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Original Article

Effectiveness and safety of reduced-dose telaprevir-based triple therapy in chronic hepatitis C patients

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Aim: To compare the early virological effectiveness, sustained virological response and safety of telaprevir 1500 mg/day with telaprevir 2250 mg/day, when combined in triple therapy with pegylated interferon and ribavirin in Japanese patients with high viral loads of genotype 1 hepatitis C virus.

Methods: The telaprevir 2250 mg/day and 1500 mg/day groups each contained 60 patients matched by age, sex and history of previous interferon-based treatment. Serum levels of genotype 1 hepatitis C virus RNA, hemoglobin levels, drug adherence and drug discontinuation rates were monitored during and after triple therapy.

Results: Patients receiving telaprevir 1500 mg/day had significantly lower telaprevir adherence and lower initial ribavirin dose but similar or superior pegylated interferon and ribavirin adherence and a lower rate of telaprevir discontinuation than

did those receiving telaprevir 2250 mg/day. The early virological responses and sustained virological response rates were similar in both groups. Hemoglobin levels decreased to a greater extent in patients treated with telaprevir 2250 mg/day.

Conclusion: Compared to triple therapy including telaprevir 2250 mg/day, that including telaprevir at a reduced dose of 1500 mg/day was associated with lower rates of anemia and similar antiviral efficacy. Such a regimen may meaningfully improve sustained virological response rates, especially among female and elderly Japanese patients.

Key words: chronic hepatitis, hepatitis C virus, pegylated interferon, ribavirin, telaprevir

INTRODUCTION

APPROXIMATELY 170 MILLION people are chronically infected with hepatitis C virus (HCV) worldwide,¹ and approximately 30% develop serious liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC).^{2,3} Currently, interferon (IFN) is the only antiviral drug capable of eliminating HCV infection. The present standard of care (SOC) for patients infected with HCV genotype 1, the most prevalent global genotype, is pegylated interferon (PEG IFN)

combined with ribavirin (RBV) for 48 weeks.⁴ However, sustained virological response (SVR), defined as the reduction of serum HCV RNA to undetectable levels 24 weeks after the completion of therapy, is achieved in only 42–52% of patients.^{5–7} Moreover, response rates are influenced by patient factors such as sex, age and ethnicity,^{8–10} as well as virological factors such as genotype and viral load.¹¹ SVR rates remain unsatisfactorily low (22%) in women aged 50 years or more who are infected with HCV genotype 1 in Japan.¹² Hence, there is a pressing need to improve the efficacy of antiviral treatment in such patients.

Recently, a new class of drugs, with a mechanism based on inhibition of the NS3/NS4 protease of the HCV polyprotein, has been investigated for the treatment of chronic hepatitis C. Of the drugs in this class, telaprevir has been selected as a clinical candidate for further development.¹³ Telaprevir combined with PEG IFN and RBV has shown potent antiviral activity in phase II^{14,15} and III clinical trials,^{16,17} SVR rates of

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approximately 70% have been reported in patients infected with HCV-1. Similarly, in Japan, a phase III study was conducted in patients with HCV-1 to compare the efficacy and safety of the telaprevir regimen with those of the current SOC in treatment-naïve patients,¹⁸ and to assess the efficacy and safety of the telaprevir regimen in relapsers and non-responders after previous IFN-based therapy.¹⁹ However, the high efficacy was offset by treatment-induced anemia: early hemoglobin levels during triple therapy decreased by up to 4 g/dL, whereas decreases with SOC were not higher than 3.0 g/dL.^{14,15} Additionally, we have previously reported that the factors associated with decreases in hemoglobin levels during triple therapy included female sex and age of more than 50 years.²⁰ Japanese patients infected with HCV genotype 1b with high viral loads are, on average, much older than Western patients infected with the same genotype, owing to a widespread HCV infection that occurred in Japan approximately 20 years ago.²¹ Therefore, we considered that triple therapy would be highly effective when combined with careful monitoring of hemoglobin levels and prompt modification of RBV dose.

Consequently, in this study, we evaluated the effectiveness and safety of telaprevir-based triple therapy, administered at an initial telaprevir dose of 2250 or 1500 mg/day, in the retrospective matched control study of 120 Japanese patients with chronic HCV-1 infection with high viral loads.

METHODS

Patients

FROM DECEMBER 2008 to August 2012, 204 patients with chronic hepatitis C were recruited to receive triple therapy with telaprevir, PEG IFN and RBV for 24 weeks at the Department of Hepatology in the Toranomon Hospital in Metropolitan Tokyo. All patients had the following characteristics: (i) positive for HCV RNA genotype 1 and antibody to HCV (anti-HCV), absence of co-infection with HCV of other genotypes; (ii) negative for hepatitis B surface antigen; (iii) HCV RNA levels of 5.0 log IU/mL or more as determined with the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (iv) platelet counts of more than $80 \times 10^3/\text{mm}^3$ without cirrhosis diagnosed by ultrasonography; (v) not pregnant or lactating; (vi) total previous alcohol intake of less than 500 kg; (vii) absence of HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune

hepatitis; and (viii) absence of antiviral or immunosuppressive treatment during the previous 3 months.

Patients were followed for liver function and virological markers at least monthly during treatment and until 24 weeks after completion of the triple therapy. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the a priori approval of the institution's human research committee.

Study design

Telaprevir (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at the dose of 2250 (750 mg three times daily) or 1500 mg/day (750 mg twice daily). We selected 60 patients per group who were matched by age, sex and history of previous IFN-based treatment from the telaprevir 2250 and 1500 mg/day groups (Table 1), because 204 patients had many differences in baseline characteristics in both groups. PEG IFN- α -2b (PEG-Intron; Schering Plough, Kenilworth, NJ, USA) was injected s.c. at a median dose of 1.5 $\mu\text{g}/\text{kg}$ (range, 1.1–1.8) once a week. RBV (Rebetol; Schering Plough) was administered at 200–1000 mg/day; RBV dose of 600 mg/day (for bodyweight ≤ 60 kg), 800 mg/day (for bodyweight >60 to ≤ 80 kg) or 1000 mg/day (for bodyweight >80 kg) in principle. Since November 2011, the initial dose of RBV was reduced by 200 mg in cases of female sex, aged 66 years or older, hemoglobin level of less than 13 g/dL, bodyweight of less than 45 kg or platelet counts of less than $150 \times 10^3/\text{mm}^3$ at baseline by the judgment of the physician. All participating patients received these three drugs for the initial 12 weeks, followed by PEG IFN and RBV for an additional 12 weeks. All patients were followed up for at least 24 weeks after the last dose of study drugs to assess SVR.

Doses of telaprevir, PEG IFN and RBV were reduced or their administration discontinued as required, based on the reduction of hemoglobin levels; reduction of white blood cell, neutrophil or platelet counts; or the development of adverse events. Thus, the total dose of each drug administered during the 12–24 weeks was calculated as the ratio of the actual administered total dose to the anticipated total dose of each drug; these ratios provided adherence measures for telaprevir, PEG IFN and RBV.

HCV RNA measurements

Blood samples were obtained at weeks 1, 2, 4, 6, 8, 12, 16, 20 and 24 after initiation of treatment and at week 24 after completion of treatment, and routine biochemical

Table 1 Baseline characteristics of the patients infected with genotype 1 HCV who received triple therapy with pegylated interferon, ribavirin and TVR

	TVR 2250 mg/day	TVR 1500 mg/day	P-value
<i>n</i>	60	60	
Sex (male/female)	30/30	30/30	Matched
Age (years)	60 (53–63)	62 (56–64)	Matched
Body mass index (kg/m ²)	22.1 (20.4–24.0)	22.7 (20.1–24.8)	0.278
<i>IL28B</i> genotype (rs8099917) TT/TG + GG	40/20	54/6	0.003
<i>ITPA</i> genotype (rs12979860) CC/CA + AA	44/16	36/23	0.175
Hemoglobin (g/dL)	14.3 (13.5–15.2)	14.2 (13.0–14.8)	0.223
Platelets (×10 ⁴ /μL)	17.6 (14.9–21.0)	16.9 (13.8–19.9)	0.227
Albumin (g/dL)	3.8 (3.7–4.0)	3.8 (3.7–4.1)	0.404
Alanine aminotransferase (IU/L)	35 (25–49)	37 (25–58)	0.437
γ-Glutamyltransferase (IU/L)	29 (18–49)	22 (17–39)	0.230
Creatinine (mg/dL)	0.7 (0.6–0.8)	0.6 (0.6–0.7)	0.333
Uric acid (mg/dL)	5.6 (4.9–6.5)	5.5 (4.7–6.3)	0.487
α-Fetoprotein (μg/L)	4 (3–7)	5 (3–8)	0.740
HCV RNA (log ₁₀ IU/mL)	6.8 (6.4–7.0)	6.7 (6.3–7.0)	0.551
Core a.a. 70 (wild/mutant)	38/22	45/15	0.235
Core a.a. 91 (wild/mutant)	28/32	36/24	0.200
Previous IFN-based treatment			
Naïve/relapsed/null response	23/25/12	23/25/12	Matched

Values are number with percentage in parentheses or median with interquartile range in parentheses. a.a., amino acid; HCV, hepatitis C virus; IFN, interferon; TVR, telaprevir.

and hematological tests were performed. The antiviral effects were assessed by measuring plasma HCV RNA levels using the COBAS TaqMan HCV test. The linear dynamic range of the assay was 1.2–7.8 log₁₀ IU/mL; undetectable samples were defined as negative.

