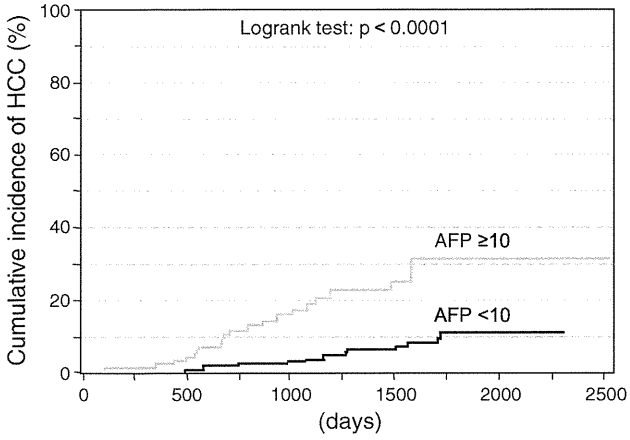

Fig. 3 Comparison of HCC rates in patients administered PegIFNα-2a ($n = 594$) with respect to alpha-fetoprotein (*AFP*) levels in the first 24 weeks after the start of therapy. *Black line* patients with AFP ≥10 ng/mL at 24 weeks, *gray line* patients with AFP <10 ng/mL at 24 weeks

Study 2

We compared the incidence of HCC between 59 patients in the control group and the same number of patients in the PegIFNα-2a group using the matched-pair test. The backgrounds of the patients are shown in Table 4. The PegIFNα-2a group had higher rates of advanced fibrosis (F3 and F4) and active inflammation (A2 and A3). No other differences were found between the two groups, except for the white blood cell count (Table 4).

Development of HCC was observed in 2 patients in the PegIFNα-2a group and 8 in the control group. The incidence of HCC was compared between the two groups, using the Kaplan–Meier method. The incidence of HCC in the PegIFNα-2a group was significantly lower than that in the control group (log-rank test, $p = 0.0187$; Fig. 4). Among the patients with advanced fibrosis of the liver (F3 and F4), those in the PegIFNα-2a group had a lower incidence of HCC than those in the control group. The independent risk factors for the development of HCC were analyzed using the stepwise Cox proportional hazard model. Only PegIFNα-2a administration and age were identified as independent risk factors for the development of HCC (Table 5).

Discussion

The number of HCC cases resulting from HCV infection continues to increase worldwide [19]. To date, IFN therapy is the most effective preventive measure against HCC in patients with chronic hepatitis C; furthermore, the incidence of HCC is reduced in patients who achieve an SVR to IFN [6–9] Therefore, achieving an SVR is the most effective approach for reducing the risk of developing HCC. In Japan, the incidence of HCC is elevated in older patients with hepatitis C. Corroborating this finding, the results of a Japanese study show a higher risk of HCC in patients aged 65 years and more [10]. Therefore, prevention of HCC in aged patients is an important challenge.

In the present multicenter, cooperative, retrospective study conducted in Japan, the incidence of HCC was reduced in patients who received 90 μg PegIFNα-2a weekly or biweekly and had AFP values of <10 ng/mL and ALT values of ≤40 IU/L 24 weeks after the start of the treatment. The results of the matched case–control study of the PegIFNα-2a group and the non-IFN control group show that the incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group, especially in patients with advanced fibrosis of the liver (F3 and F4). However, there could have been a selection bias between

Table 4 Backgrounds of the patients in the propensity-matched control study (PegIFNα-2a group, $n = 59$; control group, $n = 59$)

	PegIFNα-2a group ($n = 59$)	Control group ($n = 59$)	p value
Age (years)	60.5 ± 13.0	63.3 ± 10.5	n.s.
Gender (male/female)	24/35	25/34	n.s.
BMI	22.9 ± 3.6	22.9 ± 3.4	n.s.
Genotype (1/2)	49/10	46/13	n.s.
History of excess alcohol consumption (60 g/day; yes/no)	10/49	4/55	n.s.
Fibrosis (F0, 1, 2/F3, 4)	37/22	43/16	<0.05
Development of HCC (F0–2/F3, 4)	1/1	1/7	n.s.
Inflammatory activity (A0,1/A2, 3)	19/40	30/29	<0.05
Diabetes mellitus (no/yes)	57/2	56/3	n.s.
LDL cholesterol (mg/dL)	95.3 ± 23.8	117.0 ± 4.2	n.s.
White blood cell count (/mm^3)	4,260 ± 1,239	5,193 ± 2,078	<0.05
Red blood cell count ($\times 10^{-4}$/μL)	430 ± 57.8	441 ± 44.9	n.s.
Hemoglobin (g/dL)	13.6 ± 1.5	13.6 ± 1.9	n.s.
Platelet count ($\times 10^{-3}$/μL)	14.5 ± 5.7	15.8 ± 5.7	n.s.
Albumin (g/dL)	4.1 ± 0.5	4.1 ± 0.4	n.s.
Total bilirubin (mg/dL)	0.7 ± 0.5	0.9 ± 0.7	n.s.
AST (IU/L)	58.3 ± 47.7	49.7 ± 26.6	n.s.
ALT (IU/L)	63.6 ± 68.7	58.0 ± 39.2	n.s.
Gamma-GTP (IU/L)	78.3 ± 81.3	55.3 ± 75.1	n.s.
Baseline alpha-fetoprotein (AFP) (ng/L)	7.2 (4.3–14.2)	7.7 (3.9–13.8)	n.s.
Baseline HCV RNA level (KIU/mL)	1,230 (24–3,870)	1,024 (38–3,110)	n.s.

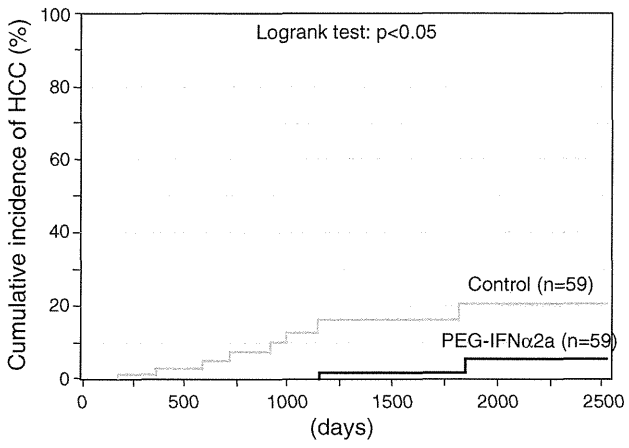

Fig. 4 Comparison of HCC rates between the long-term PegIFNα-2a administration group ($n = 59$) and non-administration group ($n = 59$) in the propensity-matched control study (Kaplan–Meier log-rank test, $p = 0.019$)

Table 5 Risk factors for HCC in the propensity-matched control study (Cox proportional hazard model)

Variables	Risk ratio	95 % CI	*p* value
PegIFN versus control	0.17	0.03–0.75	<0.05
Age (every 1 year)	1.12	1.02–1.25	<0.05
Fibrosis (F3, 4 vs. F0, 1, 2)	1.70	0.75–4.16	n.s.
Platelet count (every 10 × 10^3/μL)	0.89	0.73–1.09	n.s.
Albumin (every 1.0 g/dL)	0.80	0.10–6.68	n.s.
On-treatment AFP (<10 vs. ≥10 ng/L)	4.07	0.59–40.12	n.s.

the PegIFNα-2a group and the control group (patients who did not agree to receive IFN treatment), because this was a retrospective and non-randomized study. However, concordant with the findings of the HALT-C study [14], the present results show that PegIFNα-2a inhibits the development of HCC in patients with advanced fibrosis of the liver.

Recent studies show that polymorphisms in the host *IL28B* gene are important factors in the response to PegIFNα and ribavirin combination therapy [20, 21]. However, the mechanism of IL28B involvement in the response to PegIFNα and ribavirin has not been elucidated completely. A recent report has shown that IL28B is a significant factor in the development of HCC as well as in the response to IFN therapy [22]. Further studies are warranted to analyze the relationship between IL28B and inhibition of the development of HCC by PegIFNα in chronic hepatitis C.

