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Week 4 viral response to peginterferon and ribavirin: How should it be used in combination with a baseline predictive factor?

To the Editor:

We read with great interest the article by Marcellin *et al.* [1] evaluating the predictive value of week 4 viral response to peginterferon-alpha 2a and ribavirin combination therapy in patients with hepatitis C virus (HCV) genotype 1. They concluded that patients with a $\geqslant 3\log_{10}$ drop in HCV RNA at week 4 have a high probability of achieving sustained virologic response (SVR), which is consistent with our previous study [2].

Previous studies reported that the genetic polymorphism near the interleukin 28B (*IL28B*) gene (rs12979860 or rs8099917) is a strong baseline factor associated with the outcome of therapy [3]. However, this variable was not included in the study by Marcellin *et al.*, probably because the actual treatment period in the study predated this finding [4,5]. Given these predictors, i.e. week 4 viral response and a baseline variable, how should they be combined to predict response?

We evaluated the predictive value of week 4 viral response to combination therapy on SVR in 272 patients infected with HCV genotype 1b [6]. Overall, a $\geqslant 3\log_{10}$ drop in HCV RNA at week 4 was a strong predictor of SVR. SVR was achieved in 77.0% of patients with rapid virologic response (RVR) or a $\geqslant 3\log_{10}$ drop, whereas only 16.7% of patients with a $<3\log_{10}$ drop achieved SVR ($p<0.0001$). When patients were stratified based on the *IL28B* genetic polymorphism rs8099917, which corresponds to rs12979860 in more than 99% of Japanese ethnicity [7], a $\geqslant 3\log_{10}$ drop at week 4 was strongly predictive of SVR in patients with the favorable TT rs8099917 genotype (CC rs12979860 genotype). The SVR rate was 79.5% in patients with RVR or a $\geqslant 3\log_{10}$ drop and 15.6% in patients with a $<3\log_{10}$ drop ($p<0.0001$). In contrast, among patients with an unfavorable TG/GG rs8099917 genotype, no differences were found in the SVR rate between patients with RVR or a $\geqslant 3\log_{10}$ drop (20.0%) and those with a $<3\log_{10}$ drop (18.3%, $p=0.9265$); the predictive value of week 4 response is low in this subset. In addition, the predictive value of complete early virologic response (EVR) for SVR is lower in patients with the unfavorable TG/GG genotype. The SVR rate was 81.6% in patients with complete EVR and 21.2% in patients without ($p<0.0001$), when patients had the favorable TT rs8099917 genotype. In contrast, the rate of SVR was 25.0% in patients with complete EVR and 18.0% in patients without ($p=0.7279$), when patients had the unfavorable TG/GG genotype. Therefore, it appears to be difficult to identify patients with the unfavorable genotype of the genetic polymorphism near the *IL28B* gene who have a likelihood to achieve SVR by week 4 viral response, although it can identify patients with a high likelihood of achieving SVR in patients with the favorable genotype.

In contrast to our results, a previous large study by Thompson *et al.* [8] reported that patients who attained RVR showed high SVR rate regardless of the genetic polymorphisms near the *IL28B* gene (rs12979860), although they focused on patients with RVR and did not include patients with non-RVR but with a $\geqslant 3\log_{10}$ drop at week 4. This discrepancy between their study and ours may be partly explained by the difference in the ethnicity of the study population. The study by Thompson *et al.* was based on patients from the IDEAL study including Caucasians, African Americans, and Hispanics, whereas all patients were Japanese Asians in our study. Similarly, the ethnicity was different between the population studied by Marcellin *et al.* and ours. Accordingly, the distribution of rs12979860 or rs8099917 genotypes and the rate of concordance between rs12979860 (analyzed in a study by Thompson *et al.*) and rs8099917 (analyzed in our study) would be different. For example, the rate of favorable homozygote (CC rs12979860 genotype and TT rs8099917 genotype) was largely different: 33.0% in Thompson's study and 76.1% in our study. Moreover, our study involved only patients infected with HCV genotype 1b. These factors should be adjusted when comparing the association between the genetic polymorphisms near the *IL28B* gene and the predictive value of week 4 viral response between studies. Nonetheless, the genetic polymorphism near the *IL28B* gene appears to have a strong impact on the predictive value of early viral response to therapy; the prediction of SVR by week 4 viral response may have to be modified based on this strong baseline predictive factor.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Marcellin P, Reau N, Ferenci P, Hadziyannis S, Messinger D, Tatsch F, et al. Refined prediction of week 12 response and SVR based on week 4 response in HCV genotype 1 patients treated with peginterferon alfa-2a (40KD) and ribavirin. J Hepatol 2012;56:1276–1282.

[2] Toyoda H, Kumada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, et al. High ability to predict the treatment outcome of peginterferon and ribavirin combination therapy based on the reduction in HCV RNA levels at 4 weeks after starting therapy and amino acid substitutions in hepatitis C virus in patients infected with HCV genotype 1b. J Gastroenterol 2011;46:501–509.

[3] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.

[4] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales Jr FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.

[5] Hadziyannis SJ, Sette Jr J, Morgan TR, Bolan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis

C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346–355.
[6] Toyoda H, Kumada T, Tada T, Hayashi K, Honda T, Katano Y, et al. Predictive value of early viral dynamics during peginterferon and ribavirin combination therapy based on genetic polymorphisms near the *IL28B* gene in patients infected with HCV genotype 1b. J Med Virol 2012;84:61–70.
[7] Tanaka Y, Nishida N, Sugiyama M, Tokunaga K, Mizokami M. A-interferons and the single nucleotide polymorphisms: a milestone to tailor-made therapy for chronic hepatitis C. Hepatol Res 2010;40:449–460.
[8] Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, et al. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 2010;139:120–129.

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Encephalopathy or hepatic encephalopathy?

To the Editor:

We read with interest the paper by Ginès and co-authors on the management of critically-ill cirrhotic patients [1]. However, we have some concerns on the section on management of hepatic encephalopathy. The authors seem to base their recommendations on a 'statistical' rather than a pathophysiological definition of the syndrome, grouping under the heading 'severe hepatic encephalopathy' a set of different neuropsychiatric symptoms arising in critically-ill cirrhotic patients, to include mental abnormalities relating to sepsis, electrolyte imbalance, and even the side- or desired-effects of drugs such as opioids and benzodiazepines. Within this frame, they state that ammonia levels should not be measured, as they provide no clinical information nor do they relate to clinical outcomes. While we agree with the authors that patients with cirrhosis, especially if critically-ill, may present with more than one metabolic encephalopathy, and these may all contribute and worsen the clinical picture, it seems to us that an effort should be made to differentiate *hepatic* encephalopathy from other forms of metabolic/toxic neuropsychiatric disturbance. For example, we need to be reasonably sure that the encephalopathy we refer to in order to define fulminant hepatic failure is *hepatic* encephalopathy, as we would not want to list for transplant a patient with hepatitis who is confused because of hypoglycaemia, or opioid/benzodiazepine overdose. In this respect, ammonia levels seem useful, as they reflect hepatic failure and portal-systemic shunting [2], they correlate with recognised, quantified indices of hepatic encephalopathy, and they predict the development of hepatic encephalopathy over time [3]. Notably, sepsis, electrolyte imbalance, and psychoactive drugs cause neuropsychiatric abnormalities in critically-ill patients with no liver dysfunction [4]: we would not diagnose these patients with hepatic encephalopathy, we would not expect them to be hyperammonaemic and we would not treat them with ammonia-lowering drugs such as non-absorbable disaccharides/antibiotics. Critically-ill cirrhotic patients are no exception. Should they present with more than one potential cause for neuropsychiatric dysfunction, each cause should be identified and treated according to its pathophysiology. Finally, there seems to be some confusion in Table 2, in relation to the West Haven criteria [5]. These are clinical criteria and they are described, although not in their exact, original form [5], in columns 2 and 3 of the table. However, the table also depicts stages, characterized by parallel alterations in consciousness, cognitive/behavioural features, neurological findings, and electroencephalographic changes. Such correspondence has never been established, which is the reason why Conn and co-workers proposed the use of an index, not unlike the Child–Pugh score, combining the independent scores of five dimensions (mental state based on the West Haven criteria, Trail Making Test A, asterixis, electroencephalographic slowing and arterial ammonia levels) [5]. In addition, the classification of electroencephalographic changes reported in column 5 of the table does not correspond to either the one proposed by Conn *et al.* [5] or to more modern ones [6], most likely in relation to a typo or an alignment problem. An *errata corrige* on the involuntarily misleading information provided in Table 2 of the paper might be necessary.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Gines P, Fernandez J, Durand F, Saliba F. Management of critically-ill cirrhotic patients. J Hepatol 2012;56:S13–S24.
[2] Watanabe A. Portal-systemic encephalopathy in non-cirrhotic patients: classification of clinical types, diagnosis and treatment. J Gastroenterol Hepatol 2000;15:969–979.
[3] Montagnese S, Biancardi A, Schiff S, Carraro P, Carla V, Mannaioni G, et al. Different biochemical correlates for different neuropsychiatric abnormalities in patients with cirrhosis. Hepatology 2011;53:558–566.
[4] van den Boogaard M, Pickkers P, Slooter AJ, Kuiper MA, Spronk PE, van der Voort PH, et al. Development and validation of PRE-DELIRIC (PREdiction of DELIRium in ICu patients) delirium prediction model for intensive care patients: observational multicentre study. BMJ 2012;344:e420.
[5] Conn HO, Leevy CM, Vlahcevic ZR, Rodgers JB, Maddrey WC, Seeff L, et al. Comparison of lactulose and neomycin in the treatment of chronic portal-