Detection of amino acid substitutions in the core of HCV-1b

Amino acid (a.a.) substitutions in the HCV core region were determined using direct sequencing of polymerase chain reaction products after extraction and reverse transcription of HCV RNA. Core a.a. substitutions at positions 70 and 91 (core 70 and 91, respectively) were determined according to the methods of our previous reports.^{22,23}

Determination of *IL28B* and *ITPA* genotypes

ITPA (rs1127354) and *IL28B* (rs8099917 and rs12979860) were genotyped using the Invader assay, TaqMan assay or direct sequencing, as described.^{24,25}

Statistical analyses

Non-parametric tests, including the χ^2 -test, Fisher's exact test, Mann–Whitney *U*-test and Kruskal–Wallis tests, were used to analyze differences in the baseline clinical

profiles of patients. Kaplan–Meier analysis and the log-rank test were applied to estimate and compare serum HCV RNA elimination rates between the groups. *P* < 0.05 by two-tailed test was considered statistically significant. All analyses were performed using SPSS software version 10.1 (SPSS, Chicago, IL, USA).

RESULTS

Baseline characteristics

THE BASELINE CHARACTERISTICS of the 120 patients are listed in Table 1. There were no significant differences in the baseline characteristics between the telaprevir 2250 mg/day group and 1500 mg/day group, except for *IL28B* genotypes. Patients receiving telaprevir 1500 mg/day had a significantly higher incidence of TT in *IL28B* genotypes than did those receiving 2250 mg/day.

Initial drug doses, drug adherence and discontinuation rate up to 12 weeks

Patients receiving telaprevir 1500 mg/day had a significantly lower initial telaprevir dose and initial RBV dose than those receiving 2250 mg/day (Table 2). Telaprevir adherence was significantly lower in the 1500 mg/day

Table 2 Initial drug doses, drug adherence up to 24 weeks and discontinuation rates up to 12 weeks

	TVR 2250 mg/day	TVR 1500 mg/day	P-value
<i>n</i>	60	60	
Initial TVR dose (mg/kg per day)	38.1 (33.6–45.1)	25.6 (22.5–29.6)	<0.001
TVR adherence up to 12 weeks (%)	100 (75–100)	67 (65–67)	<0.001
Discontinuation of TVR	15 (25.0%)	6 (10.0%)	0.053
Discontinuation of TVR due to anemia	12 (20%)	3 (5%)	0.025
Initial PEG IFN dose (μ g/kg per week)	1.5 (1.4–1.6)	1.5 (1.4–1.6)	0.706
PEG IFN adherence up to 24 weeks (%)	100 (85–100)	100 (89–100)	0.062
Initial RBV dose (mg/kg per day)	11.6 (10.6–12.8)	9.9 (7.9–11.3)	<0.001
RBV adherence up to 24 weeks (%)	51 (41–61)	59 (46–68)	0.090
Discontinuation of all drugs up to 12 weeks	5 (8.3%)	1 (1.7%)	0.207

Values are number with percentage in parentheses or median with interquartile range in parentheses. PEG IFN, pegylated interferon; RBV, ribavirin; TVR, telaprevir.

group than in the 2250 mg/day group, while there were no differences in adherence for the other two drugs. Although there were no significant differences between the groups in the rates of discontinuation of telaprevir or all drugs up to 12 weeks, the rates of discontinuation of telaprevir due to anemia in the 1500 mg/day group were significantly lower than in 2250 mg/day group.

Loss of serum HCV RNA according to *IL28B* genotypes

Figure 1 compares the on-treatment virological response over the first 12 weeks for the telaprevir 2250 and 1500 mg/day groups according to *IL28B* genotypes, respectively, because there were significant differences in distribution of *IL28B* genotypes between both groups.

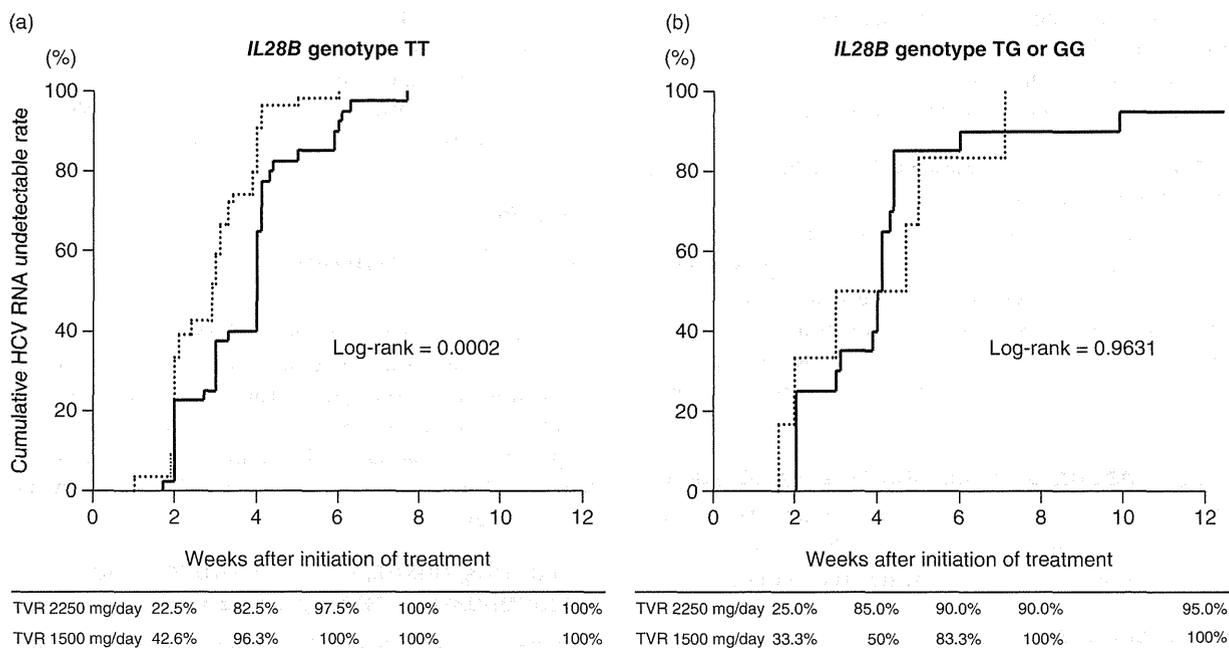


Figure 1 Cumulative rate of undetectable hepatitis C virus (HCV) RNA during triple therapy with pegylated interferon, ribavirin and telaprevir (TVR) at either 2250 mg/day or 1500 mg/day. (a) *IL28B* genotype TT, (b) *IL28B* genotype TG or GG. (—) TVR 2250 mg/day, (.....) TVR 1500 mg/day.

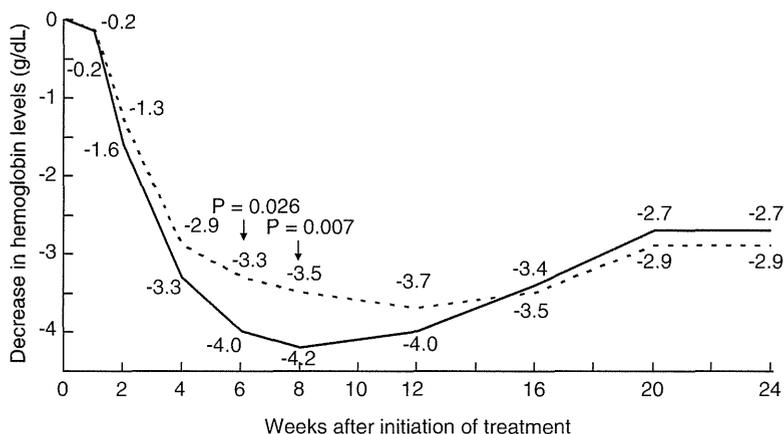


Figure 2 Decreases in hemoglobin levels during triple therapy with pegylated interferon (PEG IFN), ribavirin (RBV) and telaprevir (TVR) at either 2250 mg/day or 1500 mg/day. Each time point in this figure corresponds to median values. Patients evaluated at each time point are indicated below, with the number of patients who discontinued TVR (continued PEG IFN and RBV) in parentheses. (—) TVR 2250 mg/day, (.....) TVR 1500 mg/day.

