a]	1
Core aa 91	
Leucine	104
Methionine	89
ND	1
ISDR of NS5A	
Wild type	155
Non-wild type	17
ND	22
IRRDR of NS5A	
≤5	144
≥6	38
ND	1
V3 of NS5A	
≤2	49
≥3	144
ND	1
IL28B genotype (no.)	
rs8099917 genotype	
TT	139
Non-TT	53
ND	2
ITPA genotype (no.)	
rs112735 genotype	
CC	147
Non-CC	47
Telaprevir-resistant variants by direct sequencing (no.)[b]	
V36	1
T54	6
R155	0
A156	1
V170	0

[a] ND, not determined.

[b] Telaprevir-resistant variants, detected by direct sequencing, included V36A/C/M/L/G, T54A/S, R155K/T/I/M/G/L/S/Q, A156V/T/S/I/G, and V170A.

gen (HBsAg), (ix) no evidence of HIV infection, (x) negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C, (xi) negative history of depression, schizophrenia or suicide attempts, angina pectoris, cardiac insufficiency, myocardial infarction, severe arrhythmia, uncontrolled hypertension, uncontrolled diabetes, chronic renal dysfunction, cerebrovascular disorders, thyroidal dysfunction that is uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication, or anaphylaxis at baseline; pregnant or breastfeeding women or those open to becoming pregnant during the study and men with pregnant partners were excluded. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki and was approved by the institutional review board of Toranomon Hospital. Each patient received ample information about the goals and potential side effects of the treatment and their right to withdraw from the study. Each provided a signed consent form before participating in the trial.

The efficacy of treatment was evaluated by absence of HCV RNA at 24 weeks after the completion of therapy (i.e., SVR), as measured by the cobas TaqMan HCV test (Roche Diagnostics). Furthermore, failure to achieve an SVR was classified as nonresponse (if HCV RNA was detected during or at the end of treatment) or relapse (reelevation of viral load after the end of treatment, even when HCV RNA was negative at the end of treatment).

Twenty patients (10%) were assigned to a 12-week regimen of triple therapy (the T12PR12 group) and were randomly subdivided into two groups treated with either 1,500 mg/day or 2,250 mg/day of telaprevir to evaluate the treatment efficacy during 12 weeks of treatment. Sixty patients (31%) were allocated to a 24-week regimen of the same triple therapy described above followed by dual therapy of PEG-IFN and ribavirin for another 12 weeks (the T12PR24 group) to evaluate treatment efficacy according to the response to prior treatment. All subjects in the T12PR24

group were treated with telaprevir at 2,250 mg/day. Another group of 114 patients (59%) were treated as described above for the T12PR24 group, except for telaprevir dose, and were subdivided into two groups treated with either 1,500 mg/day or 2,250 mg/day of telaprevir, as prescribed by the attending physician. Table 1 summarizes the profiles and laboratory data of the entire group of 194 patients at the commencement of treatment. They included 117 males and 77 females, aged 23 to 65 years (median, 56 years). At the start of treatment, telaprevir was administered at a median dose of 31.8 mg/kg of body weight (range, 14.5 to 59.2 mg/kg) daily. Especially, 120 patients (62%) were treated with telaprevir at a dose of 2,250 mg/day, while the other 74 patients (38%) were treated with telaprevir at a dose of 1,500 mg/day. PEG-IFN-α-2b was injected subcutaneously at a median dose of 1.5 μg/kg (range, 0.9 to 1.7 μg/kg) once a week. Ribavirin was administered at a median dose of 11.0 mg/kg (range, 4.3 to 15.8 mg/kg) daily. Each drug was discontinued or its dose reduced, as required upon judgment of the attending physician, in response to a fall in hemoglobin level, leukocyte count, neutrophil count, or platelet count, or the appearance of side effects. Triple therapy was discontinued when the leukocyte count decreased to $<1{,}000/mm^3$, neutrophil count to $<500/mm^3$, or platelet count to $<5.0 \times 10^4/mm^3$, or when hemoglobin decreased to <8.5 g/dl.

Follow-up. Clinical and laboratory assessments were performed at least once every month before, during, and after treatment. Especially, they were performed every week in the initial 12 weeks of treatment. Adverse effects were monitored clinically by careful interviews and a medical examination at least once every month. Compliance with treatment was evaluated by a questionnaire.

Measurement of HCV RNA. The antiviral effects of triple therapy on HCV were assessed by measuring blood plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every month before, during, and after therapy. HCV RNA concentrations were determined using the cobas TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2 to 7.8 log IU/ml, and undetectable levels were defined as negative samples.

Determination of *IL28B* and *ITPA* genotypes. *IL28B* rs8099917 and *ITPA* rs112735 genotypes have been reported as predictors of treatment efficacy and side effects to PEG-IFN-ribavirin dual therapy, and they were genotyped by the Invader assay, TaqMan assay, or direct sequencing, as described previously (12–16).

Detection of amino acid substitutions in core and NS5A regions of HCV-1b. With the use of HCV-J (accession no. D90208) as a reference (17), the sequence of amino acids (aa) 1 to 191 in the core protein of HCV-1b was determined and compared with the consensus sequence constructed in a previous study to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70[/His70]) and aa 91 of leucine (Leu91) or methionine (Met91) (18). The sequence of aa 2209 to 2248 in the NS5A of HCV-1b (the interferon sensitivity determining region [ISDR]) reported by Enomoto and coworkers (19) was determined, and the numbers of aa substitutions in the ISDR were defined as wild-type (≤1) or non-wild-type (≥2) compared against HCV-J. Furthermore, the sequence of aa 2334 to 2379 in the NS5A of HCV-1b (IFN-ribavirin resistance-determining region [IRRDR]) reported by El-Shamy and coworkers (20), including the sequence of aa 2356 to 2379 referred to as variable region 3 (V3), was determined and compared with the consensus sequence constructed in a previous study. The numbers of aa substitutions in the IRRDR and V3 were divided into two groups for analysis (numbers of aa substitutions in the IRRDR of ≤5 and ≥6, and those in V3 of ≤2 and ≥3). In the present study, aa substitutions of the core region and NS5A-ISDR-IRRDR-V3 of HCV-1b were analyzed by direct sequencing.

Assessment of telaprevir-resistant variants. The genome sequence of the N-terminal 609 nucleotides (203 amino acids) in the NS3 region of HCV isolates from the patients was examined. HCV RNA was extracted from 100 μl of serum, and the nucleotide sequences were determined by direct sequencing and deep sequencing. The primers used to amplify the NS3 region were NS3-F1 (5′-ACA CCG CGG CGT GTG GGG ACA T-3′; nucleotides 3295 to 3316) and NS3-AS2 (5′-GCT CTT GCC GCT GCC AGT GGG A-3′; nucleotides 4040 to 4019) as the first (outer) primer pair and NS3-F3 (5′-CAG GGG TGG CGG CTC CTT-3′; nucleotides 3390 to 3407) and NS3-AS2 (sequence above) as the second (inner) primer pair (21). Thirty-five cycles of first and second amplifications were performed as follows: denaturation for 30 s at 95°C, annealing of primers for 1 min at 63°C, extension for 1 min at 72°C, and final extension at 72°C for 7 min. The PCR-amplified DNA was purified after agarose gel electrophoresis and then used for direct sequencing and ultradeep sequencing.

All patients were examined for telaprevir-resistant variants by direct sequencing before the start of triple therapy. Furthermore, patients who did not achieve an SVR were analyzed by ultradeep sequencing, at baseline and at the time of reelevation of viral loads. Telaprevir-resistant variants included V36A/C/M/L/G, T54A/S, R155K/T/I/M/G/L/S/Q, A156V/T/S/I/G, and V170A (22, 23).

