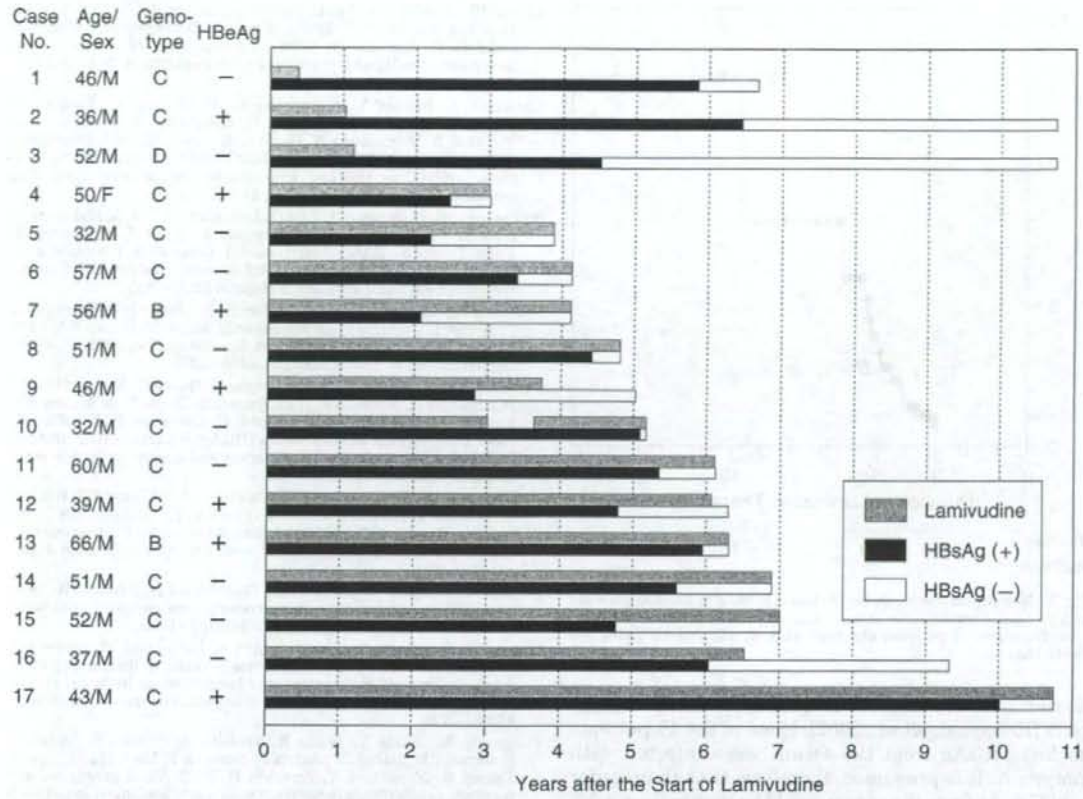

Fig. 1. Loss of HBsAg from the serum in the 17 patients followed for longer than 3 years after the start of lamivudine. Age and sex of patients, as well as genotypes of HBV and HBeAg at the baseline, are described on the left. Duration of lamivudine is indicated above, and that of HBsAg below, in columns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

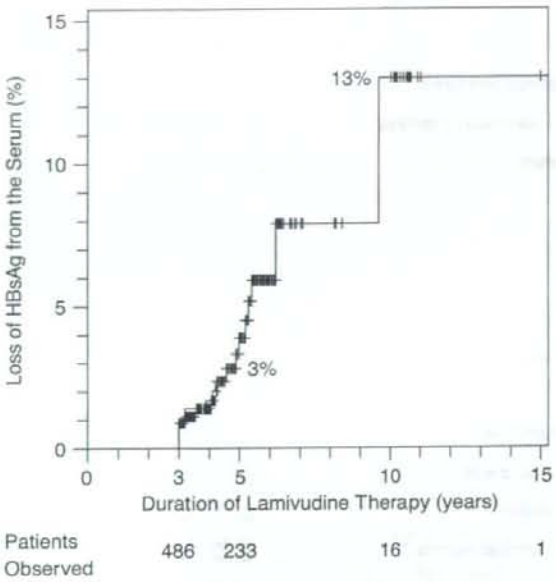

Fig. 2. Kaplan–Meier curve for the loss of HBsAg in the 486 patients who had been treated with lamivudine and followed for 3 years or longer. Numbers of patients observed at 3, 5, 10, and 15 years are indicated below.

