Table 1. Profile and laboratory data at commencement of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b

Demographic data	
Number of patients	80
Sex, M/F	43/37
Age, years*	55 (23–65)
History of blood transfusion	24 (20.0%)
Family history of liver disease	13 (16.3%)
Body mass index*	22.5 (13.2–32.4)
*Laboratory data**	
Level of viremia, log IU/ml	6.8 (5.1–7.6)
Serum aspartate aminotransferase, IU/l	34 (15–118)
Serum alanine aminotransferase, IU/l	42 (12–175)
Serum albumin, g/dl	3.9 (3.3–4.6)
Gamma-glutamyl transpeptidase, IU/l	36 (9–229)
Leukocyte count, per mm^3	4,800 (2,800–8,100)
Hemoglobin, g/dl	14.3 (11.7–16.8)
Platelet count, $\times 10^4/mm^3$	17.3 (9.5–33.8)
α-Fetoprotein, μg/l	4 (2–39)
Total cholesterol, mg/dl	180 (112–276)
Fasting plasma glucose, mg/dl	92 (64–125)
Treatment	
PEG-IFNα-2b dose, μg/kg*	1.5 (1.3–2.0)
Ribavirin dose, mg/kg*	11.5 (7.2–18.4)
Telaprevir dose, 1,500/2,250 mg/day	10/70
Treatment regimen (T12PR12 group/T12PR24 group)	20/60
Amino acid substitutions in the HCV-1b	
Core aa 70, arginine/glutamine (histidine)	47/33
Core aa 91, leucine/methionine	43/37
ISDR of NS5A, wild-type/non-wild-type	76/4
Genetic variation near IL28B *gene*	
rs8099917 genotype, TT/TG/GG/ND	46/30/2/2
rs12979860 genotype, CC/CT/TT/ND	43/31/2/4
Past history of IFN therapy	
Treatment naive	27
Relapsers to previous treatment	33
Nonresponders to previous treatment	20

Data are numbers and percentages of patients, except those denoted by *, which represent the median (range) values.
ND = Not determined.

phil count below 750/mm^3 or platelet count below 80,000/mm^3; PEG-IFN was discontinued when these counts decreased below 1,000/mm^3, 500/mm^3 or 50,000/mm^3, respectively. When hemoglobin decreased to <10 g/dl, the daily dose of ribavirin was reduced from 600 to 400, 800 to 600 and 1,000 to 600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dl. However, the dose of telaprevir (MP-424) remained the same, and its administration was stopped when the discontinuation was appropriate for the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Table 1 summarizes the profiles and laboratory data of the 80 patients at the commencement of treatment. They included 43 males and 37 females, aged 23–65 years (median 55 years).

Measurement of HCV RNA

The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every month before, during, and after therapy. Furthermore, to investigate the pretreatment predictors for viral dynamics, HCV RNA levels during treatment were evaluated at 7 time points; 24 h, 1, 2, 4, 6, 8 and 12 weeks after the commencement of treatment. HCV RNA levels during treatment were evaluated in 80 (100%), 80 (100%), 80 (100%), 79 (98.8%), 75 (93.8%), 74 (92.5%), and 69 (86.3%) of the 80 patients, at the above time intervals, respectively. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as loss of HCV RNA. Especially, falls in HCV RNA levels at 24 h relative to baseline were investigated as very early dynamics.

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 [23], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on 80 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [12]. The sequence of 2209–2248 aa in the NS5A of HCV-1b (IFN sensitivity-determining region; ISDR) reported by Enomoto et al. [24] was determined, and the numbers of aa substitutions in ISDR were defined as wild-type (0, 1) or non-wild-type (≥2). In the present study, aa substitutions of the core region and NS5A-ISDR of HCV-1b were analyzed by direct sequencing [22].

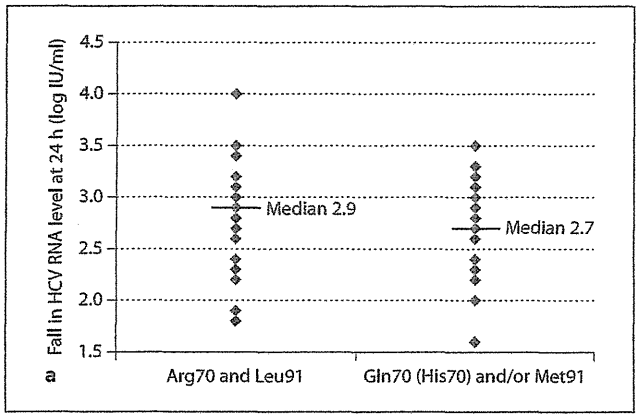
Genetic Variation near IL28B Gene

Samples for genomewide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of the Invader assay, TaqMan assay, or direct sequencing as described previously [25, 26].

In this study, genetic variations near *IL28B* gene (rs8099917, rs12979860), reported as the pretreatment predictors of treatment efficacy and clinical outcome [17–22], were investigated.

Statistical Analysis

Nonparametric tests (χ^2 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 viral dynamics and sustained virological response. The ORs and 95%CI were also calculated. All p values less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into



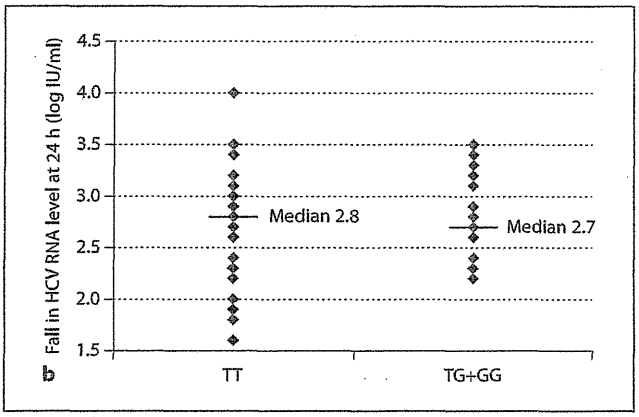


Fig. 1. a Very early dynamics according to amino acid substitutions in core region. After 24 h of commencement of the triple therapy, patients with Arg70 and Leu91 (median 2.9 log IU/ml; range 1.8–4.0 log IU/ml) significantly showed the steeper decline of HCV RNA level than those with Gln70 (His70) and/or Met91 (median 2.7 log IU/ml; range 1.6–3.5 log IU/ml). **b** Very early dynamics according to genetic variation near the *IL28B* gene. After 24 h of commencement of the triple therapy, the decline of HCV RNA level of patients with rs8099917 genotype TT (median 2.8 log IU/ml; range 1.6–4.0 log IU/ml) was not significantly different from that of patients with genotype TG and GG (median 2.7 log IU/ml; range 2.2–3.5 log IU/ml).

multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. The potential pretreatment factors associated with treatment efficacy included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, α-fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, treatment regimen of triple therapy, past history of IFN therapy, genetic variation near the *IL28B* gene, and amino acid substitution in the core region, and NS5A-ISDR. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

Virological Response to Therapy and Loss of HCV RNA during Treatment

Sustained virological response was achieved by 63.6% (49 of 77 patients). The disappearance rate of HCV RNA during treatment was 0% (0 of 80), 1.3% (1 of 80), 33.8% (27 of 80), 81.0% (64 of 79), 90.7% (68 of 75), 94.6% (70 of 74), and 89.9% (62 of 69) at 24 hours, 1, 2, 4, 6, 8, and 12 weeks, respectively.

Very Early Dynamics according to Amino Acid Substitutions in Core Region and Genetic Variation near the IL28B Gene

After 24 h of commencement of the triple therapy, the proportion of patients with Arg70 and Leu91 substitutions who showed ≥3.0 log drop in HCV RNA level (45.2%; 14 of 31 patients) was significantly higher than that of patients with Gln70 (His70) and/or Met91 (14.3%; 7 of 49) ($p = 0.004$). Thus, patients with Arg70 and Leu91 (median 2.9 log IU/ml; range 1.8–4.0 log IU/ml) significantly showed the steeper decline of HCV RNA level than those with Gln70 (His70) and/or Met91 (median 2.7 log IU/ml; range 1.6–3.5 log IU/ml) (fig. 1a).

After 24 h of commencement of treatment, the proportion of patients with rs8099917 genotype TT who showed ≥3.0 log drop in HCV RNA level (30.4%; 14 of 46 patients) was not significantly different from that of patients with genotype TG and GG (21.9%; 7 of 32). Thus, the decline of HCV RNA level of patients with genotype TT (median 2.8 log IU/ml; range 1.6–4.0 log IU/ml) was not significantly different from that of patients with genotype TG and GG (median 2.7 log IU/ml; range 2.2–3.5 log IU/ml) (fig. 1b).

Hence, the fall in HCV RNA level at 24 h was influenced by aa substitution patterns in the core region, but was independent of genetic variation near *IL28B* gene.

Rates of Loss of HCV RNA according to Amino Acid Substitutions in Core Region and Genetic Variation near the IL28B Gene

According to the substitution of core aa 70 and 91, the rate of HCV RNA loss of patients with Arg70 and Leu91 was not significantly different from that of patients with

Gln70 (His70) and/or Met91 at each time point (1, 2, 4, 6, 8 and 12 weeks).

According to genetic variation near the *IL28B* gene, the rate of HCV RNA loss at 1, 2, 4 and 6 weeks was not significantly different between rs8099917 genotype TT and non-TT (TG and GG). However, at 8 and 12 weeks, the rate of HCV RNA loss of patients with genotype TT was significantly higher than that of patients with genotype non-TT (fig. 2).

Predictive Factors Associated with ≥3.0 log Fall in HCV RNA Level at 24 Hours

Univariate analysis identified two parameters that correlated with ≥3.0 log fall in HCV RNA level at 24 h significantly: substitution of aa 70 and 91 (Arg70 and Leu91; OR 4.94, p = 0.003) and body mass index (≥25.0; OR 3.92, p = 0.022). Two factors were identified by multivariate analysis as independent parameters that either significantly ($p < 0.05$) or marginally ($p < 0.10$) influenced ≥3.0 log fall in HCV RNA level at 24 h [Arg70 and Leu91 (OR 3.99, p = 0.015) and body mass index ≥25.0 (OR 3.24, p = 0.061)] (table 2).

