

included the following 29 variables: sex, age, history of blood transfusion, family history of liver disease, body mass index, AST, ALT, albumin,  $\gamma$ GTP, leukocyte count, hemoglobin, platelet count, indocyanine green retention rate at 15 min, serum iron, serum ferritin, creatinine clearance, level of viremia, AFP, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, fasting blood sugar, fibrosis stage, hepatocyte steatosis, IFN dose per day, and presence of initial daily IFN administration. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. Variables that achieved statistical significance ( $P < 0.05$ ) or marginal significance ( $P < 0.10$ ) on univariate analysis were entered into a multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using the SPSS software (SPSS, Inc., Chicago, IL). All  $P$  values of less than 0.05 by the two-tailed test were considered significant.

## RESULTS

### Efficacy of IFN Monotherapy

The rates of ALT normalization in the intention to treat population analysis were evaluated at 0, 4, 12, 24, and 48 weeks after commencement of treatment. As a whole, the rates were 18.0% (36/200), 50.5% (94/186), 65.9% (116/176), 58.4% (90/154), and 61.7% (71/115), respectively. Thus, ALT normalization rates favorably exceeded 50% at 4 weeks. Furthermore, in 40 patients with abnormal AFP levels ( $\geq 21$   $\mu$ g/L) at the commencement of treatment, AFP levels of 92.5% (37/40)

decreased and those of 7.5% (3/40) increased within 48 weeks. Especially, 52.5% (21/40) achieved normalization of AFP within 48 weeks. These results indicate that low-dose intermittent IFN monotherapy achieved favorable biochemical response.

### Predictive Factors Associated With ALT Normalization by Univariate and Multivariate Analysis

The data for the whole population sample were analyzed to determine those factors that could predict ALT normalization within 48 weeks after the commencement of treatment. Univariate analysis identified nine parameters that tended to or significantly correlated with ALT normalization. These included AST ( $P = 0.007$ ), ALT ( $P = 0.009$ ),  $\gamma$ GTP ( $P = 0.075$ ), platelets ( $P = 0.093$ ), fibrosis stage ( $P = 0.066$ ), indocyanine green retention rate at 15 min ( $P = 0.026$ ), serum iron ( $P = 0.003$ ), high-density lipoprotein cholesterol ( $P = 0.067$ ), and AFP ( $P = 0.046$ ). These factors were entered into multivariate analysis, which then identified indocyanine green retention rate at 15 min ( $P = 0.027$ ) as the parameter that influenced significantly and independently ALT normalization (Table II).

### Efficacy of IFN Monotherapy in Patients With Predictors of Poor Response to PEG-IFN Plus RBV Combination Therapy

Figure 1 shows the prevalence with respect to ALT normalization rates in patients with predictors of poor response to PEG-IFN plus RBV combination therapy.

TABLE II. Factors Associated With ALT Normalization During Interferon Monotherapy, Identified by Univariate and Multivariate Analysis

Factor	Category	Univariate Cox proportional hazard model		Multivariate Cox proportional hazard model	
		Odds ratio (95% CI)	$P$	Odds ratio (95% CI)	$P$
Aspartate aminotransferase (IU/L)	1:<70	1		—	—
	2: $\geq$ 70	0.589 (0.400–0.867)	0.007	—	—
Alanine aminotransferase (IU/L)	1:<75	1		—	—
	2: $\geq$ 75	0.588 (0.395–0.875)	0.009	—	—
$\gamma$ -Glutamyl transpeptidase (IU/L)	1:<50	1		—	—
	2: $\geq$ 50	0.636 (0.386–1.047)	0.075	—	—
Platelets ( $\times 10^4/\text{mm}^3$ )	1:<15.0	1		—	—
	2: $\geq$ 15.0	1.397 (0.946–2.064)	0.093	—	—
Fibrosis stage	1:1.2	1		—	—
	2:3.4	0.627 (0.381–1.031)	0.066	—	—
Indocyanine green retention rate at 15 min (%)	1:<20	1		1	—
	2: $\geq$ 20	0.557 (0.333–0.932)	0.026	0.503 (0.274–0.925)	0.027
Serum iron ( $\mu$ g/dl)	1:<150	1		—	—
	2: $\geq$ 150	0.522 (0.342–0.797)	0.003	—	—
High-density lipoprotein cholesterol (mg/dl)	1:<45	1		—	—
	2: $\geq$ 45	1.468 (0.973–2.215)	0.067	—	—
Alpha-fetoprotein ( $\mu$ g/L)	1:<10	1		—	—
	2: $\geq$ 10	0.662 (0.441–0.992)	0.046	—	—

Only variables that achieved statistical significance ( $P < 0.05$ ) or marginal significance ( $P < 0.10$ ) on univariate and multivariate Cox proportional hazard model are shown.

95% CI, 95% confidence interval.

According to the substitutions of core aa 70 and aa 91, the ALT normalization rates were 15.7% (18/115) versus 22.4% (17/76) at 0 week, 42.6% (46/108) versus 61.4% (43/70) at 4 weeks, 62.1% (64/103) versus 69.7% (46/66) at 12 weeks, 59.3% (54/91) versus 56.1% (32/57) at 24 weeks, and 58.5% (38/65) versus 63% (29/46) at 48 weeks for non-double wild-type and double wild-type, respectively [not significantly different, except for 4 weeks ( $P=0.021$ ), Fig. 1A].

According to sex, the ALT normalization rates were 22.7% (22/97) versus 13.6% (14/103) at 0 week, 50.6% (44/87) versus 50.5% (50/99) at 4 weeks, 66.3% (53/80) versus 65.6% (63/96) at 12 weeks, 62.7% (42/67) versus 55.2% (48/87) at 24 weeks, and 67.3% (33/49) versus 57.6% (38/66) at 48 weeks for female and male, respectively (not significant, Fig. 1B).

According to the levels of low-density lipoprotein cholesterol, the ALT normalization rates were 15.5% (9/58) versus 20.0% (23/115) at 0 week, 37.0% (20/54) versus 54.1% (59/109) at 4 weeks, 58.8% (30/51) versus 66% (68/103) at 12 weeks, 58.5% (24/41) versus 53.8% (49/91) at 24 weeks, and 66.7% (22/33) versus 57.4% (39/68) at 48 weeks for low levels (<86 mg/dl) and high levels ( $\geq 86$  mg/dl), respectively [not significant, except for 4 weeks ( $P=0.047$ ), Fig. 1C].

The above results indicate that in low-dose intermittent IFN monotherapy, ALT normalization rates of

patients with predictors of poor response were not different from those without such predictors.