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 pegyrated 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-IFNα-2b at a median dose of 1.5 μg/kg (range, 0.8–1.8 μg/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 ($\times 10^4/mm^3$)	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-IFNα-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.
[a]Data are number and percentages of patients, except those denoted by, which represent the median (range) values.
[b]Amino 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 μg/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 μg/L; $P < 0.001$), aa substitutions of the HCV core region (double wild-type; $P = 0.001$), TC (≥170 mg/dl; $P = 0.005$), RBV dose/body weight (≥11.0 mg/kg; $P = 0.009$), AST (<60 IU/L; $P = 0.024$), LDL-C (≥86 mg/dl; $P = 0.027$), ICG R15 (<10%; $P = 0.053$), gender (male sex; $P = 0.054$), leukocyte count (≥4,500/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 (≥170 mg/dl; $P = 0.003$), and age (<55 years; $P = 0.042$). Especially, aa substitutions of HCV core region (double-wild-type) and TC (≥170 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 (≥86 mg/dl; $P < 0.001$), AFP (<11 μg/L; $P < 0.001$), aa substitutions of the HCV core region (double wild-type; $P = 0.001$), TC (≥170 mg/dl; $P = 0.005$), leukocyte count (≥4,500/mm^3; $P = 0.009$), viremia level (≥2,000 KIU/ml;

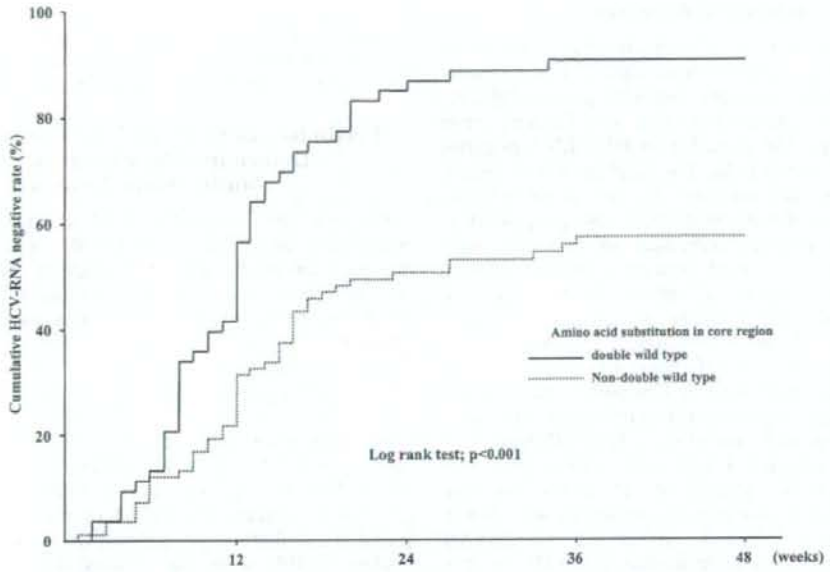

Fig. 1. Cumulative HCV-RNA negative rates during treatment, according to amino acid substitutions of the HCV core region.

$P = 0.042$), 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 (≥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 μg/L; $P < 0.001$), LDL-C (≥86 mg/dl; $P = 0.002$), viremia level (≥2,000 KIU/ml; $P = 0.009$), gender (male sex; $P = 0.011$), TC (≥170 mg/dl; $P = 0.035$), leukocyte count (≥4,500/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 (≥86b mg/dl; $P = 0.004$), AFP (<11b μg/L; $P = 0.007$), viremia level (≥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 μg/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 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	2.725 (1.686–4.405)	<0.001
Alpha-fetoprotein (μg/L)	(1) ≥11	1	
	(2) <11	2.427 (1.199–4.902)	0.014
Age (years)	(1) ≥55	1	
	(2) <55	1.767 (1.062–2.941)	0.028

Only variables that achieved statistical significance ($P < 0.05$) on multivariate Cox proportional hazard model are shown.
95% CI, 95% confidence interval.
[a]The 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	
	(2) Double wild-type	7.692 (2.421–24.39)	0.001
Total cholesterol (mg/dl)	(1) <170	1	
	(2) ≥170	5.459 (1.768–16.86)	0.003
Age (years)	(1) ≥55	1	
	(2) <55	3.165 (1.044–9.615)	0.042

