from that of the USA and EU; thus, the age of the majority of Japanese patients is high and their bodyweights are low in comparison with those in Caucasians.[4–8] As a result, the RBV dose-reduction rates and the discontinuation rates of TVR treatment due to adverse events are higher in Japan than those in the USA and EU,[4–8] though the addition of RBV increased the SVR rates in patients receiving TVR-based regimens.[9] These backgrounds call for more efficient treatment of the aged and/or lower bodyweight in patients with CHC in Japan.

The antiviral activity at different doses of TVR was examined after administration of TVR alone for 14 days at 450 mg every 8 h (q8h), 750 mg q8h or 1250 mg q12h,[10] and the greatest HCV RNA reduction and the highest plasma trough concentrations (C_{trough}) were achieved in the 750 mg q8h cohort. On the basis of this result, the TVR 750 mg q8h regimen was selected in the TVR-based triple therapy thereafter. Indeed, TVR 750 mg q8h co-administrated with PEG IFN or PEG IFN/RBV resulted in greater HCV RNA reduction than that after the administration of TVR alone. The Advisory Committee Briefing Document for NDA prepared by the TVR Review Team reports that the higher exposure to TVR was significantly associated with the increased risk of anemia and grade 2 or higher hemoglobin toxicity, defined as hemoglobin of less than 10 g/dL or any decrease from baseline of more than 3.5 g/dL.[11] In addition, the comparison of individual exposure estimated from population pharmacokinetic analysis demonstrated that age, race, sex or weight/body mass index (BMI) of subjects had no clinically relevant effects on TVR exposure.[12]

We previously reported the dynamics of HCV RNA during 12 weeks of triple therapy of TVR (q8h at two doses of 500 mg and 750 mg) with PEG IFN and RBV in Japanese CHC patients.[13] From this perspective, in this study, we explored the antiviral effects, safety and TVR pharmacokinetics in the above Japanese CHC patients.

METHODS

Study design and organization

THIS DOUBLE-ARM, RANDOMIZED, open-label study was conducted between April 2008 and March 2009 at the Department of Hepatology in the Toranomon Hospital in compliance with Good Clinical Practice Guidelines and the Declaration of Helsinki. Before the study, the protocol and informed consent forms were approved by the Institutional Review Board. All patients had given informed consent in writing after sufficient explanation before they participated in this trial.

Patients

This study was conducted using 20 CHC patients who were selected according to the following inclusion and exclusion criteria.[13] Inclusion criteria: (i) diagnosed with CHC; (ii) infected with HCV-1b confirmed by the sequence analysis in the NS5B region; (iii) HCV RNA levels of 5.0 $\log_{10}$ IU/mL or higher determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (iv) Japanese race (Mongoloid), aged 20–65 years at the entry; and (v) bodyweight of 35 kg or more but 120 kg or less at the time of registration. Exclusion criteria were the same as previously described.[13]

Study design

The 20 patients were randomly allocated to two groups with different doses of TVR by a third party institute, Bellsystem24 (Tokyo, Japan). TVR was administrated at a dose of 750 mg (group A) or 500 mg (group B) q8h intervals after meal. PEG IFN-α-2b (PegIntron; MSD, Tokyo, Japan) was injected s.c. to them at a median dose of 1.50 μg/kg (range, 1.250–1.739 μg/kg) once a week. RBV (Rebetol; MSD) was administrated at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose, 600–1000 mg). These three drugs were administrated for 12 weeks. After completion or discontinuation of the triple therapy, a follow-up observation was performed for 24 weeks. Doses of PEG IFN and RBV were reduced or their administration was discontinued, as required, based on the reduction of hemoglobin levels, white blood cell count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG IFN was reduced to half, when either leukocyte count decreased below 1500/mm^3, neutrophil count below 750/mm^3 or platelet count below 80×10^3/mm^3. PEG IFN was withdrawn when they decreased below 1000/mm^3, 500/mm^3 or 50×10^3/mm^3, respectively. When hemoglobin decreased below 10 g/dL, the daily dose of RBV was reduced from 600 to 400 mg, from 800 to 600 mg and from 1000 to 600 mg, depending on the initial dose of each patient. RBV was withdrawn when hemoglobin decreased below 8.5 g/dL. The decrease of TVR dose was not permitted, and its administration was stopped when the discontinuation was appropriate due to the development of adverse events.

In cases where the administration of TVR stopped, the administration of PEG IFN-α-2b and RBV was terminated also.

This study was registered at Clinical Trials (no. NCT00630058).

NS5A interferon-sensitivity determining region (ISDR) and core amino acid (a.a.) substitutions

Amino acid substitutions in the HCV core and NS5A ISDR regions were determined using direct sequencing of polymerase chain reaction products after extraction and reverse transcription of HCV RNA. A core a.a. substitution at positions 70 and 91 (core 70 and core 91, respectively) was determined according to the procedure of Akuta *et al.*,[14,15] and the number of ISDR substitutions was determined using the methods of Enomoto *et al.*[16,17]

Single-nucleotide polymorphism (SNP) genotyping

Interleukin (IL)-28B (rs8099917 and rs12979860) and inosine triphosphate pyrophosphatase (rs1127354) were genotyped by the Invader assay, TaqMan assay or direct sequencing, as described elsewhere.[18–20]

HCV RNA measurements

Antiviral effects of TVR on HCV were assessed by measuring plasma HCV RNA levels. Blood samples were obtained on day 1 before dosing and at 2.5, 4, 8, 16 and 24 h after the first dose (the 8- and 16-h samples were collected before administration of the second and third administration, respectively). Pre-dose samples were obtained on days 2, 3, 8, 14, 29, 43, 57, 86, 92, 99, 113, 141, 169, 197, 225 and 253. 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 $\log_{10}$ IU/mL.

Pharmacokinetic assessments

Blood samples were collected immediately before the first dose in the morning, and at 1, 2.5, 4, 6, 8, 12, 16 and 24 h after the first dose on days 1, 14 and 85 to determine the concentrations of TVR in the plasma. Samples were also taken before the first dose in the morning on days 3, 8, 29, 43, 57 and 99 for evaluation of trough concentrations of TVR.

Plasma concentrations of TVR were determined using a high-performance liquid chromatographic apparatus fitted with a mass spectrometer. Plasma concentrations and actual plasma-sampling times were used to calculate the area under the plasma concentration time curve from 0–8 h (AUC_{0-8h}) and terminal half-life ($t_{1/2}$) by the non-compartmental method using WinNonlin software Version 5.2.1. The maximum plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were directly determined from the observed values on days 1, 14 and 85.

Safety assessments

During the on-study period, patients were monitored for safety at regular intervals from the start of dosing through every hospital visit. Safety assessments included physical examinations, clinical laboratory tests and check of adverse events. After the treatment was completed or aborted, patients were monitored for safety by the standard practice of investigators.

Statistical analysis

Hepatitis C virus RNA values in $\log_{10}$ IU/mL were summarized using descriptive statistics for each treatment group and at scheduled time points. From the plasma concentrations of TVR and clinical laboratory data, the descriptive statistics were calculated. Continuous variables between groups were compared by Student's *t*-test or Mann–Whitney *U*-test. The number of patients with adverse events was summarized by MedDRA (ver. 12.0) system organ class, preferred term and relationship to study drug. All statistical analyses were performed using the validated ver. 9.1.3 of the SAS System (SAS Institute, Cary, NC, USA) or SPSS software (ver. 19.0.0; IBM, Armonk, NY, USA).

RESULTS

Baseline demographic and virological characteristics of the 20 patients with CHC who received the triple treatment

TABLE 1 LISTS the baseline demographic and virological characteristics of the 20 patients who received the triple therapy with TVR, PEG IFN and RBV for 12 weeks. All of them were infected with HCV-1b in high viral loads with a median of 6.48 $\log_{10}$ IU/mL in group A and 6.80 $\log_{10}$ IU/mL in group B. Of the 20 patients in the study, 12 (60%) were older than 50 years. The bodyweights of 10 (50%) patients were lower than 60 kg. Of the 20 patients, 10 (50%) did not receive antiviral treatments previously, six (30%) did not respond to previous monotherapy with the standard IFN and four (20%) failed to respond to PEG IFN and RBV (non-responder) previously.

