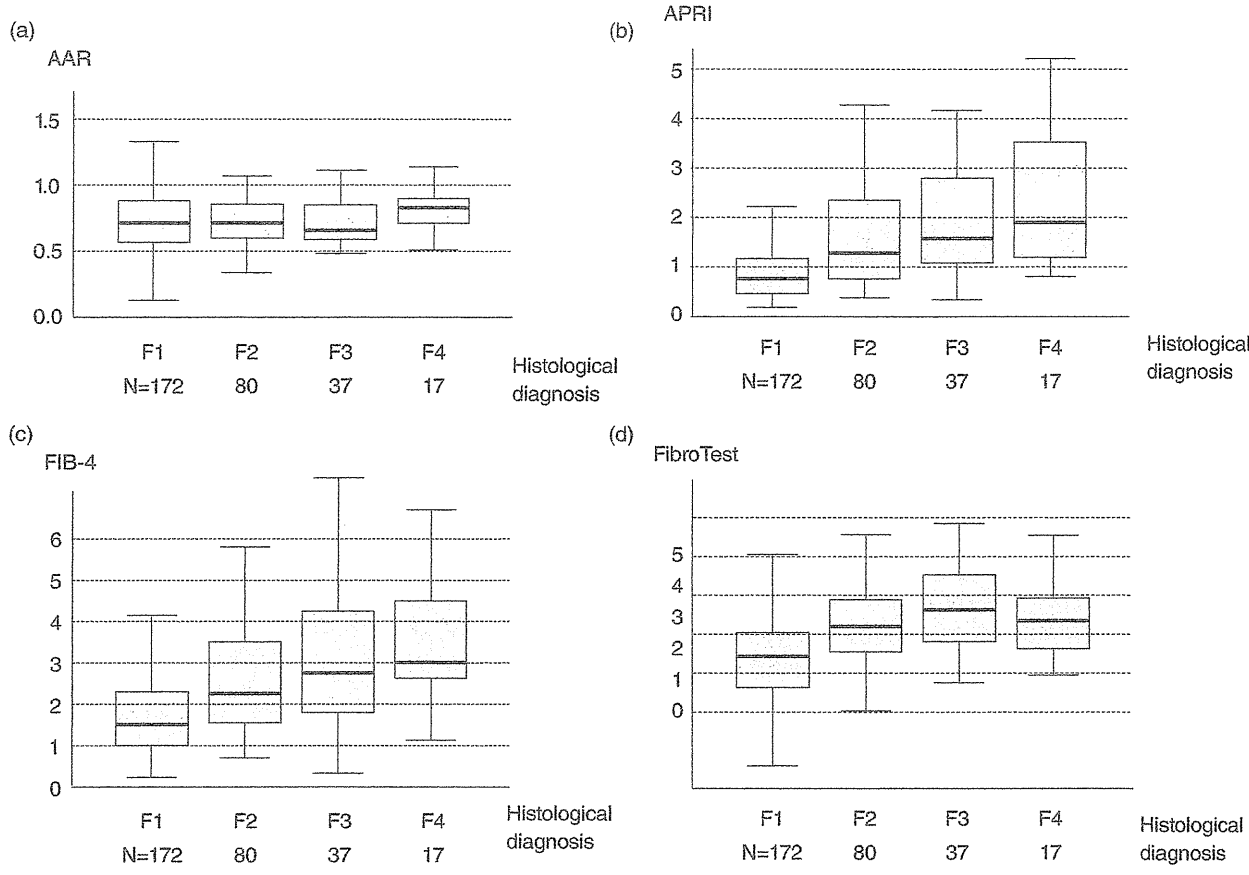

Figure 4 Previously published fibrotic scores: (a) aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR),[8] (b) AST-to-platelet ratio index (APRI), calculated by AST / (upper limit of normal of AST) / (platelet count [$\times 10^9$/L]) $\times$ 100.[12] (c) FIB-4 score, calculated by age $\times$ AST [IU/L] / (platelet count [$\times 10^9$/L] $\times$ ALT [IU/L]$^{0.5}$).[13] (d) FibroTest score regression coefficient was: $Z = 4.467 \times \log^{10}$ (α2-macroglobulin [g/L]) $- 1.357 \times \log^{10}$ (haptoglobin [g/L]) $+ 1.017 \times \log^{10}$ [γ-glutamyltransferase [GGT] [IU/L]) $+ 0.0281 \times$ (age [years]) $+ 1.737 \times \log^{10}$ (bilirubin [μm/L]) $- 1.184 \times \log^{10}$ (apolipoprotein A1 [g/L]) $+ 0.301 \times$ (sex [female = 0, male = 1]) $- 5.54$.[9]

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

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

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

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

The score can be calculated for any patients with chronic HCV infection. Although this multiple regression model dealt with appropriate logarithmic transformation for non-normal distribution parameters, the regression analysis was based on a linear regression model. Very slight fibrosis can be calculated as less than 1.00, which is commonly found with a slight degree of chronic hepatitis with a tiny fibrotic change as F0. Very severe fibrosis may be calculated as more than 4.00, which is an imaginable and nonsense number in the scoring system of fibrosis. FSC is, however, very useful and valuable in real clinical setting. Estimation of severity of liver fibrosis in outpatient clinics, evaluation of natural progression of patients' fibrosis over 10 years, and assessment of a long-term administration of interferon in patients with chronic hepatitis C from the viewpoint of fibrotic change. In this study, because certain patients actually had a history of interferon administration, regression of liver fibrosis during and after the treatment could be assessed when prior sera were available for serial evaluation of FSC. We can also expect the usefulness of evaluation of carcinogenic risk after sustained virological response, and stage progression with alcohol intake or obesity-induced steatosis. Recent development of new directly acting antiviral agents require evaluation for long-term histological advantage, for aggravation of hepatitis stage during viral and biochemical breakthrough caused by HCV mutation, estimation of future carcinogenic risk, and even for the best way of management of patients with chronic hepatitis C. FSC seems one of the ideal methods of approximation for fibrotic stage of chronic hepatitis C. Repeated measurement is quite suitable for patients with an unestablished treatment or trial, every 1 or 2 years, for example. Because the current regression function was generated from the data of HCV-related chronic liver disease, this equation would not be suitable for the recognition of HBV-related chronic liver disease,[22] alcoholic liver disease and other congenital or autoimmune liver diseases. To recognize the latter diseases, other studies about individual diseases must be performed.

We compared the usefulness of the FSC with that of other fibrotic scores.[8,9,12,13] More simple and inexpensive AAR or APRI could not well estimate fibrotic stages with poor correlation coefficients of 0.021 and 0.462, which were much lower than the coefficient of FSC of 0.572. FibroTest, which contained three costly fibrotic markers (α2-macroglobulin, haptoglobin and apolipoprotein A1), also showed a low correlation coefficient of 0.415, suggesting that the usefulness was limited in HCV positive Asian patients. Although FIB-4 demonstrated the best coefficient of 0.440 among the fibrotic scores, significant overlaps were found between neighboring stages and obtained scores were not coordinated for rea17l histological classification. Because this study also measured those special markers included in FibroTest, the ability of discrimination of fibrotic stages could be compared among the five fibrotic scoring systems.