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression are shown.
[a]The 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 μg/L; $P < 0.001$), stage of fibrosis (F1; $P = 0.001$), leukocyte count (≥4,500/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 (≥1.25 μg/kg; $P = 0.003$), AST (<60 IU/L; $P = 0.006$), platelet count (≥15×10^4/mm^3; $P = 0.012$), UA (≥7.0 mg/dl; $P = 0.029$), Hb (≥14.0 g/dl; $P = 0.032$), serum albumin (≥3.9 g/dl; $P = 0.056$), RBV dose/body weight (≥11.0 mg/kg; $P = 0.059$), and viremia level (<1,000 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 μg/L; $P = 0.005$), leukocytes (≥4,500/mm^3; $P = 0.011$), ribavirin dose (≥11.0 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 μg/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 IU/L; $P = 0.014$), age (<55 years; $P = 0.015$), leukocyte count (≥4,500/mm^3; $P = 0.020$), PEG-IFN dose/body weight (≥1.25 μg/kg; $P = 0.025$), viremia level (<1,000 KIU/ml; $P = 0.027$), AFP (<11 μg/L; $P = 0.057$), and Hb (≥14.0 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 (≥4,500/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	
	(2) Double wild-type	10.20 (2.674–38.46)	0.001
LDL cholesterol (mg/dl)	(1) <86	1	
	(2) ≥86	5.844 (1.911–17.87)	0.002
Level of viremia (KIU/ml)	(1) <2,000	1	
	(2) ≥2,000	3.359 (1.147–9.833)	0.027

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression are shown.
[a]The 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.
[a]The 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 [Voltides 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.
[a]The 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	
	(2) Male	7.576 (2.179–26.32)	0.001
Stage of fibrosis	(1) F2,3,4	1	
	(2) F1	6.944 (2.058–23.26)	0.002
Leukocytes (/mm^3)	(1) <4,500	1	
	(2) ≥4,500	4.432 (1.260–15.58)	0.020
Amino acid substitution in core region[a]	(1) Non-double wild-type	1	
	(2) Double wild-type	4.000 (1.190–13.51)	0.025

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression are shown.
[a]The 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 $P = 0.001$, 7.69 (2.42–24.4)[a]	Core region $P = 0.001$, 10.2 (2.67–38.5)[a]	Core region $P < 0.001$, 17.9 (4.13–76.9)[a]	Core region $P = 0.001$, 5.99 (2.07–17.2)[a]
Lipid metabolism	Total cholesterol $P = 0.003$, 5.46 (1.77–16.9)[a]	LDL cholesterol $P = 0.002$, 5.84 (1.91–17.9)[a]	LDL cholesterol $P = 0.004$, 6.80 (1.86–25.0)[a]	
Others			Alpha-fetoprotein $P = 0.007$, 5.53 (1.61–18.9)[a]	Alpha-fetoprotein $P = 0.005$, 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.
[a]Odds 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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Prediction of Response to Pegylated Interferon and Ribavirin in Hepatitis C by Polymorphisms in the Viral Core Protein and Very Early Dynamics of Viremia

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Key Words
Chronic hepatitis · Hepatitis C virus · HCV core · Pegylated interferon · Virological responses · Ribavirin

Abstract
Objective: To evaluate power of amino acid polymorphisms in core protein of hepatitis C virus (HCV) for predicting sustained virological response (SVR) to pegylated interferon (Peg-IFN)/ribavirin, when they were combined with virological response. ***Methods:*** Peg-IFN/ribavirin was given to 118 patients infected with HCV genotype 1b in high viral loads. Amino acid polymorphisms (Arg70 vs. Gln70 and Leu91 vs. Met91) in combination with on-treatment virological responses were correlated with SVR. ***Results:*** End-of-treatment response (ETR) was achieved in 71% and SVR in 47% of the 118 patients. In multivariate analysis, Arg70 and Leu91, and higher ribavirin dose were independently associated with ETR. In patients with Gln70 and/or Met91, SVR was more frequent in those with than without prompt virological response (PVR) for a decrease in viral load ≥1.0 log by 48 h. Specificity in predicting patients without ETR and SVR, in combination with core polymorphisms, was not different between PVR and early virological response at 12 weeks. ***Conclusion:*** Core polymorphisms combined with PVR would be useful in promptly identifying the patients who will not respond to Peg-IFN/ribavirin, thereby avoiding unrewarding side effects and high costs.

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Introduction

Worldwide, an estimated 300 million people are persistently infected with hepatitis C virus (HCV), and decompensated cirrhosis or hepatocellular carcinoma develop in 20–30% of them during the lifetime [1]. HCV is classified into six major genotypes named 1–6 that break down into many subtypes designated by lower-case letters [2]. Treatments based on interferon (IFN) have been developed to prevent morbidity and mortality caused by persistent HCV infection. Monotherapy with IFN started in 1986 [3], was followed by combined IFN and ribavirin [4, 5], and more recently, pegylated (Peg)-IFN plus ribavirin [6, 7]; they have achieved increasing efficacy in treatment of patients with chronic hepatitis C [8].