Table 1 Baseline characteristics of patients with chronic hepatitis C who received a telaprevir-based triple therapy

No. of patients	Group A (750 mg q8h) $n = 10$	Group B (500 mg q8h) $n = 10$	Total $n = 20$
Sex (male/female)	6/4	4/6	10/10
Age (years) (median [range])	47.0 (42–62)	55.0 (36–65)	53.5 (36–65)
Height (cm) (median [range])	163.00 (147.3–178.5)	160.25 (148.7–175.8)	160.75 (147.3–178.5)
Weight (kg) (median [range])	61.95 (38.0–72.6)	61.00 (44.3–79.0)	61.95 (38.0–79.0)
HCV RNA (log_{10} IU/mL) (median [range])	6.48 (5.6–7.2)	6.80 (5.5–7.2)	6.78 (5.5–7.2)
rs8099917 (TT/TG/GG)	8/2/0	5/5/0	13/7/0
rs12979860 (CC/CT/TT)	8/2/0	5/5/0	13/7/0
rs1127354 (CC/CA/AA)	8/2/0	9/1/0	17/3/0
Core a.a. 70 (W/M)	6/4	6/4	12/8
Core a.a. 91 (W/M)	9/1	6/4	15/5
ISDR (0–1/≥2)	10/0	9/1	19/1
WBC (/mm^3) (median [range])	4900 (3600–6300)	5200 (4100–7800)	4900 (3600–7800)
Plt ($\times 10^4$/mm^3) (median [range])	164 (95–248)	160 (129–243)	163 (95–248)
Hb (g/dL) (median [range])	14.20 (12.8–16.0)	14.00 (11.7–16.8)	14.20 (11.7–16.8)
ALT (IU/L) (median [range])	57.0 (36–94)	43.0 (26–167)	49.5 (26–167)
GGT (IU/L) (median [range])	45.0 (15–85)	35.0 (7–142)	38.5 (7–142)
Creatinine (g/dL) (median [range])	0.765 (0.49-0.93)	0.725 (0.45-0.89)	0.755 (0.45-0.93)
History of IFN-based therapy			
Treatment naïve	6 (60.0)	4 (40.0)	10 (50.0)
IFN monotherapy	3 (30.0)	3 (30.0)	6 (60.0)
PEG IFN/RBV	1 (10.0)	3 (30.0)	4 (40.0)

ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; Hb, hemoglobin; IFN, interferon; ISDR, interferon sensitivity-determining region; M, mutant; PEG, pegylated; Plt, platelets; RBV, ribavirin; W, wild type; WBC, white blood cell.

Pharmacokinetics

The pharmacokinetic parameters of TVR in group A (750 mg q8h) and group B (500 mg q8h) on days 1, 14 and 85 are given in Table 2. The TVR C_{trough} on days 1 and 3, and weeks 1, 2, 4, 6, 8 and 12 in both groups are shown in Figure 1(a). Because the C_{trough} did not reach the steady state until day 2 in group A and group B as shown in Figure 1(a), the parameters relating to exposure (C_{max}, AUC_{0-8h} and C_{trough}) on day 1 were lower than those on days 14 and 85 in both groups (Table 2). The mean value of $t_{1/2}$ on day 1 (4.87 and 4.03 h in groups A and B, respectively) was shorter than those on the other days (6.22 to 10.00 h), while mean t_{max} were approximately the same on these 3 days. The values of $t_{1/2}$ and t_{max} were not different between the two groups. Although the difference was not statistically significant other than the C_{trough} at week 4, the parameters of C_{max}, $AUC_{0-\infty}$ and C_{trough} tended to be higher in group A than those in group B.

Virological response and SVR

Figure 1(b) illustrates a comparison of the serum HCV RNA levels (mean ± standard deviation [SD]) in patients between group A and group B during the TVR triple therapy. Similar decreases were observed in both groups. Characteristics and clinical outcomes of the individual patients are shown in Table 3. The SVR rates were 40% (4/10 patients) in group A and 50% (5/10) in group B. The SVR rates in the naïve patients were 67% (4/6) in group A and 75% (3/4) in group B, while the SVR rates in non-responders to the IFN monotherapy were 0% (0/3) in group A and 67% (2/3) in group B, and those in non-responders to the PEG IFN and RBV therapy were 0% in both groups (0/1 vs 0/3). At week 2, the percentage of subjects with undetectable HCV RNA was 40% in group A and 60% in group B. The percentage of subjects with undetectable HCV RNA at week 4 (rapid viral response: RVR) in group A was similar to that in group B (80% vs 70%). Eight (80%) of the 10 patients with undetectable HCV RNA at week 2 achieved SVR. One patient (undetectable HCV RNA at week 2) who stopped the treatment at week 4 achieved transient response (TR).

Four of five naïve patients with IL-28B rs8099917 TT and wild-type core a.a. 70 achieved SVR. Two of four naïve patients with rs8099917 TT and mutant-type core a.a. 70 achieved SVR, and the other naïve patient with

Table 2 Pharmacokinetic parameters of plasma telaprevir

	n	C_{max} (μg/mL)	t_{max}† (h)	AUC_{0-8h} (μg·h/mL)	C_{trough}‡ (μg/mL)	$t_{1/2}$ (h)
(a) Group A (750 mg q8h)						
Day 1	10	1.62 ± 0.43	2.51 (2.25–6.00)	7.53 ± 1.93	0.846 ± 0.500	4.87 ± 2.12§,¶
Day 14	10	3.96 ± 1.10	2.50 (2.42–5.75)	26.00 ± 6.77††	2.639 ± 0.556††	9.99 ± 4.37§,‡‡
Day 85	6	3.67 ± 0.87	3.24 (2.35–7.75)	25.00 ± 5.23	2.679 ± 0.355	9.06 ± 3.98§§
(b) Group B (500 mg q8h)						
Day 1	10	1.45 ± 0.83	2.54 (2.33–8.02)	6.55 ± 3.73	0.681 ± 0.412	4.03 ± 1.63§,‡‡
Day 14	10	3.06 ± 0.90	2.45 (2.33–6.00)	19.94 ± 5.97	1.914 ± 0.717	10.00 ± 6.97§,††
Day 85	7	3.16 ± 1.10	2.43 (2.33–4.00)	21.35 ± 6.88	2.105 ± 0.819	6.22 ± 3.64¶¶

Mean values ± standard deviations.
†Medians (minimum value to maximum value).
‡C_{trough} at 8 h after the first administration.
§Calculated from measured values at 8 h after the first administration.
¶$n = 7$.
††$n = 9$.
‡‡$n = 8$.
§§Calculated from measured values at 24 h after the first administration.
¶¶Calculated from measured values at 24 h after the first administration.
AUC_{0-8h}, area under the plasma concentration time curve from 0–8 h; C_{max}, maximum plasma concentration; C_{trough}, plasma trough concentrations; $t_{1/2}$, terminal half-life; t_{max}, time to reach C_{max}.

rs8099917 TG and wild-type core a.a. 70 achieved SVR. Two of four non-responders receiving the IFN monotherapy with rs8099917 TT and wild-type core a.a. 70 achieved SVR. The other two non-responders receiving the IFN monotherapy with rs8099917 TG and wild-type core a.a. 70 achieved TR. All four non-responders receiving the PEG IFN and RBV therapy with rs8099917 TG achieved TR. However, none of the pharmacokinetic parameters (C_{trough}, C_{max}, t_{max}, $AUC_{0-\infty}$ and $t_{1/2}$) of TVR were different between patients with and without SVR. Moreover, the adherence of PEG IFN and RBV did not affect SVR (Table 3).

Safety

Adverse events were observed in all patients in groups A and B. Adverse events with a frequency of more than 20% in total patients are listed in Table 4. The overall safety profile was similar in both groups. The ratios of discontinuation of all the study drugs because of adverse events were 40% (three cases of anemia, one case of malaise and vertigo) in group A and 30% (two cases of anemia, one case of severe skin disorder) in group B. Despite the modification of RBV dose, five patients (one man and four women) developed low hemoglobinemia (<8.5 g/dL) on days 22, 31, 39, 78 and 85 after the start of triple therapy. One patient (female, aged 53 years) developed IFN-related symptoms including general malaise and vertigo, and another (female, aged 56 years) developed severe skin disorder that was unable to be treated with topical steroid ointments. There was no dose-dependent trend for adverse events. During the triple therapy for 12 weeks, the amounts of hemoglobin tended to be the same or low in group A in comparison with those in group B (Fig. 2a), while serum creatinine increased more eminently in group A than in group B, with the statistical significance at weeks 4 and 8 ($P < 0.01$ and $P < 0.05$, respectively) as shown in Figure 2(b). The serum creatinine recovered to the baseline level at the end of the follow-up period.