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Original Article

Pegylated interferon monotherapy in patients with chronic hepatitis C with low viremia and its relationship to mutations in the NS5A region and the single nucleotide polymorphism of interleukin-28B

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Aim: Previous studies have suggested that patients with chronic hepatitis C with a low pretreatment hepatitis C virus (HCV) level have a high sustained virological response (SVR) rate, and that there would be a subpopulation of patients in which HCV can be eradicated with pegylated interferon (PEG IFN) alone without a decrease in SVR. However, the efficacy of PEG IFN monotherapy in patients with low HCV RNA levels is unclear. Several studies have reported that interferon sensitivity-determining region (ISDR) and the single-nucleotide polymorphism (SNP) of interleukin-28B (IL-28B) contribute to IFN response, but these relationships are controversial. The aim of this study was to determine whether the SNP of IL-28B (rs8099917) and amino acid substitutions in the ISDR among patients with low HCV levels affect the response to PEG IFN monotherapy.

Methods: One hundred and four patients with low-level HCV infection were studied. Low HCV level was defined as 100 KIU/mL or less.

Results: SVR was achieved in 94 patients (92.2%). HCV levels (≤50 KIU/mL) and ISDR (≥2 mutations) were associated with SVR on univariate analysis. The rates of SVR in the patients with IL-28B genotypes TT, TG and GG were 94.5%, 77.8% and 100%, respectively. The G allele tended to be associated with poor response to IFN therapy ($P = 0.0623$). On multivariate analysis, the ISDR was the factor predictive of SVR ($P = 0.004$).

Conclusion: The ISDR is significantly associated with a good response to PEG IFN monotherapy in patients with low HCV levels.

Key words: hepatitis C virus, interferon sensitivity-determining region, interferon, interleukin-28B, rapid virological response

INTRODUCTION

HEPATITIS C VIRUS (HCV) is a member of the Flaviviridae family and causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma (HCC) that easily progresses to end-stage liver disease.[1] Because 170 000 000 persons are infected with HCV worldwide, HCV infection is a significant global health problem.

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The current recommended therapy for patients with chronic hepatitis C is a combination of pegylated interferon (PEG IFN) and ribavirin and/or telaprevir or boceprevir.[2–6] HCV RNA levels, as well as genotypes, are an important factor associated with sustained virological response (SVR) to IFN therapy.[3,4] Patients with low HCV RNA levels have a high SVR rate, and even standard IFN monotherapy is useful for eradication of HCV in patients with low viral loads.[7–9] Several studies have succeeded in reducing the duration of treatment without risk of relapse.[10,11] Although patients with low HCV RNA have higher response rates to IFN treatment, not all patients achieve SVR. Other factors for improving the prediction of SVR in patients with low HCV RNA levels are needed. The predictive factors for SVR in patients with genotype 1b and high HCV RNA levels have been investigated, and several studies have shown that the single nucleotide polymorphism of interleukin-28B (IL-28B) and amino acid substitutions in the core and NS5A region affect the response to IFN therapy.[12–16] However, the predictive factors for SVR among patients with low HCV RNA levels treated with PEG IFN monotherapy have been unclear.

Hepatitis C virus consists of three structural proteins (core, envelope 1 and envelope 2) and six non-structural proteins (NS2 to NS5). HCV NS5A protein was reported to have a domain associated with IFN response. This domain in the region of HCV genotype 1b is closely associated with response to IFN therapy and is known as the IFN sensitivity-determining region (ISDR).[12,15–21] IFN acts to control replication of the virus by inducing the dsRNA-dependent protein kinase (PKR). The ISDR is located in the PKR-binding domain, is inhibited by PKR *in vitro*,[22] and is useful for prediction in patients with genotypes 2a, 2b and 3a.[23–28] Therefore, ISDR heterogeneity is an important factor that may affect response to IFN in patients with low HCV RNA levels. We hypothesized that ISDR heterogeneity could be predicted in patients with low HCV RNA levels in which HCV can be eradicated with PEG IFN-α alone without a decrease in SVR.

Not only genetic heterogeneity in the HCV genome but also host genetics contribute to IFN treatment outcomes. Therefore, several studies were performed to understand the host factors associated with IFN responsiveness; these showed that IL-28B polymorphisms are strongly associated with response to PEG IFN and ribavirin combination therapy in patients with genotype 1b and high viral load.[13,14,16,29] However, the associations between ISDR and IL-28B and the effects of PEG IFN-α monotherapy in patients with low HCV RNA levels are not well known.

The aim of the present study was to determine whether genomic heterogeneity of the ISDR and the SNP of IL-28B among patients with low HCV RNA levels affects the response to PEG IFN-α-2a monotherapy.

METHODS

A TOTAL OF 295 patients with chronic hepatitis C were treated by PEG IFN-α-2a monotherapy at Nagoya University Hospital and Affiliated Hospitals; 104 patients with low HCV RNA levels were selected for this study. The patients consisted of 62 men and 42 women with a mean age of 55.1 years (range, 19–78). All patients were positive for serum anti-HCV antibody by a commercial enzyme-linked immunosorbent assay (Dinabot, Tokyo, Japan) and for HCV RNA by a commercial polymerase chain reaction (PCR) (Roche Diagnostic Systems, Tokyo, Japan).

A low HCV level was defined as 100 KIU/mL or less, as previously reported.[4,7,9,11] No patient had hepatitis B surface antigen, co-infection with HIV, autoimmune disease or chronic alcohol abuse.

Schedule of IFN therapy

Patients received PEG IFN-α-2a (Pegasys Chugai-Roche, Tokyo, Japan) at a dose of 180 μg injected s.c. once per week for 24 or 48 weeks. The patients were allocated, at the discretion of the physician in charge, to a protocol lasting either 24 or 48 weeks. Laboratory tests and evaluations of adverse events were performed once per week during treatment.

The dose of PEG IFN-α-2a was reduced to 90 μg when clinically significant adverse events or laboratory abnormalities such as neutropenia (<750 cells/mm^3) or thrombocytopenia (<50 000 cells/mm^3) occurred. PEG IFN-α-2a was discontinued when neutropenia of less than 250 cells/mm^3 or a platelet count of less than 25 000 cells/mm^3 was seen.

Hepatitis C virus RNA in serum samples was examined at 4 weeks, at the end of IFN therapy, and at 6 months after the end of treatment (ETR). Serum was stored at −80°C for virological examination at pretreatment.

Patients who were persistently negative for serum HCV RNA and who had a normal serum alanine aminotransferase (ALT) level at 24 weeks after withdrawal of IFN treatment were considered to have SVR. Patients who were HCV negative at the ETR but returned to HCV

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positive status after withdrawal of IFN were defined as virological relapsers. Patients who did not become HCV negative with IFN therapy were defined as non-virological responders.

This study was approved by the ethics committee of each institution involved. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Virological tests

Hepatitis C virus was genotyped by direct sequencing of the 5′-untranslated region and/or E1 regions, as described previously.[30,31] Genotypes were classified according to the nomenclature proposed by Simmonds *et al.*[32]

Nested PCR analysis and direct sequencing of the NS5A-ISDR were performed as previously reported for each genotype.[15,16,27,28] In brief, RNA was extracted from 140 μL serum using a QIAamp Viral RNA Kit (Qiagen, Valencia, CA, USA) and dissolved in 50 μL diethylpyrocarbonate-treated water. RNA (10 ng) was used for reverse transcription with oligo and random hexamer primers with an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). NS5A-ISDR was sequenced after amplification by nested PCR as previously described.[15,16,27,28]

The primers used were as follows: NS5A-ISDR of genotype 1b, sense 5′-TGGATGGAGTGCGGTTGCACA GGTA-3′ and antisense 5′-TCTTTCTCCGTGGAGGTGGT ATTG-3′; NS5A-ISDR of genotype 2a, sense 5′-ACGTCC ATGCTAACAGACCC-3′ and antisense 5′-GGGAATCT CTTCTTGGGGAG-3′; and NS5A-ISDR of genotype 2b, sense 5′-TCTCAGCTCCCTTGCGATCCTGA-3′ and antisense 5′-GATGGTATCGAAGGCTC-3′. Amplification conditions consisted of 10 min at 94°C, followed by 40 cycles of 94°C for 10 s, 55°C for 30 s and 72°C for 30 s in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems, Foster City, CA, USA). The second PCR was done using the following sets of primers: NS5A-ISDR of genotype 1b, sense 5′-CAGGTACGC TCCGGCGTGCA-3′ and antisense 5′-GGGGCCTTGGT AGGTGGCAA-3′; NS5A-ISDR of genotype 2a, sense from the first-round PCR and a new antisense primer 5′-CGAGAGAGTCCAGAACGACC-3′; and NS5A-ISDR of genotype 2b, sense 5′-AGCTCCTCAGCGAGCCA GCT-3′ and antisense 5′-GATGGTATCGAAGGCTC-3′. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers with a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems).

