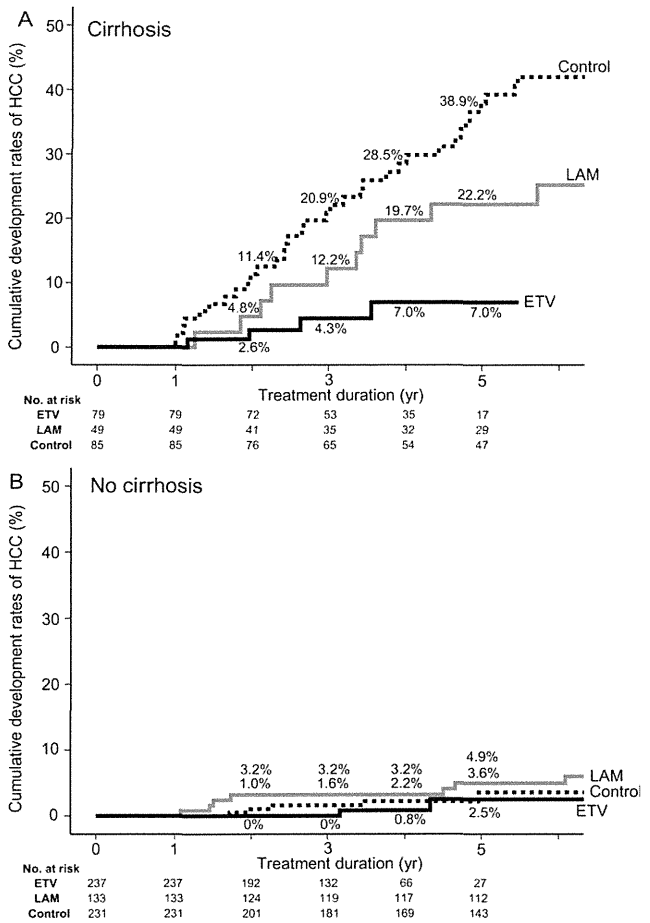

Fig. 3. Comparison of HCC cumulative incidence rates between the entecavir (ETV)-treated group, lamivudine (LAM)-treated, and the nontreated control group after PS matching stratified by cirrhosis. The log-rank test revealed a statistically significant difference in the incidence of HCC at 5 years time in cirrhosis patients: ETV versus control group ($P < 0.001$); LAM versus control ($P = 0.019$); ETV versus LAM ($P = 0.043$). The differences were not seen in the noncirrhosis patients: ETV versus control ($P = 0.440$); LAM versus control ($P = 0.879$); ETV versus LAM ($P = 0.126$).

HBV has been previously shown to influence HCC development. Ikeda et al.[20] reported that the cumulative HCC incidence rates among Japanese HBV patients were 2.1% at 5 years, 4.9% at 10 years, and 18.8% at 15 years among NA-naïve patients. Other studies, both from Japan and other countries, have reported a 5-year cumulative HCC incidence rate of 3.3% among chronic HBV, and 21.2% to 59% among cirrhosis patients.[21,22] The incidence of HCC varies significantly by country and ethnic group,[4] which seems to be attributable to diverse exposure to HCC risk factors.

Carcinogenicity related to HBV infection is somewhat complex and multifactorial when compared with carcinogenicity related to HCV infection. Known HCC risk factors among HBV-infected patients include older age, male gender, cirrhotic status, diabetes mellitus, family history, alcohol consumption, AST, HBsAg, HBeAg, and genotype C.[20,23,25] Chen et al.[5] found a dose-response relationship between pretreatment serum HBV DNA levels and the development of HCC. Baseline ALT is another risk factor for HCC, as elevated ALT levels indicate an active immune response against HBV, resulting in repetitive hepatocyte injury.[5] Our study corroborates these findings on these factors influence on HCC development.

The potential ability of ETV to reduce the risk of HCC is an additional example of a long-term NA treatment effect. Some studies have shown that ETV has low incidence of HCC but these studies did not have a control arm.[9] A meta-analysis and a systematic review showed that NAs can reduce liver complications, including HCC.[26,27] Other studies have begun to show that control of sustained viral loads through drugs such as NAs is important in preventing long-term complications. Chen et al.[28] showed that greater decreases in serum HBV DNA levels ($<10^4$ copies/mL) during follow-up were associated with a lower risk of HCC.

Our comparison among the PS-matched ETV-treated group, nonrescued LAM-treated patients, and the control showed that ETV is superior to LAM in HCC suppression. Kurokawa et al.[29] showed that treatment with lamivudine for an average of 5 years reduced the incidence of HCC in HBV-infected cirrhosis patients, who showed sustained viral response at a median HBV DNA of <4.0 log copies/mL. Unfortunately, only 48% of the patients in this study achieved sustained viral response, while 51% developed lamivudine-resistant tyrosine-methionine-aspartate-aspartate mutation (YMDD mutation) during follow-up.[29] Patients with drug resistance were reported to have a 2.6 times greater chance of developing long-term complications.[26] A systematic review of 21 studies showed that HCC occurred more (2.3% versus 7.5%, $P < 0.001$) in nonresponding patients or in patients with viral breakthrough compared with those who experienced remission.[28] On-treatment drug resistance could subject patients to a variable viral status. Suppression of HCC by NAs requires NAs that do not lead to drug resistance. Compared with other NAs, ETV shows minimal drug resistance. Our results showed that ~90% of the ETV-treated patients had sustained viral suppression at year 1, and that drug resistance was minimal (0.8%) during the median follow-up period of 3.2 years.