Predictive Factors Associated with Loss of HCV RNA at 2, 4 and 12 Weeks

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 2 weeks significantly: platelet count (≥15.0 × 10^4/mm^3; OR 6.99, p = 0.014) and level of viremia (<7.0 log IU/ml; OR 3.13, p = 0.045). One factor was identified by multivariate analysis as independent parameter that either significantly or marginally influenced loss of HCV RNA at 2 weeks (platelet count ≥15.0 × 10^4/mm^3; OR 6.99, p = 0.014) (table 2).

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 4 weeks significantly: history of blood transfusion (absence; OR 5.71, p = 0.006) and body mass index (≥20.0; OR 4.29, p = 0.019). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced loss of HCV RNA at 4 weeks (history of blood transfusion: absence; OR 4.29, p = 0.026, and body mass index ≥20.0; OR 3.47, p = 0.069) (table 2).

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 12 weeks significantly: sex (male; OR 9.52, p = 0.043) and genetic variation in rs8099917 (genotype TT; OR 9.00, p = 0.048). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced loss of HCV RNA at 12 weeks (male sex; OR 11.0, p = 0.036, and rs8099917 genotype TT; OR 10.3, p = 0.042) (table 2).

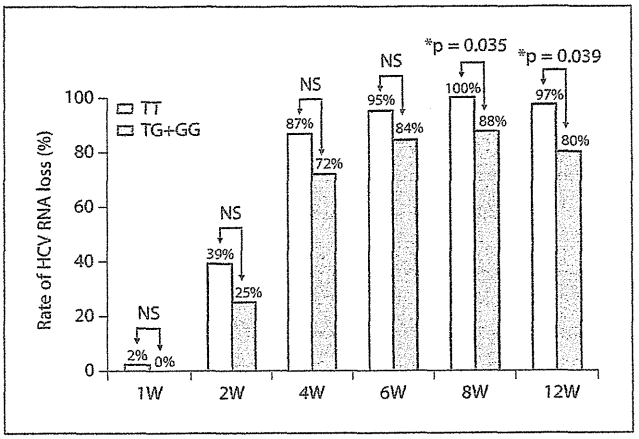


Fig. 2. Rates of loss of HCV RNA according to genetic variation near the *IL28B* gene. According to genetic variation near the *IL28B* gene, the rate of HCV RNA loss at 1, 2, 4 and 6 weeks was not significantly different between rs8099917 genotype TT and non-TT (TG and GG). However, at 8 and 12 weeks, the rate of HCV RNA loss of patients with genotype TT was significantly higher than that of patients with genotype non-TT.

Predictive Factors Associated with Sustained Virological Response

Univariate analysis identified three parameters that correlated with sustained virological response significantly: substitution of aa 70 (Arg70; OR 3.51, p = 0.011), and genetic variation in rs8099917 (genotype TT; OR 11.1, $p < 0.001$) and rs12979860 (genotype CC; OR 10.2, $p < 0.001$). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced sustained virological response (rs8099917 genotype TT; OR 9.94, $p<0.001$, and Arg70; OR 3.15, p = 0.055) (table 2).

Comparison of Factors Associated with Each Treatment Efficacy Identified by Multivariate Analysis

Table 3 shows independent parameters that either significantly or marginally influenced multivariate logistic regression for each evaluation of treatment efficacy. Multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 h (Arg70 and Leu91) and sustained virological response (Arg70), and rs8099917 (TT) as a predictor of HCV RNA loss at 12 weeks and sustained virological response. Thus, genetic variation near *IL28B* gene and aa substitution of the core region affect viral dynamics of different phases during triple therapy.

Table 2. Factors associated with treatment efficacy of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b, identified by univariate and multivariate analysis

Factor		Category	Univariate logistic regression		Multivariate logistic regression	
			OR (95% CI)	p	OR (95% CI)	p
A	≥3.0 log fall in HCV RNA at 24 h					
	Substitution of aa 70 and 91	1: Gln70 (His70) and/or Met91	1		1	
		2: Arg70 and Leu91	4.94 (1.70–14.4)	0.003	3.99 (1.31–12.2)	0.015
	Body mass index	1: <25.0	1		1	
		2: ≥25.0	3.92 (1.22–12.6)	0.022	3.24 (0.95–11.1)	0.061
B	HCV RNA loss at 2 weeks					
	Platelet count, $\times 10^4/mm^3$	1: <15.0	1		1	
		2: ≥15.0	6.99 (1.49–32.8)	0.014	6.99 (1.49–32.8)	0.014
	Level of viremia, log IU/ml	1: ≥7.0	1		–	–
		2: <7.0	3.13 (1.02–9.52)	0.045	–	–
C	HCV RNA loss at 4 weeks					
	History of blood transfusion	1: presence	1		1	
		2: absence	5.71 (1.66–19.6)	0.006	4.29 (1.86–15.6)	0.026
	Body mass index	1: <20.0	1		1	
		2: ≥20.0	4.29 (1.26–14.5)	0.019	3.47 (0.91–13.3)	0.069
D	HCV RNA loss at 12 weeks					
	Sex	1: female	1		1	
		2: male	9.52 (1.08–83.3)	0.043	11.0 (1.16–100)	0.036
	rs8099917 genotype	1: TG+GG	1		1	
		2: TT	9.00 (1.02–79.5)	0.048	10.3 (1.08–98.0)	0.042
E	Sustained virological response					
	rs8099917 genotype	1: TG+GG	1		1	
		2: TT	11.1 (3.68–33.5)	<0.001	9.94 (3.05–32.4)	<0.001
	Substitution of aa 70	1: Gln70 (His70)	1		1	
		2: Arg70	3.51 (1.33–9.26)	0.011	3.15 (0.97–10.2)	0.055
	rs12979860 genotype	1: CT+TT	1		–	–
		2: CC	10.2 (3.33–3.13)	<0.001	–	–

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 other significant predictors of HCV RNA loss were platelet count (≥15.0 × $10^4/mm^3$) at 2 weeks, history of blood transfusion (absence) at 4 weeks, and sex (male) at 12 weeks.

Discussion

Thompson et al. [27] reported that genetic variation near *IL28B* gene was also associated with increased on-treatment and sustained virological response and effectively predicted treatment outcome in treatment-naive HCV-1 patients treated with PEG-IFN plus ribavirin. However, HCV RNA loss at 4 weeks (rapid virological response) was a strong predictor of sustained virological response regardless of genetic variation near the *IL28B* gene. This phenomenon probably explains why it might be important to identify the pretreatment factors that could predict viral dynamics during treatment. The present study is the first to identify the pretreatment factors that could predict viral dynamics during triple therapy in patients infected with HCV-1. These results should be interpreted with caution since races other than Japanese and the patients infected with HCV-1a were not included. Any generalization of the results should await confirmation by studies including patients of other races and with HCV-1a to explore whether genetic variation near *IL28B* gene and aa substitution

Table 3. Comparison of factors associated with treatment efficacy of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b identified by multivariate analysis

Factor	≥3.0 log fall in HCV RNA (at 24 h)	HCV RNA loss (at 2 weeks)	HCV RNA loss (at 4 weeks)	HCV RNA loss (at 12 weeks)	Sustained virological response
Core aa 70 and 91	Arg70 and Leu91 p = 0.015 3.99 (1.31–12.2)*				Arg70 p = 0.055 3.15 (0.97–10.2)*
IL28B rs8099917				genotype TT p = 0.042 10.3 (1.08–98.0)*	genotype TT p < 0.001 9.94 (3.05–32.4)*
Others	body mass index p = 0.061 3.24 (0.95–11.1)*	platelet count p = 0.014 6.99 (1.49–32.8)*	body mass index p = 0.069 3.47 (0.91–13.3)* history of blood transfusion p = 0.026 4.29 (1.86–15.6)*	sex p = 0.036 11.0 (1.16–100)*	

Only variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) on multivariate logistic regression are shown. * OR (95% CI).

of core region also affect viral dynamics during triple therapy.

Two studies showed that aa substitution of the core region and genetic variation near *IL28B* gene affected viral dynamics during treatment, and sustained virological response to 48-week PEG-IFN plus ribavirin therapy in patients infected with HCV-1 [27, 28]. Furthermore, a recent report also showed that aa substitutions of core region might be used to predict very early dynamics (within 48 h) after the start of triple therapy of telaprevir with PEG-IFN and ribavirin [29]. In the present study, multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 hours (i.e. viral dynamics of very early phase) and sustained virological response, and rs8099917 as a predictor of HCV RNA loss at 12 weeks (i.e. viral dynamics of later phase) and sustained virological response. This study is the first to report that genetic variation near *IL28B* gene and aa substitution of the core region affect viral dynamics of different phases during triple therapy, and probably explains why the combination of these independent factors is very useful as pretreatment predictors of sustained virological response by triple therapy [22]. The underlying mechanisms of the different viral dynamics to treatment are still unclear, and further studies based on a larger number of patients are necessary to investigate the present results.

Previous data indicated that absence of advanced liver fibrosis and male gender were positive predictors of virological response to 48-week PEG-IFN plus ribavirin therapy [13, 28]. The present study also showed that higher levels of platelet count at 2 weeks, as a surrogate marker of milder liver fibrosis, and male gender at 12 weeks were significant positive predictors of HCV RNA loss during triple therapy. The other positive predictors were absence of history of blood transfusion at 4 weeks and higher levels of body mass index at 24 h and 4 weeks, but the underlying mechanisms are still unclear. Thus, this report identified the pretreatment factors that could predict viral dynamics during triple therapy, but this study, based on a small number of patients, might provide misleading results (e.g. possible type error). Further studies of a larger number of patients are required to explore predictors, including viral- and host-related factors.