We analyzed the relationship between the above adverse events and the pharmacokinetic parameters of TVR. The AUC_{0-8h} on day 1 of patients developing low hemoglobinemia (<8.5 g/dL) was significantly higher than that of the other patients ($P = 0.040$; 9.70 ± 3.29 vs 6.15 ± 2.28). There was no correlation of creatinine elevation (>0.3 or 0.5 mg/dL from baseline) or rush with the pharmacokinetic parameters of TVR. Moreover, there was no correlation between creatinine elevation and clinical factors (age, sex, bodyweight and BMI).

DISCUSSION

THE DOSE OF TVR in the triple therapy was determined based on the TVR monotherapy study[10] as described above, in which the highest TVR C_{trough} (1054 ng/mL) and the greatest reduction of HCV RNA

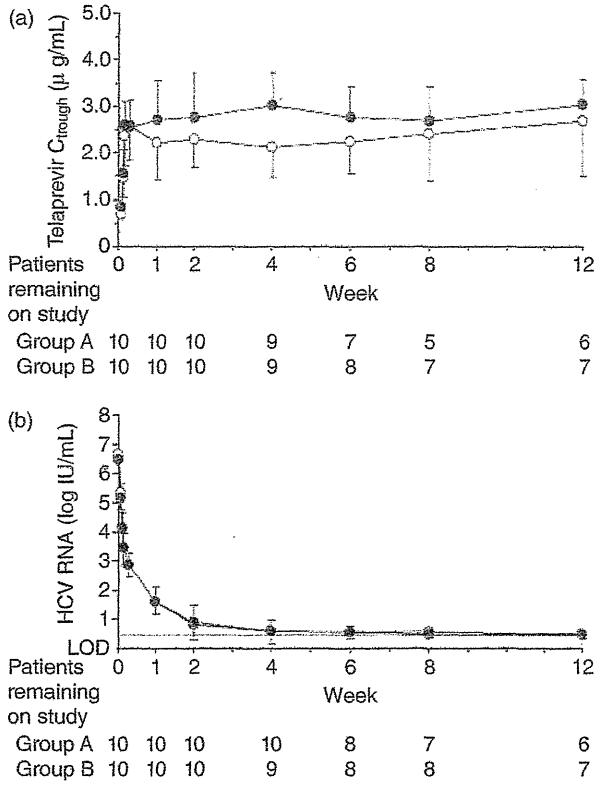

Figure 1 (a) Telaprevir C_{trough} levels and (b) change from baseline of hepatitis C virus (HCV) RNA in Japanese patients with chronic hepatitis C during the telaprevir-based triple therapy. Each circle and bar represent mean values ± standard deviations, respectively. Number of patients at each time point is indicated below. Statistical tests were performed at each point. $^*P < 0.05$ difference. 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 less than 1.2 $\log_{10}$ IU/mL (no HCV RNA detectable.). The areas below the sensitivity of detection are indicated by a shaded bar (<1.2 $\log_{10}$ IU/mL, LOD: limit of detection). –●–, Group (telaprevir 750 mg q8h); –○–, group B (telaprevir 500 mg q8h).

were achieved by a 750 mg q8h regimen. Thus, no dose-finding study of TVR was conducted based on the TVR-based triple regimen. This was the first exploratory study to evaluate the antiviral response, safety and pharmacokinetics of TVR after administration at doses of 750 mg q8h and 500 mg q8h with PEG IFN and RBV. The $t_{1/2}$ of TVR on days 14 and 85 were longer than those on day 1 in both groups, probably due to the saturation of CYP3A4 activity by the repeated administration, because CYP3A4 is the major isozyme involved in the metabolism of TVR and, in addition, TVR acts as the inhibitor of this isozyme. The mean C_{max}, $AUC_{0\text{-}8h}$ and C_{trough} of TVR at steady state increased in an approximately dose-dependent manner, and those at week 2 were 3.96 μg/mL, 26.00 μg·h/mL and 2.639 μg/mL in group A, and 3.06 μg/mL, 19.94 μg·h/mL and 1.914 μg/mL in group B, respectively. The steady state pharmacokinetic parameters of TVR were similar to those obtained in the C208 study.[21] The optimum TVR dose regimen, 750 mg q8h, in Japanese CHC patients was justified based on the overseas dose-finding study and the studies on TVR-based triple therapy, because: (i) no race-related pharmacokinetic difference has been noticed in TVR between Japanese and European patients; and (ii) co-administration with PEG IFN and RBV did not notably change the exposure to TVR.

The change of mean (±SD) $\log_{10}$ HCV RNA and viral response (HCV RNA undetectable) in group A were similar to those in group B (Fig. 1b). The SVR and TR rates were 40% and 60% in group A, and 50% and 40% in group B, respectively. Although the SVR rates of all patients in this study were lower than those in the previous reports,[7,8] the rates of naïve patients (67% in group A and 75% in group B) were similar. The SVR rate of difficult to treat patients, who had not achieved SVR in the prior IFN-based therapy, was lower (20%, 2/10) in this study; the result indicating that these patients will require the TVR-based triple therapy for 24 weeks (PEG IFN, RBV and TVR were administrated for 12 weeks followed by switching to PEG IFN and RBV therapy for an additional 12 weeks).[8] Moreover, the patients possessing the IL-28B SNP rs8099917 TT and wild-type core a.a. 70 were likely to achieve higher SVR than the patients with other genotypes, regardless of TVR dose (Table 3). Recent reports identify IL-28B genotype and a.a. substitution of the core region as predictors of SVR to TVR-based triple therapy.[22,23] Although these results indicate that the optimum regimen for the patients possessing the IL-28B SNP rs8099917 TT and wild-type core a.a. 70 may be 500 mg q8h, the number of patients in this study was too small to reach a definitive conclusion on this point and a large-scale clinical study will be required.

The overall safety profiles of the triple regimen were similar in the two groups, and the ratios of TVR discontinuation due to anemia were 30% in group A and 20% in group B. We examined concentrations of hemoglobin and serum creatinine as the indicator of anemia and renal function, respectively (Fig. 2). The concentrations of hemoglobin were the same or higher in group B than those in group A during the dosing period, but there was no significant difference in this indicator. On the

Table 3 Individual characteristics and outcomes

	Patient									
	1	2	3	4	5	6	7	8	9	10
Group A (750 mg q8h)										
Baseline characteristics										
Age/sex	60/F	42/M	53/F	47/M	46/M	47/M	54/M	46/F	62/F	44/M
Height (cm)	154.6	171.6	147.3	168.0	178.5	165.0	169.0	154.0	159.0	161.0
Weight (kg)	54.0	58.3	65.1	64.9	72.6	72.0	65.0	38.0	54.0	59.0
IL-28B SNP (rs8099917)	TT	TG	TT	TG	TT	TT	TT	TT	TT	TT
IL-28B SNP (rs12979860)	CC	CT	CC	CT	CC	CC	CC	CC	CC	CC
ITPA SNP (rs1127354)	CC	CC	CA	CC	CC	CC	CA	CC	CC	CC
Core a.a. 70 (W/M)	W	M	W	W	M	W	M	M	W	W
Core a.a. 91 (W/M)	W	W	W	W	W	W	W	W	W	M
ISDR substituted a.a. sites	0	0	1	0	0	0	0	1	1	1
History of IFN-based therapy†	IFN	IFN	Naïve	PR	Naïve	IFN	Naïve	Naïve	Naïve	Naïve
Baseline laboratory data										
HCV RNA (log_{10} IU/mL)	6.10	6.85	7.10	7.15	6.85	6.55	6.40	5.60	6.00	6.00
Hb (g/dL)	13.1	14.3	16.0	14.2	14.9	13.8	14.2	13.9	12.8	15.8
Creatinine (g/dL)	0.93	0.77	0.66	0.77	0.76	0.85	0.73	0.49	0.51	**0.83**
Dose										
RBV, max/min (mg)	600/400	600/200	800/200	800/200	800/400	800/200	800/200	600/200	600/200	600/200
Duration of treatment (weeks)	4	12	7	12	12	12	12	12	6	12
Telaprevir, adherence (%)	36.1	99.2	44.7	99.2	98.0	99.2	97.6	98.8	45.1	**101.6**
PEG IFN, adherence (%)	41.7	100	41.7	100	100	75.0	66.7	100	41.7	**100**
RBV, Adherence (%)	32.2	59.6	28.2	51.2	67.4	64.7	51.5	42.7	21.6	**45.1**
Pharmacokinetic parameter‡										
C_{trough} (μg/mL)	3.102	2.485	3.408	2.662	3.807	2.947	1.294	3.396	3.164	1.932
Outcome										
HCV RNA negativity (weeks)	2	6	2	4	4	4	4	6	2	2
Effect of therapy (SVR/BT/TR/NR)§	TR	TR	SVR	TR	SVR	TR	TR	TR	SVR	SVR