We found that the effect of ETV treatment in reducing the risk of HCC was more prominent among high-risk patients. This phenomenon was observed by examining the combination of parameters associated with the recently developed risk scores (Fig. 4). The published risk scores were developed mainly to create

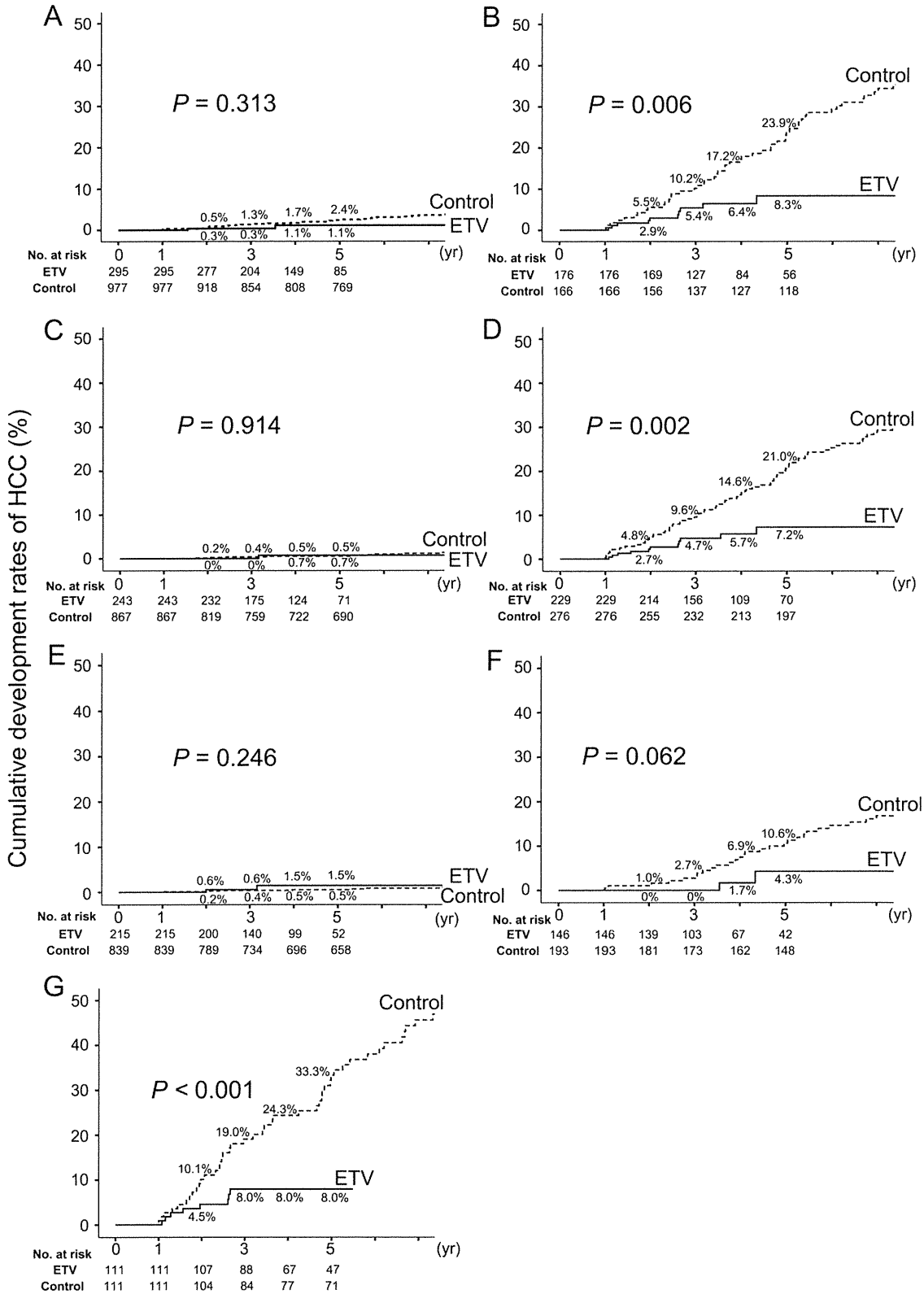

Fig. 4. Cumulative incidence of HCC by risk score scales: comparison between entecavir-treated and nontreated control patients: Risk score cutoff points were based on those presented in articles by the following: A,B (Yang et al.[10]): low-risk score cutoff point < 12; high-risk score cutoff point ≥ 12. C,D (Yuen et al.[11]): low-risk score cutoff point < 82; high-risk score cutoff point ≥ 82. E-G (Wong et al.[12]): low-risk score cutoff point < 4; medium-risk cutoff point 4-19; high-risk score cutoff point ≥ 20. A statistically significant difference in HCC incidence was seen between the ETV group and the control group in the higher-risk groups when observed the incidence of HCC over time (log-rank test $P = 0.006$ for risk score ≥ 12; $P = 0.002$ for risk score ≥ 82; $P = 0.062$ for patients with medium risk; $P < 0.001$ for patients at high risk for HCC).