Genomic analysis

Detection of the SNP of IL-28B (rs8099917) was done by a real-time PCR system, as previously reported.[16] In brief, genomic DNA was extracted from 15 μL of whole blood using a commercial kit (QIAamp DNA Blood mini Kit; Qiagen) and dissolved in 50 μL diethylpyrocarbonate-treated water. DNA (1 ng) was used for PCR with primers and probes of commercial kit (Taqman SNP Genotyping Assays; Applied Biosystems). The SNP of IL-28B (rs8099917) was amplified, and the results were analyzed by real-time PCR in a thermal cycler (7300 Real time PCR System; Applied Biosystems).

Statistical analysis

Data are expressed as mean ± standard deviation. A paired Student's *t*-test or Fisher's exact test were used to analyze differences in variables. $P < 0.05$ was considered significant. Multiple logistic regression models were used to identify factors predictive of SVR. Statview ver. 5.0 software (SAS Institute, Cary, NC, USA) was used for all analyses.

RESULTS

Background

PATIENTS' CLINICAL CHARACTERISTICS are summarized in Table 1. HCV genotypes 1b ($n = 34$), 2a ($n = 58$), 2b ($n = 9$) and unknown ($n = 3$) were detected.

Table 1 Clinical characteristics at pretreatment

Clinical characteristics	$n = 104$
Age (years)	55.1 ± 12.5
Sex: male/female	62/42
AST (IU/L)	50.0 ± 28.2
ALT (IU/L)	62.7 ± 47.3
Platelet count (10^4/uL)	18.4 ± 5.7
HCV RNA level (KIU/mL)	36 (1.6–100)
HCV genotype (1b/2a/2b/unknown)	34/58/9/3
IFN length (weeks) (24/48/<17)	49/45/10
Body mass index	22.7 ± 3.2

Data are expressed as mean ± standard deviation.
HCV RNA level was shown by median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IFN, interferon.

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Table 2 Virological response in each group

(a) Virological response according to durations of IFN therapy				
	Overall ($n = 102$)	24W ($n = 48$)	48W ($n = 45$)	<17W ($n = 9$)
RVR	81.4% ($n = 83$)	87.5% ($n = 42$)	73.3% ($n = 33$)	88.9% ($n = 8$)
ETR	100% ($n = 102$)	100% ($n = 48$)	100% ($n = 45$)	100% ($n = 9$)
SVR	92.2% ($n = 94$)	93.8% ($n = 45$)	91.1% ($n = 41$)	88.9% ($n = 8$)
(b) Virological response according to HCV genotypes				
	Overall ($n = 102$)	1b ($n = 32$)	2a ($n = 58$)	2b ($n = 9$)
RVR	81.4% ($n = 83$)	81.3% ($n = 26$)	81.0% ($n = 47$)	88.9% ($n = 8$)
SVR	92.2% ($n = 94$)	87.5% ($n = 28$)	93.1% ($n = 54$)	100% ($n = 9$)

ETR, end of treatment response; HCV, hepatitis C virus; IFN, interferon; RVR, rapid virological response; SVR, sustained virological response; W, weeks.

All patients had serum HCV RNA levels of 100 KIU/mL or less, and the median HCV RNA level was 36 KIU/mL.

One hundred and four patients were initially included in this study; 49 patients were treated with PEG IFN-α-2a for 24 weeks, and 45 patients were treated for 48 weeks. Ten patients withdrew from IFN therapy within 17 weeks, and two of these 10 patients could not be followed. The reasons for discontinuing therapy were fatigue ($n = 3$), depression ($n = 1$), rash ($n = 1$), appetite loss ($n = 1$), liver failure ($n = 1$) and unknown ($n = 3$). The two patients who withdrew from follow up were excluded from the analysis, and the remaining 102 patients were followed for 6 months after the ETR.

Virological response

Virological response is shown in Table 2. Rapid virological response (RVR), which was defined as negativity for HCV after 4 weeks of treatment, for the overall group, the 48 weeks' group, the 24 weeks' group and the under 17 weeks' group was 81.4% (83/102), 73.3% (33/45), 87.5% (42/48) and 88.9% (8/9), respectively. Virological response at the ETR was 100% among all patients. Finally, 94 (92.2%) of 102 patients achieved SVR.

There was no significant difference in virological response between patients treated for 24 weeks and those treated for 48 weeks. The virological response according to HCV genotype is shown in Table 2(b). Patients with genotype 1b had a lower SVR rate than genotypes 2a and 2b, but no significant differences in genotype were noted.

Genetic heterogeneity in NS5A-ISDR and response to IFN therapy

The prevalences of the number of amino acid substitutions in ISDR according to HCV genotypes are summarized in Figure 1. The ISDR were examined by direct sequencing, and classification involved counting the number of amino acid substitutions compared to consensus strains of each genotype, as previously reported.[15,24,27,28]

Interferon sensitivity-determining region sequences were obtained in 81 patients. Five patients did not have serum at pretreatment, and 16 patients could not be amplified by PCR. Sixty-one patients (84.7%) had one mutation or more. SVR according to the ISDR is shown in Figure 2. All patients with three or more mutations in the ISDR achieved SVR, but 18 (69.2%) of 26 patients with two or less mutations in the ISDR achieved SVR. Patients with two or less mutations in the ISDR were poor responders to IFN therapy.

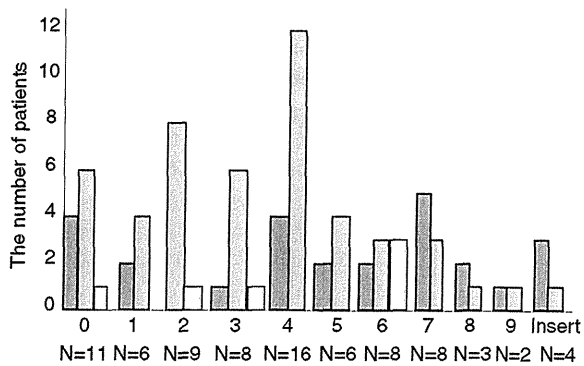

Figure 1 Number of amino acid substitutions in interferon sensitivity-determining region (ISDR) according to hepatitis C virus (HCV) genotypes. ■, HCV genotypes 1b; □, HCV genotypes 2a; □, HCV genotypes 2b.

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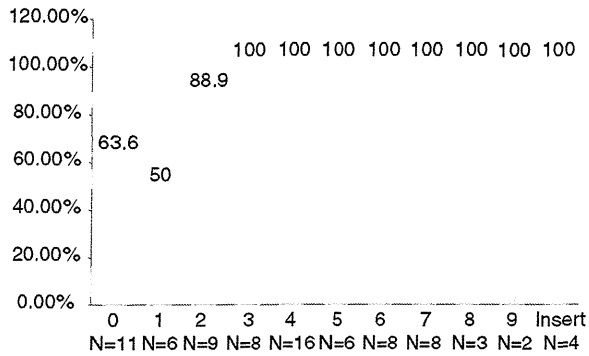

Figure 2 Sustained virological response (SVR) according to the number of amino acid substitutions in interferon sensitivity-determining region (ISDR).

Prevalence of the SNP of IL-28B (rs8099917) T (major allele) and G (minor allele) and response to IFN therapy

The frequencies of the IL-28B genotypes were: major homozygotes (TT), 73; heterozygotes (TG), 18; and minor homozygotes (GG), two. The rates of SVR in the patients with TT, TG and GG were 94.5% (69/73), 77.8% (14/18) and 100% (2/2), respectively. The SVR rate of patients with G allele of the IL-28B genotype was 80.0% (16/20), and that with T allele was 94.5% (69/73). Patients with T allele of the IL-28B genotype had a slightly higher SVR rate than did those with G allele, but there were no significant differences ($P = 0.0623$).

Analysis for factors predictive of SVR

The results of univariate analysis for factors predictive of SVR are shown in Table 3. HCV RNA levels were lower in patients with SVR than in those without SVR ($P = 0.0154$). SVR was achieved in 41.2% of patients with less than two mutations in the ISDR and 98.4% of patients with two or more mutations in the ISDR ($P = 0.0001$). HCV RNA levels and ISDR were associated with SVR on univariate analyses.

Results of multivariate analyses of factors predictive of SVR are shown in Table 4. Variables were recorded categorically as ordinal data. Background factors were age (<60 vs ≥60 years), sex (male vs female), platelet count ($<15 \times 10^4/mm^3$ vs $\geq 15 \times 10^4/mm^3$), HCV RNA level (<50 vs ≥50 KIU/mL), ALT levels (<70 vs ≥70 IU/L), aspartate aminotransferase (AST) levels (<60 vs ≥60 IU/L), HCV genotype (1 vs 2), ISDR (<2 vs ≥2 mutations), IL-28B (TT vs TG and GG) and RVR (yes vs no). As can be seen in Table 4, factors such as age, sex, platelet count, HCV RNA level, ALT levels, AST levels, HCV genotype, IL-28B and RVR did not have any effect on SVR. In contrast, the ISDR was the most influential factor.

