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Journal of Medical Virology 85:1523–1533 (2013)

New Proposal for Response-Guided Peg-Interferon-Plus-Ribavirin Combination Therapy for Chronic Hepatitis C Virus Genotype 2 Infection

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This study aimed to determine the most suitable duration of pegylated-interferon (Peg-IFN)-plus-ribavirin combination therapy in patients infected with hepatitis C virus (HCV) genotype 2 who had not achieved rapid virological response (serum HCV RNA disappearance after 4 weeks of therapy). HCV genotype 2 patients (n = 182) with a high viral load received >80% of the standard Peg-IFN-plus-ribavirin dose for at least 24 weeks, and their final virological responses were studied. Patients were classified into "rapid virological response" and "non-rapid virological response" groups. The non-rapid virological response group was further divided into a "virological response at 8 weeks" (serum HCV RNA disappearance after 8 weeks of therapy) and a "non-virological response at 8 weeks" group. Factors related to rapid virological response and optimal therapy duration in the non-rapid virological response group were evaluated. Multivariate logistic regression analysis showed that subtype HCV genotype 2a ($P = 0.0015$) and low concentration of pretreatment serum HCV RNA ($P = 0.0058$) were independent factors in a rapid virological response. In the virological response at 8 weeks group, the sustained virological response rate after 24 weeks of therapy was significantly lower than after 36 weeks ($P = 0.044$) or after 48 weeks ($P = 0.006$), and was similar for 36- and 48-weeks. The cost for achieving (CAS) one sustained virological response was lowest with 36-week therapy. Prolongation of Peg-IFN-plus-ribavirin combination therapy to 36 weeks is suitable for achieving virological response at 8 weeks, given the high, sustained virological response rate and cost benefit. ***J. Med. Virol. 85:1523–1533, 2013.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: cost per one patient achieving sustained virological response (CAS); duration of therapy; HCV genotype 2; response-guided therapy; virological response

INTRODUCTION

Approximately 200 million people worldwide are chronically infected with hepatitis C virus (HCV) [Geneva: World Health Organization, 2011]. HCV infection is the most common cause of chronic liver disease and a leading cause of cirrhosis and hepatocellular carcinoma (HCC) globally [Perz et al., 2006].

Chronic HCV infection has been treated with pegylated-interferon (Peg-IFN)-α-plus-ribavirin combination therapy. Although there are many host and viral factors affecting the efficacy of combination therapy, one of the most important viral predictors is HCV genotype [Lee and Abdo, 2003]. In Japan, approximately 70% of patients with HCV infection carry HCV genotype 1, while 30% have HCV genotype 2 [Okamoto et al., 1992]. The rates of sustained virological response, defined as undetectable serum HCV RNA at least 6 months after completion of combination therapy for 48 weeks for HCV genotype 1 and for 24 weeks for HCV genotype 2 are 42–52%

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Accepted 20 March 2013

DOI 10.1002/jmv.23626
Published online 17 June 2013 in Wiley Online Library (wileyonlinelibrary.com).

and 81–84%, respectively [Manns et al., 2001; Fried et al., 2002].

For treatment of HCV genotype 2, the duration of combination therapy has been extended to raise the sustained virological response rate in patients who do not show a rapid virological response (serum HCV RNA disappearance after 4 weeks of therapy) [Sato et al., 2012], while several trials to shorten the treatment duration have been carried out in patients who had favorable host and viral characteristics and who achieved rapid virological response with combination therapy [Toyoda et al., 2009]. The aim of shortening the duration of therapy is mainly to reduce the expense and the adverse effects of treatment [Yu et al., 2008; Berg et al., 2009]. The results of some randomized, controlled studies suggested that patients with HCV genotype 2 who achieved rapid virological response had a very high, sustained virological response rate after 12 or 16 weeks of Peg-IFN-plus-ribavirin combination therapy [Mangia et al., 2005; von Wagner et al., 2005]. However, another group reported that shortening the duration of combination therapy from 24 to 16 weeks in patients with HCV genotype 2 or 3 lowered the probability of sustained virological response, even in patients who had attained rapid virological response [Shiffman et al., 2007]. A recent meta-analysis on suitable duration of therapy concluded that HCV genotype 2 patients can be treated with 16 weeks of Peg-IFN plus weight-based ribavirin combination therapy if they achieve rapid virological response [Di Martino et al., 2011]. Therefore, the validity of shortening the duration of combination therapy appears to have been established.

In patients who did not achieve rapid virological response, the sustained virological response rate after 24 weeks of therapy decreased to approximately 50% [von Wagner et al., 2005], suggesting that extension of the duration of combination therapy is needed to acquire a higher sustained virological response rate. Response-guided extension of therapy duration (response-guided therapy) appears to be one of the most useful methods for obtaining a higher sustained virological response rate, as demonstrated for HCV genotype 1 [Pearlman et al., 2007], but the usefulness of response-guided therapy for patients with HCV genotype 2 who do not achieve rapid virological response with conventional treatment has not been definitively confirmed.

In the present study, the sustained virological response rate was examined in patients with HCV genotype 2 who had been treated with 24-week therapy and who achieved rapid virological response, and the factors contributing to this rapid virological response were identified. In addition, we evaluated the factors contributing to a sustained virological response in non-rapid virological response patients. Then, the significance of response-guided therapy was evaluated, with a focus on determining the most suitable duration of Peg-IFN-plus-ribavirin combination therapy, based on the period in which HCV RNA was not detectable during combination therapy.

METHODS

Patients

This study complied with the standards of the 1975 Declaration of Helsinki and current ethical guidelines as reflected by approval by the human ethics review committee of the Katsushika Medical Center, Jikei University School of Medicine, and Shinmatsudo Central General Hospital. Informed written consent was obtained from each patient.

A total of 245 patients chronically infected with HCV genotype 2 was treated with Peg-IFN-α-2b (PegIntron, MSD K.K., Tokyo, Japan) plus ribavirin (Rebetol, MSD K.K.) combination therapy, after informed consent for treatment was obtained, between January 2006 and August 2011 at Jikei University Katsushika Medical Center, Tokyo, Japan, or Shinmatsudo Central General Hospital, Chiba, Japan. All patients satisfied the following criteria: amount of serum HCV RNA $\geq$10,000 copies/ml (AMPLICOR HCV MONITOR Test, version 2.0, Roche Molecular Systems, Pleasanton, CA; quantification limit: 50 IU/ml) or $\geq$5 $\log_{10}$IU/ml (COBAS AmpliPrep/COBAS TaqMan HCV Test, Roche Molecular Systems; quantification limit: 1.2 $\log_{10}$IU/ml) which have been defined as "high viral load" according to the Japanese criteria [Kumada et al., 2010]; white blood cell count $\geq$3,000/mm^3; neutrophil count $\geq$1,500/mm^3; hemoglobin level $\geq$12 g/dl; and platelet count $\geq$85,000/mm^3. Patients who were positive for the hepatitis B surface antigen or anti-human immunodeficiency virus antibody, or whose current alcohol consumption was >20 g/day, had psychiatric conditions or HCC, or whose diagnosis was complicated by other liver diseases, were excluded.

All patients were scheduled to receive Peg-IFN-α-2b at a dose of 1.5 μg/kg subcutaneously once weekly, plus ribavirin at a dose of 600–1,000 mg/day according to body weight (<60 kg: 600 mg/day; 60–80 kg: 800 mg/day; >80 kg: 1,000 mg/day) for at least 24 weeks.

Of 245 patients, 182 patients who underwent liver biopsy and in whom the subtype of HCV genotype 2 was determined prior to therapy and who had received >80% of the scheduled dosage of both Peg-IFN-α-2b and ribavirin, and in whom the final virological response at 24 weeks after the end of treatment (either sustained virological response or non-sustained virological response) had been determined, were enrolled in this study. Of the 182 patients, 37 patients had received interferon monotherapy (retreatment cases), whereas 145 patients had not received interferon (naïve cases). None of the retreatment cases had received Peg-IFN-plus-ribavirin combination therapy before enrolment in this study. Establish cirrhotic cases that were easily diagnosed by image inspection, or in whom laboratory

tests did not indicate the need for liver biopsy, were not included in the present study. The baseline characteristics of the 182 patients are summarized in Table I.

Histology and HCV Subtyping

The histological grade of fibrosis was evaluated, according to the METAVIR scoring system [Bedossa and Poynard, 1996], as F1–F4. The subtype of HCV genotype 2 (2a or 2b) was examined according to the method previously reported [Ohno et al., 1997]. The pre-treatment serum HCV RNA concentration, established using the quantitative AMPLICOR HCV MONITOR (version 2.0; Roche Molecular Systems) HCV RNA assay, was converted into $\log_{10}$ IU/ml.