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

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Risk Factors for Long-Term Persistence of Serum Hepatitis B Surface Antigen Following Acute Hepatitis B Virus Infection in Japanese Adults

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The proportion of patients who progress to chronicity following acute hepatitis B (AHB) varies widely worldwide. Moreover, the association between viral persistence after AHB and hepatitis B virus (HBV) genotypes in adults remains unclear. A nationwide multicenter study was conducted throughout Japan to evaluate the influence of clinical and virological factors on chronic outcomes in patients with AHB. For comparing factors between AHB patients with viral persistence and those with self-limited infection, 212 AHB patients without human immunodeficiency virus (HIV) coinfection were observed in 38 liver centers until serum hepatitis B surface antigen (HBsAg) disappeared or a minimum of 6 months in cases where HBsAg persisted. The time to disappearance of HBsAg was significantly longer for genotype A patients than that of patients infected with non-A genotypes. When chronicity was defined as the persistence of HBsAg positivity for more than 6 or 12 months, the rate of progression to chronicity was higher in patients with genotype A, although many cases caused by genotype A were prolonged cases of AHB, rather than chronic infection. Multivariate logistic regression analysis revealed only genotype A was independently associated with viral persistence following AHB. A higher peak level of HBV DNA and a lower peak of alanine aminotransferase (ALT) levels were characteristics of AHB caused by genotype A. Treatment with nucleotide analogs (NAs) did not prevent progression to chronic infection following AHB overall. Subanalysis suggested early NA initiation may enhance the viral clearance. *Conclusion*: Genotype A was an independent risk factor for progression to chronic infection following AHB. Our data will be useful in elucidating the association between viral persistence after AHB, host genetic factors, and treatment with NAs in future studies. (HEPATOLOGY 2014;59:89-97)

Abbreviations: AHB, acute hepatitis B; ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to HBsAg; HBeAg, hepatitis B e-antigen; CLIA, chemiluminescent enzyme immunoassay; EIA, enzyme immunoassay; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IgM, immunoglobulin M; anti-HBe, antibody to HBeAg; NAs, nucleotide analogs; RPHA, reverse passive hemagglutination.

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Hepatitis B virus (HBV) infection is one of the most common persistent viral infections in humans. Approximately 2 billion people worldwide have been exposed to HBV and 350 million of them remain persistently infected.[1] The incidence of HBV infection and the patterns of transmission vary greatly worldwide among different population subgroups.[2] In Western countries, chronic HBV infection is relatively rare and acquired primarily in adulthood by way of sexual transmission or the use of injectable drugs. Meanwhile, in Asia and most of Africa, the majority of infections are the result of transmission from an infected mother to her newborn. However, very few studies of acute hepatitis B (AHB) in adults have been reported.

HBV genomic sequences vary worldwide and have been classified into at least eight genotypes (A through H) based on an intergroup divergence of 8% or more over the complete nucleotide sequence.[3,4] These genotypes have distinct geographic distributions.[5-7] In particular, genotype A is predominant in Northwestern Europe, the United States, Central Africa, and India.[8,9] The Japanese have been infected with genotypes B and C since prehistoric times.[10] Recently, many lines of evidence have revealed among the Japanese an increase in acute infection with HBV genotype A following sexual transmission.[11,12] As a result of this increasing transmission of genotype A, the distribution of HBV genotypes in Japan clearly differs among patients with acute and chronic infections.[13] Moreover, recent studies suggest that acute infection with HBV genotype A may be associated with an increased risk of progression to persistent infection.[15] Indeed, the prevalence of HBV genotype A in chronic hepatitis B patients doubled in Japan between 2000-2001 and 2005-2006 (1.7% versus 3.5%).[11]

Human immunodeficiency virus (HIV)-1 infection results in an immunodeficient state, with the virus sharing routes of transmission with HBV. HIV-related immunodepletion influences the natural history of HBV infection, and epidemiological studies have revealed that HIV-positive patients are more likely to have a prolonged acute illness following HBV infection and lower rates of hepatitis B e-antigen (HBeAg) clearance.[16] Therefore, in this study patients with coinfection of HIV were excluded to examine the influence of HBV genotype directly without the confounding influence of HIV.

From 2005 to 2010, a multicenter cohort study was conducted throughout Japan on 212 patients with AHB. The aim of this cohort study was to assess the influence of clinical and virological factors, including HBV genotypes and treatment with nucleotide analogs (NAs), on AHB patients who became persistently infected.

Patients and Methods

Patients With AHB. The multiple-source cohort included 212 randomly selected AHB patients without coinfection of HIV. From 2005 through 2010, the study participants were recruited from 38 liver centers throughout Japan. The cohort included patients who were admitted to the hospital because of AHB and who visited the hospital every month after being discharged. The diagnosis of AHB was contingent on the rapid onset of clinical symptoms accompanied by elevated serum alanine aminotransferase (ALT) levels, the detection of serum hepatitis B surface antigen (HBsAg), and a high-titer antibody to hepatitis B core antigen (anti-HBc) of the immunoglobulin M (IgM) class. Patients with initial high-titer anti-HBc (>10.0 S/CO) were diagnosed as having an exacerbation of chronic hepatitis B and were excluded. If the patient had been tested previously, the absence of serum HBsAg and anti-HBc before admission was verified from the medical record to discriminate a new infection from an acute exacerbation of a persistent infection. Patients with acute hepatitis A, hepatitis C, and drug- or alcohol-induced acute hepatitis were also excluded; hepatitis D virus infection was not determined because of its extreme rarity in Japan. The study protocol conformed to the 1975 Declaration of

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Table 1. Characteristics of Patients With Genotype A or a Non-A Genotype Acutely Infected With Hepatitis B Virus

Features	Genotype A (n = 107)	Non-A Genotypes (n = 105)*	*P* Value
Age (years)	36.3 ± 12.0	40.7 ± 14.3	0.032
Male sex	102 (95.3)	75 (71.4)	<0.001
HBeAg positive	104 (97.2)	79 (75.2)	<0.001
ALT (IU/L)	1210 ± 646	2225 ± 2851	0.045
Total bilirubin (mg/dL)	9.9 ± 9.4	7.5 ± 6.7	0.115
HBV DNA (log copies/mL)	7.0 ± 1.5	5.8 ± 1.5	<0.0001
Duration until disappearance of HBsAg (month)	6.7 ± 8.5	3.4 ± 6.5	<0.0001
Persistence of HBsAg positivity more than 6 months	25 (23.4)	9 (8.6)	0.003
Persistence of HBsAg positivity more than 12 months	8 (7.5)	1† (0.9)	0.018
Sexual transmission	81/84 (96.4)‡	71/79 (89.9)§	0.095
Treatment with NAs	61 (57.0)	42 (40.0)	0.013

Data are presented as n (%), mean ± standard deviation. HBV, hepatitis B virus; HBeAg, hepatitis B e-antigen; ALT, alanine aminotransferase; NAs, nucleotide analogs.

*Non-A genotypes include genotypes B, C, D, F and H (n = 25, 77, 1, 1, and 1, respectively).

†One patient had genotype C.

‡Transmission routes were unknown for 23 patients.

§Transmission routes were unknown for 26 patients.

Helsinki and was approved by the Ethics Committees of the institutions involved. Every patient gave informed consent for this study.

Serological Markers of HBV Infection. HBsAg, HBeAg, antibodies to HBsAg (anti-HBs), HBeAg (anti-HBe), and HBcAg, and anti-HBc of the IgM class were tested by a chemiluminescent enzyme immunoassay (CLIA) by ARCHITECT (Abbott Japan, Tokyo, Japan). HBV DNA measurements were performed using a real-time polymerase chain reaction (PCR) assay (Cobas TaqMan HBV Auto; Roche Diagnostics, Tokyo, Japan).

Genotyping of HBV. The six major HBV genotypes (A through F) were determined serologically by enzyme immunoassay (EIA) using commercial kits (HBV GENOTYPE EIA; Institute of Immunology, Tokyo, Japan). This method is based on the pattern of detection by monoclonal antibodies of a combination of epitopes on preS2-region products, which is specific for each genotype.[17,18] Samples for which EIA could not determine the genotype were examined by direct sequencing of the pre-S2/S gene, followed by phylogenetic analysis.

Treatment With NAs. Treatments with NAs were performed using lamivudine or entecavir for more than 3 months. The individual clinicians determined if NAs were administered to patients, and when the treatment was to be started. The time to onset of treatment with NAs was measured in days from onset of AHB.

