Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/j.1440-1746.2012.07171.x

HEPATOLOGY

Increase in platelet count based on inosine triphosphatase genotype during interferon beta plus ribavirin combination therapy

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Key words
chronic hepatitis C, inosine triphosphatase, natural interferon β, platelet count, ribavirin.

Accepted for publication 21 March 2012.

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Abstract

Background and Aim: The inosine triphosphatase (*ITPA*) genotype is associated with ribavirin-induced anemia and pegylated interferon α (PEG IFN-α)-induced platelet reduction during PEG IFN-α plus ribavirin combination therapy. Natural IFN-β plus ribavirin therapy is associated with increases in platelet counts during treatment. We investigated decreases in platelet counts according to *ITPA* genotype during natural IFN-β/ribavirin therapy to determine if patients with low platelet counts were eligible for this combination therapy.

Methods: A total of 187 patients with chronic hepatitis C received PEG IFN-α/ribavirin or natural IFN-β/ribavirin therapy. Decreases in platelet counts based on *ITPA* genotype were investigated during treatment through 24 weeks.

Results: Platelet counts decreased during week 1 of PEG IFN-α/ribavirin therapy, but increased during week 2, after which platelet counts decreased gradually. Platelet counts decreased until week 4 of natural IFN-β/ribavirin therapy, after which platelet counts increased. Platelet counts after week 8 were higher relative to pretreatment platelet counts. Patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts during natural IFN-β/ribavirin therapy than those with the *ITPA*-CA/AA genotype: platelet counts after week 8 of this therapy were higher than pretreatment platelet counts, regardless of pretreatment platelet counts. Multivariate logistic regression analyses showed that natural INF-β/ribavirin therapy was the only significant independent predictor for an increase in platelets through week 8.

Conclusion: Natural IFN-β/ribavirin therapy is safe for patients with the *ITPA*-CC genotype, even if their pretreatment platelet counts are low.

Introduction

The introduction of pegylated interferon-α (PEG IFN-α) plus ribavirin (PEG-IFN/RBV) combination therapy has led to an improved sustained virological response (SVR) rate in patients with chronic hepatitis C receiving IFN therapy.[1–6] However, cytopenia has been observed during PEG-IFN/RBV therapy. Specifically, cases of RBV-induced anemia and PEG-IFN-induced thrombocytopenia or neutropenia have been reported, and we have previously described cases of RBV-induced anemia.[7] A genome-wide association study (GWAS) identified the inosine triphosphatase gene (*ITPA*) single nucleotide polymorphism (SNP) as being strongly associated with RBV-induced anemia.[8–10] This *ITPA* SNP was also reported to play a role in the decreases in platelet counts that occur during PEG-IFN/RBV therapy.[11,12] In Japan, natural INF-β plus ribavirin (IFN-β/RBV) therapy has been indicated for the treatment of chronic hepatitis C. This therapy is associated with greater increases in platelet counts than seen with PEG-IFN/RBV therapy.[13] Therefore, we investigated the association between the *ITPA* genotype and decreases in platelet count during IFN-β/RBV therapy to determine if patients with a low platelet count were eligible for IFN-β/RBV therapy.

Methods

Patients. A total of 187 patients with chronic hepatitis C who received IFN therapy for at least 24 weeks at the Shinkokura Hospital between January 2009 and April 2011 were included in the study. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each

patient provided informed consent before participating in this trial.

Criteria for exclusion were as follows: (i) clinical or biochemical evidence of hepatic decomposition or advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma; (ii) IFN-β/RBV: a white blood cell count of less than 3×10^9/L and a platelet count of less than 50×10^9/L, PEG-IFN/RBV: a white blood cell count of less than 4×10^9/L and a platelet count of less than 80×10^9/L; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive); (iv) excessive active alcohol consumption exceeding 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy in the 12 months prior to enrollment.

IFN-β/RBV combination therapy. Interferon-β (Feron; Toray Industries, Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 20–44 weeks. The ribavirin (Rebetol; MSD, Tokyo, Japan) dose was adjusted according to body weight (600 mg for ≤ 60 kg; 800 mg for > 60 to ≤ 80 kg; and 1000 mg for > 80 kg), based on the guidelines of the Ministry of Health, Labor and Welfare of Japan.[5] The drug was administered orally after breakfast and dinner.

PEG-IFN/RBV combination therapy. Pegylated interferon-α-2B (PEG-Intron; MSD) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.3–1.5 μg/kg) once a week. Ribavirin was administered twice a day according to body weight, as described for IFN-β/RBV combination therapy.

This study was a prospective, nonrandomized open trial. Platelet counts and hemoglobin levels were measured at baseline and at weeks 1, 2, 4, 8, 12, and 24.

We genotyped each patient for two SNPs: rs8099917, an *IL28B* SNP previously reported to be associated with therapy outcome, and rs1127354 (14), an *ITPA* SNP reported to be associated with ribavirin-induced anemia[14] and decreases in platelet counts.[11] Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or with the Invader or TaqMan assay, as described elsewhere.[15–17]

Statistical analysis. Statistical analysis was performed using PASW Statistics, version 18 (SPSS, Chicago, IL, USA) and R, version 2.11. Categorical data were analyzed using the χ^2 test and Fisher's exact tests, and continuous data were analyzed using the nonparametric Mann–Whitney *U*-test. Univariate and multivariate logistic regression analyses were used to determine the factors that significantly contributed to the increase in platelets $> 0 \times 10^9$/L from week 0 through week 8. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P*-values found to be less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.1$) on univariate analysis were entered into a multiple logistic regression analysis to identify significant independent predictive factors. The potential pretreatment factors associated with increases in platelets $> 0 \times 10^9$/L from week 0 to week 8 included the following variables: age, sex, method of IFN treatment, hepatitis C virus (HCV) genotype, *ITPA* genotype, *IL28B* genotype, hemoglobin, platelet count, alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), and HCV RNA level.

Results

The clinical backgrounds of chronic hepatitis C patients before combination therapy with IFN-β/RBV or PEG-IFN/RBV are shown in Table 1. The mean age of patients receiving IFN-β/RBV therapy was 59.3 years and that of patients receiving PEG-IFN/RBV therapy was 57.9 years, with no difference between the two patient groups. The PEG-IFN/RBV group had more men, although the number was not significantly higher. All baseline laboratory parameters, including hemoglobin levels, platelet counts, ALT levels, γ-GTP levels, and HCV loads, showed no differences

Table 1 Clinical background before combination therapy with interferon β plus ribavirin (IFN-β/RBV) or pegylated interferon plus ribavirin (PEG-IFN/RBV) in chronic hepatitis C patients

		IFN-β/RBV	PEG-IFN/RBV	*P*-value
		n = 45	*n* = 137	
Age	Year (SD)	59.3 (14.3)	57.9 (10.4)	ns
Sex	M/F	22/23	73/64	ns
Hb	g/dL (SD)	14 (1.5)	14.2 (1.4)	ns
Platelet	10^9/L (SD)	178 (59)	183 (59)	ns
ALT	IU/L (SD)	84.1 (63.3)	76.5 (64)	ns
γ-GTP	IU/L (SD)	79.1 (56.29)	69.5 (58.5)	ns
HCV	logIU/mL (SD)	6.7 (1.1)	6.4 (0.9)	ns
HCV genotype	1/2	21/24	102/35	< 0.001
ITPA (rs1127354)	CC/CA or AA	36/9	99/38	ns
IL28B (rs8099917)	TT/TG or GG	35/10	96/41	ns
Decrease in platelet count at week 1	10^9/L (SD)	−47 (32)	−47 (43)	ns
Decrease in platelet count at week 4	10^9/L (SD)	−42 (33)	−28 (33)	< 0.05
Decrease in platelet count at week 8	10^9/L (SD)	19 (36)	−35 (43)	< 0.0001

ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HCV, hepatitis C virus; ITPA, inosine triphosphate pyrophosphatase; ns, not significant; SD, standard deviation.

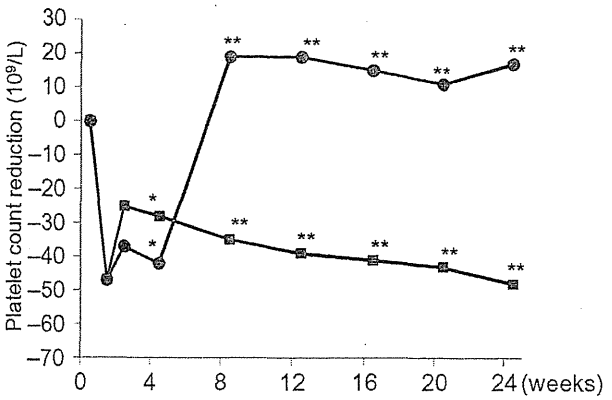

Figure 1 Decreases in platelet count during combination therapy with IFN-β/RBV or PEG-IFN/RBV (closed circle, IFN-β/RBV; closed square, PEG-IFN/RBV; $*P < 0.05$, IFN-β/RBV *versus* PEG-IFN/RBV at week 2; $**P < 0.0001$, IFN-β/RBV *versus* PEG-IFN/RBV at weeks 8, 12, 16, 20, and 24). IFN-β, interferon β; RBV, ribavirin; PEG-IFN, pegylated interferon.