Number of patients (TVR withdrawn)	
2250 mg/day	60 60 60 (1) 59 (4) 55 (10) 55 55 55
1500 mg/day	60 60 60 (1) 59 (2) 59 (3) 59 59 59

Triple therapy suppressed HCV RNA levels quickly and effectively in both groups. In the 2250 and 1500 mg/day groups of *IL28B* genotype TT, HCV RNA became undetectable in 22.5% and 42.6% of patients at 2 weeks, 82.5% and 96.3% at 4 weeks, and 100% and 100% at 8 weeks, respectively (Fig. 1a). The early virological response of the telaprevir 1500 mg/day group was significantly higher than that of the 2250 mg/day group in *IL28B* genotype TT (log-rank test = 0.0002).

In the subgroups of *IL28B* genotype non-TT patients receiving telaprevir 2250 and 1500 mg/day, HCV RNA became undetectable in 25.0% and 33.3% of patients at 2 weeks, 85.0% and 50% at 4 weeks, 90.0% and 100% at 8 weeks, and 95.0% and 100% at 12 weeks, respectively. The virological responses during the first 12 weeks in this subgroup of patients did not significantly differ between the telaprevir 2250 and 1500 mg/day groups (log-rank test = 0.9631, Fig. 1b).

Safety

Figure 2 shows the decreases in hemoglobin levels in telaprevir 2250 and 1500 mg/day recipients. Data from six patients were omitted (five receiving telaprevir 2250 mg/day and one receiving 1500 mg/day) because treatment was withdrawn between 8 and 12 weeks after initiation. Telaprevir was discontinued in 15 of the 60 (25.0%) patients receiving telaprevir 2250 mg/day (one at week 6, four at week 8 and 10 at week 12) and six of the 60 (10.0%) receiving 1500 mg/day (one at week 6, two at week 8 and three at week 12). Hemoglobin

decreased to a greater extent in patients receiving telaprevir 2250 mg/day than in those receiving 1500 mg/day at week 6 (-4.0 [-6.7 to -1.2] vs -3.3 [-5.2 to 0.2] g/dL, *P* = 0.026) and week 8 (-4.2 [-7.7 to -1.3] vs -3.5 [-6.9 to -1.3] g/dL, *P* = 0.007).

Skin disorder frequency was comparable between the telaprevir 2250 mg/day group and 1500 mg/day group (81.7% and 75%, respectively). However, skin disorders of grades 2–3 occurred more frequently in the telaprevir 2250 mg/day group than in the 1500 mg/day group (55% vs 35%, *P* = 0.043).

With respect to renal dysfunction, increases in serum creatinine (sCR) levels during therapy were not significantly different between both groups. However, blood uric acid levels increased to a greater extent in patients receiving telaprevir 2250 mg/day than in those receiving 1500 mg/day at week 1 (1.3 [-1.6 to 4.8] vs 0.9 [-2.1 to 4.3] g/dL, *P* = 0.015), week 2 (1.2 [-2.3 to 4.1] vs 0.5 [-2.3 to 2.7] g/dL, *P* = 0.004), week 4 (1.6 [-1.1 to 5.5] vs 0.7 [-2.4 to 3.8] g/dL, *P* < 0.001), week 6 (1.6 [-1.7 to 4.8] vs 0.5 [-3.5 to 3.6] g/dL, *P* < 0.001) and week 8 (1.1 [-3.6 to -4.9] vs 0.7 [-1.6 to 3.7] g/dL, *P* = 0.029).

Predictive factors associated with SVR

The overall SVR rate was 83% (169/204) in our hospital. SVR was accomplished in 106 (88%) of 120 patients selected for this study, including 50 of 60 (83%) patients in the telaprevir 2250 mg/day and 56 of 60 (93%) patients in telaprevir 1500 mg/day groups (Fig. 3).

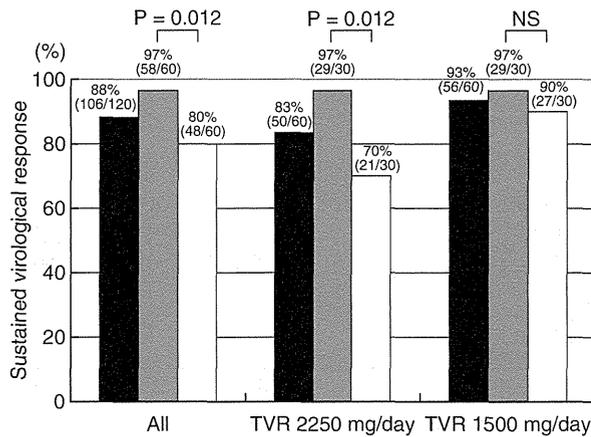


Figure 3 Sustained virological response in patients with chronic hepatitis C to triple therapy with telaprevir (TVR), pegylated interferon and ribavirin for 24 weeks. Sustained virological response was compared among all patients (men and women), TVR 2250 mg/day patients and TVR 1500 mg/day patients, respectively. (■) Total, (▒) male, (□) female.

Significant univariate predictors for SVR included male sex, *IL28B* genotype TT, and HCV core a.a. 70 wild type, except for null response to prior treatment, initial telaprevir dose of 37.5 mg/kg per day or more, telaprevir dosing period of 10 weeks or more, 100% PEG IFN adherence up to 24 weeks, PEG IFN adherence up to 12 weeks of 80% or more, RBV adherence up to 12 weeks of 50% or more, γ -glutamyltransferase of 35 IU/mL or less, and sCr of 0.6 mg/dL or more ($P < 0.05$). Of these, male sex (odds ratio [OR] = 13.7; $P = 0.028$) and *IL28B* genotype TT (OR = 44.4; $P = 4.47 \times 10^{-5}$) were identified as significant independent predictors for SVR (Table 3).

Therefore, we assessed the SVR rate of triple therapy according to sex and *IL28B* genotype. SVR was much less frequent in women than in men (48/60 [80%] vs 58/60 [97%], $P = 0.0012$, Fig. 3). Especially, in the telaprevir 2250 mg/day group, there were significant differences

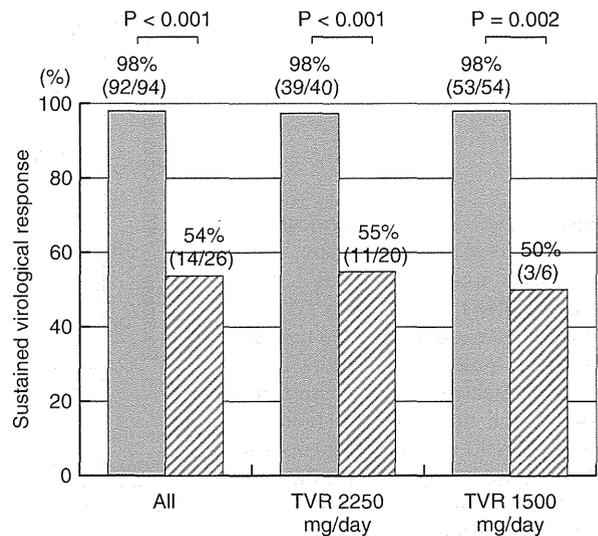


Figure 4 Sustained virological response in patients with chronic hepatitis C to triple therapy with telaprevir (TVR), pegylated interferon and ribavirin for 24 weeks. Sustained virological response was compared between *IL28B* (rs8099917) genotype TT and TG/GG in all patients, TVR 2250 mg/day patients and TVR 1500 mg/day patients, respectively. (▒) TT, (▨) TG or GG.

between men and women (29/30 [97%] vs 21/30 [70%], $P = 0.0012$). However, there were no differences between men and women in the telaprevir 1500 mg/day group (29/30 [97%] and 27/30 [90%], respectively).

Patients with *IL28B* genotype TT were significantly more likely to achieve SVR (92/94 [98%] vs 14/26 [54%], $P < 0.001$, Fig. 4), compared with patients with TG or GG genotypes. There were significant differences between *IL28B* genotype TT and non-TT in both the telaprevir 2250 and 1500 mg/day groups (39/40 [98%] vs 11/20 [55%], $P < 0.001$ and 53/54 [98%] vs 3/6 [50%], $P = 0.002$, respectively).

Table 3 Multivariate analysis of factors associated with sustained virological response of TVR, pegylated interferon and ribavirin triple therapy in Japanese patients infected with HCV

Factor	Category	Odds ratio (95% CI)	P-value
Sex	1; female	1	0.028
	2; male	13.7 (1.33–141.2)	
<i>IL28B</i> genotype (rs8099917)	1; TG or GG	1	4.47×10^{-5}
	2; TT	44.4 (7.18–274.2)	

CI, confidence interval; HCV, hepatitis C virus; TVR, telaprevir.

