

Table 3 Results of univariate analysis for SVR to IFN therapy in patients with HCV-related cirrhosis

Factor	Category	SVR (n = 48)	Non-SVR (n = 58)	P-value
Age	> 56 years	29 (60.4%)	32 (55.2%)	NS
Sex	Male	31 (64.6%)	38 (65.5%)	NS
BMI	≥ 25 kg/m ²	14 (29.2%)	17 (29.3%)	NS
Albumin	> 4.0 g/dL	17 (35.4%)	18 (31.0%)	NS
AST	> 76 IU/l	23 (47.9%)	29 (50.0%)	NS
ALT	> 100 IU/l	14 (29.2%)	22 (37.9%)	NS
Cholesterol	> 160 mg/dL	26 (54.2%)	27 (46.6%)	NS
Choline esterase	> 1.0 ΔpH	22 (46.8%)	14 (25.5%)	0.037
AFP	> 20 μg/L	11 (23.9%)	24 (43.6%)	0.058
FBS	> 126 mg/mL	10 (23.3%)	6 (11.1%)	NS
Platelets	> 10 × 10 ⁴ /mL	27 (56.3%)	27 (46.6%)	NS
HCV genotype	1b	9 (18.8%)	12 (20.7%)	NS
HCV-RNA level	High	12 (25.0%)	36 (62.1%)	< 0.001
Total dose of IFN†	≥ 400 MU	21 (45.7%)	26 (44.8%)	NS
Daily dose of IFN†	≥ 6 MU	31 (67.4%)	25 (43.1%)	0.018
Duration of IFN	≥ 52 weeks	4 (8.3%)	13 (22.4%)	0.064
Induction therapy†	Yes	31 (67.4%)	24 (41.4%)	0.010
Type of IFN	Alpha	29 (60.4%)	38 (65.5%)	NS
Combination with ribavirin	Yes	5 (10.4%)	2 (3.4%)	NS

†Patients who were treated with peglated IFN were excluded from analysis.

High viral load ≥ 100 KIU/mL or ≥ 1 Meq/mL.

AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, fasting blood sugar; HCV, hepatitis C virus; IFN, interferon; NS, not significant; SVR, sustained virological response.

therapy in patients with HCV-related cirrhosis.^{10–13} In addition, the incidence of hepatocarcinogenesis is reduced in patients with SVR to IFN.¹⁷

Previous studies of patients with chronic hepatitis C indicated that HCV genotype and HCV-RNA level are the most significant factors that contribute to SVR and it is assumed that patients with genotype 1b and high viral load respond poorly to IFN therapy.⁷ In addition, IFN is less effective in patients with advanced fibrosis than those without.^{7,18} In the present study, we retrospectively reviewed the efficacy of IFN therapy and subsequent cancer prevention effect among patients with HCV-related compensated cirrhosis. Patients with both HCV

genotype 1b and high viral load were excluded because the rate of HCV elimination among these patients was extremely low and the anticancer effect of such therapy was expected to be low.

In our study, even in patients in whom fibrosis progressed to liver cirrhosis, the SVR ratio was 42.9% with genotype 1b-low HCV concentration, 25.8% with genotype 2a-high HCV concentration, 74.3% with genotype 2a-low HCV concentration, and 23.5% with genotype 2b-high HCV concentration. Thus, the therapeutic efficacy of IFN was approximately equal for liver cirrhosis and chronic hepatitis. Univariate analysis identified four parameters that significantly influenced the SVR. These

Table 4 Results of multivariate analysis for SVR to IFN for HCV-related cirrhosis

Factors	Category	Risk ratio (95% CI)	P-value
HCV-RNA level	1: low viral load	6.99 (2.72–17.9)	< 0.001
	2: high viral load	1	
Daily dose of IFN	1: ≥ 6 MU	2.62 (1.04–6.67)	0.042
	2: < 6 MU	1	

High viral load > 100 KIU/mL or > 1 Meq/mL; low viral load < 100 KIU/mL or < 1 Meq/mL.

CI, confidence interval; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.

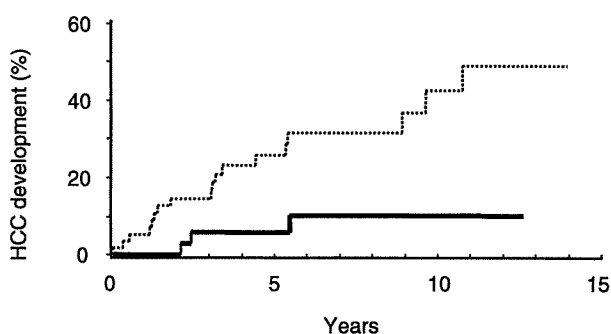


Figure 1 Cumulative rate of development of hepatocellular carcinoma (HCC) in patients treated with interferon. The rates of development of HCC in sustained virological response (SVR) patients at 5 years (5.8%) and 10 years (10.3%) were significantly lower than the respective rates in non-SVR patients (25.8%, 42.5%, $P = 0.007$). —, SVR; ·····, non-SVR.

were viral load, daily dose of IFN, presence of induction therapy and choline esterase.

According to a previous study, the SVR rate was higher among patients who received long-term and high daily dose of IFN.¹³ Although the duration of IFN

therapy was marginally longer in that study compared to the present study, the results were almost similar to those reported here. In addition, as the advantage of induction therapy among patients with chronic hepatitis has been emphasized in Japan, our result indicates that induction therapy is also important for cirrhotic patients. While the total dose of IFN was also reported previously to correlate with SVR,¹⁹ no significant effect for the dose was recognized in our study. Because the design of our study was not prospective, the treatment schedule varied among patients, and therefore many biases may exist in our results. Further prospective studies are needed to confirm our findings. Multivariate analysis showed that viral load (< 100 KIU/mL) independently influenced SVR, and the SVR rate was as high as 63.2% in those patients with low viral load. As patients with genotype 1b-high viral load were not included in our study, HCV genotype was not significant. When we compared only patients with low viral load, 9/21 (42.9%) patients with genotype 1b, 26/35 patients (74.3%) with genotype 2a, and one patient with genotype 2b showed SVR. Thus, the therapeutic effect of IFN in patients with genotype 2 was significantly high ($P = 0.023$).

Table 5 Results of univariate analysis for development of hepatocellular carcinoma after IFN therapy

Factor	Category	HCC (+) <i>n</i> = 22	HCC (-) <i>n</i> = 97	<i>P</i> -value
Age	> 56 years	16 (72.7%)	50 (51.5%)	0.080
Sex	Male	16 (72.7%)	63 (64.9%)	NS
BMI	≥ 25 kg/m ²	4 (18.2%)	32 (33.0%)	NS
Albumin	> 4.0 g/dL	3 (13.6%)	49 (50.5%)	0.043
AST	> 76 IU/L	11 (50.0%)	47 (48.5%)	NS
ALT	> 100 IU/L	11 (50.0%)	44 (45.4%)	NS
Cholesterol	> 160 mg/dL	13 (59.1%)	49 (50.5%)	NS
Choline esterase	> 1.0 ΔpH	3 (14.3%)	40 (42.1%)	0.009
AFP	> 20 μg/L	9 (42.9%)	31 (32.6%)	NS
FBS	> 126 mg/mL	2 (9.5%)	13 (14.4%)	NS
Platelets	> 10×10^4 /mL	11 (50.0%)	47 (48.5%)	NS
HCV genotype	1b	16 (72.7%)	81 (83.5%)	NS
HCV-RNA level	High	10 (45.5%)	51 (52.6%)	NS
IFN efficacy	SVR	3 (14.3%)	43 (52.4%)	0.007
Total dose of IFN	≥ 400 MU	10 (45.5%)	44 (48.9%)	NS
Daily dose of IFN	> 6 MU	8 (36.4%)	48 (52.7%)	0.100
Duration of IFN	≥ 52 weeks	7 (31.9%)	22 (22.7%)	NS
Induction therapy	Yes	8 (36.4%)	50 (54.9%)	NS
Type of IFN	Alpha	15 (68.2%)	65 (67.0%)	NS
Combination with ribavirin	Yes	0 (0.0%)	10 (10.3%)	NS

High viral load ≥ 100 KIU/mL or ≥ 1 Meq/mL.

AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, fasting blood sugar; HCV, hepatitis C virus; IFN, interferon; NS, not significant; SVR, sustained virological response.

Table 6 Results of multivariate analysis for development of hepatocellular carcinoma after IFN therapy

Factor	Category	Hazard ratio (95% CI)	P-value
IFN efficacy	1: SVR	0.185 (0.042–0.810)	0.025
	2: non-SVR	1	

CI, confidence interval; IFN, interferon; SVR, sustained viral response.

Our results showed that the incidence of HCC increased in a time-dependent manner after induction of IFN (16.8% at the fifth year, 29.1% at the 10th year, and 34.2% at the 15th year). Compared with the rates in Child A cirrhosis patients in our institution who had not been treated with IFN (32.5, 59.6 and 77.4%, respectively),¹ the rate of hepatocarcinogenesis after induction of IFN was half. These results suggest that hepatocarcinogenesis is significantly reduced in patients treated with IFN.