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HCV genotypes influence the response to IFN, of which genotype 1, as well as genotypes 4 and 6, is more resistant than genotypes 2 or 3 to antiviral treatments based on IFN [9, 10]. Response to IFN is influenced by viral loads, also [9, 10]. Thus, hepatitis C patients infected with genotype 1 in high viral loads are most resistant to IFN-based treatments. In Japan, more than half the patients with chronic hepatitis C are infected with HCV-1b ≥100 KIU/ml. Sustained virological response (SVR), defined by the loss of detectable HCV RNA 6 months after the treatment, to IFN monotherapy was dismal at merely 6%, but it increased to 20% with combined IFN and ribavirin [11]. Even by treatment with Peg-IFN and ribavirin, however, SVR has been achieved in 42–48% of the patients infected with HCV-1 in large-scale multicenter studies [6, 7]; the response would be lower in patients with HCV-1 in high viral loads [10].

Along with a high SVR, combined Peg-IFN and ribavirin accompany severe side effects and entail high costs. Hence, the patients with high-titer HCV-1 who do not achieve SVR need to be identified as early as possible, in order to free them of unnecessary side effects and high costs. Davis et al. [12] have proposed early virological response (EVR) at 12 weeks after treatment, defined by a reduction of HCV RNA by ≥2 logs, for predicting the lack of SVR to combined Peg-IFN and ribavirin. We have reported that polymorphisms of amino acid (aa) 70 of arginine or glutamine and aa 91 of leucine or methionine in the core protein are significantly associated with the lack of response to treatment with the standard or Peg-IFN combined with ribavirin [13–15]. An attempt was made in the present study for an earlier prediction of SVR in patients with HCV-1b ≥100 KIU/ml based on virological responses 48 h, 4 weeks and 12 weeks after the start of Peg-IFN plus ribavirin, when they were combined with Arg70 and Leu91 in the core protein.

Materials and Methods

Patients

During December 2001 through July 2005, 225 consecutive patients with chronic hepatitis C received combination therapy with Peg-IFN and ribavirin at the Department of Hepatology in Toranomon Hospital. The following inclusion criteria were met by 118 (52%) of them. They were: (1) positive for antibody to HCV (anti-HCV) and HCV RNA of genotype 1b by the qualitative method and not coinfected with HCV of other genotypes; (2) negative for hepatitis B surface antigen (HBsAg) or antibody to human immunodeficiency virus type-1 (HIV-1); (3) confirmed for HCV RNA ≥100 KIU/ml within the past 2 months; (4) with body weight ≥40 kg and not pregnant or lactating; (5) with the total alcohol intake <500 kg in the past; (6) without HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune hepatitis; (7) naive to ribavirin and not received antivirals or immunosuppressants during the past 3 months, and (8) compliant to the treatment protocol for 24 weeks or longer and followed at least monthly.

All the 118 patients were followed at least 6 months after completion of combination therapy with Peg-IFN and ribavirin, for the purpose of identifying virological and biochemical factors predictive of SVR as soon as possible after it was started. The study design was approved by the Ethics Committee of Toranomon Hospital, and every patient gave an informed consent on the purpose of this study.

Serum Markers of Hepatitis Viruses

Anti-HCV was determined by the third-generation enzyme-linked immunosorbent assay (ELISA) by commercial kits (Ortho HCV Ab ELISA Test 3; Chiron Corp., Emeryville, Calif., USA). HCV RNA was determined quantitatively by polymerase chain reaction (PCR) (Cobas Amplicor HCV Monitor Version 2.0, Roche Diagnostics, Tokyo, Japan) in serum diluted 10-fold at the baseline as well as at least monthly during and after treatment; it has a dynamic range between 5 and 5,000 KIU/ml. Sera negative for HCV RNA (<5 KIU/ml) by the quantitative assay were tested by qualitative PCR (Amplicor, Roche Molecular Systems, Calif., USA) with the detection limit of 100 copies/ml. HBsAg was determined by ELISA with commercial kits (F-HBsAg; Sysmex, Kobe, Japan).

Amino Acid Polymorphisms in the Core Protein

With use of HCV-J (accession No. D90208) as a reference [16], the sequence of 1–191 aa in the core protein of genotype 1b was determined, and it was compared with the consensus sequence constructed on 50 clinical samples [14] for detecting polymorphisms at aa 70 of arginine or glutamine/histidine and aa 91 of leucine or methionine. In the present study, the PCR methods with primers specific for polymorphisms at aa 70 or 91 were performed on 109 patients [17]; the remaining 9 patients were analyzed by direct sequencing [13, 14].