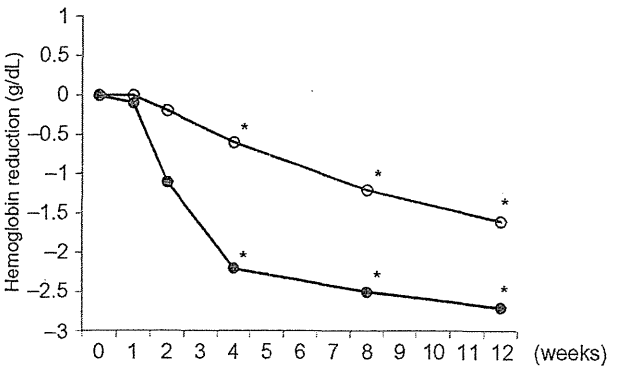

Figure 2 Decreases in hemoglobin levels according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with IFN-β/RBV (closed circle, *ITPA*-CC; open circle, *ITPA*-CA/AA; $*P < 0.01$, *ITPA*-CC *versus ITPA*-CA/AA (rs1127354) at weeks 4, 8, and 12). IFN-β, interferon β; RBV, ribavirin.

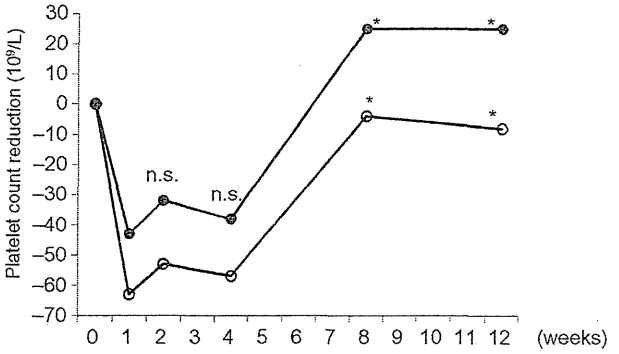

Figure 3 Decreases in platelet count according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with IFN-β/RBV (closed circle, *ITPA*-CC; open circle, *ITPA*-CA/AA; $*P < 0.05$, *ITPA*-CC *versus ITPA*-CA/AA (rs1127354) at weeks 8 and 12). IFN-β, interferon β; RBV, ribavirin.

between the two patient groups. Significantly more patients with HCV genotype 1 were in the PEG-IFN/RBV group ($P < 0.001$). A total of 74% (135/182) patients had the *ITPA*-CC genotype, while 72% of patients had the *IL28B* TT genotype. The frequencies of the *ITPA*-CC genotype and the *IL28B* TT genotype were comparable between the two patient groups. There was no difference in the decreases in platelet counts at week 1; however, at weeks 4 and 8, decreases in platelet counts differed significantly between the two patient groups ($P < 0.05$, $P < 0.0001$).

Platelet count decreases that occurred during combination therapy with IFN-β/RBV or PEG-IFN/RBV are depicted in Figure 1. A decrease in platelet counts of 47×10^9/L was observed at week 1 during IFN-β/RBV therapy. Subsequently, platelet counts transiently increased at week 2, but reduced again at week 4. Platelet counts reduced for 4 weeks after the start of treatment, as IFN-β/RBV therapy involved continuous, daily dosing with IFN-β for 4 weeks after the start of treatment. As per the treatment protocol, IFN-β administration was subsequently reduced to thrice-weekly dosing. At week 8, platelet counts increased and were significantly higher than the pretreatment platelet counts ($P < 0.001$). Platelet counts remained unchanged after week 8. A reduction of 47×10^9/L was observed at week 1 during PEG-IFN/RBV therapy, similar to the reduction that was observed during IFN-β/RBV therapy. Subsequently, platelet counts increased at week 2, decreased at week 4, and gradually decreased further after week 8. The decrease in platelet counts at week 4 during IFN-β/RBV therapy was significantly larger than the decrease observed during PEG-IFN/RBV therapy ($P < 0.05$). However, platelet counts after week 8 of IFN-β/RBV treatment were significantly higher than those during PEG-IFN/RBV therapy ($P < 0.0001$), due to a rapid increase in platelet counts after week 4 of the IFN-β/RBV regimen.

Decreases in hemoglobin levels in relation to the *ITPA* genotype (rs1127354: CC, CA/AA) are shown in Figure 2. At week 2, a large decrease in hemoglobin levels was observed in patients with the *ITPA*-CC genotype. There was no difference in hemoglobin levels based on *ITPA* genotype up to week 2 in patients receiving IFN-β/RBV therapy. Patients with the *ITPA*-CC genotype showed a significantly larger decrease in hemoglobin levels at weeks 4, 8, and 12 than those with the *ITPA*-CA/AA genotype ($P < 0.01$).

Platelet counts during combination therapy with IFN-β/RBV according to the *ITPA* genotype is shown in Figure 3. Similar changes in platelet count decreases were observed in patients with the *ITPA*-CC and *ITPA*-CA/AA genotypes. Patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts at weeks 1, 2, 4, 8, 12, 24 during therapy compared to those with the *ITPA*-CA/AA genotype. Specifically, patients with the *ITPA*-CC genotype showed a statistically lower degree of platelet decrease at weeks 8, 12, and 24 than those with the *ITPA*-CA/AA genotype ($P < 0.05$). Patients with the *ITPA*-CC genotype had significantly increased platelet counts at week 8 compared with the pretreatment platelet counts ($P < 0.0001$).

Decreases in platelet counts during combination therapy with PEG-IFN/RBV in relation to the *ITPA* genotype are shown in

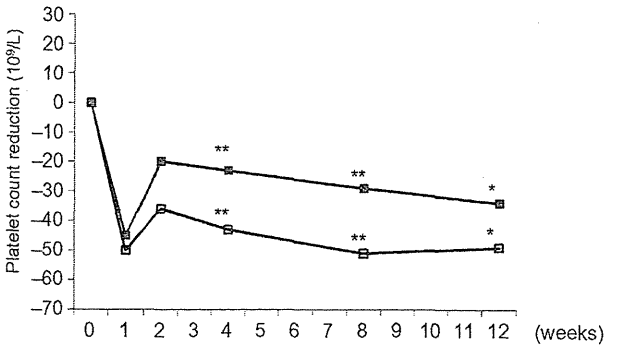

Figure 4 Decreases in platelet count according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with PEG-IFN/RBV (closed square, *ITPA*-CC; open square, *ITPA*-CA/AA; $*P<0.05$, *ITPA*-CC *versus* *ITPA*-CA/AA (rs1127354) at week 12; $**P<0.01$, *ITPA*-CC *versus* *ITPA*-CA/AA (rs1127354) at weeks 4 and 8). PEG-IFN, pegylated interferon; RBV, ribavirin.

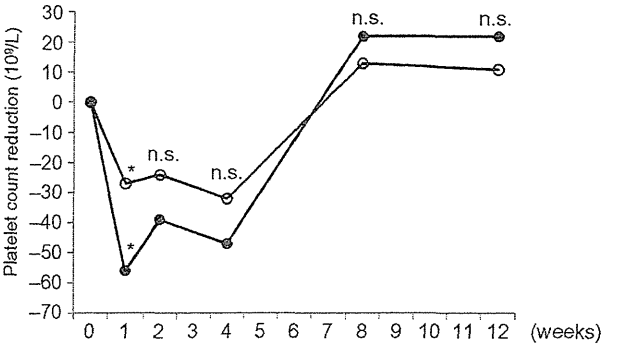

Figure 5 Decreases in platelet count relative to pretreatment platelet counts during combination therapy with IFN-β/RBV (closed circle, ≥ 150 × 10⁹/L; open circle, < 150 × 10⁹/L; $*P<0.05$, ≥ 150 × 10⁹/L *versus* < 150 × 10⁹/L at week 1). IFN-β, interferon β; RBV, ribavirin.

Figure 4. Similar changes in platelet count decreases were observed in patients with the *ITPA*-CC and *ITPA*-CA/AA genotypes. Patients with the *ITPA*-CC genotype showed a lower degree of platelet reduction at weeks 1, 2, 4, 8, 12, 24 during therapy compared to those with the CA/AA genotype. Specifically, patients with the *ITPA*-CC genotype had a significantly smaller decrease in platelet counts at weeks 4, 8, and 12 than those with the *ITPA*-CA/AA genotype ($P < 0.01$, $P < 0.05$).

Platelet reduction during combination therapy with IFN-β/RBV compared with pretreatment platelet counts is shown in Figure 5. At week 1, patients with a low pretreatment platelet count (< 150 × 10⁹/L) showed a significantly smaller decrease in platelet counts than those with a high pretreatment platelet count (≥ 150 × 10⁹/L; $P < 0.01$). Five patients had pretreatment platelet counts of ≤ 100 × 10⁹/L, and a decrease in platelet counts of ≤ 40 × 10⁹/L was observed in these patients at week 1. Patients with low pretreatment platelet counts showed a small decrease in platelet counts at week 1, after which there was no difference in platelet counts between the groups of patients with high and low

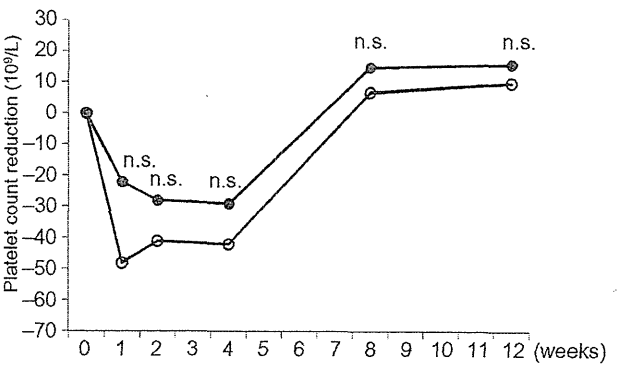

Figure 6 Decreases in platelet count according to inosine triphosphate pyrophosphatase *(ITPA)* genotype during combination therapy with IFN-β/RBV in patients with pretreatment platelet counts (< 150 × 10⁹/L) (closed circle, *ITPA*-CC; open circle, *ITPA*-CA/AA). IFN-β, interferon β; RBV, ribavirin.

pretreatment platelet counts. Among patients with both high and low pretreatment platelet counts, platelet counts at week 8 were significantly increased compared with pretreatment platelet counts ($P < 0.01$, $P < 0.05$).