Risk factors for the development of HCC have been discussed previously. Increased intrahepatic fat is involved in the development of HCC in chronic hepatitis C patients [23, 24]. In addition, diabetes-associated fat disorder [25, 26], hepatic iron overload [27], advanced fibrosis, older age, and fatty deposits in the liver are risk factors for HCC development [4]. Therefore, it is important to establish strategies to mitigate these risk factors to prevent the development of HCC and thus improve the outcomes of hepatitis C patients.

IFN therapy after HCC treatment is reported to inhibit the recurrence of tumors [28, 29], and a meta-analysis has revealed a trend toward inhibition of the recurrence of HCC [30, 31]. The prevention of HCC is an important issue that needs to be addressed to improve the survival of chronic hepatitis C patients. The findings of the present study and the HALT-C trial [14] indicate the effectiveness of long-term administration of maintenance IFN for preventing the development of HCC in chronic hepatitis C patients without an SVR. Improvement in ALT levels is also known to be an important predictor for the prevention of HCC [32]. A low AFP value during IFN administration is also recognized as a significant indicator of a lower risk of HCC [33, 34]. Recently, Osaki et al. [35] reported that a decrease of serum AFP during treatment with IFN was associated with a reduced incidence of HCC. Taking these findings and our own together, we conclude that maintenance administration of low-dose PegIFNα-2a weekly or biweekly to non-SVR patients with chronic hepatitis C decreases the incidence of HCC, especially in patients whose serum ALT and AFP levels are within the normal range 24 weeks after the start of treatment. The preventive effects of IFN against the development of HCC without elimination of the virus may be associated with its anticarcinogenic effects [16, 35]; however, the precise mechanism should be investigated.

The limitations of the present study are that it is retrospective and multicentric; therefore, potentially there may have been a selection bias. However, the reduction of the rate of development of HCC by maintenance administration of PegIFNα-2a in the patients in whom serum ALT and AFP levels were within the normal ranges 24 weeks after the start of treatment may be attributable to the anticarcinogenic effects of IFN without elimination of the virus.

Conclusion

The incidence of HCC was lower in non-SVR patients with chronic hepatitis C who were administered with maintenance low-dose PegIFNα-2a; especially in those whose serum ALT and AFP levels were within the normal ranges 24 weeks after the start of treatment.

Acknowledgments This study was supported by a Grant-in-Aid from the Japanese Ministry of Health, Welfare, and Labor.

Conflict of interest Namiki Izumi received lecture fees from Chugai Co. and MSD Co. in 2011.

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Hepatology Research

Hepatology Research 2013; 43: 35–43 doi: 10.1111/j.1872-034X.2012.01056.x

Special Report

A multicenter survey of re-treatment with pegylated interferon plus ribavirin combination therapy for patients with chronic hepatitis C in Japan

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Aim: This study aimed to clarify the factors associated the efficacy of re-treatment with pegylated interferon (PEG IFN) plus ribavirin combination therapy for patients with chronic hepatitis C who had failed to respond to previous treatment.

Methods: One hundred and forty-three patients who had previously shown relapse ($n = 79$), non-response ($n = 34$) or intolerance ($n = 30$) to PEG IFN plus ribavirin were re-treated with PEG IFN plus ribavirin.

Results: Twenty-five patients with intolerance to previous treatment completed re-treatment and the sustained virological response (SVR) rates were 55% and 80% for hepatitis C virus (HCV) genotype 1 and 2, respectively. On re-treatment of the 113 patients who completed the previous treatment, the SVR rates were 48% and 63% for genotype 1 and 2, respectively. Relapse after previous treatment and a low baseline HCV RNA level on re-treatment were associated with SVR in genotype 1 ($P < 0.001$). Patients with the interleukin-28B major genotype responded significantly better and earlier to re-treatment, but the difference in the SVR rate did not reach a significant level between the major and minor genotypes ($P = 0.09$). Extended treatment of 72 weeks raised the SVR rate among the patients who attained complete early virological response but not rapid virological response with re-treatment (72 weeks, 73%, 16/22, vs 48 weeks, 38%, 5/13, $P < 0.05$).

Conclusion: Relapse after previous treatment and a low baseline HCV RNA level have predictive values for a favorable response of PEG IFN plus ribavirin re-treatment for HCV genotype 1 patients. Re-treatment for 72 weeks may lead to clinical improvement for genotype 1 patients with complete early virological response and without rapid virological response on re-treatment.

Key words: chronic hepatitis C, pegylated interferon and ribavirin combination therapy, re-treatment

INTRODUCTION

PEGYLATED INTERFERON (PEG IFN) plus ribavirin combination therapy can show antiviral efficacy for patients with chronic hepatitis C (CH-C). However, a sustained virological response (SVR), which is defined as undetectable serum hepatitis C virus (HCV) RNA at 24 weeks after the treatment, remains at 50% for patients with HCV genotype 1 and 80% for those with HCV genotype 2 treated with PEG IFN plus ribavirin.[1–6] The number of patients who fail to achieve a SVR increases over time, requiring urgent action to eradicate HCV in them.

Recently, addition of the first-wave protease inhibitor telaprevir to PEG IFN plus ribavirin combination therapy, which has been reported to improve antiviral efficacy, has become commercially available, but this

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Received 18 April 2012; revision 19 May 2012; accepted 21 May 2012.

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triple therapy increases side-effects, especially severe anemia and skin rash.[7–11] Second-wave protease inhibitors, such as TMC435, which not only improve antiviral efficacy but also decrease side-effects, have been developed and are undergoing clinical trials.[12] Also, IFN-free regimens, such as protease inhibitor and polymerase inhibitor combination therapy, have been developed.[13,14] In Japan, HCV carriers are increasing in an aging population, and large numbers of patients are ineligible for triple therapy with telaprevir due to potential anemia. That is why re-treatment with PEG IFN plus ribavirin is a possible choice for patients who failed to achieve SVR to previous antiviral therapy or patients ineligible for triple therapy with telaprevir who must wait until next-generation antiviral therapies, such as triple therapy with second-wave protease inhibitors or IFN-free regimens, become commercially available.

As for re-treatment with PEG IFN plus ribavirin, some studies have been reported but the subjects and treatment protocols were varied.[15–20] According to past reports, the previous treatment response is associated with the efficacy of the re-treatment[17,20] and the SVR rates in re-treatment ranged 4–23%.[16–18] Recently, host factors, such as single nucleotide polymorphisms (SNP) located near the interleukin (IL)-28B gene, and virus factors, such as the amino acid substitutions in the HCV core region, were revealed to have a strong impact on SVR in PEG IFN plus ribavirin combination therapy for naïve CH-C patients.[21–26] Moreover, response-guided therapy which extends treatment duration until 72 weeks for patients with a slow virological response can raise the SVR rate for naïve CH-C patients.[27–29] However, the value of IL-28B SNP has been uncertain in re-treatment and the most appropriate treatment duration in re-treatment is still unclear. Although it remains obscure which factors are associated with SVR in re-treatment with standard PEG IFN plus ribavirin therapy as pointed out above, some patients do respond to re-treatment and it is very important to be able to identify them. Such findings will be valuable for optimizing the antiviral treatment for CH-C patients by making it possible to decide which patients should be considered for re-treatment with PEG IFN plus ribavirin therapy and which should wait for next-generation antiviral treatment.

In the present study, we tried to determine which patients could benefit from re-treatment and to identify the factors associated with SVR in re-treatment, including the host genome SNP and treatment duration.

METHODS

Patients

THIS RETROSPECTIVE, MULTICENTER study was conducted by the Study Group of Antiviral Therapy for Difficult-to-Treat Chronic Hepatitis C supported by the Ministry of Health, Labor and Welfare, Japan. This study was conducted with 143 CH-C patients, 113 patients (genotype 1, $n = 86$; genotype 2, $n = 27$) who had previously completed PEG IFN-α-2b plus ribavirin combination therapy but had failed to attain SVR, and 30 patients (genotype 1, $n = 22$; genotype 2, $n = 8$) who had previously discontinued this combination therapy due to adverse events.