easy-to-use nomograms based on clinical characteristics to predict the risk of HCC in patients with HBV. These scales have been validated, and can accurately estimate the risk of HCC up to 10 years. The cutoff scores used in these studies were based on their sensitivity to detect HCC derived and validated with non-treated HBV cohorts. The importance of our study using these risk scales in our cohorts was to see the change in risk with the initiation of therapy. We found that the ETV treatment effect to reduce the risk of HCC was more prominent among cirrhosis and high-risk patients despite the lack of interactions between ETV treatment and preexisting cirrhosis or risk factors. The lower treatment effect among lower-risk patients was somewhat not surprising. HCC development among low-risk patients is generally rare, and therefore, the treatment effect may not have occurred in large enough numbers during the treatment period allotted in our study to be able to detect a difference. In addition, HCC development differs greatly by cirrhotic status and risk factors in the control group. The treatment effect of ETV to reduce HCC is probably more likely reflected among cirrhosis or high-risk patients. A study with a longer observation period and higher patient numbers might be necessary to examine this ETV treatment effect among low-risk patients. The development of a scoring system to predict treatment effect of HBV patients with different risk levels will be useful in determining the most appropriate timing of treatment initiation in clinical settings.

Study Limitations. There were several limitations to our study. First, because our patients were recruited from one hospital, they might not have been representative of the general Japanese HBV population. Second, our control group included historically observed patients who entered the cohort long before the ETV group, resulting in treatment differences during the time gap. However, we used PS matching and a similar follow-up period between the two cohorts to minimize this bias. Third, our study was an observational study with patients having large demographic differences. Although we used a PS to match ETV-treated and control groups, our sample size did not take into account other unobserved confounding factors such as HCC family history, stage of cirrhosis, and comorbidities when determining associating factors for carcinogenesis in HBV. Finally, the observation period of the ETV group was relatively short, and patients in the ETV-treated cohort at 5 years consisted of only less than ~25% of the initial recruited patients. Because of this limitation, we censored patients who were followed for more than 5 years. The observed treatment effect would require confirmation over a longer period and a more complete follow-up.

Conducting a long-term study to examine the effect of antiviral therapy with HCC as the endpoint would be time-consuming and challenging. Such a study would require a large sample size and would, therefore, be costly. In addition, the increases in choices of therapy over time would make it difficult to conduct a long-term study using a single therapy. Owing to ethical issues, it would be difficult to recruit or follow a naïve, untreated cohort over an extended period of time. Because of these challenges, most studies have examined the relationship between antiviral treatment and the risks of HCC involved older drugs, lacked a control group, or were of relatively short duration. Consequently, the association between antiviral treatment and carcinogenesis is inferential and requires additional confirmatory studies.

In conclusion, in our study we observed the effect of HCC risk among HBV-infected patients treated by ETV by comparing them with a group of NA-naïve patients. We followed these Japanese patients for a relatively long period of time and compared them with a large pool of untreated control patients. In this long-term study among Japanese patients, ETV significantly reduced the incidence of HCC among chronic HBV-infected patients, and was more prominent among patients at higher risk for HCC.

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Original Article

Exploratory study on telaprevir given every 8 h at 500 mg or 750 mg with peginterferon-alpha-2b and ribavirin in hepatitis C patients

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Aim: The aims of this study are to assess the antiviral effects, safety and telaprevir (TVR) pharmacokinetics in two cohorts given TVR every 8 h (q8h) at doses of 500 mg and 750 mg with peginterferon-α-2b and ribavirin in chronic hepatitis C patients.

Methods: Twenty chronic hepatitis C (HCV) patients with genotype 1b in high viral loads were randomly assigned to two TVR-based regimens of 750 mg q8h (group A) and 500 mg q8h (group B) in combination with peginterferon-α-2b and ribavirin for 12 weeks.

Results: Although the difference was not statistically significant other than trough concentration (C_{trough}) at week 4, the parameters of maximum concentration (C_{max}), the area under the concentration time curve ($AUC_{0-\infty}$) and C_{trough} tended to be higher in group A than those in group B. The antiviral effects were similar in the two groups (sustained virological response rates [SVR], 40% in group A, 50% in group B). The discontinuation rates by anemia were 30% in group A and 20% in group B. Serum creatinine concentrations were lower in group B than those in group A.

Conclusion: Although the exposure to TVR tended to be lower in 500 mg q8h than that in 750 mg q8h, the SVR rates in both groups were similar. The result suggests that the 500 mg q8h dose may be one option for treatment. In addition, the present findings indicate that the development of adverse events which increase with a TVR-based regimen, specifically anemia and creatinine, could be avoided by dose adjustment of TVR.