Our retrospective analysis of 123 patients with HCV-related cirrhosis who received IFN treatment showed that the outcome of IFN therapy was significantly associated with carcinogenesis. SVR was associated with a significantly reduced risk of carcinogenesis (hazard ratio: 0.185) compared with non-SVR. Three patients with SVR developed HCC; Case 1 with genotype 2a-low viral load showed SVR after 6 weeks of 3MU IFN- β therapy, but developed HCC 28 months later. Case 2 with genotype 2a-low viral load showed SVR after 6 weeks of 3MU IFN- β therapy but developed HCC 5 years later. Case 3 was also genotype 2a-low viral load who developed HCC at the end of 24 months of 6 MU IFN- α three times per week therapy. According to our previous study, the tumor-doubling time of small HCC ranges from 227 to 607 days (median, 392).²⁰ The tumor diameter at the time of diagnosis of HCC was 20 mm in Case 1, 26 mm in Case 2 and 10 mm in Case 3. Therefore, it is not clear whether hepatocarcinogenesis developed before or after eradication of HCV.

There was no relationship between the duration of IFN therapy and the rate of hepatocarcinogenesis. Likewise, the duration of IFN therapy and the total dose of IFN did not correlate with hepatocarcinogenesis. It should be noted that 38 patients had been treated with IFN prior to the present study and, thus, we cannot exclude the influence of previous IFN therapy on the development of HCC. Unfortunately, we could not collect data on the type, dose or duration of IFN for these patients because such treatment was carried out in other hospitals. To compensate for this, we analyzed those 85 patients who received IFN for the first time. In

these patients, neither the duration of IFN therapy nor the total dose of IFN was significantly associated with the development of HCC.

Our results indicated that long-term IFN therapy in non-SVR patients does not seem to improve long-term prognosis, such as the development of HCC and progression to decompensation. We reported previously that the rate of hepatocarcinogenesis was approximately equal in non-responders and untreated patients with genotype 1b-high virus load.²¹ However, the rate was lower in BR cases who were maintained on long-term IFN therapy.²¹ In three patients who developed HCC during IFN therapy in the present study, ALT normalization was noted after giving twice or thrice per week IFN- α (3MU), a therapeutic course aimed at reducing the risk of hepatocarcinogenesis. Although the duration of IFN therapy was 3.3, 4.2 and 5.3 years and serum ALT was almost below the upper limit of normal, HCC appeared in these patients during the course of IFN therapy. It is difficult to conclude from the present study that SVR is the only factor that reduced the risk of hepatocarcinogenesis. Although the result of statistical analysis was not significant in our patients, hepatocarcinogenesis was less frequent in patients with BR than in those with NR (the rate of HCC development at 5 years was 17.5% vs 29.4%, respectively, data not shown). A larger study is needed to determine the impact of BR on hepatocarcinogenesis among non-SVR patients.

In the present study, only seven of 123 patients (5.7%) discontinued IFN therapy. None developed life-threatening serious adverse effects. This outcome is probably similar to that reported in chronic hepatitis.²¹ However, careful follow up is necessary in such cirrhotic patients including liver function tests, platelet and leukocyte counts.

In summary, the SVR rate to IFN therapy was 48.0% in patients with HCV-related compensated cirrhosis except for those patients infected with genotype 1b-high virus load. The SVR was especially high (63.2%) among patients with low viral loads. The risks of hepatocarcinogenesis decreased to almost 50% in all patients treated with IFN and to only 20% among patients with SVR.

Therefore, IFN therapy is strongly recommended for patients with compensated cirrhosis with genotype 1b-low viral load or genotype 2.

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Loss of Hepatitis B Surface Antigen From the Serum of Patients With Chronic Hepatitis Treated With Lamivudine

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Although loss of hepatitis B e antigen (HBeAg) from the serum is sought by treatment with lamivudine, clearance of hepatitis B surface antigen (HBsAg) is the eventual goal of any antiviral therapy. In a single hepatology center in the Metropolitan Tokyo, 486 patients with chronic hepatitis B were followed up for longer than 3 years after they started treatment with lamivudine. HBsAg disappeared from the serum in 17 (3.5%). Age ≥ 50 years and low HBsAg levels (hemagglutination titer $\leq 2^7$) at the start of lamivudine were significantly more frequent in the patients who did than did not lose HBsAg from the serum. Except for these two factors, there were no differences between the two groups of patients in the prevalence of HBeAg and HBV DNA levels at the baseline, as well as the development of YMDD mutants and breakthrough hepatitis during lamivudine treatment. Using multivariate analysis, age ≥ 50 years at the start of lamivudine was the only factor predicting the loss of HBsAg (hazard ratio: 2.96 [95% confidence interval: 1.14–7.68], $P=0.028$). By the method of Kaplan–Meier performed on the 486 patients, the loss of HBsAg was estimated to occur in 3% and 13% of patients, respectively, who had received lamivudine therapy for 5 and 10 years. These results indicate that loss of HBsAg occurs in a minority (3.5%) of patients with chronic hepatitis B who receive lamivudine therapy and more frequently in those with lower HBsAg titers and older ages at the start of treatment. *J. Med. Virol.* 79:1472–1477, 2007. © 2007 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis; hepatitis B virus; hepatitis B surface antigen; hepatitis B e antigen; lamivudine

INTRODUCTION

Over the world, an estimated 400 million people are infected persistently with hepatitis B virus (HBV), which may progress to chronic hepatitis, cirrhosis and hepatocellular carcinoma [Lai et al., 2003]. Many antiviral drugs have been used for preventing the development of liver disease. Among these, lamivudine was approved for clinical use in 1995 and has gained popularity for the treatment of chronic hepatitis B [Lai et al., 1998; Dienstag et al., 1999; Schalm et al., 2000]. The major goal of lamivudine therapy is loss of hepatitis B e antigen (HBeAg) from the serum, because it reflects decreased HBV replication in the liver [Magnius and Espmark, 1972]. HBV mutants resistant to lamivudine, however, elicits in recipients in parallel with the duration of treatment; they have mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the DNA polymerase/reverse transcriptase [Honkoop et al., 1997; Chayama et al., 1998; Liaw et al., 1999]. Although YMDD mutants induce breakthrough hepatitis, they can be treated by rescue treatments with other antiviral drugs [Suzuki et al., 2002; Hosaka et al., 2004], thereby enabling long-term treatment with lamivudine for 7 years or longer [Kumada, 2003; Akuta et al., 2005].

Loss of hepatitis B surface antigen (HBsAg) from the serum is regarded as the eventual goal of antiviral therapy, because it improves long-term clinical outcomes of HBV infection [Fattovich et al., 1998; de Franchis et al., 2003; Lok and McMahon, 2004]. Data

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are lacking, however, on the efficacy of lamivudine in achieving loss of HBsAg from the sera of patients. In a single hepatology center in Metropolitan Tokyo, 486 patients with chronic hepatitis B were followed-up for the loss of HBsAg. Pretreatment factors influencing the loss of HBsAg were evaluated by univariate and multivariate analyses.

MAERIALS AND METHODS

Patients

During 10 years from 1995 to 2004, 486 patients with chronic hepatitis B received 100 mg of lamivudine daily, and had been followed for 3 years or longer at the Department of Hepatology, Toranomon Hospital in Tokyo, Japan. They had a median age of 43 years (range: 18–76 years) and included 399 (82.1%) men. Chronic hepatitis was diagnosed in 385 (79.2%) by liver biopsies performed under laparoscopy and/or ultrasonic imaging, and cirrhosis in the remaining 101 (20.7%) by liver biopsy and/or ultrasonography plus laparoscopic findings. They had a median serum level of HBV DNA of 7.2 log genome equivalents (LGE) per milliliter, and 338 (69.5%) of them were positive for HBeAg. They received lamivudine for the median of 4.8 years (range: 0.1–15.8 years), and were followed up for the median of 5.0 years (3–15) after the treatment had been started. Lamivudine was discontinued in only 95 (19.5%) patients. Reasons for withdrawal were a change to another antiviral drug in 41, the wish of patients in 29, loss of HBeAg accompanied by normalized ALT levels in 19 and side effects in 6. HBV genotypes were A in 10 (2.1%) patients, B in 32 (6.6%), C in 438 (90.1%), F in 2 (0.4%) and not typeable in the remaining 4 (0.8%) patients.

The 502 patients were followed-up for loss of HBsAg, at least 3 years or longer after treatment with lamivudine had been started, and pretreatment factors predictive of the loss were evaluated by univariate and multivariate analyses. The study design conformed to the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of the institution. Every patient gave an informed consent on the purpose of this study.

Markers of HBV Infection

HBsAg and the corresponding antibody (anti-HBs) were determined by hemagglutination (MyCell; Institute of Immunology Co., Ltd., Tokyo, Japan) and HBeAg by enzyme-linked immunosorbent assay (ELISA) (F-HBe; Sysmex, Kobe, Japan). HBV DNA was determined by transcription-mediated amplification (TMA; Chugai Diagnostics, Tokyo, Japan) and the results were expressed in LGE/ml over a range from 3.7 to 8.7. The six major genotypes of HBV (A–F) were determined serologically by ELISA (HBV GENOTYPE EIA; Institute of Immunology) and the PCR-Invader method with genotype-specific probes [Tadokoro et al., 2006]. YMDD mutants were determined by polymerase chain reaction

(PCR) followed by restriction fragment length polymorphism (RFLP) after the method of Chayama et al. [1998].