Single Nucleotide Polymorphism Analysis

Additionally, the rs8099917 single nucleotide polymorphism (SNP) near the interleukin-28B (IL-28B) gene, which was reported to be an independent predictive factor for a rapid virological response to Peg-IFN-plus-ribavirin combination therapy in Asian chronic HCV genotype 2 infection [Yu et al., 2011], was examined in 74 of the patients by using TaqMan SNP Genotyping Assays and a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) [Tanaka et al., 2009].

Detection of HCV RNA

Presence or absence of serum HCV RNA was evaluated every 4 weeks during combination therapy, at the end of therapy, and at 24 weeks after the end of therapy. HCV RNA in the sera was evaluated by qualitative AMPLICOR HCV MONITOR (Roche Molecular Systems) HCV RNA assay until November 2007, and by COBAS AmpliPrep/COBAS TaqMan HCV test (Roche Molecular Systems) since December 2007. To prevent errors due to differences in the measurement method, in cases in which serum HCV RNA had originally been examined by qualitative AMPLICOR MONITOR HCV RNA assay, HCV RNA was re-determined using the COBAS AmpliPrep/COBAS TaqMan HCV test in serum stocks that had been stored at −30°C.

TABLE I. Baseline Characteristics of Study Patients (n = 182)

Age (years)	53.9 ± 12.4
Sex (male/female)	74/108
History of interferon therapy (naïve/retreatment)	145/37
Body mass index (kg^2/cm)	23.5 ± 3.7
Pretreatment HCV RNA concentration ($\log_{10}$ IU/ml)	5.9 ± 1.0
Genotype (2a/2b/not detect)	99/71/12
Histological fibrosis of liver (F0/1/2/3/4)	3/26/95/46/12
Platelet count ($\times 10^3$/ml)	18.3 ± 5.6
Alanine aminotransferase (IU/L)	79.9 ± 100.5
Total cholesterol (mg/dl)	179.9 ± 34.5
LDL-cholesterol (mg/dl)	104.6 ± 30.1
Duration of treatment (24/36/48 weeks)	122/23/37

Naïve, patients had not have interferon therapy; retreatment, patients had haven interferon mono-therapy.

Study Design

The 182 patients were classified into two groups according to the virological response to Peg-IFN-plus-ribavirin therapy after 4 weeks of therapy as the rapid virological response group and the non-rapid virological response group. The group in whom serum HCV RNA was undetectable by COBAS AmpliPrep/COBAS TaqMan HCV Test at 4 weeks after the end of therapy was defined as the rapid virological response group, whereas the group in whom HCV RNA was still detected at this time-point was defined as the non-rapid virological response group. All rapid virological response group patients were treated with 24-week therapy. Patients in the non-rapid virological response group were further divided into two groups according to the virological response at 8 weeks after the end of therapy. Those in whom HCV RNA was undetectable at 8 weeks after the end of treatment was defined as the "virological response at 8 weeks" group, while those in whom HCV remained detectable at this time-point was defined as the "non-virological response at 8 weeks" group. The "virological response at 8 weeks" group was further sub-divided into three groups according to the duration of Peg-IFN-α-2b-plus-ribavirin combination therapy (24-, 36-, and 48-week group). Duration of therapy was selected freely by the patients. Similarly, the "non-virological response at 8 weeks" group was sub-divided into two groups according to the duration of therapy (36- and 48-week group; Fig. 1).

The sustained virological response rate with 24-week therapy was compared between the rapid virological response group and the "virological response at 8 weeks" group and factors significantly associated with rapid virological response were extracted. Then, factors affecting a sustained virological response in the non-rapid virological response patients were examined. In the "virological response at 8 weeks" group and the "non-virological response at 8 weeks" group, the sustained virological response rate was examined according to the duration of combination therapy. Additionally, the cost–benefit of extension of combination therapy in the "virological response at 8 weeks" group of patients was evaluated by comparing the cost for attaining one sustained virological response patient in the 24-week group, the 36-week group, and the 48-week group, using the following formula.

Cost per one patient achieving sustained virological response (CAS) = (sum of drug costs in each group)/(number of patients who achieved sustained virological response in each group) and was expressed as the ratio when the CAS of the 24-week group was adjusted to 1. Finally, the effect of the HCV genotype 2 subtype (HCV genotype 2a or 2b) on the sustained virological response rate was determined.

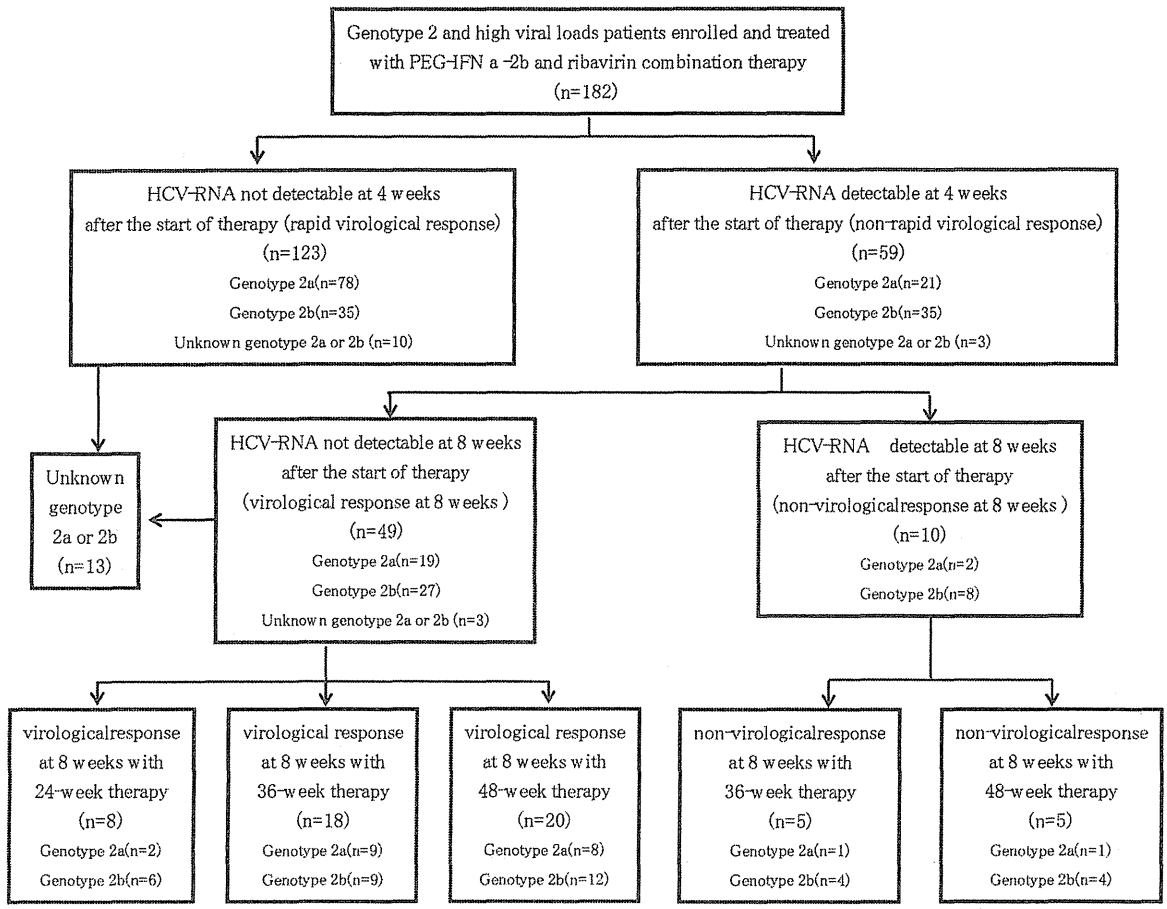

Fig. 1. Study flow chart: PEG-IFN, peg-interferon; rapid virological response, serum HCV RNA disappearance at 4 weeks of therapy; virological response at 8 weeks, serum HCV undetectable at 8 weeks of treatment was defined.

Statistical Analyses

The Mann–Whitney *U*-test was used to analyze differences in continuous variables. Fisher's exact tests were used to analyze differences in categorical data.

To determine the baseline factors associated with a rapid virological response, univariate analyses and a multivariate logistic regression model were used after categorizing the continuous data into two groups by the median values of the parameters. The grade of liver fibrosis was categorized into two groups as 1: F0–F2 or 2: F3–F4.

All tests of significance were two-tailed, and a *P*-value <0.05 was considered significant, while *P*-values <0.1 was considered marginal. To identify independent predictive factors, variables that were significant or marginal on univariate analysis were considered as candidates for multivariate logistic regression analysis. All statistical analyses were carried out using STATISTICA for Windows version 6 (StatSoft, Tulsa).