DISCUSSION

IN JAPANESE PATIENTS, virological response to triple therapy with telaprevir, PEG IFN and RBV was excellent. We have previously reported that in 20 patients with chronic HCV-1b infection with high viral load who received triple therapy for 12 weeks, HCV RNA became undetectable in 50% at 2 weeks, 79% at 4 weeks, 88% at 6 weeks, 94% at 8 weeks and 100% at 12 weeks.²⁶ This previous study was a randomized open-label study in which telaprevir was administered at doses of 2250 or 1500 mg/day. Early virological response at 7 and 14 days was similar for both telaprevir doses, suggesting that virological response to triple therapy is not affected by lowering the telaprevir dose. Therefore, to expand the dataset, we retrospectively evaluated HCV RNA response and safety during 12 weeks of triple therapy including the two different telaprevir doses followed by PEG IFN and RBV for an additional 12 weeks: we analyzed 204 cases in total. However, because of the non-random nature of treatment allocation, there was a preponderance of women, elderly and anemic patients in the group receiving telaprevir 1500 mg/day. Because there were many differences in baseline characteristics between telaprevir 2250 and 1500 mg/day groups, we selected 60 patients per group who were matched by age, sex and history of previous IFN-based treatment. Therefore, there were no differences in baseline characteristics between both groups in this analysis, except for *IL28B* genotype. Although we tried to match the distribution of *IL28B* genotypes between both groups, this was not possible because of the small number of cases. Therefore, we matched the groups by the history of previous IFN-based treatment, which we considered a similarly strong predictive factor of triple therapy. Moreover, there was a significant difference in the initial dose of RBV between both groups. A significant number of patients underwent RBV dose reductions at the beginning of treatment in the telaprevir 1500 mg/day group because we considered that such patients were likely to experience hemoglobin decrements during triple therapy, but before November 2011, we could not reduce the initial dose of telaprevir and RBV. Nine patients (15.0%) receiving telaprevir 2250 mg/day and 32 cases (53.3%) receiving 1500 mg/day underwent RBV dose reduction at the beginning of treatment. In other words, the group receiving telaprevir 1500 mg/day had a significantly lower initial dose of telaprevir and RBV dose than did the group receiving 2250 mg/day (Table 2).

However, in the present study, HCV RNA became undetectable during the 12 weeks of treatment at

similar or higher rates in the telaprevir 1500 mg/day group than in the 2250 mg/day group (Fig. 1). In the *IL28B* TT genotype, the early virological response of the telaprevir 1500 mg/day group was significantly higher than that of the 2250 mg/day group. Although we assessed baseline factors, drug adherence and drug discontinuation rates only in the *IL28B* TT genotype, there were no significant differences between both groups, except for lower telaprevir adherence up to 12 weeks and a greater number of cases of PEG IFN and RBV dose reductions at the beginning of treatment in the telaprevir 1500 mg/day group. Therefore, the reason for significant differences in the early virological response between both groups is unclear. However, we considered that these results did not affect the SVR rate because HCV RNA became undetectable in all patients in both groups at 8 weeks after the start of triple therapy. In all cases, *IL28B* TT cases and non-TT cases, there were no significant differences in SVR rates after triple therapy between those receiving telaprevir 2250 and 1500 mg/day (Figs 3,4). By examining the detailed course of drug administration from 12–24 weeks (Table 2), we found that the group receiving telaprevir 1500 mg/day had a lower discontinuation rate of telaprevir and higher adherence to RBV and PEG IFN up to 24 weeks in spite of the low initial RBV dose. Furthermore, hemoglobin levels showed greater reductions during triple therapy with telaprevir 2250 mg/day than with telaprevir 1500 mg/day, and the group receiving telaprevir 2250 mg/day had a significantly higher discontinuation rate of telaprevir due to anemia than did the group receiving telaprevir 1500 mg/day (Fig. 2). Therefore, telaprevir 1500 mg/day may be a safe option as part of triple therapy, while maintaining PEG IFN and RBV adherence.

Viral breakthrough or relapse can occur during telaprevir monotherapy or telaprevir plus PEG IFN dual therapy (without RBV) because of the development of mutations that confer resistance to telaprevir.^{14,27–29} Furthermore, in a Japanese phase III trial of triple therapy in relapsers and non-responders who had not achieved SVR to a previously administered IFN-based regimen, SVR rates increased as RBV adherence increased, particularly in previous non-responders.¹⁹ In triple therapy with telaprevir, PEG IFN and RBV, we consider that telaprevir could be important for early virological response, but it could also be important for maintaining high adherence to PEG IFN and RBV, which is a key factor for achieving SVR. We speculate that triple therapy including telaprevir at the reduced dose of 1500 mg/day could maintain high levels of adherence

to PEG IFN and RBV, and consequently achieve high SVR rates.

In this study, we investigated the independent predictors for SVR in the multivariate analysis (Table 3). As reported in previous studies, *IL28B* genotype remained the strongest predictor of SVR.^{30,31} The next strongest predictive factor was sex: women had significantly lower SVR rates than did men (Fig. 3). However, when we investigated the SVR rates of the telaprevir 2250 mg/day group and 1500 mg/day group, we found that there were significant differences in SVR rates between men and women in the telaprevir 2250 mg/day group but no differences in the telaprevir 1500 mg/day group. In the previous study, we reported that female sex was one of the factors influencing decreases in hemoglobin levels during triple therapy administered 2250 mg/day of initial telaprevir dose.²⁰ In the present study, the discontinuation rates of telaprevir due to anemia were significantly higher in women in the telaprevir 2250 mg/day group as compared with men (36.7% vs 3.3%, $P=0.002$, data not shown), but there were no differences in the discontinuation rates of telaprevir due to anemia between men and women in the telaprevir 1500 mg/day group (0% vs 10%, $P=0.237$, data not shown). Therefore, we speculate that there were significant differences in SVR rates between men and women because of high telaprevir discontinuation rates owing to anemia in women.

In conclusion, after the completion of 24 weeks of therapy, triple therapy including telaprevir at a reduced dose of 1500 mg/day was as effective as triple therapy including telaprevir 2250 mg/day at suppressing HCV RNA to undetectable levels and achieving SVR. Of note, we found that telaprevir 1500 mg/day was associated with lower levels of anemia and discontinuation of telaprevir owing to anemia, and higher PEG IFN and RBV adherence during triple therapy. These results suggest that the telaprevir 1500 mg/day regimen is an effective and safe alternative for the treatment of elderly and female Japanese patients. This study is a retrospective study. Prospective randomized controlled studies with longer follow-up periods are required to fully assess the efficacy and safety of an initial telaprevir dose of 1500 mg/day.

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Original Article

Discrimination of fibrotic staging of chronic hepatitis C using multiple fibrotic markers

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Aim: In order to evaluate and judge a fibrotic stage of patients with chronic hepatitis C, multivariate regression analysis was performed using multiple fibrotic markers.

Methods: A total of 581 patients from eight hepatology units and institutes were diagnosed by needle biopsy as having chronic liver disease caused by hepatitis C virus. Twenty-three variables and their natural logarithmic transformation were employed in the multivariate analysis.

Results: Multivariate regression analysis finally obtained the following function: $z = 2.89 \times \ln(\text{type IV collagen 7S (ng/mL)}) - 0.011 \times (\text{platelet count}) (\times 10^9/\text{mm}^3) + 0.79 \times \ln(\text{total bilirubin (mg/dL)}) + 0.39 \times \ln(\text{hyaluronic acid (}\mu\text{g/L)}) - 1.87$. Median values of the fibrotic score of F1 ($n = 172$), F2 ($n = 80$),

F3 ($n = 37$) and F4 ($n = 16$) were calculated as 1.00, 1.45, 2.82 and 3.83, respectively. Multiple regression coefficient and coefficient of determination were 0.56 and 0.320, respectively. Validation with patient data from other institutions demonstrated good reproducibility of the fibrotic score for hepatitis C (FSC), showing 1.10 in F1 ($n = 156$), 2.35 in F2 ($n = 73$), 3.16 in F3 ($n = 36$) and 3.58 in F4 ($n = 11$).

Conclusion: A concise multiple regression function using four laboratory parameters successfully predicted pathological fibrotic stage of patients with hepatitis C virus infection.

Key words: chronic hepatitis, hepatitis C virus, liver cirrhosis, liver fibrosis, multiple regression analysis, stage

INTRODUCTION

WHEN HEPATITIS C virus (HCV)-related chronic liver disease was found by biochemical and virological examination, peritoneoscopy and/or liver biopsy can establish the definitive diagnosis of chronic hepatitis and liver cirrhosis. Although these pathological procedures are reliable and informative both in diagnosis and treatment, they sometimes require medical invasion and financial costs, including the risk of bleeding from needle puncture, some pain experienced during the examination, medical expenses and hospitalization for a

few days. The pathological examination is, therefore, rarely performed repeatedly in a short period of time, even when disease activity is severe and progression of liver disease is highly suspected. Recently, many authors described the usefulness of ultrasonographic elastography and magnetic resonance imaging technology in the estimation of staging of chronic hepatitis and cirrhosis.^{1–4} These ways of estimation using the imaging apparatuses seem truly useful for current patients, but it cannot evaluate and compare with past fibrotic states of patients retrospectively. Moreover, the same apparatus for elastometry will not be available for repeated measurement for a follow-up examination, several years later for example.