Treatment

For the previous treatment, patients had been treated with PEG IFN-α-2b (PEGINTRON; MSD, Whitehouse Station, NJ, USA) plus ribavirin (REBETOL; MSD). For re-treatment with PEG IFN plus ribavirin, patients were treated PEG IFN-α-2a (PEGASYS; Roche, Basel, Switzerland) plus ribavirin (COPEGUS; Roche) or PEG IFN-α-2b plus ribavirin. In principle, as a starting dose, PEG IFN was given once weekly at a dose of 180 μg of PEG IFN-α-2a and 1.5 μg/kg of PEG IFN-α-2b and ribavirin was given at a total dose of 600–1000 mg/day based on bodyweight (bodyweight, ≤60 kg, 600 mg; 60–80 kg, 800 mg; ≥80 kg, 1000 mg), according to the standard treatment protocol for Japanese patients and the decision of the investigator at the participating clinical center. Dose modification followed, as a rule, the manufacturer's drug information on the intensity of the hematological adverse effects.

Laboratory tests and virological assessment

Examination of peripheral blood, transaminase and the serum HCV RNA level were tested at the start of treatment, weeks 4, 12 and 24, end of treatment (EOT), and 24 weeks after the treatment. Sequences of the IFN-sensitivity determining region (ISDR) and the core region of HCV were determined at start of the previous treatment, and the number of mutations in the ISDR, the amino acid substitutions at core 70 and 91, glutamine (Gln) or histidine (His) at core 70 and methionine (Met) at core 91, were analyzed. Genetic polymorphisms located near the IL-28B gene (rs8099917) and ITPA gene (rs1127354) were determined. As for the IL-28B gene, homozygosity for the major sequence (TT) was defined as having the IL-28B major allele, whereas homozygosity (GG) or heterozygosity (TG) of the minor sequence was defined as having

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the IL-28B minor allele. As for the ITPA gene, homozygosity for the major sequence (CC) was defined as having the ITPA major allele, whereas homozygosity (AA) or heterozygosity (CA) of the minor sequence was defined as having the ITPA minor allele. The serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test ver. 2.0 (detection range, 6–5000 KIU/mL; Roche Diagnostics, Branchburg, NJ, USA) or COBAS TaqMan HCV test (detection range, 1.2–7.8 $\log_{10}$ IU/mL) and qualitatively analyzed using the COBAS AMPLICOR HCV test ver. 2.0 (lower limit of detection, 50 IU/mL). When the serum HCV RNA level quantified by the COBAS TaqMan HCV test was less than 1.7 $\log_{10}$ IU/mL, which was equivalent to 50 IU/mL of HCV RNA, that case was judged as HCV RNA negativiation against the lower limit of detection of the COBAS AMPLICOR HCV test.

Definition of virological response

A rapid virological response (RVR) was defined as undetectable serum HCV RNA level at week 4, partial early virological response (p-EVR) as a more than 2-log decrease in the HCV RNA level at week 12 compared with the baseline, complete EVR (c-EVR) as undetectable serum HCV RNA at week 12, late virological response (LVR) as detectable serum HCV RNA at week 12 and undetectable at week 24, and SVR as undetectable serum HCV RNA at 24 weeks after the treatment. Relapse was defined as undetectable serum HCV RNA at the EOT but a detectable amount after the treatment. Patients without p-EVR or without clearance of HCV RNA at week 24 were considered to be showing non-response (NR), and treatment was stopped in both the previous treatment and this re-treatment. A patient who attained HCV RNA negativiation during the re-treatment continued to be treated for 48 weeks or 72 weeks according to response-guided therapy or the decision of the investigator at the participating clinical center.

Statistical analysis

Baseline data of the patients are expressed as means ± standard deviation or median values. In order to analyze the difference between baseline data or the factors associated with SVR, univariate analysis using the Mann–Whitney *U*-test or χ^2-test and multivariate analysis using logistic regression analysis were performed. A two-tailed *P*-value of less than 0.05 was considered significant. The analysis was conducted with SPSS ver. 17.0J (IBM, Armonk, NY, USA).

RESULTS

THE PATIENT FLOW in this study is shown in Figure 1. Among the patients who had previously discontinued PEG IFN-α-2b plus ribavirin combination therapy, two patients underwent splenectomy to increase platelet count prior to re-treatment, 25 completed re-treatment of PEG IFN plus ribavirin combination therapy and 15 achieved SVR (genotype 1, $n = 11$; genotype 2, $n = 4$).

All of the patients who completed previous treatment also completed re-treatment and the baseline characteristics of those patients are shown in Table 1. Of the 86 genotype 1 patients, 54 were relapsers and 32 had shown NR to previous treatment. Of the 27 patients with genotype 2, 25 were relapsers and two had shown NR to previous treatment. Thirty-seven patients with genotype 1 and 14 patients with genotype 2 were assessed as IL-28B genotype, and 27 patients with genotype 1 and 10 patients with genotype 2 were assessed as ITPA genotype. There was no significant difference in the baseline characteristics between the previous treatment and the re-treatment with respect to peripheral blood cell counts, amino transaminase level and serum HCV RNA at the start of treatment (Table 1).

The baseline characteristics of patients with genotype 1 according to antiviral efficacy of the previous treatment are shown in Table 2. Among those with NR in the previous treatment, the rate of the minor allele of IL-28B was significantly higher than those with relapse in the previous treatment ($P < 0.01$). For genotype 1, the HCV RNA negative rate on re-treatment was 20% (17/86) at week 4, 61% (52/85) at week 12 and 76% (65/86) at week 24, and the SVR rate was 48% (41/86). The factors associated with SVR were assessed by univariate analysis and the factors of relapse after previous treatment and the serum HCV RNA level at the start of re-treatment were selected as being significant (Table 3). The SVR

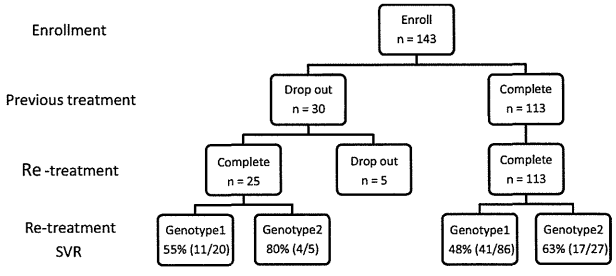

Figure 1 Patient flow for this study. SVR, sustained virological response.

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Table 1 Baseline characteristics of patients and treatment factors in previous treatment and re-treatment

Factor	Genotype 1		Genotype 2	
No.	86		27	
Sex: male/female	46/40		15/12	
Effect of previous treatment: relapse/NR	54/32		25/2	
	Previous treatment	Re-treatment	Previous treatment	Re-treatment
PEG IFN type: α-2a/α-2b	0/86	41/45	0/27	6/21
Age (years)	58.1 ± 8.3	60.0 ± 8.5	58.9 ± 8.2	60.0 ± 8.1
White blood cells (/mm^3)	4779 ± 1383	4610 ± 1443	5195 ± 1473	4724 ± 1266
Neutrophils (/mm^3)	2478 ± 930	2355 ± 1071	2561 ± 827	2389 ± 941
Hemoglobin (g/dL)	13.7 ± 1.2	13.5 ± 1.7	14.4 ± 1.3	14.0 ± 1.2
Platelets (×10^4/mm^3)	16.0 ± 5.9	16.6 ± 6.2	18.0 ± 5.7	16.8 ± 5.2
ALT (IU/L)	75 ± 51	73 ± 72	57 ± 46	42 ± 32
Histology: activity, 0–1/2–3	29/29		11/7	
Fibrosis, 0–2/3–4	45/14		17/1	
Serum HCV RNA (KIU/mL)	1600	850	1500	700
IL-28B SNP: rs8099917; TT/TG	26/11		10/4	
ITPA SNP: rs1127354; CC/CA	20/7		9/1	
Core 70: wild/mutant	11/11			
Core 91: wild/mutant	15/7			
ISDR: 0–1/≥2	15/1			

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism.

rates of relapsers were significantly higher than those of patients with NR in the previous treatment (relapse, 67%, 36/54 vs NR, 16%, 5/32, $P < 0.0001$). As for the serum HCV RNA level at the start of re-treatment, although the SVR rate of those patients with 5 $\log_{10}$ IU/mL or more of HCV RNA was 38% (26/69), all patients with less than 5 $\log_{10}$ IU/mL of HCV RNA attained SVR (11/11) ($P = 0.0001$). As for the IL-28B genotype, among the patients with the major allele, the p-EVR rate was significantly higher and the EOT response rate showed marginal significance compared to that with the minor allele (p-EVR rate, 100%, 23/23 vs 30%, 3/10, $P < 0.0001$, EOT rate, 92%, 24/26 vs 64%, 7/11, $P = 0.05$). There was no significant difference of the SVR rate between major and minor alleles (major, 65%, 17/26 vs minor, 36%, 4/11, $P = 0.15$).