RESULTS

Virological Responses During Peg-IFN-Plus-Ribavirin Combination Therapy and the Sustained Virological Response Rate After 24 Weeks of Therapy

Serum HCV RNA was undetectable on at least one test in the 24-week period after the start of treatment, and reduced to undetectable during therapy in all 182 patients. Of the 182 patients, 123 (67.6%) achieved a rapid virological response. Of the 145 naïve cases, 97 (66.9%) achieved a rapid virological response. In 49 of the 59 patients (83%) who did not achieve rapid virological response, serum HCV RNA was undetectable at 8 weeks after the start of treatment ("virological response at 8 weeks" group), whereas in 179 of the 182 patients serum HCV RNA was undetectable at 12 weeks after therapy commenced (Fig. 2).

Of the 123 rapid virological response patients, 114 (94.5%) achieved sustained virological response with 24-week therapy, while only four of nine (44.4%)

patients in "virological response at 8 weeks" group achieved sustained virological response with 24-week therapy (Fig. 3).

Factors Affecting Rapid Virological Response to Peg-IFN-Plus-Ribavirin Combination Therapy

Subtypes of HCV genotype 2 (2a vs. 2b; $P = 0.0001$) and pre-treatment serum HCV RNA concentration (<6.2 vs. $\geq 6.2\log_{10}$ IU/ml; $P = 0.0002$) were factors significantly related to a rapid virological response on univariate analysis. There were no marginal differences. On multivariate logistic regression analysis, the subtype of HCV genotype 2 (2a vs. 2b; $P = 0.0015$) and pretreatment HCV RNA concentration (<6.2 vs. $\geq 6.2\log_{10}$ IU/ml; $P = 0.0058$), were again identified as independent factors related to a rapid virological response.

These findings were similar when the subjects were limited to the 146 naïve cases; subtype of HCV genotype 2 (2a vs. 2b; $P = 0.0003$) and pre-treatment serum HCV RNA concentration (<6.2 vs. $\geq 6.2\log_{10}$ IU/ml; $P = 0.0026$) were the factors significantly related to a rapid virological response on univariate analysis. Marginal differences were identified as fibrosis of the liver on histology (1: F0–F2 vs. 2: F3–F4; $P = 0.0656$). On multivariate logistic regression analysis, the subtype of HCV genotype 2 (2a vs. 2b; $P = 0.0093$) and pretreatment HCV RNA concentration (<6.2 vs. $\geq 6.2\log_{10}$ IU/ml; $P = 0.0382$) were again revealed as independent factors related to a rapid virological response (Table II).

As to the rs8099917 genotype, although only 74 of the 182 patients could be examined, the ratio of the major (TT) genotype was similar between the rapid virological response patients (36 of 46 patients; 78.3%) and non-rapid virological response patients (22 of 28 patients; 78.6%).

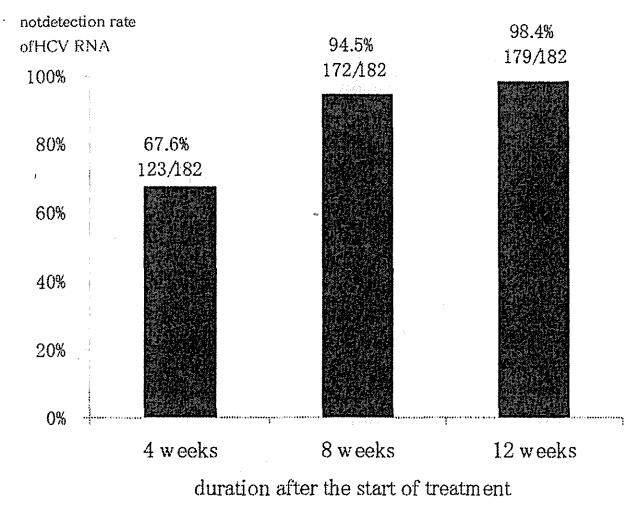

Fig. 2. Not detection rate of HCV RNA according to duration after the start of treatment (n = 182).

Factors Affecting Sustained Virological Response During Peg-IFN-Plus-Ribavirin Combination Therapy in Non-Rapid Virological Response Patients

In non-rapid virological response patients, duration of treatment (24-week vs. 36- or 48-week; $P = 0.0038$) and HCV undetectable at 8 weeks of treatment (yes vs. no; $P = 0.0017$) were the factors significantly related to sustained virological response upon univariate analysis. No marginal differences were detected. On multivariate logistic regression analysis, duration of treatment (24-week vs. 36- or 48-week; $P = 0.0021$) and HCV undetectable at 8 weeks of treatment (yes vs. no; $P = 0.0006$), were again identified as significant independent factors related to sustained virological response (Table III).

Sustained Virological Response Rate and Cost–Benefit According to Duration of Peg-IFN-Plus-Ribavirin Combination Therapy in the "Virological Response at 8 Weeks" Group

The sustained virological response rate was significantly lower in the 24-week therapy group than in the 36-week therapy group ($P = 0.044$) or the 48 weeks group ($P = 0.006$). However, the sustained virological response rate was similar between the 36-week therapy group and the 48-week therapy group (Fig. 3). The baseline characteristics of these three groups were not significantly different.

CAS was the highest in the 24-week therapy group and lowest in the 48-week therapy group. CAS was 0.89-fold lower in the 48-week therapy group and 0.69-fold lower in the 36-week group than in the 24-week therapy group. These findings are summarized in Table IV.

Sustained Virological Response Rate According to Therapy Duration in the "Non-Virological Response at 8 Weeks" Group

With 36 weeks of therapy, none of the five patients achieved sustained virological response, while three of the five patients (60%) achieved sustained virological response with 48 weeks of therapy.

Differences in Sustained Virological Response Rate Between Patients Infected With HCV Genotypes 2a and 2b

In rapid virological response patients, 72 of 78 (92.3%) with HCV genotype 2a and 34 of 35 (97.1%) with HCV genotype 2b achieved sustained virological response. Although the sustained virological response rate was slightly higher in HCV genotype 2b patients than in HCV genotype 2a patients, there was no significant difference. In "virological response at 8 weeks" patients, sustained virological response was not achieved in HCV genotype 2a (0/2), but it was achieved in 66.7% (2/3) of HCV genotype 2b patients by 24-week therapy. The sustained virological response rate was

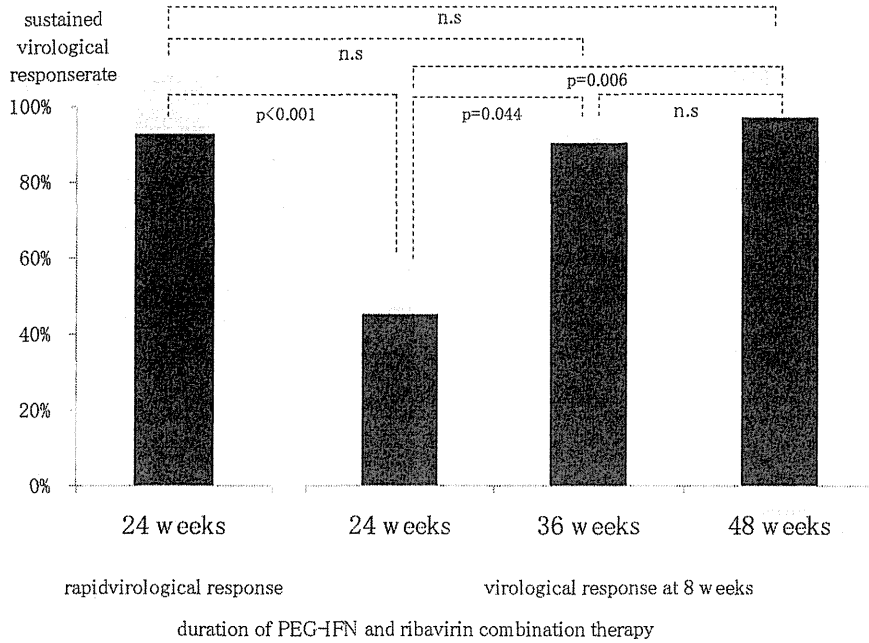

Fig. 3. Relation between sustained virological response rate in non-rapid virological response patients whose serum HCV RNA were not detectable at 8 weeks ("virological response at 8 weeks") and duration of PEG-IFN and ribavirin combination therapy.

similar and exceeded 87.5% in HCV genotype 2a and HCV genotype 2b patients after 36- or 48-week therapy (Fig. 4).

Of 10 "non-virological response at 8 weeks" patients, none of those with HCV genotype 2a, but 37.5% (3/8) of those with HCV genotype 2b, achieved a sustained virological response. In HCV genotype 2b patients, the sustained virological response rate was higher with 48-weeks therapy (3/4; 75%) than with 36-weeks therapy (0/4; 0%). Furthermore, "non-virological response at 8 weeks" patients were not frequently judged to be F3 or F4 on histological evaluation.