DISCUSSION

THE HCV RNA level is one of the most important factors affecting response to IFN therapy. Patients with high HCV RNA levels respond poorly to IFN therapy, whereas patients with low HCV RNA levels have a high SVR rate to IFN therapy. Thus, most patients with low HCV RNA levels have achieved SVR, but other therapeutic options for patients who fail IFN therapy are needed. Several studies have attempted to reduce the duration of treatment, reduce the dose of IFN and/or ribavirin, or use standard IFN without risk of relapse.[8–10] The present study confirmed the high SVR rate (92.2%) in patients with low HCV RNA levels (≤100 KIU/mL)

Table 3 Univariate analysis: factors predictive of SVR

Factors	SVR ($n = 94$)	Non-SVR ($n = 8$)	*P*-value
Age (years)	54.6 ± 12.6	57.4 ± 8.8	0.5528
Sex: male/female	58/36	2/6	0.0619
ALT (IU/L)	63.2 ± 48.3	56.3 ± 32.5	0.7126
AST (IU/L)	50.7 ± 28.6	41.4 ± 21.6	0.4043
PLT ($\times 10^4/mm^3$)	18.5 ± 5.8	18.0 ± 5.0	0.8292
HCV RNA level (KIU/mL)	42.5 ± 34.8	75.0 ± 45.7	0.0154
HCV genotype: 1/2	29/63	4/3	0.4337
ISDR: <2/≥2	10/63	7/1	0.0001
IL-28B: TT/TG, GG	69/16	4/4	0.0623
RVR: yes/no	78/16	5/3	0.1661

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IL-28B, interleukin-28B; ISDR, interferon sensitivity-determining region; PLT, platelets; RVR, rapid virological response; SVR, sustained virological response.

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Table 4 Multivariate analysis: factors predictive of SVR

Factors	*P*-value	Risk ratio	95% CI	
Age: <60 years	0.4556	2.837	0.183	43.891
Sex: male	0.8712	0.756	0.026	22.166
AST: <60 IU/L	0.7806	2.131	0.010	438.334
ALT: <70 IU/L	0.6063	0.239	0.001	55.563
Platelet count: $<15 \times 10^4$/uL	0.6873	0.463	0.011	19.680
HCV RNA: <50 KIU/mL	0.1046	13.170	0.585	296.318
Genotype: 2	0.1693	14.110	0.324	614.872
ISDR: <2	0.0074	0.004	0.001	0.235
IL-28B: TT	0.2684	5.978	0.252	141.852
RVR: yes	0.7495	1.756	0.055	55.696

95% CI, 95% confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IL-28B, interleukin 28B; ISDR, interferon sensitivity-determining region; RVR, rapid virological response; SVR, sustained virological response.

treated by PEG IFN-α-2a monotherapy. Although the effects of shortened treatment duration of PEG IFN-α with ribavirin for patients with low HCV RNA levels are unclear, PEG IFN-α-2a monotherapy could reduce the cost and adverse events of ribavirin while maintaining a high SVR rate. This treatment would be a good therapeutic option for patients with low HCV RNA levels. However, selection by HCV RNA level alone was insufficient to predict IFN responsiveness completely, and other factors would be necessary to improve the positive predictive values for SVR in patients infected with low HCV RNA levels.

Hepatitis C virus genotype is another major factor, in addition to HCV RNA levels, that is associated with response to IFN therapy. In the present study, the SVR rates of genotypes 1 and 2 were 87.5% and 94.0%, respectively. Patients infected with genotypes 2 had a slightly higher SVR rate than did those with genotype 1, but there were no significant differences in our small study. The difference in SVR according to genotype may exist, but HCV genotype did not have enough power to be a determinant of IFN response completely among patients with low HCV RNA levels because of the bias for HCV RNA levels. However, patients infected with low HCV RNA levels respond differently to IFN therapy, suggesting that an additional factor associated with resistance to IFN exists.

The heterogeneity of the HCV NS5A region is an important factor that may affect response to IFN in patients with HCV genotype 1b and was named the ISDR.[17] Mutations in the ISDR affect the interaction with PKR and may inhibit viral replication. Therefore, ISDR of other HCV genotypes, in addition to 1b, could be used as predictors of IFN responsiveness.[23–28] In the present study, it was hypothesized that the amino acid substitutions in the ISDR would explain differences in IFN resistance in patients infected with low HCV RNA levels. Therefore, the utility of substitutions of amino acids in the ISDR for predicting IFN responsiveness was investigated. The ISDR was the most influential factor for SVR on multivariate analyses. All patients with three or more mutations in the ISDR achieved SVR, and 18 of 26 patients with less than three mutations in the ISDR achieved SVR. Thus, patients with less than three mutations in the ISDR would be resistant to PEG IFN-α-2a monotherapy and may need to receive much more powerful treatment, even if they have low HCV RNA levels. The ISDR system could be used as a diagnostic tool to predict SVR in patients infected with low HCV RNA levels. An individualized treatment strategy based on HCV RNA levels and the ISDR in patients with chronic hepatitis C would be an important consideration to achieve optimal therapy and avoid unnecessary treatment.

Some studies of SVR to PEG IFN-α-2b and ribavirin and/or telaprevir combination therapy for chronic hepatitis C patients with genotype 1 and high viral load identified genetic variation near the IL-28B gene associated with IFN responsiveness.[13,14,16] However, the effects of genetic variation near the IL-28B gene on SVR in patients with low HCV RNA levels treated with PEG IFN monotherapy are unknown. Therefore, the utility of the SNP of IL-28B for predicting IFN responsiveness was investigated. Patients with IL-28B (rs8099917) genotypes TG and GG had a lower SVR rate than genotype TT, but no significant differences in genotype were found in this study. The SNP of IL-28B would be associated with the response to IFN, especially for poor responders, and

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was partially associated with SVR in a study of patients with HCV genotype 2 who were treated with PEG IFN-α-2b and ribavirin.[13,14,16,33,34] The clear suggestion of a correlation between the SNP of IL-28B with IFN responsiveness would not be supported in patients with low HCV RNA levels because of the high SVR rate and predominant genotype 2.

Viral factors associated with SVR have been studied, and several regions, including 5′-untranslated region, core, E2, NS5A and NS5B, have been suggested to play important roles in IFN responsiveness.[14,16,35–38] Further studies need to investigate whether these other viral factors, especially interferon and ribavirin resistance-determining region of NS5A and core amino acid substitutions, among patients with low HCV RNA levels affect the response to PEG IFN monotherapy.

Hepatitis C virus RNA levels could be easy to measure using commercial kits and would be useful for clinical practice, but sequencing analysis, which involves much effort and cost, would be needed to characterize the ISDR. SVR was achieved in 95.1% of patients with lower HCV RNA levels (<50 KIU/mL) and 98.4% of patients with mutant type. ISDR was a better factor, but HCV RNA level might be used as a predictive factor instead of measurement of ISDR.

The definition of the low HCV RNA level that was related to a good response to IFN therapy has varied widely, from 100–600 KIU/mL.[7,9–11] Zeuzem *et al.* reported that 24 weeks of therapy with PEG IFN-α-2b plus ribavirin is insufficient for the treatment of patients with HCV genotype 1 and a HCV RNA level of 600 KIU/mL or less.[10] They suggested that patients with HCV RNA of 250 KIU/mL or less would have a good response to PEG IFN-α-2b and ribavirin combination therapy for 24 weeks. Most reports from Japan defined 100 KIU/mL as the cut-off level for low HCV levels and used standard IFN monotherapy.[4,7,9,11] The outcome that would maximize the efficacy of IFN therapy would depend on the relationships between the cut-off HCV RNA level and therapeutic regimens. The optimal cut-off level for low HCV levels and the matching therapeutic regimens are not well understood, and further studies are needed to clarify these issues.

Based on the SVR in patients receiving therapy for 24 weeks compared to those treated for 48 weeks, there was no difference in IFN responsiveness by duration in this small study. However, this study was not a randomized study. Further studies are needed to investigate the optimal duration of PEG IFN-α-2a monotherapy for patients with low HCV RNA levels.

Pascu *et al.* performed a meta-analysis for the correlation between SVR and ISDR in patients with HCV genotype 1b infection who received standard IFN therapy.[19] They found that 11 of 21 European patients with mutant type ISDR and HCV RNA levels of less than 6.6 log copies/mL achieved SVR, but 67 of 69 Japanese patients with mutant type ISDR and HCV RNA levels of less than 6.6 log copies/mL achieved SVR. The mode of HCV infection and geographical and racial differences would have effects on the prediction of SVR by ISDR.[39,40] As a result, the ISDR system is more suitable for predicting SVR in Asian than in European patients. Although validation of these observations in larger cohorts is required, mutations in the ISDR were useful for predicting the response to PEG IFN-α-2a monotherapy in patients with low HCV levels.