In spite of the accuracy of biopsy and of convenience of elastography in chronic liver disease, clinical diagnosis based on biochemistry and hematology is still indispensable for the daily practice of many patients with

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HCV-related liver disease. Recently, several studies were published about estimation of hepatitis stages, using one or more serum biomarkers. Discriminant functions or multivariate analyses demonstrated that approximately 60–90% of patients with chronic hepatitis C were correctly classified as mild hepatitis and severe hepatitis with advanced fibrosis.^{5–16} The usefulness of the discriminant functions was, however, less valuable up to the present time for a few reasons. First, these functions were made for the purpose of discrimination of severe hepatic fibrosis from mild fibrosis, and four histological classifications (F1, F2, F3 and F4) were selected in almost of the studies. Second, some studies analyzed both hepatitis B virus and HCV infection, although the significance and actual values of each liver function test in the evaluation of the severity of liver disease were not similar among each viral hepatitis and alcoholic liver disease. Third, biochemical markers for liver fibrosis (e.g. hyaluronic acid, type IV collagen, procollagen III peptide)^{17–19} were not always included in those previous studies.

We tried to generate a function estimating fibrotic stages of HCV-related chronic hepatitis, which were objectively diagnosed by liver biopsy. The purpose of this study is, therefore, to make a reliable multiple regression function and to obtain practical coefficients for significant variables also using fibrotic markers.

METHODS

Patients

A TOTAL OF 605 Japanese patients with chronic hepatitis C were recruited for the study from eight hospitals in Japan: Toranomon Hospital, Hiroshima University Hospital (K. Chayama, M.D.), Ehime University Hospital (M. Onji, M.D.), Musashino Red Cross Hospital (N. Izumi, M.D.), Shishu University Hospital (E. Tanaka, M.D.), Showa University Hospital (M. Imawari, M.D.), Osaka University Hospital (T. Takehara, M.D.) and Kagoshima University Hospital (H. Tsubouchi, M.D.). Inclusion criteria for this study were: (i) positive HCV antibody for more than 6 months; (ii) persistent or intermittent elevation in aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels; and (iii) liver biopsy showing chronic hepatitis (F1, F2, F3 or F4). We excluded those patients with overt alcoholic liver disease or fatty liver, association of other types of liver disease (e.g. hepatitis B, primary biliary cirrhosis, autoimmune hepatitis), or those associated with hepatocellular carcinoma or other malignancy. Among the patients, 603 fulfilled the conditions for the

study: complete demographic data, basic laboratory data of hematology and biochemistry, required liver biopsy specimens, and sufficient amount of frozen sera. We also excluded an additional 22 patients with eventual histological diagnosis of F0 stage.

Finally, a total of 581 patients who were diagnosed as having chronic hepatitis or cirrhosis (F1, F2, F3 or F4) were analyzed for the following hematological, biochemical and histopathological examination. There were 305 males and 276 females aged 15–78 with a median of 55 years.

All the patients presented written informed consent in individual hospitals and medical centers, and the study was approved by each ethical committee.

Hematological and biochemical examination

Hematological and standard biochemical evaluation had been performed in each medical institution: white blood cell, red blood cell count, hemoglobin, platelet count, total bilirubin, AST, ALT, AST/ALT ratio (AAR), γ -glutamyltransferase (GGT), total protein, albumin and γ -globulin.

Special biochemical examinations including fibrotic markers were carried out using stored frozen sera at -20°C or lower: α 2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipoprotein A1, hyaluronic acid, tissue inhibitor of matrix metalloproteinase (TIMP)-1, TIMP-2, procollagen III peptide and type IV collagen 7S.

Histological diagnosis of chronic hepatitis and cirrhosis

All of the 581 cases fulfilled required standards of histological evaluation: sufficient length of specimen, hematoxylin–eosin staining and at least one specimen with fiber staining. Four independent pathologists (Y. T., J. F., F. K. and T. F.), who were not informed of patients' background and laboratory features except for age and sex, evaluated the 581 specimens regarding the stages of fibrosis and activity. Pathological classification of chronic hepatitis staging was based on Desmet *et al.*²⁰

Before judgment of histological staging of individual specimens, the pathologists discussed objective and reproducible judgment of pathological diagnosis of hepatitis. They made a panel for obvious criteria using typical microscopic pictures for each stage, and it was always referred to during the procedure of pathological judgment. When inconsistent results were found in the diagnosis of stage of hepatitis among the pathologists, the final judgment was accepted as the majority rule among them.

Statistical analysis

Non-parametric procedures were employed for the analysis of background characteristics and laboratory data among patients in each stage, including Mann-Whitney *U*-test, Kruskal-Wallis test and χ^2 -test.

The normality of the distribution of the data was evaluated by Kolmogorov-Smirnov one-sample test. Because certain variables partly did not conform to a normal distribution, natural logarithmic transformation of bilirubin, AST, ALT, GGT, α 2-macroglobulin, hyaluronic acid, type IV collagen 7S and TIMP-2 were also analyzed in the following calculation. The natural logarithmic transformation of the results yielded a normal distribution or symmetrical distribution for all the analyzed factors. After the procedures, the following multiple regression analysis became rationally robust against deviations from normal distribution. In order to avoid introducing into the model any variables that were mutually correlated, we checked the interaction between all pairs of the variables by calculating variance of inflation factors. Of the highly correlated variables, less significant factors were removed from the viewpoint of multicollinearity.

Multivariate regression analysis was performed using 305 patient data from Toranomon Hospital (training dataset), to generate training data of predicting function. We used a stepwise method for selection of informative subsets of explanatory variables in the model. Multiple regression coefficient and coefficient of determination are also taken into account in the selection of variables. Next, we validated the obtained predictive function using the remaining 276 patient data from the other seven liver institutions (validation dataset).

A *P*-value of less than 0.05 with two-tailed test was considered to be significant. Data analysis was performed using the computer program SPSS version 19.²¹

For evaluation of the efficiency and usefulness of obtained function for estimation of fibrosis, we compared various fibrotic scores for hepatitis C, including AAR,⁸ AST-to-platelet ratio index (APRI),¹² FIB-4¹³ and FibroTest.⁹

RESULTS

Pathological diagnosis

FOUR PATHOLOGISTS INDEPENDENTLY judged the fibrotic stages and inflammatory activity for 581 specimens of chronic hepatitis/cirrhosis caused by HCV. A total of 328 patients (56.5%) had a fibrotic stage of F1, 153 (26.3%) F2, 73 (12.6%) F3 and 27 (4.6%) F4. In

the training subgroup (*n* = 305), judgment of F1 was made in 172, F2 in 80, F3 in 37 and F4 in 16. In the validation group (*n* = 276), judgment as F1 was made in 156, F2 in 73, F3 in 36 and F4 in 11.

According to hepatitis activity classification, A0 was found in nine patients (1.52%), A1 in 350 (60.2%), A2 in 198 (34.1%) and A3 in 24 (4.1%).

Laboratory data of each hepatitis stage in training group

There were 161 males and 144 females with a median age of 54 years (range, 22–69). Laboratory data of the 305 patients in the training group are shown in Table 1. Although several individual items were well correlated with the severity of hepatic fibrosis, significant overlap values were noted among F1 to F4 stages: platelet count, GGT, γ -globulin, hyaluronic acid and type IV collagen 7S.

Regression function generated from training patient group

After stepwise variable selection, multivariate regression analysis finally obtained the following function: $z = 2.89 \times \ln(\text{type IV collagen 7S (ng/mL)}) - 0.011 \times (\text{platelet count}) (\times 10^3/\text{mm}^3) + 0.79 \times \ln(\text{total bilirubin (ng/mL)}) + 0.39 \times \ln(\text{hyaluronic acid } (\mu\text{m/L}) - 1.87$. Median values of the fibrotic score of F1 (*n* = 172), F2 (*n* = 80), F3 (*n* = 37) and F4 stages (*n* = 16) were calculated as 1.00, 1.45, 2.82 and 3.83, respectively (Fig. 1). The multiple regression coefficient and coefficient of determination were 0.56 and 0.32, respectively.

A 55-year-old man with F1 fibrotic stage (Fig. 2a) showed serum type IV collagen concentration as 3.8 ng/mL, platelet as 152×10^3 count/mm³, total bilirubin as 0.8 mg/dL and hyaluronic acid as 16 μ g/L. The regression function provided his fibrotic score as 1.16. Another man aged 43 years had F3 fibrosis with severe hepatitis activity of A3 on histological examination (Fig. 2b). His type IV collagen was 11.0 ng/mL, platelet 162×10^3 count/mm³, total bilirubin 0.7 mg/dL and hyaluronic acid 189 μ g/L, and regression function calculated his fibrotic score as 4.98.