Statistical Analysis. Categorical variables were compared between groups by the chi-squared test and noncategorical variables by the Mann-Whitney *U* test. A *P* value less than 0.05 was considered significant. Multivariate analysis was performed using a backward stepwise logistic regression model to determine independent factors for viral persistence following AHB. Variables in the multivariate analysis were selected based on variables that were marginally significant with $P < 0.1$ in univariate analysis. Maintenance of HBsAg positivity was analyzed using the Kaplan-Meier method and significance was tested with the log-rank test. STATA Software (StataCorp, College Station, TX) v. 11.0 was used for analyses.

Results

Comparison of Characteristics Between Genotype A and Non-A Genotype AHB Patients. A total of 107 AHB patients (50.5%) were infected with genotype A while 105 AHB patients (49.5%) were infected with non-A genotypes, including genotypes B (25 [11.8%]), C (76 [35.8%]), D (1 [0.5%]), F (1 [0.5%]), and H (1 [0.5%]). Compared to those infected with non-A genotypes, genotype A patients were significantly younger (36.3 ± 12.0 versus 40.7 ± 14.3 years, $P = 0.032$), predominantly men (95.3% versus 71.4%, $P < 0.001$), and more frequently positive for HBeAg (97.2% versus 75.2%, $P < 0.001$). Moreover, genotype A patients had a lower peak ALT levels (1,210 ± 646 versus 2,225 ± 2,851 IU/L, $P = 0.045$) and a higher peak level of HBV DNA (6.7 ± 8.5 versus 3.4 ± 6.5 log copies/mL, $P < 0.0001$). A significantly higher percentage of genotype A patients were treated with NAs (57% versus 40%, $P = 0.013$). These data are summarized in Table 1.

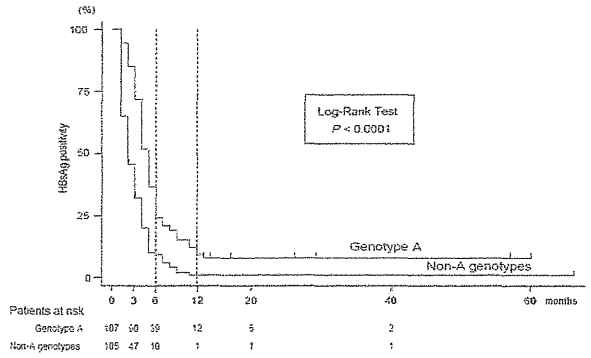

Fig. 1. Comparison of the cumulative proportion of AHB patients maintaining HBsAg positivity between genotype A and non-A genotypes, analyzed using the Kaplan-Meier test. $P < 0.0001$, genotype A: red line, non-A genotypes: blue line.

Cumulative Maintenance of HBsAg Positivity During Follow-up in Patients With Genotype A and Non-A Genotypes. In the patients infected with genotype A and non-A genotypes, the mean durations of HBsAg positivity maintenance were 6.7 ± 8.5 and 3.4 ± 6.5 months, respectively ($P < 0.0001$; Table 1, Fig. 1). For 6 months after AHB onset, the number of patients with genotype A and non-A genotypes maintaining HBsAg positivity were 39/107 (36.4%) and 10/105 (9.5%), respectively ($P < 0.001$). However, in many patients HBsAg disappeared between 7 and 12 months after AHB onset; that is, HBsAg disappeared in 31/107 (29.0%) of patients with genotype A and in 9/105 (8.6%) of patients with non-A genotypes during this time period. However, in some patients HBsAg never disappeared after persisting for more than 12 months following AHB onset. When chronicity after AHB was defined as the persistence of HBsAg for more than 12 months, chronicity developed in 7.5% (8/107) of patients with genotype A and in 0.9% (1/105) of patients with non-A genotypes ($P = 0.018$).

Comparison of Characteristics Between Patients in Whom HBsAg Persisted More Than 6 or 12 Months and Those With Self-Limited AHB Infection. Table 2 compares the demographic and clinical characteristics between patients in whom HBsAg disappeared within 6 months and those in whom HBsAg persisted for more than 6 months from AHB. The peak ALT levels ($1,882 \pm 2,331$ versus $1,018 \pm 696$ IU/L, $P = 0.0024$) and peak HBV DNA levels (6.3 ± 1.6 versus 7.4 ± 1.6 mg/dL, $P = 0.0004$) were significantly higher and lower in the former group than in the latter group, respectively. Moreover, marked differences were present in the distribution of genotypes between the two groups. The percentage of the HBV genotype A (46.1% versus 73.5%, $P = 0.003$) was significantly higher among patients in whom HBsAg was persistent for more than 6 months. In addition, we compared the demographic and clinical characteristics between patients in whom HBsAg disappeared within 12 months and those in whom HBsAg persisted for more than 12 months from AHB. Peak ALT ($1,787 \pm 2,118$ versus 775 ± 513 IU/L, $P = 0.0089$) and peak total bilirubin (8.7 ± 8.2 versus 3.8 ± 6.6 mg/dL, $P = 0.0039$) levels were significantly higher in the former group than in the latter group. In contrast, the peak HBV DNA levels (6.4 ± 1.6 versus 7.9 ± 1.4 mg/dL, $P = 0.0046$) were significantly lower

Table 2. Comparison Between Patients With Chronicity Following Acute Hepatitis B and Those With Self-Limited Acute Infections Determined by the Persistence of HBsAg for More Than 6 or 12 Months

Features	Disappearance of HBsAg Within 6 Months ($n = 178$)	Persistence of HBsAg for More Than 6 Months From AHB ($n = 34$)	P Value	Disappearance of HBsAg Within 12 Months ($n = 203$)	persistence of HBsAg for More Than 12 Months From AHB ($n = 9$)	P Value
Age (years)	38.2 ± 13.1	40.0 ± 14.5	0.454	38.1 ± 13.2	46.7 ± 14.0	0.061
Male sex	147 (82.6)	30 (88.2)	0.416	169 (83.3)	8 (88.9)	0.677
HBeAg positive	150 (84.3)	32 (94.1)	0.131	175 (86.2)	8 (88.9)	0.815
ALT (IU/L)	1882 ± 2331	1018 ± 696	0.0024	1787 ± 2118	775 ± 513	0.0089
Total bilirubin (mg/dL)	8.6 ± 7.5	8.7 ± 11.3	0.137	8.7 ± 8.2	3.8 ± 6.6	0.0039
HBV DNA (log copies/mL)	6.3 ± 1.6	7.4 ± 1.6	0.0004	6.4 ± 1.6	7.9 ± 1.4	0.0046
HBV genotype						
Non-A	96 (53.9)	9 (26.5)		104 (51.2)	1 (11.1)	
A	82 (46.1)	25 (73.5)	0.003	99 (48.8)	8 (88.9)	0.018
Sexual transmission	128/137 (93.4)*	24/26 (92.3)†	0.711	146/157 (93.0)‡	6/6 (100.0)§	0.356
NAs treatment (+)	82 (46.1)	21 (61.8)	0.093	98 (48.3)	8 (88.9)	0.017

Data are presented as n (%) and mean ± SD. HBsAg, hepatitis B surface antigen; AHB, acute hepatitis B, HBeAg, hepatitis B e-antigen; ALT, alanine aminotransferase; HBV, hepatitis B virus; NAs, nucleotide analogs.

*Transmission routes of 41 patients were unknown.

†Transmission routes of 8 patients were unknown.

‡Transmission routes of 46 patients were unknown.

§Transmission routes of 3 patients were unknown.

Table 3. Multivariate Analysis of Factors Independently Associated With Persistence of HBsAg Positivity Following Acute Hepatitis B

	Persistence of HBsAg More Than 6 Months From AHB		
Factors	Odds Ratio	95% CI	*P* Value
ALT (per 1 IU/L increase)	1.000	0.999-1.000	0.035
HBV DNA (per 1 log copy/mL increase)	1.176	0.931-1.484	0.173
Genotypes			
Non-A	1.00		
A	4.224	1.853-9.631	0.001

95% CI, 95% confidence interval; ALT, alanine aminotransferase; HBV, hepatitis B virus.

in the former group than in the latter group. The percentages of HBV genotype A (48.8% versus 88.9%, $P = 0.018$) and NAs treatment (+) (48.3% versus 88.9%, $P = 0.017$) were significantly higher among patients in whom the HBsAg persisted for more than 12 months.