In conclusion, in patients with HCV infection, low HCV levels and more than two mutations in the ISDR are significantly associated with a good response to PEG IFN-α-2a monotherapy. An individualized treatment strategy based on HCV RNA levels and the ISDR in patients with chronic hepatitis C would be useful in clinical practice.

REFERENCES

1 Seeff LB. Natural history of chronic hepatitis C. *Hepatology* 2002; **36**: S35–46.
2 Hoofnagle JH, Seeff LB. Peginterferon and ribavirin for chronic hepatitis C. *N Engl J Med* 2006; **355**: 2444–51.
3 Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335–74.
4 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40**: 8–13.
5 McHutchison JG, Everson GT, Gordon SC *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360**: 1827–38.
6 Kwo PY, Lawitz EJ, McCone J *et al.* Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010; **376**: 705–16.
7 Yokosuka O, Iwama S, Suzuki N *et al.* High sustained virologic response rate after interferon monotherapy in Japanese hepatitis C patients with a low HCV RNA titer and/or HCV genotype 2. A prospective study. *Intervirology* 2004; **47**: 328–34.

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8 Tabaru A, Narita R, Hiura M, Abe S, Otsuki M. Efficacy of short-term interferon therapy for patients infected with hepatitis C virus genotype 2a. *Am J Gastroenterol* 2005; **100**: 862–7.
9 Kawamura Y, Arase Y, Ikeda K *et al.* The efficacy of short-term interferon-beta therapy for chronic hepatitis C patients with low virus load. *Intern Med* 2008; **47**: 355–60.
10 Zeuzem S, Buti M, Ferenci P *et al.* Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. *J Hepatol* 2006; **44**: 97–103.
11 Arase Y, Suzuki F, Akuta N *et al.* Combination therapy of peginterferon and ribavirin for chronic hepatitis C patients with genotype 1b and low-virus load. *Intern Med* 2009; **48**: 253–8.
12 Okanoue T, Itoh Y, Hashimoto H *et al.* Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study. *J Gastroenterol* 2009; **44**: 952–63.
13 Tanaka Y, Nishida N, Sugiyama M *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105–9.
14 Akuta N, Suzuki F, Hirakawa M *et al.* Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. *Hepatology* 2010; **52**: 421–9.
15 Hayashi K, Katano Y, Ishigami M *et al.* Mutations in the core and NS5A region of hepatitis C virus genotype 1b and correlation with response to pegylated-interferon-alpha 2b and ribavirin combination therapy. *J Viral Hepat* 2011; **18**: 280–6.
16 Hayashi K, Katano Y, Honda T *et al.* Association of interleukin 28B and mutations in the core and NS5A region of hepatitis C virus with response to peg-interferon and ribavirin therapy. *Liver Int* 2011; **31**: 1359–65.
17 Enomoto N, Sakuma I, Asahina Y *et al.* Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; **334**: 77–81.
18 Nakano I, Fukuda Y, Katano Y, Nakano S, Kumada T, Hayakawa T. Why is the interferon sensitivity-determining region (ISDR) system useful in Japan? *J Hepatol* 1999; **30**: 1014–22.
19 Pascu M, Martus P, Höhne M *et al.* Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. *Gut* 2004; **53**: 1345–51.
20 Yen YH, Hung CH, Hu TH *et al.* Mutations in the interferon sensitivity-determining region (nonstructural 5A amino acid 2209-2248) in patients with hepatitis C-1b infection and correlating response to combined therapy of pegylated interferon and ribavirin. *Aliment Pharmacol Ther* 2008; **27**: 72–9.
21 Muñoz de Rueda P, Casado J, Patón R *et al.* Mutations in E2-PePHD, NS5A-PKRBD, NS5A-ISDR, and NS5A-V3 of hepatitis C virus genotype 1 and their relationships to pegylated interferon-ribavirin treatment responses. *J Virol* 2008; **82**: 6644–53.
22 Gale M Jr, Blakely CM, Kwieciszewski B *et al.* Control of PKR protein kinase by hepatitis C virus nonstructural 5A protein: molecular mechanisms of kinase regulation. *Mol Cell Biol* 1998; **18**: 5208–18.
23 Sáiz JC, López-Labrador FX, Ampurdanés S *et al.* The prognostic relevance of the nonstructural 5A gene interferon sensitivity determining region is different in infections with genotype 1b and 3a isolates of hepatitis C virus. *J Infect Dis* 1998; **177**: 839–47.
24 Murakami T, Enomoto N, Kurosaki M, Izumi N, Marumo F, Sato C. Mutations in nonstructural protein 5A gene and response to interferon in hepatitis C virus genotype 2 infection. *Hepatology* 1999; **30**: 1045–53.
25 Sarrazin C, Kornetzky I, Rüster B *et al.* Mutations within the E2 and NS5A protein in patients infected with hepatitis C virus type 3a and correlation with treatment response. *Hepatology* 2000; **31**: 1360–70.
26 Dal Pero F, Tang KH, Gerotto M *et al.* Impact of NS5A sequences of Hepatitis C virus genotype 1a on early viral kinetics during treatment with peginterferon- alpha 2a plus ribavirin. *J Infect Dis* 2007; **196**: 998–1005.
27 Nagase Y, Yotsuyanagi H, Okuse C *et al.* Effect of treatment with interferon alpha-2b and ribavirin in patients infected with genotype 2 hepatitis C virus. *Hepatol Res* 2008; **38**: 252–8.
28 Hayashi K, Katano Y, Honda T *et al.* Mutations in the interferon sensitivity-determining region of hepatitis C virus genotype 2a correlate with response to pegylated-interferon-alpha 2a monotherapy. *J Med Virol* 2009; **81**: 459–66.
29 Ge D, Fellay J, Thompson AJ *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399–401.
30 Otagiri H, Fukuda Y, Nakano I *et al.* Evaluation of a new assay for hepatitis C virus genotyping and viral load determination in patients with chronic hepatitis C. *J Virol Methods* 2002; **103**: 137–43.
31 Hayashi K, Fukuda Y, Nakano I *et al.* Prevalence and characterization of hepatitis C virus genotype 4 in Japanese hepatitis C carriers. *Hepatol Res* 2003; **25**: 409–14.
32 Simmonds P, Bukh J, Combet C *et al.* Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology* 2005; **42**: 962–73.
33 Mangia A, Thompson AJ, Santoro R *et al.* An IL28B polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. *Gastroenterology* 2010; **139**: 821–7.

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34 Akuta N, Suzuki F, Seko Y *et al.* Association of IL28B genotype and viral response of hepatitis C virus genotype 2 to interferon plus ribavirin combination therapy. *J Med Virol* 2012; **84**: 1593–9.
35 Akuta N, Suzuki F, Sezaki H *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; **48**: 372–80.
36 Watanabe K, Yoshioka K, Yano M *et al.* Mutations in the nonstructural region 5B of hepatitis C virus genotype 1b: their relation to viral load, response to interferon, and the nonstructural region 5A. *J Med Virol* 2005; **75**: 504–12.
37 Katano Y, Hayashi K, Ishigami M *et al.* Association with 5′-untranslated region and response to interferon in chronic hepatitis C. *Hepatogastroenterology* 2007; **54**: 854–7.
38 El-Shamy A, Nagano-Fujii M, Sasase N *et al.* Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. *Hepatology* 2008; **48**: 38–47.
39 Layden-Almer JE, Kuiken C, Ribeiro RM *et al.* Hepatitis C virus genotype 1a NS5A pretreatment sequence variation and viral kinetics in African American and white patients. *J Infect Dis* 2005; **192**: 1078–87.
40 Jenke AC, Moser S, Orth V, Zilbauer M, Gerner P, Wirth S. Mutation frequency of NS5A in patients vertically infected with HCV genotype 1 predicts sustained virological response to peginterferon alfa-2b and ribavirin combination therapy. *J Viral Hepat* 2009; **16**: 853–9.

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RESEARCH ARTICLE Open Access

Significance of a reduction in HCV RNA levels at 4 and 12 weeks in patients infected with HCV genotype 1b for the prediction of the outcome of combination therapy with peginterferon and ribavirin

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Abstract

Background: The importance of the reduction in hepatitis C virus (HCV) RNA levels 4 and 12 weeks after starting peginterferon (PEG-IFN) and ribavirin combination therapy has been reported to predict a sustained virologic response (SVR) in patients infected with HCV genotype 1. We conducted a multicenter study to validate this importance along with baseline predictive factors in this patient subpopulation.

Methods: A total of 516 patients with HCV genotype 1 and pretreatment HCV RNA levels ≥5.0 $\log_{10}$ IU/mL who completed response-guided therapy according to the AASLD guidelines were enrolled. The reduction in serum HCV RNA levels 4 and 12 weeks after starting therapy was measured using real-time PCR, and its value in predicting the likelihood of SVR was evaluated.