DISCUSSION

In the present cohort study, the rapid virological response rate and the sustained virological response rate with 24-week therapy for HCV genotype 2 patients were similar to those reported by several previous studies [Toyoda et al., 2009; Inoue et al., 2010]. As the sustained virological response rate exceeded 90% in patients who achieved rapid virological response in the present study, a therapy duration of 24 weeks was sufficient for patients who achieved a rapid virological response. However, the use of 24-week therapy for patients who did not achieve rapid virological response is not justified, as the sustained virological response rate with 24-week therapy is reported to be very low [Andriulli et al., 2008], and was only 44.4% in our study. For these patients, a novel therapeutic agent [Asselah and Marcelin, 2011] may be needed. Unfortunately, a considerable time will be needed for the approval of such a drug. Recently, response-guided extension of therapy from 24 to 48 weeks has been used to improve the efficacy of antiviral therapy in chronic HCV genotype 2 patients who failed to achieve a rapid virological response [Sato et al., 2012; Yamaguchi et al., 2012]. However, the validity of 48 weeks of therapy has not been verified.

In the present study, factors associated with failure to achieve rapid virological response were examined, because prediction of capacity for rapid virological response is important, to give a patient notice of the possibility of extending the duration of therapy. Multivariate logistic analysis indicated that infection with HCV genotype 2b and/or high serum HCV load may be associated with failure to achieve rapid virological response. Therefore, patients infected with HCV genotype 2b and a high viral load should be aware of the possibility of the need for prolonged therapy.

In order to examine the appropriate duration of therapy in patients who did not achieve a rapid virological response, the patients were classified into two groups according to the length of the period required to achieve disappearance of HCV RNA from the serum, the "virological response at 8 weeks" and the "non-virological response at 8 weeks" group. In the "virological response at 8 weeks" group, the results suggested that prolongation of Peg-IFN-plus-ribavirin therapy to 36 weeks was optimal. In these

TABLE II. Univariate and Multivariate Logistic Regression Analysis of Factors Related to Rapid Virological Response

	All cases (n = 182), rapid virological response (123/182; 67.6%)			Naïve cases (n = 145), rapid virological response (96/145; 66.2%)		
	Odds ratio	95% Confidence interval	*P*-value	Odds ratio	95% Confidence interval	*P*-value
Univariate analysis						
Age (1: 55/2: ≥55 years old)	0.70	0.38–1.32	0.2685	0.75	0.37–1.50	0.4135
Sex (1: male/2: female)	0.84	0.45–1.60	0.5992	0.89	0.44–1.79	0.7356
Histological fibrosis of liver (1: F 0, 1, 2/2: F 3, 4)	0.54	0.25–1.18	0.1196	0.42	0.17–1.06	0.0656
Body mass index (1: ≥23/2: 23 kg^2/cm)	0.98	0.52–1.86	0.9514	0.92	0.45–1.86	0.8117
HCV genotype (1: 2a/2: 2b)	0.25	0.13–0.49	0.0001	0.25	0.12–0.54	0.0003
Pretreatment HCV RNA concentration (1: 6.2/2: ≥6.2 $\log_{10}$ IU/ml)	0.28	0.14–0.55	0.0002	0.32	0.16–0.68	0.0026
Alanine aminotransferase (1: 45/2: ≥45 IU/L)	0.98	0.52–1.88	0.9604	0.78	0.39–1.57	0.4799
Platelet count (1: 18/2: ≥18 × 10^3/ml)	1.35	0.71–2.55	0.3599	1.40	0.69–2.82	0.3454
Total cholesterol (1: 176/2: ≥176 mg/dl)	1.19	0.62–2.29	0.6082	1.22	0.59–2.50	0.5907
LDL-cholesterol (1: 102/2: ≥10^2 mg/dl)	1.13	0.56–2.27	0.7259	1.18	0.55–2.52	0.6641
Dose of peg-interferon (1: ≥1.53/2: 1.53 mg/kg/week)	0.73	0.38–1.39	0.3377	0.77	0.38–1.57	0.4667
Dose of ribavirin (1: ≥11.8/2: 11.8 mg/kg/day)	0.66	0.34–1.26	0.2000	0.84	0.42–1.71	0.6306
History of interferon therapy (1: retreatment/2: naïve)	1.69	0.71–4.02	0.2340			
Multivariate logistic regression analysis						
Histological fibrosis of liver						
1: F0, 1, 2				1		
2: F3, 4				0.38	0.13–1.09	0.0692
HCV genotype						
1: 2a	1			1		
2: 2b	0.31	0.15–0.64	0.0015	0.31	0.13–0.76	0.0093
Pretreatment HCV RNA concentration						
1: 6.2$\log_{10}$ IU/ml	1			1		
2: ≥6.2$\log_{10}$ IU/ml	0.35	0.17–0.74	0.0058	0.45	0.18–0.99	0.0382

Rapid virological response, serum HCV RNA disappearance at 4 weeks of therapy; naïve cases, patients had not have interferon therapy.

TABLE III. Univariate and Multivariate Logistic Regression Analysis of Factors Related to Sustained Virological Response in Non-Rapid Virological Response

	Non-rapid virological response cases (n = 59), sustained virological response (44/59; 74.6%)		
	Odds ratio	95% Confidence interval	*P*-value
Univariate analysis			
Age (1: 55/2: ≥55 years old)	0.87	0.26–2.89	0.8145
Sex (1: male/2: female)	2.21	0.65–7.46	0.1919
Histological fibrosis of liver (1: F 0, 1, 2/2: F 3, 4)	0.86	0.22–3.41	0.8253
Body mass index (1: ≥23/2: 23 kg^2/cm)	0.62	0.17–2.34	0.4709
HCV genotype (1: 2a/2: 2b)	1.00	0.27–3.64	1.0000
Pretreatment HCV RNA concentration (1: 6.2/2: ≥6.2log_{10} IU/ml)	0.84	0.21–3.38	0.8004
Alanine aminotransferase (1: 45/2: ≥45 IU/L)	0.65	0.17–2.44	0.5164
Platelet count (1: 18/2: ≥18 × 10^3/ml)	1.83	0.46–7.18	0.3778
Total cholesterol (1: 176/2: ≥176 mg/dl)	1.22	0.33–4.59	0.7608
LDL-cholesterol (1: 102/2: ≥10^2 mg/dl)	1.13	0.56–2.27	0.7259
Dose of peg-interferon (1: ≥1.53/2: 1.53 mg/kg/week)	0.63	0.16–2.50	0.5024
Dose of ribavirin (1: ≥11.8/2: 11.8 mg/kg/day)	0.69	0.18–2.75	0.5950
History of interferon therapy (1: retreatment/2: naïve)	1.35	0.76–2.85	0.9992
Duration of treatment (1: 24 weeks/2: 36 or 48 weeks)	5.00	1.10–22.83	0.0038
HCV undetectable at 8 weeks of treatment (1: yes/2: no)	11.96	2.45–58.31	0.0017
Multivariate logistic regression analysis			
Duration of treatment			
1: 24 weeks	1		
2: 36 or 48 weeks	1.19	1.06–1.34	0.0021
HCV undetectable at 8 weeks of treatment			
1: Yes	1		
2: No	0.02	0.002–0.18	0.0006

Sustained virological response, undetectable serum HCV RNA at least 6 months after completion of therapy; rapid virological response, serum HCV RNA disappearance at 4 weeks of therapy.

patients, the sustained virological response rates were significantly higher with 36-week therapy and 48-week therapy than with 24-week therapy, whereas the sustained virological response rate was similar between 36-week therapy and 48-week therapy. These results suggested that a treatment period of 48 weeks is not requisite, and that 36 weeks is sufficient in the "virological response at 8 weeks" group, to which most "non-rapid virological response" patients belonged. Thus, 36-week therapy was more beneficial than 48-week therapy in terms of cost and reducing the possibility of side effects of combination therapy in patients in the "virological response at 8 weeks" group. However, in the "non-virological response at 8 weeks" group, the duration of combination therapy should be extended to at least 48 weeks

TABLE IV. Baseline Characteristics of Non-Rapid Virological Response Patients Whose Serum HCV RNA Were Not Detectable at 8 Weeks After the Start of Treatment

Duration of treatment	24 Weeks (n = 9)	36 Weeks (n = 18)	48 Weeks (n = 22)	*P*-value
Age (years)	53.1 ± 6.4	53.9 ± 14.6	57.2 ± 12.5	n.s
Sex (male/female)	4/5	8/10	6/16	n.s
History of interferon therapy (naïve/retreatment)	8/1	16/2	18/4	n.s
Body weight (kg)	61.4 ± 11.4	60.9 ± 12.2	60.1 ± 13.3	n.s
Body mass index (kg^2/cm)	23.0 ± 1.9	24.3 ± 5.0	24.1 ± 3.5	n.s
Pretreatment HCV RNA concentration (log_{10} IU/ml)	6.1 ± 1.8	6.4 ± 0.5	6.3 ± 0.7	n.s
Genotype (2a/2b/not detect)	2/6/1	9/9/0	8/12/2	n.s
Histological fibrosis of liver (F 0–2/3–4)	7/2	14/4	14/8	n.s
Platelet count (×10^3/μl)	21.1 ± 7.1	18.6 ± 5.9	16.9 ± 5.9	n.s
Alanine aminotransferase (IU/L)	84.7 ± 78.2	66.7 ± 83.5	66.0 ± 55.5	n.s
Total cholesterol (mg/dl)	163.9 ± 30.3	181.7 ± 33.0	169.4 ± 40.4	n.s
LDL-cholesterol (mg/dl)	98.0 ± 29.5	109.6 ± 25.0	94.1 ± 32.4	n.s
Dose of peg-interferon-α-2b (μg/kg/week)	1.51 ± 0.13	1.57 ± 0.17	1.52 ± 0.13	n.s
Dose of ribavirin (mg/kg/day)	11.6 ± 1.4	11.5 ± 1.6	11.5 ± 1.9	n.s
The rate of CAS# (vs. 24 weeks)	1	0.69	0.82	

#CAS: cost for a patient achieving sustained virological response in each groups = (sum of drug costs in each groups)/(number of patients achieved sustained virological response in each groups); non-rapid virological response, serum HCV RNA appearance at 4 weeks of therapy; sustained virological response, undetectable serum HCV RNA at least 6 months after completion of therapy.