Factors Independently Associated With Viral Persistence Following AHB. A stepwise logistic regression model was used to perform multivariate analysis which explains relationships between some factors and persistence of HBsAg positivity more than 6 months following AHB. Peak ALT level, peak HBV DNA level, genotype A, and treatment with NAs were retained in the final multivariate logistic model in a backward stepwise manner ($P < 0.1$). For predicting the persistence of HBsAg for more than 6 months, only genotype A was independently associated with progression of AHB to the persistence of HBsAg (odds ratio [OR]: 4.224, $P = 0.001$, Table 3).

Characteristics of Patients Who Progressed to Chronicity That Was Defined as the Persistence of HBsAg for More Than 12 Months Following Acute Hepatitis B. Table 4 shows the clinical and virological characteristics of nine patients who progressed to chronicity defined as the persistence of HBsAg for more than 12 months following AHB. Among the nine patients who progressed to chronicity from AHB, eight (88.9%) were men and eight (88.9%) were HBeAg-positive. In general, among the patients who progressed to chronicity following AHB, the peak HBV DNA levels were high, and the peak total bilirubin and ALT levels were low. In eight (88.9%) patients, entecavir was administered; however, the duration until the onset of NA treatment from AHB onset was long (75-570 days).

Early Onset of Treatment With NAs Was Able to Prevent Viral Persistence After AHB Caused by Genotype A. The cumulative proportion maintaining HBsAg positivity during follow-up, expressed in terms of time after AHB onset, were significantly longer in patients with NAs treatment than in those without NAs treatment ($P = 0.046$, Fig. 2A). Table 5 shows the percentages of patients in whom HBsAg persisted for more than 6 or 12 months among patients categorized based on the period of time (i.e., duration) until the onset of NAs treatment. For patients in whom the onset of NAs treatment was less than 4 weeks from the onset of AHB, 12.7% of the patients showed persistent HBsAg for more than 6 months, while none showed HBsAg positivity for more than 12 months. For patients in whom the onset of NAs treatment was at 5-8 weeks, 37.5% of the patients showed persistent HBsAg for more than 6 months, whereas none showed persistent HBsAg for more than 12 months. For all groups, the period of HBsAg positivity in patients starting NAs treatment within 8 weeks from AHB onset was significantly shorter than that in patients beginning NAs treatment after more than 8 weeks from AHB onset ($P < 0.0001$, Fig. 2B). Patients starting NAs treatment within 8 weeks from AHB onset never progressed to chronicity after AHB caused by genotype A.

Table 4. Characteristics of Patients Who Progressed to Chronicity Following Acute Hepatitis B

Case	Age	Gender	HIV	HBeAg	HBV DNA (log copies/mL)	Total Bilirubin (mg/dL)	ALT (IU/L)	Observation Period (Months)	NAs Treatment	Duration Until NAs Treatment (Days)	Transmission Routes	Genotype
1	23	Male	(−)	(+)	7.6	1.7	1271	26	ETV	570	Heterosexual	A
2	40	Male	(−)	(−)	8.8	1.4	568	13	ETV	240	Heterosexual	A
3	45	Male	(−)	(+)	7.7	0.9	867	57	ETV	135	Heterosexual	A
4	37	Male	(−)	(+)	7.6	3.4	384	29	ETV	75	Unknown	A
5	54	Male	(−)	(+)	9	2	455	17	ETV	155	Homosexual	A
6	45	Male	(−)	(+)	4.8	21.2	512	60	(−)	(−)	Homosexual	A
7	61	Male	(−)	(+)	9.1	1.5	804	17	ETV	88	Unknown	A
8	56	Male	(−)	(+)	9.0	1.1	1820	14	ETV	118	Unknown	A
9	31	Female	(−)	(+)	7.4	0.8	296	66	ETV	150	Blood transfusion	C

HIV, human immunodeficiency virus; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; NAs, nucleotide analogs; ETV, entecavir.

Table 5. Proportion of Patients in Whom HBsAg Persisted for More Than 6 or 12 Months Among Patients Categorized Based on the Number of Weeks Until the Onset of NAs Treatment

Duration Until Onset of NAs Treatment (Weeks)	Persistence of HBsAg for More Than 6 Months	Persistence of HBsAg for More Than 12 Months	Total Patients
<4 weeks (n, %)	9 (12.7)	0 (0)	71
5-8 weeks (n, %)	6 (37.5)	0 (0)	16
9-12 weeks (n, %)	1 (33.3)	1 (33.3)	3
13-16 weeks (n, %)	4 (100)	1 (25.0)	4
>17 weeks (n, %)	9 (100)	6 (66.7)	9
Total	29	8	103

HBsAg, hepatitis B surface antigen; NAs, nucleotide analogs.

Discussion

A multicenter nationwide study was conducted throughout Japan to evaluate the influence of clinical and virological factors on chronic outcomes in Japanese patients who contracted AHB in adulthood. The study was feasible in Japan, where a universal vaccination program for HBV has not been implemented because of the extremely high efficacy of the immunoprophylaxis that is given to babies born to carrier mothers. The implementation of this program has resulted in a decrease in the persistent HBV carrier rate from 1.4% to 0.3%.[19] Selective vaccination means that Japanese are more likely to be infected with HBV by way of horizontal transmission since the percentage of the population possessing anti-HBs is much lower than that in countries in which universal vaccination programs have been established.[20] In addition, Japan is faced with the ever-increasing impacts of globalization: as many as 17 million Japanese travel abroad and over 7 million people visit Japan from overseas each year. This "population mixing" may help to explain the increased prevalence in Japan of AHB due to genotype A, which is transmitted through indiscriminate sexual contact. Consequently, Japan may be the only country in the world where the influences of HBV genotypes, including genotype A (as is predominant in Western countries) and genotypes B and C (as are predominant in Asian countries), on chronic outcomes after AHB can be compared.

Currently, the persistence of HBsAg in serum for more than 6 months is considered to represent a progression to chronic infection.[21] However, our data showed that HBsAg frequently disappeared between 7 to 12 months after the onset of AHB in patients with genotype A (31/107 [29.0%]) and non-A genotypes (9/105 [8.6%]) (Fig. 1). These patients were considered to exhibit prolonged cases of AHB, rather than persistent infection. This finding reflects the higher sensitivity of the most up-to-date assays for HBsAg as compared with previous methods. In the present study, HBsAg was measured by CLIA, which has been reported to be about 150 times more sensitive in the detection of HBsAg than reverse passive hemagglutination (RPHA)-HBsAg, which has been used for the last 30 years in Japan.[22] The use of a more sensitive assay for HBsAg results in a longer period during which HBsAg may be detected. In this study, HBsAg did not disappear in nine patients after remaining continuously detectable for more than 12 months. Therefore, the persistence of HBsAg for more than 12 months, as measured with a highly sensitive method for detecting HBsAg, may be suitable for defining the progression of AHB to chronicity; however, further study is necessary to determine whether this definition is appropriate worldwide.