### Efficacy of IFN Monotherapy in Non-Responders to IFN Plus RBV Combination Therapy

The rates of ALT normalization were evaluated in 27 patients who had received prior therapy with adequate IFN plus RBV for at least 24 weeks but showed no sustained virological response. The rates were 14.8% (4/27), 29.2% (7/24), 60.9% (14/23), 60% (12/20), and 40.0% (4/10) at 0, 4, 12, 24, and 48 weeks, respectively. Thus, ALT normalization rates favorably exceeded 60% at 12 weeks. These results indicate that non-responders to IFN plus RBV treated again with low-dose intermittent IFN monotherapy can achieve a favorable biochemical response.

## DISCUSSION

For chronic HCV infection, PEG-IFN plus RBV combination therapy is expensive, associated with severe side effects but results in a high-sustained virological response. However, it is desirable to identify those patients who do not achieve sustained virological response before the start of the combination therapy in order to free them of unnecessary side effects and high costs. One alternative option for such patients is IFN

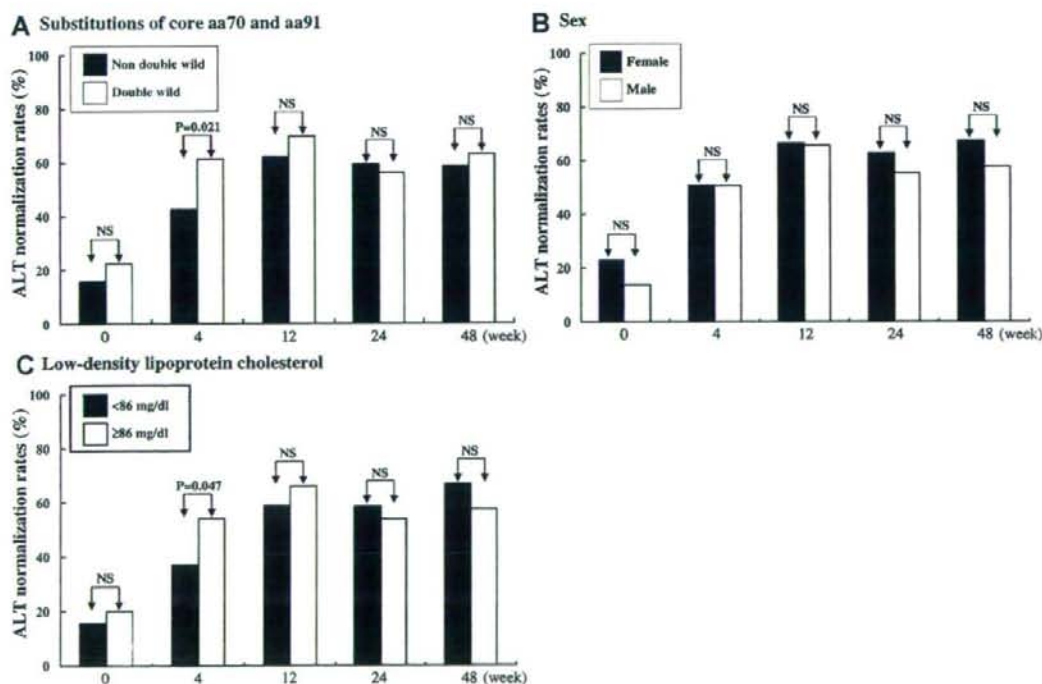


Fig. 1. The ALT normalization rates at 0, 4, 12, 24, and 48 weeks after commencement of IFN monotherapy, according to (A) substitutions of core aa 70 and aa 91, (B) sex, and (C) the levels of low-density lipoprotein cholesterol.



monotherapy, which could reduce the risk of hepatocarcinogenesis [Arase et al., 2001, 2007; Akuta et al., 2005a,b; Donlin et al., 2007; Nomura et al., 2007; McHutchison et al., 2008].

The efficacy of IFN monotherapy in patients who are predicted to respond poorly to IFN plus RBV combination therapy is still unknown. Previous results identified substitutions of aa 70 and/or 91 in the core region, female sex, and lower levels of low-density lipoprotein cholesterol as independent and significant pretreatment negative predictors associated with virological response [Akuta et al., 2005a, 2006, 2007a,b,c]. These studies also showed that substitutions of aa 70 and/or 91 are risk factors for hepatocarcinogenesis in the same patients [Akuta et al., 2007d]. The present study of low-dose intermittent IFN monotherapy showed that ALT normalization rates of patients who were predicted to have a poor response to the combination therapy were not different from others. It is important to achieve a better biochemical response regardless of core aa substitutions as risk factor of hepatocarcinogenesis. Thus, a low-dose intermittent IFN monotherapy for patients predicted to fail to respond to PEG-IFN plus RBV is an efficacious therapeutic regimen for normalization of ALT and thus reduction of risk of hepatocarcinogenesis.

The efficacy of IFN monotherapy for non-responders to IFN plus RBV combination therapy is still controversial. In the present study of low-dose intermittent IFN monotherapy, ALT normalization rates in non-responders to combination therapy favorably exceeded 60% at 12 weeks. Di Bisceglie et al. [2007] reported recently that maintenance therapy with PEG-IFN alpha-2a for non-responder to combination therapy was associated with significant decreases in ALT levels, but the rate of disease progression was similar in treated (34.1%) and untreated (33.8%) groups. The discrepancy between the present results and those of HALT-C trial may be due to one or more factors. The first reason for the difference is probably the large number of cirrhotic patients (about 40% of all) in HALT-C trial. The second reason is probably related to the difference in the efficacy measures used in HALT-C trial, which evaluated decreases in ALT levels regardless of ALT normalization or not. The third reason is probably related to the study design of HALT-C trial with PEG-IFN alpha-2a for non-responders to combination therapy [Di Bisceglie et al., 2007]. Large-scale prospective randomized controlled trials should be conducted in the future to confirm whether low-dose intermittent IFN monotherapy could reduce the risk of hepatocarcinogenesis based on ALT normalization for non-responders to PEG-IFN plus RBV combination therapy.