Validation of discriminant function

Validation data of 276 patients (Table 2) were collected from the other seven institutions in Japan. When applying the regression function for the validation set, the fibrotic score for hepatitis C (FSC) demonstrated good reproducibility, showing 1.10 in patients with chronic hepatitis of F1 (*n* = 156), 2.35 in F2 (*n* = 73), 3.16 in F3 (*n* = 36) and 3.58 in F4 (*n* = 11) (Fig. 3). Although F4

Table 1 Demography and laboratory data of 305 patients in training group

	F1 (n = 172)	F2 (n = 80)	F3 (n = 37)	F4 (n = 16)
Demography				
Males : females	97:75	38:42	20:17	6:10
Age (median, range)	51 (22–69)	55 (29–68)	55 (27–69)	56.5 (29–65)
Laboratory data (median, range)				
WBC ($\times 10^3/\text{mm}^3$)	4.7 (2.0–10.1)	4.3 (2.3–8.5)	4.5 (2.9–6.8)	4.7 (3.3–6.9)
Hemoglobin(g/dL)	14.6 (11.0–18.2)	14.4 (9.3–17.4)	14.6 (11.5–17.7)	14.55 (12.1–16.5)
Platelet ($\times 10^3/\text{mm}^3$)	183 (52–364)	161 (82–387)	131 (74–237)	124 (77–191)
Albumin (g/dL)	4.1 (2.3–4.9)	4.0 (3.5–4.6)	3.9 (3.1–4.6)	3.8 (3.3–4.3)
Bilirubin (mg/dL)	0.8 (0.2–1.9)	0.7 (0.3–1.7)	0.9 (0.4–7.5)	0.8 (0.5–7.4)
AST (IU/L)	42 (16–386)	61 (16–332)	63 (13–238)	71 (30–160)
ALT (IU/L)	60.5 (12–1664)	84.5 (10–647)	108 (27–415)	90.5 (36–264)
γ -GTP (IU/L)	40 (7–383)	48 (10–262)	54 (13–209)	58 (21–195)
γ -Globulin (g/dL)	1.47 (0.58–3.40)	1.61 (1.02–2.41)	1.69 (0.66–2.64)	1.79 (1.22–2.73)
γ -Globulin (%)	19.4 (10.0–40.5)	20.9 (14.0–28.3)	21.3 (8.1–30.4)	22.7 (16.5–36.9)
α 2-Macroglobulin (mg/dL)	269 (123–505)	335 (154–551)	369 (183–627)	317 (207–511)
Haptoglobin (mg/dL)	94.5 (<5–265)	75.5 (<5–263)	56 (<5–2031)	75 (30–142)
Apolipoprotein A1 (mg/dL)	132 (71–209)	131 (73–207)	124 (98–166)	121 (83–153)
Hyaluronic acid ($\mu\text{g/L}$)	25 (<5–407)	41.5 (<5–263)	71 (<5–326)	89.5 (5–246)
TIMP-1 (ng/mL)	165 (73–291)	173 (97–302)	182 (126–308)	192.5 (128–260)
TIMP-2 (ng/mL)	77.5 (31–210)	80 (34–307)	76 (46–143)	78 (58–110)
Procollagen III peptide (U/mL)	0.75 (0.47–1.50)	0.805 (0.61–1.70)	0.86 (0.53–1.50)	1.05 (0.66–1.60)
Type IV collagen 7S (ng/mL)	4.0 (1.7–73)	4.3 (2.1–11.0)	5.2 (3.2–11.0)	5.8 (4.3–9.4)

γ -GTP, γ -glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TIMP, tissue inhibitor of matrix metalloproteinase; WBC, white blood cell.

fibrotic stage consisted of only 11 patients and the score 3.58 was regarded as a rather low value, the scores of other stages of fibrosis were concordant with histological fibrosis.

Fibrosis score of hepatitis C

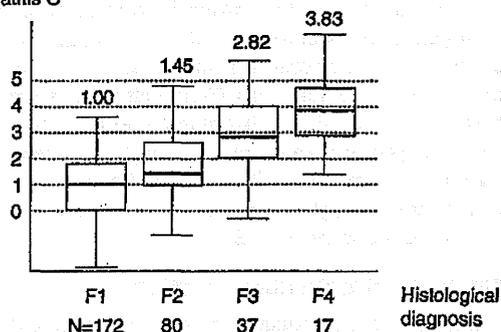


Figure 1 Box and whisker plots of fibrotic score of each group of histological fibrosis in the training dataset. Fibrotic score of hepatitis C (FSC) was generated by the function, $z = 2.89 \times \ln(\text{type IV collagen 7S}) - 0.011 \times (\text{platelet count}) + 0.79 \times \ln(\text{total bilirubin}) + 0.39 \times \ln(\text{hyaluronic acid}) - 1.87$.

Comparisons of efficacy with various fibrotic scores (Fig. 4)

In order to evaluate the efficacy and usefulness of the obtained FSC, we compared with previously reported fibrotic scores using training data. AAR, APRI, FIB-4 and FibroTest showed only slight correlation with actual histological stage. APRI and FIB-4 demonstrated increasing trends of the score associated with histological fibrosis, but significant overlapping scores were found through F1 to F4. Spearman's correlation coefficients of AAR, APRI, FIB-4 and FibroTest were 0.021 ($P = 0.707$), 0.462 ($P < 0.001$), 0.440 ($P < 0.001$) and 0.415 ($P < 0.001$), respectively. Our FSC showed Spearman's correlation coefficient of 0.572 ($P < 0.001$), and was of much higher value than the others.

DISCUSSION

RECOGNITION OF SEVERITY of chronic hepatitis is essential in managing patients with chronic HCV infection; estimation of length of infection, existence of any previous hepatitis activity, presumption of current fibrotic stage, and prediction of future fibrotic progression and hepatocarcinogenesis. Differential diagnosis of cirrhosis from chronic hepatitis is especially important

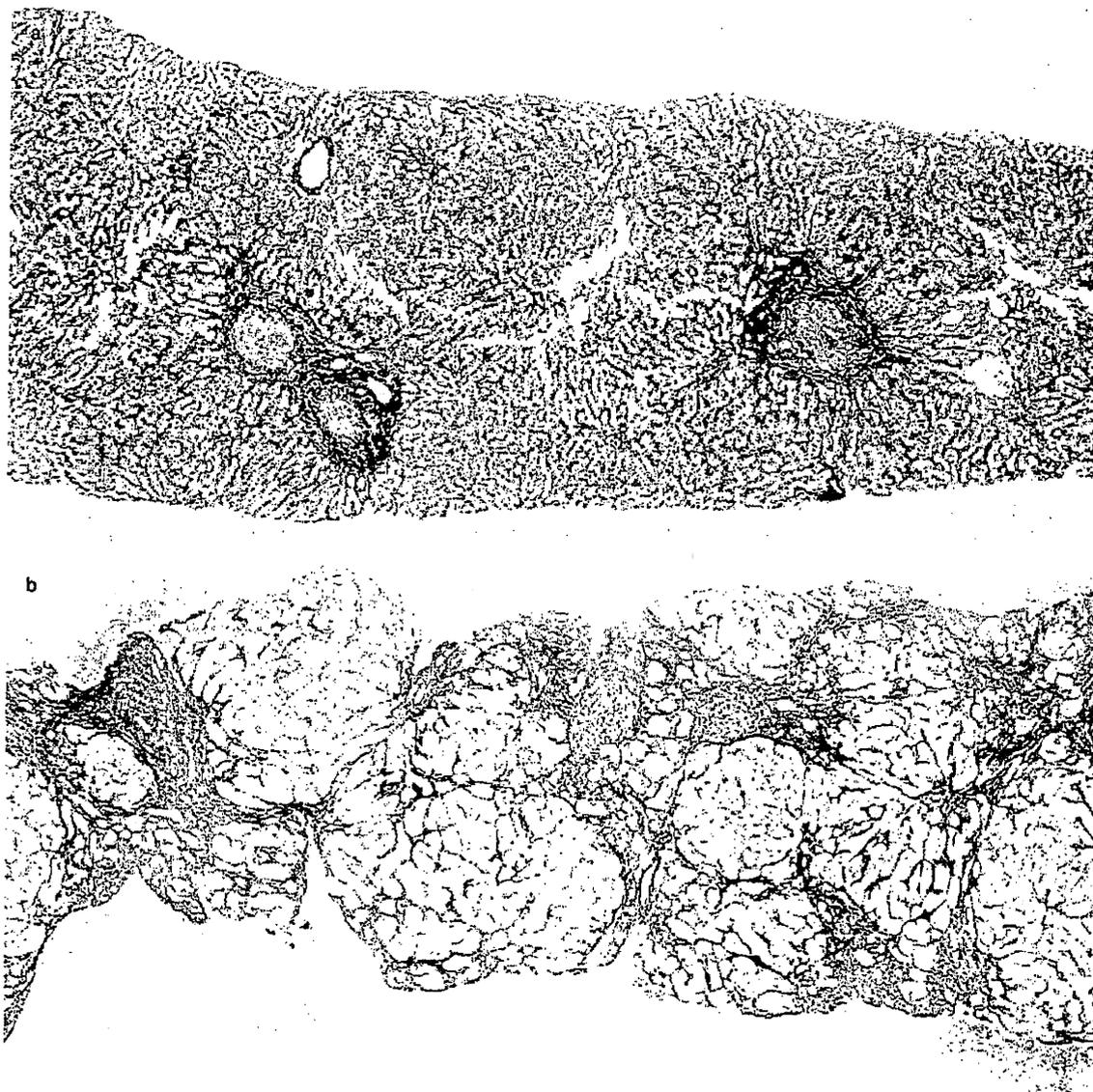


Figure 2 Case presentations of the training set. (a) A 55-year-old man with F1 fibrosis. Final regression function provided his fibrotic score as 1.16. (b) A 43-year-old man with F3 fibrosis with severe hepatitis activity. His regression coefficient was calculated as 4.98 (silver stain, $\times 40$).