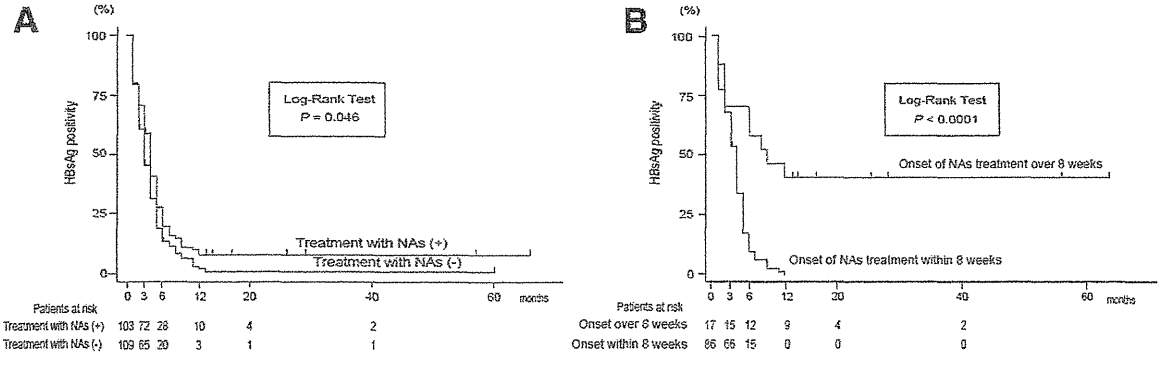

Fig. 2. (A) Comparison of the cumulative proportion of AHB patients maintaining HBsAg positivity between treatment with NAs (+) and treatment with NAs (−), as analyzed using the Kaplan-Meier test. $P = 0.046$, treatment with NAs (+): red line, treatment with NAs (−): blue line. (B) Comparison of the cumulative proportion of AHB patients in genotype A maintaining HBsAg positivity between treatment onset with NAs within 8 weeks and treatment onset with NAs over 8 weeks after onset of AHB, as analyzed using the Kaplan-Meier test. $P < 0.0001$, treatment onset with NAs over 8 weeks: red line, treatment onset with NAs within 8 weeks: blue line.

It has been reported that ~10% of patients who contract HBV as adults do not clear HBsAg from their serum and become carriers.[23] Meanwhile, a wide variation has been seen in the rate of persistence after AHB infection in adults. For example, viral persistence following AHB was seen in 0.2% (1/507) of adults in Greece,[24] 7.7% (5/65) of adult Alaskan Eskimos, and 12.1% (7/58) of adults in Germany.[25] The difference in the proportion of patients progressing from AHB to chronicity in different regions may be attributable to virological and host factors. In this study, 4.2% (9/212) of patients progressed to chronicity after AHB: 7.5% (8/107) of those infected with genotype A and 0.9% (1/105) of those infected with non-A genotypes. The non-A genotypes included genotypes B, C, D, F, and H (n = 25, 77, 1, 1, and 1, respectively). Genotypes B and C are predominant in eastern Asian countries, where the majority of those infected with HBV acquired the virus during the perinatal period by way of vertical transmission.[26] On the other hand, genotype A is predominant in Western countries, where the main route is horizontal transmission later in life.[26,27] Because HBeAg persists long after the infection in the genotype C as compared to other genotypes, this genotype has been shown to be a risk factor for perinatal and horizontal transmission in newborns and children.[28] The predominance of genotype A in Western countries may be attributable to a higher chronicity rate following AHB by way of horizontal transmission in adults.

In this study the characteristics of AHB associated with genotype A were a higher peak level of HBV DNA and a lower peak level of ALT. These findings were similar to those for patients with HBV-HIV coinfection.[29] Such characteristics of genotype A or coinfection with HIV are assumed to be attributable to milder hepatitis associated with weaker cellular immune responses. More slowly replicating viruses have been reported to evoke weaker cellular responses, enhancing the likelihood of persistence.[30] Indeed, our prior study showed that the replication of genotype A was significantly slower than that of genotype C in immunodeficient, human hepatocyte chimeric mice.[31] Moreover, variation among genotypes in the expression pattern of HBeAg may affect the progression of AHB to chronicity. Another previous study of ours revealed that a single form of HBeAg was detected by western blot analysis in serum samples from patients infected with genotypes B through D, but that two additional larger forms of HBeAg were detected in patients with genotype A.[32] Milich and Liang[33] reported that HBeAg may modulate the host immune response as a tolerogen to promote chronicity. Therefore, the different expression pattern of HBeAg by genotype A HBV may contribute to chronicity following AHB.

Early NAs initiation appeared to enhance the viral clearance across genotypes, although treatment with NAs did not show any overall benefit in duration of HBsAg. Previous studies examining the efficacies of NAs for preventing progression to chronic infection after AHB have reported conflicting results. Some small-scale studies have suggested the efficacy of lamivudine and entecavir in preventing the progression of AHB to chronic hepatitis.[34,35] Another study showed a lower seroconversion rate of HBsAg in lamivudine users.[36] Further, a randomized placebo-controlled trial showed no significant difference in clinical outcomes.[37] However, these previous studies did not mention the prevalence of HBV genotypes in the respective study populations. Although this was a retrospective study, our study included data on the prevalence of HBV genotypes. Additionally, our findings suggested that larger prospective randomized studies for every HBV genotype should be performed to determine whether early treatment with NAs prevented the progression of AHB to a chronic state.

In conclusion, in Japan genotype A was an independent risk factor for progression to chronic infection following AHB in adults. Confirmation of this association in patients with AHB in other countries is desirable and may provide insight into the pathogenetic mechanisms underlying this association. Early NA treatment appeared to reduce the likelihood of chronicity but this potentially important intervention needs to be prospectively studied before recommendations can be made.

Appendix

Members of the Japanese AHB Study Group include Yasuharu Imai (Ikeda Municipal Hospital), Norie Yamada, Hideaki Takahashi (St. Marianna University School of Medicine), Koji Ishii (Toho University School of Medicine), Hideyuki Nomura (Shin-Kokura Hospital), Jiro Nishida (Tokyo Dental Collage Ichikawa General Hospital), Shigeru Mikami (Kikkoman Hospital), Tsuneo Kitamura (Juntendo University Urayasu Hospital), Akihito Tsubota (Kashiwa Hospital Jikei University School of Medicine), Noritomo Shimada (Shinmatsudo Central General Hospital), Tetsuya Ishikawa (Nagoya University Graduate School of Medicine), Yoshiyuki Ueno (Tohoku University Graduate School of Medicine), Tomoyoshi Ohno (Social Insurance Chukyo Hospital), Etsuro Orito (Nagoya

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ORIGINAL ARTICLE

Use of simeprevir following pre-emptive pegylated interferon/ribavirin treatment for recurrent hepatitis C in living donor liver transplant recipients: a 12-week pilot study

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Abstract

Background The management of recurrent hepatitis C following liver transplantation remains a challenge.

Methods We prospectively investigated the efficacy and safety of simeprevir in combination with pegylated interferon and ribavirin in five patients undergoing living donor liver transplantation (LDLT) with recurrent hepatitis due to hepatitis C virus (HCV) genotype 1b.

Results As the immunosuppressive regimen, four received cyclosporine A (CsA) and one received tacrolimus (FK); no dose adjustment was made prior to the introduction of simeprevir, but the dose was accordingly modified afterwards. All five patients completed the intended 12-week treatment course without significant adverse events greater than grade 2, and no episodes of rejection were detected during the study period. The trough levels of CsA and FK were stably maintained. At week 12, HCV-RNA was not detectable in three of the five patients, whereas the HCV titer of the other two patients, including one with Q80L and V170I mutations at the HCV NS3 position, was at the lower level of quantification (1.2 $\log_{10}$ IU/ml).

Conclusions Based on this pilot study, simeprevir-based triple therapy is safe and somewhat effective within the first 12 weeks in LDLT recipients with HCV recurrence. Further studies are warranted to obtain robust conclusions.

Keywords Direct-acting antiviral drugs · Hepatitis C · Living donor liver transplantation · Simeprevir

Introduction

Compared with liver transplant patients not infected with hepatitis C virus (HCV), those with HCV have a poorer post-transplant prognosis [1–3], especially when the virologic response is inadequate [4, 5]. The lower antiviral response in liver transplant recipients, however, limits the efficacy of conventional interferon-based antiviral treatment (pegylated interferon [Peg-IFN] and ribavirin [RBV]) for recurrent hepatitis C following liver transplantation [6].