The present results based on multivariate analysis showed that a high level of indocyanine green retention rate at 15 min is a negative predictor of ALT normalization during the course of low-dose intermittent IFN monotherapy. Previous data indicated that absence of advanced liver fibrosis is a positive predictor of sustained virological response to IFN monotherapy and IFN plus RBV combination therapy [Jouet et al.,

1994; Poynard et al., 2000; Bruno et al., 2004]. Akuta et al. [2005a, 2007a] also showed previously that high levels of indocyanine green retention rate at 15 min or low levels of serum albumin are associated with advanced liver fibrosis, and that they are independent and significant negative predictors of the virological response to PEG-IFN plus RBV. To our knowledge, this is the first report that describes the relationship between indocyanine green retention rate at 15 min level and biochemical response during IFN monotherapy. Di Bisceglie et al. [2007] recently reported that maintenance therapy with PEG-IFN alpha-2a failed to halt liver disease progression in patients with advanced hepatic fibrosis. Further studies of large number of patients are required to investigate the relationship between severity of histopathological liver changes and biochemical response during low-dose intermittent IFN monotherapy.

One limitation of the present study was that viral factors other than the core region of HCV genome were not examined, such as the interferon sensitivity determining region (ISDR) and the interferon/ribavirin resistance determining region (IRDR) of NS5A region [Enomoto et al., 1995, 1996; El-Shamy et al., 2007]. Biochemical response during low-dose intermittent IFN monotherapy 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.

In conclusion, the present study showed that one therapeutic regimen that can reduce the risk of hepatocarcinogenesis based on ALT normalization is low-dose intermittent IFN monotherapy, for patients unsuitable for PEG-IFN plus RBV including non-responders. Large-scale prospective randomized controlled trials should be conducted to confirm this finding.

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# Efficacy in Patients with Dose Reduction in Combination Therapy of Peginterferon and Ribavirin for Chronic Hepatitis C

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## Key Words

Chronic hepatitis C · Peginterferon · Ribavirin ·  
Dose reduction

## Abstract

**Objective:** The aim of this study was to elucidate efficacy after dose reduction in combination therapy of peginterferon and ribavirin for chronic hepatitis C. **Methods:** Inclusion criteria were hepatitis C virus (HCV) genotype 1b, serum HCV RNA level of  $\geq 100$  KIU/ml, dose reduction of peginterferon and/or ribavirin between the first 4 weeks and 20 weeks after the initiation of treatment. 164 patients were enrolled in this retrospective cohort study. Predictive factors for sustained viral response (SVR) after dose reduction were examined. **Results:** Out of the 146 patients treated with dose reduction, 57 had SVR. Multivariate analysis showed that SVR occurred when serum HCV RNA at the time of dose reduction was negative ( $p < 0.001$ ) and total ribavirin dose was  $\geq 100\%$  of the anticipated total dose ( $p < 0.001$ ). 57% (55/97) of patients with undetectable serum HCV RNA at the time of dose reduction had SVR. In contrast, only 4% (2/49) of patients with detectable serum HCV RNA at the time of dose reduction had SVR. **Conclusions:** On dose reduction of com-

bination therapy for chronic hepatitis C, undetectable serum HCV RNA at the time of dose reduction and attainment of the total ribavirin dose of  $\geq 100\%$  enhance SVR.

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## Introduction

Combination therapy of peginterferon and ribavirin for hepatitis C virus (HCV) induces sustained virological response (SVR) in 50–60% of cases with genotype 1 and in 80–90% of cases with genotype 2. These SVR rates in patients treated with combination of peginterferon and ribavirin were higher than those treated with interferon (IFN) alone [1–6]. Thus, combination therapy of peginterferon and ribavirin has been recommended as a first choice for chronic hepatitis C. However, combination therapy has been associated with various adverse events, such as psychological disturbances, poor appetite, skin rash, infection, anemia and leukopenia [1, 2, 5, 7]. Dose reduction or even discontinuation of treatment often becomes necessary in combination therapy for chronic hepatitis C. In several studies, the reduction rate due to severe side effects was reported to be about 25–40%.

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Some authors have reported that adherence to combination therapy enhanced sustained virological eradication in genotype 1 with chronic hepatitis C [8–12]. In the present study, we evaluated the efficacy after dose reduction of combination therapy in Japanese patients. The study design is non-randomized retrospective cohort study.

## Materials and Methods

### Patients

Eligibility criteria for entry into the study included the following: (1) HCV genotype 1b; (2) serum level of HCV RNA of  $\geq 100$  KU/ml; (3) dose reduction of peginterferon and ribavirin between the first 4 weeks and 20 weeks after the initiation of combination therapy; (4) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; (5) no hepatitis B surface antigens, antinuclear antibodies, or antimitochondrial antibodies detectable in serum, determined by radioimmunoassay, and (6) leukocytes  $>2,000/\text{mm}^3$ , platelet count  $>80,000/\text{mm}^3$ , and bilirubin  $<2.0$  mg/ml. We excluded from the study all the patients with the following: (1) a history of alcohol abuse; (2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The Institutional Ethics Review Board of our hospital approved our study. The physician in charge explained the purpose and method of this clinical trial, as well as the potential adverse reactions, to each patient, who later gave his/her informed consent for participation.

### Combination Therapy of Pegylated-IFN and Ribavirin

For the treatment regimen, the peginterferon (Peg-intron; Schering-Plough Pharmaceutical Co., Osaka, Japan) and ribavirin (Rebetol; Schering-Plough Pharmaceutical Co.) were given based on body weight. At the initiation of combination therapy, patients received peginterferon at a median dose of 1.5  $\mu\text{g}/\text{kg}$  (range 1.2–1.6  $\mu\text{g}/\text{kg}$ ) subcutaneously each week and oral ribavirin at a median dose of 11.9 mg/kg (range 10.0–16.3 mg/kg) daily. The peginterferon dose was adjusted according to body weight (60  $\mu\text{g}$  for  $\leq 40$  kg, 80  $\mu\text{g}$  for  $>40$  and  $\leq 60$  kg, 100  $\mu\text{g}$  for  $>60$  and  $\leq 80$  kg, and 120  $\mu\text{g}$  for  $>80$  and  $\leq 100$  kg). The ribavirin dose was adjusted according to body weight (600 mg for  $\leq 60$  kg, 800 mg for  $>60$  and  $\leq 80$  kg, and 1,000 mg for  $>80$  kg).

The physician in charge reduced the dose of treatment when the blood cell count decreased. Peginterferon was stepwise reduced 20  $\mu\text{g}/\text{week}$  if the WBC declined to  $<1,500/\text{mm}^3$ , leukocyte count to  $<750/\text{mm}^3$  or platelet count to  $<80,000/\text{mm}^3$ . Ribavirin was stepwise reduced 200 mg if the hemoglobin level declined to  $\leq 10$  g/dl. The doses of peginterferon and ribavirin could be increased back to starting doses if these adverse events resolved. The amounts of both medications taken by each patient were expressed as a percentage of the anticipated total dose in the 48-week treatment regimen based on body weight. The median duration of treatment was 48 weeks (range 8–96 weeks). A SVR to therapy was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV, Version 2.0; Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy [13].