Results: The area under the receiver operating characteristics (ROC) curve was 0.852 for 4-week reduction and 0.826 for 12-week reduction of HCV RNA levels, respectively. When the cut-off is fixed at a 2.8-$\log_{10}$ reduction at 4 weeks and a 4.9-$\log_{10}$ reduction at 12 weeks on the basis of ROC analysis, the sensitivity and specificity for SVR were 80.9% and 77.9% at 4 weeks and were 89.0% and 67.2% at 12 weeks, respectively. These variables were independent factors associated with SVR in multivariate analysis. Among 99 patients who showed a delayed virologic response and completed 72-week extended regimen, the area under ROC curve was low: 0.516 for 4-week reduction and 0.482 for 12-week reduction of HCV RNA levels, respectively.

Conclusions: The reduction in HCV RNA levels 4 and 12 weeks after starting combination therapy is a strong independent predictor for SVR overall. These variables were not useful for predicting SVR in patients who showed a slow virologic response and experienced 72-week extended regimen.

Keywords: Chronic hepatitis C, Peginterferon, Ribavirin, Reduction in HCV RNA levels, Four and twelve weeks, Baseline factors, Response-guided therapy, Extended treatment

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Background

Many investigators have sought to identify factors that can predict the treatment outcome of peginterferon (PEG-IFN) and ribavirin combination therapy in patients infected with HCV genotype 1. Previous studies reported baseline host and viral factors that are associated with the treatment outcomes. The genetic polymorphisms near the *IL28B* gene (rs12979860 or rs8099917) reportedly constitute a host factor that is strongly associated with treatment outcome [1-5], and studies from Japan have reported that amino acid substitutions at residue 70 of the HCV core region and residues 2209–2248 of the NS5A region of HCV (i.e., interferon sensitivity-determining region, ISDR) are viral factors associated with treatment outcome in patients infected with HCV genotype 1 [6-10]. In addition to the baseline predictive factors, the response to HCV during therapy, i.e., the changes in serum HCV RNA levels after initiation of therapy, has also been shown to be an important predictor of treatment outcome [11-14]. Especially, the disappearance or the reduction in serum HCV RNA levels at 4 and 12 weeks after starting therapy have been reported to be important, therefore, rapid virologic response (RVR) or early virologic response (EVR) defined at 4 and 12 weeks after starting therapy, respectively, is a pivotal criteria in predicting treatment response [11-23].

There are adverse effects associated with PEG-IFN and ribavirin antiviral therapy, and the treatment course is costly. For these reasons, it is important to predict the likelihood that a patient will achieve SVR during early stages of therapy with high reliability, in order to prevent unnecessary treatment. This will become increasingly important with the emergence of new antiviral drugs against HCV [24-28]. In the present study, we conducted a multicenter cohort study to examine whether the reduction in HCV RNA levels 4 and 12 weeks after starting PEG-IFN and ribavirin combination therapy, along with baseline predictive factors, has any value in predicting SVR.

Methods

Patients, treatments, and evaluation of responses

The inclusion criteria for this multicentre study were (i) infection with HCV genotype 1 without co-infection with hepatitis B virus or human immunodeficiency virus; (ii) pretreatment HCV RNA levels ≥5.0 $\log_{10}$ IU/mL, based on a quantitative real-time PCR-based method (COBAS AmpliPrep / COBAS TaqMan HCV Test; Roche Molecular Systems: Pleasanton, CA, US.; lower limit of quantification, 1.6 $\log_{10}$ IU/ mL: lower limit of detection, 1.2 $\log_{10}$ IU/ mL) [29,30]; (iii) standard PEG-IFN and ribavirin therapy according to the American Association for the Study of the Liver Diseases (AASLD) guidelines [31] started between December 2004 and January 2010; (iv) completed treatment regimen of 48- or 72-week duration with virologic outcomes available for evaluation; and (v) 100% medication adherence for both PEG-IFN and ribavirin during the initial 4 weeks of therapy and 80% or more throughout the treatment period. With regard to inclusion criterion (i), this study did not include any patients infected with HCV genotype 1a because this genotype is usually not found in the Japanese general population. With regard to criterion (ii), we focused on patients with pretreatment HCV RNA level ≥5.0 $\log_{10}$ IU/mL because the use of ribavirin along with PEG-IFN is not allowed by Japanese National Medical Insurance System for patients with pretreatment HCV RNA levels <5.0 $\log_{10}$ IU/mL. With regard to criterion (iv), the treatment duration was determined based on the response-guided therapy according to AASLD guidelines. Patients in whom serum HCV RNA disappeared until 12 weeks after starting therapy (complete EVR) underwent 48-week treatment regimen. Patients in whom serum HCV RNA disappeared after 12 weeks but until 24 weeks after starting therapy (delayed virologic response) underwent 72-week extended treatment regimen. Patients whose treatment was discontinued due to the presence of serum HCV RNA at 24 weeks of therapy (partial responders or null responders as per the AASLD guidelines), or due to viral breakthrough were also included in the study.

A total of 808 patients underwent the combination therapy with PEG-IFN and ribavirin between December 2004 and January 2010 in one of the following five Liver Centers: Musashino Red Cross Hospital, Kurume University Hospital, Ogaki Municipal Hospital, Shinmatsudo Central General Hospital, and Kagawa Prefectural Central Hospital. For 126 patients, the treatment regimen consisted of weekly PEG-IFN alpha-2a (Pegasys, Chugai Pharmaceutical, Tokyo, Japan) and daily ribavirin (Copegus, Chugai Pharmaceutical). The other 682 patients were treated with weekly PEG-IFN alpha-2b (Pegintron, MSD Co., Tokyo, Japan) and daily ribavirin (Rebetol, MSD Co.). We excluded patients who had been treated with PEG-IFN alpha-2a and ribavirin in order to avoid the influence of PEG-IFN subtype on the association between viral dynamics and treatment outcome. In 682 patients who received PEG-IFN alpha-2b, 516 patients fulfilled the eligibility criteria and were included for analysis (Figure 1). The doses of PEG-IFN alpfa-2b and ribavirin were adjusted based on the patient's body weight. Patients ≤ 45 kg were given 60 μg of PEG-IFN alpha-2b weekly, those > 45 kg and ≤ 60 kg were given 80 μg, those > 60 kg and ≤ 75 kg were given 100 μg, those > 75 kg and ≤ 90 kg were given 120 μg, and those > 90 kg were given 150 μg. Patients ≤ 60 kg were given 600 mg of ribavirin daily, those > 60 kg and ≤ 80 kg were given 800 mg, and those > 80 kg were given 1000 mg per

F1

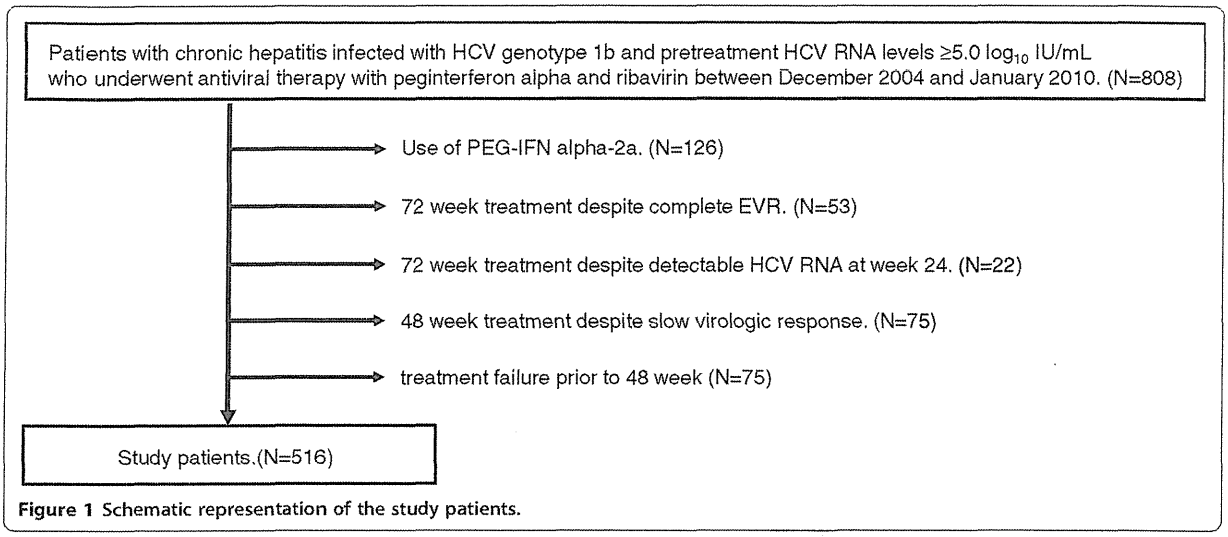

Figure 1 Schematic representation of the study patients.

day. Dose modifications of PEG-IFN or ribavirin were based on the manufacturer's recommendations.