Statistical Analysis

Factors influencing the loss of HBsAg were evaluated by the log-rank test. Independent factors associated with clearance of HBsAg from the serum by lamivudine treatment were analyzed with a stepwise Cox regression analysis. The relationship between loss of HBsAg and duration of lamivudine therapy was assessed by the method of Kaplan–Meier. Analysis of all data was performed with the computer program SPSS software (SPSS, Inc., Chicago, IL).

RESULTS

Comparison of Baseline Characteristics Between Patients Who Did and Did Not Lose HBsAg From the Serum

During follow-ups for 3 years or longer after the start of lamivudine treatment, 17 of the 486 (3.5%) patients lost HBsAg from the serum. All the 17 patients had lost HBV DNA detectable by a semiquantitative method before HBsAg loss, while only two (12%) patients seroconverted to anti-HBs. Table I compares demographical, clinical and virological characteristics between the patients who did and did not lose HBsAg from the serum by univariate analysis. Older age (≥ 50 years) and lower HBsAg levels (hemagglutination titer $\leq 2^7$) at the start of lamivudine were significantly more frequent in the patients who did than did not lose HBsAg from the serum. Except for these two factors, there were no differences between them in ALT levels, HBeAg, HBV DNA, and genotypes, and emergence of YMDD mutants as well as breakthrough hepatitis during lamivudine treatment. Among the 14 factors listed in Table I, however, only the age ≥ 50 years at the start of lamivudine therapy was found to increase the chance for HBsAg loss by multivariate analysis (Table II).

Loss of HBsAg From the Serum of the 17 Patients

Figure 1 illustrates loss of HBsAg from the serum of the 17 patients who received treatment with lamivudine. HBsAg was cleared after the withdrawal of lamivudine treatment in three (18%) of them (cases 1–3). In the remaining 14 patients, HBsAg was cleared from the serum during 3.2–10.8 years while they received lamivudine. Lamivudine was withdrawn in one patient (case 4) after he had received it for 3 years, but it was resumed 6 months thereafter. Anti-HBs developed in sera of cases 2 and 6 after they lost HBsAg.

Within 3 years after the start of lamivudine therapy, HBsAg was cleared from the serum more frequently in the patients who did than did not possess HBeAg at the baseline (57% [4/7] vs. 10% [1/10], $P = 0.036$). Genotypes of HBV were B in two, C in 14 and D in the remaining one; none were infected with HBV genotype A.

TABLE I. Univariate Analysis for Factors Influencing the Loss of HBsAg in Patients Treated With Lamivudine

Factors	Category	HBsAg		Differences (P-value) ^a
		Lost (n = 17)	Persisted (n = 469)	
Age	≥50 years	9 (83%)	121 (26%)	0.017
Gender	Male	16 (94%)	382 (82%)	NS
HBV infection in mother	Positive	6 (32%)	180 (38%)	NS
History of liver disease	Chronic hepatitis	14 (82%)	371 (79%)	NS
ALT (IU/l)	≥60 IU/l	13 (76%)	360 (77%)	NS
Total bilirubin	≥0.7 mg/dl	14 (82%)	292 (62%)	NS
Cholin esterase	≥1.2 ΔpH	7 (41%)	181 (34%)	NS
HBsAg titer (2 ^N) ^b	≤2 ⁷	8 (47%)	84 (18%)	0.032
HBeAg	Positive	7 (41%)	240 (51%)	NS
HBV DNA	≥7.1 LGE ^c /ml	8 (47%)	270 (58%)	NS
HBV genotypes	Genotype C	14 (82%)	438 (93%)	NS
Duration of lamivudine	>4.1 years	13 (76%)	348 (74%)	NS
YMDD mutants	Present	9 (53%)	264 (56%)	NS
Breakthrough hepatitis	Occurred	4 (24%)	158 (34%)	NS

NS, Not significant.

^aEvaluated by the log-rank test.^bDetermined by the passive hemagglutination method.^cLog genome equivalents.

Time Course of HBsAg Loss During Treatment With Lamivudine

Figure 2 shows the loss of HBsAg in the 486 patients who had been treated with lamivudine by the method of Kaplan–Meier. HBsAg was estimated to be cleared from the serum in 3% and 13% of the patients, respectively, at 5 and 10 years.

DISCUSSION

Conventionally, the therapeutic efficacy of antiviral treatment in patients with chronic liver disease has been evaluated by loss of HBeAg from the serum [Wong et al., 1993]. However, HBeAg reappears in the sera of some patients from whom lamivudine therapy had been withdrawn [Song et al., 2000; Lee et al., 2002; van Nunen et al., 2003]. Hence, loss of HBeAg from the serum is not a reliable indicator of the antiviral response to lamivudine. Loss of HBsAg from the serum, instead, is usually durable and would be the gold standard for valid virological responses to lamivudine treatment. It has been absent or rare in studies on small series of patients treated with lamivudine [Lai et al., 1998; Dienstag et al., 1999].

TABLE II. Multivariate Analysis for Factors Influencing the Loss of HBsAg^a

Factor	Category	Hazard ratio	Significance
Age at the start of lamivudine	1: ≤49 years	1	P = 0.028
	2: ≥50 years	2.96 (1.14–7.68) ^b	

^aEvaluated by a stepwise Cox regression analysis on factors listed in Table I.^b95% confidence interval.

In the present study, HBsAg was lost from the serum, during or after treatment with lamivudine, in 17 of the 487 (3.5%) patients who had been followed up at least 3 years after the start of treatment. Three patients lost HBsAg after lamivudine had been withdrawn; it is not certain whether or not lamivudine influenced HBsAg loss. The observed incidence is much lower than that in the six patients with de novo HBV reactivator, five (83%) of whom cleared HBsAg from the serum and developed anti-HBs after short-term lamivudine treatment [Umeda et al., 2006]. The efficacy of lamivudine would be much different between patients with ongoing HBV infection and those who have resolved infection and in whom HBV is reactivated following immunosuppressive treatments.

Older age and lower HBsAg levels at the start of lamivudine therapy increased significantly the likelihood of the loss of HBsAg from serum. Anti-HBs developed in only two of the 17 (12%) patients who had cleared HBsAg from the serum. It is not certain, therefore, whether HBsAg disappeared from the circulation, or complexed with anti-HBs and escaped detection, in the patients who lost HBsAg from the serum without developing detectable anti-HBs. When lamivudine treatment can be withdrawn is a matter of conjecture. It would be reasonable to stop lamivudine therapy in the patients who seroconverted to anti-HBs. The patients who lost HBsAg but remain negative for anti-HBs, however, would need to receive lamivudine for at least 6 months, and lamivudine therapy may be stopped in those in whom HBsAg remains negative, provided that they are followed for virological markers at regular intervals.

In previous studies, HBsAg was cleared from the serum in none of the 272 patients treated with lamivudine for 48 weeks and followed for an additional