Table 3 *Continued*

	Patient									
	1	2	3	4.	5	6	7	8	9	10
Group B (500 mg q8h)										
Baseline characteristics										
Age/sex	64/M	54/F	36/F	60/F	52/M	46/F	56/F	65/M	56/F	54/M
Height (cm)	173.2	151.0	148.7	160.5	175.8	160.0	160.0	167.0	158.0	170.0
Weight (kg)	75.0	47.6	44.3	67.9	71.8	52.0	57.0	79.0	65.0	55.0
IL-28B SNP (rs8099917)	TT	TG	TG	TT	TG	TT	TG	TT	TT	TG
IL-28B SNP (rs12979860)	CC	CT	CT	CC	CT	CC	CT	CC	CC	CT
ITPA SNP (rs1127354)	CC	CC	CC	CC	CC	CC	CC	CC	CC	CA
Core a.a. 70 (W/M)	M	W	W	W	M	W	M	W	W	M
Core a.a. 91 (W/M)	W	W	W	W	M	W	M	W	M	M
ISDR substituted a.a. sites	6	0	1	0	0	0	0	0	0	0
History of IFN-based therapy†	Naïve	IFN	Naïve	Naïve	PR	Naïve	PR	IFN	IFN	PR
Baseline laboratory data										
HCV RNA (log_{10} IU/mL)	5.50	7.15	6.15	6.80	6.80	7.00	6.10	7.20	6.85	6.75
Hb (g/dL)	16.1	11.7	12.1	13.6	14.5	12.3	16.8	14.3	13.7	14.8
Creatinine (g/dL)	0.78	0.50	0.45	0.56	0.87	0.58	0.80	0.89	0.75	0.70
Dose										
RBV, max/min (mg)	800/400	600/200	600/200	800/400	800/200	600/200	600/600	800/200	800/200	600/200
Duration of treatment (weeks)	12	12	11	12	12	3	12	12	5	12
Telaprevir, adherence (%)	98.0	99.2	91.0	99.2	101.6	25.5	98.8	99.2	43.1	98.4
PEG IFN, adherence (%)	98.3	66.7	87.5	100	91.7	25.0	100	100	41.7	100
RBV, adherence (%)	68.5	44.7	39.2	54.4	48.8	24.3	99.2	36.5	28.2	64.3
Pharmacokinetic parameter‡										
C_{trough} (μg/mL)	1.950	2.763	3.276	1.690	1.478	1.939	2.955	4.065	1.962	1.846
Outcome										
HCV RNA negativity (weeks)	2	6	2	2	4	-	2	2	2	8
Effect of therapy (SVR/BT/TR/NR)	SVR	TR	SVR	SVR	TR	NR	TR	SVR	SVR	TR

†Naïve, treatment naïve, IFN, IFN monotherapy, PR, PEG IFN/RBV.

‡Pharmacokinetic parameters of the patients who received triple therapy at weeks 2.

a.a., amino acid; ALT, alanine aminotransferase; C_{trough}, plasma trough concentrations; GGT, γ-glutamyltransferase; Hb, hemoglobin; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, interferon sensitivity-determining region; M, mutant; PEG, pegylated; Plt, platelets; RBV, ribavirin; SNP, single nucleotide polymorphism; SVR, sustained virological response, BT, breakthrough, TR, transient response, NR, non-response; W, wild type; WBC, white blood cell.

Table 4 Adverse events developing in more than 20% of patients in total

MedDRA/J (ver. 12.0) PT	Group A (750 mg q8h) $n = 10$ n (%)	Group B (500 mg q8h) $n = 10$ n (%)	Total $n = 20$ n (%)
Platelet count decreased	10 (100.0)	10 (100.0)	20 (100.0)
Anemia	10 (100.0)	9 (90.0)	19 (95.0)
White blood cell count decreased	9 (90.0)	10 (100.0)	19 (95.0)
Rash	7 (70.0)	7 (70.0)	14 (70.0)
Pyrexia	6 (60.0)	8 (80.0)	14 (70.0)
Malaise	6 (60.0)	5 (50.0)	11 (55.0)
Blood triglycerides increased	6 (60.0)	5 (50.0)	11 (55.0)
Headache	3 (30.0)	7 (70.0)	10 (50.0)
Blood lactate dehydrogenase increased	3 (30.0)	7 (70.0)	10 (50.0)
Anorexia	3 (30.0)	6 (60.0)	9 (45.0)
Blood uric acid increased	4 (40.0)	4 (40.0)	8 (40.0)
Nausea	3 (30.0)	5 (50.0)	8 (40.0)
Pruritus	3 (30.0)	5 (50.0)	8 (40.0)
Protein total decreased	0 (0.0)	8 (80.0)	8 (40.0)
Hyperuricaemia	5 (50.0)	2 (20.0)	7 (35.0)
Blood creatinine increased	5 (50.0)	2 (20.0)	7 (35.0)
Nasopharyngitis	3 (30.0)	4 (40.0)	7 (35.0)
Neutrophil percentage decreased	3 (30.0)	4 (40.0)	7 (35.0)
Influenza-like illness	4 (40.0)	2 (20.0)	6 (30.0)
Abdominal discomfort	2 (20.0)	3 (30.0)	5 (25.0)
Vomiting	2 (20.0)	3 (30.0)	5 (25.0)
Dizziness	0 (0.0)	5 (50.0)	5 (25.0)
Dysgeusia	3 (30.0)	1 (10.0)	4 (20.0)
Stomatitis	3 (30.0)	1 (10.0)	4 (20.0)
Lymphocyte percentage increased	2 (20.0)	2 (20.0)	4 (20.0)
Diarrhea	1 (10.0)	3 (30.0)	4 (20.0)
Alopecia	1 (10.0)	3 (30.0)	4 (20.0)

contrary, there was observed a difference in serum creatinine concentrations between group A and group B; thus, the serum creatinine concentrations in group A were higher than those in group B at all of the time points examined with a statistical significance at weeks 4 and 8 ($P < 0.01$ and $P < 0.05$, respectively) as shown in Figure 2(b). The TVR Review Team confirms that higher exposure of TVR and RBV was significantly associated with increased risk of anemia and grade 2 or higher hemoglobin toxicity.[11] The behaviors of hemoglobin and creatinine in the triple therapy shown in Figure 2 are of interest from the viewpoints of development of anemia with TVR-based regimen and could be explained by the following possibilities: (i) the increase of plasma concentration of TVR may directly affect the renal function to cause the increase of creatinine especially in group A and the decrease of hemoglobin; (ii) TVR first caused the increase of systemic exposure to RBV which in turn additively or synergistically resulted in renal dysfunction. The decrease of renal function reportedly leads to the increase of RBV concentration in plasma, because RBV is mainly excreted via the renal route.[24,25] In this study, the $AUC_{0\text{-}8h}$ on day 1 of patients who developed low hemoglobinemia (<8.5 g/dL) were significantly higher than those of the other patients. The pharmacokinetic parameters of TVR on day 14, at which plasma concentrations of TVR were in the steady state, did not affect low hemoglobinemia. The timing of reducing RBV dose may cause development of low hemoglobinemia, because the RBV dose reduction set in the protocol of this study was less strict than that in the previous reports.[7,8]

Because the present data show that the TVR exposure tended to be increased in a dose-dependent manner, there is a possibility that the triple therapy with TVR 500 mg q8h is advantageous in aged patients whose renal function, body water content or both are lower than those of younger patients. It should be noted,