Combined Peg-IFN and Ribavirin Therapy

Patients received subcutaneous Peg-IFN-α_{2b} (PEG-Intron, Schering Corp, N.J., USA) weekly at the median dose of 1.5 μg/kg, along with oral ribavirin daily at the median dose of 11.0 mg/kg for 48 weeks. The dose of ribavirin was adjusted by the body weight: 600 mg for the patients weighing ≤60 kg, 800 mg for those between >60 kg and <80 kg, and 1,000 mg for those ≥80 kg. It was tapered in the 37 patients in whom levels of hemoglobin decreased during the combination therapy.

Evaluation of Virological Responses

Therapeutic efficacy of combined Peg-IFN and ribavirin was evaluated by the disappearance of HCV RNA from serum detectable by qualitative PCR (<100 copies/ml) at the end of treatment (ETR) and 6 months after the completion of therapy (SVR). Dynamics of on-treatment HCV was assessed by prompt virological response (PVR) defined by a decrease in HCV RNA by ≥1.0 log at 48 h, rapid virological response (RVR) by that of ≥2.0 log or disappearance at 4 weeks, and by that of >2.0 log or disappearance at 12 weeks (EVR) after the combination therapy was initiated.

Table 1. Baseline characteristics of the 118 patients infected with HCV of genotype 1b who received combination therapy with Peg-IFN and ribavirin

Demographic data	
Male	76 (64%)
Age, years	55 (30–70)
History of blood transfusion	42 (36%)
Family history of liver disease	36 (31%)
Body mass index, kg/m^2	23.3 (17.6–31.2)
Laboratory data	
Leukocytes, /mm^3	4,800 (2,300–8,800)
Hemoglobin, g/dl	14.6 (10.6–17.6)
Platelets, × 10^4/mm^3	17.2 (6.6–30.9)
Albumin, g/dl	3.7 (3.0–4.5)
Alanine aminotransferase, IU/l	83 (25–504)
Aspartate aminotransferase, IU/l	59 (17–266)
γ-Glutamyl transpeptidase, IU/l	62 (15–393)
Ferritin, μg/l	154 (<10–927)
Iron, μg/dl	147 (18–308)
Retention of indocyanine green at 15 min, %	15 (4–49)
Creatinine clearance, ml/min	99 (51–146)
Histological findings	
Fibrosis stage (F1/F2/F3/F4/ND)	52 (44%)/28 (24%)/18 (15%)/1 (1%)/19 (16%)
Steatosis (none–mild/moderate–severe/ND)	87 (74%)/11 (9%)/20 (17%)
HCV RNA, KIU/ml	2,100 (100–>5,000)
Polymorphism of amino acids in the core protein	
Arg70 and Leu91	40 (34%)
Gln70 (His70) and/or Met91	78 (66%)
Treatment for 48 weeks	
Peg-IFN-α2b (≥1.25 μg/kg)	90 (76%)
Ribavirin (≥11.0 mg/kg)	57 (48%)

Number of patients with percentage in parentheses or the median value with a range in parentheses are shown. ND = Not determined.

Statistical Analysis

Non-parametric variables were compared between groups by the χ^2 test, Fisher's exact probability test and Mann-Whitney U test. Uni- and multivariate analyses for factors influencing the response to combination therapy were performed by the χ^2 test and logistic regression, respectively.

Results

Factors Predictive of ETR to Combination Therapy

Table 1 lists demographic, biochemical and virological characteristics of the 118 patients at the baseline. They all were infected with HCV-1b in high titers with the median of 2,100 KIU/ml. More than half (66%) of them possessed Gln70 and/or Met91 in the core protein. A total of 22 factors in table 1 were evaluated for association with the response to combination treatment. Factors influencing ETR to combination therapy were evaluated by univariate analysis (table 2). There were three factors with significant difference ($p < 0.05$) and one with marginal significance (p between 0.05 and 0.1). Among them, Gln70 and/or Met91 in the core protein influenced the response to combination therapy with the highest significance ($p < 0.001$), followed by γ-GTP and ribavirin dose. Multivariate analysis identified only two factors predictive of ETR (table 3). They were Arg70 and Leu91 (i.e., without Gln70 or Met91) in the core protein and ribavirin dose ≥11.0 mg/kg. They increased chances for ETR by 8.55- and 3.22-fold, respectively.

Predicting ETR and SVR by Core Polymorphisms in Combination with Early Dynamics of HCV

Based on a strong power of Arg70 and Leu91 in predicting ETR (table 3), it was evaluated how they increase the predictive value when they were combined with PVR, RVR and EVR. The results are schematically depicted in

Table 2. Factors associated with ETR to combined Peg-IFN and ribavirin for 48 weeks in 118 patients with HCV-1b by univariate analysis

Factor	Category	ETR	p value[a]
Gln70 and Met91	1: either or both	60% (47/78)	
	2: none	93% (37/40)	<0.001
γ-Glutamyl transpeptidase, IU/l	1: ≥110	50% (11/22)	
	2: <110	76% (73/96)	0.020
Ribavirin dose, mg/kg	1: <11.0	62% (38/61)	
	2: ≥11.0	81% (46/57)	0.041
Leukocytes, /mm^3	1: <4,500	61% (28/46)	
	2: ≥4,500	78% (56/72)	0.061

[a] Evaluated by χ^2 test.