Blood samples were obtained just before and 6 months after combination therapy. The samples were stored at  $-80^\circ$  until analyzed. Using these blood samples, the HCV RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0; Roche Molecular Systems) [14]. HCV genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously [15]. The start of the follow-up period was defined as the first day of dose reduction in combination therapy. Clinical evaluation and biochemical and hematological tests were performed at 1- to 4-weekly intervals. We evaluated the following: (1) SVR after dose reduction, and (2) predictive factors for SVR after reduction based on combination therapy-related side effects.

### Liver Histology before IFN Therapy

Liver biopsy specimens were obtained percutaneously under the observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The biopsy specimens were scored according to the system of Desmet et al. [16].

### Statistical Analysis

A Cox proportional hazards model was used to analyze the factors contributing to the stop of treatment and dose reduction due to combination therapy: factors examined included age, sex, body mass index, histological findings, HCV load, ALT, hemoglobin, WBC, platelet count, HCV RNA at the time of dose reduction, total ribavirin dose and peginterferon dose. Significance of trends in values was determined with a Cochran-Armitage Trend Test.  $p < 0.05$  was considered statistically significant. The SPSS software package (SPSS 11.0 for Windows; SPSS Inc., Chicago, Ill., USA) was used for analyses.

## Results

### Clinical Characteristics of the Patients

A total of 146 patients were enrolled in the present study. The clinical characteristics of patients before combination therapy are shown in table 1. In 15 patients, a liver biopsy was not available because the patients declined to have a biopsy taken. Reduction time after the initiation of combination therapy was  $12.0 \pm 12.7$  weeks (mean  $\pm$  SD).

### Predictors for SVR after Dose Reduction in Combination Therapy

Out of the 146 patients treated with dose reduction, 57 had SVR. Univariate analysis showed that the following seven factors significantly affected the SVR rate in all the patients: HCV RNA at the time of dose reduction ( $p < 0.001$ ), HCV RNA at week 12 ( $p < 0.001$ ), peginterferon

**Table 1.** Clinical characteristics before combination therapy of peginterferon and ribavirin in chronic hepatitis C patients (n = 146)

Characteristics	Patients, n or median (range)
Age, years	55 (20–69)
Male/female	81/65
Body weight, kg/height, cm	64.9 (36.7–96.6)/ 163.2 (135.2–185.5)
Body mass index	23.1 (16.6–32.0)
History of interferon therapy (-/+)	79/67
Liver histology (fibrosis, mild/moderate/severe)	61/47/23
HCV load, KIU/ml	1,500 (105–5,000)
AST/ALT, IU/l	55 (20–324)/76 (13–580)
Hemoglobin, g/dl	14.4 (10.4–17.9)
Platelets, $\times 10^3/\text{mm}^3$	14.1 (8.4–26.0)
WBC, $\times 10^3/\text{mm}^3$	4,200 (2,000–8,800)

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; WBC = white blood cells.

and/or ribavirin dose ( $p < 0.001$ ), sex ( $p < 0.001$ ), HCV RNA level before treatment ( $p = 0.024$ ), histopathological staging ( $p = 0.031$ ), and HCV RNA at week 24 ( $p = 0.042$ ) (table 2A). The variables were mutually correlated and multivariate Cox regression analysis was performed with the seven statistically significant variables in the model. As shown in table 2B, multivariate analysis showed that SVR occurred when serum HCV RNA at the time of dose reduction after the initiation of combination therapy was negative ( $p < 0.001$ ) and total ribavirin dose was  $\geq 100\%$  ( $p < 0.001$ ).

#### SVR Rate Based on Adherence of Combination Therapy in Patients with Dose Reduction

The SVR rate based on adherence of combination therapy in patients with dose reduction was evaluated. Patients were divided into two groups based on the negativity or positivity of HCV RNA at the time of dose reduction after the initiation of combination therapy. Table 3A shows the SVR rate based on adherence of combination

**Table 2.** Predictive factors for SVR after dose reduction, based on combination therapy-related side effects

#### A Univariate analysis

Factor	Category	Odds ratio	95% CI	p value
HCV RNA at the time of reduction	-/+	1/0.09	0.04–0.21	<0.001
HCV RNA at week 12	-/+	1/0.09	0.04–0.22	<0.001
Total ribavirin dose, %	<100/ $\geq 100$	1/8.58	3.99–18.47	<0.001
Total peginterferon dose, %	<100/ $\geq 100$	1/3.37	1.672–7.04	<0.001
Sex	male/female	1/0.20	0.10–0.42	<0.001
HCV RNA, MEq/ml	<1,000/ $\geq 1,000$	1/0.43	0.21–0.90	0.024
Liver histology, fibrosis	mild/moderate or severe	1/0.46	0.23–0.93	0.031
HCV RNA at week 24	-/+	1/0.12	0.01–0.92	0.042
Hemoglobin, g/dl	<13/ $\geq 13$	1/2.29	0.87–5.98	0.092
Platelets, $\times 10^3/\text{mm}^3$	<10/ $\geq 10$	1/2.55	0.86–7.61	0.092
WBC, $\text{mm}^3$	<3,000/ $\geq 3,000$	1/1.14	0.40–1.27	0.062
ALT, IU/l	$\geq 100$ / $< 100$	1/1.31	0.59–2.93	0.164
Body mass index	<25/ $\geq 25$	1/1.24	0.59–2.62	0.566
Age, years	<55/ $\geq 55$	1/1.13	0.35–3.69	0.838

#### B Multivariate analysis

Factor	Category	Odds ratio	95% CI	p value
HCV RNA at the time of reduction	-/+	1/0.10	0.04–0.28	<0.001
Total ribavirin dose, %	<100/ $\geq 100$	1/6.90	2.54–19.05	<0.001

ALT = Alanine aminotransferase; WBC = white blood cells.