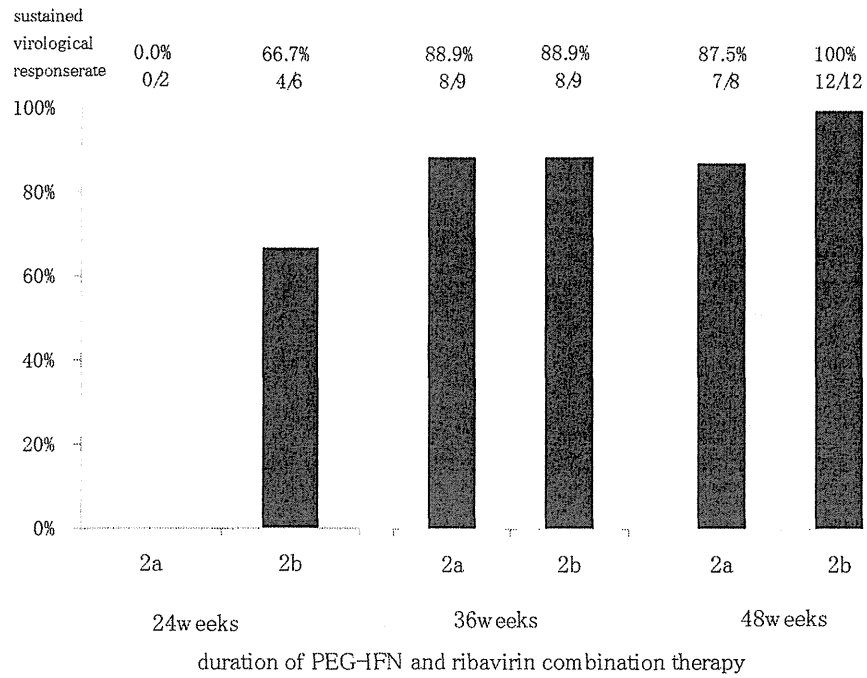

Fig. 4. Association between HCV genotype and duration of treatment in sustained virological response rate in non-rapid virological response patients whose HCV RNA were not detectable at 8 weeks ("virological response at 8 weeks").

if serum HCV RNA disappeared during therapy, because a sustained virological response was not found within the 36 weeks of therapy. In our experience, however, the sustained virological response rate in this group was only 60% even when therapy was extended to 48 weeks. As the number of patients in the "non-virological response at 8 weeks" group is fairly low, a larger scale study is required to identify a suitable duration of prolonged combination therapy in patients in the "non-virological response at 8 weeks" group. In a previous study [Huang et al., 2010], the sustained virological response rate of HCV genotype 2 patients without a rapid virological response was high with 24-week therapy; this finding was inconsistent with those of the present study. Differences in the weight-based dose of ribavirin between the two studies may have influenced these results. In the present study, as with a previous Japanese study [Inoue et al., 2010], the dose of ribavirin tended to be lower in order to alleviate the side effects of ribavirin, including severe anemia.

The difference in the virological response to Peg-IFN-plus-ribavirin combination therapy between patients with HCV genotype 2a and 2b has not been extensively studied. The present findings suggested that patients infected with HCV genotype 2a are more likely to achieve a rapid virological response than those with HCV genotype 2b. However, the sustained virological response rate of HCV genotype 2a patients tended to be lower not only in the sustained virological response group, but also in the "virological response at 8 weeks" group and the "non-virological response at 8 weeks" group. In particular, in the "virological response at 8 weeks" group, none of the HCV genotype 2a patients achieved sustained virological response with 24-week therapy. Therefore, for patients infected with HCV genotype 2a and belonging to the "virological response at 8 weeks" group, extension of the therapy duration to 36 weeks is strongly recommended. These findings of different virological responses may suggest that an early response to Peg-IFN-plus-ribavirin therapy is more promising, while eradication of HCV is less likely in patients infected with HCV genotype 2a than in those infected with HCV genotype 2b.

As an alternative strategy to overcome resistance to Peg-IFN-plus-ribavirin combination therapy, addition of telaprevir to Peg-IFN-plus-ribavirin combination therapy has been reported to achieve a sustained virological response in all enrolled patients with HCV genotype 2, but not in those with HCV genotype 3 [Foster et al., 2011]. Adding a currently available direct-acting antiviral drug to Peg-IFN-plus-ribavirin combination therapy could be an option for HCV genotype 2 patients in the non-rapid virological response group. However, further clinical trials are needed to establish the validity of this strategy.

In the present study, the relationship between HCV RNA clearance and genotypes near the IL-28B gene was not fully examined. Previous reports showed that IL-28B genotype was associated with a

rapid virological response and a sustained virological response in HCV genotype 2 patients [Kawaoka et al., 2011; Sakamoto et al., 2011]. On the other hand, the usefulness of IL-28B genotyping was restricted in the management of response-guided therapy for HCV genotype 1 patients [Mangia et al., 2011]. Therefore, the validity of IL-28B genotyping in the management of response-guided therapy for HCV genotype 2 patients and on viral response in these patients should be resolved in future.

In conclusion, determining the duration of Peg-IFN-plus-ribavirin combination therapy for HCV genotype 2 patients by monitoring the clearance of HCV RNA is a useful strategy for attaining a high sustained virological response rate and achieving cost benefits. In particular, in the "virological response at 8 weeks" group, 36 weeks of therapy is more suitable than 48 weeks of therapy in terms of efficacy and cost–benefits.

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World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/esps/
wjh@wjgnet.com
doi:10.4254/wjh.v5.i4.182

World J Hepatol 2013 April 27; 5(4): 182-188
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Platelet count and sustained virological response in hepatitis C treatment

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Supported by A grant from the Chiba University Young Research-Oriented Faculty Member Development Program in Bioscience Areas, to Kanda T
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Received: September 20, 2012 Revised: December 26, 2012
Accepted: January 29, 2013
Published online: April 27, 2013

Abstract

AIM: To examine the epidemiological data, hematological safety and treatment responses of peginterferon-alpha 2a plus ribavirin therapy for hepatitis C.

METHODS: Between March 2008 and February 2011, 196 hepatitis C virus (HCV) genotype 1 infected Japanese (127 treatment-naive and 69 treatment-experienced patients) patients treated with peginterferon-alpha 2a plus ribavirin were enrolled. We examined the epidemiological data and treatment responses were retrospectively analyzed in terms of hematological safety. HCV RNA was measured by the COBAS TaqMan HCV test.

RESULTS: Overall sustained virological response (SVR) rates of treatment-naive and treatment-experienced patients were 56% and 39%, respectively. Multivariate logistic regression analysis showed that SVR was attained independently of early virological response in both treatment-naive and treatment-experienced patients. SVR rates did not differ between the pretreatment hemoglobin < 13 g/dL and ≥ 13 g/dL groups. However, in treatment-naive patients, the SVR rate of the pretreatment platelet count < 130000/μL group was significantly lower than that of the pretreatment platelet count ≥ 130000/μL group.

CONCLUSION: Attention should be paid to potential thrombocytopenia in the treatment of chronic hepatitis C patients.