Direct sequencing was analyzed by the dye-terminator method. Dideoxynucleotide termination sequencing was performed with the BigDye deoxy terminator v1.1 cycle sequencing kit (Life Technologies, Carlsbad, CA) (21). Sequence data were deposited in GenBank. Ultradeep sequencing was performed using the Ion personal genome machine (PGM) sequencer (Life Technologies). An Ion Torrent adapter-ligated library was prepared using an Ion Xpress Plus fragment library kit (Life Technologies). Briefly, 100 ng of fragmented genomic DNA was ligated to the Ion Torrent adapters P1 and A. The adapter-ligated products were nick translated and PCR amplified for a total of 8 cycles. Subsequently, the library was purified using AMPure beads (Beckman Coulter, Brea, CA), and the concentration was determined using the StepOnePlus real-time PCR (Life Technologies) and Ion Library quantitation kit, according to the instructions provided by the manufacturer. Emulsion PCR was performed using Ion OneTouch (Life Technologies) in conjunction with Ion OneTouch 200 template kit v2 (Life Technologies). Enrichment for templated Ion Sphere particles (ISPs) was performed using the Ion OneTouch enrichment system (Life Technologies), according to the instructions provided by the manufacturer. Templated ISPs were loaded onto an Ion 314 chip and subsequently sequenced using 130 sequencing cycles according to the Ion PGM 200 sequencing kit user guide. Total output read length per run is >10 Mb (0.5 m-TAG, 200-base read) (24). The results were analyzed with the CLC Genomics Workbench software (CLC bio, Aarhus, Denmark) (25).

We also included a control experiment to validate the error rates in ultradeep sequencing of the viral genome. In this study, the amplification products of the second-round PCR were ligated with plasmid and transformed in *Escherichia coli* in a cloning kit (TA Cloning; Invitrogen, Carlsbad, CA). A plasmid-derived NS3 sequence was used as the template by the control experiment. The fold coverage values evaluated per position for aa 36, aa 54, aa 155, aa 156, and aa 170 in the NS3 region were 359,379×, 473,716×, 106,435×, 105,979×, and 49,058×, respectively. Thus, using the control experiment based on a plasmid encoding an HCV NS3 sequence, amino acid mutations were defined as amino acid substitutions at a frequency of >0.2% of the total coverage. This frequency ruled out putative errors caused by the ultradeep sequence platform used in this study (26).

Statistical analysis. Nonparametric tests (chi-square test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to SVRs. The odds ratios (OR) and 95% confidence intervals (CI) were also calculated. All P values of <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. The potential pretreatment factors associated with SVR included sex, age, body mass index, levels of viremia, aspartate aminotransferase, alanine aminotransferase, albumin, total bilirubin, gamma-glutamyl transpeptidase (GGT), and creatinine, leukocyte count, hemoglobin level, platelet count, alpha-fetoprotein level, total cholesterol, high-density lipoprotein cholesterol, low-density lipo-

protein cholesterol, triglycerides, uric acid, fasting blood plasma glucose, PEG-IFN dose/kg body weight, ribavirin dose/kg body weight, telaprevir dose/kg body weight, telaprevir dose/day, kind of treatment regimen, response to prior treatment, amino acid substitutions in the core region and NS5A-ISDR-IRRDR, *IL28B* genotype, *ITPA* genotype, and telaprevir-resistant variants. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, IL). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated to determine the reliability of the predictors of response to therapy.

RESULTS

Virological response to therapy. Analysis of the entire group showed that 78% (151 of 194 patients) achieved an SVR. According to the treatment regimen, an SVR was achieved by 45% (9 of 20 patients) and 82% (142 of 174 patients) of the T12PR12 group and T12PR24 groups, respectively. Taking into consideration the response to prior treatment, in the 173 patients of the T12PR24 group, an SVR was achieved in 89% (54 of 61 patients), 89% (66 of 74 patients), and 55% (21 of 38 patients) of treatment-naive patients, patients who showed relapse after prior treatment, and nonresponders to prior treatment, respectively. Furthermore, SVRs were achieved by 100% (8 of 8 patients) and 43% (13 of 30 patients) of the nonresponders to prior IFN monotherapy and IFN-ribavirin dual therapy, respectively.

Predictors of SVR. Univariate analysis of the data of the entire group identified seven parameters that correlated significantly with SVR: *IL28B* rs8099917 (genotype TT) ($P < 0.001$), substitution of aa 70 (Arg70) ($P = 0.001$), response to prior treatment (naive or relapse) ($P < 0.001$), PEG-IFN dose (≥1.3 μg/kg) ($P = 0.004$), treatment regimen (T12PR24 group) ($P = 0.001$), platelet count ($\geq 15.0 \times 10^4/mm^3$) ($P = 0.013$), and GGT (<50 IU/liter) ($P = 0.001$). Multivariate analysis that included the above variables identified 5 parameters that independently influenced SVR: *IL28B* rs8099917 (genotype TT) (OR, 9.52; $P < 0.001$), substitution of aa 70 (Arg70) (OR, 2.67; $P = 0.038$), response to prior treatment (naive or relapse) (OR, 3.80; $P = 0.007$), PEG-IFN dose (≥1.3 μg/kg) (OR, 35.5; $P < 0.001$), and treatment regimen (T12PR24 group) (OR, 12.8; $P < 0.001$) (Table 2).

TABLE 2 Multivariate analysis of factors associated with sustained virological response to telaprevir, pegylated interferon, and ribavirin triple therapy in patients infected with HCV genotype 1b

SVR-influencing factor	OR (95% CI)[a]	*P*
IL28B rs8099917 genotype		
Non-TT	1	
TT	9.52 (3.36–27.0)	<0.001
Substitution of aa 70		
Gln70 (His70)	1	
Arg70	2.67 (1.05–6.76)	0.038
Response to prior treatment		
Nonresponse	1	
Naive or relapse	3.80 (1.44–10.1)	0.007
PEG-IFN-α−2b dose (μg/kg of body weight)		
<1.3	1	
≥1.3	35.5 (6.37–198)	<0.001
Treatment regimen		
T12PR12 group	1	
T12PR24 group	12.8 (3.44–48.1)	<0.001

[a] OR, odds ratio; CI, confidence interval.

Host, viral, and treatment factors for prediction of non-SVR. Using data of the 172 patients of the T12PR24 group, we evaluated the ability to predict non-SVR by host factor (*IL28B* rs8099917 genotype), viral factor (substitution of aa 70), and treatment factor (response to prior treatment). With the combination of the rs8099917 non-TT genotype, Gln70 (His70), and nonresponse to ribavirin combination therapy, the sensitivity, specificity, PPV, and NPV for non-SVR were 38% (12 of 32 patients), 99% (138 of 140 patients), 86% (12 of 14 patients), and 87% (138 of 158 patients), respectively. These results indicate that using the combination of the above three predictors has high specificity, PPV, and NPV for the prediction of non-SVR.

The SVR rates using the combination of rs8099917 genotype, substitution of aa 70, and response to prior treatment are shown in Fig. 1. In 126 patients with the rs8099917 TT genotype, the degree of SVR was not significantly different between Arg70 (91% [86 of 95 patients]) and Gln70 (His70) (97% [29 of 30 patients]). In contrast, in 46 patients with the rs8099917 non-TT genotype, a significantly higher proportion of patients with Arg70 (76% [16 of 21 patients]) achieved an SVR than did patients with Gln70 (His70) (32% [8 of 25 patients]) ($P = 0.004$). Furthermore, in 25 patients with the rs8099917 non-TT genotype and Gln70 (His70), a lower proportion of nonresponders to ribavirin combination therapy (14% [2 of 14 patients]) tended to achieve an SVR than did other patients (55%[6 of 11 patients]) ($P = 0.081$). These results highlight three properties of triple therapy: (i) a high efficacy of triple therapy was seen in patients with the TT genotype who achieved an SVR at 92%, irrespective of substitution of aa 70, (ii) among patients with the non-TT genotype, 76% of those with Arg70 achieved an SVR, and (iii) only 14% of the patients with the three factors of the rs8099917 non-TT genotype, Gln70 (His70), and nonresponders to ribavirin combination therapy achieved an SVR.