In the past several years, the development of direct-acting antiviral drugs (DAA), telaprevir (TVR) and boceprevir (BOC), for the treatment of HCV genotype 1 has provided a promising treatment option [7, 8]. Although the feasible efficacy of triple therapy, including such "1st generation protease inhibitors", has been demonstrated by several groups, the likelihood and severity of adverse events seem to be inevitable and have limited its use as the first choice for recurrent hepatitis C post-liver transplantation [9]. In addition, it is difficult to maintain the levels of calcineurin inhibitors such as cyclosporine A (CsA) or tacrolimus (FK)

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in combination with the 1st generation DAA, which are primarily metabolized by the cytochrome P450 3A4 pathway [10].

In December 2013, simeprevir (SMV), which is a one-pill, once-daily, oral HCV NS3/4A protease inhibitor, a so-called "2nd generation protease inhibitor", was approved for clinical use in Japan. SMV is associated with few adverse events, but the antiviral effects in patients with hepatitis C are as good or better than those of DAA [8]. In liver transplant recipients, SMV is likely superior to prior DAAs in terms of drug interactions, based on its small impact on the blood levels of calcineurin inhibitors when used simultaneously [10].

We conducted this prospective pilot study to evaluate the feasibility of SMV-based triple therapy in liver transplant recipients with hepatitis C, mainly with respect to the antiviral response, adverse events, and drug interactions with immunosuppressants by week 12 (namely by the cessation of SMV).

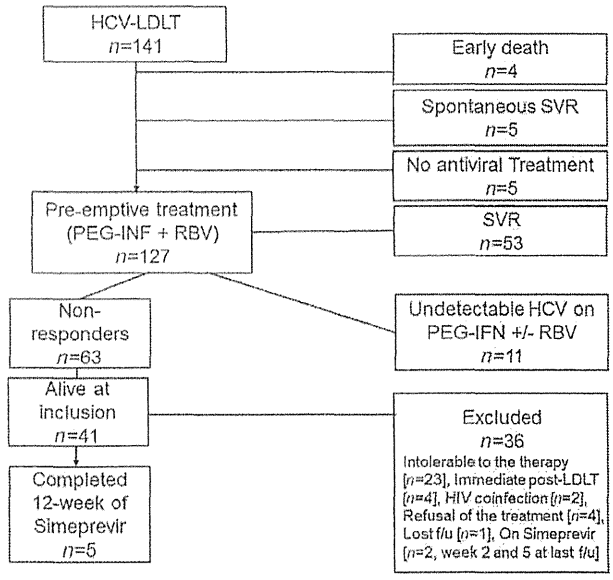


Fig. 1 Flow diagram of the patients enrolled in the simeprevir-based triple therapy

Materials and methods

Antiviral treatment regimen and patient selection

Between January 1996 and December 2013, 141 adult-to-adult living donor liver transplantations (LDLTs) were performed for HCV-positive recipients at the University of Tokyo Hospital. As previously reported [11], antiviral treatment was generally initiated with low-dose Peg-IFN alpha-2b and RBV 200–400 mg/day promptly after improvement of the general condition following liver transplantation in our institution. Recovery of hematologic and renal function was considered crucial, with a leukocyte number >4000/ml, platelet count >50000/ml, hemoglobin >8 g/l, and serum creatinine levels <2 mg/dl. During conventional dual treatment, flexible dose adjustments were made as necessary to avoid serious adverse events. A fixed overall treatment period length was not defined. Splenectomy was performed at the time of LDLT to prevent the progression of thrombocytopenia under IFN-based antiviral therapy [12].

Pre-emptive Peg-IFN /RBV treatment was administered to 127 of our 141 HCV-positive LDLT recipients, excluding cases of early death (within 3 months) after LDLT (n = 4), cases with spontaneous sustained virologic response (SVR) (n = 5), and cases without antiviral treatment due to clinical decision (n = 5). SVR was achieved in 53 patients, 11 had undetectable HCV-RNA on Peg-IFN and RBV therapy (dual treatment) upon inclusion; the remaining 63 were classified as non-responders. We selected patients for the current study among the 41 non-responders who were alive with sustainably positive HCV-RNA at the time of inclusion in this study. Patients who had either not tolerated or were not expected to tolerate conventional dual treatment were excluded. The current study protocol was not intended for those who were immediately post-transplant or were coinfected with human immunodeficiency virus (HIV) because of the lack of a detailed profile of SMV-based triple therapy in the transplant setting, considering the risk of unknown adverse events that could be fatal in this population, but only for those who survived the perioperative period and tolerated dual therapy for recurrent hepatitis C. Patient selection is shown in the flowchart in Figure 1.

SMV (100 mg daily) was intended to be continued for 12 weeks in combination with Peg-IFN and RBV (triple-antiviral treatment), followed by 36 weeks of dual treatment. The patients were generally admitted for 1 week, both to undergo liver biopsy pre-induction of SMV and to carefully monitor the daily change in the trough levels of calcineurin inhibitors (CNIs) following the induction of SMV.

Here we prospectively studied the 12-week clinical courses of all five patients who met the inclusion criteria and in whom triple-antiviral therapy with SMV was initiated by the end of March 2014, and followed up by the end of June 2014.

Laboratory test and histopathology assessment

Conventional blood work for the management of the patients with post-transplant hepatitis was checked as necessary. The estimated glomerular filtration rate

(eGFR; ml/min per 1.73 m^2) was calculated using the following formula: 194 × serum creatinine (−1.094) × age (−0.287) × 0.739 (if female), Japanese equation (equation 4) [13]. HCV RNA was measured quantitatively by reverse-transcriptase polymerase chain reaction (Amplicor HCV; Roche Molecular Systems, Pleasanton, CA, USA). Before liver transplantation, the HCV genotype was determined: the HCV genotype in all the five patients was 1b. In addition, the nucleotide sequences of the core and the number of amino acid substitutions in the interferon sensitivity-determining region (ISDR) in the NS5A gene were determined using a direct sequencing method [14]. The interleukin 28B (IL28B) genotype rs8099917 was also examined using the Invader assay (Third Wave Technologies, Madison, WI, USA) [15]. Prior to the induction of SMV, HCV NS3 and NS5A sequencing was determined, and liver biopsy was performed and evaluated by a pathologist based on the Metavir score [16].

Immunosuppression

Our post-transplant strategy for immunosuppression is documented elsewhere [11, 17]: briefly, it comprises steroid induction with CsA or FK, and the doses of each drug are gradually tapered for 6 months after LDLT. Methylprednisolone is tapered from 3 mg/kg on the first postoperative day to 0.05 mg/kg at the sixth postoperative month, and a maintenance dose of 2–4 mg of methylprednisolone is continued in all recipients. Mycophenolate mofetil (MMF) is added mainly for recipients requiring CNI dose reduction.

Ethics statement

The study protocol was approved as project number 2032, and human subject research regarding the IL28 polymorphism was particularly approved as project number G3514 by the Graduate School of Medicine and Faculty of Medicine at the University of Tokyo Research Ethics Committee; and the Human Genome, Gene Analysis Research Ethics Committee.

Statistical analysis

We used SPSS 17.0 statistical software (SPSS, Chicago, IL, USA) to analyze the relevant data. Differences between groups were analyzed by the Mann–Whitney *U*-test or ANOVA for continuous variables as appropriate, and the χ^2 test for categorical variables. *P*-values <0.05 were considered significant.

Results

The clinical characteristics of those five LDLT recipients are shown in Table 1. The median Model for End-Stage Liver Disease score was 15 (range 9–23). None of the five was coinfected with HIV, and four (80%) had hepatocellular carcinoma within the Milan criteria [18]. The details of each patient, including the HCV profile and the single nucleotide polymorphisms of IL28B rs8099917, are shown in Table 1. The Q80L/V170I and S122T/V170 mutations in NS3 were detected in patient #2 and 3, respectively. Q54H, F37L, Q54H, F37L/Q54H/Q62E, F37L mutations in NS5A were detected in patient #1 to 5, respectively.