**Table 3.** SVR rate based on adherence of combination therapy in patients with **A** negativity and **B** positivity of serum HCV RNA at the time of dose reduction<sup>1</sup>

**A**

Total peginterferon dose, %	Total ribavirin dose, %				Total
	<60	61–80	81–100	101–130	
<60	25 (1/4)	40 (2/5)	75 (2/4)	none	38 (5/13)
61–80	0 (0/2)	50 (2/4)	60 (6/10)	100 (2/2)	56 (10/18)
81–100	33 (2/6)	36 (5/14)	50 (7/14)	82 (9/11)	52 (23/44)
101–130	none	none	67 (6/9)	79 (11/14)	77 (17/22)
Total	30 (3/10)	39 (9/23)	57 (21/37)	81 (22/27)	57 (55/97)

**B**

Peginterferon dose, %	Ribavirin dose, %				Total
	<60	61–80	81–100	101–130	
<60	0 (0/4)	0 (0/2)	0 (0/2)	none	0 (0/8)
61–80	0 (0/2)	0 (0/7)	0 (0/1)	0 (0/4)	0 (0/14)
81–100	0 (0/2)	0 (0/7)	0 (0/2)	20 (1/5)	6 (1/16)
101–130	none	none	0 (0/7)	25 (1/4)	9 (1/11)
Total	0 (0/8)	0 (0/16)	0 (0/12)	15 (2/13)	4 (2/49)

<sup>1</sup>  $p = 0.08$  for comparison of the 4 peginterferon groups and  $p < 0.001$  for comparison of the 4 ribavirin groups (Cochran-Armitage Trend Test).

therapy in patients with negativity of serum HCV RNA at the time of dose reduction. 55 (57%) of 97 patients with undetectable serum HCV RNA at the time of dose reduction had SVR. A stepwise increase in SVR was observed when the dose of ribavirin was increased ( $p < 0.001$ , Cochran-Armitage Trend Test). SVR was 42.8% (27/63) in patients who had negativity of serum HCV RNA at the time of dose reduction and had adherence of <100% in both peginterferon and ribavirin. Relapse rate after termination of combination therapy was 43% (42/97) in patients with negativity of serum HCV RNA at the time of dose reduction.

Table 3B shows the SVR rate based on adherence of combination therapy in patients with positivity of serum HCV RNA at the time of dose reduction. Only 2 (2%) of 49 patients with positivity of serum HCV RNA at the time of dose reduction had SVR.

#### Reasons for Dose Reduction

Of 146 patients with dose reduction, 40 had dose reduction of peginterferon only. 53 patients had dose reduction of ribavirin and 53 patients had both reduction of

peginterferon and ribavirin. The cause of reduction accounted for the following: anemia 87 (59.6%), leukopenia 39 (26.7%), thrombocytopenia 22 (15.1%) and other reason, such as general fatigue, 46 (31.5%). 55 of 146 patients with dose reduction had two causes for dose reduction.

#### Discussion

We have described the efficacy in patients with dose reduction after the initiation of combination therapy of peginterferon and ribavirin for chronic hepatitis C. The present study was limited to patients with genotype 1 and HCV load of  $\geq 100$  KIU/ml because previous studies have suggested that SVR in patients with genotype 2 or 3 is not adversely affected by dose reduction [5, 6, 17]. Another limitation of the study was that patients were treated for different durations. This heterogeneity makes it slightly difficult to interpret the results of the study.

However, several findings from the present study have direct implications for dose reduction of the combination therapy of chronic hepatitis C in the future. First, unde-



tectable serum HCV RNA at the time of dose reduction and attainment of total ribavirin dose of  $\geq 100\%$  enhanced SVR in patients with dose reduction. For now, the gold standard of treatment for chronic hepatitis C is a 48-week regimen of combination therapy. Many studies have suggested that reducing the ribavirin dose within the first 12–20 weeks of treatment in patients with genotype 1 was associated with a decline in SVR [8, 9, 18]. The present study indicated that the treatment for  $>48$  weeks and the total ribavirin dose of  $\geq 100\%$  enhanced SVR when patients with a dose reduction showed negativity of serum HCV RNA at the time of dose reduction. Second, most patients with detectable HCV RNA at the time of dose reduction did not have SVR regardless of a peginterferon and ribavirin dose of  $\geq 100\%$ . In patients with detectable HCV RNA at the time of dose reduction, prolonged combination therapy could not enhance SVR.

Several predictive factors of SVR to combination therapy in patients without dose reduction have been identified, and these include amino acid (aa) substitutions in HCV CR (double wild-type; arginine at aa 70/leucine at aa 91), low-density lipoprotein cholesterol ( $\geq 86$  mg/dl), male gender,  $\gamma$ -glutamyl transpeptidase ( $<109$  IU/l), indocyanine green retention test at 15 min ( $<10\%$ ), and ribavirin dose ( $\geq 11.0$  mg/kg) [19]. The present study indicates that HCV RNA at the time of dose reduction and total ribavirin dose were good indicators for predicting SVR in patients with dose reduction.

Some studies have suggested that SVR is increased when patients receive a higher dose of peginterferon and/or ribavirin according to body weight [1, 20]. However, in the present study, when the patients had detectable HCV RNA at the time of dose reduction, they had a slight chance of achieving a SVR regardless of prolonged combination therapy with a total dose of  $\geq 100\%$ . Thus, the suitable strategies of combination therapy for chronic hepatitis C patients with dose reduction within the first 20 weeks of treatment are as follows: (1) When the patients show undetectable serum HCV RNA at the time of dose reduction, they should be given combination therapy aimed at SVR; they should be treated with combination therapy of peginterferon and total ribavirin dose of  $\geq 100\%$ . (2) When they show a detectable serum HCV RNA at the time of dose reduction, they should not be given combination therapy aimed at SVR.

In conclusion, on dose reduction of combination therapy in patients with genotype 1b and high virus load, undetectable serum HCV RNA at the time of dose reduction and attainment of the total ribavirin dose of  $\geq 100\%$  enhance SVR.

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# **The Efficacy of Short-Term Interferon-Beta Therapy for Type C Cirrhotic Patients with Genotype 2a and Low Virus Load**

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## The Efficacy of Short-Term Interferon-Beta Therapy for Type C Cirrhotic Patients with Genotype 2a and Low Virus Load

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### Abstract

**Objective** The aim of this study was to elucidate the efficacy of short-term interferon (IFN) therapy for type C cirrhotic patients with genotype 2a and low virus load.

**Methods** The present study was retrospective cohort study. Inclusion criteria were liver cirrhosis, hepatitis C virus (HCV) genotype 2a, the serum HCV RNA level of less than 100 KIU/mL, and IFN period of 6 or 8 weeks. Twenty-five consecutive patients who satisfied the above criteria were treated with IFN-beta daily at the dosage of 6 MU for 6 or 8 weeks. Independent factors that might have influenced sustained virologic response (SVR) were studied using multiple logistic regression analysis.