Decreases in platelet counts according to *ITPA* genotype during combination therapy with IFN-β/RBV for patients with pretreatment platelet counts (< 150 × 10⁹/L) are shown in Figure 6. For patients with pretreatment platelet counts of < 150 × 10⁹/L, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts than those with the *ITPA*-CA/AA genotype.

The results of univariate and multivariate logistic regression analyses of factors associated with the increase in platelets > 0 × 10⁹/L from week 0 to 8 are shown in Table 2. Univariate and multivariate logistic regression analyses revealed that IFN-β/RBV therapy was the only significant independent predictor for the increase in platelets > 0 × 10⁹/L from week 0 to week 8.

Only one patient in the IFN-β/RBV group was withdrawn from the study by week 24. The reason for discontinuation was proteinuria. The dose of IFN was reduced only in the one patient. The dose of ribavirin was reduced in four of 45 patients, all of whom had the *ITPA*-CC genotype.

Discussion

This study showed that the platelet counts of patients undergoing IFN-β/RBV combination therapy for chronic hepatitis C infection after week 8 are higher than those before treatment. Moreover, patients with the *ITPA*-CC genotype showed a smaller decrease in their platelet counts not only during IFN-β/RBV, but also with PEG-IFN/RBV therapy, compared to those with the *ITPA*-CA/AA genotype. In particular, the results demonstrated that platelet counts after week 8 during IFN-β/RBV therapy were higher than pretreatment platelet counts, regardless of pretreatment platelet counts. Compared with pretreatment platelet counts, patients with the *ITPA*-CC genotype had markedly increased platelet counts after week 8 of IFN-β/RBV therapy. Multivariate logistic regression analyses showed that IFN-β/RBV therapy was the factor that contributed to increased platelet counts at week 8 relative to pretreatment platelet counts.

Table 2 Results of univariate and multivariate logistic regression analyses of factors associated with the increase in platelets > 0 (10^9/L) from week 0 to week 8

			Simple regression		Multiple logistic regression	
Factor	Range		Odds ratio	*P*-value	Odds ratio	*P*-value
Age (years)	≥ 60/< 60		1.219	0.389	–	–
Sex	Male/Female		1.219	0.554	–	–
Genotype	1/2		1.303	0.451	–	–
Method of IFN therapy	IFN-β/RBV/PEG-IFN/RBV		20.797	< 0.0001	23.596	< 0.0001
ITPA	CC/CA or AA		0.468	0.073	–	–
IL28B	TT/TG or GG		0.569	0.153	–	–
Baseline hemoglobin	< 14/≥ 14	g/dL	0.569	0.153	–	–
Baseline platelet count	< 150/≥ 150	10^9/L	0.737	0.399	–	–
Baseline ALT	≥ 50/< 50	IU/L	1.646	0.140	–	–
Baseline γ-GTP	≥ 45/< 45	IU/L	1.603	0.166	–	–
Baseline viral load	≥ 6.0/< 6.0	LogIU/mL	1.833	0.091	–	–

ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; IFN-β, interferon-β; ITPA, inosine triphosphate pyrophosphatase; RBV, ribavirin; PEG-IFN, pegylated interferon.

A GWAS identified several new host genetic variants that may be important for PEG-IFN/RBV therapy in chronic hepatitis C. One of these was the SNP in the *IL28B* gene that was strongly associated with therapy outcome,[18–21] and another was the *ITPA* gene that was associated with RBV-induced anemia during PEG-IFN/RBV therapy in chronic hepatitis C.[8–10]

Tanaka *et al.* reported that one SNP (rs11697186) located on the *DDRGK1* gene on chromosome 20 showed strong associations with a decrease in platelet counts in response to PEG-IFN/RBV therapy, and fine mapping with 22 SNPs around the *DDRGK1* and *ITPA* genes showed that rs11697186 had strong linkage disequilibrium with rs1127354, known as a functional variant of the *ITPA* gene.[11] We investigated the changes in platelet count decreases during IFN-β/RBV or PEG-IFN/RBV therapy relative to the *ITPA* genotype (CC, CA/AA). PEG-IFN/RBV therapy was associated with a larger decrease in hemoglobin levels among patients with the *ITPA*-CC genotype than those with the *ITPA*-CA/AA genotype.[8–10] A reactive increase in platelet counts was observed from week 1 through week 4 of treatment, with patients with the *ITPA*-CC genotype showing a higher degree of a reactive increase in platelet counts. This trend was similar to findings reported by Tanaka *et al.*, who reported that a reactive increase in platelet counts occurred secondary to RBV-induced anemia through week 4.[11]

In this investigation, decreases in hemoglobin levels were also observed from weeks 2 through 4 during IFN-β/RBV therapy. Secondarily, a temporary reactive increase in platelet counts occurred. IFN-β/RBV therapy involves continuous daily dosing of IFN-β for 4 weeks, and therefore, platelet counts typically decrease up until week 4, after which platelet counts rapidly increase following a reduction in the dosing frequency of IFN-β to thrice-weekly dosing. However, patients receiving IFN-β/RBV therapy had higher platelet counts at week 8 than pretreatment platelet counts. Arase *et al.* reported that platelet counts increased following a reduction in the dosing frequency of IFN-β from continuous daily dosing to thrice-weekly dosing.[15] We could demonstrate evidence of a relationship between the reduction of the dosing frequency of IFN-β and increases in platelet counts because we developed a treatment protocol using a 4-week continuous daily dosing of IFN-β and complied strictly with the protocol-defined duration of continuous daily dosing of 4 weeks. A higher degree of these recurrent increases in platelet counts was observed in patients with the *ITPA*-CC genotype than in those with the *ITPA*-CA/AA genotype. As with PEG-IFN/RBV therapy, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts during IFN-β/RBV therapy. In the present study, our results demonstrated that the *ITPA* genotype was strongly involved in platelet reduction during IFN therapy, in both PEG-IFN RBV and IFN-β/RBV therapy.

The *ITPA* genotype is strongly associated with ribavirin-induced anemia and IFN-induced platelet reduction, although the reasons for these associations are not clear. Erythropoietin (EPO) is produced when hemoglobin reduction occurs as a result of ribavirin-induced anemia. The sequence homology of thrombopoietin (TPO) and EPO may explain the synergy of the physiological roles of TPO and EPO in platelet production. When EPO is elevated, as in iron deficiency anemia, an amino acid sequence similar to TPO may increase the platelet count.[22]

In Japan, the IFN-β/RBV regimen used in the present study has been indicated for chronic hepatitis C patients receiving IFN-based therapy. The SVR rate among patients with HCV genotype 1 who were treated with IFN-β/RBV was lower (approximately 40%) than that among those treated with PEG-IFN/RBV.[13] We reported that IFN-β/RBV therapy was associated with a lower incidence of depressive symptoms or sleep disorders than PEG-IFN/RBV therapy.[23] Therefore, we have also used IFN-β/RBV therapy in elderly patients or patients with concurrent depression. Patients with HCV genotype 2 who were treated with IFN-β/RBV had an SVR rate of approximately 87%, which was similar to that observed in those treated with PEG-IFN/RBV.[24] This study is a prospective, nonrandomized open trial. Thus, the SVR rate among patients with HCV genotype 1 who were treated with PEG-IFN/RBV was higher than the SVR rate of those treated with IFN-β/RBV. IFN-β/RBV therapy was performed only in patients with depression or sleep disorder, thus the number of enrolled patients with HCV genotype 1 who were treated with IFN-β/RBV was small. As for patients with HCV genotype 2, since there was no difference in the SVR rate between IFN-β/RBV and PEG-IFN/RBV therapies, the number of enrolled patients was not different.

Therefore, more patients with HCV genotype 1 were included in the PEG-IFN/RBV group.

In this investigation, there were few discontinuations, dose reductions of IFN, and dose reductions of ribavirin in the IFN-β/RBV group. This is likely due to the fact that few patients developed anorexia, no patients showed weight loss, and dietary intake was adequate during the IFN-β/RBV therapy.

In the present study, patients with the *ITPA*-CC genotype showed a higher increase in platelet counts after week 8 during IFN-β/RBV therapy than those with the *ITPA*-CA/AA genotype. Platelet counts after week 8 were increased compared with pretreatment platelet counts, regardless of pretreatment platelet counts. In patients with low pretreatment platelet counts, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet count than those with the CA/AA genotype, and the platelet counts were increased after week 8. The IFN-β/RBV regimen appears to be a safe strategy for IFN therapy for patients with the *ITPA*-CC genotype, even if they have low pretreatment platelet counts.

The present study demonstrated that as with PEG-IFN/RBV therapy, patients with the *ITPA*-CC genotype showed a smaller decrease in platelet counts during IFN-β/RBV therapy. Platelet counts after week 8 of IFN-β/RBV therapy were increased compared with pretreatment platelet counts, regardless of pretreatment platelet counts. Therefore, we concluded that IFN-β/RBV therapy is safe for patients with the *ITPA*-CC genotype, even if their pretreatment platelet counts are low.