Evolution of telaprevir-resistant variants over time detected by ultradeep sequencing. Between May 2008 and the end of September 2009, 17 patients (4 treatment-naive patients, 3 who had a relapse after prior ribavirin combination therapy, and 10 nonresponders to ribavirin combination therapy) did not achieve SVR with triple therapy, and they were analyzed for telaprevir-resistant variants by ultradeep sequencing at baseline and at the time of reelevation of viral load.

In 6 of 17 patients (35%), telaprevir-resistant variants were detected at baseline by ultradeep sequencing. In 4 of these 6 patients, a very low frequency of variants at baseline (0.2% of 32,413× coverage for V36A, 0.2% of 27,915× coverage for V36A, 0.2% of 26,230× coverage for T54A, and 0.4% of 29,881× coverage for V170A) were replaced after treatment by *de novo* high-frequency variants (97.2% of 36,757× coverage for V36C, 27.7% of 5,032× coverage for T54A, 50.2% of 15,487× coverage for A156S, and 99.6% of 14,757× coverage for A156T), respectively. In one of the 6 patients, very high-frequency variants of T54S (99.9% of 33,830× coverage) at baseline persisted during treatment as very high-frequency variants of T54S (99.7% of 26,348× coverage), and *de novo* very high-frequency variants of R155K (96.1% of 20,630× coverage) also emerged during treatment. In another patient, variants of T54A increased from very low frequency at baseline

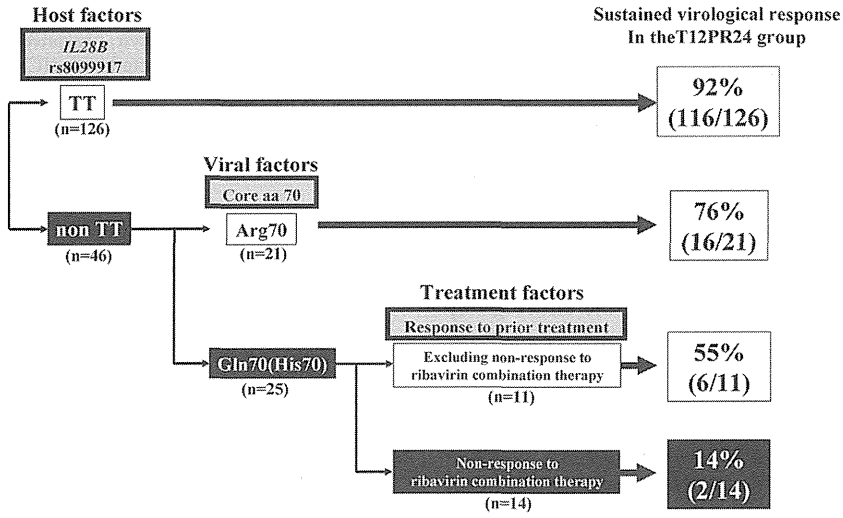

FIG 1 Prediction of sustained virological response (SVR) by the combination of *IL28B* rs8099917 genotype, substitution of aa 70, and response to prior treatment. In the T12PR24 group, treatment efficacy was high in patients with the TT genotype who achieved an SVR (92%), irrespective of the substitution of aa 70. In patients with the non-TT genotype, those with Arg70 achieved a high SVR (76%). Patients with the non-TT genotype, Gln70 (His70), and nonresponse to ribavirin combination therapy achieved the lowest frequency of an SVR (14%).

(0.2% of 53,127× coverage) to high frequency during treatment (99.9% of 45,240× coverage).

In the other 11 of 17 patients (65%), telaprevir-resistant variants were not detected by ultradeep sequencing at baseline, but *de novo* resistant variants were detected according to treatment (4 patients with V36A/C/M [median 41.5% of median 27,769× coverage], 8 with T54A/S [median 40.2% of median 27,067× coverage], 3 with R155K/Q [median 0.3% of median 17,847× coverage], and 8 with A156S/T [median 2.1% of median 18,150× coverage]).

Thus, in 16 of 17 patients (94%), *de novo* resistant variants were detected according to treatment. In other words, using ultradeep sequencing, the present study detected the emergence of *de novo* telaprevir-resistant variants regardless of variant frequencies at baseline, and the emergence of variants after the start of treatment could not be predicted at baseline.

DISCUSSION

Along with resulting in a high SVR, triple therapy is expensive and associated with serious side effects. Furthermore, employing ultradeep sequencing, the present study demonstrated the emergence of *de novo* telaprevir-resistant variants regardless of variant frequencies at baseline, and that the emergence of variants after the start of triple therapy could not be predicted at baseline. Hence, patients who failed to achieve an SVR with triple therapy need to be identified beforehand to avoid unnecessary side effects, high costs, and the emergence of telaprevir-resistant variants. Host genetic factors (e.g., *IL28B* genotype), and viral factors (e.g., amino acid substitutions in the core-NS5A region) have often been used as pretreatment predictors of poor virological response to PEG-IFN-ribavirin dual therapy (12, 14, 16, 18, 20) and telaprevir-PEG-IFN-ribavirin triple therapy (27, 28). The present study identified that the treatment efficacy of triple therapy could be predicted by the combination of host (*IL28B* rs8099917 genotype), viral (substitution of aa 70), and treatment (response to prior treatment, PEG-IFN dose, and T12PR24 regimen) factors. Especially, the use of the combination of rs8099917 non-TT genotype, Gln70 (His70), and nonresponse to ribavirin combination therapy had high specificity, PPV, and NPV for the prediction of non-SVR in the T12PR24 group. Unfortunately, the lowest frequency of an SVR (14%) was in patients who possessed the above three factors (namely, the treatment-resistant group). Previous studies showed that IFN monotherapy reduced the risk of HCC (29–31). Furthermore, analysis of the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) cohort recently showed that long-term PEG-IFN monotherapy reduced the incidence of HCC among patients with cirrhosis who did not achieve an SVR after previous IFN treatment, with or without ribavirin (32). Thus, the present study suggests that the treatment-resistant group should be selected for IFN monotherapy to overcome the problem of telaprevir-resistant variants, and to reduce the risk of hepatocarcinogenesis.

Interestingly, ultradeep sequencing identified telaprevir-resistant variants at baseline in 5 patients (2 patients with V36A [0.2%], 2 with T54A [0.2%], and t1 with V170A [0.4%]) at a very low frequency, but the frequency of resistant variants did not increase over time, except for one patient with T54A in whom it increased from 0.2% at baseline to 99.9% during treatment. This finding may be due to one or more reasons. One reason is probably related to the high susceptibility of telaprevir-resistant variants to IFN. A previous study indicated that mice infected with a resistant strain (A156F [99.9%]) developed only low-level viremia, and the virus was successfully eliminated with IFN therapy (9). Furthermore, this finding probably suggests that a small number of mutant-type viral RNAs may be incomplete or defective, since a large proportion of viral genomes are thought to be defective due to the high replication and mutation rates of the virus (33). Further studies should be performed to evaluate the significance of the presence of low-frequency variants detected by ultradeep sequencing.

A recent study using the human hepatocyte chimeric mouse model and deep sequencing reported that the rapid emergence of *de novo* telaprevir-resistant HCV quasispecies was induced by mu-

tation of the wild-type strain of HCV *in vivo* (9). In the present study, ultradeep sequencing did not detect any telaprevir-resistant variants at baseline in 11 patients, although *de novo* resistant variants emerged in all 11 patients over time. The present clinical results based on patients who did not achieve an SVR provide evidence in support of a *de novo* emergence of telaprevir resistance that is induced by viral mutation.

The results of the present study should be interpreted with caution, since the study was performed in a small number of Japanese patients infected with HCV-1b. Any generalization of the results should await confirmation by a multicenter randomized trial based on a larger number of patients, including patients of other races and those infected with HCV-1a. Furthermore, the other limitation of the present study is that the existence of very low-frequency telaprevir-resistant variants was not investigated long after the cessation of therapy by ultradeep sequencing. Further large-scale studies using ultradeep sequencing should be performed to investigate the effects of telaprevir-resistant variants on the response to treatment using new drugs, including direct-acting antiviral agents.