The limitations of the present study were that aa substitutions in areas other than the core region and NS5A-ISDR of the HCV genome, such as the interferon/ribavirin resistance determining region (IRRDR) [30], were not examined. Furthermore, HCV mutants with aa conversions for resistance to telaprevir during triple therapy, such as the 156S mutation [31], were also not investigated. In this regard, telaprevir-resistant HCV mutants were reported to be susceptible to IFN in both in vivo and in vitro studies [32, 33]. Thus, viral factors before and during triple therapy should be investigated in

future studies, and identification of these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, this study identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of viral dynamics during triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV-1b. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of the triple therapy, and further understanding of the complex interaction between virus- and host-related factors should facilitate the development of more effective therapeutic regimens.

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Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan

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Background & Aims: To evaluate the efficacy and safety of telaprevir in combination with peginterferon-α2b (PEG-IFN) and ribavirin (RBV) in patients with chronic hepatitis C.

Methods: In a multi-center randomized clinical trial in Japan, on patients infected with HCV of genotype 1, 126 patients were assigned to telaprevir for 12 weeks along with PEG-IFN and RBV for 24 weeks (Group A), while 63 to PEG-IFN and RBV for 48 weeks (Group B).

Results: HCV RNA disappeared more swiftly in patients in Group A than B, and the frequency of patients without detectable HCV RNA at week 4 (rapid virological response (RVR)) was higher in Group A than B (84.0% *vs.* 4.8%, p <0.0001). Grade 3 and 4 skin disorders, including Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms, as well as Grade 3 anemia (<8.0 g/dl), occurred more frequently in Group A than B (skin disorders, 11.9% *vs.* 4.8%; anemia, 11.1% *vs.* 0.0%). The total RBV dose was smaller in Group A than B (47.0% *vs.* 77.7% of the target, p <0.0001). Despite these drawbacks, sustained virological response (SVR) was achieved more frequently in Group A than B (73.0% *vs.* 49.2%, p = 0.0020).

Conclusions: Although the triple therapy with telaprevir-based regimen for 24 weeks resulted in more adverse events and less total RBV dose than PEG-IFN and RBV for 48 weeks, it was able to achieve higher SVR within shorter duration by carefully monitoring adverse events and modifying the RBV dose as required.

Keywords: Telaprevir; Chronic hepatitis C; Peginterferon; Ribavirin; Sustained virological response; Genotypes.

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Abbreviations: PEG-IFN, peginterferon; RBV, ribavirin; SVR, sustained virological response; SOC, standard of care; DAA, direct acting antiviral.

Introduction

Over the world, an estimated 170 million people are persistently infected with hepatitis C virus (HCV) [1]. Most individuals with persistent HCV infection can fulfill the life expectancy, while about 30% of them develop life-threatening liver disease such as decompensated cirrhosis and hepatocellular carcinoma [2,3].

Currently, interferon (IFN) is the only antiviral drug capable of terminating HCV infection. The present standard-of-care (SOC) therapy for patients infected with HCV of genotype 1, the most prevalent genotype over the world, is peginterferon (PEG-IFN) combined with ribavirin (RBV) for 48 weeks. However, sustained virological response (SVR), judged by the loss of detectable HCV RNA from serum 24 weeks after the completion of therapy, can be achieved in only 42–52% of the patients [4–6]. To cope with this grim situation, a number of direct acting antivirals (DAAs) have been designed and developed, represented by NS3/4A protease inhibitors and NS5B polymerase or NS5A inhibitors [7]. Among them, telaprevir has shown promising results, when combined with PEG-IFN and RBV, in the phase 2 [8,9] and 3 clinical trials [10,11], by improving SVR to ~70% in patients infected with HCV-1.

Previous trials with the triple therapy were conducted in Europe and the United States, respectively. Hence, Asians were under-represented, accounting only for 1.6–2.1% of studied patients, and distributions of genotypes 1a (44–67%) and 1b (27–55%) varied widely [8–10]. In view of ethnic differences in response to IFN-based treatments [12,13], as well as profiles of resistance to telaprevir difference between genotypes 1a and 1b [14], a multicenter, randomized, and treatment-controlled clinical trial was conducted for comparison of therapeutic efficacy between the triple therapy and SOC in patients infected with HCV-1b in Japan.

Patients and methods

Patients

From November 2008 through August 2010, 220 patients, who were infected with HCV-1 and had not received antiviral treatments before, were recruited at 41 institutions in Japan. They joined the study for finding differences in the

Table 1. Baseline characteristics of patients.

Features[a]	Group A: T12PR24 (n = 126)	Group B: PR48 (n = 63)
Men (%)	66 (52.4%)	33 (52.4%)
Age (years)	53.0 (20-65)	55.0 (20-65)
Weight (kg)	60.2 (40.7-87.5)	64.1 (42.1-84.9)
BMI (kg/m^2)	22.6 (16.2-31.1)	23.3 (17.9-30.8)
Hemoglobin (g/dl)	14.3 (12.1-17.1)	14.5 (12.3-17.5)
White blood cells (/mm^3)	5300 (2900-10,670)	5130 (2950-11,050)
Platelets (x10^4/mm^3)	19.2 (9.0-36.2)	20.2 (8.7-37.0)
ALT (IU/L)	36.5 (12-252)	45.0 (18-259)
AST (IU/L)	34.0 (18-170)	38.0 (17-142)
Total bilirubin (mg/dl)	0.70 (0.3-1.9)	0.80 (0.4-1.8)
Total cholesterol (mg/dl)	182 (111-299)	180 (116-263)
HCV RNA ($\log_{10}$ IU/ml)	6.7 (5.1-7.5)	6.9 (5.1-7.4)
HCV genotypes		
1a	2 (1.6%)	0 (0.0%)
1b	124 (98.4%)	63 (100.0%)

[a]Values are the median with the range in parentheses, or number with the percentage in parentheses.

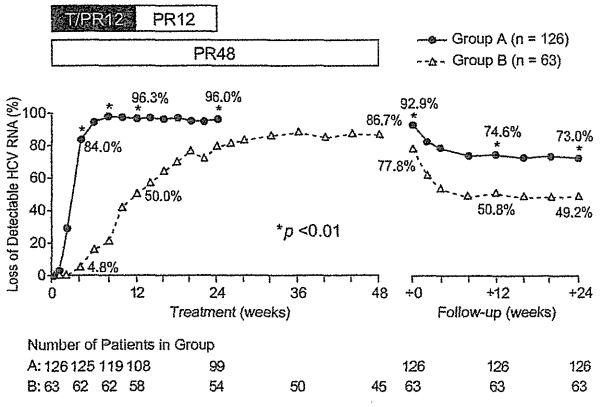

Fig. 1. Loss of detectable HCV RNA in patients in Groups A and B. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and weeks 12 and 24 in the follow-up period. An asterisk (*) indicates p <0.01 differences. The number of patients at each time point is indicated below the graph.

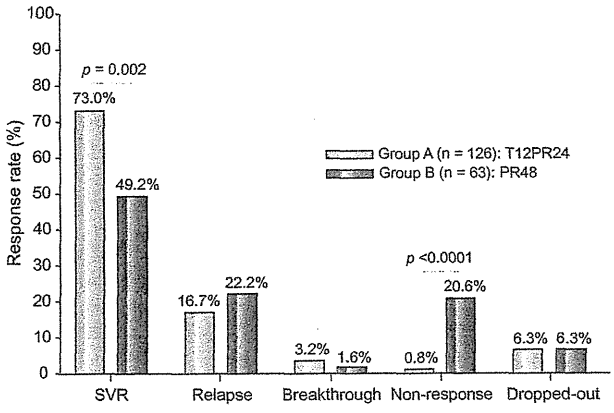

Fig. 2. Comparison of treatment responses between patients in Groups A and B. SVR, sustained virological response (HCV RNA negative 24 weeks after the completion of treatment); relapse, reappearance of HCV RNA in serum during follow-up period; breakthrough, reappearance of HCV RNA in serum during treatment period; non-response, HCV RNA continuously detectable in serum during treatment period.

treatment response and adverse events between the triple therapy involving telaprevir, PEG-IFN and RBV, and SOC with PEG-IFN and RBV. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the review board of each institution. Each patient gave a written informed consent before participating in this study.

Study design

This prospective, multi-center, and randomized study was planned on Japanese patients with chronic hepatitis C who met inclusion and did not meet exclusion criteria. Main inclusion criteria were: (a) diagnosed with chronic hepatitis C, and had not received antiviral treatments before; (b) infected with HCV-1 confirmed by the sequence analysis in the NS5B region; (c) had HCV RNA levels $\geqslant$5.0 $\log_{10}$ IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics K.K. Tokyo, Japan); (d) Japanese aged from 20 to 65 years at the entry; (e) had the body weight between >40 and $\leqslant$120 kg; (f) were not pregnant and capable of contraception till 24 weeks after the treatment; and (g) agreed on the admission for 15 days since the treatment start. Main exclusion criteria were: (a) decompensated liver cirrhosis; (b) hepatitis B surface antigen; (c) hepatocellular carcinoma or other malignancy, or its history; (d) autoimmune hepatitis, alcoholic liver disease, hemochromatosis or chronic liver disease other than chronic hepatitis C; (e) depression or schizophrenia, or its history, or history of suicide attempts; (f) chronic renal disease or creatinine clearance $\leqslant$50 ml/min at the baseline; (g) hemoglobin <12 g/dl, neutrophil counts <1500/mm^3 or platelet counts <100,000/mm^3 at the baseline; and (h) pregnancy in progress or planned during the study period of either partner.

Patients were randomly assigned to either of the following two treatment groups in a 2:1 ratio, with stratification to balance sex and age: (1) the triple therapy with telaprevir, PEG-IFN, and RBV for 12 weeks, followed by PEG-IFN and RBV for an additional 12 weeks (Group A: T12PR24); and (2) SOC with PEG-IFN and RBV for 48 weeks (Group B: PR48). After the treatment was completed or discontinued, they were followed for $\geqslant$24 weeks for SVR evaluation. Patients were followed regularly for subjective symptoms and objective signs, as well as blood

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Table 2. Comparison of SVR stratified by demographic and virological factors as well as discontinuation of study drugs between two groups with different therapeutic regimens.