Key words: Anemia; Antiviral treatment; Chronic hepatitis C; Platelet count; Sustained virological response

Kanda T, Kato K, Tsubota A, Takada N, Nishino T, Mikami S, Miyamura T, Maruoka D, Wu S, Nakamoto S, Arai M, Fujiwara K, Imazeki F, Yokosuka O. Platelet count and sustained virological response in hepatitis C treatment. *World J Hepatol* 2013; 5(4): 182-188 Available from: URL: http://www.

wjgnet.com/1948-5182/full/v5/i4/182.htm DOI: http://dx.doi.org/10.4254/wjh.v5.i4.182

INTRODUCTION

Chronic hepatitis C virus (HCV) infection leads to cirrhosis and hepatocellular carcinoma[1]. The combination of pegylated interferon alpha-2a or alpha-2b plus ribavirin is the standard of care (SOC) for HCV-infected patients[2]. This therapy leads to sustained virological response (SVR) in approximately 50% of patients[2]. In 2011, two HCV NS3/4A protease inhibitors, boceprevir and telaprevir, became available for HCV genotype 1 patients in United States and some other countries[3-6]. The addition of boceprevir or telaprevir to standard therapy with pegylated interferon plus ribavirin, compared with standard therapy alone, significantly increased the SVR rates in patients infected with HCV genotype 1[3-6].

Thrombocytopenia occasionally accompanies advanced chronic liver diseases[7] and is associated with the natural history of HCV infection and anti-viral therapy[8]. Thrombocytopenia is also one of the major obstacles when treating patients infected with HCV by pegylated interferon plus ribavirin with or without direct-acting antivirals, including boceprevir and telaprevir[9,10]. Diagnosis of thrombocytopenia in chronic hepatitis C patients is associated with increased incidences of certain comorbidities, complications and medical interventions, significantly increasing medical resource utilization[11].

Genome-wide association studies have recently revealed that interleukin 28B (IL28B) single nucleotide polymorphisms are significantly associated with the response to pegylated interferon-alpha plus ribavirin therapy for chronic hepatitis C[12-15] and that inosine triphosphatase (*ITPA*) gene variant protects against anemia during pegylated interferon-alpha plus ribavirin therapy for chronic hepatitis C[16]. However, severe hemoglobin decline, which is mainly found in ITPA-CC patients, was inversely correlated with thrombocytopenia, contributing to the association between severe anemia and a relative reactive increase in platelet count[17,18].

It is well known that improved adherence to medication will favorably affect SVR rates in pegylated interferon-alpha 2a plus ribavirin therapy for chronic hepatitis C[19,20]. Because the use of erythropoietin or hematopoietic growth factors was not allowed in these treatments by Japanese health insurance plans, hematological adverse events are the most common laboratory abnormalities, leading to dose modification or discontinuation. In the present study, we retrospectively analyzed the epidemiological data and treatment responses were retrospectively analyzed in terms of hematological safety.

MATERIALS AND METHODS

Patients

From March 2008 through October 2011, patients were recruited from Chiba University and 30 hospitals in Chiba, Ibaraki and Saitama prefectures[21-23]. Patients were eligible if they met the following inclusion criteria: (1) infected with HCV genotype 1 alone; (2) age $\geqslant$ 20 years; (3) diagnosis of chronic hepatitis C based on positive HCV RNA; (4) negative for HBs antigen; (5) negative for human immunodeficiency viral antibody; (6) no high autoantibody titers; (7) no severe renal disease; (8) no severe heart disease; (9) no mental disorders; (10) no current intravenous drug abuse; and (11) no pregnancy[21].

Study design

The design of this study has been partly described[21-23]. 196 patients, who could be judged with SVR or non-SVR, were enrolled. In this study, 180 μg of pegylated interferon-alpha 2a per week plus 400-1200 mg of ribavirin daily comprised the usual treatment protocol for as long as 48 or 72 wk. Clinical and laboratory assessments were performed at least every 4 wk during treatment and a 24 wk follow-up period[22]. Adverse reactions were documented by oral inquiry, physical examinations and laboratory tests.

Measurement of HCV RNA in serum

The COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan), with a range from 1.2 to 7.8 log IU/mL, was used for the measurement of HCV RNA levels every 4 wk before, during and for 24 wk after the end of treatment.

Measurement of serum alanine aminotransferase levels, other liver function and hematological tests

Serum alanine aminotransferase, other liver function and hematological tests were carried out by standard methods every 4 wk before, during and for 24 wk after the end of treatment.

Definition of treatment response

SVR was defined as undetectable serum HCV RNA at 24 wk after the end of treatment. Patients with undetectable HCV RNA within the initial 4 wk of treatment were considered to have demonstrated a rapid virological response (RVR). Patients who had undetectable HCV RNA within the initial 12 wk of treatment were considered to have had a complete early virological response (cEVR) (described as EVR here).

Ethics

This work was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The Ethics Committee of Chiba University School of Medicine approved the study protocol. Informed consent was obtained from all patients prior to enrollment.

Statistical analysis

Data were expressed as mean ± SD. Differences were evaluated by Student's t test, χ^2 test or Fisher's exact test. $P < 0.05$ was considered statistically significant. Multivariate logistic regression analysis was used to determine

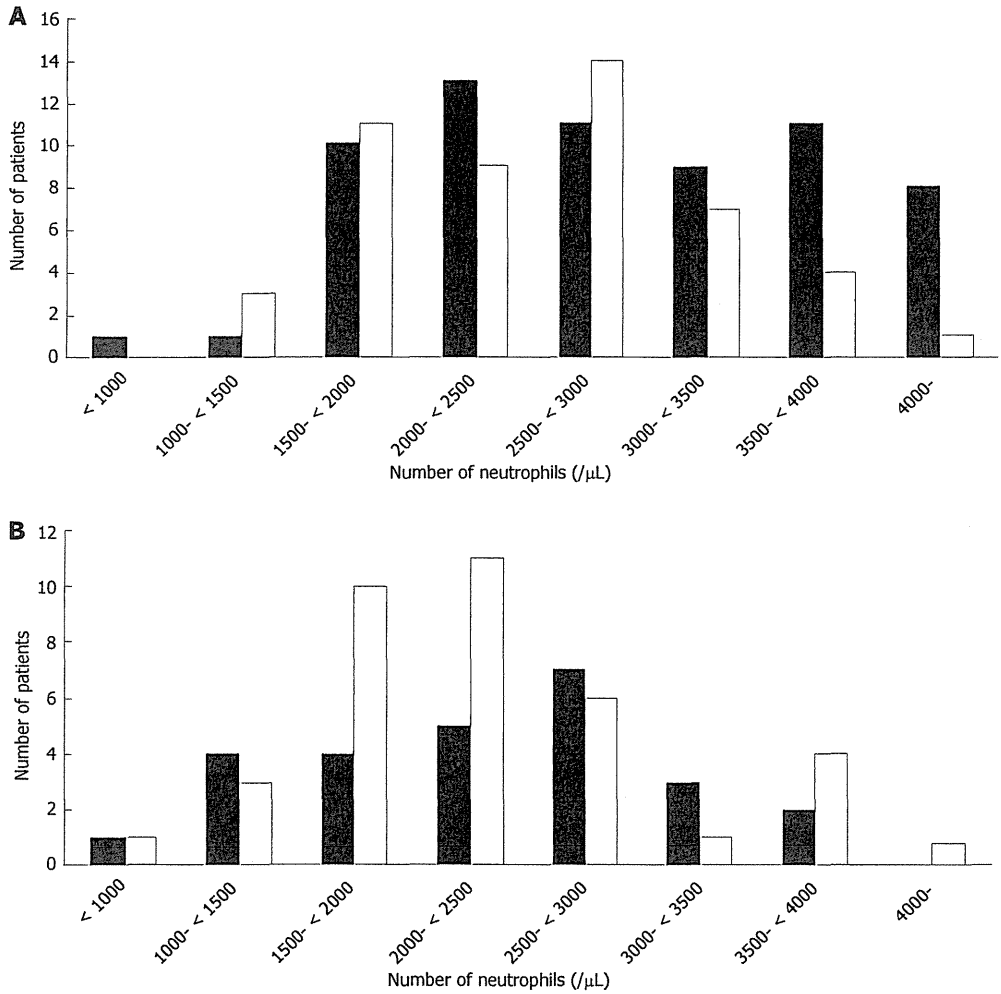

Figure 1 Distribution of pre-treatment neutrophil counts in treatment-naive and treatment-experienced patients. A: Results from 64 sustained virological response (SVR, black column) and 49 non-SVR (white column) of 113 treatment-naive patients are shown; B: Results from 26 SVR (black column) and 41 non-SVR (white column) of 67 treatment-experienced patients are shown.