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J Gastroenterol (2011) 46:545–555
DOI 10.1007/s00535-010-0358-6

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Factors predictive of sustained virological response following 72 weeks of combination therapy for genotype 1b hepatitis C

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Received: 1 November 2010 / Accepted: 25 November 2010 / Published online: 19 January 2011

Abstract
Background Treatment of genotype 1b chronic hepatitis C virus (HCV) infection has been improved by extending peg-interferon plus ribavirin combination therapy to 72 weeks, but predictive factors are needed to identify those patients who are likely to respond to long-term therapy.
Methods We analyzed amino acid (aa) substitutions in the core protein and the interferon sensitivity determining region (ISDR) of nonstructural protein (NS) 5A in 840 genotype 1b chronic hepatitis C patients with high viral load. We used logistic regression and classification and regression tree (CART) analysis to identify predictive factors for sustained virological response (SVR) for patients undergoing 72 weeks of treatment.
Results When patients were separately analyzed by treatment duration using multivariate logistic regression, several factors, including sex, age, viral load, and core aa70 and ISDR substitutions ($P = 0.0003$, $P = 0.02$, $P = 0.01$, $P = 0.0001$, and $P = 0.0004$, respectively) were significant predictive factors for SVR with 48 weeks of treatment, whereas age, previous interferon treatment history, and ISDR substitutions ($P = 0.03$, $P = 0.01$, and $P = 0.02$,

Electronic supplementary material The online version of this article (doi:10.1007/s00535-010-0358-6) contains supplementary material, which is available to authorized users.

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respectively) were the only significant predictive factors with 72 weeks of treatment. Using CART analysis, a decision tree was generated that identified age, cholesterol, sex, treatment length, and aa70 and ISDR substitutions as the most important predictive factors. The CART model had a sensitivity of 69.2% and specificity of 60%, with a positive predictive value of 68.4%.

Conclusions Complementary statistical and data mining approaches were used to identify a subgroup of patients likely to benefit from 72 weeks of therapy.

Keywords CART analysis · Core protein · Decision tree · ISDR · LDL cholesterol

Abbreviations

HCV	Hepatitis C virus
ISDR	Interferon sensitivity determining region
CART	Classification and regression tree analysis
SVR	Sustained virological response
NR	Non-viral response

Introduction

Chronic hepatitis C virus (HCV) infection is a major global cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1–3]. The treatment of chronic hepatitis C has improved with the advent of peg-interferon (IFN) plus ribavirin combination therapy [4–7], but fewer than half of the patients with high viral loads of genotype 1b show a sustained virological response (SVR), defined as testing negative for HCV RNA 24 weeks after cessation of the therapy. To overcome this limitation, recent therapeutic regimens have extended the treatment period to 72 weeks [8–11]. This extension is especially effective in patients whose HCV RNA declines relatively slowly [9–11]. Accordingly, recent treatment protocols have recommended extending the treatment period to 72 weeks in patients who become negative for HCV RNA after 12 weeks of treatment but before 24 weeks [10, 11]. This response-guided decision-making approach to therapy has resulted in improvements of the SVR rate [10, 11]. Following this approach, patients with a non-viral response (NR), i.e., patients who show very poor response to the therapy (defined as less than 2-log decline of HCV RNA during 12 weeks of treatment), should be advised to discontinue therapy because SVR is rare in such patients. While response-guided therapy is useful in determining the appropriate duration of treatment for patients who are likely to respond eventually, predictors that can be assessed before the start of therapy will aid in differentiating which difficult-to-treat patients are likely to achieve an SVR with extended therapy and which may be better served by considering alternative therapy options.

To predict NR, recent studies recommend analysis of amino acid (aa) substitutions in the HCV core protein at positions 70 and 91 [12, 13]. The substitution of arginine with glutamine or other amino acids at core protein aa 70 has been reported to be associated with NR, and this finding was confirmed by several other groups [14–16]. Analysis of core aa 70 has also been shown to be useful to predict the outcome of 72 weeks of combination therapy [17]. While many factors have been reported to be useful predictors of the effect of combination therapy [18–26], many of these factors are mutually interdependent. Furthermore, because almost all of these factors have been reported under conditions in which a majority of patients were receiving 48 weeks of treatment, it is necessary to consider the effect of the treatment period.

In this study, we compiled a database of clinical data from 840 patients from 16 national centers in Japan. We used logistic regression and classification and regression tree analysis (CART) to identify factors predictive of SVR for 48- and 72-week therapy and to assess which patients are most likely to benefit by long-term 72-week therapy.

Methods

Study subjects

In this retrospective study, data from 840 patients with chronic hepatitis C treated at 16 different hospitals in Japan were analyzed for predictive factors for SVR based on

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Table 1 Patient characteristics for 48- and 72-week treatments

	All patients (n = 840)	48-Week therapy (n = 619) 73.69%	72-Week therapy (n = 221) 25.12%
Age (years)	54.4 ± 10.73	53.8 ± 11.21	56.2 ± 9.03
Gender (male/female)	449/391	357/262	92/129
Body weight (kg)	60.9 ± 10.8	61.3 ± 10.6	59.8 ± 11.4
Height (cm)	162.2 ± 9.1	162.7 ± 9.1	160.7 ± 9.0
BMI	23.0 ± 3.05	23.0 ± 2.92	23.0 ± 3.4
HCV core protein aa 70 (wild/mutant)	539/301	396/223	143/78
HCV core protein aa 91 (wild/mutant)	504/336	369/250	135/86
ISDR (0–1/≥2)	714/126	513/106	201/20
Hypertension (present/absent/ND)	538/113/189	395/78/146	143/35/43
Diabetes (present/absent/ND)	634/47/159	457/38/124	177/9/35
Transfusion (present/absent/ND)	505/227/108	379/162/78	126/65/30
Fibrosis stage (0–2/3–4/ND)	604/128/108	448/90/81	156/38/27
Activity stage (0–1/2–3/ND)	382/343/115	287/245/87	95/98/28
Steatosis (present/absent/ND)	158/344/338	119/250/250	39/94/88
AST (IU/l)	65 ± 49	66 ± 47	63 ± 53
ALT (IU/l)	68 ± 56	68 ± 56	66 ± 55
White blood cell count (/mm^3)	4832 ± 1455	4882 ± 1488	4693 ± 1352
Hemoglobin (g/dl)	14.2 ± 1.36	14.3 ± 1.39	14.1 ± 1.29
Platelets (×10^4/mm^3)	16.9 ± 5.18	17.0 ± 5.11	16.8 ± 5.35
γGTP (IU/l)	56 ± 59	59 ± 64	49 ± 42
Albumin (g/dl)	4.02 ± 0.348	4.01 ± 0.350	4.03 ± 0.343
Uric acid (mg/dl)	5.41 ± 1.29	5.46 ± 1.27	5.25 ± 1.35
Iron (μg/dl)	147.0 ± 69.65	151.0 ± 75.71	136.1 ± 47.45
Ferritin (μg/l)	173.9 ± 167.9	181.7 ± 175.7	153.0 ± 143.7
Fasting blood sugar (mg/dl)	99.8 ± 19.8	99.3 ± 19.1	101.2 ± 21.5
Alpha-fetoprotein (μg/l)	16.3 ± 50.4	14.2 ± 44.8	22.0 ± 62.7
Total cholesterol (mg/dl)	175 ± 32.3	173 ± 31.8	179 ± 33.4
LDL cholesterol (mg/dl)	100.8 ± 29.8	100.2 ± 30.3	102.5 ± 28.4
HDL cholesterol (mg/dl)	52.1 ± 15.5	51.4 ± 15.0	53.9 ± 16.6
Triglycerides (mg/dl)	103.2 ± 48.8	103.8 ± 46.1	101.7 ± 55.1
HCV-RNA (KIU/ml)	3239 ± 4669	3170 ± 4828	3427 ± 4205
Response to treatment (SVR/TR/NR)	465/246/129	341/164/114	124/82/15

BMI body mass index, *HCV* hepatitis C virus, *aa* amino acid, *ISDR* interferon sensitivity determining region, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γGTP* γ-glutamyl transpeptidase, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *SVR* sustained virological response, *TR* transient response/relapsers, *NR* non-viral response, *ND* not determined

treatment duration. Inclusion criteria included testing positive for HCV RNA for longer than 6 months and testing negative for both hepatitis B virus surface antigen and anti-HIV antibody. Patients with confounding conditions such as hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease were excluded. We excluded patients who were lost for follow up and those who did not show a high level of viremia for genotype 1b, as well as patients for whom we failed to determine both core and IFN sensitivity determining region (ISDR) of nonstructural protein (NS) 5A sequences; 385 patients were treatment-naïve. All subjects gave their written informed consent to participate in the study according to the process approved by the ethics committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki. Patient profiles are listed in Table 1.

All patients initially received weekly injections of peg-IFN-alpha-2b for 48 weeks (60 μg for body weight (BW) 35–45 kg, 80 μg for BW 46–60 kg, 100 μg for BW 61–75 kg, 120 μg for BW 76–90 kg, and 150 μg for BW 91–120 kg). Ribavirin was administered orally, and the dosage was determined based on the patient's BW (600 mg for <60 kg, 800 mg for 60–80 kg, and 1,000 mg

for >80 kg). Ribavirin dosage was reduced when hemoglobin levels were reduced to 10.0 g/dl and stopped if hemoglobin levels reached 8.5 g/dl. Successful treatment was ascertained based on SVR, defined as HCV RNA-negative 6 months after cessation of therapy. Using response-guided therapy, slow viral responders, i.e., patients for whom HCV RNA levels became negative after 12 weeks of therapy but before 24 weeks, and some non-responders were recommended for extension of therapy to 72 weeks.