SVR was defined as undetectable serum HCV RNA 24 weeks after the end of therapy. A patient was considered to have relapsed when serum HCV RNA levels became detectable between the end of treatment and 24 weeks after completion of therapy, although serum HCV RNA levels were undetectable at the end of therapy. A non-response was defined as detectable serum HCV RNA at 24 weeks after initiation of therapy (i.e., null response or partial non-response according to the AASLD guidelines). RVR was defined as undetectable serum HCV RNA 4 weeks after starting therapy. EVR was defined as the disappearance or a decrease in serum HCV RNA levels by at least 2 $\log_{10}$ at 12 weeks after starting therapy. Patients were considered to have a complete EVR if the serum HCV RNA levels were undetectable 12 weeks after starting therapy and a partial EVR if the serum HCV RNA levels were detectable but had decreased by at least 2 $\log_{10}$ at 12 weeks of therapy. A non-EVR was defined as a lack of a decrease of HCV RNA by more than 2 $\log_{10}$ at 12 weeks when compared to pretreatment levels. Patients were considered to have a delayed virologic response if serum HCV RNA levels became undetectable after 12 weeks but until 24 weeks on treatment.

The study protocol was in compliance with the Helsinki Declaration and was approved by the ethics committee of each participating institution, i.e., the ethics committee of Musashino Red Cross Hospital, the ethics committee of Kurume University Hospital, the ethics committee of Ogaki Municipal Hospital, the ethics committee of Shinmatsudo Central General Hospital, and the ethics committee of Kagawa Prefectural Central Hospital. Prior to initiating the study, written informed consent was obtained from each patient to use their clinical and laboratory data and to analyze stored serum samples.

Measurements of serum HCV RNA levels, amino acid substitution at residue 70 in the HCV core, amino acid sequence of HCV NS5A-ISDR, and genetic polymorphisms near the IL28B gene

After a patient gave informed consent, serum samples were obtained during the patient's regular hospital visits, just prior to beginning treatment, and every 4 weeks during the treatment period and the 24-week follow-up period after treatment. Serum samples were stored at −80°C until they were analyzed. HCV RNA levels were measured using a quantitative real-time PCR-based method (COBAS AmpliPrep/ COBAS TaqMan HCV Test) [29,30]. The reduction in HCV RNA 4 and 12 weeks after initiation of therapy was calculated. When calculating the decrease in serum HCV RNA, HCV RNA level was defined as 0 when HCV RNA was undetectable.

Amino acid 70 of the HCV core region and the amino acid sequence of ISDR region (residues 2209–2248 of the NS5A region) were analyzed by direct nucleotide sequencing of each region as previously reported [6,7]. The following PCR primer pairs were used for direct sequencing of the HCV core region:

5′-GCCATAGTGGTCTGCGGAAC-3′ (outer, sense primer),
5′-GGAGCAGTCCTTCGTGACATG-3′ (outer, antisense primer),
5′-GCTAGCCGAGTAGTGTT-3′ (inner, sense primer), and
5′-GGAGCAGTCCTTCGTGACATG-3′ (inner, antisense primer).

The following PCR primers were used for direct sequencing of ISDR:

5′-TTCCACTACGTGACGGGCAT-3′ (outer, sense primer),
5′-CCCGTCCATGTGTAGGACAT-3′ (outer, antisense primer),
5′-GGGTCACAGCTCCCTGTGAGCC-3′ (inner, sense primer), and
5′-GAGGGTTGTAATCCGGGCGTGC-3′ (inner, antisense primer).

When evaluating ISDR, HCV was defined as wild-type when there were 0 or 1 amino acid substitutions in residues 2209–2248 as compared with the HCV-J strain [32], and as non-wild-type when there was more than 1 substitutions.

Genotyping of rs 8099917 polymorphisms near the *IL28B* gene was performed using the TaqMan SNP assay (Applied Biosystems, Carlsbad, CA) according to the manufacturer's guidelines. A pre-designed and functionally tested probe was used for rs8099917 (C__11710096_10, Applied Biosystems). Genetic polymorphism of rs8099917 reportedly corresponds to rs12979860 in more than 99% of individuals of Japanese ethnicity [33]. The TT genotype of rs8099917 corresponds to the CC genotype of rs12979860, the GG genotype of rs8099917 corresponds to the TT genotype of rs12979860, and the TG heterozygous genotype of rs8099917 corresponds to the CT of rs12979860.

Statistical analyses

Quantitative values are reported as medians and ranges. Differences in percentages between groups were analyzed with the chi-square test. Differences in mean quantitative values were analyzed by the Mann–Whitney U test. The receiver-operating characteristics (ROC) analyses were performed to determine the cut-offs of the reduction in HCV RNA levels at 4 and 12 weeks after starting therapy to evaluate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for predicting SVR. Univariate and multivariate analyses using a logistic regression model were performed to identify factors that predict SVR. The factors that are potentially associated with SVR were included in the analyses, i.e., age, sex, body mass index (BMI), serum alanine aminotransferase activity, serum gamma-glutamyl transpeptidase level, total-cholesterol levels, neutrophil count, hemoglobin, platelet count, grade of activity and fibrosis of the liver, pretreatment HCV RNA levels, reduction in HCV RNA levels 4 and 12 weeks after starting therapy, amino acid substitution at residue 70 in the HCV core (arginine vs. glutamine or histidine), amino acid mutations in ISDR (non-wild-type vs. wild-type), and genetic polymorphisms near the *IL28B* gene (rs8099917, genotype TT vs. genotype TG or GG). Data analyses were performed using StatFlex statistical software, version 6 (Artech Co., Ltd., Osaka, Japan). All p values were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

Patient characteristics and treatment outcome

The characteristics of the patients are shown in Table 1. Genotyping of rs8099917 near the *IL28B* gene was performed in 396 patients. Amino acid substitutions at residue 70 in the HCV core region were measured in 361 patients. Amino acid sequences in the ISDR were evaluated in 416 patients. Among 516 patients who were included in the analysis, treatment was completed at 48 weeks in 268 patients who underwent the standard regimen because they showed complete EVR. Treatment was extended from 48 weeks to 72 weeks in 99 patients who yielded delayed virologic response. Treatment was discontinued until 48 weeks in 149 patients because

Table 1 Characteristics of study patients

Age (years), median (range)	60.0 (20.0–80.0)
Sex (male/female) (%)	245 (47.5)/ 271 (52.5)
Body weight (kg), median (range)	58.0 (36.35–107.6)
BMI, median (range)	22.7 (15.8–37.0)
Prior treatment for HCV (no/yes) (%)	359 (69.6)/ 157 (30.4)
Initial dose of PEG-IFN (μg), median (range)	80.0 (40.0–150.0)
Initial dose of ribavirin (mg), median (range)	600 (400–1000)
Pretreatment HCV RNA levels (log^{10} IU/mL), median (range)	6.1 (5.0–7.7)
Platelet count ($\times 10^3/\mu L$)	161 (43–352)
Hemoglobin (g/dL)	13.9 (9.7–17.9)
Neutrophil count (/μL)	2489 (578–7480)
Alanine aminotransferase (IU/L)	47 (10–485)
LDL-cholesterol (mg/dL)	99 (25–226)
Total-cholesterol (mg/dL)	171 (29–325)
γ-glutamyl transpeptidase (IU/L)	34.5 (7.0–579)
Alfa fetoprotein (ng/mL)	5.0 (0.8–584)
Fibrosis score (F1/F2/F3/F4) (%)	208(45.9)/139(30.7)/69(15.2)/37(8.2)
Activity score (A1/A2/A3/A4) (%)	258(56.1)/178(38.7)/24(5.2)/0(0)
Genetic polymorphisms of rs8099917 (TT/GG or TG) (%)	288 (72.7)/ 108(27.3)
Amino acid at residue 70 of HCV core (arginine/glutamine or histidine) (%)	242 (67.0)/ 119 (33.0)
Amino acid sequence of ISDR (non-wild-type/wild-type) (%)	110 (26.4)/ 306 (73.6)

BMI, body mass index; *HCV*, hepatitis C virus; *PEG-IFN*, peginterferon; *ISDR*, interferon sensitivity-determining region.
(N = 516).

serum HCV RNA remained positive 24 weeks after starting therapy (partial response or null response), or because patients experienced viral breakthrough during therapy.

As a final outcome, 272 patients (52.7%) achieved SVR, 90 patients (17.5%) relapsed, and 128 patients (24.8%) had a non-response (48 patients with partial response and 80 patients with null-response). Viral breakthrough was observed in 26 patients (5.0%). The rate of SVR was 79.9% (214 of 268 patients) among patients with complete EVR in whom treatment was completed at 48 weeks and 58.6% (58 of 99 patients) among patients with delayed virologic response who underwent the extended 72-week regimen.

Baseline factors affecting SVR in all patients who underwent response-guided therapy according to AASLD guidelines

In all patients who underwent treatment according to the AASLD guidelines, the rate of SVR was significantly higher in patients with the TT genotype of rs8099917 near the *IL28B* gene (179 of 288 patients [62.3%] with TT genotype vs. 15 of 108 patients [13.9%] with TG/GG genotype, $p < 0.0001$). In addition, SVR rate was significantly higher in patients with HCV with arginine at residue 70 in the HCV core region (145 of 242 patients [59.9%] with arginine vs. 34 of 119 patients [28.6%] with glutamine or histidine, $p < 0.0001$). SVR was significantly higher in patients with HCV with non-wild type ISDR (75 of 110 patients [68.2%] with non-wild-type ISDR vs. 139 of 306 patients [45.4%] with wild-type ISDR, $p < 0.0001$). SVR was significantly higher in patients with pretreatment HCV RNA levels <6.0 $\log_{10}$ IU/mL (127 of 199 patients [63.8%] with pretreatment HCV levels <6.0 $\log_{10}$ IU/mL vs. 145 of 317 patients [45.7%] with pretreatment HCV RNA levels ≥6.0 $\log_{10}$ IU/mL, $p < 0.0001$).