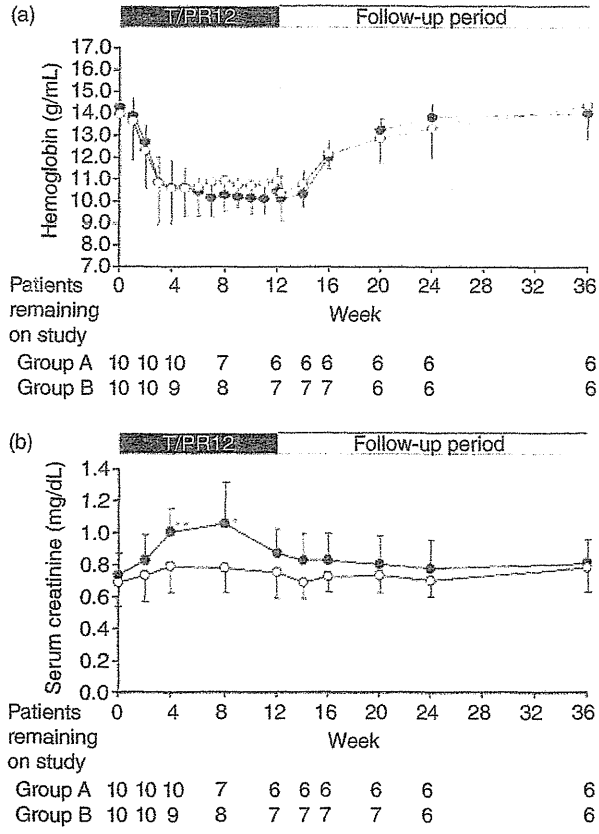

Figure 2 (a) Change from baseline of hemoglobin and (b) serum creatinine levels in Japanese patients with chronic hepatitis C during the telaprevir-based triple therapy. Each circle and bar represent mean values ± standard deviations, respectively. Number of patients at each time point is indicated below. Statistical tests were performed at each point. $*P < 0.05$ and $**P < 0.01$ difference. —●—, Group A (telaprevir 750 mg q8h); —○—, group B (telaprevir 500 mg q8h). T/PR12, triple therapy of telaprevir with peginterferon and ribavirin for 12 weeks.

however, that the small number of patients per arm in this study limits conclusions that can be drawn, and a future larger study is essential.

In conclusion, although the exposure to TVR tended to be lower in 500 mg q8h than that in 750 mg q8h in the TVR-based triple therapy, relatively high exposure of TVR was observed in Japanese CHC patients given TVR at the lower dose. The result suggests that the lower dose regimen may be one of the options for the treatment of Japanese patients. In addition, in the view of antiviral effects, TVR pharmacokinetics and safety profiles, the present findings indicate that development of adverse events, specifically anemia and creatinine increase in the treatment with TVR-based regimen, could be avoided by dose adjustment of TVR as well as RBV.

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Hepatology Research

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Original Article

Fibrosis score consisting of four serum markers successfully predicts pathological fibrotic stages of chronic hepatitis B

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

Method: A total of 227 patients from seven hepatology units and institutes were diagnosed by needle biopsy as having chronic liver disease caused by hepatitis B virus. Twenty-three variables and their natural logarithmic transformation were employed in the multivariate analysis. Multiple regression function was generated from data of 158 patients in one hospital, and validation was performed using the other data of 69 patients from six other hospitals.

Results: After stepwise variable selection, multivariate regression analysis finally obtained the following function: $z = 1.40 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.017 \times$ (platelet count) ($\times 1000^3/mm^3$) $+ 1.24 \times ln$ (tissue inhibitor of matrix metalloproteinase-2) (ng/mL) $+ 1.19 \times ln$ (α-2-macroglobulin) (mg/dL) $- 9.15$. Median values of fibrosis scores of F1 ($n = 73$), F2 ($n = 42$), F3 ($n = 31$) and F4 stages ($n = 12$) were calculated as 0.95, 2.07, 2.98 and 3.63, respectively. Multiple regression coefficient and coefficient of determination were 0.646 and 0.418, respectively. Validation with patient data from other institutions demonstrated good reproducibility of fibrosis score for hepatitis B (FSB), showing 1.33 in F1 ($n = 27$), 2.20 in F2 ($n = 20$), 3.11 in F3 ($n = 20$) and 5.30 in F4 ($n = 2$), respectively.

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

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

INTRODUCTION

WHEN HEPATITIS B virus (HBV)-related chronic liver disease is found by biochemical and virological examination, liver biopsy can establish the definitive diagnosis of chronic hepatitis and its fibrotic staging. 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 procedure and hospital stays of a few days. The pathological examination is, therefore, rarely performed repeatedly in a short period of time, unless disease activity is severe or progression of liver disease is highly suspected. Recently, many authors described the usefulness of ultrasonographic elastography and multiple resonance imaging technology in the estimation of staging of chronic hepatitis and cirrhosis.[1–5] These ways of estimation using the imaging apparatuses seem truly useful for current patients, but they cannot evaluate and compare with past fibrotic states of patients retrospectively. Moreover,

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the same apparatus for elastometry will not be available for repeated measurement for a follow-up examination, for example, several years later.

In spite of the accuracy of biopsy and 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 HBV-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 B were correctly classified as having mild hepatitis and severe hepatitis with advanced fibrosis.[2,6–13] Up to the present time, however, the usefulness of the discriminant functions are less valuable 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–F4) were neglected in almost of the studies. Second, some studies analyzed both hepatitis B and hepatitis C virus 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)[14–16] were not always included in those previous studies.

We tried to generate a function estimating fibrotic stages of HBV-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 fibrosis markers.

METHODS

Patients

A TOTAL OF 273 Japanese patients with chronic hepatitis B were recruited for the study from seven 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, MD), Shishu University Hospital (E. Tanaka, M.D.), Showa University Hospital (M. Imawari, M.D.) and Osaka University Hospital (T. Takehara, M.D.). Inclusion criteria for this study were: (i) positive hepatitis B surface antigen 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–F4). We excluded those patients with overt alcoholic liver disease or fatty liver, association of other types of liver disease (e.g. hepatitis C, primary biliary cirrhosis, autoimmune hepatitis), or those associated with hepatocellular carcinoma or other malignancy. Among the patients, 244 patients 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. Also, we excluded additional 17 patients with eventual histological diagnosis as F0 stage.

Finally, a total of 227 patients who were diagnosed as having chronic hepatitis or cirrhosis (F1–F4) were analyzed for the following hematological, biochemical and histopathological examination. There were 172 males and 55 females aged 16–70 years (median, 39 years).

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

Hematological and biochemical examination

Hematological and standard biochemical evaluation had been performed in each medical institution: white blood cells, red blood cells, hemoglobin, platelets, total bilirubin, AST, ALT, AST/ALT ratio (AAR), γ-glutamyl transpeptidase (γ-GTP), total protein, albumin and γ-globulin.

Special biochemical examinations including "fibrosis 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 the 227 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 227 specimens regarding the stages of fibrosis and activity. Pathological classification of chronic hepatitis staging was based on Desmet *et al.*[17]

Before judgment of histological staging of individual specimens, the pathologists discussed the objective and reproducible judgment of pathological diagnosis of

hepatitis. They made a panel about 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 hepatitis stage among the pathologists, the final judgment accepted 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 a Kolmogorov–Smirnov one-sample test. Because certain variables partly did not conform to a normal distribution, natural logarithmic transformation of bilirubin, AST, ALT, γ-GTP, 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 inflation factors. Of the highly correlated variables, less significant factors were removed from the viewpoint of multicollinearity.

Multivariate regression analysis was performed using 158 patient data from Toranomon Hospital (training dataset) to generate a 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 were also taken into account in the selection of variables. Next, we validated the obtained predictive function using the remaining 69 patient data from the other six 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 ver. 19.[18]

For evaluation of the efficiency and usefulness of obtained function for fibrosis estimation, we compared various fibrosis scores for hepatitis B and C, including AAR,[19] AST-to-platelet ratio index (APRI),[20] FIB-4,[21] FibroTest[22] and discrimination function of cirrhosis from hepatitis in Japanese patients.[23]

RESULTS

Pathological diagnosis

FOUR PATHOLOGISTS INDEPENDENTLY judged the fibrotic stages and inflammatory activity for 227 specimens of chronic hepatitis/cirrhosis caused by HBV. One hundred patients (44.1%) had a fibrosis stage of F1, 62 (27.3%) F2, 51 (22.5%) F3 and 14 (6.2%) F4. In the subgroup of the 158 patients in the training group, judgment as F1 was made in 73 cases, F2 in 42, F3 in 31 and F4 in 12. Of the 69 patients in the validation group, judgment as F1 was made in 27, F2 in 20, F3 in 20 and F4 in two.