Figure 2(a) shows the result of stratified analysis according to the previous treatment response and HCV RNA at the start of re-treatment. The significant difference in SVR observed between high (≥5 $\log_{10}$ IU/mL) and low (<5 $\log_{10}$ IU/mL) baseline viral loads was still found in both previous relapsers ($P = 0.02$) and previous non-responders ($P = 0.02$). In patients with a high baseline viral load, previous relapsers achieved a higher SVR rate than previous non-responders ($P < 0.0001$). Next, the results of stratified analyses according to IL-28B genotype and previous treatment response or HCV RNA at the start of re-treatment showed no significant difference in SVR rates between the IL-28B genotype in patients with relapse after previous treatment ($P = 0.63$) (Fig. 2b). All patients with less than 5 $\log_{10}$ IU/mL of HCV RNA achieved SVR despite their IL-28B genotype and the SVR rates of patients with 5 $\log_{10}$ IU/mL or more of HCV RNA did not differ between IL-28B genotypes (Fig. 2c). Multivariate analysis among the factors of relapse to previous treatment response, HCV RNA at the start of re-treatment and IL-28B genotype showed that relapse after previous treatment response bore the most predictable relationship to SVR in re-treatment ($P = 0.074$).

As for the efficacy of re-treatment according to treatment duration among patients with HCV RNA negativity during re-treatment, the SVR rate of 72-week treatment was significantly higher than that of 48-week treatment (72 weeks, 73%, 29/40, vs 48 weeks, 52%, 12/25, $P < 0.05$). This significant difference was especially found in patients who attained c-EVR but not RVR on re-treatment (72 weeks, 73%, 16/22, vs 48 weeks,

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Table 2 Baseline characteristics of patients and treatment factors according to the virological response in previous treatment among patients with genotype 1

Factor	Relapser in previous treatment		NR in previous treatment	
No.	54		32	
Sex: male/female	28/26		18/14	
	Previous treatment	Re-treatment	Previous treatment	Re-treatment
PEG IFN type: α-2a/α-2b	0/54	29/25	0/32	12/20
Age (years)	58.1 ± 8.1	60.3 ± 8.4	57.9 ± 8.9	59.6 ± 8.8
White blood cells (/mm^3)	4917 ± 1290	4692 ± 1035	4546 ± 1520	4462 ± 1993
Neutrophils (/mm^3)	2618 ± 846	2479 ± 805	2225 ± 1033	2105 ± 1454
Hemoglobin (g/dL)	13.9 ± 1.2	13.7 ± 1.6	13.5 ± 1.3	13.1 ± 1.9
Platelets (×10^4/mm^3)	17.1 ± 6.3	17.7 ± 6.1	14.1 ± 4.7	14.7 ± 6.2
ALT (IU/L)	75 ± 57	70 ± 76	75 ± 39	78 ± 64
Histology: activity, 0–1/2–3	20/18		9/11	
Fibrosis, 0–2/3–4	31/8		14/6	
Serum HCV RNA (KIU/mL)	1600	980	1550	800
IL-28B SNP: rs8099917; TT/TG	24/5		2/6	
ITPA SNP: rs1127354; CC/CA	15/6		5/1	
Core 70: wild/mutant	6/6		5/5	
Core 91: wild/mutant	9/3		6/4	
ISDR: 0–1/≥2	9/0		6/1	

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism.

38%, 5/13, $P < 0.05$) but not in patients who attained RVR or LVR (Fig. 3).

In genotype 2, the HCV RNA negative rate on re-treatment was 59% (16/27) at week 4, 85% (23/27) at week 12 and 93% (25/27) at week 24, and the SVR rate was 63% (17/27). The two patients with NR in previous treatment did not attain SVR with re-treatment. The factors associated with SVR were assessed by univariate analysis and only the factor of younger age at the start of re-treatment showed marginal significance ($P = 0.06$) (Table 4). Among the patients with RVR on re-treatment, the SVR rates were similar at 75% (6/8) to those with 24-week and 48-week treatment.

DISCUSSION

PAST STUDIES HAVE revealed that the factors of age, sex, progression of liver fibrosis, value of HCV RNA, number of mutations in the ISDR, amino acid substitutions in the core region, drug adherence and treatment duration show association with HCV eradication in PEG IFN plus ribavirin combination for naïve patients with CH-C.[3–5,25–33] Recently, the IL-28B genotype has been reported to be the most powerful factor associated with the antiviral effect of this combination therapy.[21–25] While the predictive factors for SVR in PEG IFN plus ribavirin combination therapy for naïve patients have been actively analyzed, those factors for patients who had already experienced this therapy are still unclear. Especially needing assessment is the correlation between IL-28B SNP or the previous treatment response and the antiviral effect in re-treatment. In this study, we tried to determine which factors could most effectively predict the antiviral effect in re-treatment.

In the present study, patients with relapse after the previous treatment and patients with a low serum HCV RNA level at the start of re-treatment showed significantly different results in this study of re-treatment of CH-C patients who had previously failed to attain SVR with PEG IFN plus ribavirin therapy. This result was similar to those of the EPIC[3] study on relapse and NR[17] and the SYREN trial of NR.[18] On the other hand, there was no significant difference between the influence of the IL-28B genotype and SVR. More specifically, if the previous treatment response was the same, there was no difference regardless of the IL-28B genotype. Considering this result, in re-treatment, the previous treatment response was a more effective predictive factor than IL-28B genotype. However, further investigation is needed to clarify the association between IL-28B

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Table 3 Factors associated with a sustained virological response in re-treatment with PEG IFN plus ribavirin in patients with genotype 1

Factor		SVR	Non-SVR	*P*-value
No. of patients		41	45	
Age (years)		60.2 ± 7.1	59.9 ± 9.6	0.71
Sex: male/female		24/17	22/23	0.40
Serum HCV RNA (log IU/mL)		5.8 ± 1.4	6.4 ± 0.6	0.11
Serum HCV RNA: <5 log/≥5 log		11/28	0/43	<0.001
White blood cells (/mm^3)		4656 ± 1029	4566 ± 1763	0.42
Neutrophils (/mm^3)		2443 ± 804	2259 ± 1301	0.16
Hemoglobin (g/dL)		13.5 ± 1.6	13.4 ± 1.8	0.80
Platelets (×10^4/mm^3)		16.9 ± 5.7	16.3 ± 6.7	0.36
ALT (IU/L)		68 ± 69	78 ± 75	0.43
IL-28B SNP: TT/TG		17/4	9/7	0.15
ITPA SNP: CC/CA		13/3	7/4	0.39
Core 70: wild/mutant		5/4	6/7	1.00
Core 91: wild/mutant		7/3	8/5	1.00
ISDR: 0–1/≥2		9/0	6/1	0.44
PEG IFN: α-2a/α-2b		16/25	25/20	0.14
PEG IFN dose (μg/kg per week)	α-2a	2.91 ± 0.77	2.74 ± 0.69	0.61
	α-2b	1.25 ± 0.39	1.20 ± 0.32	0.59
Ribavirin dose (mg/kg per day)		9.34 ± 2.72	9.64 ± 3.20	0.51
1st treatment virological response	Relapse/NR	36/5	18/27	<0.001

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; NR, non-response; PEG, pegylated; SNP, single nucleotide polymorphism; SVR, sustained virological response.