	A: T12PR24 n = 126	B: PR48 n = 63	Differences *p* value
Gender			
Men	50/66 (75.8%)	18/33 (54.5%)	0.0400
Women	42/60 (70.0%)	13/30 (43.3%)	0.0214
Age (years)			
≤49	35/41 (85.4%)	13/21 (61.9%)	0.0543
≥50	57/85 (67.1%)	18/42 (42.9%)	0.0125
HCV RNA (log_{10} IU/ml)			
≥7	18/26 (69.2%)	5/18 (27.8%)	0.0132
<7	74/100 (74.0%)	26/45 (57.8%)	0.0556
Discontinuation of study drugs			
Not discontinued	66/79 (83.5%)	27/46 (58.7%)	0.0030
All drugs discontinued	14/27 (51.9%)	4/17 (23.5%)	0.1143

counts and chemistry. HCV RNA levels were monitored at day −28, days 1 (pre-dose), 2, and 3, weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 (both groups), as well as weeks 26, 28, 32, 36, 40, and 48 (Group B), during the treatment period; they were monitored at weeks 2, 4, 8, 12, 16, 20, and 24 in the follow-up period (both groups).

HCV RNA and genotypes

HCV RNA was quantified using the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). The linear dynamic range of this assay was 1.2–7.8 log_{10} IU/ml, and samples with no HCV RNA detected were reported as: <1.2 log_{10} IU/ml (no HCV RNA detectable). Genotypes of HCV were determined by direct sequencing followed by phylogenetic analysis of the NS5B region [15].

Antiviral treatments

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) 750 mg was administered three times a day at an 8-h interval (q8h) after each meal. Peginterferon-α2b (PegIntron®, MSD, Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.250–1.739 μg/kg) once a week. Ribavirin (Rebetol®, MSD, Tokyo, Japan) 200–600 mg was administered after breakfast and dinner. The daily dose of RBV was adjusted to the body weight: 600 mg for ≤60 kg; 800 mg for >60 kg ~≤80 kg; and 1000 mg for >80 kg.

RBV dose was diminished by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1000 mg) when hemoglobin decreased <12 g/dl, and by extra 200 mg when it lowered <10 g/dl. In addition, RBV was reduced by 200 mg in patients with hemoglobin <13 g/dl at baseline or in those in whom it decreased by 1 g/dl within a week and below 13 g/dl. Dose modification of RBV in Group B was conducted in accordance with SOC. PEG-IFN dose was reduced to one half, when leukocyte counts decreased <1500/mm^3, neutrophil counts <750/mm^3 or platelet counts <8 × 10^4/mm^3; PEG-IFN was discontinued when they decreased <1000/mm^3, 500/mm^3 or 5 × 10^4/mm^3, respectively. The triple therapy was discontinued or interrupted when hemoglobin decreased <8.5 g/dl. In patients whose hemoglobin increased ≥8.5 g/dl within 2 weeks after the interruption, treatment was resumed with PEG-IFN and RBV 200 mg. The reduction of telaprevir dose was not permitted.

Statistical analysis

SVR was evaluated in the full analysis set. The difference in SVR between Groups A and B with the 2-sided 95% confidence interval (CI) was calculated with the adjustment for sex and age, and *p* value was evaluated by the Wald-test. Continuous variables between groups were compared by the Mann–Whitney test (*U*-test), and categorical variables by the Fisher's exact test. Statistical analyses were performed using the statistical software SAS Version 9.1 (SAS Institute Inc., Cary, NC), and a *p* value <0.05 was considered significant.

Results

Patient cohorts

Of the 220 Japanese patients from whom an informed consent was obtained, 31 (14.1%) were found not eligible for the study entry. The remaining 189 patients were randomly assigned to T12PR24 (Group A [n = 126]) or PR48 (Group B [n = 63]). Overall, 114 out of the 126 (90.0%) patients in Group A and 54 out of the 63 (85.7%) in Group B completed the full study period. Table 1 compares baseline characteristics of studied patients in Groups A and B. There were no differences in demographic characters, hematology, biochemistry, or virology between the two groups of patients.

Loss of HCV RNA during treatment

Dynamics of HCV RNA during treatment was much different between Groups A and B. HCV RNA disappeared more frequently (98.4% *vs.* 79.4%, *p* <0.001) and swiftly (within 8 *vs.* 38 weeks) in patients in Group A than B. Time courses of the loss of HCV RNA are compared in Fig. 1. The loss of HCV RNA increased constantly, sharply, and swiftly in Group A. By contrast, in Group B, it gradually increased during the first 24 weeks of treatment. Rapid virological response at 4 weeks (RVR) occurred more frequently in Group A than B (84.0% *vs.* 4.8%, *p* <0.0001). HCV RNA was undetectable in >90% of patients in Group A, while it stayed undetectable in <80% of patients in Group B at the start of follow-up. After treatment completion, HCV RNA re-appeared in patients in both Groups A and B (16.7% *vs.* 22.2%, *p* = 0.4272).

Responses to treatments

Fig. 2 compares treatment responses between Groups A and B. SVR was achieved more frequently in Group A than B (73.0% *vs.* 49.2%, *p* = 0.0020). By contrast, non-response was less frequent in Group A than B (0.8% *vs.* 20.6%, *p* <0.0001). The difference in SVR between Groups A and B, adjusted for sex and age, was 23.8% (95% CI: 9.4–38.2%, *p* = 0.0012, Wald-test).

Table 3. Adverse events developing in more than 15% of patients in either Groups A or B.

	A: T12PR24 (n = 126)		B: PR48 (n = 63)	
Anemia	115	(91.3%)	46	(73.0%)
Pyrexia	98	(77.8%)	46	(73.0%)
Leukocytopenia	86	(68.3%)	46	(73.0%)
Thrombocytopenia	81	(64.3%)	23	(36.5%)
Malaise	73	(57.9%)	30	(47.6%)
Serum uric acid increased	65	(51.6%)	5	(7.9%)
Serum hyaluronic acid increased	64	(50.8%)	25	(39.7%)
Alopecia	51	(40.5%)	29	(46.0%)
Headache	48	(38.1%)	32	(50.8%)
Skin rashes	48	(38.1%)	18	(28.6%)
Anorexia	42	(33.3%)	17	(27.0%)
Insomnia	40	(31.7%)	17	(27.0%)
Vomiting	37	(29.4%)	9	(14.3%)
Drug eruption	37	(29.4%)	2	(3.2%)
Arthralgia	36	(28.6%)	15	(23.8%)
Serum triglycerides increased	36	(28.6%)	11	(17.5%)
Dysgeusia	34	(27.0%)	10	(15.9%)
Diarrhoea	34	(27.0%)	19	(30.2%)
Nausea	32	(25.4%)	7	(11.1%)
Serum creatinine increased	32	(25.4%)	0	
Erythema at the injection site	33	(26.2%)	21	(33.3%)
Reactions at the injection site	29	(23.0%)	16	(25.4%)
Stomatitis	24	(19.0%)	12	(19.0%)
Abdominal discomfort	23	(18.3%)	12	(19.0%)
Pruritus	23	(18.3%)	13	(20.6%)
Nasopharyngitis	23	(18.3%)	18	(28.6%)
Influenza-like symptoms	22	(17.5%)	16	(25.4%)
Serum bilirubin increased	22	(17.5%)	13	(20.6%)
Back pain	21	(16.7%)	12	(19.0%)
Hyperuricemia	20	(15.9%)	2	(3.2%)
Serum phosphorus decreased	16	(12.7%)	13	(20.6%)
Constipation	14	(11.1%)	13	(20.6%)
Erythema	9	(7.1%)	13	(20.6%)

Factors influencing the treatment response are compared in Table 2. SVR was higher in Group A than B, irrespective of different genders, age ranges, or HCV RNA loads. Of note, SVR in women in Group A was higher than that in Group B (70.0% *vs.* 43.3%, $p = 0.0214$). Likewise, SVR in patients ⩾50 years was higher in Group A than B (67.1% *vs.* 42.9%, $p = 0.0125$), and that in patients with high HCV RNA loads (⩾7 $\log_{10}$ IU/ml) at the baseline was higher in Group A than B (69.2% *vs.* 27.8%, $p = 0.0132$).

Adverse events

Adverse events occurred in all patients in both Groups A and B. Adverse events with a frequency >15% in either group are listed in Table 3. Of them, frequencies of anemia, thrombocytopenia, malaise, and elevated serum levels of uric acid as well as hyaluronic acid were >10% higher in Group A than B. Most of them were mild, and severe and serious adverse events occurred in small proportions of patients (9.5% and 11.9% in Group A, respectively, and 9.5% and 9.5% in Group B). All drugs were discontinued due to adverse events comparatively frequently in Groups A and B (16.7% and 22.2%, respectively), and telaprevir alone in 19.0% of patients in Group A. The total dose of RBV was less in Group A than B (47.0% *vs.* 77.7% of the target, $p < 0.0001$). Doses of antiviral treatments were reduced or discontinued in some patients with moderate to severe adverse events, patients were taken care of by specialists, and received specific therapies when necessary. Eventually, all patients recovered from adverse events.

Hematological disorders

Anemia occurred in 91.3% and 73.0% of patients in Groups A and B, respectively. Table 4 compares the severity of anemia between Groups A and B. Combined, Grade 1 and 2 anemia developed more frequently in Group A than B (38.1% *vs.* 17.5%, $p = 0.0045$). Grade 3 anemia occurred in 11.1% in Group A only. During the follow-up, hemoglobin increased both in Groups A and B, and returned to pretreatment levels 12 weeks after the completion of therapy and thereafter (Fig. 3A). Platelet counts decreased more extensively in Group A than B (Fig. 3B). They rebounded after the completion of therapy, and then returned to pretreatment values. Decreases in neutrophil counts were milder in Group A than B (Fig. 3C). Both in Groups A and B, neutrophils started to increase immediately after the treatment completion, and returned to pretreatment levels within 12 weeks.