In conclusion, this study, which is based on Japanese patients infected with HCV genotype 1b, indicated that the efficacy of triple therapy could be predicted by the combination of host, viral, and treatment factors. However, the present results show that it might be difficult to predict at baseline the emergence of telaprevir-resistant variants during triple therapy, even with the use of ultradeep sequencing. Further large-scale prospective studies are needed to investigate the pretreatment predictors of treatment efficacy and the emergence of telaprevir-resistant variants after triple therapy, and to develop more effective therapeutic regimens in patients infected with HCV genotype 1.

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A Pilot Study of Triple Therapy With Telaprevir, Peginterferon and Ribavirin for Elderly Patients With Genotype 1 Chronic Hepatitis C

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The prevalence of hepatitis C virus (HCV) infection in elderly patients has been increasing in Japan. However, there are no reports on the safety and efficacy of the triple therapy of telaprevir, peginterferon, and ribavirin for elderly patients with chronic HCV infection. This study evaluated the safety and efficacy of triple therapy [12 weeks of telaprevir 1,500 mg/day, reduction dose, and 24 weeks of peginterferon and ribavirin] in 18 elderly Japanese patients aged >65 years, with chronic infection with HCV genotype 1b. Four patients received triple therapy with telaprevir 2,250 mg/day and the other 14 patients received telaprevir 1,500 mg/day. Sustained virological response-12 (HCV RNA negativity at 12 weeks after completion of therapy) was 50% (9 of 18 patients); while 4 of 18 (22%) patients discontinued triple therapy due to adverse events (skin rashes, anemia, poor appetite). The dose of telaprevir did not affect HCV RNA clearance rates. Regardless of the dose, 50% of the treated patients achieved sustained virological response-12, evaluated by intention-to-treat analysis. Furthermore, the fall in hemoglobin and the rise in serum creatinine were significantly milder in the telaprevir 1,500 mg group than the telaprevir 2,250 mg/day group. Further analysis showed that 67% (6 of 9 elderly patients) with IL28B gene (rs8099917) genotype TT, treated with telaprevir 1,500 mg, achieved sustained virological response-12. These results suggest that 24-week triple therapy with telaprevir 1,500 mg seems safe and efficacious for elderly Japanese patients infected with HCV genotype 1b. ***J. Med. Virol. 85:1746–1753, 2013.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: HCV; telaprevir; peginterferon; ribavirin; elderly patient

INTRODUCTION

Hepatitis C virus (HCV) often causes chronic liver infection, and can potentially cause liver cirrhosis and hepatocellular carcinoma (HCC) [Niederau et al., 1998; Kenny-Walsh, 1999]. There is a growing need for treatment of chronic HCV in elderly patients with increased proportion of such patients in the last few decades. This is important since Japanese patients infected with HCV are much older than Western patients due to the widespread HCV infection that affected Japan about 20 years ago [Yoshizawa et al., 2006].

Sustained virological responders who are negative for serum HCV RNA at 24 weeks after the completion of interferon therapy are likely to remain in virological and biochemical remission and show histological improvement [Marcellin et al., 1997; Shiratori et al., 2000]. In addition, interferon therapy reduces the risk of HCC in virological or biochemical responders [Imai et al., 1998; Ikeda et al., 1999; Yoshida et al., 1999]. Especially, HCV in elderly patients is associated with hepatocarcinogenesis and poor survival [Ikeda et al., 2009], and sustained virological response to interferon therapy is associated with improved clinical outcome [Asahina et al., 2010]. However, the sustained virological response rate tends to be lower in elderly patients with chronic hepatitis C, due in part to less tolerability and efficacy of interferon (IFN) combination therapy compared with adult patients [Iwasaki et al., 2006; Honda et al., 2010].

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Several direct acting antiviral agents have been designed and developed recently, represented by NS3/4A or NS5A protease inhibitors and NS5B polymerase or NS5A inhibitors [Asselah and Marcellin, 2011]. Among them, telaprevir has shown more effective results when combined with peginterferon and ribavirin in the treatment of chronic hepatitis C than peginterferon and ribavirin combination therapy [McHutchison et al., 2009, 2010; Hézode et al., 2010; Kumada et al., 2011]. However, there are no reports about the safety and efficacy of the triple therapy, which are combined with telaprevir, peginterferon, and ribavirin for elderly patients with chronic HCV infection. Clinically, it is important to determine whether elderly patients with HCV infection can be treated with triple therapy of telaprevir, peginterferon, and ribavirin.

The aim of this pilot study was to evaluate the safety and efficacy of triple therapy with telaprevir, peginterferon, and ribavirin for elderly patients with chronic HCV infection genotype 1b.

PATIENTS AND METHODS

Study Population

From May 2008 through November 2012, 297 patients with chronic hepatitis C were selected for treatment with telaprevir, peginterferon, and ribavirin at the Department of Hepatology, Toranomon Hospital (located in metropolitan Tokyo). Subsequently, 18 of these patients received the triple therapy based on the following inclusion criteria: (1) diagnosis of chronic HCV infection; (2) infection with HCV genotype 1b confirmed by sequence analysis in the NS5B region; (3) HCV RNA levels >5.0 $\log_{10}$ IU/ml, determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (4) Japanese aged ≥66 years at the start of treatment; (5) agreed to be treated with telaprevir, peginterferon, and ribavirin; (6) no evidence of liver cirrhosis; (7) no evidence of HCC; (8) negative for hepatitis B surface antigen; (9) no evidence of human immunodeficiency virus infection; (10) no evidence of autoimmune hepatitis, alcoholic liver disease, hemochromatosis or chronic liver disease other than chronic HCV infection; and (11) no history of cardiac disease, cerebral disorder, and pulmonary disease. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave an informed consent before participating in this trial.

Table I summarizes the profiles and laboratory data of the 18 patients at the time of commencement of treatment. Treatment efficacy was evaluated by intention-to-treat analysis classified as treatment failure in patients who could not complete the treatment regimen. HCV RNA levels and hemoglobin were monitored at baseline and weeks 1, 2, 4, 8, 12, 16, 20, and 24 during treatment.

Four patients were treated with telaprevir 750 mg every 8-hr (q8h) (2,250 mg/day group), while the other 14 patients were treated with telaprevir 750 mg twice daily at 12-hr interval (q12h) (1,500 mg/day group). Peginterferon-α-2b was injected subcutaneously at a median dose of 1.5 μg/kg

TABLE I. Characteristics of Patients at Baseline

Number of patients	18
Age (years)*	68 (66–73)
Male/female	10/8
Body mass index (kg/m^2)*	22.8 (18.9–26.3)
Viral load of HCV ($\log_{10}$ IU/ml)	6.5 (5.1–7.3)
Serum aspartate aminotransferase (IU/L)	36 (11–95)
Serum alanine aminotransferase (IU/L)	38 (19–80)
Serum albumin (g/dl)	3.8 (3.3–4.1)
Gamma-glutamyl transpeptidase (IU/L)	27 (10–62)
Leukocyte count ($/mm^3$)	4,000 (2,500–7,300)
Hemoglobin (g/dl)	14.0 (12.5–16.1)
Platelet count ($\times 104/mm^3$)	15.5 (9.6–21.4)
Alpha-fetoprotein (μg/L)	4 (1–18)
Treatment	
Peginterferon α-2b dose (μg/kg)*	1.5 (1.0–1.8)
Ribavirin dose (mg/kg)*	7.7 (5.8–13.2)
Telaprevir dose(1,500/2,250 mg/day)	14/4
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 (arginine/glutamine)	10/8
Core aa 91 (leucine/methionine)	11/7
ISDR of NS5A (wild-type/non wild-type/ND)	17/0/1
Genetic variation near IL28B gene rs8099917 genotype (TT/TG/GG)	11/6/1
Past history of interferon therapy Treatment-naïve/relapsers to previous treatment/nonresponders to previous treatment	3/10/5
Comorbidities[a]	
Diabetes mellitus	3 (17%)
Hypertension	9 (50%)

Data are numbers (percentages) of patients, except those denoted by *, which represent the median (range) values.
[a]All patients were not on medications.