Key words: anemia, chronic hepatitis C, creatinine increase, pharmacokinetics, telaprevir

INTRODUCTION

THE WORLD HEALTH organization (WHO) estimates that approximately 170 million people are infected with hepatitis C virus (HCV).[1] Decompensated cirrhosis and hepatocellular carcinoma (HCC) develop in approximately 30% of individuals infected with HCV and result in a fatal outcome.[2,3] In Japan, it is estimated that more than 1.5 million people are chronically infected with hepatitis C. Telaprevir (TVR), a potent HCV protease inhibitor, has recently been approved for the treatment of people suffering from chronic genotype 1 HCV infection in the USA, European Union (EU) and Japan. The overseas phase 3 studies demonstrate that patients who received TVR in combination with peginterferon (PEG IFN)-α-2b and ribavirin (RBV) achieved significantly higher rates of sustained virological response (SVR) than those who received only PEG IFN and RBV, regardless of their prior treatment experience with the anti-HCV agents.[4–6] The high SVR rates were also observed in the Japanese phase 3 studies of the TVR-based triple regimen.[7,8] In Japanese patients, anemia was the most common side-effect in the TVR-based triple regimen. The epidemiology of chronic hepatitis C (CHC) in Japan takes on a different aspect

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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

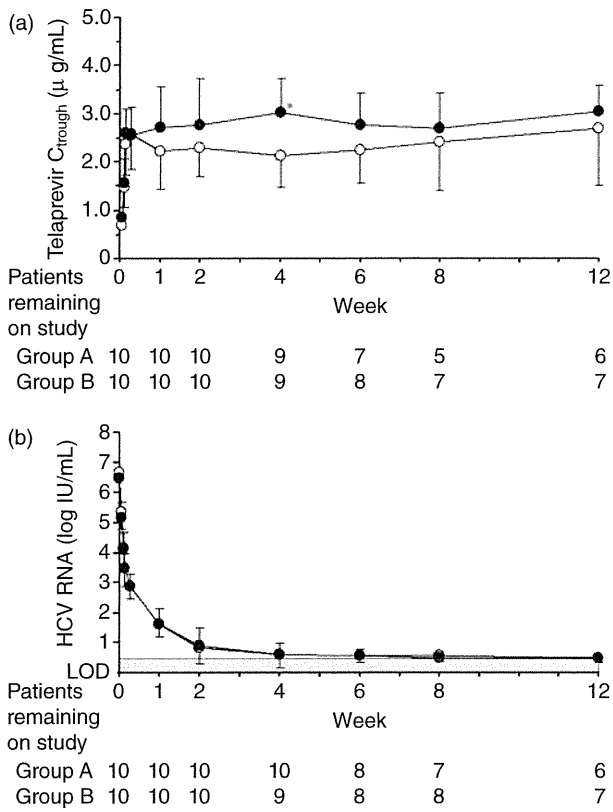

Figure 1 (a) Telaprevir C_{trough} levels and (b) change from baseline of hepatitis C virus (HCV) RNA in Japanese patients with chronic hepatitis C during the telaprevir-based triple therapy. Each circle and bar represent mean values ± standard deviations, respectively. Number of patients at each time 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,

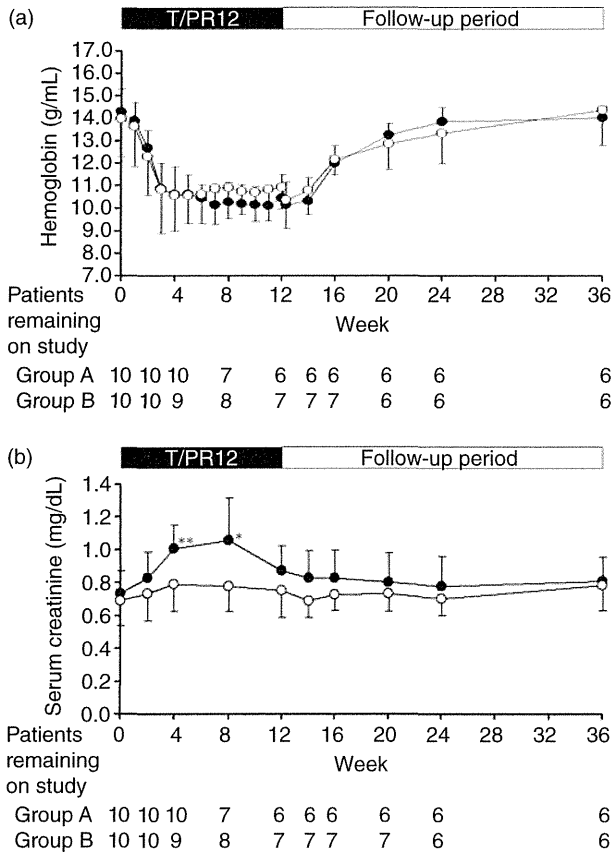

Figure 2 (a) Change from baseline of hemoglobin and (b) serum creatinine levels in Japanese patients with chronic hepatitis C during the telaprevir-based triple therapy. Each circle and bar represent mean values ± standard deviations, respectively. Number of patients at each time point is indicated below. 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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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Clearance of hepatitis B surface antigen during long-term nucleot(s)ide analog treatment in chronic hepatitis B: results from a nine-year longitudinal study