Biochemical tests were performed at the individual hospitals, and pathological diagnosis was made by pathologists in each hospital according to the criteria of Desmet et al. [27]. Fibrosis and activity data were compared among hospitals to ensure that there were no systematic differences.

Analysis of viral titer and amino acid sequences in the core and ISDR region

The HCV RNA level was analyzed using reverse transcription polymerase chain reaction (RT-PCR)-based methods (Amplicor™ high-range test; Roche Diagnostics, Basel, Switzerland, or TaqMan RT-PCR test; Applied Biosystems, CA). The measurement ranges of these assays were 5–5000 KIU/ml and 1.2–7.8 log IU/ml, respectively. For values exceeding the measurable range, the limit value was used as an approximation. The values obtained by the Amplicor test were converted to logarithmic values [28].

Nucleotide and amino acid sequences of the core and the ISDR region were determined by direct sequencing of cDNA fragments amplified by PCR. Arginine and leucine were considered wild-type for core protein aa 70 and aa 91, respectively [12, 13]. The number of aa substitutions in the ISDR was determined by comparison with the reference sequence reported by Kato et al. [29] using the method of Enomoto et al. [30, 31].

Statistical analysis

Statistical analysis was performed using the R software package (http://www.r-project.org). The χ^2 or Fisher's exact and Mann–Whitney *U*-tests were used to detect significant associations. All statistical analyses were two-sided, and $P < 0.05$ was considered significant. Simple and multiple logistic regression analyses were used to examine the association between viral substitutions and clinical factors, using $P < 0.05$ as the criterion for inclusion in the initial multivariate model. Multivariate logistic regression analysis was performed using forward/backward stepwise selection based on the akaike information criterion (AIC) score and validated by bootstrapping, using the rms package in R. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each factor.

CART analysis

CART analysis was used to generate a decision tree by classifying patients by SVR, based on a recursive partitioning algorithm with minimal cost-complexity pruning to identify optimal classification factors. The SimpleCart classifier in the WEKA data mining package [32] was used with a minimal terminal node size of 4 and trained with the variables listed in Table 1. Performance was assessed using tenfold cross-validation, and the sensitivity, specificity, and precision of the model were calculated. Receiver operating characteristic (ROC) curves were generated and results were compared with the logistic regression model.

Results

Patient characteristics

Patients were partitioned into two groups based on whether they received 48 or 72 weeks of therapy (Table 1). In this study 465 patients achieved an SVR, whereas 375 patients were either non-responders or relapsers, yielding an overall SVR rate of 55.4%. The rate of SVR did not differ significantly between the 48- and 72-week treatment groups (55.3 vs. 56.4%, respectively; $P = 0.81$), but the NR rate was significantly lower in patients who were treated for 72 weeks (18.3 vs. 6.4%; $P = 9.3 \times 10^{-6}$).

Predictive factors for SVR

The association between SVR and individual clinical factors was assessed using logistic regression. A number of factors were significant at the $P < 0.05$ level, including age, sex, viral load, aa70/ISDR substitutions, hypertension, fibrosis, steatosis, prior IFN treatment, low-density lipoprotein (LDL) cholesterol, total cholesterol, white blood cell count, platelet count, hemoglobin, γ-glutamyl transpeptidase (γGTP), and albumin (Table 2). On multivariate logistic regression, only age, sex, core aa70, ISDR, LDL, and γGTP were identified as significant independent predictors of SVR. Although length of treatment was not identified as a significant predictor in this analysis, exploratory analysis suggests the presence of potential interactions between treatment length and age and/or sex that are not captured by the first-order terms in the model. When second-order terms were selected a posteriori, however, a significant interaction was found between sex and treatment length ($P = 0.0034$). When analyzed separately, independent predictive factors for SVR for 48 weeks

Table 2 Factors associated with sustained virological response to combination therapy

Variable	Simple			Multiple			
	n	OR	*P*	*n*	OR	(95% CI)	*P*
Age	840	0.393	3.16×10^{-11}***	517	0.386	(0.27–0.56)	5.08×10^{-7}***
Sex (male vs. female)	840	0.521	3.61×10^{-6}***	517	0.52	(0.35–0.78)	0.001459**
BMI (kg/m^2)	834	0.8	0.1094				
Viral load (Log IU/ml)	840	0.761	0.001828**				
Core aa70 substitution	840	0.537	1.98×10^{-5}***	517	0.507	(0.35–0.74)	0.000521***
Core aa91 substitution	840	0.818	0.1568				
ISDR (0–1 vs. ≥2)	840	2.36	5.19×10^{-5}***	517	2.12	(1.19–3.77)	0.01037*
Hypertension	651	0.625	0.02389*				
Diabetes	681	0.794	0.4464				
Blood transfusion	732	1	0.9788				
Fibrosis (F0–1 vs. F2–4)	732	0.674	0.008287**				
Activity (A0–1 vs. A2–4)	725	0.779	0.09567				
Steatosis	502	0.645	0.03413*				
Prior IFN treatment	830	1.37	0.02648*				
HDL cholesterol (mg/dl)	493	0.761	0.1333				
LDL cholesterol (mg/dl)	529	1.46	0.03223*	517	1.61	(1.10–2.38)	0.01521*
Triglyceride (mg/dl)	726	0.913	0.5412				
Total cholesterol (mg/dl)	814	1.25	0.11				
AST (IU/l)	783	0.933	0.6316				
ALT (IU/l)	840	0.972	0.837				
WBC (/mm^3)	836	1.55	0.001831**				
Hemoglobin (g/dl)	838	1.34	0.00276**				
Platelets ($\times 10^4$/mm^3)	838	1.74	7.92×10^{-5}***				
Gamma-GTP (IU/l)	823	0.735	0.0288*	517	0.656	(0.43–0.99)	0.04588*
Albumin (g/dl)	809	1.41	0.01699*				
Ferritin (μg/l)	532	0.898	0.5404				
Treatment period (weeks)	840	1.02	0.6095				

Simple and multiple logistic regression was used to examine the association between SVR and patient and viral factors. Factors with $P < 0.05$ were considered for inclusion in the multiple regression model and the best model selected by backwards stepwise selection using AIC

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

IFN interferon, *OR* odds ratio, *CI* confidence interval, *AIC* akaike information criterion

of treatment included age, sex, viral load, core aa70, LDL, platelets, and white blood cell counts, whereas for 72 weeks of treatment only age, ISDR, and prior IFN treatment were significant, although LDL cholesterol was marginally significant (Table 3).

Among patients who underwent 48 weeks of therapy, 61% of patients with core aa 70 wild-type achieved an SVR compared to only 44% of patients with mutant core aa 70 ($P = 1.8 \times 10^{-5}$, Fig. 1a), whereas for 72-week patients, the ratio was 1:1 (Fig. 3a). Conversely, in the 48-week group, 71% of patients with two or more mutations in the ISDR were able to achieve an SVR compared to 52% with the wild-type ISDR, and in the 72-week group (Fig. 1b), 80% of patients with two or more ISDR mutations achieved an SVR compared to 54% with zero or one ISDR mutations (Fig. 3b). Median baseline viral load was significantly lower in 48-week SVR patients compared to that in non-SVR patients ($P = 0.001$, Fig. 1c), whereas there was no significant difference between viral load and SVR in 72-week therapy patients ($P = 0.625$, Fig. 4c). There was a significant effect of age and treatment outcome among 48-week patients ($P = 9.3 \times 10^{-6}$, Fig. 2), but the difference was not significant among 72-week therapy patients. However, the proportion of patients achieving an SVR tended to decrease with age in both groups, particularly in females over age 70 years in the 72-week group (Figs. 2, 4).

Table 3 Independent factors associated with sustained virological response to 48- and 72-week peg-interferon plus ribavirin combination therapy

Variable	48 Weeks			72 Weeks			
	n	OR	*P*	*n*	OR	(95% CI)	*P*
Age	535	0.642	0.0165*	133	0.4	(0.176–0.91)	0.02877*
Sex (male vs. female)	535	0.481	0.000284**				
Viral load (Log IU/ml)	535	0.738	0.01033*				
Core aa70 substitution	535	0.454	9.95×10^{-5}**				
ISDR (0–1 vs. ≥2)	535	2.75	0.000358**	133	7	(1.35–36.2)	0.02047*
Fibrosis (F0–1 vs. F2–4)	535	0.66	0.03954*				
Prior IFN treatment				133	2.67	(1.22–5.85)	0.01431*
LDL cholesterol (mg/dl)				133	2.04	(0.952–4.35)	0.06673
WBC ($/mm^3$)	535	1.53	0.03342*				
Platelets ($\times 10^4/mm^3$)	535	1.54	0.03707*				

Simple and multiple logistic regression analysis was used to examine the association between SVR and patient/viral factors separately for patients receiving 48 and 72 weeks of treatment