(range: 1.1–1.8 μg/kg) once a week. Ribavirin was administered at a median dose of 8.3 mg/kg body weight (range: 5.8–13.2 mg/kg) twice a day every 12 hr. Each drug was discontinued or its dose reduced, as required upon judgment of the attending physician, in response to a fall in hemoglobin level, leukocyte count, neutrophil count or platelet count, or the appearance of side effects. The triple therapy was discontinued when the leukocyte count decreased to $<1{,}000/mm^3$, neutrophil count $<500/mm^3$, or platelet count $<5 \times 10^4/mm^3$, or when hemoglobin decreased to <8.5 g/dl.

Measurement of HCV RNA

The virological response was assessed using the COBAS TaqMan HCV test. The linear dynamics range of this assay is 1.2–7.8 $\log_{10}$ IU/ml and samples with undetectable HCV RNA were defined as negative. The response to treatment was divided into the following: sustained virological response-12 (negative HCV RNA at 12 weeks after completion of therapy), which is relevant to sustained virological response-24 defined by Martinot-Peignoux et al. [2010] and Mauss et al. [2012], relapse (rise in viral load after the end of treatment, even when HCV RNA was negative at the end of treatment), and viral breakthrough (rise in viral load before the end of treatment, even when HCV RNA was temporarily negative during treatment).

Detection of Amino Acid Substitutions in Core and NS5A Regions of HCV-1b

With the use of HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 amino acids (aa) in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed in a previous study to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. The sequence of 2,209–2,248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al. [1996] was determined and the numbers of aa substitutions in the ISDR were defined as wild-type (0, 1) or non wild-type (≥2), compared with HCV-J. In the present study, aa substitutions of the core region and NS5A-ISDR of HCV-1b were analyzed by direct sequencing.

Determination of IL-28B Genotype

IL-28B (rs8099917) was genotyped by the Invader assay, Taq Manassay, or direct sequencing, as described previously [Ohnishi et al., 2001; Suzuki et al., 2003].

Statistical Analysis

The χ^2 test, Fisher's exact probability test, and Mann–Whitney's *U*-test were used to compare the background characteristics of the groups. All *P* values were two-tailed, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL).

RESULTS

Efficacy of Triple Therapy

Figure 1a illustrates the negative rates of HCV RNA at different time points. The disappearance rate of HCV RNA during treatment was 11% (2/18), 33% (6/18), 83% (15/18), 89% (16/18), 78% (14/18), 78% (14/18), 67% (12/18), and 67% (12/18) at 1, 2, 4, 8, 12, 16, 20, and 24 weeks, respectively. Furthermore, 50% (9/18) of elderly patients achieved sustained virological response-12.

Four of the 18 patients discontinued triple therapy because of side effects, but in the remaining 14 patients, HCV RNA level was below the detection limit of the test during treatment. Two patients experienced viral breakthrough at 20 weeks after the commencement of treatment and three patients experienced relapse. Four patients discontinued the triple therapy due to the appearance of side effects [two developed skin disease (at 4th and 10th week), one developed anemia (at second week), and one patient discontinued due to poor appetite (at the 11th week)]. Figure 1b shows changes in hemoglobin level in patients who received the triple therapy. During the administration of telaprevir to 12 weeks, hemoglobin decreased steadily, with a maximum of 3.7 g/dl (mean value) at 12 week. However, hemoglobin tended to increase after the end of telaprevir medication, during treatment with peginterferon and ribavirin. Figure 1c shows changes in serum creatinine level in patients who received the triple therapy. During administration of telaprevir to 12 weeks, creatinine increased steadily, with a maximum of 0.14 g/dl (mean value) at 8 week. Similar to the pattern described above for hemoglobin, serum creatinine tended to decrease after the end of telaprevir medication, during treatment with peginterferon and ribavirin.

Response to Treatment as a Function of Telaprevir Dose

Table II summarizes the profiles and laboratory data of the 18 patients according to the dose of telaprevir. At baseline, leukocyte count in patients treated with telaprevir 1,500 mg/day was lower than in those treated with 2,250 mg/day. None of the female patients received telaprevir at 2,250 mg/day. The HCV RNA clearance rate was similar in the 2,250 and 1,500 mg/day groups (Fig. 2a). Both doses of telaprevir resulted in fall in hemoglobin, but the falls in the 2,250 mg/day group at 2, 4, 8, weeks after the start of treatment were significantly more profound compared with the 1,500 mg/day group (Fig. 2b). Furthermore, both doses of telaprevir

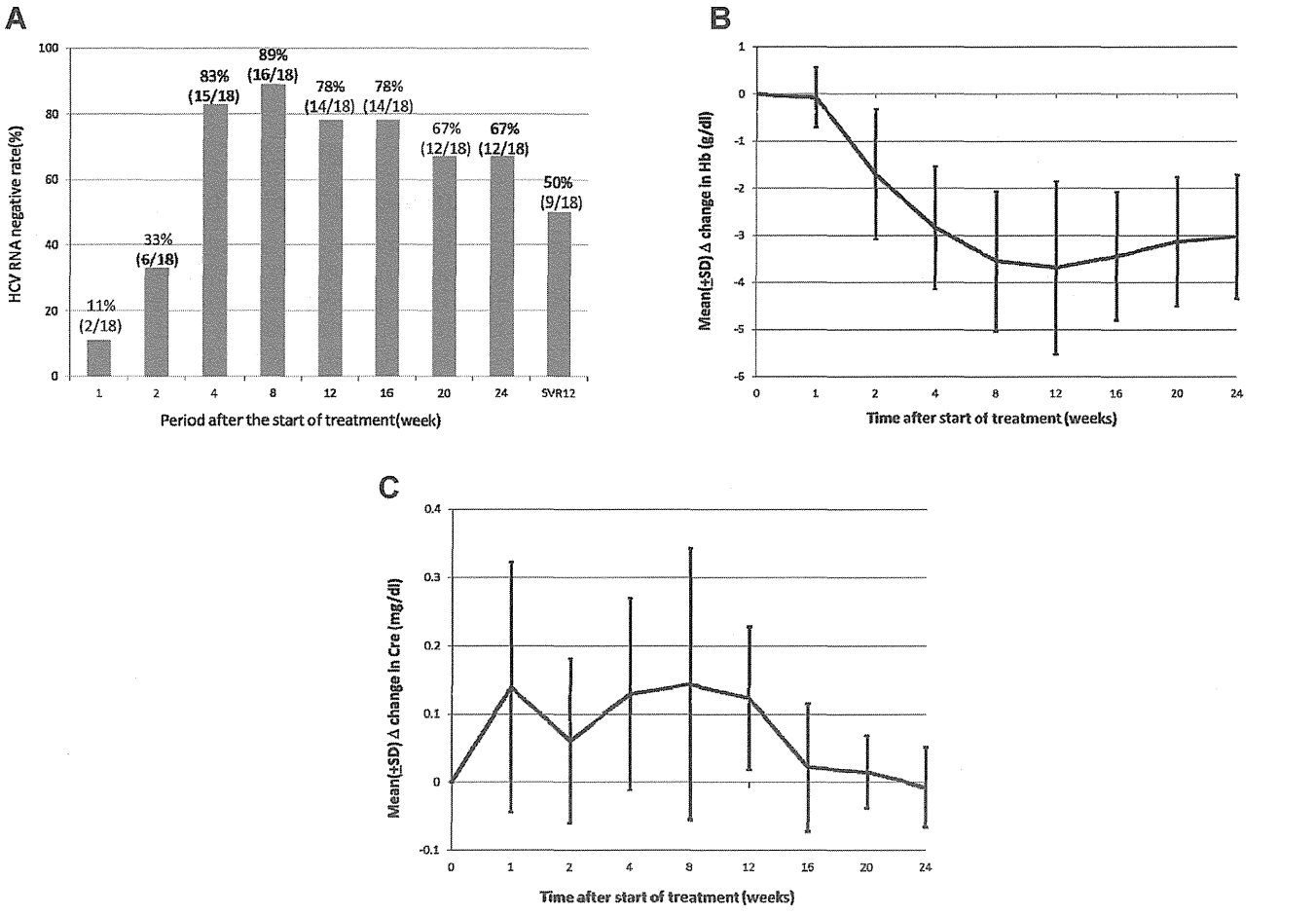

Fig. 1. **a**: HCV RNA clearance rate at different time points after the start of triple therapy of telaprevir with peginterferon and ribavirin. The sustained virological response-12 rate was 50% and the end-of-treatment response rate was 67%. **b**: Fall in hemoglobin in patients who received triple therapy of telaprevir, with peginterferon and ribavirin. **c**: Rise in creatinine in patients who received triple therapy of telaprevir, with peginterferon and ribavirin.