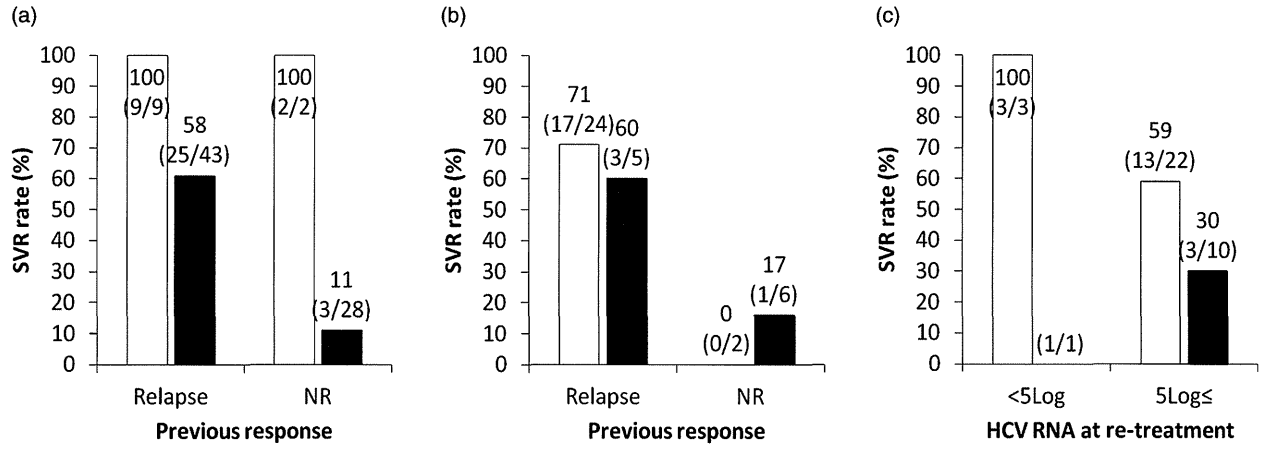

Figure 2 Sustained virological response (SVR) rates according to previous virological response, hepatitis C virus (HCV) RNA at start of re-treatment and genotype of interleukin (IL)-28B single nucleotide polymorphism (SNP) in patients with genotype 1. (a) Stratified analysis of previous virological response and HCV RNA at start of re-treatment. □, HCV RNA <5 log IU/mL at start of re-treatment; ■, HCV RNA ≥5 log IU/mL at start of re-treatment. (b) Stratified analysis of previous virological response and genotype of IL-28B SNP. □, Patients with major allele of IL-28B SNP; ■, patients with minor allele of IL-28B SNP. (c) Stratified analysis of HCV RNA at start of re-treatment and genotype of IL-28B SNP. □, Patients with major allele of IL28B SNP; ■, patients with minor allele of IL-28B SNP.

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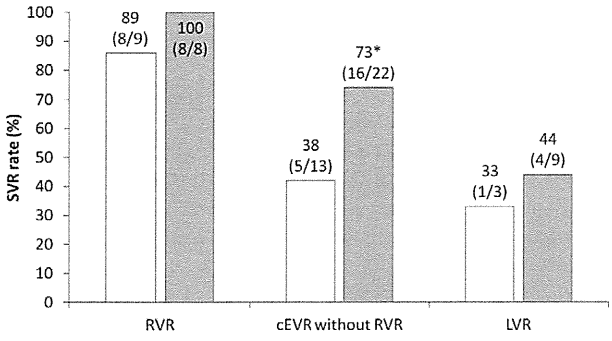

Figure 3 Sustained virological response (SVR) rates according to virological response in re-treatment and treatment duration in patients with genotype 1. □, Patients treated for 48 weeks; ▩, patients treated for 72 weeks. RVR, rapid virological response; cEVR, complete early virological response; LVR, late virological response. *$P < 0.05$; compared to 48 weeks of treatment.

genotype and antiviral effect of re-treatment because of their small number in this study. In this study, only one patient with the minor allele of IL-28B and NR in previous treatment could start and continue with the increased dose of PEG IFN (from 1.37 μg/kg in the previous treatment to 1.79 μg/kg in re-treatment) and ribavirin (from 10.3 mg/kg per day in the previous treatment to 11.1 mg/kg per day in re-treatment) and attained SVR by extended treatment. If the drug adherence does not improve, patients with the minor allele of IL-28B who show NR in the previous treatment should be treated with new drugs.

The next question is how the patients should be re-treated in order to attain SVR on re-treatment. In this study, the patients with a low serum HCV RNA level (<5 $\log_{10}$ IU/mL) at the start of re-treatment showed a significant rate of cure on re-treatment, and this is almost the same result as that previously reported.[16,17] In this study, the two patients with NR in the previous treatment and with less than 5 $\log_{10}$ IU/mL of HCV RNA level (20 KIU/mL and 52 KIU/mL of HCV RNA) at the start of re-treatment attained SVR. On the other hand, even if the previous treatment response was a relapse, the SVR rates were 58% (25/43) among the patients with 5 $\log_{10}$ IU/mL or more of HCV RNA. Because the HCV RNA level changed after the antiviral treatment, it is important to not miss the timing of when the HCV RNA level is low.

With respect to treatment duration among patients with HCV RNA negativiation during re-treatment, 72 weeks of treatment significantly increased the SVR rate compared to 48 weeks. This result was almost the same as that of the REPEAT study.[16] In our present study, the SVR rate among the patients with c-EVR but not RVR in re-treatment was significantly high by 72 weeks of treatment. On the other hand, the SVR rates among the

Table 4 Factors associated with a sustained virological response in re-treatment with PEG IFN plus ribavirin in patients with genotype 2

Factor		SVR	Non-SVR	*P*-value
No. of patients		17	10	
Age (years)		57.7 ± 8.8	63.7 ± 5.1	0.06
Sex: male/female		7/10	8/2	0.11
Serum HCV RNA (log IU/mL)		5.4 ± 1.4	6.1 ± 0.8	0.15
Serum HCV RNA: <5 log/≥5 log		5/11	1/9	0.35
White blood cells (/mm^3)		5049 ± 1355	4171 ± 910	0.10
Neutrophils (/mm^3)		2556 ± 1064	1999 ± 404	0.24
Hemoglobin (g/dL)		14.1 ± 1.3	13.8 ± 1.6	0.51
Platelets (×10^4/mm^3)		17.9 ± 5.4	14.8 ± 4.3	0.17
ALT (IU/L)		38 ± 19	48 ± 47	0.71
IL-28B SNP: TT/TG		6/2	4/2	1.00
ITPA SNP: CC/CA		5/1	4/0	1.00
PEG IFN: α-2a/α-2b		4/13	2/8	1.00
PEG IFN dose (μg/kg per week)	α-2a	3.23 ± 0.34	2.24 ± 2.25	1.00
	α-2b	1.32 ± 0.28	1.18 ± 0.23	0.21
Ribavirin dose (mg/kg per day)		10.4 ± 2.21	10.1 ± 1.31	0.44
1st treatment virological response	RVR/non-RVR	4/13	3/7	1.00

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; PEG, pegylated; RVR, rapid virological response; SNP, single nucleotide polymorphism; SVR, sustained virological response.

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patients with RVR in re-treatment were similar between the patients with 48 weeks and 72 weeks of treatment. Thus, patients with c-EVR but not RVR in re-treatment should be re-treated for a longer period. In order to attain better SVR, extended treatment duration is generally recommended for patients with on-treatment LVR, whereas standard treatment duration is considered to be sufficient for patients with on-treatment c-EVR. However, the present study revealed that, even if patients achieved c-EVR on re-treatment, 72 weeks of treatment seems to be better than 48 weeks for treatment-experienced patients. The majority of naïve patients showing on-treatment c-EVR could eradicate HCV with 48 weeks of treatment while some could not. In a treatment-experienced setting, patients who are able to respond early but not eradicate HCV would be selected, and therefore extended treatment may be needed.

With genotype 2, the SVR rate was relatively high (63%). The patients who could not attain SVR in re-treatment (two patients) showed NR in the previous treatment. Thus, the patients with genotype 2 and showing NR in previous treatment seemed to be difficult to treat and could be treated with other drugs. Among the patients with RVR in re-treatment, the SVR rates were similar among those with RVR in re-treatment between 24 weeks and 48 weeks of treatment. The effectiveness of extended treatment for the patients with genotype 2 in re-treatment could not be demonstrated because of their small number in this study. Further investigation is needed to clarify this.

In conclusion, this study shows that the efficacy of re-treatment for genotype 1 patients who failed to show SVR to previous treatment with PEG IFN plus ribavirin could be predicted from the previous treatment response and a low HCV RNA level at the start of re-treatment. Re-treatment for 72 weeks led to clinical improvement for genotype 1 patients with c-EVR and without RVR on re-treatment.