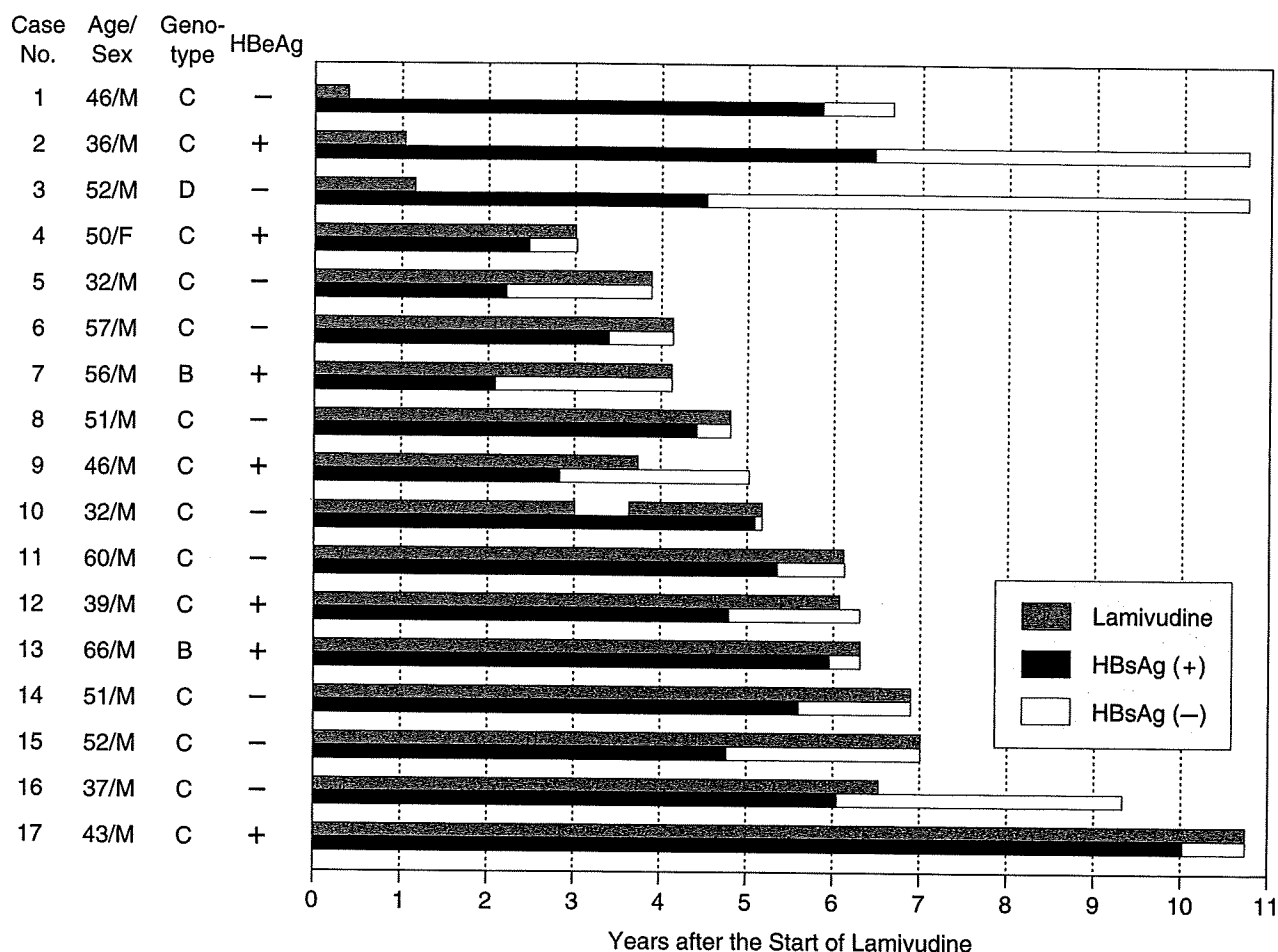


Fig. 1. Loss of HBsAg from the serum in the 17 patients followed for longer than 3 years after the start of lamivudine. Age and sex of patients, as well as genotypes of HBV and HBsAg at the baseline, are described on the left. Duration of lamivudine is indicated above, and that of HBsAg below, in columns for each patient. White columns represent periods negative for HBsAg by the hemagglutination method.

24 weeks [Lau et al., 2005], in only 1 of the 313 (0.3%) patients [Lai et al., 2006] and in 4 of the 355 (1%) patients who had received lamivudine therapy for at least 52 weeks [Chang et al., 2006]. By contrast, HBsAg was lost from the serum in 17 of the 487 (3.5%) patients treated with lamivudine in the present study. Such differences may be explained by the long-term treatment with lamivudine given to patients with chronic hepatitis B since 1995 in the Department of Hepatology, Toranomon Hospital [Kumada, 2003; Akuta et al., 2005]; lamivudine treatment has been continued in some patients for longer than 10 years. It is naturally presumed that chances for HBsAg seroconversion will increase in parallel with the duration of lamivudine therapy. This view would be supported by the loss of HBsAg in the 486 patients simulated by the method of Kaplan-Meier (Fig. 2).

With careful monitoring for YMDD mutants and breakthrough hepatitis, as well as intervention with rescue therapy by other antiviral drugs as required [Suzuki et al., 2002; Hosaka et al., 2004], long-term treatment with lamivudine has been carried out in

Toranomon Hospital [Suzuki et al., 2002; Kumada, 2003; Akuta et al., 2005]. Some patients had histological improvement [Suzuki et al., 1999, 2003]. Long-term treatment with lamivudine would be justified, because antiviral effects are shown only during therapy, and rebound of HBV DNA in the serum accompanied by hepatitis flare can develop after the withdrawal of lamivudine. Furthermore, it can suppress the development of hepatocellular carcinoma [Liaw et al., 2004; Matsumoto et al., 2005]. Combined with an excellent tolerability [Lok et al., 2003], the merit of long-term treatment with lamivudine would far outweigh its demerits [Kumada, 2003; Ryu et al., 2003].

The loss of HBsAg would be influenced by the route by which patients were infected with HBV, as well as the genotype. Loss of HBsAg from the serum may occur less frequently in the patients who were infected perinatally and were infected with HBV for many decades than in those who contracted infection during the adulthood by sexual contacts or intravenous drug use. Most Oriental carriers have been infected perinatally and possess HBV genotype B or C, in contrast to those with the adult

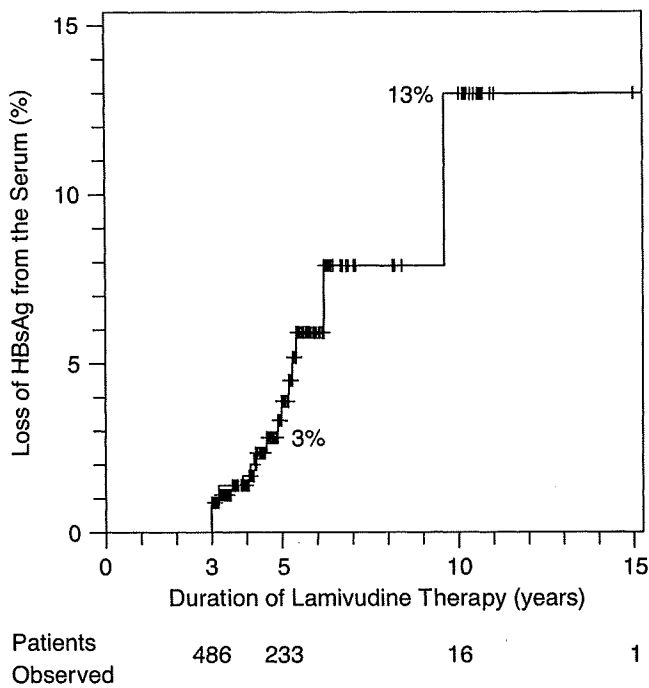


Fig. 2. Kaplan-Meier curve for the loss of HBsAg in the 486 patients who had been treated with lamivudine and followed for 3 years or longer. Numbers of patients observed at 3, 5, 10, and 15 years are indicated below.

infection who are infected frequently with HBV genotype A [Kobayashi et al., 2002]. None of the 17 patients who lost HBsAg from the serum were infected with genotype A. It is presumed, therefore, that chances for loss of HBsAg from the serum would be higher than 3.5% in the present study, among the patients in Western countries where genotype A is prevalent [Miyakawa and Mizokami, 2003].

The incidence of HBsAg clearance during long-term lamivudine, as well as factors influencing it, will provide the basis for comparison with those of other antiviral treatments including adefovir [Hadziyannis et al., 2003; Marcellin et al., 2003], entecavir [Chang et al., 2006; Lai et al., 2006], and standard as well as pegylated interferons [Wong et al., 1993; Chan et al., 2005; Lau et al., 2005], for achieving the eventual goal of clearing HBsAg from the serum. HBsAg would be useful as a practical marker, for evaluating the efficacy of antiviral therapies, since HBV DNA will rarely, if ever be cleared from the circulation of patients, once they are infected with this enduring blood-borne virus [Rehermann et al., 1996; Yotsuyanagi et al., 1998].

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Predictors of Viral Kinetics to Peginterferon Plus Ribavirin Combination Therapy in Japanese Patients Infected With Hepatitis C Virus Genotype 1b

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For chronic hepatitis C virus (HCV) infection, evaluation of response to peginterferon (PEG-IFN) plus ribavirin (RBV) therapy based on viral kinetics is useful as an early predictor of treatment efficacy, but the underlying mechanisms of the different viral kinetics to treatment are still unclear. The response to 48-week PEG-IFN-RBV combination therapy was evaluated in 160 Japanese adult patients infected with HCV genotype 1b and determined the rapid virological response (at 4 weeks), early virological response (at 12 weeks), end-of treatment response, and sustained virological response (6 months after end of treatment). The proportion of patients who showed rapid, early and sustained virological, and end-of treatment responses were 50%, 73%, 47%, and 71%, respectively. Furthermore, 66% of patients who achieved early virological response also showed sustained virological response. Multivariate analysis identified substitutions of amino acid (aa) 70 and 91 in the HCV core region (double-wild-type) as a predictor of early HCV-RNA negativity, rapid, early, and sustained virological responses and end-of treatment response, and lipid metabolic factors (high levels of LDL cholesterol and total cholesterol) as predictors of early and rapid virological responses and end-of treatment response. Male sex and low levels of alpha-fetoprotein were other predictors of sustained virological response. Furthermore, female sex and severity of liver fibrosis were determinants of lack of sustained virological response in spite of early virological response. This study identified predictors of efficacy of PEG-IFN-RBV therapy based on viral kinetics in Japanese patients infected with HCV genotype 1b. **J. Med. Virol. 79:1686–1695, 2007.** © 2007 Wiley-Liss, Inc.

KEY WORDS: HCV; viral kinetics; peginterferon; ribavirin; core region; lipid metabolism; sex; alpha-fetoprotein

INTRODUCTION

Treatment of chronic hepatitis C virus (HCV) infection with peginterferon (PEG-IFN) combined with ribavirin (RBV) carries potential serious side effects and is costly especially when used long enough to achieve a high sustained virological response. For these reasons, we need to identify those patients who do not achieve sustained virological response to free them of unnecessary side effects and reduce costs, preferably as early as possible after the start of the combination therapy. In this regard, previous studies showed that the rapid virological response at 4 weeks and the early virological response at 12 weeks after the commencement of 48-week treatment with PEG-IFN plus RBV are important predictors of sustained virological response [Fried et al., 2002; Jensen et al., 2006]. In fact, the observation that patients lacking early virological response following PEG-IFN- α -2a-RBV combination therapy are highly unlikely to develop sustained virological response was adopted as an assessment criterion by the National Institutes of Health Consensus Development Conference [National Institutes of Health Consensus Development Conference Statement, 2002]. The predictive potential of early virological response

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was also confirmed in patients treated with PEG-IFN- α -2b-RBV [Davis et al., 2003]. Thus, it is useful to evaluate the response to treatment based on viral kinetics as an early predictor of treatment efficacy. However, the underlying mechanisms of the different viral kinetics to treatment are still unclear.