induced a rise in serum creatinine, but the rises in the 2,250 mg/day group at 12, 16, 24, weeks after the start of treatment were significantly more profound compared with the 1,500 mg/day group (Fig. 2c).

Relation between Loss of HCV RNA and IL-28B (rs8099917) Genotype TT

Figure 3a illustrates the negative rates of HCV RNA in patients with the rs8099917 genotype TT/non TT at different time points. The HCV RNA disappearance rate in patients with the rs8099917 genotype TT during treatment was 9% (1/11), 36% (4/11), 82% (9/11), 100% (11/11), 91% (10/11), 91% (10/11), 73% (8/11), and 73% (8/11) at 1, 2, 4, 8, 12, 16, 20, and 24 weeks, respectively. Furthermore, 64% (7/11) of the elderly patients achieved sustained virological response-12.

Figure 3b illustrates the HCV RNA clearance rates in patients with the rs8099917 genotype TT/non TT during treatment with telaprevir 1,500 mg. The HCV RNA clearance rates in patients with the rs8099917 genotype TT during treatment was 11% (1/9), 44% (4/9), 89% (8/9), 100% (9/9), 100% (9/9), 100% (9/9), 78% (7/9), and 78% (7/9) at 1, 2, 4, 8, 12, 16, 20, and 24 weeks, respectively. Furthermore, 67% (6/9) of the elderly patients achieved sustained virological response-12. These results highlight the safety and efficacy of telaprevir 1,500 mg, peginterferon, and ribavirin in elderly patients with the rs8099917 genotype TT.

DISCUSSION

With the aging society in Japan, it is important to evaluate the efficacy of interferon therapy in elderly patients with chronic HCV infection. This is important especially due to the lack of information on the safety and efficacy of triple therapy of telaprevir, peginterferon, and ribavirin. In the study of Suzuki

TABLE II. Characteristics of Patients at Baseline According to Telaprevir Dose and Adherence to Each Drug

Characteristics	Telaprevir dose		*P*-value
	2,250 mg	1,500 mg	
Number of patients	4	14	
Age (years)*	67 (66–68)	69 (66–73)	0.079
Male/female	4/0	10/4	0.023
Body mass index (kg/m^2)*	23.1 (22.3–24.1)	22.6 (18.9–26.3)	NS
Viral load of HCV (log_{10} IU/ml)	5.9 (5.3–7.0)	6.5 (5.1–7.3)	NS
Serum aspartate aminotransferase (IU/L)	43 (37–48)	27 (11–95)	NS
Serum alanine aminotransferase (IU/L)	36 (23–44)	36 (19–80)	NS
Serum albumin (g/dl)	3.9 (3.6–4.0)	3.7 (3.3–4.1)	NS
Gamma-glutamyl transpeptidase (IU/L)	31 (19–62)	22 (10–61)	NS
Leukocyte count ($/mm^3$)	5,400 (4,000–7,300)	3,900 (2,500–5,300)	0.035
Hemoglobin (g/dl)	14.4 (13.5–16.1)	13.9 (12.5–14.9)	NS
Platelet count ($\times 10^4/mm^3$)	16.9 (15.1–21.0)	14.9 (9.6–21.4)	NS
Alpha-fetoprotein (μg/L)	6 (5–7)	3 (1–18)	NS
Treatment			
Peginterferon α-2b dose (μg/kg)*	1.4 (1.3–1.6)	1.5 (1.0–1.8)	
Ribavirin dose (mg/kg)*	12.4 (11.6–13.2)	7.0 (5.8–12.9)	0.005
Amino acid substitutions in the HCV genotype 1b			NS
Core aa 70 (arginine/glutamine)	1/3	9/5	NS
Core aa 91 (leucine/methionine)	2/2	9/5	NS
ISDR of NS5A (wild-type/non wild-type/ND)	4/0/0	13/0/1	NS
Genetic variation near IL28B gene rs8099917 genotype (TT/TG/GG)	2/1/1	9/5/0	NS
Past history of interferon therapy Treatment-naïve/relapsers to previous treatment/nonresponders to previous treatment	2/2/0	1/8/5	0.087
PegIFN adherence (%)	78.7 (55.6–100)	80.0 (8.3–100)	NS
RBV adherence (%)	33.9 (17.7–68.8)	50.0 (6.7–79.2)	NS
TVR adherence (%)	68.4 (36.7–100)	66.7 (11.2–66.7)	NS
Comorbidities[a]			
Diabetes mellitus	1 (25%)	2 (14%)	NS
Hypertension	2 (50%)	7 (50%)	NS

Date are number (percentage) of patients, except those denoted by *, which represent the median (range) values.
[a]All patients were not on medications.

et al. [2012], 20 patients with chronic HCV infection and high viral load of genotype 1b were randomly assigned to two telaprevir-based regimens of 2,250 and 1,500 mg/day in combination with peginterferon and ribavirin for 12 weeks. The sustained virological response rates were not different between the 1,500 and 2,250 mg groups, while serum creatinine increased more extensively in the 2,250 mg group than in the 1,500 mg group. However, their patients were <65 years old and treated for only 12 weeks. In the present study, the response to triple therapy with telaprevir for 12 weeks, peginterferon, and ribavirin for 24 weeks was examined in a pilot study that included 18 elderly patients infected with HCV-1b with high viral loads. Four of the 18 patients were treated with telaprevir 2,250 mg/day and the other 14 patients were treated with telaprevir 1,500 mg/day. The results showed no tolerance to the triple therapy in 4 of 18 (22%) patients due to skin rashes, anemia, and poor appetite. However, 9 of 18 (50%) elderly patients who received the triple therapy were able to achieve sustained virological response-12. Furthermore, even when treated for 24 weeks, elderly patients of the 1,500 mg group showed reduction in the elevated serum creatinine that was similar to that seen in patients aged <65 years.

The IL-28B genotype is identified as a pretreatment predictor of virological response to 48-week peginterferon plus ribavirin combination therapy in individuals infected with HCV-1 [Ge et al., 2009; Tanaka et al., 2009; Suppiah et al., 2009], and also as a predictor of response to triple therapy with telaprevir, peginterferon, and ribavirin in Japanese patients infected with HCV-1 [Akuta et al., 2010, 2012; Chayama et al., 2011]. In the present study, among patients with the rs8099917 genotype TT who were treated with telaprevir 1,500 mg, 6 of 9 (67%) could achieve sustained virological response-12, and none discontinued the triple therapy because of side effects. Thus, for elderly patients with the rs8099917 genotype TT, triple therapy with telaprevir 1,500 mg, peginterferon, and ribavirin was safe and efficacious, especially in patients with the rs8099917 genotype TT.