Efficacy

All five patients completed the 12-week course of triple therapy with SMV. All of them were treated with dual therapy with Peg-IFN and RBV afterward.

Three of the five patients achieved an undetectable viral load of HCV at week 4, 8, and 12 weeks, and the viral titer of the remaining two patients was at the lower level of quantification (LLOQ, <1.2 $\log_{10}$ IU/ml) at week 4; one patient achieved an undetectable viral load at week 8, but the viral load became detectable again at week 12. The HCV titer of the remaining patient remained around LLOQ at weeks 8 and 12 (Table 1). At the last follow up (median 22 [range 16–27] weeks since the initiation of triple therapy), HCV viral load of those with undetectable HCV-RNA at week 12 were sustained to be below detectable level, although those with positive HCV-RNA at week 12 were both positive then (1.4 and 7.5 $\log_{10}$ IU/ml). HCV-RNA levels in the five patients are shown in Figure 2.

Safety profile and immunosuppression levels with SMV

No significant adverse events were observed other than grade 2 diarrhea in patient #1 on day 26, which was resolved immediately (within 1 week) after the reduction of mycophenolate mofetil (MMF) from 3000 mg/day to 1500 mg/day. None of the five patients required a dose reduction of Peg-IFN or RBV, use of granulocyte-colony stimulating factor for neutropenia, or blood transfusion for anemia. Renal function was well preserved during the study period, with no significant change in eGFR before or after the introduction of SMV (median 68 [range, 39.1–97.2] to 64.9 [range, 44.5–102] ml/min, $P = 0.84$). Bilirubin levels were not increased in any of the five patients. Immunosuppression was not modified before the initiation of SMV. The CsA trough levels before (median 78 [range 48–113] ng/ml), 1 week after (median 68.5 [67–104] ng/ml) and 12 weeks after (median 72.5 [65–92] ng/ml) initiating the triple therapy did not differ significantly ($P = 0.72$), and the FK

Table 1 Patient characteristics

Patient #	1	2	3	4	5
Age (years)	51	64	66	49	59
Sex	M	F	M	M	F
Height (cm) / weight (kg)	170/65	147/54	166/56	168/63	156/53
Donor age (years)	50	30	24	44	60
Donor relationship	Spouse	Daughter	Son	Spouse	Spouse
Calcineurin inhibitor (mg/day)	CsA (40)	CsA (75)	CsA (60)	FK (2)	CsA (60)
MMF (mg/day)	3000	None	1000	1500	None
Histopathological activity and fibrosis at triple therapy[a]	A2 / F1	A0-1 / F0-1	A1 / F1	A0 / F0	A1 / F1
Baseline clinical chemistry at triple therapy					
Total bilirubin (mg/dl)	1.9	0.8	0.9	0.9	0.7
Alanine aminotransferase (IU/ml)	68	31	47	25	29
Creatinine (mg/dl) and Estimated GFR (ml/min)	0.65 / 100.5	0.64 / 70.5	1.43 / 39.4	1.33 / 46.2	0.61 / 76
International normalized ratio	1.29 (on warfarin)	0.90	0.85	0.95	0.84
Hemoglobin (g/dl)	9.0	8.5	12.3	13.5	9.6
Leukocytes (/ul)	5900	5000	4900	5900	4600
Platelets (/ul)	476000	145000	186000	192000	262000
NS3 mutation	Non	Q80L/V170I	S122T/V170I	Non	Non
NS5A mutation	Q54H	F37L	Q54H	F37L/Q54H/Q62E	F37L
Pre-transplant antiviral therapy	Relapse	Non responder	Not applicable	Not applicable	Not applicable
Baseline HCV-RNA pre-LT (log_{10} IU/ml)	3.1	6.4	7.1	6.7	5.7
TPV therapy post -LT	Relapse	Not applicable	Relapse	Not applicable	Not applicable
Pre-triple treatment interferon (mo) since LT	23	16	118	26	16
Dose of Peg-IFN α2b (μg/week)	80	70	100	100	100
RBV dose (mg/day)	200	200	200	200	200
%CNI after the triple therapy	50%	67%	100%	75%	100%
CNI trough at triple therapy (ng/ml)	113	73	48	9.8	83
CNI trough 1 week after initiation (ng/ml)	104	69	67	9.5	68
CNI trough 12 week after initiation (ng/ml)	92	79	66	9.0	65
ISDR mutation (number)	Mutant (9)	Wild (0)	Wild (0)	Intermediate (1)	Undeterminable
Core 70	Undeterminable	Wild	Mutant	Wild	Wild
Core 91	Undeterminable	Wild	Mutant	Wild	Wild
IL28B Recipient /Donor[b]	TT/TT	TG/TT	TG/TT	GG/TG	TT/TT

CNI calcineurin inhibitor, *CsA* cyclosporine A, *FK* tacrolimus, *GFR* glomerular filtration rate, *HCV* hepatitis C virus, *IFN* interferon, *MMF* mycophenolate mofetil, *RBV* ribavirin, *LT* liver transplantation

[a] As per Metavir

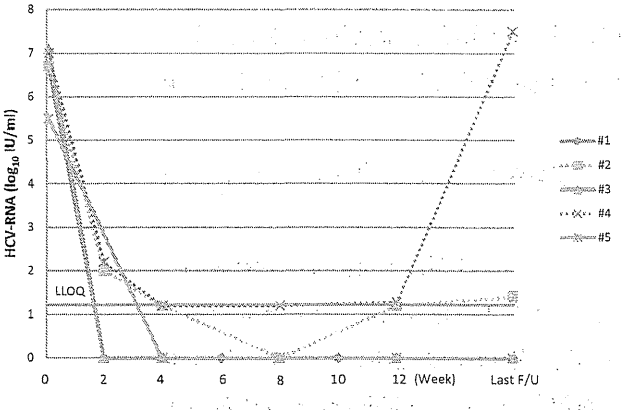
[b] Genotype rs8099917

trough level only moved from 9.8 to 9.0 ng/ml following the initiation of SMV.

After the completion of SMV, the CNIs were not restored to the original dose automatically, but modified according to the trough levels. Those without dose adjustment during the triple therapy (patient #3 and 5), the trough level at the last follow up were stable (67 and 61 ng/ml, respectively) with the same dose of CsA. Patient #2 showed lower trough level at week 20, and the dose of the CsA was re-increased to the original dose (75 to 100 ng/ml). The CNI dose of the remaining two patients (patient #1 and 4) were not changed since the completion of SMV to the last follow up with stable trough levels. The dose/use of MMF was not changed during the triple therapy throughout the follow up period, other than patient #1 who experienced diarrhea as noted above. There were no episodes of acute cellular or chronic (ductopenic) rejection observed during the study period.

Discussion

Here we present the results of a pilot study to reveal the characteristics of SMV-based triple anti-HCV treatment for



	w0	w4	w8	w12	Last F/U
#1	6.7	Undetectable	Undetectable	Undetectable	(w27) Undetectable
#2	6.7	<1.2	Undetectable	<1.2	(w23) 1.4
#3	7.1	Undetectable	Undetectable	Undetectable	(w22) Undetectable
#4	6.7	<1.2	<1.2	1.2	(w21) 7.5
#5	5.5	Undetectable	Undetectable	Undetectable	(w16) Undetectable

Fig. 2 Hepatitis C virus (HCV) RNA levels in five patients with simeprevir-based triple antiviral treatment. Each solid line represents an individual patient with an on-treatment virological response. Each dashed line represents an individual patient who did not achieve undetectable HCV RNA at week 12. The lower level of quantification (LLOQ) was 1.2 log10 IU/ml

LDLT recipients with recurrent hepatitis C. SMV became available after the introduction of TVR, which we have used in a selected patient group before the SMVs were introduced, and BOC into the liver transplant setting, thus a primary aim of the present study was to provide a preliminary report of the clinical experience with SMV in the liver transplant setting. Compared with TVR and BOC, the result of the current study suggested that the treatment with SMV was acceptably effective, with a rapid virologic response in three out of all five patients. In addition, importantly, no fatal adverse events, such as rejection, renal impairment, or severe cytopenia were observed.