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Abstract
Background Clearance of hepatitis B surface antigen (HBsAg) is considered the ultimate goal in chronic hepatitis B treatment. One treatment option is long-term nucleot(s)ide analog (NA) therapy. We followed a group of long-term NA therapy patients to evaluate the efficacy of this treatment in promoting clearance and longitudinal declines of HBsAg.
Method The study included 791 NA therapy patients who received lamivudine as their first drug. At the baseline, 442 patients were hepatitis B e antigen (HBeAg)+ and 349 were HBeAg−. All analyses were performed after separating the HBeAg+ and HBeAg− cohorts. Cox proportional hazards models were used to determine which factors were associated with HBsAg clearance.
Results HBsAg clearance was observed in 18 (4.1 %) of the HBeAg+ patients and 20 (5.7 %) of the HBeAg− patients at baseline, giving seroclearance rates of 6.4 and 6.9 %, respectively, over the nine-year study period. HBsAg clearance was influenced by several independent factors that varied according to HBeAg cohort. For HBeAg+ patients, these included previous interferon therapy, infection with hepatitis B virus (HBV) genotype A, a ≥0.5 log IU/mL decline in HBsAg level within six months, and clearance of HBeAg at six months. For HBeAg− patients, these included infection with HBV genotype A, decline in HBsAg at six months, and a baseline HBsAg level of <730 IU/mL.
Conclusion This study suggests that both direct antiviral potential and host immune response are needed to achieve HBsAg clearance by NA therapy. Viral genotype strongly influenced HBsAg clearance during NA therapy.

Keywords Hepatitis B surface antigen · Nucleot(s)ide analog · Lamivudine · Interferon

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Introduction

Worldwide, an estimated 400 million people are infected with hepatitis B virus (HBV) persistently, and one million people die of decompensated cirrhosis and/or hepatocellular carcinoma (HCC) annually [1, 2]. Recently, oral nucleot(s)ide analogs (NAs) have been used as a mainstay therapeutic strategy against chronic hepatitis B. Five such antiviral agents—lamivudine (LAM), entecavir (ETV), telbivudine, adefovir dipivoxil (ADV), and tenofovir disoproxil fumarate—which inhibit viral replication [e.g., hepatitis B virus DNA (HBV DNA) priming, reverse transcription of negative-stranded HBV DNA, and synthesis of positive-stranded HBV DNA] have been approved; these NAs vary in both the strength and the rapidity with which they suppress HBV DNA [3–10]. Sustained viral suppression by NA therapy can improve liver fibrosis and clinical outcomes of patients [11, 12]. LAM was the first NA to be approved to treat chronic hepatitis B in Japan, followed by ADV and ETV.

Responses to antiviral treatments can be evaluated by monitoring serum HBV DNA levels, hepatitis B e antigen (HBeAg) and antibody levels, and hepatitis B surface

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antigen (HBsAg) and antibody levels. Serum HBsAg levels appear to reflect the amount of intrahepatic covalently closed circular DNA (cccDNA), which acts as a template for the transcription of viral genes [13–15]. Previous studies have shown that both interferon (IFN) and NA therapy result in a reduction of intrahepatic cccDNA [16, 17], suggesting that these treatments may be helpful in achieving the ultimate therapeutic goal of antiviral therapy for chronic hepatitis B (i.e., total clearance of HBsAg).

Very low rates of HBsAg clearance have been reported in the past [18–22]. Recent work has shown that over a one-year period, pegylated (PEG)-IFN therapy is more successful than ETV at reducing serum HBsAg [23]; furthermore, PEG-IFN therapy has also been reported to promote the complete clearance of HBsAg [24–27]. Several studies have detailed similar successes achieved by NA therapy but over relatively short (<5 years) treatment durations [18–20, 22, 28, 29]. The kinetics of HBsAg during long-term (>5 years) treatment remain unknown. NA therapy leads to time-dependent decreases in intrahepatic cccDNA and serum HBsAg levels if sustained viral suppression is longer term, and may therefore increase the rates of HBsAg clearance.

In order to evaluate this possibility empirically, we conducted a ten-year-long study in which we followed patients who received NA therapy initiated by the administration of LAM. We evaluated the resulting clearance and longitudinal declines of HBsAg using highly sensitive assays. Our aim was to determine whether long-term NA therapy can lead to HBsAg clearance, as suggested; if so, we also wished to elucidate the factors associated with its success.

Methods

Study population

Over a period of 12 years (September 1995 to September 2007), 949 consecutive patients who were chronically monoinfected with HBV (confirmed HBsAg positivity for at least six months), were treated with LAM monotherapy at the Department of Hepatology, Toranomon Hospital, Metropolitan Tokyo. The indication for antiviral therapy was abnormal ALT levels accompanying the increase in HBV DNA (over 4 log copies/mL) as a rule. However, in cases where ALT levels were normal, patients with advanced fibrosis were administered LAM. We did not treat patients without fibrosis who had low HBV DNA and normal ALT levels as a rule. We selected 791 patients for the final study after we had excluded all those who had been treated with LAM for <6 months, were co-infected with hepatitis C virus, had not provided sufficient serum samples, and/or had insufficient clinical records (Fig. 1). No patient was co-infected with human immunodeficiency virus in this cohort. Seven hundred ninety-one patients were enrolled in this cohort study. Of these 791 patients, 442 were HBeAg+ and 349 were HBeAg− at baseline. All analyses were performed after separating the HBeAg+ and HBeAg− cohorts. Written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved a priori by the institution's human research committee. This study has been registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN CTR) as the number UMIN000007993.