Skin disorders

Skin disorders were monitored at every hospital visit for severity and extent, and they were categorized into four Grades (Table 4). When skin disorders of Grades 2–4 occurred, the attendant physician was instructed to consult with a dermatologist in each institution for the diagnosis and specific cares, and telaprevir was discontinued, while PEG-IFN and RBV were reduced or discontinued, as required. Skin disorders were mainly rash, drug eruptions, and erythema. They occurred comparably frequently in Groups A and B (89.7% and 84.1%, respectively). Most skin disorders were mild and categorized into Grade 1 in 75.4% and 76.2% of patients in Groups A and B, respectively. Combined, skin disorders of Grades 2–4 occurred more frequently in Group A than B (46.8% *vs.* 23.8%, $p = 0.0026$). Due to skin disorders, at least one drug was discontinued in merely 9.5% and 3.2% of patients in Groups A and B, respectively, and most skin disorders were controllable by anti-histamine and/or steroid ointments.

Serious skin disorders developed in three patients in Group A, but none in Group B. Stevens–Johnson syndrome occurred in one patient 35 days after the treatment start, and led to the discontinuation of treatment. Erythema spread widely in the trunk (Fig. 4A), as well as limbs and the face. Erosion of oral mucosae, epidermal detachment, conjunctival redness, high fever to reach 39.3 °C, and lymphadenopathy were also noted. Histopathology showed the epidermal necrosis, satellite-cell necrosis, and perivascular dermatitis with infiltration of lymphocytes, neutrophils, and eosinophils in the superficial dermis (Fig. 4B). The patient was admitted and received steroids intravenously, and recovered completely within 9 weeks. Drug rash with eosinophilia and

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Table 4. Decreases in hemoglobin levels and skin disorders according to the grade.

Grade	A: T12PR24 n = 126	B: PR48 n = 63	Differences *p* value
A Hemoglobin levels			
Grade 1 (9.5- <11.0 g/dl)	50 (39.7%)	32 (50.8%)	0.1631
Grade 2 (8.0- <9.5 g/dl)	34 (27.0%)	11 (17.5%)	0.2043
Grade 3 (<8.0 g/dl)	14 (11.1%)	0	0.0055
Total	98 (77.8%)	43 (68.3%)	0.1613
B Skin disorders			
Grade 1[a]	95 (75.4%)	48 (76.2%)	1.0000
Grade 2[b]	44 (34.9%)	12 (19.0%)	0.0282
Grade 3[c]	13 (10.3%)	3 (4.8%)	0.2709
Grade 4[d]	2 (1.6%)	0 (0.0%)	0.5532
Any grade	113 (89.7%)	53 (84.1%)	0.3451

[a]Localized skin lesions.
[b]Diffuse or multiple skin lesions.
[c]Skin lesions covering >50% of the body surface or rashes with some characteristics such as bullae, ulceration of mucous membrane, epidermal detachment, target lesion or significant systemic signs.
[d]Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms (DRESS) were categorized in Grade 4.

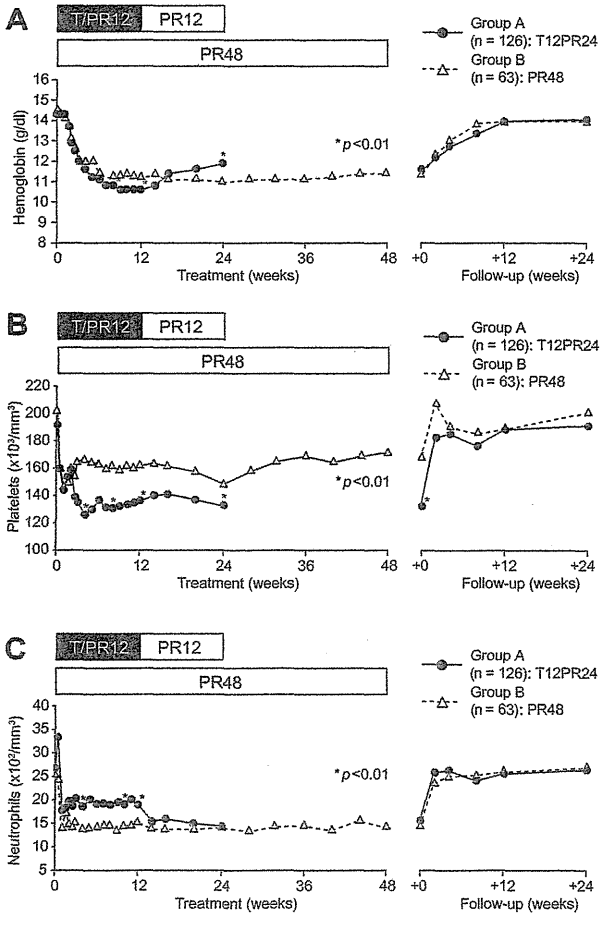

Fig. 3. Comparison of hematopoietic disorders between patients in Groups A and B. (A) Median hemoglobin levels, (B) platelet counts, and (C) neutrophil counts are plotted during treatment and follow-up. Ranges from 25% to 75% are omitted for visual clarity. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and at weeks 12 and 24 in the follow-up period. An asterisk (*) indicates p <0.01 difference.

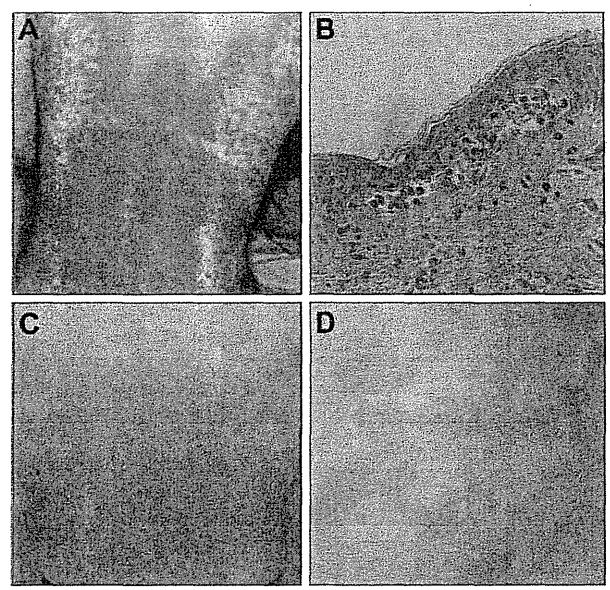

Fig. 4. Grade 4 skin regions in patients who received the triple therapy. (A) Erythema and (B) histopathology of the skin in the patient with Stevens–Johnson syndrome, as well as (C and D) generalized erythema in the patient developing drug rashes with eosinophilia and systemic symptoms (DRESS), are shown.

systemic symptoms (DRESS or drug-induced hypersensitivity syndrome) occurred in another patient. Fresh red erythema appeared on the whole body, and fresh red-colored target lesions (up to 3–4 cm in diameter) were also observed (Fig. 4C and D). Edema in the face, lymphadenopathy, fever up to 39.7 °C, and erosion of oral mucosae were noted, also. Maximum levels of white blood cells, eosinophils, and atypical lymphocytes were 46,300/mm^3, 45.7%, and 23.3%, respectively. Titers of IgG antibody to human herpes virus 6 were ×160 (29 days after the onset) and ×2560 (57 days). The remaining patient developed erythema multiforme. These two patients received steroids orally and recovered completely within 14 weeks.

Discussion

A prospective, randomized, and treatment-controlled clinical trial was planned and conducted in Japan to compare the therapeutic efficacy and safety profiles between the triple therapy with T12PR24 and the SOC treatment with PR48. In this trial, 126 patients were assigned to receive T12PR24 (Group A) and the 63 to receive PR48 (Group B). They all were treatment-naive, and infected with HCV-1 in high viral loads ($\geqslant 5$ $\log_{10}$ IU/ml) and of genotype 1b in the great majority (98.9%). Randomization was not adopted due to ethnical concerns against giving intravenous placebo weekly for 24 weeks to patients in Group A.

Dynamics of circulating HCV RNA during treatment was quite different between Groups A and B. HCV RNA disappeared more frequently (98.4% *vs.* 79.4%, $p < 0.001$) and swiftly (within 8 *vs.* 38 weeks) in patients in Group A than B. Accordingly, SVR was achieved more frequently in patients with T12PR24 than PR48 (73.0% *vs.* 49.2%, $p = 0.0020$), while rates of relapse (16.7% *vs.* 22.2%) and breakthrough (3.2% and 1.6%) were not different between them. Due to the higher therapeutic efficacy and shorter treatment duration, T12PR24 would be more suitable for treatment of HCV-1 patients than the standard PR48, and lessen the total economic burden of patients and the nation.

Previous clinical trials with telaprevir were conducted in Europe or the United States and combined with PEG-IFN-α2a [8–11]. In the present study, Japanese patients have responded to a triple therapy with PEG-IFN-α2b, with an efficacy of 73% in comparison with 72–75% in phase 3 clinical trials [10,11]. In a recent report, PEG-IFN-α2a and -α2b were equally effective in triple therapies in combination with telaprevir and RBV [16]. Frequency of side effects demanding the discontinuation of all drugs is comparable between patients receiving the triple therapy with PEG-IFN-α2a in phase 3 trials [10,11] and -α2b in the present study (7–17% and 17%, respectively).

In our previous report [17], the IFN-responsive C/C genotype of *IL28B* at rs12979860 was detected in 42 out of the 72 (55%) patients infected with HCV-1 in Japan; the prevalence was not much different from that in 336 out of the 769 (44%) European-Americans [18]. The susceptibility to telaprevir depends on HCV genotypes, and is higher for genotypes 1 and 2 than genotypes 4 and 5 in *in vitro* experiments [19]. Further, it may differ between 1a and 1b, due to dissimilar evolution patterns of drug-resistant mutations [14]. Nevertheless, present patients infected with HCV-1b in the great majority (98.4%) were equally responsive to the triple therapy with telaprevir as those infected with HCV-1a [8,9,11].