Determinants of the response to the PEG-IFN-RBV therapy were studied previously in patients with high titers of genotype 1b (≥ 100 kiloIU [KIU]/ml), which is dominant in Japan [Akuta et al., 2005, 2006, 2007a,b]. The results identified aa substitutions of aa 70 and/or 91 in the HCV core region, LDL cholesterol (LDL-C), and sex as independent and significant pretreatment predictors of the response to PEG-IFN-RBV therapy. Whether these factors are also useful as pretreatment predictors of viral kinetics await further investigation.

The aim of the present study was to analyze the pretreatment viral kinetics in order to determine those factors that could predict the early response to 48-week PEG-IFN-RBV therapy in Japanese patients with HCV genotype 1b.

PATIENTS AND METHODS

Study Population

A total of 342 HCV-infected Japanese patients were recruited consecutively into the study protocol between December of 2001 and August of 2005 at Toranomon Hospital, Tokyo. Among these, 160 patients were selected based on the following criteria. (1) Negativity for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positivity for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emerville, CA), and positivity for HCV RNA qualitative analysis with PCR (Amplicor, Roche Diagnostic Systems, CA). (2) Infection with HCV genotype 1b only. (3) A high viral load (≥ 100 KIU/ml) by quantitative analysis of HCV RNA with PCR (Cobas Amplicor HCV monitor v 2.0 using the 10-fold dilution method, Roche Diagnostics, Tokyo, Japan) within the preceding 2 months of enrolment. (4) No hepatocellular carcinoma. (5) Body weight >40 kg. (6) Lack of coinfection with human immunodeficiency virus. (7) No previous treatment with antiviral or immunosuppressive agents within the preceding 3 months of enrolment. (8) None was an alcoholic; lifetime cumulative alcohol intake was <500 kg. (9) None had other forms of liver diseases, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, or autoimmune liver disease. (10) None of the females was pregnant or a lactating mother. (11) All accepted treatment for ≥ 24 weeks as outlined in the study protocol, as well as repeated evaluation of HCV-RNA levels during the treatment (at least once every month). (12) All patients have completed a 24-week follow-up program after cessation of treatment, and sustained virological response could be evaluated. (13) Each signed a consent form of the study protocol that had been approved by the Human Ethics Review Committee of Toranomon Hospital.

Patients received PEG-IFN α -2b at a median dose of 1.5 μ g/kg (range, 0.8–1.8 μ g/kg) subcutaneously

each week plus oral RBV at a median dose of 11.0 mg/kg (range, 3.4–14.2 mg/kg) daily for 48 weeks. The RBV dose was adjusted according to body weight (600 mg for ≤ 60 kg, 800 mg for >60 kg and ≤ 80 kg, and 1000 mg for >80 kg), except for 40 patients who started at a reduced dose based on low pretreatment levels of hemoglobin (Hb). Furthermore, the dose of RBV was reduced during treatment in another group of 47 patients due to falls in Hb concentration.

Table I summarizes the profiles of the patients. They included 103 men and 57 women. The median duration of treatment was 48 weeks (range, 24–48 weeks). The efficacy of the combination therapy was evaluated by HCV-RNA negativity based on qualitative PCR analysis at the end of treatment (end-of treatment response) and 6 months after the completion of therapy (sustained virological response). The dynamics of on-treatment HCV was assessed by the rapid virological response defined as a decrease in HCV RNA of >2.0 log based on quantitative PCR analysis or HCV-RNA negativity based on qualitative PCR analysis at 4 weeks, and by that of >2.0 log or HCV-RNA negativity at 12 weeks (early virological response) since the commencement of combination therapy. The rapid virological response, early virological response, end-of treatment response, and sustained virological response could be evaluated in 115 (71.9%), 151 (94.4%), 160 (100%), 160 (100%) of the 160 patients, respectively.

Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for alanine aminotransferase (ALT) and HCV-RNA levels. The serum samples were frozen at -80°C within 4 hr of collection and then thawed at the time of measurement. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of NS5 region [Chayama et al., 1993]. HCV-RNA level was measured quantitatively by PCR (Cobas Amplicor HCV monitor v 2.0 using the 10-fold dilution method, Roche) before, during, and after therapy. The lower detection limit of the assay was 5 KIU/ml. Samples collected during and after therapy that showed no detectable levels of HCV-RNA (<5 KIU/ml) were checked also by qualitative PCR (Amplicor, Roche), which has a higher sensitivity than quantitative analysis, and the results were labeled as positive or negative. The lower limit of this assay was 50 IU/ml. For evaluation of the rapid virological response and early virological response, we used the \log_{10} of the cut-off value (5 KIU/ml) was used for HCV-RNA values below the limit of detection.

Histopathological Examination

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle (Tohoku University style, Kakinuma Factory, Tokyo), fixed in 10% formalin, and subsequently stained

TABLE I. Patient Profile and Laboratory Data at Commencement of 48-Week Combination Therapy of Peginterferon Plus Ribavirin in 160 Patients Infected With HCV Genotype1b

Demographic data	
Number of patients	160
Sex (M/F)	103/57
Age (years) ^a	54 (24–70)
History of blood transfusion	56 (35.0%)
Family history of liver disease	43 (26.9%)
Body mass index (kg/m ²) ^a	23.1 (17.6–32.7)
Laboratory data ^a	
Serum aspartate aminotransferase (IU/L)	56 (17–266)
Serum alanine aminotransferase (IU/L)	77 (24–504)
Serum albumin (g/dl)	3.8 (3.0–4.5)
Gamma-glutamyl transpeptidase (IU/L)	50 (14–393)
Leukocytes (/mm ³)	4,600 (2,200–9,400)
Hemoglobin (g/dl)	14.4 (10.6–17.6)
Platelets (×10 ⁴ /mm ³)	16.9 (6.6–40.2)
ICG R15 (%)	15 (3–49)
Serum iron (μg/dl)	138 (18–308)
Serum ferritin (μg/L)	150 (<10–1,104)
Creatinine clearance (ml/min)	99 (51–146)
Level of viremia (KIU/ml)	1,800 (12 to >5,000)
Alpha-fetoprotein (μg/L)	6 (2–167)
Total cholesterol (mg/dl)	168 (98–236)
High density lipoprotein cholesterol (mg/dl)	45 (10–83)
Low density lipoprotein cholesterol (mg/dl)	97 (46–162)
Triglycerides (mg/dl)	98 (33–362)
Uric acid (mg/dl)	5.6 (2.3–8.8)
Fasting blood sugar (mg/dl)	97 (67–257)
Histological findings	
Stage of fibrosis (F1/F2/F3/F4/ND)	79/34/22/1/24
Hepatocyte steatosis (none to mild/moderate to severe/ND)	116/15/29
Treatment	
PEG-IFN α -2b dose (μg/kg)	1.5 (0.8–1.8)
Ribavirin dose (mg/kg)	11.0 (3.4–14.2)
Amino acid substitutions in the core region ^b	
aa 70 (wild/non wild/ND)	79/53/9
aa 91 (wild/non wild/ND)	85/53/3
aa 70 and aa 91 (double wild/non double wild/ND)	53/84/4

Two patterns of mutant and competitive are indicated as non-wild. The pattern of wild at aa 70 and wild at aa 91 was evaluated as double wild-type, and the other patterns were non-double wild-type.
ND, not determined.

^aData are number and percentages of patients, except those denoted by, which represent the median (range) values.

^bAmino acid substitutions were evaluated in 141 patients using pretreatment sera by PCR method with mutation specific primers.

with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens contained 6 or more portal areas. Histopathological diagnosis was confirmed by an experienced liver pathologist (H.K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on the histological scoring system of Desmet et al. [1994]. Hepatocyte steatosis was graded as none (absent), mild (<33% of hepatocytes involved), moderate (>33% but <66% of hepatocytes involved), or severe (>67% of hepatocytes involved) [D'Alessandro et al., 1991].

Detection of Amino Acid Substitutions in the Core Region

We developed a simple and low-cost PCR method for detecting substitutions of aa 70 or aa 91 in the HCV core region of genotype 1b using mutation-specific primer, as an alternative to the direct sequencing method. The major protein type was determined based on the relative

intensity of the bands for wild (aa 70: arginine, aa 91: leucine) and mutant (aa 70: glutamine/histidine, aa 91: methionine) HCV in agarose gel electrophoresis. If the intensities of the bands were similar, the case was regarded as competitive. The detection rate was 94.4%, the sensitivity was 10 KIU/ml, the reproducibility was high, and consistency with direct sequencing was 97.1% in positive cases [Okamoto et al., 2007]. In this study, the pattern of arginine (wild) at aa 70 and leucine (wild) at aa 91 was evaluated as double wild-type, while the other patterns were non-double-wild-type. The mutation in this study refers to substitution from consensus sequence. In previous studies, HCV-J was considered as a prototype and the aa substitution was evaluated by comparison with the consensus sequence prepared from 50 clinical trial samples [Kato et al., 1990; Akuta et al., 2005]. In the present study, the PCR genotyping could be performed in 141 patients; the remaining 19 patients could not be analyzed due to the lack of adequate serum samples obtained before treatment.