Table 3. Factors associated with ETR to combined Peg-IFN and ribavirin for 48 weeks on 118 patients with HCV-1b in multivariate analysis

Factor	Category	Odds ratio (95% CI)	p value[a]
Gln70 and Met91	1: either or both	1	
	2: none	8.55 (2.34–31.26)	0.001
Ribavirin dose mg/kg	1: <11.0	1	
	2: ≥11.0	3.22 (1.28–8.09)	0.013

[a] Evaluated by logistic regression analysis.

figures 1–3, respectively. Together they demonstrate three points: (1) efficacy of combination therapy was high in the patients with Arg70 and Leu91 who accomplished ETR at 93% and SVR at 63%, irrespective of any virological response; (2) even in the patients having Gln70 and/or Met91, those achieving PVR (fig. 1), RVR (fig. 2) and EVR (fig. 3) gained high ETR (89–100%) and considerable SVR (48–78%), and (3) ETR (10–25%) and SVR (7–14%) were the worst in patients who possessed Gln70 and/or Met91 and failed to achieve PVR, RVR or EVR (differences significant in all comparisons).

Performance of Core Polymorphisms Combined with On-Treatment Virological Responses in Predicting Non-ETR and Non-SVR

Table 4 evaluates performances, in predicting the lack of ETR, of Gln70 and/or Met91 combined with on-treatment HCV RNA decreases at three time points: 48 h (PVR), 4 weeks (RVR) and 12 weeks (EVR) after the start of therapy. They were largely comparable, except for the specificity that was lower for RVR than EVR (86 vs. 96%, $p = 0.049$). Likewise, the performance in predicting the lack of SVR by combined factors was evaluated in table 5. The sensitivity was lower for PVR than RVR (38 vs. 62%, $p = 0.026$).

Discussion

Some patients infected with HCV-1 do not respond to combined therapy with Peg-IFN and ribavirin. Lest they should suffer from unrewarding side effects and high costs through 48 weeks, it is necessary to predict the lack of SVR for timing the withdrawal of treatment in them (stopping rule). The most popular stopping rule is based on two large-scale multinational studies involving 348 and 296 patients, respectively [6, 7]. Accordingly, Davis [18] has proposed the prediction of SVR by a fall of HCV RNA by ≥2 logs or to undetectable levels 12 weeks after the start of therapy (EVR) as the optimal predictor of SVR. Since only 3 of the 187 (1.6%) patients without EVR established SVR, he recommends to discontinue combination therapy in the patients infected with HCV-1 who fail to gain EVR. By applying his stopping rule, treatment costs are reduced by <16% and side effects avoided in 19% of HCV-1 patients [18].

Previous works have not established the optimal time for predicting non-SVR in patients with HCV-1 who receive the 48-week Peg-IFN/ribavirin therapy. Patients who gain SVR have cleared HCV RNA from serum by 2 weeks after the treatment start [19]. HCV RNA decreased by 3 logs within the first 4 weeks of treatment in all patients who have accomplished SVR [20, 21]; decreases in HCV RNA were almost identical at 1 and 3 months, however [22]. It is reported that lowering HCV RNA levels during the first phase is essential for efficient elimination of HCV during the second phase [23]. Decreases in HCV RNA titers within the first 24–48 h after the start of IFN, therefore, would be dependable estimates on the antiviral efficacy [24–27]. Early HCV dynamics may differ between patients receiving Peg-IFN and standard IFN [28], because it takes 3 days before the maximum serum level is reached with Peg-IFN. In our previous study [29], however, decreases in HCV RNA levels were significantly greater in patients with than without SVR from 24 h to 12 weeks after the start of combined Peg-IFN and ribavirin; they all were infected with HCV-1b in high loads.