in the evaluation of chronic HCV infection. Identification of liver cirrhosis often leads to an important change in management of the patients: needs for fiberoptic examination for esophageal varices, ultrasonographic exploration for the association of liver cancer, and prediction of hepatic decompensation.

Recently, non-invasive estimation of severity of liver fibrosis has been reported in patients with HCV-related chronic hepatitis.^{6–14} However, these studies were principally aimed at differentiation of advanced fibrotic stages of F3 or F4 from mild fibrotic stages of F1 or F2. Those discriminative functions were insufficient to

Table 2 Demography and laboratory data of 276 patients in validation group

	F1 (n = 156)	F2 (n = 73)	F3 (n = 36)	F4 (n = 11)
Demography				
Males : females	83:73	42:31	13:23	6:5
Age (median, range)	55 (15–74)	58 (32–77)	62.5 (30–78)	51 (38–73)
Laboratory data (median, range)				
WBC ($\times 10^3/\text{mm}^3$)	5.1 (2.1–10.5)	4.8 (2.6–9.0)	4.85 (2.3–14.2)	3.9 (3.2–6.0)
Hemoglobin (g/dL)	14.2 (8.9–17.7)	14.4 (11.8–17.4)	14.1 (10.1–16.4)	13.6 (8.9–16.3)
Platelet ($\times 10^3/\text{mm}^3$)	183 (59–440)	153 (80–265)	136 (64–348)	135 (79–153)
Albumin (g/dL)	4.3 (3.1–5.3)	4.3 (3.3–5.2)	4.05 (3.0–5.5)	3.9 (3.0–4.7)
Bilirubin (mg/dL)	0.7 (0.2–8.7)	0.7 (0.2–1.7)	0.8 (0.2–2.5)	0.8 (0.4–11.0)
AST (IU/L)	35 (11–1390)	49 (19–183)	80 (20–190)	96 (29–257)
ALT (IU/L)	49 (11–1635)	62 (12–575)	84 (14–218)	115 (29–303)
γ -GTP (IU/L)	35 (11–600)	52 (10–497)	51 (14–236)	112 (17–312)
γ -Globulin (g/dL)	1.47 (0.70–2.14)	1.60 (0.80–2.37)	1.71 (0.63–2.62)	2.19 (1.70–2.82)
γ -Globulin (%)	19.5 (9.2–26.4)	20.8 (10.8–30.8)	22.4 (9.5–29.9)	27.4 (21.8–35.3)
α 2-Macroglobulin (mg/dL)	271.5 (126–572)	381 (172–573)	405.5 (196–594)	468 (242–655)
Haptoglobin (mg/dL)	95 (<5–305)	80 (<5–223)	63.5 (<5–192)	65 (<5–130)
Apolipoprotein A1 (mg/dL)	126 (45–198)	127 (63–191)	116 (46–172)	108 (62–171)
Hyaluronic acid ($\mu\text{g/L}$)	37.5 (<5–1260)	68 (5–1000)	140.5 (23–2610)	159 (33–364)
TIMP-1 (ng/mL)	157.5 (77–301)	172 (89–355)	188.5 (99–430)	192 (112–320)
TIMP-2 (ng/mL)	70 (21–294)	73 (21–207)	89 (27–280)	76 (36–120)
Procollagen III peptide (U/mL)	0.73 (0.52–8.30)	0.81 (0.53–1.60)	1.00 (0.63–1.90)	1.00 (0.68–1.60)
Type IV collagen 7S (ng/mL)	3.9 (1.2–12.0)	4.5 (2.3–9.9)	5.8 (2.8–16.0)	6.1 (4.6–10.0)

γ -GTP, γ -glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TIMP, tissue inhibitor of matrix metalloproteinase; WBC, white blood cell.

recognize the stepwise progression of viral hepatitis from F1 through F4. This dichotomy (mild or severe) of chronic hepatitis C seemed less valuable in the study of disease progression, disease control abilities of antiviral

drugs and estimation of histological improvement after anti-inflammatory drugs. A histology-oriented, practical and reliable formula is therefore required for the diagnosis and investigation of chronic hepatitis C.

This study was aimed to establish non-invasive evaluation and calculation of liver fibrosis for patients with chronic HCV infection. Although it was retrospectively performed as a multicenter study of eight institutions, judgment of histological diagnosis was independently performed by four pathologists in the other hospital, informed of nothing except for the patient's age, sex and positive HCV infection. Objective judgment of the histological staging and grading in sufficient biopsy specimens could be obtained.

As many as 581 patients with chronic hepatitis C were analyzed in this study, who had been diagnosed as having chronic hepatitis or cirrhosis by liver biopsy performed in experienced liver units in Japan. To obtain the most suitable equation approximating histological fibrotic stage, multivariate analysis was performed using two demographic parameters (age and sex) and 21 hematological and biochemical markers with or without logarithmic transformation. They included many kinds of fibrotic markers: α 2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipo-

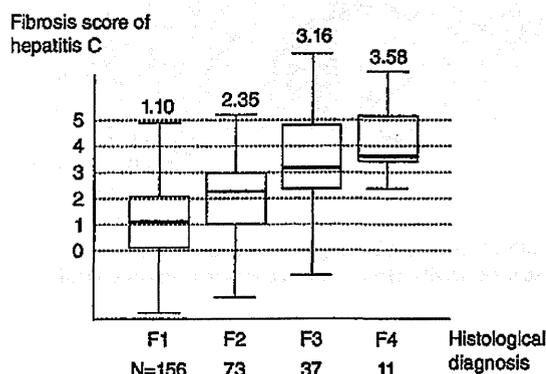


Figure 3 Box and whisker plots of fibrotic score of each group of histological fibrosis in the validation dataset. Fibrotic score of hepatitis C (FSC) was generated by the function, $z = 2.89 \times \ln(\text{type IV collagen 7S}) (\text{ng/mL}) - 0.011 \times (\text{platelet count}) (\times 10^3/\text{mm}^3) + 0.79 \times \ln(\text{total bilirubin}) (\text{ng/mL}) + 0.39 \times \ln(\text{hyaluronic acid}) (\mu\text{g/L}) - 1.87$.