Iwasaki et al. [2006] and Honda et al. [2010] reported that the sustained virological response rates at the completion of the 48-week interferon and ribavirin combination therapy for elderly patients were only 16% and 31%, respectively. However, in the present study, of 18 elderly patients, 12 (67%) were negative for HCV RNA at the end of the triple therapy, and sustained virological response-12 was

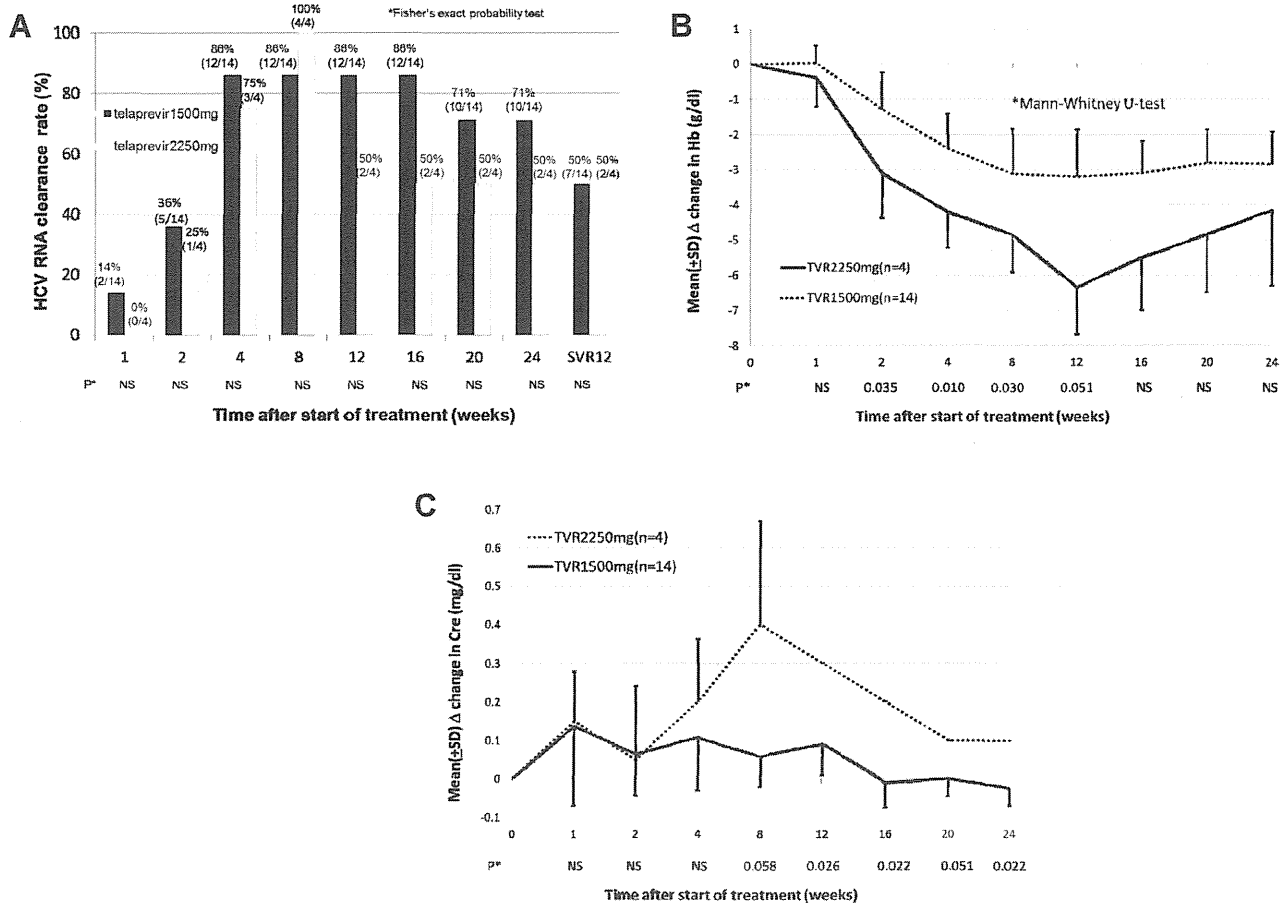

Fig. 2. **a**: HCV RNA clearance rate according to the dose of telaprevir (1,500 and 2,250 mg/day), combined with peginterferon and ribavirin. The sustained virological response-12 rate was 50% in both dose groups and the end-of-treatment response rates were 71% and 50%, respectively. **b**: Fall in hemoglobin according to the dose of telaprevir (1,500 and 2,250 mg/day), in combination with peginterferon and ribavirin. The fall was more profound in the 2,250 mg/day group at 2, 4, and 8 weeks compared with the 1,500 mg/day group. **c**: Rise in serum creatinine according to the dose of telaprevir (1,500 and 2,250 mg/day), in combination with peginterferon and ribavirin. The rise was more profound in the 2,250 mg/day group at 12, 16, and 24 weeks compared with the 1,500 mg/day group.

achieved by 9 patients (50%). Analysis of the data of the 14 elderly patients showed sustained virological response-12 was achieved in seven (50%) patients who received triple therapy with telaprevir 1,500 mg, peginterferon, and ribavirin, seven (50%). These results indicate that triple therapy with telaprevir 1,500 mg, peginterferon, and ribavirin, is safe and efficacious. Further studies are needed to determine if such treatment can be shortened to 24 weeks.

This study is not without limitations. The number of patients who received triple therapy was small and the study failed to show statistical significance in any comparison of various factors, especially between telaprevir 1,500 mg and telaprevir 2,250 mg treatment groups. This study is retrospective in nature; therefore, selection bias may have affected the results. We did not estimate sustained virological response-24 in the present study. Martinot-Peignoux et al. [2010] and Mauss et al. [2012] reported sustained virological response-12 as endpoint for future trials because HCV relapse usually occurs within the first 12 weeks after the end of treatment. Accordingly, in this study, we estimated sustained virological response-12. To generalize medical treatment for elderly patients with chronic HCV infection, further large scale randomized control clinical trials for telaprevir 1,500 mg and 2,250 mg are necessary to investigate the sustained virological response-24.

In conclusion, triple therapy with telaprevir 1,500 mg, peginterferon, and ribavirin, is safe and efficacious in elderly patients with chronic HCV infection. The triple therapy could be selected as

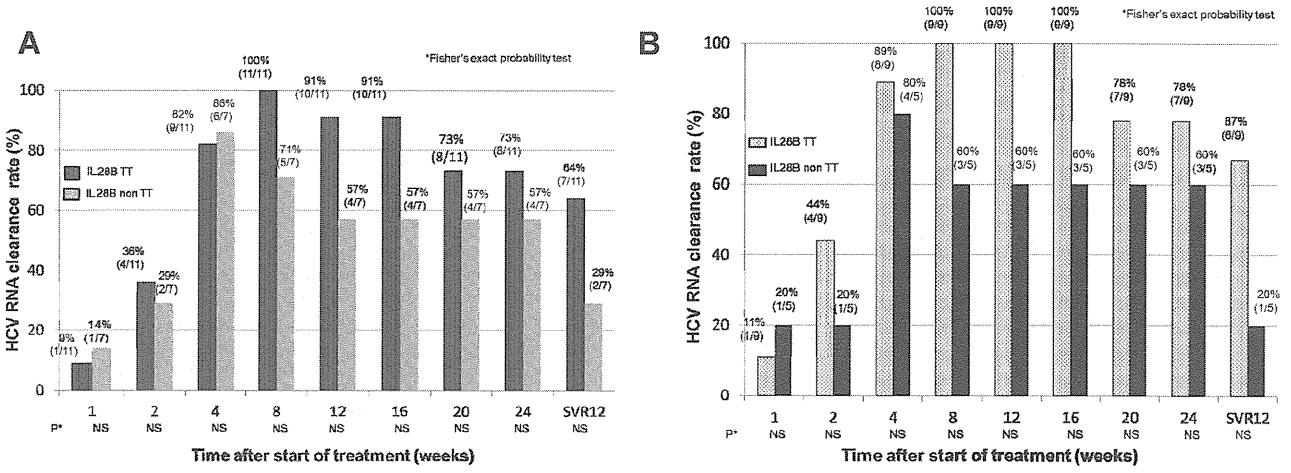

Fig. 3. **a**: HCV RNA clearance rate according to rs8099917 genotype TT. The sustained virological response-12 rate was 64% and the end-of-treatment response rate was 73% in patients with rs8099917 genotype TT. **b**: HCV RNA clearance rate according to rs8099917 genotype TT after the start of triple therapy of telaprevir 1,500 mg with peginterferon and ribavirin. The sustained virological response-12 rate was 78% and the end-of-treatment response rate was 67%.

potentially suitable therapy for elderly Japanese patients aged >66 years with chronic HCV of genotype 1b.