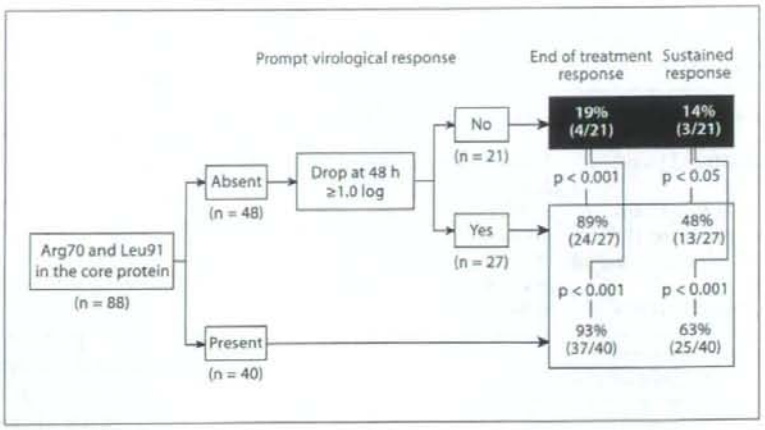

Fig. 1. ETR and SVR to combined Peg-IFN and ribavirin in patients with high-titer HCV-1b stratified by amino acid polymorphism and prompt virological response at 48 h for a decrease in HCV RNA by ≥1.0 log.

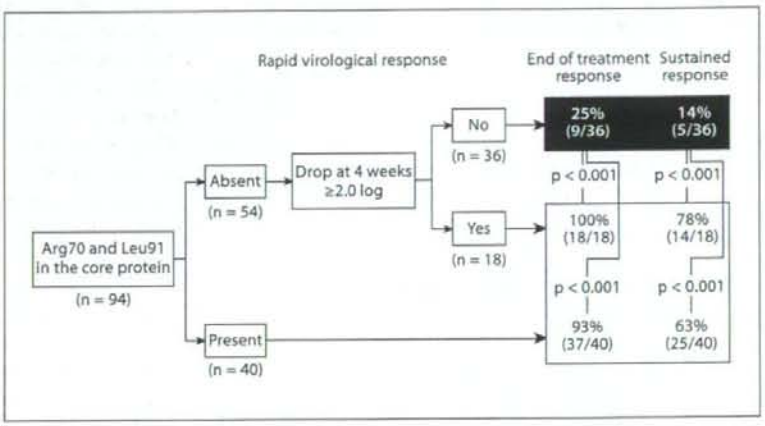

Fig. 2. ETR and SVR to combined Peg-IFN and ribavirin in patients with high-titer HCV-1b stratified by amino acid polymorphism and rapid virological response at 4 weeks for a decrease in HCV RNA by ≥2.0 logs.

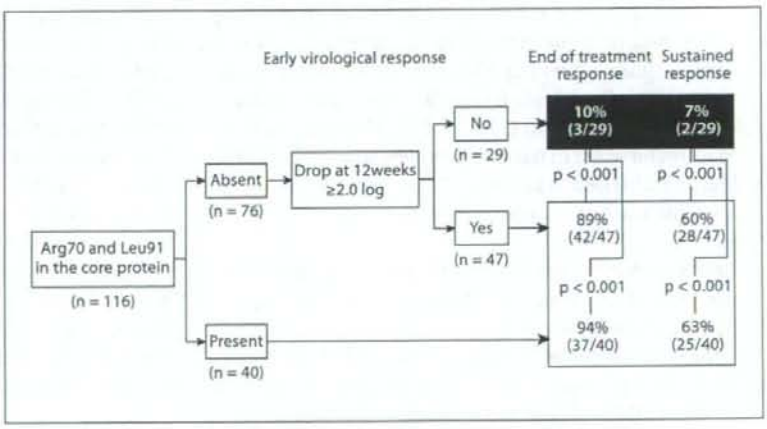

Fig. 3. ETR and SVR to combined Peg-IFN and ribavirin in patients with high-titer HCV-1b stratified by amino acid polymorphisms and early virological response at 12 weeks for a decrease in HCV RNA by ≥2.0 logs.

Table 4. Prediction of the lack of end-of-treatment response by polymorphism in the core protein combined with virological responses 48 h (PVR), 4 weeks (RVR) and 12 weeks (EVR) after the start of 48-week Peg-IFN and ribavirin in the patients with high-titer HCV-1b

Performance	(1) Gln70 and/or Met91 plus non-response at 48 h (PVR [–]) (n = 88)	(2) Gln70 and/or Met91 plus non-response at 4 weeks (RVR [–]) (n = 94)	(3) Gln70 and/or Met91 plus non-response at 12 weeks (EVR [–]) (n = 116)	Differences (p value)[a]		
				(1) vs. (2)	(2) vs. (3)	(1) vs. (3)
Sensitivity	17/23 (74%)	27/30 (90%)	26/34 (76%)	NS	NS	NS
Specificity	61/65 (94%)	55/64 (86%)	79/82 (96%)	NS	0.049	NS
Predictive value						
Positive	17/21 (81%)	27/36 (75%)	26/29 (90%)	NS	NS	NS
Negative	61/67 (91%)	55/58 (95%)	79/87 (91%)	NS	NS	NS

PVR = Prompt virological response; RVR = rapid virological response; EVR = early virological response; NS = not significant.
[a] Evaluated by Fisher's exact probability test.