**Results** Background of clinical profiles were as follows: median (range) age=64 (53-76) years, male/female=13/12, and median (range) HCV-RNA=31 (8-90) KIU/mL. Out of 25, 14 patients (56.0%) had SVR by the intention-to-treat analysis. The SVR was significantly associated with serum HCV RNA level. Logistic analysis showed that SVR occurred when HCV RNA level was <50 KIU/mL ( $p=0.047$ ). Based on the difference of the serum HCV RNA level, the SVR rate was 68.4% (13/19) in patients with a serum HCV RNA level of <50 KIU/mL and 16.7% (1/6) in patients with a serum HCV RNA level of  $\geq 50$  KIU/mL.

**Conclusions** The 6 or 8-week IFN-beta therapy is a possible selection of therapy for cirrhotic patients with HCV genotype 2a and a serum HCV RNA level of <50 KIU/mL.

**Key words:** liver cirrhosis, hepatitis C virus, genotype 2a, low virus load, interferon, sustained viral response

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### Introduction

Current interferon (IFN) therapy for patients with chronic hepatitis C viral infection has been directed at viral clearance. Recent studies have reported improvement of therapeutic efficacy when IFN is combined with ribavirin (1-8). However, IFN is expensive and has a number of serious side effects. Therefore, if the treatment period would become shorter, it could be preferable.

On the other hand, several predictive factors of sustained viral response (SVR) to IFN have been identified, and these

include short duration of disease, young age, absence of liver cirrhosis, genotype 2a, low hepatitis C virus (HCV)-RNA levels, HCV and mutant type of nonstructural5A region (9-15). Patients with liver cirrhosis (LC) have a high development of hepatocellular carcinoma (HCC) and progression to decompensated state. Thus, patients with a cirrhotic state should be treated for protection of progression of LC stage. In particular, LC patients with genotype 2a and low HCV-RNA levels might have the possibility of eradication of HCV RNA with a small dose or a short period of interferon (IFN). However, there is also controversy over how long the IFN therapy should be continued to eradicate HCV RNA in

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**Table 1. Clinical Characteristics before Short-term Interferon Therapy in Type C Liver Cirrhosis with Genotype 2a and Low Virus Load**

Characteristics	(n=25)
Age (years old)*	64 (53-76)
Male/female†	13/12
Period of IFN therapy (6w/8w)†	19/6
Total dose of IFN (MU)*	246 (123-336)
HCV load (KIU/mL)*	31 (8-90)
AST (IU/L)*	83 (39-203)
ALT (IU/L)*	74 (27-412)
Hemoglobin (g/dL)*	12.6 (9.7-16.3)
Platelet( $10^4/mm^3$ )*	11.4 (8.0-17.0)
WBC( $10^3/mm^3$ )*	3.8 (3.0-6.9)

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HCV: hepatitis C virus, IFN: interferon, MU: million unit, WBC: white blood cell count.

\*Data are expressed as median (range), †Data are number of patients.

LC patients with genotype 2a and low HCV-RNA.

Thus, in this study, we evaluated the efficacy of short-term interferon (IFN) therapy for type C cirrhotic patients with genotype 2a and a low virus load.

## Materials and Methods

### Patients

A total of 25 consecutive cirrhotic type C patients treated with IFN-beta for HCV RNA clearance at Toranomon Hospital in Tokyo, Japan between 2002 and 2006 were enrolled in this study. This study was a retrospective cohort study. Enrollment criteria were: repeated alanine aminotransferase (ALT) elevation of greater than the upper normal limits (ALT normal range: 12-50 IU/L) for more than six months; histological evidence of liver cirrhosis at the time of entry into the trial by the use of distinction equation between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection (16); positive serum HCV RNA; serum HCV RNA level of less than 100 KIU/mL; genotype 2a. We excluded from the study all the patients: 1) with concurrent hepatitis B virus (HBV); 2) with a history of IFN therapy; 3) Leukocytes  $<3,000/mm^3$ , platelets  $<80,000/mm^3$  and bilirubin  $>1.5$  mg/mL before IFN therapy.

Twenty-five patients received IFN at a dose of 6 million units (MU) of natural IFN-beta (Toray Industries or Daiichi Pharmaceutical Co., Tokyo, Japan) daily for 6 or 8 weeks. In general, patients were treated with IFN for 6 weeks and six patients who were treated for 8 weeks were assigned by randomized controlled trial. We regarded sustained virologic response (SVR) to therapy as clearance of HCV RNA by amplicor method (17) for more than 6 months after cessation of therapy. Our study was approved by the institutional ethics review board of our hospital. The physician in charge explained the purpose and method of the clinical trial as well as the potential adverse reactions to each patient, who later gave his/her informed consent for participation.

### Blood testing

Blood samples were obtained just before IFN therapy and stored at  $-80^{\circ}C$ . Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems, USA) (18).

On the other hand, serum HCV-RNA at 6 months after the termination of IFN therapy was analyzed by the qualitative PCR assay. The lower detection limit of the qualitative assay is 100 copies/mL. HCV genotype was examined by the PCR assay, using a mixture of primers for the six sub-

**Table 2. Predictive Factors for SVR in Short-term Interferon Therapy in Type C Liver Cirrhosis with Genotype 2a and Low Virus Load**

Factor	Category	Odds ratio	95% CI	p value*
HCV RNA (KIU/mL)	<50 / ≥50	1/0.09	0.01-0.97	.047
AST (IU/L)	≥76 / <76	1/0.46	0.18-1.17	.102
Age (years)	<60 / ≥60	1/0.22	0.04-1.42	.112
Platelet(10 <sup>4</sup> /mm <sup>3</sup> )	<10 / ≥10	1/3.00	0.57-15.76	.306
WBC(10 <sup>3</sup> /mm <sup>3</sup> )	<4 / ≥4	1/2.33	0.46-11.81	.367
Sex	Male / Female	1/0.71	0.14-3.58	.682
ALT (IU/L)	<100 / ≥100	1/0.75	0.13-4.29	.746
Total dose of IFN (IU/L)	<200 / ≥200	1/1.29	0.23-7.05	.772
Period of IFN therapy (week)	6 / 8	1/1.19	0.20-6.99	.851

ALT: alanine aminotransferase, AST: aspartate aminotransferase, CI: confidence interval, HCV: hepatitis C virus, IFN: interferon, WBC: white blood cell count.

\*p value calculated by logistic regression analysis.

types known to exist in Japan, as reported previously (19).

### Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test. Independent factors that might have influenced SVR were studied using multiple logistic regression analysis, and the following variables were evaluated as prognostic factors: sex, age, HCV RNA level, liver histology, biochemical factors (AST (aspartate aminotransferase), ALT) before IFN therapy and methods of IFN administration. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

## Results

### Patients' characteristics

Table 1 shows the characteristics of the 25 patients who

had performed IFN therapy. Clinical profiles were as follows: median (range) age=64 (53-76) years, male/female=13/12, and median (range) HCV-RNA=31 (8-90) KIU/mL. All the patients were categorized as Child-Pugh-Turcotte score class A. Of the 25 patients originally included in this study, in five patients the dose of the IFN therapy was reduced from 6 MU to 3 MU because of general fatigue and thrombocytopenia at the time of 1-3 weeks after the initiation of IFN. Thus, the total dose of IFN was 228.0±79.2 million units (MU). The median (range) leukocyte and platelet count in patients with dose reduction were 3,400 (3,100-4,800)/mm<sup>3</sup> and 95,000 (8.8-11.4)/mm<sup>3</sup>, respectively, while those in patients without dose reduction were 4,600 (3,000-6,900)/mm<sup>3</sup> and 120,000 (80,000-120,000)/mm<sup>3</sup>. Both leukocyte and platelet count in patients without dose reduction were higher than those in patients with dose reduction (leukocyte; p=0.013, platelet; p=0.011).

### Efficacy of treatment

Out of twenty-five patients enrolled on present study, 14 patients (56.0%) had SVR by the intention-to-treat analysis.



**Table 3. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR**

	SVR	Non-SVR	p value
Age (years old) †	7/7	2/9	0.183
(<60/≥60)			
Sex (male/female) †	8/6	5/6	0.647
Period of IFN therapy †	9/5	8/3	0.986
(6 week/8 week)			
Total dose of IFN (MU) † (<200/≥200)	5/9	5/6	0.698
HCV-load (KIU/mL) *	16 (8-69)	66 (23-98)	0.021
AST (IU/L) *	63 (39-203)	85 (53-141)	0.730
ALT (IU/L) *	75 (27-434)	88 (34-230)	0.557
Hemoglobin (g/dL) *	13.7 (10.1-16.3)	11.7 (9.7-16.1)	0.139
Platelet(10 <sup>4</sup> /mm <sup>3</sup> ) *	12.2 (8.7-17.0)	10.0 (8.0-16.0)	0.096
WBC(10 <sup>3</sup> /mm <sup>3</sup> ) *	4.0 (3.1-6.9)	3.8 (3.0-5.3)	0.841

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HCV:

hepatitis C virus, IFN: interferon, MU: million unit, SVR: sustained virologic response, WBC: white blood cell count.

\*Data are expressed as median (range), †Data are number of patients,

‡p value calculated by the Mann-Whitney U test.

The SVR was significantly associated with serum HCV RNA level. The patients with a HCV RNA level of <50 KIU/mL tend to have high SVR compared to those with higher than that in patients with HCV RNA level of ≥50 KIU/mL (Table 2). Based on the difference of serum HCV RNA level, the SVR rate was 68.4% (13/19) in patients with a serum HCV RNA level of <50 KIU/mL and 16.7% (1/6) in patients with a serum HCV RNA level of ≥50 KIU/mL. Table 3 shows the differences in the clinical background between patients with SVR and those without SVR. The serum level of HCV RNA in patients with SVR was lower than that in patients without SVR.

#### Adverse events

Within one week after the initiation of treatment, flu-like symptoms appeared in all the patients. The leukocyte count was 4,320±1,370/mm<sup>3</sup> and the platelet count was 119,000±23,000/mm<sup>3</sup> before the initiation of IFN therapy, whereas the values were 2,670±830/mm<sup>3</sup> and 71,000±17,000/mm<sup>3</sup>,

respectively, two weeks after the initiation of the therapy. None of the patients withdrew from this treatment due to IFN-related side effects.

## Discussion

The present study was limited by non-randomized controlled trial. Another limitation of the study was that the number of the patients was small. However, several findings from the present study have direct implications for the short-term IFN treatment of LC patients with genotype 2a and low virus load.

First, more than 50% of patients cleared HCV RNA. This result indicates that the 6- or 8-week regimen of IFN therapy was preferable to eradicate HCV RNA in LC patients with genotype 2a and low virus load. Second, the patients with HCV RNA level of <50 KIU/mL tend to have high SVR compared to those with higher than that in patients with HCV RNA level of ≥50 KIU/mL. On the treatment

period, the efficacy of the 6-week regimen of IFN therapy was almost the same as that of the 8-week regimen. Moreover, the efficacy of the total dose of IFN of <200 MU was not different from that by the total dose of  $\geq 200$  MU. These results indicate that in about two-thirds of LC patients with a genotype 2a and serum HCV RNA level of <50 KIU/mL and low virus load, HCV was eradicated by the 6-week regimen or total dose of IFN of <200 MU.

Regarding the side effects of IFN, no patient withdrew the treatment due to IFN-related side effect. Okanoue et al (20) reported that side effects occurred when the daily IFN dose was increased. In the present study, five patients had to reduce the IFN dose due to IFN side effects. On the IFN therapy for LC patients, the physician in charge should check the clinical findings compared to the patients with chronic hepatitis C.

At present, the combined IFN and ribavirin therapy is a standard therapy for chronic hepatitis C patients with genotype 1b and a high load of HCV-RNA. However, prolonged

combination therapy of IFN and ribavirin is associated with various side effects. If the total dose of IFN is decreased and the period of IFN therapy is short, it would be desirable from two points of cost and side effect. Fortunately, in patients with low HCV-RNA levels, HCV RNA tends to be eradicated with a small dose of IFN (21-24). The present study indicates that in patients with a low HCV-RNA, HCV RNA can be eradicated with a small dose of IFN.

## Conclusion

The present study indicates that the 6 or 8-week of IFN therapy is a possible selection of therapy for liver cirrhotic type C patients with genotype 2a and low virus load.

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# **Suitable Treatment Period in Patients with Virological Response during Combination Therapy of Peginterferon and Ribavirin for Chronic Hepatitis C**

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## Suitable Treatment Period in Patients with Virological Response during Combination Therapy of Peginterferon and Ribavirin for Chronic Hepatitis C

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### Abstract

**Objective** The aim of this study was to determine the suitable treatment period in patients who achieve virological response during combination therapy of peginterferon and ribavirin for chronic hepatitis C virus infection.

**Methods** Inclusion criteria were HCV-genotype 1b, serum HCV RNA level of  $\geq 100$  KIU/ml before treatment, and negativity of serum HCV RNA during treatment. The 366 patients were enrolled in this retrospective cohort study. Patients were classified into four groups according to difference of response: rapid-virological response (RVR) at week 4 after the initiation of treatment ( $n=37$ ), early-virological response (EVR) at week 5-12 ( $n=161$ ), late-virological response (LVR) at week 13-24 ( $n=131$ ), and superlate-virological response (SLVR) at week 25-48 ( $n=37$ ). A non-relapse in patients with undetectable HCV RNA during therapy was defined as clearance of HCV RNA 6 month after the cessation of therapy.