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Efficacy and Anticarcinogenic Activity of Ribavirin Combination Therapy for Hepatitis C Virus-Related Compensated Cirrhosis

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Key Words
Hepatitis C virus · Interferon · Ribavirin · Hepatocellular carcinoma · Cirrhosis · Biochemical response

Abstract
Objective: Anticarcinogenic activity of ribavirin combination therapy for hepatitis C virus (HCV)-related compensated cirrhosis is still unclear. ***Methods:*** In study 1, in 157 consecutive patients with HCV-related compensated cirrhosis, treatment efficacy with interferon plus ribavirin therapy was evaluated for 48 weeks of HCV genotype 1b (HCV-1b) or 24 weeks of HCV-2a/2b. In study 2, in 185 consecutive patients with HCV-related compensated cirrhosis, who showed no sustained virological response following the first course of interferon monotherapy, hepatocarcinogenesis rates were evaluated according to the additional treatment, and they were classified into three groups: no treatment, interferon monotherapy, and ribavirin combination therapy. ***Results:*** In study 1, in HCV-1b, rates of sustained virological response and sustained biochemical response were 21 and 56%, respectively. In HCV-2a/2b, rates of sustained virological response and sustained biochemical response were 70 and 78%, respectively. In HCV-1b, sustained biochemical response rates were significantly higher than those of sustained virological response. In study 2, the hepatocarcinogenesis rates in ribavirin combination therapy were significantly lower than those in interferon monotherapy and no treatment, respectively. ***Conclusion:*** Ribavirin combination therapy for HCV-related compensated cirrhosis reduces the risk of hepatocarcinogenesis in comparison with interferon monotherapy, and higher rates of sustained biochemical response might be associated with lower hepatocarcinogenesis rates.

Introduction

Hepatitis C virus (HCV) usually causes chronic infection, which can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1–5]. The life expectancy of patients with HCV-related cirrhosis is largely influenced by the development of hepatocellular carcinoma during the clinical course [3]. Because an effective and curative therapy for hepatocellular carcinoma remains

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Table 1. Profile and laboratory data at the start of ribavirin combination therapy in 157 patients with HCV-related compensated cirrhosis (study 1)

Demographic data	
Patients, n	157[1]
Sex (male/female), n	105/52
Age, years	58 (34–74)
Laboratory data	
Serum aspartate aminotransferase, IU/l	69 (7–235)
Serum alanine aminotransferase, IU/l	70 (14–585)
Leukocytes, /mm^3	4,100 (1,600–8,800)
Hemoglobin, g/dl	14.0 (9.4–17.6)
Platelet count, $\times 10^4$/mm^3	11.3 (6.1–32.2)
HCV genotype (1b/2a/2b), n	120/27/10
Levels of viremia, log IU/ml	6.1 (3.9–7.5)
Treatment	
Past history of interferon-based therapy, n	95 (60.5%)
PEG-IFNα-2b/IFNα-2b, n	110/47
Ribavirin dose, mg/kg	10.7 (2.7–15.1)
Duration of treatment, weeks	
Genotype 1b	48 (1–48)
Genotype 2a or 2b	24 (5–24)

Unless otherwise indicated, values represent median (range).

[1] 24 of the 157 patients with HCV-related compensated cirrhosis in study 1 were also included in study 2. They showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks) and were treated additionally with ribavirin combination therapy (≥24 weeks).

limited at best, primary prevention of hepatocellular carcinoma in patients with chronic liver disease is of great importance at present.

Treatment of HCV-chronic hepatitis with interferon can induce viral clearance and marked biochemical and histological improvement [6, 7]. Furthermore, previous studies showed that interferon monotherapy reduced the risk of hepatocellular carcinoma [8–10]. However, an extended analysis of the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) cohort recently showed that long-term peginterferon (PEG-IFN) monotherapy could not reduce the incidence of hepatocellular carcinoma among patients with advanced hepatitis C who did not achieve sustained virological response, and patients with cirrhosis who received PEG-IFN monotherapy had a lower risk of hepatocellular carcinoma than controls [11]. Thus, it is controversial whether interferon monotherapy for patients with liver cirrhosis might reduce hepatocarcinogensis. Furthermore, it is still unclear whether ribavirin combination therapy for patients with liver cirrhosis might reduce the risk of hepatocellular carcinoma, and there are also no reports on whether ribavirin combination therapy could reduce the risk in comparison with interferon monotherapy.

The present study investigated the efficacy and anticarcinogenic activity of ribavirin combination therapy for HCV-related compensated cirrhosis, especially in comparison with interferon monotherapy.

Materials and Methods

Study Population

Two retrospective cohort studies were performed to investigate treatment efficacy and anticarcinogenic activity of ribavirin combination therapy for HCV-related compensated cirrhosis.

In the study 1 cohort, 157 consecutive patients of HCV-related compensated cirrhosis were recruited into the study protocol of interferon (PEG-IFNα-2b or IFNα-2b) plus ribavirin combination therapy for 48 weeks of HCV genotype 1b (HCV-1b) or 24 weeks of HCV-2a/2b, from 2001 to 2010 at Toranomon Hospital. In this retrospective study the rates of sustained virological response [HCV-RNA negativity at 24 weeks after the completion of therapy based on the COBAS TaqMan HCV test (Roche Diagnostics)] were evaluated as well as sustained biochemical response [normal level of serum alanine aminotransferase at 24 weeks after the completion of therapy (6–50 IU/l)]. Treatment efficacy was evaluated by intention-to-treat (ITT) analysis classified as treatment failure in patients who could not complete the treatment regimen and per protocol (PP) analysis. Table 1 summarizes the profiles and data of the 157 patients at the commencement of combination therapy with interferon plus ribavirin in study 1. They included 105 men and 52 women aged 34–74 years (median 58 years). 110 (70.1%) patients received PEG-IFNα-2b plus ribavirin, and the remaining 47 (29.9%) patients received IFNα-2b plus ribavirin. They received PEG-IFNα-2b at a median dose of 1.3 μg/kg (range 0.5–1.9 μg/kg) subcutaneously each week or IFNα-2b at a median dose of 6 million units (range 3–6 million units) intramuscularly each day (7 times per week for the initial 2 weeks followed by 3 times per week). They also received oral ribavirin at a median dose of 10.7 mg/kg (range 2.7–15.1 mg/kg) daily. In 56 of the 157 (35.7%) patients, the dose of ribavirin was reduced during treatment due to a fall in hemoglobin concentration. The median total duration of treatment in 120 patients of HCV-1b was 48 weeks (range 1–48 weeks), and that in 37 patients of genotype 2a or 2b was 24 weeks (range 5–24 weeks).

In the study 2 cohort (fig. 1), 185 consecutive patients of HCV-related compensated cirrhosis, who showed no sustained virological response following at the first course of interferon monotherapy (≥24 weeks) from 1987 to 2010 at Toranomon Hospital, were recruited. Hepatocarcinogenesis rates were evaluated according to the additional treatment (second course of treatment), and were classified into three groups: no treatment (106 patients), interferon monotherapy (≥24 weeks; 55 patients), and ribavirin combination therapy (≥24 weeks; 24 patients). 106 patients without treatment did not receive the additional treatment because of concerns about adverse effects, lack of time for treatment, physician recommendation based on the appearance of depression and car-