Statistical Analysis

The sustained virological response was analyzed on an intention to treat basis. Non-parametric tests were used to compare variables between groups (Mann-Whitney *U* test, chi-squared test and Fisher's exact probability test). The cumulative HCV-RNA negative rates were calculated using the Kaplan-Meier technique, and differences between the curves were tested using the log-rank test. Statistical analyses of HCV-RNA negative periods according to variables were calculated using the period from the commencement of the combination treatment. Univariate and multivariate logistic regression analyses were used to determine the predictors of rapid virological response, early virological response, end-of treatment response, and sustained virological response. Univariate analysis and multivariate Cox proportional hazard model were used to determine the predictors of early HCV-RNA negativity. We also calculated the odds ratios and 95% confidence intervals (95%CI). All *P* values less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into multiple logistic regression analysis and multivariate Cox proportional hazard model to identify significant independent factors. Potential predictive factors associated with rapid virological response, early virological response, end-of treatment response, sustained virological response, and early HCV-RNA negativity included the following variables: sex, age, history of blood transfusion, familial history of liver disease, BMI, aspartate aminotransferase (AST), ALT, albumin, γ -glutamyl transpeptidase (GGT), leukocyte count, Hb, platelets, indocyanine green retention rate at 15 min (ICG R15), serum iron, serum ferritin, creatinine clearance, viremia level, alpha-fetoprotein (AFP), TC, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), TG, uric acid (UA), FBS, hepatocyte steatosis, pathological staging, PEG-IFN dose/body weight, RBV dose/body weight, and aa substitutions in HCV core region. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, IL).

RESULTS

Response to Therapy

A rapid virological response was achieved by 57 of 115 (49.6%) patients, early virological response by 110 of 151 (72.8%), end-of treatment response by 113 of 160 (70.6%), and sustained virological response by 75 of 160 (46.9%). Furthermore, 47.7% (72/151 patients) achieved both early and sustained virological responses, 26.5% (40/151) were considered to have neither achieved an early nor sustained virological response, 25.2% (38/151) achieved early virological response but not a sustained virological response, and 0.7% (1/151) did not achieve an early virological response but showed sustained virological response. Thus, 65.5% (72/110) of

those who achieved early virological responses also achieved sustained virological responses, and 2.4% (1/41) of those who did not show early virological response later achieved sustained virological responses.

Predictors of Early HCV-RNA Negativity as Determined by Univariate and Multivariate Analyses

Overall, the cumulative HCV-RNA negative rates were 3.8%, 30.8%, 65.4%, and 71.0% at the end of 4, 12, 24, and 48 weeks after the start of treatment. The potential predictive factors associated with early HCV-RNA negativity during treatment were explored in all 160 patients. In univariate analyses, the following 13 factors tended to or significantly influenced the early HCV-RNA negativity: HCV core region ($P < 0.001$), AFP ($P < 0.001$), sex ($P = 0.002$), LDL-C ($P = 0.002$), leukocyte count ($P = 0.015$), UA ($P = 0.015$), AST ($P = 0.026$), age ($P = 0.035$), TC ($P = 0.044$), stage of fibrosis ($P = 0.050$), ICG R15 ($P = 0.071$), RBV dose/body weight ($P = 0.076$), and Hb ($P = 0.077$). In multivariate analysis using these factors, aa substitutions of the HCV core region (double wild-type; $P < 0.001$), AFP ($< 11 \mu\text{g/L}$; $P = 0.014$), and age (< 55 years; $P = 0.028$) were independent significant predictors of early HCV-RNA negativity during treatment (Fig. 1; Table II).

Predictors of Rapid Virological Response as Determined by Univariate and Multivariate Analyses

Univariate analysis identified 10 parameters that correlated with the rapid virological response: AFP ($< 11 \mu\text{g/L}$; $P < 0.001$), aa substitutions of the HCV core region (double wild-type; $P = 0.001$), TC ($\geq 170 \text{ mg/dl}$; $P = 0.005$), RBV dose/body weight ($\geq 11.0 \text{ mg/kg}$; $P = 0.009$), AST ($< 60 \text{ IU/L}$; $P = 0.024$), LDL-C ($\geq 86 \text{ mg/dl}$; $P = 0.027$), ICG R15 ($< 10\%$; $P = 0.053$), gender (male sex; $P = 0.054$), leukocyte count ($\geq 4,500/\text{mm}^3$; $P = 0.058$), and age (< 55 years; $P = 0.063$). Multivariate analysis that included the above variables identified three parameters that independently influenced the rapid virological response: aa substitutions of the HCV core region (double-wild-type; $P = 0.001$), TC ($\geq 170 \text{ mg/dl}$; $P = 0.003$), and age (< 55 years; $P = 0.042$). Especially, aa substitutions of HCV core region (double-wild-type) and TC ($\geq 170 \text{ mg/dl}$) were three parameters that increased the likelihood of a rapid virological response fivefold or more (Table III).

Predictors of Early Virological Response as Determined by Univariate and Multivariate Analyses

Univariate analysis identified eight parameters that influenced the early virological response: LDL-C ($\geq 86 \text{ mg/dl}$; $P < 0.001$), AFP ($< 11 \mu\text{g/L}$; $P < 0.001$), aa substitutions of the HCV core region (double wild-type; $P = 0.001$), TC ($\geq 170 \text{ mg/dl}$; $P = 0.005$), leukocyte count ($\geq 4,500/\text{mm}^3$; $P = 0.009$), viremia level ($\geq 2,000 \text{ KIU/ml}$;

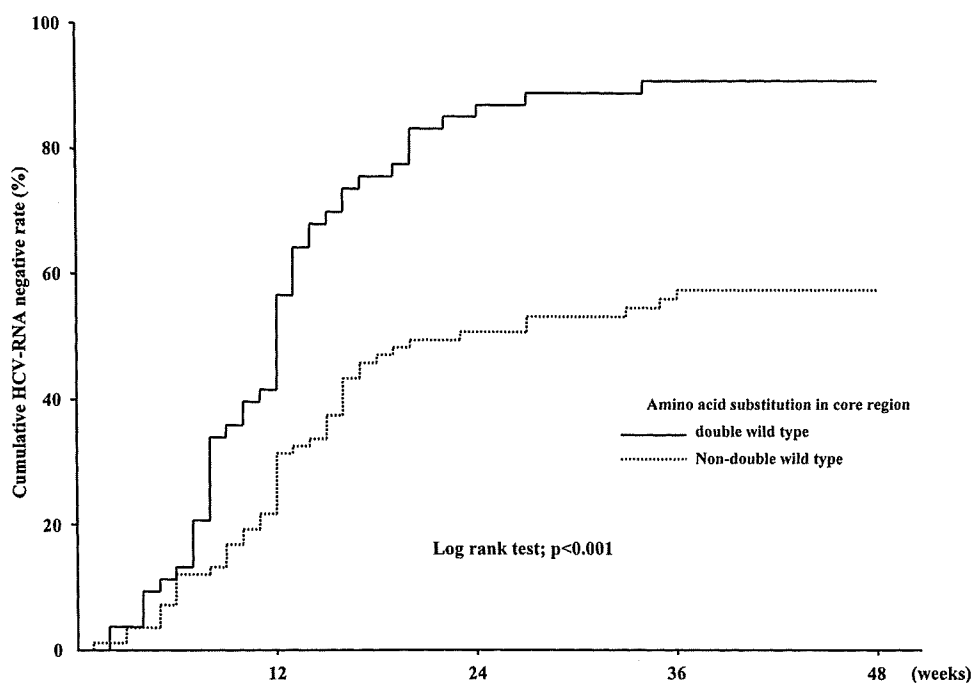


Fig. 1. Cumulative HCV-RNA negative rates during treatment, according to amino acid substitutions of the HCV core region.

$P = 0.042$), AST (<60 IU/L; $P = 0.068$), and gender (male sex; $P = 0.087$). Multivariate analysis that included the above variables identified three parameters that independently influenced the early virological response: aa substitutions of the HCV core region (double-wild-type; $P = 0.001$), LDL-C (≥ 86 mg/dl; $P = 0.002$), and viremia level ($\geq 2,000$ KIU/ml; $P = 0.027$). Especially, aa substitutions of the HCV core region (double-wild-type) and LDL-C (≥ 86 mg/dl) were the two parameters that increased the likelihood of early virological response fivefold or more (Table IV).