According to hepatitis activity classification, A0 was found in five (2.2%), A1 in 100 (44.1%), A2 in 107 (47.1%) and A3 in 15 (6.6%).

Laboratory data of each hepatitis stage in the training group

There were 124 men and 34 women with a median age of 39 years ranged 16–70 years. Laboratory data of 158 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–F4 stages: platelet count, γ-globulin, α-2-macroglobulin, haptoglobin, hyaluronic acid, TIMP-2 and type IV collagen 7S.

Significant variables serving staging of hepatitis

Univariate analyses using trend analysis with the Cochran–Armitage method showed that the fibrotic stage of chronic hepatitis B (FSB) was significantly correlated with platelet count (Spearman: $r = -0.45$, $P < 0.001$), γ-GTP ($r = 0.19$, $P = 0.017$), γ-globulin ($r = 0.29$, $P < 0.001$), α-2-macroglobulin ($r = 0.32$, $P < 0.001$), hyaluronic acid ($r = 0.36$, $P < 0.001$), TIMP-2 ($r = 0.16$, $P = 0.043$), procollagen III peptide ($r = 0.30$, $P < 0.001$) and type IV collagen 7S ($r = 0.55$, $P < 0.001$).

Regression function generated from training patient group

After stepwise variable selection, multivariate regression analysis finally obtained the following function: $z = 1.40 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.017 \times$ (platelet count) ($\times 1000^3/mm^3$) $+ 1.24 \times ln$ (TIMP-2) (ng/mL) $+ 1.19 \times ln$ (α-2-macroglobulin) (mg/dL) $-$ 9.15. Median values of the fibrosis score of F1 ($n = 73$), F2 ($n = 42$), F3 ($n = 31$) and F4 stages ($n = 12$) were calculated as 0.95, 2.07, 2.98 and 3.63, respectively

Table 1 Demography and laboratory data of 158 patients in training group

	F1 ($n = 73$)	F2 ($n = 42$)	F3 ($n = 31$)	F4 ($n = 12$)
Demographics				
Men : women	58:15	33:9	23:8	10:2
Age (median, range)	36 (16–70)	39.5 (18–66)	39 (25–64)	43 (32–59)
Laboratory data (median, range)				
WBC (×1000/mm^3)	5.4 (2.5–10.6)	5.1 (2.4–8.7)	4.9 (3.0–8.7)	4.1 (3.7–6.6)
Hemoglobin (g/dL)	15.3 (10.3–18.8)	15.4 (12.5–17.9)	15.2 (11.5–17.2)	14.45 (12.1–18.2)
Platelet (×1000/mm^3)	204 (124–341)	173 (82–308)	155 (96–220)	130 (86–230)
Albumin (g/dL)	4.1 (3.2–4.9)	4.0 (3.2–5.1)	4.0 (3.3–4.9)	3.95 (3.4–4.6)
Bilirubin (mg/dL)	0.8 (0.2–1.7)	0.8 (0.3–2.3)	0.9 (0.4–5.4)	0.85 (0.6–2.3)
AST (IU/L)	48 (16–450)	55 (17–588)	54 (17–1446)	76.5 (27–396)
ALT (IU/L)	102 (10–839)	90 (12–886)	85 (19–2148)	89 (18–809)
γ-GTP (IU/L)	37 (7–247)	55 (8–687)	44 (14–564)	69 (33–262)
γ-Globulin (g/dL)	1.29 (0.78–2.11)	1.495 (0.62–3.20)	1.43 (0.90–2.30)	1.735 (0.92–2.47)
γ-Globulin (%)	17.3 (10.8–26.1)	19.3 (8.5–35.6)	19.9 (12.9–28.6)	22.55 (13.9–30.2)
α-2-Macroglobulin (mg/dL)	226 (116–446)	276 (148–495)	261 (202–565)	286.5 (166–425)
Haptoglobin (mg/dL)	77 (<5–318)	59 (<5–238)	61 (<5–151)	48.5 (<5–145)
Apolipoprotein A-I (mg/dL)	134 (89–212)	143 (78–250)	133 (87–189)	125 (73–169)
Hyaluronic acid (μg/L)	16 (<5–130)	32.5 (<5–204)	38 (<5–418)	49 (24–335)
TIMP-1 (ng/mL)	168 (93–271)	172 (116–314)	157 (119–365)	192 (145–365)
TIMP-2 (ng/mL)	80 (41–135)	80.5 (35–121)	92 (38–251)	85.5 (70–123)
Procollagen III peptide (U/mL)	0.75 (0.53–1.90)	0.835 (0.45–1.20)	0.89 (0.58–2.50)	1.05 (0.71–2.20)
Type IV collagen 7S (ng/ml)	4.0 (2.7–7.7)	4.6 (2.6–9.6)	5.6 (2.3–15.0)	7.2 (4.2–14.0)

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

(Fig. 1). The multiple regression coefficient and coefficient of determination were 0.646 ($P < 0.001$) and 0.418 ($P < 0.001$), respectively.

Because the generated regression function was obtained by multivariate analysis with stepwise variable selection, several variables were removed from the function due to multicollinearity among them. Mutual correlation among the fibrosis predictors are shown in Table 2.

A 28-year-old man of F1 fibrotic stage (Fig. 2a) had a serum type IV collagen concentration of 4.4 ng/mL, platelet 221 × 10^3 count/mm^3, TIMP-2 75 ng/mL and α-2-macroglobulin 226 mg/dL. The regression function provided a fibrosis score of 0.99. Another man aged 46 years had F3 fibrosis on histological examination (Fig. 2b). His type IV collagen was 5.3 ng/mL, platelet 137 × 10^3 count/mm^3, TIMP-2 92 ng/mL and α-2-macroglobulin 255, and the regression function calculated his fibrosis score as 3.10.

Validation of discriminant function

Validation data of 69 patients (Table 3) were collected from the other six institutions in Japan. When applying

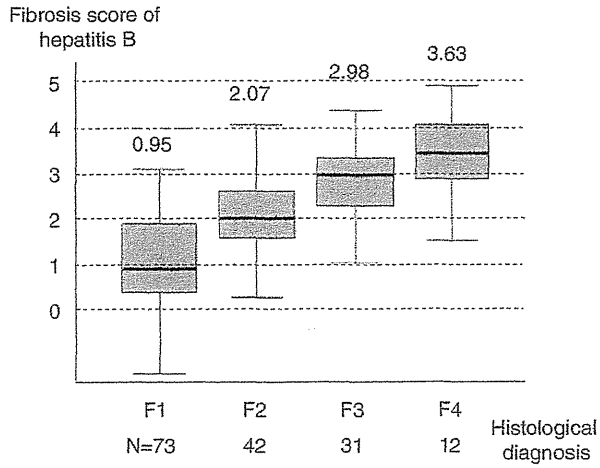

Figure 1 Box and whisker plots of fibrotic score of each histological fibrosis group in the training dataset. The fibrosis score of hepatitis B was generated by the function, z = 1.40 × *ln* (type IV collagen 7S) (ng/mL) − 0.017 × (platelet count) (×1000^3/mm^3) + 1.24 × *ln* (tissue inhibitor of matrix metalloproteinase-2) (ng/mL) + 1.19 × *ln* (α-2-macroglobulin) (mg/dL) − 9.15.