** $P < 0.001$, * $P < 0.05$

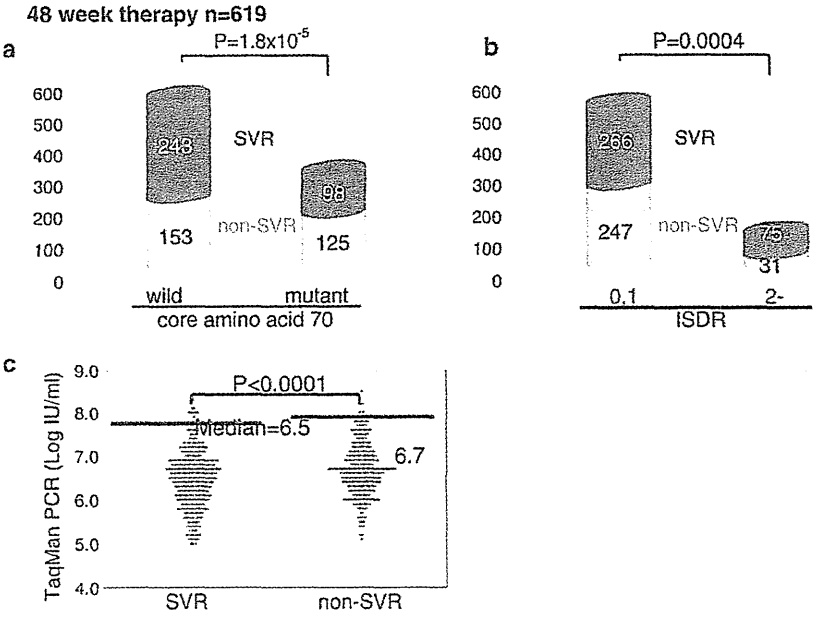
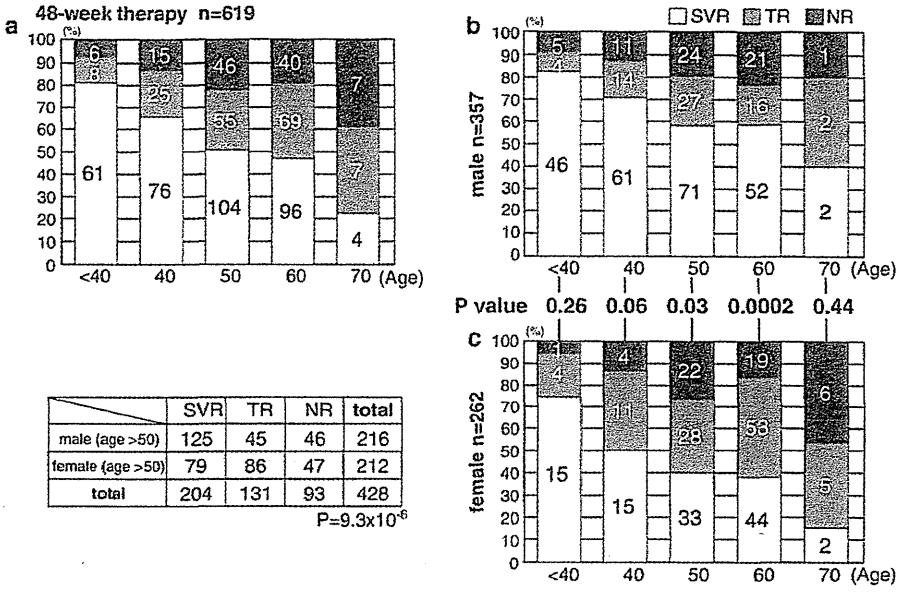


Fig. 1 Viral factors for 48-week treatment. Relationships between sustained virological response (*SVR*) and **a** core amino acid 70 substitutions, **b** amino acid substitutions in the interferon sensitivity determining region, and **c** baseline viral titers grouped by SVR and non-SVR for patients treated for 48 weeks. *PCR* Polymerase chain reaction

CART analysis

Figure 5 shows the decision tree generated by CART analysis. All variables were included during model construction, and the SimpleCart algorithm generated a tree based on the following fields: age, cholesterol, sex, γGTP, 48 versus 72 weeks of treatment, and aa substitutions in the ISDR and at core aa70. Age was used as the first cutoff, and patients younger than 46.5 years were classified as having a high probability for SVR (78%). Total cholesterol was identified as the next decision point, and patients with cholesterol higher than 211.5 mg/dl were classified as SVR if they were younger than 62.5 years (84%) and NR (65%) otherwise. Patients with cholesterol lower than 211.5 mg/dl were subdivided next by sex. Females who received 48 weeks of treatment were classified as NR (71%), whereas females receiving 72 weeks of treatment were classified as SVR if they were younger than 58.5 years (71%) or NR otherwise (64%). Males who were infected with aa70 wild-type were classified as SVR (62%), whereas males with aa70 substitutions were classified as NR if total cholesterol was less than 130 mg/dl (97%). Males with ISDR substitutions were classified as SVR (75%), and those with wild-type ISDR were classified



	SVR	TR	NR	total
male (age >50)	125	45	46	216
female (age >50)	79	86	47	212
total	204	131	93	428

$P=9.3\times10^{-6}$

Fig. 2 Relationship between age and response to treatment for 48-week therapy. Treatment outcomes by age in 10-year intervals are shown for **a** all patients, **b** males only, and **c** females only. *NR* non-viral response

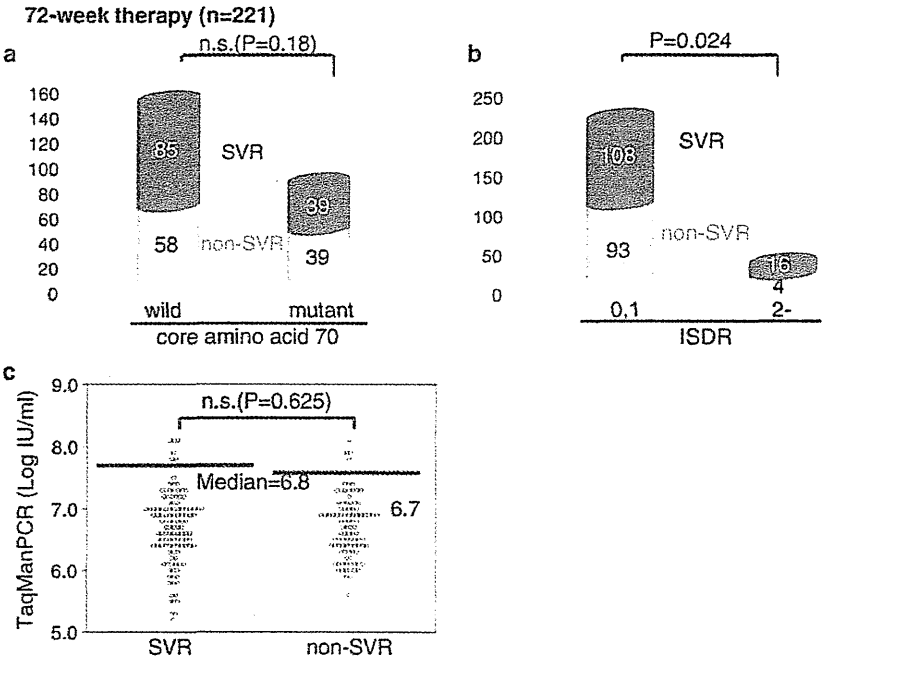
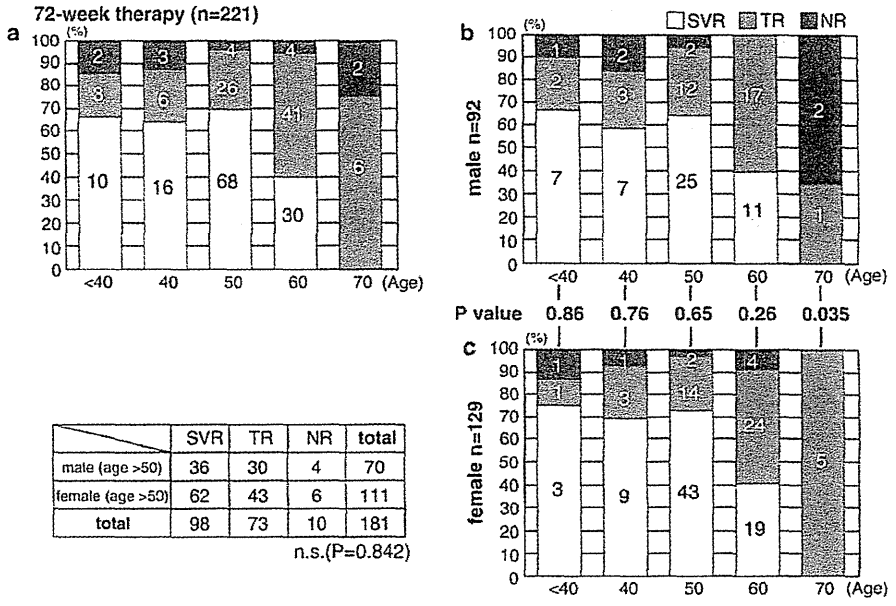


Fig. 3 Viral factors for 72-week treatment. Relationships between sustained virological response and **a** core amino acid 70 substitutions, **b** amino acid substitutions in the interferon sensitivity determining region, and **c** baseline viral titers grouped by SVR and non-SVR for patients treated for 72 weeks. *n.s.*, Not significant

as SVR if γGTP was less than 48.5 IU/l (57%) and NR otherwise (77%).

All factors selected during tree construction were found to be significant in univariate analysis, except for treatment length and cholesterol, and each remained significant in multivariate logistic regression. Although LDL was included in the multivariate logistic model, it was not selected during tree construction. Tenfold cross-validation resulted in 65.2% correctly classified instances with a kappa statistic of 0.29. The true positive rate was 69.2%, the false positive rate was 39.7%, and precision was 68.4%.