Table 1 Clinical characteristics of chronic hepatitis C patients treated with pegylated interferon alpha-2a plus ribavirin in the present study

	Previous treatment		*P* value[1]
	(-)	(+)	
Number of patients	127	69	
Age (yr)	56.1 ± 10.7	59.0 ± 10.1	0.064
Gender (male/female)	62/65	34/35	NS
Body mass index (kg/m^2)	23.4 ± 3.2	23.3 ± 3.9	NS
LDL cholesterol (mg/dL)	107 ± 52.3	102 ± 31.5	NS
ALT (IU/L)	72.0 ± 53.7	66.4 ± 48.5	NS
Gamma-glutamyl transferase (IU/L)	55.6 ± 74.8	67.0 ± 77.2	NS
Alpha-fetoprotein (ng/mL)	12.7 ± 30.0	25.9 ± 79.5	NS
HCV RNA (log IU/mL)	6.5 ± 0.7	6.4 ± 0.7	NS
White blood cells (/mL)	5213 ± 1519	4619 ± 1273	0.006
Neutrophils (/mL)	2752 ± 924	2474 ± 981	0.058
Hemoglobin (g/dL)	14.0 ± 1.4	13.7 ± 1.6	NS
Platelets (× 10^4/mL)	16.5 ± 5.0	15.6 ± 5.2	NS
RVR (+/-)	18/109	7/62	NS
EVR (+/-)	66/61	24/45	0.021

Values are expressed as mean ± SD. [1]*P* value indicates those between two groups with and without pretreatment by Student's *t* test or χ^2 test. NS: Not significant; LDL: Low-density lipoprotein; ALT: Alanine aminotransferase; HCV: Hepatitis C virus; RVR: Rapid virological response; EVR: Early virological response.

factors that significantly contributed to SVR. Statistical analysis was performed using the Excel statistics program for Windows ver.7 (SSRI, Tokyo, Japan) and DA Stats software (O. Nagata, Nifty Serve: PAF01644).

RESULTS

Patients' baseline background factors

Baseline characteristics of the patients are shown in Figure 1 and Table 1. Of 196 patients, 127 were treatment-naive and 69 had a history of interferon therapy with or without ribavirin. Higher HCV viral load (HCV RNA ≥ 5.0 log IU/mL) was seen in 95.2% (121/127) treatment-naive and 98.5% (68/69) treatment-experienced patients. In the 69 patients previously treated, 4 had received pegylated interferon-alpha 2a monotherapy, 14 standard interferon monotherapy, 6 standard interferon plus ribavirin, 38 pegylated interferon-alpha 2b plus ribavirin, 2 pegylated interferon-alpha 2a plus ribavirin, and 5 with unknown details. Concerning the virological response of the 69 patients to their previous treatment, 25 were relapsers, 26 were null-responders, and 18 were unknown. In the present study, of the 127 treatment-naive patients, 89 and 38 patients were treated for as long as 48 and 72

Table 2 Comparison of factors between chronic hepatitis C patients with and without sustained virological response in the present study

	Previous treatment					
	(-)			(+)		
	SVR	Non-SVR	*P* value[1]	SVR	Non-SVR	*P* value[1]
Number of patients	72	55		27	42	
Age (yr)	54.6 ± 10.9	58.0 ± 10.3	NS	58.5 ± 9.9	59.3 ± 10.3	NS
Gender (male/female)	41/31	21/34	0.036	11/16	23/19	NS
Body mass index (kg/m^2)	23.4 ± 3.2	23.3 ± 3.9	NS	23.6 ± 3.6	22.8 ± 2.6	NS
LDL cholesterol (mg/dL)	107 ± 52.3	102 ± 31.5	NS	100 ± 31.3	92.8 ± 26.7	NS
ALT (IU/L)	67.6 ± 42.0	77.8 ± 65.9	NS	52.9 ± 35.4	75.1 ± 54.0	NS
Gamma-glutamyl transferase (IU/L)	45.1 ± 43.1	69.8 ± 102	NS	50.2 ± 51.1	76.9 ± 88.0	NS
Alpha-fetoprotein (ng/mL)	8.8 ± 9.7	17.4 ± 43.1	NS	13.3 ± 26.3	32.6 ± 96.5	NS
HCV RNA (log IU/mL)	6.4 ± 0.7	6.6 ± 0.6	NS	6.3 ± 0.9	6.4 ± 0.6	NS
White blood cells (/mL)	5363 ± 1582	5015 ± 1423	NS	4561 ± 1175	4657 ± 1345	NS
Neutrophils (/mL)	2910 ± 1006	2546 ± 767	NS	2337 ± 813	2562 ± 1074	NS
Hemoglobin (g/dL)	14.2 ± 1.5	13.8 ± 1.4	NS	13.8 ± 1.1	13.7 ± 1.9	NS
Platelets (× 10^4/mL)	17.5 ± 4.9	15.3 ± 4.8	0.013	16.5 ± 4.9	15.0 ± 5.3	NS
RVR (+/-)	18/54	0/55	< 0.001	7/20	0/42	< 0.001
EVR (+/-)	56/16	10/45	< 0.001	8/19	5/37	< 0.001

Values are expressed as mean ± SD. [1]*P* value indicates those between two groups with and without Sustained virological response (SVR) by Student's *t* test or χ^2 test. NS: Not signi- cant; LDL: Low-density lipoprotein; ALT: Alanine aminotransferase; HCV: Hepatitis C virus; RVR: Rapid virological response; EVR: Early virological response.

wk, respectively, and of the 69 treatment-experienced patients, 36 and 33 patients were treated for as long as 48 and 72 wk, respectively.

Characteristics of SVR and non-SVR patients

In the 127 treatment-naive patients, SVR was achieved in 56.6% (72/127) and non-SVR was seen in 43.3% (55/127) (27 relapsers, 11 null-responders and 17 stopped treatment due to adverse events) (Table 2). In these treatment-naive patients, there were significantly more male patients and higher neutrophil and platelet counts in the SVR group than in the non-SVR group at baseline (Table 2). Lower HCV viral load (HCV RNA < 5.0 log IU/mL) was seen in 8.3% (6/72) treatment-naive and 0% (0/55) treatment-experienced patients. RVR and EVR were significantly higher in the SVR group [25.0% (18/72) and 77.7% (56/72), respectively] than in the non-SVR group [0% (0/55) and 18.1% (10/45), respectively].

In the 69 patients previously treated, SVR was achieved in 39.1% (27/69) and non-SVR was seen in 60.8% (42/69) (23 relapsers, 14 null-responders and 5 stopped treatment due to adverse events) (Table 2). In these previously treated patients, the baseline backgrounds between the SVR and non-SVR groups did not differ (Table 2). RVR and EVR were significantly higher in the SVR group [25.9% (7/27) and 70.3% (19/27), respectively] than in the non-SVR group [0% (0/42) and 11.9% (5/42), respectively].

Multivariate analysis showed that EVR was significantly associated with SVR in treatment-naive patients and in treatment-experienced patients. For the EVR in the treatment-naive patients, odds ratio (OR) is 15.01 (95%CI: 5.72-44.56, $P < 0.001$); for the EVR in the treatment-experienced patients, OR is 21.7 (95%CI: 6.12-96.35, $P < 0.001$). Category is the same in the two groups. In treatment-naive patients, platelet count tended to be an independent factor in multivariate analysis. For the platelet counts, category > 16.1 × 10^4/μL, OR is 2.79 (95%CI: 0.98-8.57, $P = 0.061$).

Effects of anemia on SVR

Hematological adverse events are the most common laboratory abnormalities leading to dose modification or discontinuation[24]. First, we examined the effects of hemoglobin at baseline on the SVR rates.

In the 127 treatment-naive patients, there were no differences in SVR rates between the hemoglobin < 13 g/dL and hemoglobin ≥ 13 g/dL groups [55.2% (16/29) and 57.1% (56/98), respectively]. We also did not observe any difference in SVR rates between the hemoglobin < 13 g/dL and hemoglobin ≥ 13 g/dL groups in 48 wk treatment [55.6% (10/18) and 57.7% (41/71), respectively] or 72 wk treatment [54.5% (6/11) and 55.6% (15/27), respectively] in these patients.

In the 69 previously treated patients, there were no differences in SVR rates between the hemoglobin < 13 g/dL and hemoglobin ≥ 13 g/dL groups [30.0% (6/20) and 42.9% (21/49), respectively]. We also did not observe any difference in SVR rates between the hemoglobin < 13 g/dL and hemoglobin ≥ 13 g/dL groups in 48 wk treatment [38.5% (5/13) and 39.1% (9/23), respectively] or 72 wk treatment [14.3% (1/7) and 46.2% (12/26), respectively] ($P = 0.126$) in these patients.

Effects of thrombocytopenia on SVR

Next, we examined the effects of platelet counts at baseline on the SVR rates. In the 127 treatment-naive patients, the SVR rate of the platelet count < 130000/μL group

Table 3 Treatment outcomes in treatment-naive and treatment-experienced patients according to platelet counts at baseline

	< 130000/μL	≥ 130000/μL	*P* value
Proportion of SVR-archived in treatment-naive patients			
Total patients	10/27 (37.0%)	62/100 (62.0%)	0.020
48 wk treatment	7/20 (35.0%)	44/69 (63.8%)	0.022
72 wk treatment	3/7 (42.9%)	18/31 (58.1%)	NS
Treatment-experienced patients			
Total patients	7/24 (29.2%)	20/45 (44.4%)	NS
48 wk treatment	7/20 (33.3%)	9/21 (42.9%)	NS
72 wk treatment	2/9 (22.2%)	11/24 (45.8%)	NS

NS: Not signi‧cant; SVR: sustained virological response.