ACKNOWLEDGMENT

THIS WORK WAS supported by a Grant-in-Aid for Research on Hepatitis from Ministry of Health Labor and Welfare of Japan, and Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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Journal of Viral Hepatitis, 2013, **20**, 72–76 doi:10.1111/j.1365-2893.2012.01635.x

Noninvasive estimation of fibrosis progression overtime using the FIB-4 index in chronic hepatitis C

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Received February 2012; accepted for publication May 2012

SUMMARY. The FIB-4 index is a simple formula to predict liver fibrosis based on the standard biochemical values (AST, ALT and platelet count) and age. We here investigated the utility of the index for noninvasive prediction of progression in liver fibrosis. The time-course alteration in the liver fibrosis stage between paired liver biopsies and the FIB-4 index was examined in 314 patients with chronic hepatitis C. The average interval between liver biopsies was 4.9 years. The cases that showed a time-course improvement in the fibrosis stage exhibited a decrease in the FIB-4 index, and those that showed deterioration in the fibrosis stage exhibited an increase in the FIB-4 index with a significant correlation ($P < 0.001$). Increase in the ΔFIB-4 index per year was an independent predictive factor for the progression in liver fibrosis with an odds ratio of 3.90 ($P = 0.03$). The area under the receiver operating characteristic curve of the ΔFIB-4 index/year for the prediction of advancement to cirrhosis was 0.910. Using a cut-off value of the ΔFIB-4 index/year <0.4 or ≥0.4, the cumulative incidence of fibrosis progression to cirrhosis at 5 and 10 years was 34% and 59%, respectively in patients with the ΔFIB-4 index/year ≥0.4, whereas it was 0% and 3% in those with the ΔFIB-4 index/year <0.4 ($P < 0.001$). In conclusion, measurement of the time-course changes in the FIB-4 index is useful for the noninvasive and real-time estimation of the progression in liver fibrosis.

Keywords: FIB-4, fibrosis, HCV, noninvasive.

INTRODUCTION

Advanced stage of liver fibrosis in chronic hepatitis C is associated with failure of interferon therapy or development of major concomitant disease such as variceal bleeding, liver failure and hepatocellular carcinoma [1–3]. Therefore, evaluation of the stage of liver fibrosis is essential in clinical practice. Liver biopsy is the gold standard for diagnosis of liver fibrosis [4,5], but inaccuracy in evaluation of fibrosis because of sampling errors [6–8] or by the inter-observer variation has been reported [9]. Real-time assessment of liver fibrosis may be clinically useful, but the invasiveness of liver biopsy precludes repeated examinations.

A variety of noninvasive methods to diagnose liver fibrosis have been proposed. Recently, transient elastography [10–13] and real-time tissue elastography [14] using ultrasonography have been developed, but these modalities are not widely available. For blood tests, the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio [15], the AST/platelet ratio index (APRI) [16,17] and the Fibrotest [18,19] have been reported to be useful. The FIB-4 index is another prediction value of liver fibrosis in chronic hepatitis C based on the standard biochemical values and age. The FIB-4 index has been reported to be markedly useful for the prediction of advanced liver fibrosis [20,21]. Given its noninvasiveness and simplicity, the FIB-4 index has the advantage of an easy follow-up of the time-course changes by repeated measurements.

In the present study, we investigated the utility of the real-time assessment of the FIB-4 index for the prediction of time-course progression in liver fibrosis.

Abbreviations: ALT, alanine aminotransferase; AST, aspirate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

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PATIENTS AND METHODS

Patients

A total of 421 patients with chronic hepatitis C who had repeated liver biopsies between 1991 and 2010 at the Musashino Red Cross hospital were consecutively investigated. All patients received interferon therapy after the first biopsy and had nonsustained virological response. A second

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biopsy was performed at least 6 months after the completion of interferon therapy. Exclusion criteria were as follows: (i) co-infection with HBV or HIV (n = 1), (ii) alcohol abuse (intake of alcohol equivalent to pure alcohol 40 g/day or more) (n = 8), (iii) the presence of nonalcoholic steatohepatitis (n = 14), (iv) the presence of hepatocellular carcinoma (n = 15), (v) interval between paired biopsies was <1.5 years (n = 41) and (vi) length of biopsy sample <15 mm (n = 28). The demographic characteristics of the 314 patients enrolled are shown in Table 1.

Assessment of liver fibrosis stage

Liver biopsy was carried out under laparoscopic or ultrasonographic guidance. A sample 15 mm or larger was collected and evaluated. The fibrosis stage was categorized according to the METAVIR score: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Two pathologists examined all samples and determined the fibrosis stage. When staging was inconsistent between the two pathologists, an appropriate stage was determined by discussion between the two.

Calculation of FIB-4 index

The FIB-4 index at the time of each liver biopsy was calculated based on the blood test results within 1 month before liver biopsy according to the following formula: The FIB-4 index = (age [years] × AST [IU/L])/(platelet count [10^9/L] × (ALT [IU/L])$^{1/2}$). Change in the FIB-4 index per year (ΔFIB-4 index/year) was calculated by the following formula: ΔFIB-4 index/year = (the FIB-4 index at the second liver biopsy − the FIB-4 index at the first liver biopsy)/interval between paired biopsies (years). Change in AST, ALT, platelet counts per year (ΔAST/year, ΔALT/year, ΔPlatelet counts/year) and the degree of changes in the fibrosis stage per year were calculated similarly.

Table 1 Clinical background of patients

	First biopsy	Second biopsy
Age (years)	53.7 ± 9.8	58.7 ± 9.4
Gender (male/female)	149/165	
AST (IU/L)	64.5 ± 36.7	58.5 ± 37.7
ALT (IU/L)	87.7 ± 58.9	69.9 ± 53.9
Platelet counts (× 10^9/L)	165 ± 48	159 ± 48
Histological findings		
Activity: 0/1/2/3	38/143/117/16	10/147/131/26
Fibrosis: 0–1/2/3/4	139/107/61/7	134/101/63/16
Interval of between biopsies (years)	4.9 ± 2.9	–

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Statistical analysis

The SPSS software package 15.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Categorical data were analysed using Fisher's exact test. Continuous variables were compared with Student's t-test. Factors associated with the progression in liver fibrosis were analysed by multivariate logistic regression analysis. Association between progression in fibrosis stage and changes in the FIB-4 was analysed by Spearman's rank correlation test. Kaplan–Meier method and log-rank test were used to analyse time to occurrence of fibrosis progression to cirrhosis. A P-value of < 0.05 was considered statistically significant.

RESULTS

Changes in liver fibrosis stage overtime

The clinical backgrounds of patients at the first and second biopsies are shown in Table 1. The average interval was 4.9 years between the two liver biopsies. The fibrosis stage progressed over time in 23%, regressed in 17% and remained unchanged in 60%. Changes of fibrosis stage stratified by the fibrosis stage at the first liver biopsy are shown in Table 2.

Comparison of FIB-4 index and liver fibrosis stage

For the prediction of advanced liver fibrosis (F3–4), a FIB-4 index <1.45 had a negative predictive value of 97%, whereas a FIB-4 > 3.25 had a positive predictive value of 49% at first biopsy. Similarly, a FIB-4 < 1.45 had a negative predictive value of 98%, and a FIB-4 > 3.25 had a positive predictive value of 54% at second biopsy (Fig. 1).

Table 2 Changes of fibrosis stage over time

	Fibrosis stage at second biopsy				
Fibrosis stage at first biopsy	F0–1 (%)	F2 (%)	F3 (%)	F4 (%)	Total
F0–1	98 (71)	33 (24)	8 (5)	–	139
F2	33 (31)	50 (47)	21 (20)	3 (2)	107
F3	3 (5)	18 (29)	33 (55)	7 (11)	61
F4	–	–	1 (14)	6 (86)	7

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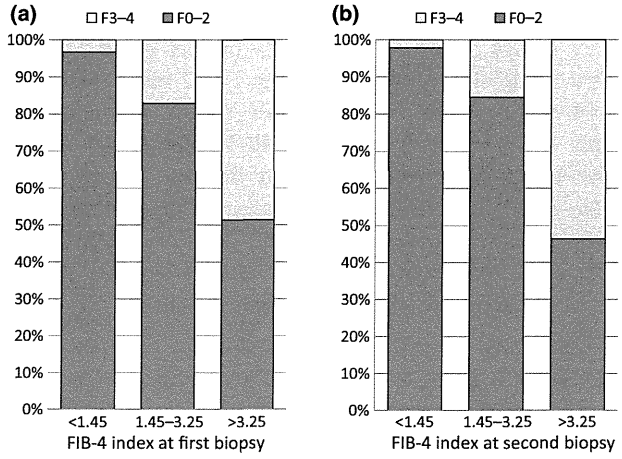

Fig. 1 Comparison of the FIB-4 index and liver fibrosis stage. Patients were categorized into three groups according to the FIB-4 index using cut-off values of < 1.45, 1.45–3.25, > 3.25 at liver biopsy. The lower bar chart (dark grey) indicates patients with F0–2, while the upper bar chart (light grey) indicates patients with F3–4. (a) comparison of the FIB-4 index and liver fibrosis stage at first biopsy and (b) at second biopsy.