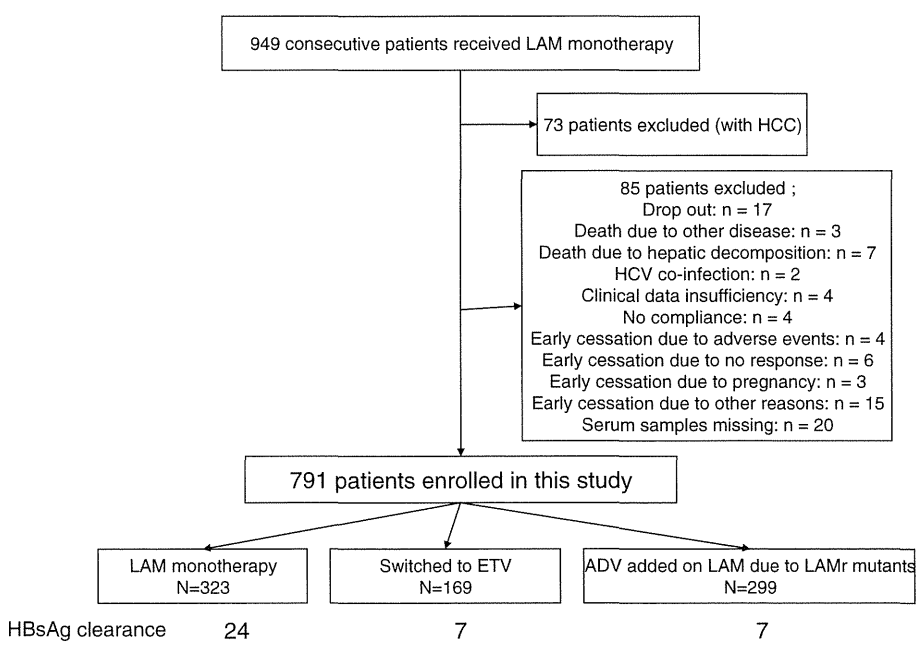

Fig. 1 Schematic of study protocol. *LAM* lamivudine, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *ETV* entecavir, *ADV* adefovir dipivoxil, *HBsAg* hepatitis B surface antigen

Antiviral therapy and drug resistance

All 791 patients received 100 mg LAM daily as an initial therapy, but a LAM-resistant rtM204I/V mutation developed in 439 (55 %) of these patients. Over time, 334 (42 %) individuals experienced an increase in HBV DNA (≥1 log copies/mL) [e.g., virological breakthrough (VBT)] and, as a result, 299 (98.5 %) individuals were also provided with ADV treatment (10 mg) added onto LAM as a rescue therapy. The remaining patients continued to receive LAM monotherapy and were lost to follow-up before the administration of ADV because of the lack of approval for ADV administration in Japan at the time. The resistant mutation for rtM204I/V was detected in 312 of 334 patients who experienced VBT using a commercial kit (as described below). Patients who had achieved an optimal or suboptimal virological response or who wished to participate in the clinical trial of ETV for LAM-refractory patients (ClinicalTrials.gov: NCT 1037166)—152 and 17 patients, respectively—switched from LAM to ETV (0.5 mg/day). Additionally, patients in whom subsequent ADV- or ETV-resistant mutants emerged received an optimal rescue therapy with other NAs (ETV + ADV combination for ADV resistance, and LAM + ADV combination for ETV resistance).

NA treatment was continued as a rule; median NA treatment duration was 75 months (25th–75th percentile, 55–102) in the HBeAg+ cohort and 92 months (67–119) in the HBeAg− cohort. Ultimately, 55 (7 %) of the 791 patients discontinued treatment; 16 of these individuals terminated treatment after achieving HBsAg seroclearance. Follow-ups were conducted for all patients, regardless of length of treatment, for as long as possible.

Clinical data collection and follow-ups

Data on patient characteristics, biochemistry, hematology, virology, histology, and previous treatments were collected and registered in our institute's database at the time of patient enrollment. Prior to beginning LAM, all patients were surveyed about the presence of a family history of HBV infection. Data on treatment dose and duration of previous IFN therapy were collected from our hospital's IFN therapy database or requested from other hospitals as necessary. Complete details on the previous treatment were lacking for 29 (9.7 %) of 297 patients who received IFN therapy before starting LAM.

At least every 1–3 months, liver function and virological markers of HBV infection were measured in all patients. All serum HBsAg titers were measured from frozen serum samples collected at six months, one year, three years, five years, and once annually for 6–10 years, and then stored at −80 °C. The day of HBsAg clearance was defined by the measurement in consecutive available serum samples before it was undetected in subsequent samples. A genotypic analysis of drug resistance was performed in cases of insufficient virological response or VBT, defined as an increase in serum HBV DNA levels ≥1 log above the nadir measured after the initial virological response. Cirrhosis was diagnosed by laparoscopy, liver biopsy, or clinical data such as imaging modalities and portal hypertension. The primary outcome for this study was HBsAg clearance. The endpoint of the follow-up was HBsAg clearance or last visit before January 2011.