Predictors of End-of Treatment Response as Determined by Univariate and Multivariate Analyses

Univariate analysis identified ten parameters that correlated with the end-of treatment response: aa substitutions of the HCV core region (double wild-type; $P < 0.001$), AFP (<11 μ g/L; $P < 0.001$), LDL-C (≥ 86 mg/dl; $P = 0.002$), viremia level ($\geq 2,000$ KIU/ml; $P = 0.009$),

gender (male sex; $P = 0.011$), TC (≥ 170 mg/dl; $P = 0.035$), leukocyte count ($\geq 4,500/\text{mm}^3$; $P = 0.035$), UA (≥ 7.0 mg/dl; $P = 0.081$), age (<55 years; $P = 0.082$), and AST (<60 IU/L; $P = 0.083$). Multivariate analysis that included the above variables identified five parameters that independently influenced the end-of treatment response: aa substitutions of the HCV core region (double-wild-type; $P < 0.001$), LDL-C (≥ 86 mg/dl; $P = 0.004$), AFP (<11 μ g/L; $P = 0.007$), viremia level ($\geq 2,000$ KIU/ml; $P = 0.012$), and UA (≥ 7.0 mg/dl; $P = 0.020$). Especially, aa substitutions of the HCV core region (double-wild-type), LDL-C (≥ 86 mg/dl), and AFP (<11 μ g/L) were the four parameters that increased the likelihood of end-of treatment response fivefold or more (Table V).

Predictors of Sustained Virological Response as Determined by Univariate and Multivariate Analyses

Univariate analysis identified 14 parameters that correlate with the sustained virological response:

TABLE II. Factors Associated With Early HCV-RNA Negativity During 48-Week Peginterferon Plus Ribavirin Combination Therapy in Patients Infected With HCV Genotype 1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	P
Amino acid substitution in core region ^a	(1) Non-double wild-type	1	<0.001
	(2) Double wild-type	2.725 (1.686–4.405)	
Alpha-fetoprotein (μ g/L)	(1) ≥ 11	1	0.014
	(2) <11	2.427 (1.199–4.902)	
Age (years)	(1) ≥ 55	1	0.028
	(2) <55	1.767 (1.062–2.941)	

Only variables that achieved statistical significance ($P < 0.05$) on multivariate Cox proportional hazard model are shown. 95% CI, 95% confidence interval.

^aThe pattern of wild at aa 70 and wild at aa 91 was evaluated as double wild-type, and the other patterns as non-double wild-type.

TABLE III. Factors Associated With Rapid Virological Response to 48-Week Peginterferon Plus Ribavirin Combination Therapy in Patients Infected With HCV genotype1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	P
Amino acid substitution in core region ^a	(1) Non-double wild-type	1	0.001
	(2) Double wild-type	7.692 (2.421–24.39)	
Total cholesterol (mg/dl)	(1) <170	1	0.003
	(2) ≥170	5.459 (1.768–16.86)	
Age (years)	(1) ≥55	1	0.042
	(2) <55	3.165 (1.044–9.615)	

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression are shown.

^aThe pattern of wild at aa 70 and wild at aa 91 was evaluated as double wild-type, and the other patterns as non-double wild-type.

gender (male sex; $P < 0.001$), AFP ($<11 \mu\text{g/L}$; $P < 0.001$), stage of fibrosis (F1; $P = 0.001$), leukocyte count ($\geq 4,500/\text{mm}^3$; $P = 0.001$), age (<55 years; $P = 0.002$), aa substitutions of the HCV core region (double wild-type; $P = 0.003$), PEG-IFN dose/body weight ($\geq 1.25 \mu\text{g/kg}$; $P = 0.003$), AST ($<60 \text{ IU/L}$; $P = 0.006$), platelet count ($\geq 15 \times 10^4/\text{mm}^3$; $P = 0.012$), UA ($\geq 7.0 \text{ mg/dl}$; $P = 0.029$), Hb ($\geq 14.0 \text{ g/dl}$; $P = 0.032$), serum albumin ($\geq 3.9 \text{ g/dl}$; $P = 0.056$), RBV dose/body weight ($\geq 11.0 \text{ mg/kg}$; $P = 0.059$), and viremia level ($<1,000 \text{ KIU/ml}$; $P = 0.084$). Multivariate analysis that included the above variables identified six parameters that independently influenced the sustained virological response: aa substitutions of the HCV core region (double-wild-type; $P = 0.001$), gender (male sex; $P = 0.002$), AFP ($<11 \mu\text{g/L}$; $P = 0.005$), leukocytes ($\geq 4,500/\text{mm}^3$; $P = 0.011$), ribavirin dose ($\geq 11.0 \text{ mg/kg}$; $P = 0.029$), and age (<55 years; $P = 0.030$). Especially, aa substitutions of the HCV core region (double-wild-type), gender (male sex), and AFP ($<11 \mu\text{g/L}$) were three parameters the increased the likelihood of sustained virological response fivefold or more (Table VI).

Predictors of Sustained Virological Response in Patients Who Achieved Early Virological Response as Determined by Univariate and Multivariate Analyses

Univariate analysis identified ten parameters that influenced the sustained virological response in patients who were able to achieve early virological response: gender (male sex; $P < 0.001$), stage of fibrosis (F1; $P = 0.002$), AST ($<60 \text{ IU/L}$; $P = 0.014$), age (<55 years; $P = 0.015$), leukocyte count ($\geq 4,500/\text{mm}^3$; $P = 0.020$), PEG-IFN dose/body weight ($\geq 1.25 \mu\text{g/kg}$; $P = 0.025$),

viremia level ($<1,000 \text{ KIU/ml}$; $P = 0.027$), AFP ($<11 \mu\text{g/L}$; $P = 0.057$), and Hb ($\geq 14.0 \text{ g/dl}$; $P = 0.058$). Multivariate analysis that included the above variables identified four parameters that independently influenced the sustained virological response of patients who achieved early virological response: gender (male sex; $P = 0.001$), stage of fibrosis (F1; $P = 0.002$), leukocyte count ($\geq 4,500/\text{mm}^3$; $P = 0.020$), and aa substitutions of the HCV core region (double wild-type; $P = 0.025$). Especially, gender (male sex) and stage of fibrosis (F1) were the two parameters that increased fivefold or more the chance for sustained virological response among the patients who achieved early virological response (Table VII).

Comparison of Factors Associated With Each of Treatment Efficacy Identified by Multivariate Analysis

Table VIII shows the variables that achieved excellent statistical significance ($P < 0.01$ and Odds ratio >5.0) on multivariate logistic regression for each evaluation of treatment efficacy. With regard to viral factors, the HCV core region was the most important predictor of rapid and early virological responses, end-of treatment response, and sustained virological response. For the host factors, lipid metabolic factors including LDL-C and TC were the two most important predictors of rapid and early virological responses as well as end-of treatment response. Thus, the HCV core region and lipid metabolic factors were important predictors of viral kinetics and treatment efficacy. Furthermore, sex and AFP were also identified as other important predictors of sustained virological response, in addition to viral and lipid metabolic factors.

TABLE IV. Factors Associated With Early Virological Response to 48-Week Peginterferon Plus Ribavirin Combination Therapy in Patients Infected With HCV Genotype1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	P
Amino acid substitution in core region ^a	(1) Non-double wild-type	1	0.001
	(2) Double wild-type	10.20 (2.674–38.46)	
LDL cholesterol (mg/dl)	(1) <86	1	0.002
	(2) ≥86	5.844 (1.911–17.87)	
Level of viremia (KIU/ml)	(1) <2,000	1	0.027
	(2) ≥2,000	3.359 (1.147–9.833)	

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression are shown.

^aThe pattern of wild at aa 70 and wild at aa 91 was evaluated as double wild-type, and the other patterns as non-double wild-type.

TABLE V. Factors Associated With End-of-Treatment Response to 48-Week Peginterferon Plus Ribavirin Combination Therapy in Patients Infected With HCV Genotype1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	P
Amino acid substitution in core region ^a	(1) Non-double wild-type	1	
	(2) Double wild-type	17.86 (4.132–76.92)	<0.001
LDL cholesterol (mg/dl)	(1) <86	1	
	(2) ≥86	6.803 (1.859–25.00)	0.004
Alpha-fetoprotein (μg/L)	(1) ≥11	1	
	(2) <11	5.525 (1.608–18.87)	0.007
Level of viremia (KIU/ml)	(1) <2,000	1	
	(2) ≥2,000	4.098 (1.359–12.35)	0.012
Uric acid (mg/dl)	(1) <7.0	1	
	(2) ≥7.0	9.259 (1.414–58.82)	0.020

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression are shown.

^aThe pattern of wild at aa 70 and wild at aa 91 was evaluated as double wild-type, and the other patterns as non-double wild-type.

DISCUSSION

Using multivariate analysis, the present study identified viral- (HCV core region) and host-related factors (lipid metabolism and sex) that influenced the rapid and early virological responses to PEG-IFN-RBV combination therapy. The same analysis also identified male sex, stage of fibrosis, leukocyte count, and aa substitutions of the HCV core region as determinants of sustained virological response of patients who achieved early virological response. Identification of these viral and host factors in the early period of PEG-IFN-RBV therapy (4–12 weeks) should help design better therapeutic regimens for those patients who are less likely to achieve sustained virological response.