We treated patients with SMV-based triple therapy as part of the pre-emptive therapy for recurrent hepatitis C. The rationale for this pre-emptive therapy is to strike at a time when histologic damage is minimal regardless of the clinical symptoms of recurrent HCV following transplantation [11, 19, 20]; thus we initiated SMV for those with even minimal or no graft injury due to recurrent hepatitis C, as long as the HCV remains persistent with dual treatment.

We investigated HCV polymorphisms at the NS3 position in all patients before the introduction of SMV. At baseline, none of the patients had mutations reported to reduce the antiviral effects of SMV *in vitro* [21]. Patient #2 had Q80L and V170I mutations at baseline; she achieved an undetectable HCV titer at week 8, whereas the other three patients achieved an undetectable HCV titer within the first 4 weeks, including two patients who relapsed with TPV-based triple therapy prior to the current study. The HCV-RNA of patient #2 became positive again at week 12, although it was around the LLOQ and not regarded as a breakthrough.

We also checked baseline polymorphisms at the NS5A position at the same time in anticipation of the coming treatment option with Daclatasvir (first-in-class, NS5A replication complex inhibitor) combined with Asunaprevir (NS3 protease inhibitor), which has been well tested in phase 3 clinical trial in Japan [22]. Patient #1, 3 and 4 had the Q54H mutation in NS5A, which might be associated with low-level resistance to an NS5A replication complex inhibitor [23]. Two out of those three patients achieved early virologic response. It seems feasible to introduce SMV-based triple therapy for such patients especially with some doubts about the potential efficacy of dual therapy with Daclatasvir and Asunaprevir in the liver transplant setting.

Importantly, there were no treatment cessations due to side-effects. One patient experienced grade 2 diarrhea, but this was resolved soon after the reduction of MMF: thus, it is difficult to determine whether SMV was the risk factor for diarrhea. Otherwise, no significant adverse events were observed, including elevation of serum total bilirubin. Necessary modifications in immunosuppression, especially CNIs, were also minimal. Technically it was not difficult for us to safely modify the dose of CNIs without a dose adjustment prior to the introduction of SMV, and comparatively mild modifications (50% to none) were required during the triple therapy. None of the five patients experienced renal dysfunction, infection, or rejection due to the uncontrolled trough level of CNIs, as noted above.

The introduction of TVR and BOC was anticipated to greatly improve virologic effects, even in liver transplant recipients with recurrent hepatitis C. The efficacy of TVR- or BOC-based triple therapy, however, was somewhat unsatisfactory; approximately 50% of the patients receiving such treatment achieved SVR [9, 24–27]. TVR- or BOC-based triple therapy was also associated with challenges in controlling the CNI trough levels and unignorable adverse events, such as cytopenic events, renal impairment, or skin rash [9]. In contrast, the previously reported profile of SMV is promising for liver transplant recipients with recurrent hepatitis C for the following reasons: first, the virologic effect is much greater than that of only Peg-IFN and RBV, with few side-effects by SMV itself [8, 28, 29], and second, SMV has few drug interactions with CNIs [10]. As demonstrated in the present study, the reported advantages of SMV in addition to TVR or BOC seem to be applicable to the management of post-transplant recurrent hepatitis C, with its safety and feasible virologic effect compared to TPV and BOC.

The present study has several limitations. The number of patients included was limited to only five, and all five patients were selected from among those receiving

pre-emptive antiviral therapy following liver transplantation with a poor virologic response. In addition, the five patients showed minimal or no graft damage when SMV was started. Hence, this study does not allow us to draw a robust conclusion regarding the use of SMV for liver transplant recipients, especially in evaluating the potential efficacy of SMV as a first-line treatment for recurrent hepatitis C. In addition, patients were followed only during the SMV-based triple therapy, and the actual virologic response after completing the treatment (i.e., 36 more weeks of dual therapy with Peg-IFN and RBV) should be evaluated. Further studies are warranted to address those concerns.

In conclusion, the present pilot study revealed the feasibility and safety of SMV in combination with Peg-IFN and RBV in LDLT recipients with recurrent hepatitis C. This combination therapy produced fewer side-effects and drug interactions with CNIs than prior DAAs. Recipients who were tolerant to dual therapy (Peg-IFN with RBV) but could not achieve a satisfactory viral response should be considered candidates for SMV. The actual profile of the current SMV-based antiviral treatment for recurrent hepatitis C post-liver transplantation, however, should be evaluated after the completion of a full course of therapy followed by 36 weeks of dual therapy with Peg-IFN plus RBV. In addition, future studies including a larger number of liver transplant recipients in diverse situations, such as those undergoing first-line treatment for established recurrence of HCV post-liver transplantation, are crucial.

Conflict of interest None declared.

Author contribution Study design: Tomohiro Tanaka, Yasuhiko Sugawara and Norihiro Kokudo. Acquisition of data: Nobuhisa Akamatsu, Junichi Kaneko, Sumihito Tamura, Taku Aoki, Yoshihiro Sakamoto, Kiyoshi Hasegawa. Analysis and interpretation: Tomohiro Tanaka, Masayuki Kurosaki, Namiki Izumi and Yasuhiko Sugawara. Manuscript drafted by: Tomohiro Tanaka, Nobuhisa Akamatsu, Masayuki Kurosaki and Yasuhiko Sugawara. Study supervision: Norihiro Kokudo.

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KIR3DL1-HLA-Bw4 combination and *IL28B* polymorphism predict response to Peg-IFN and ribavirin with and without telaprevir in chronic hepatitis C

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ABSTRACT

Natural killer cells play a key role in the immune control of viral infections. Killer immunoglobulin-like receptors (KIRs) regulate natural killer cell activation and inhibition through the recognition of their cognate HLA class I ligands. We assessed the predictive factors of a sustained virological response (SVR) in 200 Japanese patients with chronic genotype 1b hepatitis C who were treated with telaprevir (TVR), pegylated-interferon-α2b (PEG-IFN), and ribavirin (RBV) triple therapy (92 patients) or PEG-IFN/RBV therapy alone (108 patients). Sixteen KIR genotypes, HLA-A, -B and -C ligands, and an interleukin (IL) 28B polymorphism (rs8099917) were analyzed. We observed that triple therapy, white blood cell count, hemoglobin value, hepatitis C viral load, a rapid virological response (RVR), *IL28B* TT genotype, and *KIR3DL1-HLA-Bw4* genotype were associated with an SVR. In multivariate regression analysis, we identified an RVR ($P < 0.000001$; odds ratio [OR] = 20.95), the *IL28B* TT genotype ($P = 0.00014$; OR = 5.53), and *KIR3DL1-HLA-Bw4* ($P = 0.004$, OR = 3.42) as significant independent predictive factors of an SVR. In conclusion, *IL28B* and *KIR3DL1/HLA-Bw4* are independent predictors of an SVR in Japanese patients infected with genotype 1b HCV receiving TVR/PEG-IFN/RBV or PEG-IFN/RBV therapy.

1. Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide. More than half of patients with acute HCV infections progress to chronic hepatitis, which leads to liver cirrhosis and/or hepatocellular carcinoma (HCC) in at least 20% of cases [1]. HCC is a leading cause of death from malignant neoplasms in Japan [2]. Since approximately 70–80% of Japanese HCC patients are infected with HCV, viral eradication is considered important to decrease the incidence of HCC. Interferon (IFN)-based therapy can reduce HCV to undetectable levels and improve prognosis. The primary aim of antiviral therapy in HCV patients is a

Abbreviations: CI, confidence interval; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HLA, human leukocyte antigen; IL, interleukin; KIR, killer immunoglobulin-like receptors; OR, odds ratio; PEG-IFN, pegylated-interferon-α2b; RBV, ribavirin; RVR, rapid virological response; SVR, sustained virological response; TVR, telaprevir.

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