High efficacy of T12PR24 was accompanied by increased adverse events, of which anemia and skin lesions were worrisome. Moderate and severe anemia (<9.5 g/dl) developed more frequently in Group A than B (38.1% *vs.* 17.5%, $p = 0.0045$). Since Japanese patients with chronic hepatitis C are older by >10 years than those in Western countries, with a higher proportion of women, they are prone to develop anemia during treatment with telaprevir. Stringent precaution had to be taken, therefore, by deducting the RBV dose in patients in whom hemoglobin levels decrease <12 g/dl, higher than the conventional threshold of <10 g/dl. The total RBV dose was lower in Group A than B (47.0% *vs.* 77.7% of the target, $p < 0.0001$). However, decreased doses of RBV or PEG-IFN did not influence substantially the therapeutic efficacy of T12PR24.

Skin disorders of Grades 2–4 occurred more frequently in Group A than B (46.8% *vs.* 23.8%, $p = 0.0026$). It has to be noted that Grade 4 skin lesions, such as Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms (DRESS), developed exclusively in patients in Group A. Since studied patients were monitored carefully and received immediate care by dermatologists, if and when skin lesions of Grades 2–4 developed, all patients eventually recovered. In the area of DAAs, potentially accompanying severe skin disorders, physicians would need close cooperation with dermatologists for the care of patients with hepatitis C.

In conclusion, this multicenter, randomized, and treatment-controlled study of T12PR24 in Japanese patients infected with HCV-1b has proven the efficacy and safety comparable to those in previous phase 3 studies [10,11]. Due to the excellence of T12PR24 over the standard PR48, we hope it will be used widely in patients with chronic hepatitis C over the world, who are expected to increase rapidly in the foreseeable future [20].

Conflict of interest

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

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Large-Scale Long-Term Follow-Up Study of Japanese Patients With Non-Alcoholic Fatty Liver Disease for the Onset of Hepatocellular Carcinoma

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OBJECTIVES: **The aim of this study was to determine the incidence and risk factors of hepatocellular carcinoma (HCC), and to elucidate the utility of two non-invasive predictive procedures for liver fibrosis: the aspartate aminotransferase (AST) to platelet ratio index (APRI) and the BARD score (which includes the following three variables: body mass index, AST/alanine aminotransferase ratio, and diabetes) in the prediction of HCC in a large population of Japanese patients with non-alcoholic fatty liver disease (NAFLD).**

METHODS: **This was a retrospective cohort study conducted at a public hospital. Study subjects included 6,508 patients with NAFLD diagnosed by ultrasonography. The median follow-up period was 5.6 years. The primary end point was the onset of HCC. Evaluation was performed using Kaplan–Meier methodology and Cox's proportional hazards analysis.**

RESULTS: **In all, 16 (0.25%) new cases with HCC were diagnosed during the study. The cumulative rates of NAFLD-related HCC were 0.02% at year 4, 0.19% at year 8, and 0.51% at year 12. The annual rate of new HCC was 0.043%. Multivariate analysis identified serum AST level ≥40 IU/L (hazard ratio (HR): 8.20; 95% confidence interval (95% CI): 2.56–26.26; $P<0.001$), platelet count $<150\times10^3/\mu l$ (HR: 7.19; 95% CI: 2.26–23.26; $P=0.001$), age ≥60 years (HR: 4.27; 95% CI: 1.30–14.01; $P=0.017$), and diabetes (HR: 3.21; 95% CI: 1.09–9.50; $P=0.035$) as independent risk factors for HCC. With regard to the APRI, 184 patients (2.83%) were considered to have significant fibrosis (equivalent to non-alcoholic steatohepatitis (NASH) stage 3–4). The cumulative rate of HCC was significantly higher in this group (HR: 25.03; 95% CI: 9.02–69.52; $P<0.001$). In contrast, regarding the BARD score, 3,841 (59%) patients were considered to have advanced fibrosis (NASH stage 3–4). However, no significant associations between the BARD score and the incidence of HCC were observed (HR: 1.16; 95% CI: 0.40–3.37; $P=0.780$).**

CONCLUSIONS: **This retrospective study indicates that the annual incidence rate of HCC among Japanese NAFLD patients is low. Elderly NAFLD patients with diabetes, elevated serum AST, and especially thrombocytopenia (suggested to be associated with advanced liver fibrosis) should be monitored carefully during follow-up that includes using the APRI to ensure early diagnosis and treatment of HCC.**

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignancy worldwide, and its incidence is increasing in Asia and in the United States (1–3). Chronic viral hepatitis and liver cirrhosis after infection with hepatitis B and C viruses have important roles in the development of HCC (4–5). However, a substantial proportion (5–10%) of Japanese patients with HCC are negative for markers of hepatitis B and C viruses (6–8). In addition to viral infection, non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease in western countries (9–12),

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and more recently in many Asian nations (13,14). NAFLD is sometimes considered to be the liver component of metabolic syndrome (15–17). It is associated with obesity, dyslipidemia, pituitary dysfunction, hypertension, sleep apnea, and type 2 diabetes mellitus (18–24). In particular, patients with non-alcoholic steatohepatitis (NASH), a subcategory of NAFLD, are at a higher risk for the incidence of HCC (25). At this stage, NASH can only be diagnosed by histopathology. Despite being common and potentially serious, the natural history of NAFLD remains poorly defined. Most of the studies reported to date included limited numbers of highly selected patients, i.e., patients with histopathologically confirmed NAFLD who were referred to specialized tertiary care centers (26–31). However, in reality, larger numbers of NAFLD patients are diagnosed by ultrasonography (US) alone. To our knowledge, no information about the incidence and risk factors of HCC in Japanese individuals with NAFLD diagnosed by US has been published.

The number of patients with NAFLD is predicted to increase in the future, and it is unlikely that all NAFLD patients will be diagnosed by histopathological examination of liver biopsy due to the potential risks associated with fat deposition and fibrosis in the liver (e.g., risk of bleeding, allergy to local anesthetics, and patient refusal). Therefore, there is a need to define the clinical impact of NAFLD and risk factors for the incidence of HCC. One aim of this retrospective study was to determine the incidence and risk factors of HCC in patients with US-diagnosed NAFLD. The aspartate aminotransferase (AST) to platelet ratio index (APRI), a non-invasive index for prediction of significant fibrosis in patients with chronic hepatitis C, has been previously reported (32), and its utility in NAFLD has also been reported (33). More recently, the BARD score (which includes the three following variables: body mass index (BMI), AST/alanine aminotransferase (ALT) Ratio, and Diabetes), a non-invasive estimation formula for predicting advanced fibrosis in patients with NALFD, has also been reported (34). The other purpose of this study was to elucidate the utility of these non-invasive predictive procedures for liver fibrosis in the prediction for incidence of HCC in NAFLD patients.

METHODS

Study population

In this retrospective cohort study, we obtained the medical records of all patients in our database who were diagnosed with NAFLD by US (35) between January 1997 and December 2010 at the Department of Hepatology and the Health Management Center (Toranomon Hospital, Tokyo, Japan). Of these, 6,508 patients satisfied the following criteria: (i) past daily alcohol intake of <20 g/day; (ii) negativity for hepatitis C virus antibodies, hepatitis B surface antigen, antinuclear antibodies, and anti-mitochondrial antibodies in serum, as determined by radioimmunoassay or spot hybridization; (iii) no underlying systemic disease, such as systemic lupus erythematosus and rheumatic arthritis; (iv) no underlying metabolic disease, such as hemochromatosis, α-1-antitrypsin deficiency, and Wilson's disease; (v) no evidence of HCC on US and/or computed tomography; and (vi) follow-up period of ≥48 weeks. Clinical and laboratory data were collected from the medical records of all 6,508 patients and analyzed. The study was approved by the Institutional Review Board of our hospital.

Clinical background and laboratory data

Table 1 summarizes the clinical profile and laboratory data of NAFLD patients. The male:female ratio was 7.15:1, and the median BMI was 24.8 kg/m^2. Of the total population, 841 (12.9%) patients were hypertensive, and 536 (8.2%) patients had diabetes at the time of diagnosis of NAFLD. Hypertension was defined as seated systolic/diastolic blood pressure of $>140/>90$ mm Hg measured after 5 minutes of rest (36). Diabetes was diagnosed based on the 2003 criteria of the American Diabetes Association (37). These criteria include: (i) casual plasma glucose ≥200 mg/dl; (ii) fasting plasma glucose ≥126 mg/dl; and (iii) 2-h post-glucose (oral glucose tolerance test) ≥200 mg/dl.

Hepatitis C virus antibodies and hepatitis B surface antigen were examined at study entry. Hepatitis C virus antibodies were detected using a third-generation enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, IL). Hepatitis B surface antigen was tested by radioimmunoassay (Abbott Laboratories).

Table 1. Characteristics of 6,508 patients with non-alcoholic fatty liver disease

Gender, M:F	5,709:799
Age, years[a]	49 (23–86)
Body mass index, kg/m^2 [a]	24.8 (15.9–45.1)
Hypertension, yes/no	841:5,667
Albumin, g/dl[a]	4.2 (2.9–5.1)
Total bilirubin, mg/dl[a]	0.8 (0.2–4.3)
AST, IU/L[a]	26 (11–516)
ALT, IU/L[a]	30 (7–803)
LDH, IU/L[a]	145 (49–392)
γ-GTP, IU/L[a]	53 (8–2,376)
Platelet count, $\times10^3/\mu l$[a]	226 (27–554)
Fasting plasma glucose, mg/dl[a]	99 (71–377)
Diabetes mellitus, yes/no	536:5,972
Uric acid, mg/dl[a]	6.3 (0.7–11.5)
Total cholesterol, mg/dl[a]	210 (100–521)
Triglyceride, mg/dl[a]	138 (22–1,758)
LDL cholesterol, mg/dl[a]	131 (29–270)
HDL cholesterol, mg/dl[a]	46 (5–106)
Follow-up period, days[a]	2,051 (366–11,190)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; F, female; γ-GTP, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; M, male.
[a]These are expressed as median (minimum, maximum).