Predictive factors for the progression of fibrosis

Higher level of ΔAST/year, lower level of ΔALT/year, lower level of ΔPlatelet counts/year and higher level of the ΔFIB-4/year were significantly associated with the progression of fibrosis overtime (Table 3). Multivariate analysis demonstrated that only the ΔFIB-4 index/year was an independent predictive factor for the progression of fibrosis stage ($P = 0.03$) with an odds ratio of 3.70 (95% CI:1.07–12.5).

Correlation between the degree of changes in the fibrosis stage and the ΔFIB-4 index per year

When the patients were categorized into five groups according to the degree of changes in the fibrosis stage per year (< −0.2, −0.2 – < 0, 0, > 0 – 0.2 and > 0.2), median value of the ΔFIB-4 index/year was −0.29, −0.02, 0.04, 0.16 and 0.47, respectively. The FIB-4 index reduced along the regression of the fibrosis stage, while the FIB-4 index increased along the progression of the fibrosis stage, which showed a significant correlation ($P < 0.001$) (Fig. 2).

Prediction of progression to cirrhosis by the changes in the FIB-4 index per year

The area under the receiver operating characteristic curve of the ΔFIB-4 index/year for the prediction of advancement to cirrhosis was 0.910. By the ΔFIB-4 index/year of 0.4, the sensitivity and specificity for the prediction of advancement to cirrhosis was 80% and 91%. The cumulative incidence of fibrosis progression to cirrhosis, at 5 and 10 years, was 34% and 59%, respectively, in patients with the ΔFIB-4 index/year ≥0.4, whereas it was 0% and 3% in those with the ΔFIB-4 index/year <0.4 ($P < 0.001$) (Fig. 3).

DISCUSSION

Recently, noninvasive markers of liver fibrosis have been used as a predictive factor of liver-related outcome such as

Table 3 Factors associated with the progression of liver fibrosis

	Progression of Liver fibrosis	Nonprogression of Liver fibrosis	*P*-value
Gender (male/female)	31/42	118/123	0.33
Age at first biopsy (years)	54.4 ± 8.7	53.5 ± 10.2	0.50
AST at first biopsy (IU/L)	63.9 ± 35.0	64.8 ± 37.3	0.85
ALT at first biopsy (IU/L)	86.5 ± 58.4	88.1 ± 59.2	0.84
Platelet counts at first biopsy (10^9/L)	15.8 ± 4.6	16.7 ± 4.8	0.16
Change between biopsies			
ΔAST (IU/L)/year	3.8 ± 19.5	−4.1 ± 14.8	<0.001
ΔALT (IU/L)/year	−1.9 ± 28.4	7.2 ± 22.6	0.005
ΔPlatelet counts (10^9/L)/year	−4.1 ± 9.5	−0.002 ± 9.5	0.001
ΔFIB-4 index/year	0.31 ± 0.52	−0.005 ± 0.37	<0.001

ΔAST/year: (AST at the second liver biopsy – AST at the first liver biopsy) /interval between paired biopsies (years); ΔALT/year: (ALT at the second liver biopsy – ALT at the first liver biopsy) /interval between paired biopsies (years); ΔPlatelet counts/year: (platelet counts at the second liver biopsy –platelet counts at the first liver biopsy) /interval between paired biopsies (years); ΔFIB-4 index /year: (the FIB-4 index at the second liver biopsy – the FIB-4 index at the first liver biopsy) /interval between paired biopsies (years).

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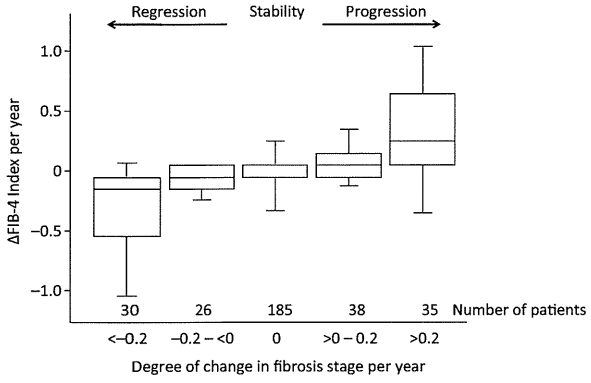

Fig. 2 Correlation between the degree of changes in the fibrosis stage and the ΔFIB-4 index per year. Boxplot of the ΔFIB-4 index/year is shown according to the degree of changes in the fibrosis stage per year. The bottom and top of each box represent the 25 and 75th percentiles, giving the interquartile range. The line through the box indicates the median value, and the error bar indicates the 5 and 95th percentiles.

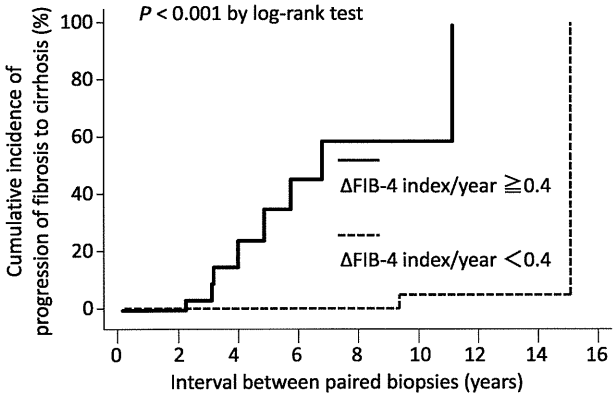

Fig. 3 Cumulative incidence of fibrosis progression to cirrhosis. Patients were categorized into two groups according to the ΔFIB-4 index/year using cut-off value of < 0.4 or ≥ 0.4.

mortality [22–24] or HCC development [24–26] in patients with chronic liver disease. There have been few studies that investigated the association between changes of noninvasive markers and liver-related outcome [27–29]. However, it is still unclear whether there is a relation between the time-course changes in the value of noninvasive markers and progression of liver fibrosis.

The aim of the study was to evaluate the utility of the real-time assessment of the FIB-4 index for the prediction of time-course progression in liver fibrosis. We have shown that the FIB-4 index reduced along the regression of the fibrosis stage, while the FIB-4 index increased along the progression of the fibrosis stage. These results indicate that the measurement of the time-course changes in the FIB-4 index may be useful for the noninvasive and real-time estimation of the progression in liver fibrosis overtime.

Although the gold standard for diagnosis of liver fibrosis is liver biopsy, there are a variety of problems including invasiveness and sampling errors [6]. Diagnostic methods of liver fibrosis by measurement of elasticity of the liver by ultrasonography [10–14] have been developed, but these modalities are not widely available.

The FIB-4 index has an advantage among these noninvasive liver fibrosis diagnostic methods. Firstly, it is quite easily calculated. The parameters required for calculation are only age, AST, ALT and platelet counts, which are measured at the routine examination of patients with liver disease. Therefore, additional blood collection is unnecessary, and the index can be calculated at no cost. Secondly, because of its simple calculation, it is possible to evaluate the clinical conditions in a real-time manner. Repeated measurements of the FIB-4 index make it possible to predict deterioration in liver fibrosis continuously over time. Because no special equipment or system is necessary, and objective data on the clinical conditions are provided in a real-time manner, the FIB-4 index is simple and convenient compared with other noninvasive liver fibrosis diagnostic methods.