To compare the performance of SVR prediction between the logistic and CART models, the WEKA Logistic classifier was used to perform tenfold validation based on the



	SVR	TR	NR	total
male (age >50)	36	30	4	70
female (age >50)	62	43	6	111
total	98	73	10	181

n.s.(P=0.842)

Fig. 4 Relationship between age and response to treatment for 72-week therapy. Treatment outcomes by age in 10-year intervals are shown for a all patients, b males only, and c females only

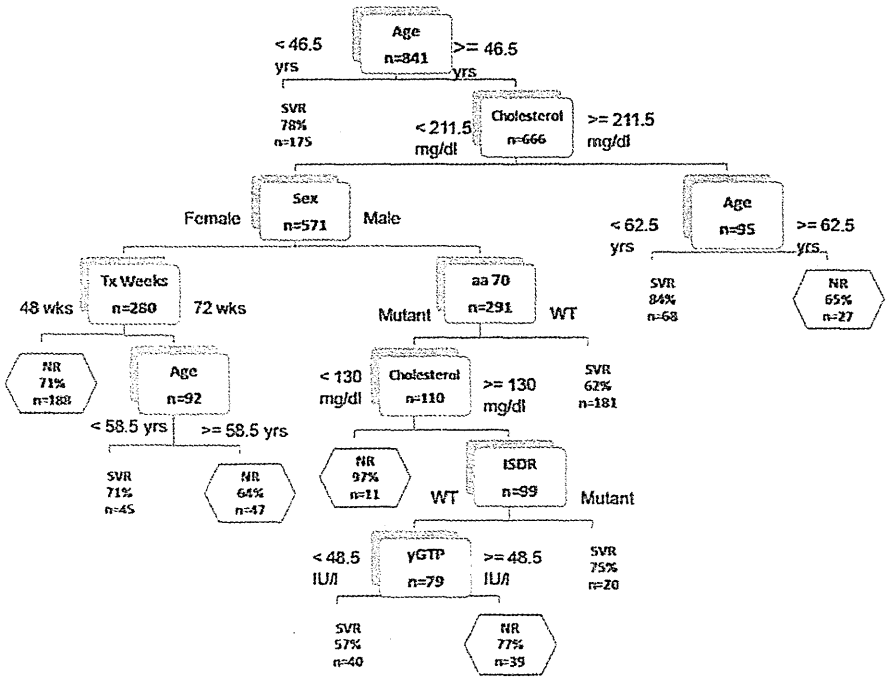


Fig. 5 Decision tree for SVR prediction. *Boxes* represent branch points based on cutoff values for factors determined by the tree generation algorithm. Each branch contains two choices, and each path ends in a prediction for either SVR or NR with an associated probability. *yrs* Years, *Tx* treatment, *ISDR* interferon sensitivity determining region, *aa* amino acid, *WT* wild-type, *γGTP* γ-glutamyl transpeptidase

multivariate logistic regression model above. The true positive rate for the logistic classifier was somewhat higher, at 73.1%, but with a slightly worse false-positive rate of 48%, and 63.7% correctly classified instances with a kappa statistic of 0.25 and precision 0.65. Receiver operating characteristic (ROC) curves were very similar, and the area under the curve was 0.677 for the CART model and 0.696 for the logistic model.

Discussion

Using two complementary approaches we identified several pretreatment factors predictive for SVR in patients treated for 48 and 72 weeks. Logistic regression and CART analysis both suggest that sex, age, cholesterol, and substitutions at core aa70 and ISDR are associated with SVR in patients with a high viral load of genotype 1b. Based on

the decision tree topology and a significant interaction between sex and treatment duration, it appears that 72 weeks of treatment may be most beneficial in women between the ages of 46 and 58 years who have low cholesterol. In general, patients who are younger, male, have cholesterol over 130 mg/dl, or who have wild-type core aa70 or mutant ISDR are the most likely to achieve an SVR.

Because each of the above values can be determined prior to treatment and are interpretable by clinicians, they may be useful as a guide when establishing a treatment regimen in the case of potentially difficult-to-treat patients. Once IFN treatment has been started, early and/or rapid viral response is likely to be the strongest predictor of SVR [33], and slow responders have been shown to be the most likely to benefit from extended treatment [34, 35]. However, because of the expense, low success rate, and potential side effects of IFN-based therapy, predictors available prior to treatment are also needed. Factors predictive of NR may help guide the decision to avoid or discontinue IFN therapy in patients with a low probability of SVR, and factors predictive of SVR may help identify subsets of patients who are likely to achieve an SVR if treated longer than the standard 48-week regimen.

Several other recent studies have examined predictors for SVR for 72 weeks of treatment, although nearly all focus on on-treatment predictors and conclude that 72-week therapy significantly improves SVR rates in slow responders [9, 10, 35]. Ferenci et al. [11] also showed that extension to 72 weeks decreased the relapse rate among early viral responders. In a large retrospective cohort study, Watanabe et al. [36] dissected a complex relationship between SVR and age, sex, and viral load similar to that reported here, although results are difficult to compare because they did not measure cholesterol or viral substitutions. While they recommend 72-week therapy for all slow-responding patients regardless of sex or age, they note that the SVR rate was surprisingly high among elderly female patients following 72-week treatment, noting that the SVR for 48-week treatment was typically low among older female patients in Japan, which they suggest could be related to the development of insulin resistance associated with menopause [36]. Other studies discourage the use of 72-week therapy for all patients except in the specific case of slow responders [8]. Moreover, in a large prospective study, Buti et al. [34] conclude that 48-week combination therapy should remain the standard of care even for slow responders, due to the increased cost and incidence of adverse events relative to a modest increase in the SVR rate. They clarify, however, that patients with a less than 2 log decline at week 8 and undetectable HCV RNA at week 24 are the most likely to benefit from 72-week treatment. Unfortunately they did not examine other predictors in a multivariate analysis. Because each of these studies hinges on rapid versus slow viral response and an on-treatment predictor requiring up to 24 weeks of treatment to establish, pretreatment predictors of early viral kinetics, including those presented here (e.g., viral substitutions and baseline cholesterol levels [12]), may be useful for predicting the outcome of extended therapy prior to treatment [17].

The combination of multiple approaches to identify predictive factors should help improve confidence in the results and partially protect against the bias inherent in any single approach. Comparing the results of a standard analysis with an alternative technique may reveal which variables are robust and which are sensitive to methodological differences. There are many different classification tools, including neural networks, Bayesian networks, and support vector machines, but models based on these may be more difficult to interpret or apply in clinical practice. On the other hand, decision tree approaches such as C4.5 and CART are widely used in biomedical studies [37–39] and provide a simple and intuitive hierarchical format that in many cases can be used without a computer.

The lack of randomized assignment of patients to duration of treatment limits the conclusions that can be drawn from the present study, and additional predictive factors, particularly interleukin (IL) 28B single-nucleotide polymorphism (SNP) genotype and viral kinetics, should be included in future prospective studies. Comparison of ROC curves suggests that the performance of the two models in the present study is similar, although neither is sufficiently sensitive or specific for accurate clinical prediction based on the number of patients analyzed. Nonetheless the strong overlap between the variables selected by each method suggests that several patient factors, including age, sex, and cholesterol level, as well as several viral factors, including core aa70 and ISDR substitutions, are robust predictors for SVR. Differences in the variables selected between the two approaches suggest that several models with similar predictive ability are also possible. In the regression model, LDL cholesterol but not total cholesterol was an independent factor associated with SVR, whereas in the CART analysis total cholesterol was selected instead. This may be due to the hierarchical nature of decision tree models, which may yield better results in the face of missing data, higher-order interactions, or nonlinear relationships. Comparison of separate models for 48 and 72 weeks also suggests that age and ISDR substitutions are important predictors of SVR for patients undergoing 72 weeks of treatment, whereas the decision tree suggests that the 72-week treatment length is important mainly for a subgroup of female patients. Without greater understanding of the role of HCV core and ISDR substitutions, it is difficult to interpret the role of these predictors, as well as

potential interactions with cholesterol level and other clinical factors. Further studies should be performed to investigate these interactions and to better characterize the subgroup of patients who are most likely to respond to long-term IFN therapy.

Acknowledgments This work was supported in part by Grants-in-Aid for scientific research and development from the Ministry of Health, Labor and Welfare and Ministry of Education, Culture, Sports, Science and Technology, Government of Japan. We thank Sakura Akamatsu and Mika Tsuzuno for their assistance.

Conflict of interest None of the authors have conflicts of interest to declare.

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Hepatology Research

Hepatology Research 2011; 41: 505–511 doi: 10.1111/j.1872-034X.2011.00807.x

Original Article

Recommendation of lamivudine-to-entecavir switching treatment in chronic hepatitis B responders: Randomized controlled trial

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Aim: In the 2007–2008 guidelines of the study group (Ministry of Health, Labor and Welfare of Japan), lamivudine (LAM)-continuous treatment was recommended in patients treated with LAM for more than 3 years who maintained hepatitis B virus (HBV) DNA less than 2.6 log copies/mL, because in these patients LAM resistance might exist and switching treatment to entecavir (ETV) might cause ETV resistance. However, there was no evidence on whether switching treatment to ETV- or LAM-continuous treatment was better in those patients. In the present study, we performed a randomized controlled trial of LAM-to-ETV switching treatment.

Methods: Twenty-seven patients treated with LAM for more than 3 years whose HBV DNA levels were less than 2.6 log copies/mL were enrolled and randomly divided into two groups, LAM-continued group or switching to ETV group. Then, we examined incidence of virological breakthrough (VBT) and breakthrough hepatitis (BTH) in each group.