Markers of HBV infection

Serum HBsAg titers were measured using ARCHITECT HBsAg QT assay kits (Abbott Laboratories, Tokyo, Japan), which have a lower limit of detection of 0.05 IU/mL and an upper limit of detection of 250 IU/mL. To expand the upper range from 250 to 125,000 IU/mL, serum samples, going off the scale, were diluted stepwise to 1:20 and 1:500 with ARCHITECT diluents as the product document described. HBeAg was determined by enzyme-linked immunosorbent assay with a commercial kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). HBV DNA was quantified using the Amplicor monitor assay (Roche Diagnostics, Tokyo, Japan), which has a dynamic range of 2.6–7.6 log copies/mL, or COBAS TaqMan HBV v.2.0 (Roche Diagnostics, Tokyo, Japan), which has a dynamic range of 2.1–9.0 log copies/mL. A commercial kit (HBV Genotype EIA; Institute of Immunology) was used to serologically determine HBV genotypes using the combination of epitopes expressed on the pre-S2 region product, which is specific to each of the seven major genotypes (A–G). YMDD mutants (rt M204I/V) were determined by polymerase chain reaction-based enzyme-linked mini-sequence assay with a commercial kit (Genome Science Laboratories, Tokyo, Japan).

Statistical analyses

Categorical data were compared between groups using chi-square or Fisher's exact tests. Continuous variables with a nonparametric distribution were analyzed with Mann–Whitney *U* tests, while those with a parametric distribution were analyzed with Student's *t* tests. When appropriate, Kruskal–Wallis tests were used to conduct pairwise comparisons of specific variables. Cox regression analyses were used to assess which variables were significantly associated with HBsAg clearance. Cut-off values were provided using the area under the receiver operating characteristic curve (ROC) only after rejecting the null hypothesis for the ROC curve. All baseline factors that were found to be significantly associated with HBsAg clearance by univariate analysis

were entered into a multivariate analysis. Independent baseline factors associated with clearance of HBsAg were calculated using a stepwise Cox regression analysis. We then performed a time-dependent Cox regression to analyze independent factors associated with HBsAg while adjusting for on-treatment factors and independent baseline factors. Three covariates of the on-treatment response factors—emergence of rtM204I/V mutants, VBT, and biochemical breakthrough—were set as the time-dependent covariates. Cumulative HBsAg clearance rates were analyzed using the Kaplan–Meier method; differences in the resulting curves were tested using log-rank tests. We performed Cox regression analysis, Kaplan–Meier curve analysis, and HBsAg kinetics analysis for no more than nine years, as the number of patients with a long-term follow-up of over ten years was too small to permit analysis [30]. Bonferroni adjustments were used to correct for the number of different ways a single predictor variable can be split. Significance was defined as $P < 0.05$ for all two-tailed tests. Data analysis was performed with IBM SPSS version 19.0 software (IBM Corp., Armonk, NY, USA).

Results

Patient characteristics

Thirty-eight (4.8 %) of 791 patients successfully cleared HBsAg. Of these, 24 had received LAM, 7 had switched to ETV treatment, and 7 had been treated with both LAM and ADV (Fig. 1). Of the 38 patients who achieved HBsAg clearance, 18 were HBeAg+, whereas 20 were HBeAg− at baseline. Table 1 provides a comparison of the baseline and on-treatment characteristics between patients who were and were not able to successfully clear HBsAg (all patients, HBeAg+ and − cohorts, respectively). In the HBeAg+ cohort, baseline characteristics that were significantly associated with HBsAg clearance included previous IFN therapy, HBV genotype, HBV DNA, and AST and ALT levels; in the HBeAg− cohort, significant characteristics included HBV genotype and HBsAg levels. Significant on-treatment characteristics in the HBeAg+ cohort included decline in HBsAg, clearance of HBeAg, and decline in HBV DNA to <2.6 log copies/mL at six months;

Table 1 Baseline, demographic, and on-treatment characteristics of patients with and without HBsAg seroclearance

Characteristics	All patients ($n = 791$)	HBeAg+ at baseline ($n = 442$)			HBeAg− at baseline ($n = 349$)		
		Persistently HBsAg+ ($n = 424$)	HBsAg seroclearance ($n = 18$)	P	Persistently HBsAg+ ($n = 329$)	HBsAg seroclearance ($n = 20$)	P
Baseline							
Age[a] (years) (SD)	43 (11.1)	41 (11.2)	44 (10.5)	0.177	47 (10.3)	46 (10.3)	0.899
Gender (male:female)	627:164	329:95	16:2	0.385	265:64	16:4	1.000
Race				0.446			
Japanese	768 (97)	411 (97)	17 (94)		320 (97)	20 (100)	1.000
Non-Japanese (%) (Asian:Caucasian)	23 (3) (21:2)	13 (3) (20:2)	1 (3) (1:0)		9 (3) (20:2)	0 (3) (1:0)	
Family history of HBV infection	539 (68)	311 (73)	10 (56)	0.107	208 (63)	10 (50)	0.238
Previous IFN therapy	297 (38)	167 (39)	15 (83)	**<0.001**	106 (32)	9 (45)	0.326
IFN duration (weeks)	27 (20–58)	26 (18–53)	52 (21–79)	0.214	32 (22–89)	23 (14–72)	0.457
Duration from the end of IFN to start of lamivudine (weeks)	50 (3–189)	26 (7–124)	37 (2–89)	0.505	119 (3–316)	102 (18–289)	0.746
Previous NA therapy	34 (4)	21 (5)	2 (11)	0.239	10 (3)	1 (5)	0.483
Presence of cirrhosis	169 (21)	76 (18)	2 (11)	0.752	87 (26)	4 (20)	0.610
HBV genotype				**<0.001**			**<0.001**
A	28 (3.5)	14 (3.3)	6 (33)		6 (1.8)	2 (10)	
B	67 (8.5)	16 (3.8)	0 (0)		48 (14.6)	3 (15)	
C	664 (83.9)	374 (88.2)	12 (67)		265 (80.5)	13 (65)	
D	3 (0.4)	2 (0.4)	0 (0)		0 (0)	1 (5)	
F	2 (0.3)	2 (0.4)	0 (0)		0 (0)	0 (0)	
Unclassified/missing	27 (3.4)	16 (3.8)	0 (0)		10 (3.0)	1 (5)	