Table 2 Correlation coefficients (Spearman's ρ) among fibrosis predictors used in multivariate analysis

	Platelet	gamma-globulin	*ln* (α-2-macroglobulin)	*ln* (hyaluronate)	*ln* (P-III-P)	*ln* (IV collagen)	*ln* (TIMP-2)
Platelet ($\times 10^3/mm^3$)	1.000	−0.214 ($P = 0.008$)	−0.260 ($P = 0.001$)	−0.384 ($P < 0.001$)	−0.045 ($P = 0.58$)	−0.297 ($P < 0.001$)	0.094 ($P = 0.24$)
γ-Globulin (g/dL)		1.000	0.276 ($P = 0.001$)	0.349 ($P < 0.001$)	0.342 ($P < 0.001$)	0.414 ($P < 0.001$)	0.268 ($P = 0.001$)
ln (α-2-macroglobulin) (mg/dL)			1.000	0.281 ($P < 0.001$)	0.141 ($P = 0.078$)	0.171 ($P = 0.032$)	−0.079 ($P = 0.32$)
ln (hyaluronic acid) (mg/L)				1.000	0.373 ($P < 0.001$)	0.493 ($P < 0.001$)	0.089 ($P = 0.27$)
ln (procollagen III peptide) (U/mL)					1.000	0.600 ($P < 0.001$)	0.145 ($P = 0.071$)
ln (type IV collagen) (mg/L)						1.000	0.358 ($P < 0.001$)
ln (TIMP-2) (mg/L)							1.000

TIMP, tissue inhibitor of matrix metalloproteinase.

the regression function for the validation set, the fibrosis score demonstrated good reproducibility, showing 1.33 in patients with chronic hepatitis of F1 ($n = 27$), 2.20 of F2 ($n = 20$), 3.11 of F3 ($n = 20$) and 5.30 of F4 ($n = 2$), respectively (Fig. 3). Although F4 fibrosis stage consisted of only two patients and the score 5.30 was regarded as of rather higher value, the scores of other stages of fibrosis were concordant with histological fibrosis.

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

In order to evaluate the efficacy and usefulness of the obtained FSB, we compared it with previously reported fibrosis scores using training data. AAR, APRI and FibroTest showed only slight correlation with actual histological stage. FIB-4 demonstrated an increasing trend of the score associated with histological fibrosis, but significant overlapping scores were found in F1–F4. Spearman's correlation coefficients of AAR, APRI, FIB-4 and FibroTest were 0.199 ($P = 0.012$), 0.265 ($P = 0.001$), 0.412 ($P < 0.001$) and 0.330 ($P < 0.001$), respectively. Our FSB showed a Spearman's correlation coefficient of 0.625 ($P < 0.001$), and was a much higher value than the others. The dichotomous discrimination function for cirrhosis and hepatitis C in Japanese patients[23] showed good differentiation also in patients with hepatitis B virus.

DISCUSSION

RECOGNITION OF SEVERITY of chronic hepatitis is essential in managing patients with chronic HBV infection: estimation of length of infection, existence of any previous hepatitis activity, presumption of current fibrotic stage, and prediction of future fibrosis progression and hepatocarcinogenesis. Differential diagnosis of cirrhosis from chronic hepatitis is especially important in the evaluation of chronic HBV infection. Identification of liver cirrhosis often leads to an important change in management of the patient: need for fiberscopic examination for esophageal varices, ultrasonographic exploration for the association of liver cancer, and prediction of hepatic decompensation. Guidelines published by the American Association of Study of Liver Disease[24] recommend liver biopsy for HBV carriers with aminotransferase elevation or for any candidates of antiviral therapy, because hepatic fibrosis sometimes shows unexpectedly far advancement to cirrhosis, and because it is very difficult to evaluate and translate the liver function tests or ultrasonographic findings compared to chronic hepatitis type C.

Table 3 Demography and laboratory data of 69 patients in training group

	F1 ($n = 27$)	F2 ($n = 20$)	F3 ($n = 20$)	F4 ($n = 2$)
Demographics				
Men : women	18:9	15:5	13:7	2:0
Age (median, range)	36 (13–64)	45 (14–64)	36.5 (24–59)	32 (25–39)
Laboratory data (median, range)				
WBC ($\times 1000/mm^3$)	5.0 (2.8–8.7)	5.8 (2.8–11.6)	5.3 (3.2–8.1)	3.85 (2.7–5.0)
Hemoglobin (g/dL)	14.8 (12.4–17.4)	15.0 (12.4–16.9)	14.4 (11.1–16.4)	14.4 (12.5–16.3)
Platelet ($\times 1000/mm^3$)	204 (86–322)	180 (90–275)	147 (90–276)	130 (67–183)
Albumin (g/dL)	4.4 (2.8–5.2)	4.2 (3.5–5.1)	4.3 (3.4–4.9)	4.45 (4.0–4.9)
Bilirubin (mg/dL)	0.9 (0.4–6.4)	0.8 (0.2–1.6)	0.75 (0.4–1.7)	1.15 (1.1–1.2)
AST (IU/L)	52 (17–575)	50.5 (21–272)	65 (22–284)	248.5 (51–446)
ALT (IU/L)	84 (16–1101)	101.5 (19–554)	86.5 (16–1113)	453.5 (74–833)
γ-GTP (IU/L)	42 (14–332)	54 (16–205)	52.5 (13–191)	193 (57–329)
γ-Globulin (g/dL)	1.30 (1.04–1.59)	1.35 (1.18–2.53)	1.62 (1.16–1.97)	1.545 (1.51–1.58)
γ-Globulin (%)	17.9 (14.3–22.1)	19.6 (15.5–30.8)	22.0 (16.5–24.6)	20.15 (19.3–21.0)
α-2-Macroglobulin (mg/dL)	287 (160–687)	270 (89–452)	272.5 (211–463)	389 (313–465)
Haptoglobin (mg/dL)	58 (<5–229)	74 (<5–154)	56.5 (<5–198)	<5 (<5–<5)
Apolipoprotein A-I (mg/dL)	146 (95–216)	137 (87–162)	120 (88–170)	100.5 (74–127)
Hyaluronic acid (μg/L)	27 (<5–113)	36 (10–1050)	59 (14–439)	331 (225–437)
TIMP-1 (ng/mL)	168.5 (83–302)	176 (127–408)	182 (104–303)	390.5 (283–498)
TIMP-2 (ng/mL)	76 (25–143)	86.5 (28–154)	77.5 (32–141)	100.5 (91–110)
Procollagen III peptide (U/mL)	0.71 (0.27–2.20)	0.88 (0.63–2.80)	0.995 (0.60–2.10)	1.75 (1.50–2.00)
Type IV collagen 7S (ng/ml)	3.6 (2.7–17.0)	5.25 (3.3–13.0)	5.7 (3.0–16.0)	15.5 (15.0–16.0)

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

Recently, non-invasive estimation of severity of liver fibrosis has been reported in patients with HBV-related chronic hepatitis.[2,6–13] 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 discrimination functions were insufficient to recognize the stepwise progression of viral hepatitis from F1–F4. This dichotomy (mild or severe) of chronic hepatitis B 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 B.

This study aimed to establish non-invasive evaluation and calculation of liver fibrosis for patients with chronic hepatitis B virus infection. Although it was retrospectively performed as a multicenter study of eight institutions, judgment of histological diagnosis was independently performed by four pathologists in another hospital, who were informed only of the patient's age, sex and positive HBV infection. Objective judgment of the histological staging and grading in sufficient biopsy specimens could be obtained.

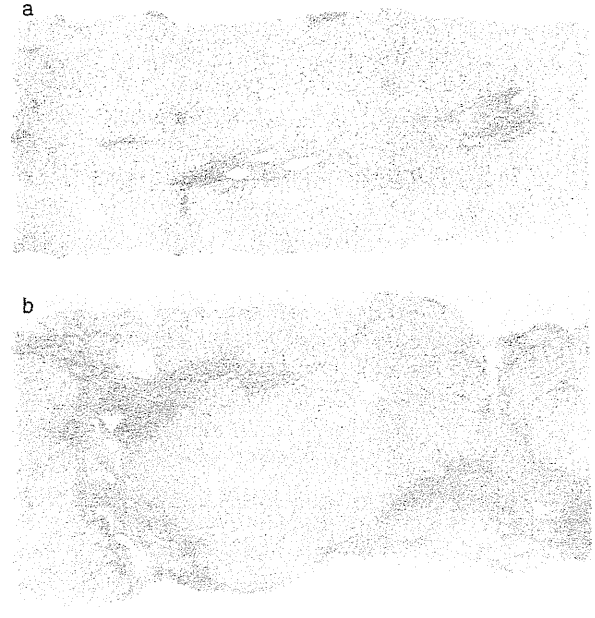

Figure 2 Case presentations of the training set. (a) A 28-year-old man with F1 fibrosis. Final regression function provided his fibrosis score as 0.99. (b) A 45-year-old man with F3 fibrosis. His regression coefficient was calculated as 3.10. Silver stain, ×40.