It was reported previously that substitutions of aa 70 and/or 91 in the HCV core region is an independent and significant predictor of the response to treatment [Akuta et al., 2005, 2006, 2007a,b]. Based on a larger number of patients, the present study also identified aa substitutions in the HCV core region as a predictor of the response to PEG-IFN-RBV therapy. Previous studies reported that the HCV core region might be associated with resistance to IFN monotherapy involving the Jak-STAT signaling cascade [Blindenbacher et al., 2003; Bode et al., 2003; Melén et al., 2004; de Lucas et al.,

2005]. The result could be also interpreted to mean that aa substitutions in the HCV core region is associated with those proteins involved in resistance to IFN monotherapy, such as SOCS proteins, which is known to inhibit IFN- α -induced activation of the Jak-STAT pathway and expression of the antiviral proteins 2',5'-OAS and MxA [Voldides et al., 2004]. Furthermore, the result also indicates that aa substitutions in the HCV core region might serve as a surrogate marker for other proteins associated with resistance to the antiviral actions of IFN. Further studies that examine the structural and functional impact of aa substitutions during combination therapy should be conducted to confirm the above finding.

It was shown previously that LDL-C is an independent and significant predictor of the response to PEG-IFN-RBV therapy [Akuta et al., 2007a]. The present study also identified lipid metabolic factors including LDL-C or TC as predictors of the treatment response, and is in agreement with similar findings of a recent study [Gopal et al., 2006]. Previous studies reported that endocytosis of HCV via the LDL receptor(s) is mediated by the formation of a complex between HCV and VLDL or LDL [Agnello et al., 1999; Andre et al., 2002]. Furthermore, there is evidence that intracellular cholesterol level modulates LDLr expression, and thus a

TABLE VI. Factors Associated With Sustained Virological Response to 48-Week Peginterferon Plus Ribavirin Combination Therapy in Patients Infected With HCV Genotype1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	P
Amino acid substitution in core region ^a	(1) Non-double wild-type	1	
	(2) Double wild-type	5.988 (2.070–17.24)	0.001
Sex	(1) Female	1	
	(2) Male	5.882 (1.901–18.18)	0.002
Alpha-fetoprotein (μg/L)	(1) ≥11	1	
	(2) <11	7.576 (1.828–31.25)	0.005
Leukocytes (/mm ³)	(1) <4,500	1	
	(2) ≥4,500	4.031 (1.374–11.83)	0.011
Ribavirin dose (mg/kg)	(1) <11.0	1	
	(2) ≥11.0	3.156 (1.128–8.825)	0.029
Age (years)	(1) ≥55	1	
	(2) <55	3.125 (1.120–8.696)	0.030

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression are shown.

^aThe pattern of wild at aa 70 and wild at aa 91 was evaluated as double wild-type, and the other patterns as non-double wild-type.

TABLE VII. Factors Associated With Sustained Virological Response in Patients Who Achieved Early Virological Response to 48-Week Peginterferon Plus Ribavirin Combination Therapy in Patients Infected With HCV Genotype1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	P
Gender	(1) Female	1	0.001
	(2) Male	7.576 (2.179–26.32)	
Stage of fibrosis	(1) F2,3,4	1	0.002
	(2) F1	6.944 (2.058–23.26)	
Leukocytes (/mm ³)	(1) <4,500	1	0.020
	(2) ≥4,500	4.432 (1.260–15.58)	
Amino acid substitution in core region ^a	(1) Non-double wild-type	1	0.025
	(2) Double wild-type	4.000 (1.190–13.51)	

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression are shown.

^aThe pattern of wild at aa 70 and wild at aa 91 was evaluated as double wild-type, and the other patterns as non-double wild-type.

high LDL-C could downregulate LDLr and diminishes the spread of hepatocyte HCV infection. Thus, the correlation between treatment efficacy and lipid metabolic factors may be explained by the role of LDL-C in transporting the HCV-LDL complex into the hepatocyte. Other mechanisms could also explain the role of LDL-C and the response to PEG-IFN-RBV therapy. For example, high LDL-C levels could act by modulating cytokine release [Netea et al., 1996] and antiviral cellular immune response [Muldoon et al., 1997; Ludewig et al., 2001]. Further studies of a large number of patients are required to explore the relationship between various lipid metabolic factors and the response to PEG-IFN-RBV therapy.

The results showed that higher levels of AFP are a negative predictor of the response to PEG-IFN-RBV therapy. Previous data indicated that absence of advanced liver fibrosis is a positive predictor of sustained virological response to IFN monotherapy and IFN-RBV dual therapy [Jouet et al., 1994; Poynard et al., 2000; Bruno et al., 2004], and that advanced liver fibrosis is usually associated with elevation of serum AFP [Bayati et al., 1998; Chu et al., 2001; Hu et al., 2004]. We showed previously that high rates of ICG R15 or low levels of serum albumin are also associated with advanced liver fibrosis, and that they are independent and significant negative predictors of the response to PEG-IFN-RBV [Akuta et al., 2005, 2007a]. To our knowledge, this is the first report that describes the relationship between serum AFP levels and the response to PEG-IFN-RBV therapy. Further studies of

large number of patients are required to explore the most important indicator of histopathological changes in the liver (including stage of fibrosis, ICG R15, albumin, and AFP), and to investigate the relationship between severity of histopathological changes and response to the combination therapy.

Similar to recent findings [Akuta et al., 2007a], the present study also identified female sex as a negative predictor of sustained virological response to PEG-IFN-RBV therapy. It should be noted, however, that other studies also showed that male sex is also a negative predictor of sustained virological response to combination therapy [Poynard et al., 1998; Conjeevaram et al., 2006]. The discrepancy between our results and such findings may be explained by differences of age by sex. The age of the female patients in the present study (median; 60 years) was higher than that of the males (median; 51 years). Other mechanisms could be also explained by the small number of patients in our study, differences in host factors including sex [Schott et al., 2007] and race [McHutchison et al., 2000; Kaplan et al., 2005], and/or differences in viral factors, such as the distribution of genotype 1a or 1b, and geographic diversities of genotype 1b [Nakano et al., 1999]. Further studies of larger number of patients matched for age, race, and HCV genotype are required to explore the relationship between sex and the response to PEG-IFN-RBV therapy.

In this study, multivariate analysis identified sex and stage of fibrosis as parameters that independently influenced the sustained virological response of patients

TABLE VIII. Comparison of Factors Associated With Efficacy of 48-Week Peginterferon Plus Ribavirin Combination Therapy in Patients Infected With HCV Genotype1b, Identified by Multivariate Analysis

Factor	Rapid virological response (at 4 weeks)	Early virological response (at 12 weeks)	End-of treatment response (at 48 weeks)	Sustained virological response
Virus	Core region	Core region	Core region	Core region
	$P = 0.001, 7.69 (2.42-24.4)^a$	$P = 0.001, 10.2 (2.67-38.5)^a$	$P < 0.001, 17.9 (4.13-76.9)^a$	$P = 0.001, 5.99 (2.07-17.2)^a$
Lipid metabolism	Total cholesterol	LDL cholesterol	LDL cholesterol	
	$P = 0.003, 5.46 (1.77-16.9)^a$	$P = 0.002, 5.84 (1.91-17.9)^a$	$P = 0.004, 6.80 (1.86-25.0)^a$	
Others			Alpha-fetoprotein	Alpha-fetoprotein
			$P = 0.007, 5.53 (1.61-18.9)^a$	$P = 0.005, 7.58 (1.83-31.3)^a$
			Gender	$P = 0.002, 5.88 (1.90-18.2)^a$

Only variables that achieved excellent statistical significance ($P < 0.01$ and Odds ratio > 5.0) on multivariate logistic regression are shown.

^aOdds ratio (95% confidence interval).

who achieved early virological response, but these two factors were not identified as significant predictors of early HCV-RNA negativity during treatment. Interestingly, sex was not a predictor of rapid and early virological responses, end-of treatment response, or early HCV-RNA negativity when evaluated for treatment efficacy based on viral kinetics, but was remarkable significant predictor of sustained virological response to PEG-IFN-RBV. Thus, the underlying mechanisms of failure to develop a sustained virological response in those patients who show HCV-RNA negativity remain unclear. Further prospective studies of a large number of patients should be performed to determine whether it would be worth to extend the Peg-IFN-RBV therapy to 72 weeks in those patients who do not achieve sustained virological responses in spite of early virological response by 48 weeks therapy [Buti et al., 2003; Berg et al., 2006].

Pretreatment prediction of a sustained virological response to PEG-IFN-RBV therapy is still incomplete, and evaluation of treatment response based on viral kinetics is useful as early predictor of treatment efficacy. Especially, in this study, viral factors (e.g., aa substitutions in the HCV core region) and host factors (e.g., lipid metabolic factors, sex, and AFP) were important predictors of response to PEG-IFN-RBV therapy in Japanese patients infected with HCV genotype 1b, to say nothing of treatment-related factors (e.g., RBV dose) [Manns et al., 2001; Akuta et al., 2006, 2007a]. One limitation of our study was that we did not examine amino acid substitutions in areas other than the core region of HCV genome, such as the interferon sensitivity determining region (ISDR) and the interferon/ribavirin resistance determining lesion (IRRDR) of NS5A region, although they should be investigated in future studies [Enomoto et al., 1995, 1996; El-Shamy et al., 2007]. It is concluded that viral kinetics to PEG-IFN-RBV therapy seems to be based on a dynamic tripartite interaction of virus, host, and treatment regimen. Further understanding of the complex interaction between these factors should facilitate the development of more effective therapeutic regimens.

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