Table 1 continued

Characteristics	All patients (n = 791)	HBeAg+ at baseline (n = 442)			HBeAg− at baseline (n = 349)		
		Persistently HBsAg+ (n = 424)	HBsAg seroclearance (n = 18)	P	Persistently HBsAg+ (n = 329)	HBsAg seroclearance (n = 20)	P
Baseline HBV DNA (log copies/mL)	7.0 (5.8–8.0)	7.6 (6.7–8.2)	8.0 (7.5–8.4)	**0.027**	6.3 (5.2–7.2)	6.1 (5.0–7.0)	0.652
Baseline HBsAg level (IU/mL)	2530 (907–6590)	3910 (1690–12300)	5280 (943–67600)	0.331	1590 (599–3050)	529 (58–1610)	**0.004**
Baseline AST level (IU/L)	74 (48–135)	81 (52–165)	201 (78–666)	**0.011**	66 (42–113)	57 (39–96)	0.694
Baseline AST level (×ULN)	2.2 (1.5–4.1)	2.5 (1.6–5.0)	6.1 (2.3–20.2)	**0.011**	2.0 (1.3–3.4)	1.7 (1.2–2.9)	0.736
Baseline ALT level (IU/L)	115 (63–252)	130 (72–290)	326 (104–775)	**0.021**	101 (56–194)	101 (55–215)	0.904
Baseline ALT level (×ULN)	3.0 (1.7–6.4)	3.5 (1.9–7.8)	7.8 (2.5–20.3)	**0.040**	2.6 (1.4–5.2)	2.6 (1.4–5.2)	0.955
Baseline total bilirubin level (mg/dL)	0.8 (0.6–1.1)	0.8 (0.5–1.1)	0.9 (0.6–1.9)	0.117	0.7 (0.6–1.0)	0.8 (0.6–0.9)	0.556
Platelet count[a] (10^5/mm^3) (SD)	16.1 (5.7)	16.5 (6.1)	14.7 (3.5)	0.221	15.6 (5.1)	17.7 (6.9)	0.216
On-treatment response							
Decline of HBsAg level (≥0.5 log IU/mL within six months)	97 (1)	67 (16)	13 (72)	**<0.001**	11 (3)	6 (30)	**<0.001**
HBeAg positive → clearance within six months	109 (14)	94 (22)	10 (56)	**0.005**	NA	NA	
Undetectable HBV DNA (<400 copies/mL) at six months	532 (67)	221 (52)	15 (83)	**0.014**	277 (84)	19 (95)	0.330
Emergence of rtM204I/V mutants	439 (55)	251 (59)	9 (50)	0.469	170 (52)	9 (45)	0.646
Viral breakthrough due to mutants	334 (42)	216 (51)	5 (28)	0.055	108 (33)	5 (25)	0.473
Biochemical breakthrough due to mutants	318 (40)	200 (47)	5 (28)	0.146	108 (33)	5 (25)	0.473

Except where marked with a superscript letter a, values are expressed as the median and 25th–75th percentiles (parenthetically), or number and percentage (parenthetically). ULN; AST = 33 IU/L, ALT = 42 IU/L (male), and 27 IU/L (female). *Asterisks* indicate data displayed as mean values and standard deviations. *Bold text* indicates statistically significant P values

the only significant characteristic in the HBeAg− cohort was a decline in HBsAg within six months. ROC curve analysis confirmed a cut-off value of 0.5 log IU/mL for a decline in HBsAg level within six months in the HBeAg+ and − cohorts [area under the curve = 0.810 (95 % CI 0.673–0.947) (HBeAg+ cohort) and 0.760 (95 % CI 0.611–0.909) (HBeAg− cohort)].

LAM-resistant rtM204I/V mutants were detected in 439 (55.5 %) of 791 patients. Of these, 334 (42.2 % of all patients) also developed VBT accompanied by an increase in HBV DNA (≥1 log copies/mL). The rate of VBT was marginally significantly lower in the HBsAg clearance group in the HBeAg+ cohort (Table 1).

Factors associated with HBsAg clearance

The overall cumulative rates of HBsAg clearance were 0.2 % at one year, 1.2 % at three years, 2.6 % at five years, 4.2 % at seven years, and 6.4 % at nine years in the HBeAg+ cohort; and 0.6 % at one year, 0.9 % at three - years, 2.2 % at five years, 5.2 % at seven years, and 6.9 % at nine years in the HBeAg− cohort. Univariate Cox