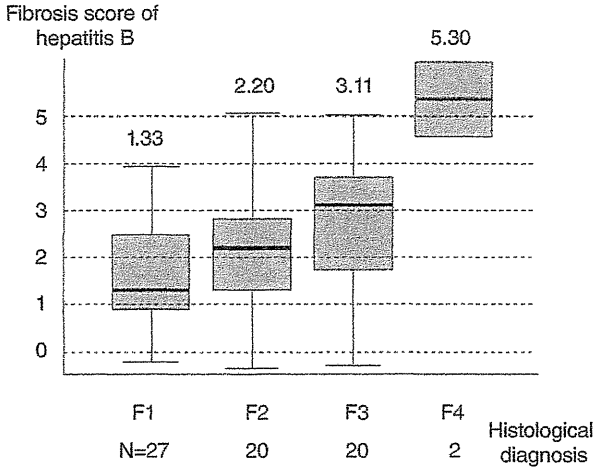

Figure 3 Box and whisker plots of fibrotic score of each group of histological fibrosis in the validation dataset. The fibrosis score of hepatitis B was generated by the function, $z = 1.40 \times ln$ (type IV collagen 7S) (ng/mL) − 0.017 × (platelet count) ($\times 1000^3/mm^3$) + 1.24 × *ln* (tissue inhibitor of matrix metalloproteinase-2) (ng/mL) + 1.19 × *ln* (α-2-macroglobulin) (mg/dL) − 9.15.

As many as 227 patients with chronic hepatitis B 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 fibrosis markers: α-2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipoprotein 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, TIMP-2 and α2-macroglobulin. A constant numeral (−9.15) was finally adjusted in the regression equation in order to obtain fitted figures for a fibrotic stage of F1–F4. From the magnitude of the standardized partial regression coefficient of individual variable in the function, platelet count demonstrated the most potent contribution toward the prediction of liver fibrosis. Type IV collagen 7S and *ln* (TIMP-2) proved to be the second and third distinctive power in the model, respectively.

The FSB was sufficiently fitted to actual fibrotic stages with certain overlapping as is usually found in histological ambiguity judged by pathologists. Because judgment of fibrosis in chronic hepatitis often shows a transitional histological staging, pathological examination cannot always make a clear-cut diagnosis discriminating F1–F4. Considering the limitation of the pathological difficulty in differentiating the four continuous disease entities, the obtained regression function showed satisfactory high accuracy rates in the prediction of liver disease severity. The FSB can provide one or two decimal places (e.g. 3.2 or 3.24) and the utility of the score is possibly higher than the mere histological stage of F1–F4. The reproducibility was confirmed by the remaining 67 patients' data obtained from the other six hospitals. Although the validation data were collected from a different geographic area and different chronological situation, the FSB showed similar results in prediction of histological staging.

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

The score can be calculated for any patients with chronic HBV 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 to a slight degree in chronic hepatitis with tiny fibrotic change as F0. Very severe fibrosis might be calculated as more than 4.00, which is an imaginary and nonsense number in the scoring system of fibrosis. The FSB is, however, very useful and valuable in a real clinical setting: estimation of severity of liver fibrosis in an outpatient clinic, evaluation of the natural progression of a patient's fibrosis over 10 years and assessment of a long-term administration of interferon in patients with chronic hepatitis B from the viewpoint of fibrotic change. Recent development of new nucleoside/nucleotide analogs requires evaluation for long-term histological advantage, for aggravation of hepatitis stage during viral and biochemical breakthrough caused by HBV mutation, and even for

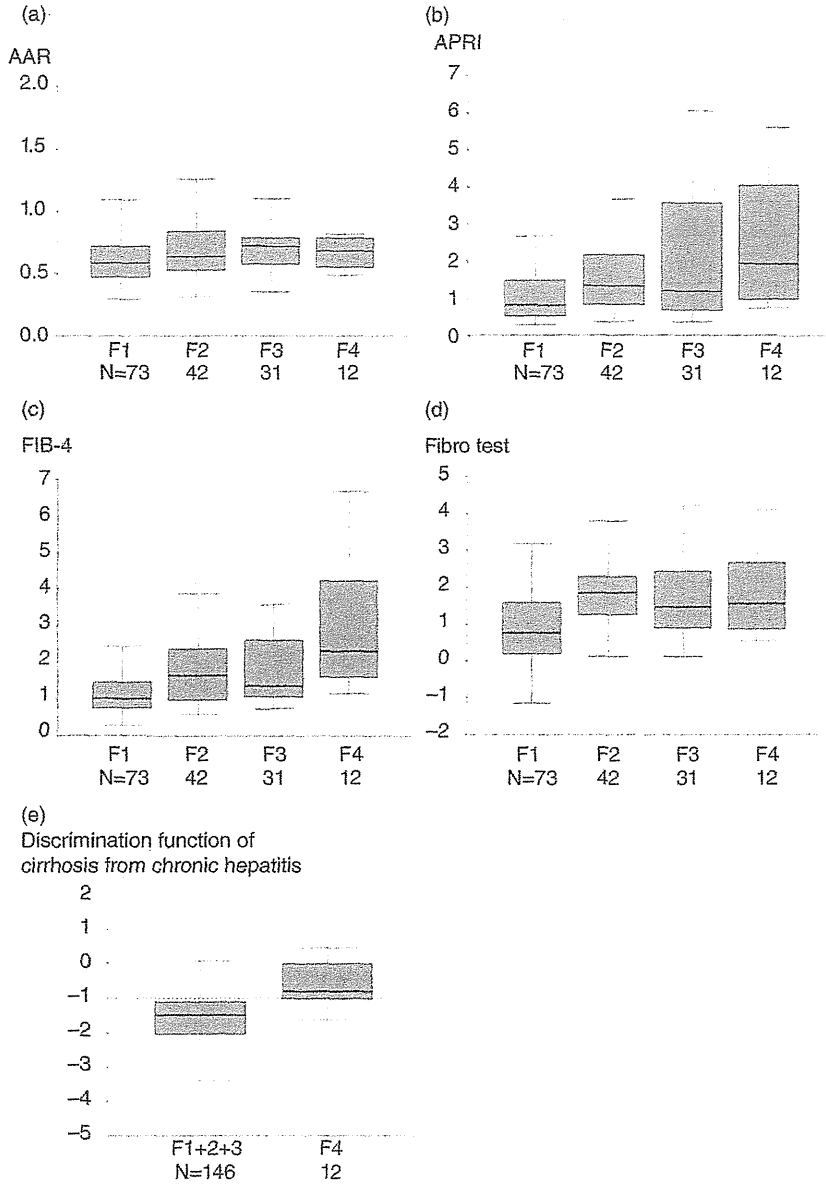

Figure 4 Previously published fibrosis scores. (a) Aspartate aminotransferase/alanine aminotransferase ratio (AAR),[19] (b) aspartate aminotransferase-to-platelet ratio index (APRI),[20] (c) FIB-4,[21] (d) FibroTest[22] and (e) discrimination function of cirrhosis from hepatitis in Japanese patients.[23]

the best management of patients with chronic hepatitis B. The FSB seems one of the ideal methods of approximating the fibrotic stage of chronic hepatitis B. 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 HBV-related chronic liver disease, this equation would not be suitable for the recognition of hepatitis C virus-related chronic liver disease, alcoholic liver disease, and other congenital or autoimmune liver diseases. To recognize the latter diseases, other studies of individual diseases must be performed.

We compared the usefulness of the FSB with that of other fibrosis scores.[19–23] The more simple and less expensive AAR or APRI could not estimate fibrotic stages with poor correlation coefficients of 0.199 and 0.265, which are much lower than the coefficient of the FSB of 0.625. FibroTest, which contained three costly fibrosis markers (α-2-macroglobulin, haptoglobin and apolipo-

protein A1), also showed a low correlation coefficient of 0.330, suggesting that its usefulness was limited in HBV positive oriental patients. Although FIB-4 demonstrated the best coefficient of 0.412 among the fibrosis scores, significant overlaps were found between neighboring stages and obtained scores were not coordinated for real histological classification.

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

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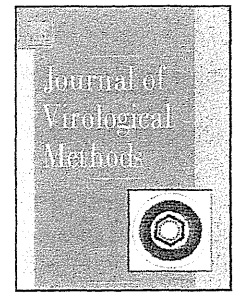

Title: Comparative quantitative analysis of hepatitis C mutations at amino acids 70 and 91 in the core region by the Q-Invader assay

Authors: Kenichi Tadokoro, Mariko Kobayashi, Fumitaka Suzuki, Chie Tanaka, Toshikazu Yamaguchi, Makoto Nagano, Toru Egashira, Hiromitsu Kumada

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