Table 5. Prediction of the lack of sustained virological response by polymorphism in the core protein combined with virological responses 48 h (PVR), 4 weeks (RVR) and 12 weeks (EVR) after the start of Peg-IFN and ribavirin in the patients with high-titer HCV-1b

Performance	(1) Gln70 and/or Met91 plus non-response at 48 h (PVR [–]) (n = 88)	(2) Gln70 and/or Met91 plus non-response at 4 weeks (RVR [–]) (n = 94)	(3) Gln70 and/or Met91 plus non-response at 12 weeks (EVR [–]) (n = 116)	Differences (p value)[a]		
				(1) vs. (2)	(2) vs. (3)	(1) vs. (3)
Sensitivity	18/47 (38%)	31/50 (62%)	27/61 (44%)	0.026	NS	NS
Specificity	38/41 (93%)	39/44 (89%)	53/55 (96%)	NS	NS	NS
Predictive value						
Positive	18/21 (86%)	31/36 (86%)	27/29 (93%)	NS	NS	NS
Negative	38/67 (57%)	39/58 (67%)	53/87 (61%)	NS	NS	NS

PVR = Prompt virological response; RVR = rapid virological response; EVR = early virological response; NS = not significant.
[a] Evaluated by Fisher's exact probability test.

We have reported that polymorphisms of aa 70 and/or aa 91 in the core protein are associated with SVR in patients infected with HCV-1b in high loads (≥100 KIU/ml) and useful in predicting response to Peg-IFN and ribavirin prior to the institution of therapy [13, 14]. Both in uni- and multivariate analyses, Arg70 and Leu91 predicted ETR with the highest significance among 22 pretreatment variables in the present study (tables 2, 3); ribavirin dose ≥11 mg/kg was the only other factor predictive of ETR. A substantial weakness of Arg70 and Leu91 in predicting SVR, however, is their unsatisfactory sensitivity and specificity [13, 14]. Hence, Arg70 and Leu91 were combined with on-treatment virological responses in an attempt to achieve the maximum sensitivity and specificity in predicting SVR in the patients infected with high-titer HCV-1b.

As the results, PVR with a decrease in HCV RNA level ≥1.0 log by 48 h after the first dose of Peg-IFN and ribavirin, when it was combined with Arg70/Leu91, was highly specific in predicting SVR; it was comparable with RVR at 4 weeks or EVR at 12 weeks (93 vs. 89 or 96% (table 5)). Predicting SVR to Peg-IFN plus ribavirin in patients with high-titer HCV-1b, by combination with Arg70/Leu91 and PVR, would have clinical advantages. Patients with Arg70/Leu91 at the baseline can be continued aggressively on the 48-week combination therapy. Prediction of SVR would be particularly beneficial in the patients in old age or with hypertension [30], as well as those with insulin resistance [31, 32], who poorly respond to combination therapy. It would be particularly helpful in countries where HCC in the aged infected with HCV is coming to the fore [33, 34].

There are some theoretical bases for the association of polymorphisms in the HCV core protein with resistance to IFN. Both IFN-α and IFN-β bind to type-I IFN receptor, and one major pathway in type-I IFN signaling involves the signal transducer and activator of transcription (STAT) cascade [35]. Previous studies indicate possible association of HCV core region with the resistance to antiviral actions of IFN [36, 37]. Recently, de Lucas et al. [38] showed that the HCV core protein inhibits IFN-α-induced transcription of antiviral genes by reducing the binding of IFN-stimulated gene factor 3 to IFN-stimulated response element in HepG2 cells. Combined, polymorphisms of amino acids in the HCV core protein might be associated with resistance to the antiviral action of IFN therapy by influencing the Jak-STAT signaling cascade. Further studies for the structural and functional impact of aa 70 and/or aa 91 in the core protein on combined IFN and ribavirin therapy are needed to verify this mechanism.

In conclusion, Arg70/Leu91 in combination with PVR at 48 h are predictive of SVR in the patients infected with high-titer HCV-1b. It would be useful for promptly lifting the burden of severe side effects and high costs from some of them who have virtually no chances of gaining benefit by continuing combination treatment, even before they receive the second dose of Peg-IFN. Insofar as Arg70/Leu91 and PVR act complementarily in predicting SVR, it would be useful to include these pre- and on-treatment predictors in the management of patients with HCV-1b in high loads.

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