Medical evaluation

The diagnosis of NAFLD was based on the US finding of bright liver with stronger echoes in the hepatic parenchyma than in the renal or spleen parenchyma. US was performed using a high-resolution, real-time scanner (model SSD-2000; Aloka, Tokyo, Japan, or Mode Logic-700 MR; GE-Yokokawa Medical Systems, Tokyo, Japan). Body weight was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline and BMI was calculated as weight (in kg)/height (in m^2). All patients were interviewed at the Toranomon Hospital using a questionnaire that collected information on demographic characteristics, medical history, and health-related habits, including questions about alcohol intake at the time of diagnosis of NAFLD.

Follow-up and diagnosis of HCC

The observation starting point (study entry) was the time of diagnosis of NAFLD by US. After that, patients were followed up monthly to every 6 months at the Toranomon Hospital. In this cohort, 5,657 (86.9%) patients underwent US every 6 months. A blood sample was taken for routine analysis. Overall, 585 patients were lost to follow-up; these were considered as censored data in statistical analysis as the appearance of HCC was not identified in these 585 patients (38).

Histopathological examination of the liver

In patients who underwent histological examination of the liver, specimens were fixed in 10% formalin and stained with hematoxylin–eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. Fibrosis was scored using a five-grade scale proposed by Brunt *et al.* (39): stage 0, normal connective tissue; stage 1, pericellular or perivenular fibrosis in zone 3 (pericentral vein area); stage 2, pericellular or perivenular fibrosis confined to zones 2 and 3 with or without periportal fibrosis; stage 3, bridging or septal fibrosis; and stage 4, cirrhosis.

A total of 104 patients underwent histological examination, and 10 (9.6%) patients received a histological diagnosis at the time of treatment of HCC. As a result of histological diagnosis, 73 (70.2%) patients were diagnosed with NASH, 30 (28.8%) patients were diagnosed with fatty liver without fibrosis, and 1 (1.0%) patient was diagnosed with liver cirrhosis without steatosis.

APRI calculation method and prevalence of significant fibrosis

The APRI was calculated according to the following formula:

$$\text{APRI} = \frac{\text{AST level (/ULN*)}}{\text{Platelet count } (10^9/\text{l})} \times 100$$

*ULN, AST upper level of normal (33 IU/l)

As previously reported, an APRI > 1.50 is predictive of significant fibrosis (positive predictive value, 88%; negative predictive value, 64%). In association with the APRI, hepatic fibrosis was assessed using the Ishak fibrosis score (40). Significant fibrosis was defined as an Ishak score of ≥3 (presence of occasional bridging fibrosis) (32). In this study, 184 of 6,508 patients (2.83%) had an APRI > 1.50 and were therefore considered to have significant fibrosis.

BARD score calculation method and prevalence of advanced fibrosis

The BARD score consists of three variables: BMI ≥28 kg/m^2, AST/ALT ratio ≥0.8, and diabetes. The following points are given to each variable: BMI, 1 point; AST/ALT ratio, 2 points; and presence of diabetes, 1 point; thus, scores range from 0 to 4. As previously reported, a BARD score of 2–4 is associated with an odds ratio for advanced fibrosis of 17 (positive predictive value, 43%; negative predictive value, 96%) (34). In association with the BARD score, advanced fibrosis was defined as NASH stage 3–4, and in this study, 3,841 of 6,508 (59.0%) patients had a BARD score of ≥2 points, and were therefore considered to have advanced fibrosis.

Statistical analysis

The cumulative incidence rate of HCC (new cases of HCC) was calculated from study entry to diagnosis of HCC using the Kaplan–Meier method. Differences in the development of HCC between groups were tested using the log-rank test. Independent factors associated with the incidence of HCC were analyzed by Cox's proportional hazards model. The following 17 variables were analyzed as potential covariates for incidence of HCC at the time of study entry: sex, age, BMI, hypertension, diabetes, serum concentration of albumin, total bilirubin, AST, ALT, lactate dehydrogenase, γ-glutamyl transpeptidase, uric acid, total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and platelet count. Several variables were transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. All factors found to be at least marginally associated with the incidence of HCC ($P < 0.15$) in univariate analysis were entered into a multivariate Cox's proportional hazards model. A P value < 0.05 in a two-tailed test was considered significant. Data analysis was performed using The Statistical Package for Social Sciences version 16.0 for Windows (SPSS, Chicago, IL).

RESULTS

Incidence of HCC in patients with NAFLD

The follow-up period for all patients ranged from 366 to 11,190 days (median, 2,051 days). Of the 6,508 NAFLD patients, 16 (0.25%) patients developed HCC. The cumulative rate of HCC was 0.02% at the end of the 4th year, 0.19% at the end of the 8th year, and 0.51% at the end of the 12th year (**Figure 1**). The annual incidence of HCC in patients with NAFLD was 0.043%.

Effect of diabetes mellitus on the incidence of HCC in NAFLD patients

During the follow-up period, 9 of the 5,972 (0.15%) non-diabetic patients developed HCC, whereas 7 of the 536 (1.31%) diabetic patients developed HCC. The cumulative rate of HCC in non-diabetic patients was 0.0% at the end of the 4th year, 0.10% at the end of the 8th year, and 0.10% at the end of the 12th year. For diabetic patients, these rates were 0.22, 0.83, and 3.42%,

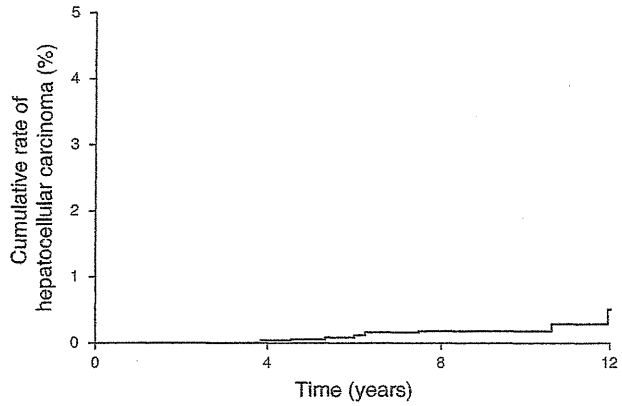

Figure 1. Cumulative rate of development of hepatocellular carcinoma in Japanese patients with non-alcoholic fatty liver disease diagnosed by ultrasonography.

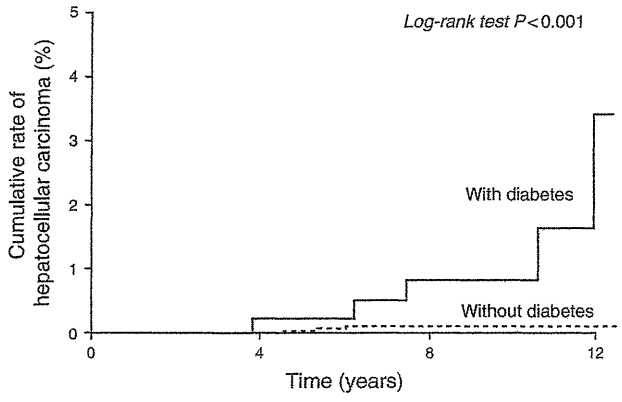

Figure 2. Cumulative rate of development of hepatocellular carcinoma in Japanese patients with or without diabetes mellitus diagnosed with non-alcoholic fatty liver disease by ultrasonography.

respectively (**Figure 2**). The cumulative rate of HCC was significantly higher in patients with diabetes than in non-diabetic patients ($P<0.001$).

Factors associated with the incidence of HCC

Multivariate Cox's proportional hazards analysis identified AST level ≥40 IU/L (hazard ratio (HR): 8.20; 95% confidence interval (95% CI): 2.56–26.26; $P<0.001$), platelet count $<150\times10^3/\mu l$ (HR: 7.19; 95% CI: 2.26–23.26; $P=0.001$), age ≥60 years (HR: 4.27; 95% CI: 1.30–14.01; $P=0.017$), and diabetes (HR: 3.21; 95% CI: 1.09–9.50; $P=0.035$) to be independent factors for development of HCC in Japanese NAFLD patients diagnosed by US (**Table 2**).

Incidence of HCC in patients with APRI-estimated significant fibrosis

On the basis of APRI estimation, 10 of the 6,324 (0.16%) non-significant fibrotic patients developed HCC during the follow-up period, whereas 6 of the 184 (3.26%) significant fibrotic patients developed HCC. The cumulative rate of HCC in non-significant fibrotic patients was 0.02% at the end of the 4th year, 0.06% at the end of the 8th year, and 0.39% at the end of the 12th year. For significant fibrotic patients, these rates were 0, 4.03, and 4.03%, respectively (**Figure 3**). The cumulative rate of HCC was significantly higher in patients with significant fibrosis than in patients without significant fibrosis (HR: 25.03; 95% CI: 9.02–69.52; $P<0.001$).

Incidence of HCC in patients with BARD score-estimated advanced fibrosis

On the basis of BARD score estimation, 5 of the 2,667 (0.19%) non-advanced fibrotic patients developed HCC during the follow-up period, whereas 11 of the 3,841 (0.29%) advanced fibrotic patients developed HCC. The cumulative rate of HCC in non-advanced fibrotic patients was 0% at the end of the 4th year, 0.06% at the end of the 8th year, and 0.06% at the end of the 12th year. For advanced fibrotic patients, these rates were 0.04, 0.27, and 0.76%, respectively (**Figure 4**). However, no significant associations between the BARD score and the incidence of HCC were observed (HR: 1.16; 95% CI: 0.40–3.37; $P=0.780$).

Clinicopathological features of NAFLD patients with HCC

Table 3 summarizes the characteristics and clinical features of the 16 patients with NAFLD-related HCC. In these patients, the median period from study entry to diagnosis of HCC was 12.5 years. In 12 of these 16 (75.0%) patients, platelet count decreased from study entry to diagnosis of HCC. Furthermore, the pathological diagnosis of background liver disease, which was performed in 11 of the 16 (68.8%) patients at the time of treatment of HCC, was NASH stage 4 (cirrhosis) in 3 (27.3%) patients, NASH stage 3 (pre-cirrhosis) in 2 (18.2%) patients, NASH stage 1–2 (slight-to-moderate fibrosis) in 3 (27.3%) patients, liver cirrhosis without fatty deposition in 1 (9.1%) patient, and fatty liver without fibrosis in 2 (18.2%) patients. Thus, 8 (72.7%) of the 11 patients had NASH. In case 4 (**Table 3**), splenectomy was performed because of associated thrombocytopenia, although the platelet count was increased at the time of diagnosis of HCC.