Results: There was no BTH in any of the patients. VBT was observed in six patients of the LAM group (6/15, 40%), and no patient of the ETV group (0/11, 0%) ($P = 0.02$). The differences of the proportion of cumulated VBT using a log–rank test with Kaplan–Meier analysis were significant between the LAM and ETV groups ($P = 0.025$).

Conclusion: In patients treated with LAM for more than 3 years maintaining HBV DNA less than 2.6 log copies/mL, switching treatment to ETV is recommended at least during the 2 years' follow-up period.

Key words: chronic hepatitis B, entecavir, lamivudine, lamivudine resistance, randomized controlled trial, switching treatment

INTRODUCTION

OVER THE PAST two decades, treatment of chronic hepatitis B (CHB) has greatly improved with the availability of nucleos(t)ide analogs (NA), including lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine, clevudine and tenofovir. NA target

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Received 22 January 2011; revision 5 March 2011; accepted 21 March 2011.

the reverse transcriptase of hepatitis B virus (HBV), and are highly effective in suppressing HBV replication and clinical progression to liver cirrhosis and hepatocellular carcinoma in CHB patients.[1–4]

Lamivudine, ADV and ETV are commonly available in Japan. LAM, the first approved NA, has been shown to provide benefit for CHB patients with respect to the reduction of HBV DNA, normalization of alanine aminotransferase (ALT) and improvement of liver histology.[5,6] However, a serious problem of LAM is the high incidence of drug resistance during long-term treatment. The detection rate of LAM resistance has been reported to be 24% at 1 year and 70% after 5 years of treatment.[7–10] Even when the HBV DNA level was maintained at less than 2.6 log copies/mL, the accumulated incidence of LAM resistance reached 65% in patients treated with LAM for a long period (3 to ~10 years).[11] LAM resistance is caused by amino acid substitution(s) at rtM204V/I within the reverse transcriptase domain of the HBV polymerase gene.[12–14] The emergence of a LAM-resistant strain leads to virological breakthrough (VBT) and breakthrough hepatitis (BTH).

Recently, ETV has been demonstrated to exert antiviral efficacy in both NA-naïve and LAM-resistant CHB patients.[15–17] The frequency of ETV resistance has been reported to be 1.2% after 5 years of treatment in NA-naïve CHB patients.[18,19] On the other hand, in switching treatment to ETV for LAM-resistant CHB patients, the cumulative probability of ETV resistance increases.[17,20] After 5 years of treatment, 51% of LAM-refractory patients treated with ETV showed genotypic ETV resistance.[21]

The 2007–2008 guidelines of the study group (Ministry of Health, Labor and Welfare of Japan) for patients on LAM therapy are summarized in Table 1.[22] Regardless of duration of LAM administration, in cases where HBV DNA is more than 2.6 log copies/mL with BTH, ADV add-on treatment was recommended. In patients treated with LAM for less than 3 years who maintained HBV DNA of less than 2.6 log copies/mL or HBV DNA of 2.6 log copies/mL or more without BTH, switching to ETV was recommended. On the other hand, in patients treated with LAM for more than 3 years who maintained HBV DNA of less than 2.6 log copies/mL or HBV DNA of 2.6 log copies/mL or more without BTH, LAM-continuous treatment was recommended because in these patients LAM resistance might exist, and switching treatment to ETV might cause ETV resistance. However, there is insufficient evidence on whether switching treatment to ETV- or LAM-continuous treatment is better for CHB patients treated with LAM for more than 3 years with HBV DNA of less than 2.6 log copies/mL.

In the present study, we performed a randomized controlled trial of LAM-to-ETV switching treatment in CHB patients treated with LAM for more than 3 years who maintained HBV DNA of less than 2.6 log copies/mL.

METHODS

Patients

A TOTAL OF 27 CHB patients (mean age 55 ± 9 years, 17 men) from 11 institutions all over Japan (Hokkaido University Hospital, Tohoku University Hospital, Akita City Hospital, Kuramitsu Clinic, Juntendo University Hospital, Chukyo Hospital, Nagoya City University Hospital, Okayama University Hospital, Kawasaki Medical University Hospital, Ehime University Hospital, Shin-Kokura Hospital) were enrolled from April 2008. All the patients were followed at least 6 months after they were diagnosed with CHB. Their characteristics are shown in Table 2. They were treated with LAM (100 mg/day) for more than 3 years (median 50 months, range 36–106 months). Before starting LAM administration, all patients were positive for hepatitis B surface antigen (HBsAg) in serum, abnormal for ALT, detectable for HBV DNA, and were not

Table 1 2007–2008 guidelines of the study group (Ministry of Health, Labor and Welfare of Japan) for patients on lamivudine treatment

Duration of lamivudine treatment HBV DNA		<3 years	≥3 years
<2.6 log copies/mL, persistently		May be switched to ETV 0.5 mg/day	LAM 100 mg/day
≥2.6 log copies/mL	No BTH†	May be switched to ETV 0.5 mg/day	LAM 100 mg/day
	With BTH	Add on ADV 10 mg/day	Add on ADV 10 mg/day

†After checking for absence of LAM resistance.
ADV, adefovir; BTH, breakthrough hepatitis; ETV, entecavir; HBV, hepatitis B virus; LAM, lamivudine.

Table 2 Characteristics of LAM continuous group and ETV switch group at baseline

	LAM ($n = 15$)	ETV ($n = 11$)	*P*-value
Male	10	6	NS
Age	53 ± 7	57 ± 7	NS
Duration of LAM administration (month)	59 ± 23	55 ± 18	NS
ALT (IU/L)	33 ± 29	28 ± 22	NS
HbeAg positive	1	1	NS

ALT, alanine aminotransferase; ETV, entecavir; HBeAg, hepatitis B e-antigen; LAM, lamivudine; NS, not significant.

infected with hepatitis C virus and HIV. Patients diagnosed with alcoholism, primary biliary cirrhosis or autoimmune hepatitis were excluded.

Study design

The overview of this study design is shown in Figure 1. Twenty-seven patients treated with LAM for more than 3 years were enrolled, who showed HBV DNA of less than 2.6 log copies/mL at entry. They were randomly divided into two groups by each institution, the LAM-continued group (LAM group) or switching to the ETV group (ETV group). The primary end-points were the incidences of VBT and BTH in each group. VBT was defined as having more than 1 log copies/mL increase of HBV DNA level from the nadir on at least two occasions after initial virological response. BTH was defined as showing abnormal ALT level due to LAM or ETV resistance. All subjects were monitored at least every 3-month intervals. At every visit, routine examination with biochemical (ALT, bilirubin, albumin) and virological (HBV DNA level, hepatitis B e-antigen [HBeAg], anti-HBe) assessments took place. The mean follow-up period was 24 ± 3 months.

This study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) on 4 April 2008 as "A randomized trial of lamivudine continuous therapy and entecavir switching therapy for chronic hepatitis B patients treated with lamivudine monotherapy" (no. UMIN000001120).

The study protocol conformed to the Declaration of Helsinki, and was approved by the Committee for Ethics of Medical Experiments on Human Subjects of all the institutions, and written informed consent was obtained from every participant.

Serological and virological markers of HBV

Hepatitis B surface antigen, antibody against HBsAg (anti-HBs), HBeAg and antibody against HBeAg (anti-HBe) were determined using commercially available enzyme immunoassays. HBV DNA was determined by an Amplicor HBV Monitor (Roche Molecular Systems, Branchburg, NJ, USA; detection limit 2.6 log copies/mL)

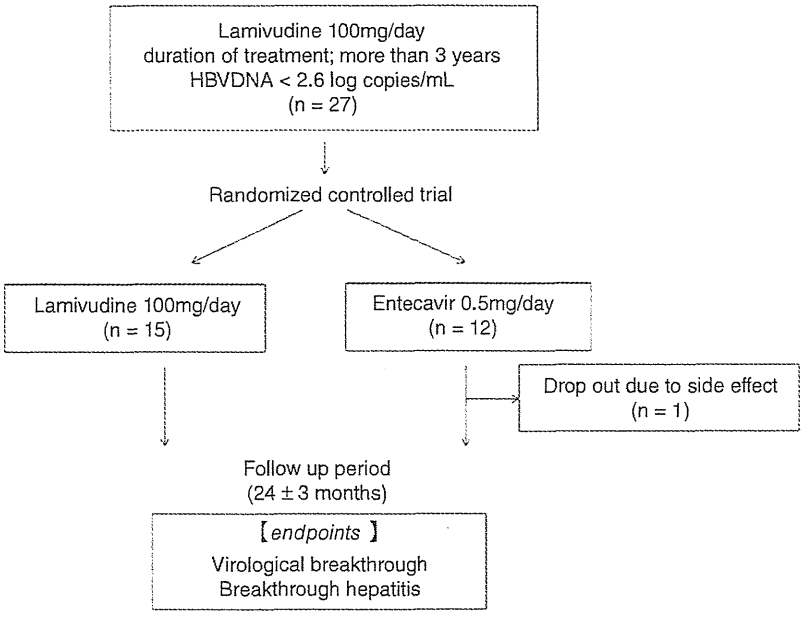

Figure 1 Overview of this study design. Twenty-seven patients treated with lamivudine for more than 3 years whose hepatitis B virus (HBV) DNA was maintained at <2.6 log copies/mL were enrolled. They were randomly divided into two groups by each institution, lamivudine-continued group or switching to entecavir group. We examined the incidence of virological breakthrough and breakthrough hepatitis in each group.