[37.0% (10/27)] was significantly lower than that of the platelet count ≥ 130000/μL group [62.0% (62/100)] (Table 3). We also observed a significantly lower SVR rate in the platelet count < 130000/μL group [35.0% (7/20)] than in the platelet count ≥ 130000/μL group [63.8% (44/69)] with 48 wk treatment. The RVR rate of the platelet count < 130000/μL group [15.0% (3/20)] was similar to that of the platelet count ≥ 130000/μL group [18.8% (13/69)] with 48 wk treatment, but the EVR rate of the platelet count < 130000/μL group [30.0% (6/20)] was significantly lower than that of the platelet count ≥ 130000/μL group [69.5% (48/69)] with 48 wk treatment ($P = 0.0033$). In contrast, there were no differences in SVR rates between the platelet count < 130000/μL [42.9% (3/7)] and ≥ 130000/μL groups with 72 wk treatment [58.1% (18/31)] (Table 3). The RVR and EVR rates of the platelet count < 130000/μL group [14.2% (1/7) and 57.1% (4/7), respectively] were similar to those of the platelet count ≥ 130000/μL group [3.2% (1/31) and 25.8% (8/31), respectively] with 72 wk treatment.

In the 69 previously treated patients, there were no differences in SVR rates between the platelet count < 130000/μL and ≥ 130000/μL groups [29.2% (7/24) and 44.4% (20/45), respectively] (Table 3). We also did not observe any differences in SVR rates between the platelet count < 130000/μL and ≥ 130000/μL groups with 48 wk [33.3% (5/15) and 42.9% (9/21), respectively] or 72 wk treatment [22.2% (2/9) and 45.8% (11/24), respectively] in these patients (Table 3).

DISCUSSION

After HCV NS3/4A protease inhibitors began to be used in clinical practice, resulting side effects of their application for chronic hepatitis C were also expected to appear[6,24]. In fact, hematological adverse events became the most common laboratory abnormalities, leading to dose modifications or even discontinuation during SOC for chronic hepatitis C[25-30]. In the present study, we observed that EVR was significantly associated with SVR in both treatment-naive and treatment-experienced patients. According to multivariate analysis, RVR was not associated with SVR in either of the patient types, although the reason for this might be that RVR was obtained in only 25 patients. We also examined the epidemiological data and treatment responses were retrospectively analyzed in terms of hematological safety. We observed that the SVR and EVR rates of the platelet count ≥ 130000/μL group were better than those of the platelet count < 130000/μL group in treatment naive-patients (Table 3).

Unexpectedly, we did not observe any difference in SVR rates between the hemoglobin < 13 and ≥ 13 g/dL groups (see Results section) or according to neutrophil counts (Figure 1), although the hemoglobin level or WBC level was supposedly an important factor affecting adherence to treatment. We did not observe any association between baseline platelet count below 130000/μL and SVR in the treatment-experienced patients in the present study (Table 3). It may be possible that thrombocytopenia reflects the fact that patients with low platelet counts are more prone to being cirrhotic and therefore should have a lower response rate to therapy.

The significant lower SVR in patients with baseline platelet counts below 130000/μL in treatment-naive patients treated for 48 wk, but not for 72 wk, likely reflects the major role of liver stage in patients with presumably favorable viral kinetics (considering that patients treated for 48 wk will have had an EVR), whereas in patients with slower decay of viral load and presumably treated for 72 wk, liver stage could have had a lower impact on SVR. In the present study, liver biopsy was performed in 67 patients and fibrosis stages 1, 2, 3 and 4 were seen in 30, 11, 5 and 3 of the platelet count ≥ 130000/μL group and 3, 7, 6, 2 of the < 130000/μL group, respectively. We also observed that 3 of 5 cirrhotic patients obtained SVR. Perhaps IL28B polymorphism played a major role in these patients, possibly explaining why some cirrhotics achieved SVR. Further studies will be needed to clarify this point.

So far, anemia and neutropenia are well-recognized effects of higher-dose peginterferon alpha plus ribavirin regimens, but it was also reported that no patient had to discontinue therapy owing to thrombocytopenia[27]. We observed a greater number of lower SVR rates in the low platelet group [35.2% (12/34); $P = 0.037$] of the higher hemoglobin group than in the high platelet group [57.5% (65/113)] of the higher hemoglobin group (hemoglobin ≥ 13 g/dL). We also observed that lower SVR rates tended to occur more in the low platelet group [29.4% (5/17)] than in the high platelet group [53.1% (17/32)] of the lower hemoglobin group (hemoglobin < 13 g/dL). Thus, the present study suggested that thrombocytopenia is an important factor for SVR.

In the present study, we also experienced only two treatment-naive patients discontinuing treatment due to neutropenia. One was a 70-year old male who discontinued treatment at 1 wk after its commencement, and the other was a 51-year old female who discontinued treatment at 9 wk (Figure 1). Further study will be needed as the numbers of samples in the current study were limited.

In conclusion, SVR was attained independently of EVR in both treatment-naive and treatment-experienced patients. The SVR rates between the pretreatment hemoglobin < 13 and ≥ 13 g/dL groups did not differ. However, in treatment-naive patients, the SVR rate of the pretreatment platelet count < 130000/μL group was significantly lower than that of the pretreatment platelet count ≥ 130000/μL group. Patients with low platelet counts were subject to dose and/or treatment duration reductions. In fact, if these subjects required such treatment adjustments, this may provide a partial explanation for the difference in SVR rates between naive and experienced patients. Attention should be paid to thrombocytopenia in the treatment of chronic hepatitis C patients.

ACKNOWLEDGMENTS

We thank Dr. Yutaka Yonemitsu, Dr. Hiroshige Kojima, Dr. Michikazu Abe, Dr. Kenichi Fukai, Dr. Fumihiko Kanai, Dr. Susumu Nakahori, Dr. Shigenobu Kawai, Dr. Yasushi Maru, Dr. Takeshi Nihei, Dr. Norio Kikuchi, Dr. Noritomo Shimada, Dr. Yasuo Hirai, Dr. Shuuichi Saito, Dr. Shinichi Hino, Dr. Masaaki Saito, Dr. Kazuhiko Kita, Dr. Shinichi Sato, Dr. Yutaka Natsuki, Dr. Hidetaka Terabayashi, Dr. Masahiko Sanada, Dr. Noriaki Suzuki, Dr. Ryosaku Azemoto, Dr. Hideki Takanashi, Dr. Michio Kimura, Dr. Nobuyuki Sugiura, Dr. Motohide Takashi, Dr. Shinnen Kin, Dr. Satoru Kaneda, Dr. Hikaru Nagahara, Dr. Kinki Rin, Dr. Ei Itobayashi and all other investigators for coordinating this work.

COMMENTS

Background

It is well known that improved adherence to medication will favorably affect sustained virological response (SVR) rates in peginterferon-alpha 2a plus ribavirin therapy for chronic hepatitis C. Because the use of erythropoietin or hematopoietic growth factors was prohibited in these treatments by Japanese health insurance plans, hematological adverse events are the most common laboratory abnormalities, leading to dose modi·cation or discontinuation.

Research frontiers

Peginterferon-alpha 2a plus ribavirin therapy for chronic hepatitis C leads to hematological adverse events, some of which are unknown. The authors examined the epidemiological data and treatment responses were retrospectively analyzed in terms of hematological safety. In this study, the authors demonstrate that attention should be paid to potential thrombocytopenia in the treatment of chronic hepatitis C patients.

Innovations and breakthroughs

Recent reports have highlighted the importance of inosine triphosphatase gene variants that protect against anemia in patients treated for chronic hepatitis C. In treatment-naive patients, the SVR rate of the pretreatment platelet count < 130000/μL group was signi·cantly lower than that of the pretreatment platelet count ≥ 130000/μL group. The authors also observed that 3 of 5 biopsy-proven cirrhotic patients obtained SVR.

Applications

With the use of standard of care, attention should be paid to thrombocytopenia in the treatment of chronic hepatitis C patients.

Peer review

It is well known that improved adherence to medication will favorably affect SVR rates in pegylated interferon-alpha 2a plus ribavirin therapy for chronic hepatitis C. In the present study, the authors retrospectively analyzed the epidemiological data and treatment responses were retrospectively analyzed in terms of hematological safety. In treatment-naive patients, the SVR rate of the pretreatment platelet count < 130000/μL group was signi·cantly lower than that of the pretreatment platelet count ≥ 130000/μL group, despite the existence of cirrhosis. Of particular interest was the fact that the results suggested that attention should be paid to thrombocytopenia in the treatment of chronic hepatitis C patients.

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