DISCUSSION

Previous retrospective studies have reported that the incidence of HCC from NASH ranges from 4 to 27% after development of cirrhosis, although the development of HCC in the setting of NAFLD remains a rare complication (41,42). The incidence of HCC in patients with NAFLD reported in several longitudinal follow-up studies ranged from 0 to 0.5%, whereas that in patients with NASH ranged from 0 to 2.8% over a follow-up period of 19.5 years (25,43–45). According to Japanese annual health check reports, 9–30% of Japanese adults demonstrate evidence of NAFLD by US (46–48). As it is known that almost 10–20% of individuals with NAFLD have NASH, the prevalence of NASH is estimated to be 1–3% of the adult Japanese population, which represents an extremely large number of potential patients. To our knowledge, no information about the incidence of HCC after

Table 2. **Predictors of hepatocellular carcinoma in patients with non-alcoholic fatty liver disease**

Variables	Category	Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender	1: Female	1			
	2: Male	2.02 (0.69–5.93)	0.198		
Age	1: <60	1		1	
	2: ≥60	9.98 (2.73–36.49)	0.001	4.27 (1.30–14.01)	0.017
Body mass index (kg/m^2)	1: <25	1			
	2: ≥25	1.69 (0.63–4.55)	0.300		
Hypertension	1: No	1			
	2: Yes	10.26 (3.78–27.83)	<0.001		
Albumin (g/dl)	1: ≥4.0	1			
	2: <4.0	2.18 (0.78–6.17)	0.139		
Total bilirubin (mg/dl)	1: ≥1.0	1			
	2: <1.0	1.06 (0.37–3.70)	0.907		
AST (IU/L)	1: <40	1		1	
	2: ≥40	16.28 (5.65–46.96)	<0.001	8.20 (2.56–26.26)	<0.001
ALT (IU/L)	1: <50	1			
	2: ≥50	12.31 (4.24–35.70)	<0.001		
LDH (IU/L)	1: <160	1			
	2: ≥160	3.35 (1.25–8.99)	0.017		
γ-GTP (IU/L)	1: <70	1			
	2: ≥70	2.10 (0.79–5.60)	0.140		
Platelet count (×10^3/μl)	1: ≥150	1		1	
	2: <150	18.18 (6.49–50.00)	<0.001	7.19 (2.26–23.26)	0.001
Diabetes	1: No	1		1	
	2: Yes	6.08 (2.26–16.36)	<0.001	3.21 (1.09–9.50)	0.035
Uric acid (mg/dl)	1: <6.0	1			
	2: ≥6.0	1.55 (0.56–4.30)	0.397		
Total cholesterol level (mg/dl)	1: ≥220	1			
	2: <220	1.04 (0.38–2.87)	0.936		
Triglyceride level (mg/dl)	1: ≥150	1			
	2: <150	4.31 (0.98–19.23)	0.054		
LDL cholesterol level (mg/dl)	1: <140	1			
	2: ≥140	1.07 (0.40–2.89)	0.889		
HDL cholesterol level (mg/dl)	1: <40	1			
	2: ≥40	1.34 (0.38–4.75)	0.648		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; F, female; γ-GTP, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; HR, hazard ratio; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; M, male.

long-term follow-up in a large number of Japanese patients with NAFLD has been previously published.

This study revealed several findings about the development of HCC in Japanese NAFLD patients. This is the first study to determine the annual rate and risk factors of newly developed HCC in a large number of Japanese patients with NAFLD diagnosed by US. In this study, the incidence of HCC calculated after long-term follow-up in NAFLD patients was 0.25%, with an annual rate of 0.043%. These low rates are similar to those reported by other groups in other countries (25,43–45). However, a total of

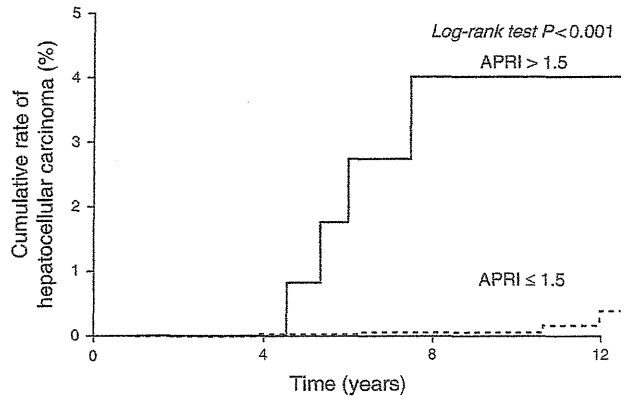

Figure 3. Cumulative rate of development of hepatocellular carcinoma in Japanese patients with non-alcoholic fatty liver disease diagnosed by ultrasonography according to the APRI. APRI, aspartate aminotransferase to platelet ratio index.

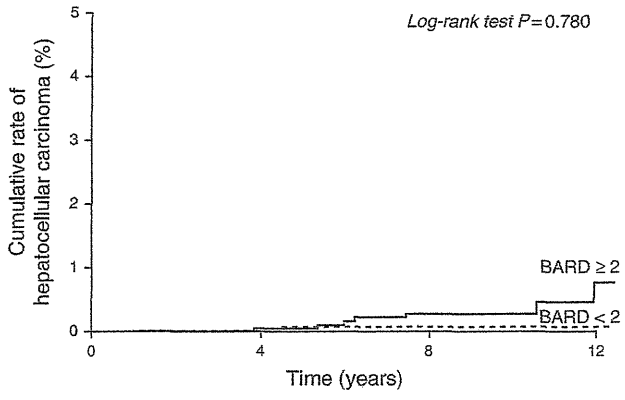

Figure 4. Cumulative rate of development of hepatocellular carcinoma in Japanese patients with non-alcoholic fatty liver disease diagnosed by ultrasonography according to the BARD score. BARD, body mass index, AST/alanine aminotransferase ratio, and diabetes.

15,944 patients were diagnosed as having a non-alcoholic history (past daily alcohol intake of <20 g/day) and without complicated fatty liver by US between January 1997 and December 2010 at the Department of Hepatology and the Health Management Center (Toranomon Hospital, Tokyo, Japan), and in this large population at the same institute, HCC occurred in only 2 of 15,944 (0.013%) patients during the follow-up period. In this study, the incidence of HCC in NAFLD patients was 0.25%, which is higher than that in the non-alcoholic, non-fatty liver population.

In this study, advanced age, high AST level, thrombocytopenia (marker of progression of liver fibrosis), and diabetes were identified as risk factors for the development of HCC in Japanese patients with US-diagnosed NAFLD. These results are in agreement with the previously reported risk factors of NASH-related HCC, namely advanced age, advanced fibrosis, cirrhosis, and diabetes (49). In this regard, a high serum ALT level was reported to be a surrogate for histopathological diagnosis of NAFLD (50). Clinically, most patients with NAFLD are known to have high ALT levels. Our analysis identified elevated AST levels, but not elevated ALT levels, as a risk factor for NAFLD-related HCC. The exact reason for this finding is not clear, but we speculate the following: based on the pathological features of NASH, necroinflammatory changes and perisinusoidal fibrosis usually appear around zone 3, i.e., the pericentral vein area of the liver. Among liver enzymes, the distribution of AST is closer to zone 3 than distributions of other enzymes. Thus, the correlation with high AST levels observed in this study may reflect the significance of AST as a factor related to NASH disease progression, in contrast to serum ALT levels.

Advanced liver fibrosis in NASH is considered to be an important etiological factor for the incidence of HCC. In this study, we identified a ≥10% decrease in platelet counts (relative to baseline) in 9 of the 16 patients whose NAFLD progressed to HCC. Thrombocytopenia has also been previously reported to be a risk factor for the incidence of HCC (39). Thus, it seems that the decrease in platelet count during progression is also an important etiological factor in the incidence of HCC, as it is in viral-induced hepatitis, and may indicate advancing liver fibrosis in NAFLD.

The results of this study revealed that with respect to APRI, the incidence of HCC was significantly higher in patients with an APRI of >1.5; however, no significant associations between the BARD score and the incidence of HCC were observed. **Table 3** shows the change of APRI and BARD scores from the beginning of follow-up to the time of diagnosis of HCC. At the beginning of follow-up, 5 of 16 (31.3%) patients had a >1.5 APRI. However, at the time of diagnosis of HCC, only 2 (12.5%) patients had a >1.5 APRI. Furthermore, in 8 of 16 (50.0%) patients, the APRI had improved at the time of diagnosis of HCC. Of these 8 patients, 1 patient underwent splenectomy due to associated thrombocytopenia, although the platelet count had increased at the time of diagnosis of HCC; however, 2 patients in whom the platelet count had decreased ≥10% since the beginning of follow-up were included. In contrast, with respect to the BARD score, 12 of 16 (75.0%) patients had a BARD score of ≥2, and BARD scores were maintained or increased in all cases. On the basis of this result, the BARD score may be more useful for evaluating disease progression in NAFLD patients than the APRI. Thus, although each of these fibrosis estimation procedures were previously believed to have both strengths and weaknesses, these results demonstrated that both estimations can be clinically applied for early detection of patients at high risk for HCC. Interestingly, two patients in this study with fatty liver but without fibrosis developed HCC. This finding differs from that of another large-scale study of NAFLD patients (25,43–45), which did not report the development of HCC from fatty liver without fibrosis. The above findings emphasize the need for further studies to identify factors that trigger the onset of HCC process in NAFLD patients without fibrosis, including single-nucleotide polymorphisms.

This study has certain limitations. First, this was a retrospective cohort trial. Second, the male:female ratio was strongly biased toward males. This heterogeneity makes it difficult to interpret the study results. Third, this study was not performed as a comparison to the background incidence of HCC in the Japanese general population without NAFLD and alcoholic liver disease.