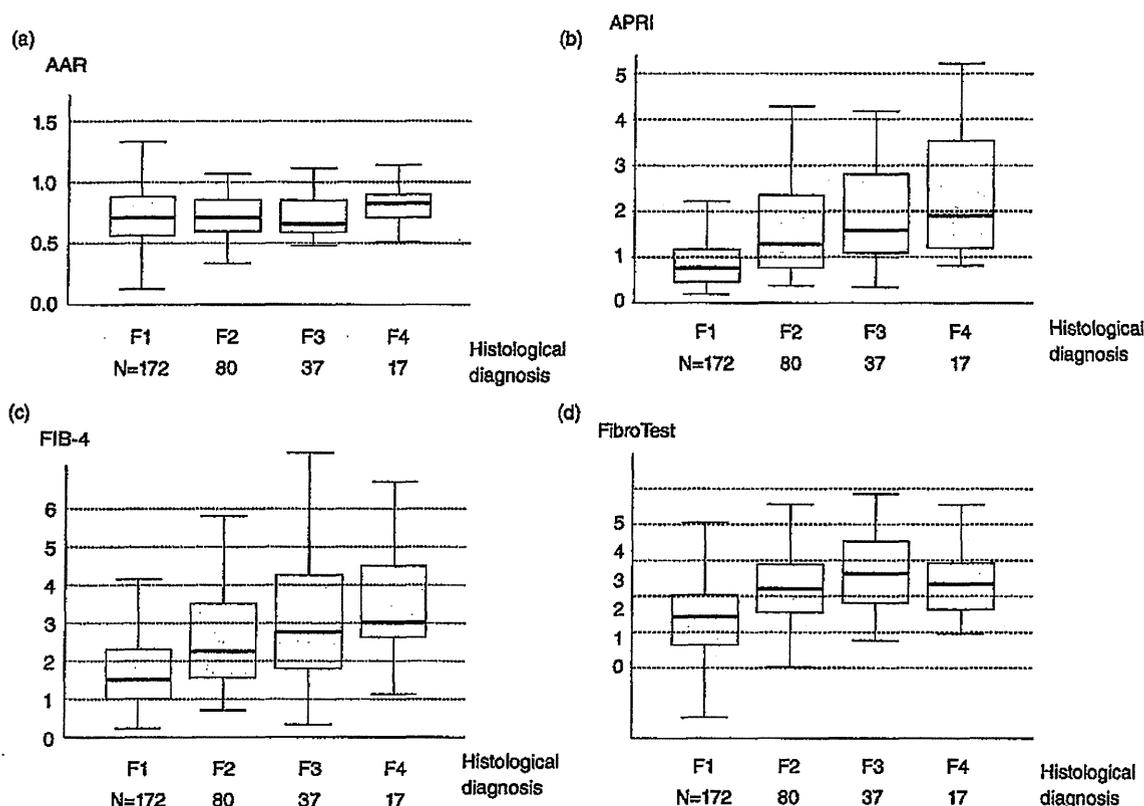


Figure 4 Previously published fibrotic scores: (a) aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR),⁸ (b) AST-to-platelet ratio index (APRI), calculated by $AST / (\text{upper limit of normal of } AST) / (\text{platelet count } [\times 10^9/L]) \times 100$.¹² (c) FIB-4 score, calculated by $\text{age} \times AST [\text{IU/L}] / (\text{platelet count } [\times 10^9/L] \times ALT [\text{IU/L}]^{0.5})$.¹³ (d) FibroTest score regression coefficient was: $Z = 4.467 \times \log^{10} (\alpha 2\text{-macroglobulin } [\text{g/L}]) - 1.357 \times \log^{10} (\text{haptoglobin } [\text{g/L}]) + 1.017 \times \log^{10} [\gamma\text{-glutamyltransferase } [\text{GGT}] [\text{IU/L}]] + 0.0281 \times (\text{age } [\text{years}]) + 1.737 \times \log^{10} (\text{bilirubin } [\mu\text{m/L}]) - 1.184 \times \log^{10} (\text{apolipoprotein A1 } [\text{g/L}]) + 0.301 \times (\text{sex } [\text{female} = 0, \text{male} = 1]) - 5.54$.⁹

protein A1, hyaluronic acid, TIMP-1, TIMP-2, procollagen III peptide and type IV collagen 7S. Multiple regression analysis finally generated a first-degree polynomial function consisting of four variables: type IV collagen 7S, platelet count, bilirubin and hyaluronic acid. A constant numeral (-1.87) was finally adjusted in the regression equation in order to obtain fitted figures for fibrotic stages of F1, F2, F3 and F4. From the magnitude of the standardized partial regression coefficient of individual variable in the function, *ln* (type IV collagen 7S) demonstrated the most potent contribution toward the prediction of liver fibrosis. Platelet count and *ln* (bilirubin) proved to be the second and third distinctive power in the model, respectively.

The obtained figure of FSC was generated to imitate actual "F factor" of histological staging. FSC was sufficiently fitted to actual fibrotic stages with certain overlapping as was usually found in histological ambiguity judged by pathologists. Because judgment of fibrosis in chronic hepatitis often shows a transitional histological staging, pathological examination could not always achieve a clear-cut diagnosis discriminating F1, F2, F3 or F4. Considering the limitation of pathological difficulty in differentiation of the four continuous disease entities, the obtained regression function showed satisfactory high accuracy rates in the prediction of liver disease severity. FSC can provide one or two decimal places (e.g. 2.4 or 2.46) and the utility of the score is possibly higher

than mere histological staging of F1, F2, F3 or F4. The reproducibility was confirmed by the remaining 276 patients' data obtained from the other seven hospitals. Although the validation data were collected from different geographic area and different chronologic situation, FSC showed similar results in prediction of histological staging.

Fibrotic score for hepatitis C seemed a very useful quantitative marker in evaluating severity of fibrotic severity of hepatitis C patients without invasive procedures and without any specialized ultrasonography or magnetic resonance imaging. FSC also has an advantage of measurement, in which old blood samples are available for retrospective assessment of varied clinical settings: old sera from 20 years ago at the time of initial liver biopsy, or paired sera before and after a long-term anti-inflammatory therapy, for example. These kinds of retrospective assessments of fibrotic staging will be valuable in estimating a long-term progression of liver disease, in evaluating efficacy of a long-term medication or other medical intervention, or in making a political judgment from the viewpoint of socioeconomic efficacy.

The score can be calculated for any patients with chronic HCV infection. Although this multiple regression model dealt with appropriate logarithmic transformation for non-normal distribution parameters, the regression analysis was based on a linear regression model. Very slight fibrosis can be calculated as less than 1.00, which is commonly found with a slight degree of chronic hepatitis with a tiny fibrotic change as F0. Very severe fibrosis may be calculated as more than 4.00, which is an imaginable and nonsense number in the scoring system of fibrosis. FSC is, however, very useful and valuable in real clinical setting. Estimation of severity of liver fibrosis in outpatient clinics, evaluation of natural progression of patients' fibrosis over 10 years, and assessment of a long-term administration of interferon in patients with chronic hepatitis C from the viewpoint of fibrotic change. In this study, because certain patients actually had a history of interferon administration, regression of liver fibrosis during and after the treatment could be assessed when prior sera were available for serial evaluation of FSC. We can also expect the usefulness of evaluation of carcinogenic risk after sustained virological response, and stage progression with alcohol intake or obesity-induced steatosis. Recent development of new directly acting antiviral agents require evaluation for long-term histological advantage, for aggravation of hepatitis stage during viral and biochemical breakthrough caused by HCV mutation, estimation of future carcinogenic risk, and even for the best

way of management of patients with chronic hepatitis C. FSC seems one of the ideal methods of approximation for fibrotic stage of chronic hepatitis C. Repeated measurement is quite suitable for patients with an unestablished treatment or trial, every 1 or 2 years, for example. Because the current regression function was generated from the data of HCV-related chronic liver disease, this equation would not be suitable for the recognition of HBV-related chronic liver disease,²² alcoholic liver disease and other congenital or autoimmune liver diseases. To recognize the latter diseases, other studies about individual diseases must be performed.

We compared the usefulness of the FSC with that of other fibrotic scores.^{8,9,12,13} More simple and inexpensive AAR or APRI could not well estimate fibrotic stages with poor correlation coefficients of 0.021 and 0.462, which were much lower than the coefficient of FSC of 0.572. FibroTest, which contained three costly fibrotic markers (α 2-macroglobulin, haptoglobin and apolipoprotein A1), also showed a low correlation coefficient of 0.415, suggesting that the usefulness was limited in HCV positive Asian patients. Although FIB-4 demonstrated the best coefficient of 0.440 among the fibrotic scores, significant overlaps were found between neighboring stages and obtained scores were not coordinated for real histological classification. Because this study also measured those special markers included in FibroTest, the ability of discrimination of fibrotic stages could be compared among the five fibrotic scoring systems.

In conclusion, FSC was a useful and reliable biomarker for prediction of liver fibrosis in patients with chronic HCV infection. FSC is expected to be introduced and utilized in varied kinds of studies and trials. Its accuracy and reproducibility require further validation using more numbers of patients in several countries other than Japan.

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Recent Progress in Radiofrequency Ablation Therapy for Hepatocellular Carcinoma

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Key Words

Hepatocellular carcinoma · Radiofrequency ablation · Bipolar radiofrequency ablation · Dexmedetomidine · Sonazoid · Fusion imaging · Microwave · Surgery

Abstract

In order to attain better ablation and more effective management of hepatocellular carcinoma (HCC), new approaches and devices in radiofrequency ablation (RFA) therapy were presented and discussed in a workshop at the 50th Annual Meeting of the Liver Cancer Study Group of Japan. A novel bipolar RFA apparatus was introduced in Japan in January 2013. Hundreds of subjects with HCC were treated with multipolar RFA with varied devices and

plans. Among these, no-touch ablation was one of the most useful procedures in the treatment of HCC with the apparatus. In RFA therapy, a few assisting devices and techniques were applied for convenience and improvement of the thermal ablation procedure. Contrast-enhanced ultrasonography and three-dimensional fusion imaging technique using volume data of CT or MRI could improve exact targeting and shorten the treatment time for RFA procedures under ultrasonographic guidance. A more complicated method using a workstation was also reported as being helpful in planning the ablated shape and volume in multineedle RFA. The effective use of sedatives and antianalgesics as well as a novel microwave apparatus with a cooled-tip electrode was also discussed.

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