**Results** Of the 366 patients, 241 had non-relapse and the non-relapse rate in each group was 89% (33/37) in RVR, 79% (127/161) in EVR, 54% (71/131) in LVR, and 27% (10/37) in SLVR. In RVR, 26 of 27 patients with continuance of negative HCV RNA of  $\geq 30$  weeks during treatment had non-relapse. In EVR, patients with period of negative HCV RNA of  $\geq 40$  weeks had non-relapse rate of 90% (71/79). In LVR and SLVR, all nine patients with continuance of negative HCV RNA of  $\geq 60$  weeks had non-relapse.

**Conclusion** A suitable treatment period of combination therapy for chronic hepatitis C should be determined based on the time of attainment of negative HCV RNA.

**Key words:** chronic hepatitis C, peginterferon, ribavirin, suitable treatment period

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### Introduction

Current evidence indicates that combination therapy of peginterferon and ribavirin for hepatitis C virus (HCV) is associated with a higher rate of sustained virological response (SVR) compared with interferon (IFN) alone (1-7). Hence, combination therapy of peginterferon and ribavirin for HCV has been recommended as a first choice for chronic hepatitis C. During combination therapy, the serum

level of HCV RNA becomes negative in about 70 to 80% of patients with genotype 1 and a high virus load. However, about 20 to 30% of patients with negativity of HCV-RNA during combination therapy relapse after termination of 48 week treatment. Thus, there is an ongoing need to refine treatment strategies because many patients do not respond or relapse after treatment.

Jensen et al reported that a rapid virological response (RVR), defined as undetectable HCV RNA at week 4 of treatment with peginterferon  $\alpha$ -2a plus ribavirin, was the

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single best predictor of SVR in genotype 1 patients treated for either 24 or 48 weeks in a randomized, multinational trial (8). This indicates that patients with RVR should be treated for a short course-regimen. In contrast, it may be necessary to treat patients without RVR using a long course-regimen.

The selection of duration of treatment and optimum doses of combination therapy is an area of active investigation (9-15). When the patients with chronic hepatitis C have an undetectable HCV RNA during combination therapy, it is not clear how long the physician should continue the treatment. Thus, in this present study, we performed a retrospective study to examine the relationship between attainment time of negativity of serum HCV RNA after the initiation of combination therapy and the continuance period of negative HCV RNA in patients with undetectable HCV-RNA during treatment.

## Materials and Methods

### Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 1b; 2) serum level of HCV RNA of  $\geq 100$  KIU/ml before treatment; 3) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 4) no hepatitis B surface antigens (HBsAg), antinuclear antibodies, or antimitochondrial antibodies detectable in serum, determined by radioimmunoassay; 5) leukocytes  $>2,000/\text{mm}^3$ , platelet count  $>80,000/\text{mm}^3$ , and bilirubin  $<2.0$  mg/ml; 6) negativity of serum HCV RNA within 48 weeks after initiation of combination therapy; 7) duration of treatment of  $\geq 8$  weeks; 8) the initiation time of treatment was from December 2004 to September 2006. We excluded from the study all the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The institutional ethics review board of our hospital approved our study. The physician in charge explained the purpose and method of this clinical trial, as well as the potential adverse reactions, to each patient, who later gave his/her informed consent for treatment. Informed consent was obtained after confirming the negativity of serum HCV RNA during combination therapy. A total of 366 HCV patients were enrolled in this retrospective cohort study at the study hospital.

Patients were classified into four groups according to the response of combination therapy: RVR, defined as undetectable HCV RNA at week 4 after the initiation of combination therapy; early virological response group (EVR), defined as undetectable HCV RNA at week of 5 to 12 of combination therapy; late virological group (LVR), defined as undetectable HCV RNA at week 13 to 24 of combination therapy; and super late virological group (SLVR), defined as undetectable HCV RNA at week 25 to 48 of combination therapy. A non-relapse in patients with undetectable HCV

RNA during combination therapy was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver.2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 month after the cessation of combination therapy (16).

Patients were classified into four groups and evaluated according to the difference of clinical and biochemical backgrounds. Next, predictors of no-relapse in patients with undetectable HCV RNA in serum during treatment were assessed by the multiple logistic regression analysis. Finally, the no-relapse rate based on the attainment time of negativity of HCV RNA and continuance of negative HCV RNA during combination therapy was examined.

### Combination therapy of peginterferon and ribavirin

For the treatment regimen, the peginterferon (Peg-intron, Schering-Plough Pharmaceutical Co., Osaka, Japan) and ribavirin (Rebetol, Schering-Plough Pharmaceutical Co.) were given at the dose described based on body weight. At the initiation of combination therapy, patients received peginterferon at a median dose of 1.4  $\mu\text{g}/\text{kg}$  (range, 1.3-1.8  $\mu\text{g}/\text{kg}$ ) subcutaneously each week and oral ribavirin at a median dose of 11.9 mg/kg (range, 9.5-15.4 mg/kg) daily. The peginterferon dose was adjusted according to body weight (60  $\mu\text{g}$  for  $\leq 40$  kg, 80  $\mu\text{g}$  for  $>40$  kg and  $\leq 60$  kg, 100  $\mu\text{g}$  for  $>60$  kg and  $\leq 80$  kg, 120  $\mu\text{g}$  for  $>80$  kg and  $\leq 100$  kg, and 150  $\mu\text{g}$  for  $>100$  kg). The ribavirin dose was adjusted according to body weight (600 mg for  $\leq 60$  kg, 800 mg for  $>60$  kg and  $\leq 80$  kg, and 1,000 mg for  $>80$  kg).

A total of 202 patients were treated with 48 week regimen and 113 patients were given combination therapy for  $> 48$  week regimen. Treatment for the remaining 51 patients was discontinued because of treatment-related side effects within 48 weeks after the initiation of combination therapy. On the duration of treatment, 126 patients were given combination therapy based on regimen of clinical trial, 142 patients were treated for 48 weeks of within health insurance, and 98 patients were decided by physician's judgement.

Blood samples were obtained just before and 6 months after combination therapy. The samples were stored at  $-80^\circ\text{C}$  until analysis. Using these blood samples, the HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (17). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (18). Serum alanine aminotransferase (ALT), aspartic aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay.

### Follow-up protocol

The start of follow-up period was defined as the first day that showed negative HCV RNA after the initiation of com-