It is widely known that a decrease in platelet counts is useful for the prediction of the progression of fibrosis stage [30]. We have reported that elevated AST or ALT is also associated with the progression of liver fibrosis [31]. However, the results of this study showed that a change in the FIB-4 index over time was a more useful factor for the prediction of the progression of fibrosis stage than AST, ALT and changes in platelet counts.

Liver biopsy is still an important examination as the gold standard for diagnosis of liver fibrosis, but time-course changes cannot be readily observed by repeated biopsies because of its invasiveness. On the other hand, it is possible to estimate the progression of liver fibrosis by repeated measurement of the FIB-4 index. Therefore, two examinations should be combined: liver biopsy may be utilized to determine the baseline of fibrosis stage, and the serial measurement of the FIB-4 index may be utilized to predict changes of fibrosis stages overtime in a real-time manner.

In conclusion, we believe that measurement of the time-course changes in the FIB-4 index is useful for the noninvasive and real-time estimation of the progression in liver fibrosis.

ACKNOWLEDGEMENTS

This study was supported by a grant-in-aid from Ministry of Health, Labor and Welfare, Japan.

CONFLICT OF INTEREST

No conflicts of interest exist for all authors.

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Digestive Diseases

Dig Dis 2012;30:554–560
DOI: 10.1159/000343064

Retreatment with Peginterferon α-2a + Ribavirin in Patients Who Failed Previous Peginterferon α-2b + Ribavirin Combination Therapy

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Key Words
Hepatitis C · Interleukin-28B · Peginterferon · Retreatment · Sustained viral response

Abstract
Background/Aims: Peginterferon (PEG-IFN) + ribavirin (RBV) combination therapy is the current standard of care for chronic hepatitis C. However, more than half of the patients cannot achieve sustained viral response (SVR). In Japan, the clinical benefit of retreatment with PEG-IFN + RBV combination retreatment is still unknown. ***Methods:*** We collected clinical data in 106 chronic hepatitis C patients who failed to achieve SVR with PEG-IFNα-2b + RBV combination therapy and were retreated with PEG-IFNα-2a + RBV. This retrospective study examined the efficacy of retreatment with PEG-IFNα-2a + RBV by evaluating the time to eradication of hepatitis C virus RNA, early virological response (EVR), and SVR. We compared the results of the previous therapy and retreatment in terms of efficacy and analyzed the factors influencing SVR. ***Results:*** The SVR rates in the non-responders and relapsers were 11 and 53%, respectively. EVR and prolonged treatment duration were associated with SVR. We also found that a prior response to PEG-IFN + RBV therapy was more important than the Interleukin-28B genotype for predicting the response to retreatment. ***Conclusions:*** Retreatment with PEG-IFNα-2a + RBV should be considered for relapsers and partial responders. Our results suggest that prolonged administration is also favorable for EVR cases to attain a higher SVR.

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Introduction

The development of a combination therapy consisting of peginterferon (PEG-IFN) and ribavirin (RBV) has increased the hepatitis C virus (HCV) RNA response rate to 65–69% at the end of therapy with a sustained HCV RNA response (sustained viral response, SVR) in 54–56% of chronic hepatitis C (CHC) patients. Conversely, this

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indicates that PEG-IFN + RBV therapy does not induce a response in 31–35% of such patients either because HCV RNA is not eliminated during therapy or the therapy is not completed. Furthermore, HCV RNA reappears in 11–13% of patients after the end of therapy [1, 2].

Retreatment with PEG-IFN + RBV for non-responding or relapse patients has been studied in Western countries [3, 4], but no large-scale studies have been performed in Japan. The AASLD guidelines [5] do not recommend retreatment with PEG-IFN + RBV. However, the Japanese guidelines [6] state that 'after examining cases with no effect in previous treatment, a treatment for SVR or for maintenance should be selected'; thus, retreatment with PEG-IFN + RBV is not completely excluded according to the Japanese guidelines.

A high SVR rate is observed after the addition of telaprevir to PEG-IFN + RBV [7–10]. However, some patients in Japan have not benefited from the launch of telaprevir, because CHC patients in Japan are older than those in Western countries [11, 12] and are often anemic. Thus, we retrospectively analyzed the results of retreatment with PEG-IFN + RBV in Japanese relapse and non-responding patients who previously received therapy with PEG-IFN + RBV. Core amino acid substitutions at position 70 [13] and host genome single-nucleotide polymorphism (SNP) genotyping of rs8099917, an interleukin-28B (IL28B) SNP [14, 15], are related to the efficacy of PEG-IFN + RBV therapy. Therefore, these factors were also examined in the study.

Materials and Methods

A total of 106 patients received combination therapy with PEG-IFNα-2a + RBV at 12 medical facilities in Japan from 2007 to 2009. We retrospectively evaluated the data of CHC patients who failed to achieve SVR (i.e. non-responders) or became HCV RNA negative on PEG-IFNα-2b + RBV therapy but relapsed again after the end of treatment (i.e. relapsers). The non-responders were divided into two groups according to the maximum decrease in HCV RNA titer during the initial treatment. Retreatment with PEG-IFNα-2a + RBV was performed to examine the relationships between SVR and patient background factors, timing of the HCV RNA response, and treatment duration.

For the previous treatment, PEG-IFNα-2b (PegIntron; MSD, Tokyo, Japan) at a dose of 1.5 μg kg^{-1} per week subcutaneously and RBV (Rebetol; MSD) were used. PEG-IFNα-2a (Pegasys; Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) + RBV (Copegus; Chugai Pharmaceutical Co., Ltd) was started between 2007 and 2009. In principle, as a starting dose, PEG-IFN was given once weekly at 180 μg PEG-IFNα-2a while RBV was given at 600–1,000 mg/day based on body weight (body weight <60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 1,000 mg), according to the standard treatment protocol in Japan.

Serum HCV RNA after PEG-IFNα-2b + RBV treatment was assessed by quantification using a Cobas Amplicor HCV monitor test (high range method: detection range, 5–5,000 KIU ml^{-1}, or version 2.0: limit of quantitation, 500 IU ml^{-1}; Roche Diagnostics Co. Ltd, Tokyo, Japan).

HCV RNA in retreatment was measured using a Cobas TaqMan HCV test (Roche Diagnostics Co. Ltd) at 4-week intervals; the linear dynamic range was 1.2–7.8 log IU ml^{-1}. Samples with undetectable HCV RNA levels were reported as <1.2 log IU ml^{-1} (i.e. no detectable HCV RNA). Patients were judged to have attained SVR status if HCV RNA was not detected for 24 weeks after the end of treatment. Rapid viral response (RVR) was defined when HCV RNA was not detected at week 4; early virological response (EVR) was defined when HCV RNA was not detected at week 12 of treatment.

Univariate analysis was performed using a χ^2 test and Fisher's exact test. Multivariate analysis was performed using logistic regression using the stepwise method.

As a rule, dose modification followed the manufacturer's drug information on the intensity of potential adverse hematologic effects. The PEG-IFN doses were reduced to 50% of the original dose if the neutrophil count fell below 750/mm^3 and discontinued if the neutrophil count fell below 500/mm^3 or the platelet count (PLT) fell below 50,000/mm^3. RBV was also reduced from 1,000 to 600, 800 to 600, or 600 to 400 mg when hemoglobin (Hb) was below 10 g dl^{-1} and was discontinued when the Hb was below 8.5 g dl^{-1}. Both PEG-IFN and RBV were discontinued if there was a need to discontinue one of the drugs.

The baseline data of the patients are expressed as median values and ranges. In order to analyze the differences between baseline data and the factors associated with SVR, univariate analysis using the Mann-Whitney U test or a χ^2 test was performed; multivariate analysis was performed using stepwise and multiple logistic-regression models. p values <0.05 were considered significant.

This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki amended in 2008, and informed consent was obtained from each patient.

Results

Characteristics

The median age of the 106 subjects was 60 years. There were 63 non-responders and 43 relapsers who received previous treatment. Non-responders were divided into two groups according to their virological response to the previous treatment: partial responders, maximum HCV RNA decrease of >2 log; null responders, maximum HCV RNA decrease <2 log. HCV RNA genotypes 1 and 2 were detected in 101 and 5 subjects, respectively. The baseline characteristics of the study patients are shown in table 1.

Efficacy

Retreatment of the 106 subjects with PEG-IFNα-2a + RBV therapy resulted in an SVR rate of 28% (30/106). The