Association between reduction of serum HCV RNA levels 4 and 12 weeks after starting therapy and SVR in all patients who underwent response-guided therapy according to the AASLD guidelines

The ROC analysis was performed in 516 patients who underwent the response-guided therapy according to the AASLD guidelines in order to evaluate the association between the reduction in serum HCV RNA levels 4 and 12 weeks after starting therapy and SVR (Figure 2). The area under the ROC curve was 0.852 and the best cut-off was calculated as 2.8 $\log_{10}$ IU/mL, when evaluated with the reduction of serum HCV RNA levels 4 weeks after starting therapy. The rate of SVR was significantly higher in patients with greater than 2.8-$\log_{10}$ reduction at 4 weeks (220 of 274 patients [80.3%] with > 2.8-$\log_{10}$ reduction vs. 52 of 242 patients [21.5%] with ≤ 2.8-$\log_{10}$ reduction, $p < 0.0001$). The sensitivity, specificity, PPV, NPV, and accuracy were 80.9%, 77.9%, 80.3%, 78.5%, and 79.5%, respectively, at this cut-off level. When evaluated with the reduction of serum HCV RNA levels 12 weeks after starting therapy, the area under the ROC curve was

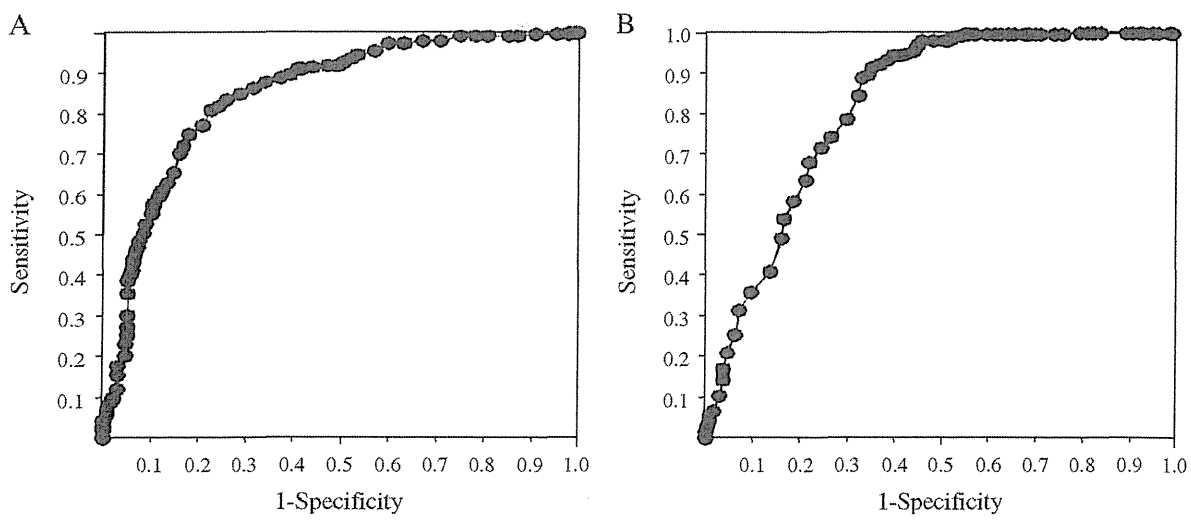

Figure 2 The receiver operating characteristics (ROC) analysis for the prediction of the sustained virologic response to combination therapy with peginterferon alpha-2b and ribavirin according to the reduction in serum HCV RNA levels in all patients who underwent response-guided therapy based on the AASLD guidelines. **A)** According to the reduction in serum HCV RNA levels 4 weeks after starting therapy. The area under the ROC curve was 0.852. **B)** According to the reduction in serum HCV RNA levels 12 weeks after starting therapy. The area under the ROC curve was 0.826.

0.826 and the best cut-off was calculated as 4.9 $\log_{10}$ IU/mL. The rate of SVR was significantly higher in patients with greater than 4.9-$\log_{10}$ reduction at 12 weeks (242 of 321 patients [75.4%] with > 4.9-$\log_{10}$ reduction vs. 30 of 194 patients [15.5%] with ≤ 4.9-$\log_{10}$ reduction, $p < 0.0001$). The sensitivity, specificity, PPV, NPV, and accuracy were 89.0%, 67.2%, 75.4%, 84.5%, and 78.7%, respectively, at this cut-off level.

A multivariate analysis showed that the reductions in serum HCV RNA levels at 4 and 12 weeks after starting therapy were independent factors associated with SVR, along with pretreatment HCV RNA levels, platelet counts, polymorphisms of rs8099917 near the *IL28B* gene, and amino acid mutations in the HCV NS5A-ISDR (Table 2). T2

Association between reduction of serum HCV RNA levels 4 and 12 weeks after starting therapy and SVR in patients with delayed virologic response who underwent an extended 72-week regimen according to response-guided therapy

The ROC analysis was performed in 99 patients with delayed virologic response who underwent an extended 72-week treatment regimen according to the response-guided therapy of the AASLD guidelines to evaluate the association between reduction in serum HCV RNA levels 4 and 12 weeks after starting therapy and SVR (Figure 3). The area under the ROC curve was 0.516 and the best cut-off was calculated as 2.3 $\log_{10}$ IU/mL, when evaluated with the reduction of serum HCV RNA levels 4 weeks after starting therapy. There was no significant difference in the rate of SVR according to the reduction at 4 weeks (21 of 33 patients [63.6%] with > 2.3-$\log_{10}$ reduction vs. 37 of 66 patients [56.1%] with ≤ 2.3-$\log_{10}$ reduction, $p = 0.6120$). The area under the ROC curve was 0.482 and the best cut-off was calculated as 5.1 $\log_{10}$ IU/mL, when evaluated with the reduction of serum HCV RNA levels 12 weeks after starting therapy. There was no significant difference in the rate of SVR according to the reduction at 12 weeks (24 of 42 patients [57.1%] with > 5.1-$\log_{10}$ reduction vs. 34 of 57 patients [59.6%] with ≤ 5.1-$\log_{10}$ reduction, $p = 0.9634$). F3

Discussion

Several previous studies have reported that patients who achieved RVR, in whom serum HCV RNA levels become undetectable 4 weeks after starting the therapy, had a high likelihood of achieving SVR [15-18]. However, there are relatively few patients infected with treatment-resistant HCV genotype 1 who achieve RVR. A considerable percentage of patients achieve SVR even without RVR. Therefore, RVR has high specificity but low

Table 2 Univariate and multivariate analyses for sustained virologic response to the combination therapy with peginterferon and ribavirin in patients who underwent response guided therapy according to the AASLD guidelines

	Univariate analysis	Multivariate analysis*	Odds ratio (95% confidence interval)
Age (years)	< 0.001	N.S.	
Sex (male/female)	0.005	N.S.	
BMI, median (range)	N.S.		
Prior treatment for HCV (no/yes)	N.S.		
Pretreatment HCV RNA levels ($\log_{10}$ IU/mL), (≤6.0 vs. 6.0<)	0.015	0.013	2.235 (1.189-4.203)
Platelet count (×10^3/μL)	< 0.001	0.011	1.007 (1.002-1.013)
Hemoglobin (g/dL)	0.002	N.S.	
Neutrophil count (/μL)	0.003	N.S.	
Alanine aminotransferase (IU/L)	N.S.		
Total-cholesterol (mg/dL)	0.001	N.S.	
γ-glutamyl transpeptidase (IU/L)	0.014	N.S.	
Fibrosis score (F1 or F2/F3 or F4)	< 0.001	N.S.	
Activity score (A1 or A2/A3 or A4)	0.002	N.S.	
Genetic polymorphisms of rs8099917 (TT/GG or TG)	< 0.001	< 0.001	5.782 (2.298-14.552)
Amino acid at residue 70 of HCV core (arginine/glutamine or histidine)	< 0.001	N.S.	
Amino acid sequence of ISDR (non-wild-type/wild-type)	< 0.001	0.038	2.077 (1.041-4.147)
Reduction of HCV RNA [Pre - 4 week] ($\log_{10}$ IU/mL), (≤2.8 vs. 2.8<)	< 0.001	< 0.001	3.911 (1.935-7.908)
Reduction of HCV RNA [Pre - 12 week] ($\log_{10}$ IU/mL), (≤4.9 vs. 4.9<)	< 0.001	0.013	2.578 (1.220-5.448)

*Multivariate analysis was performed on 314 patients in whom all variables were available. (N = 516).

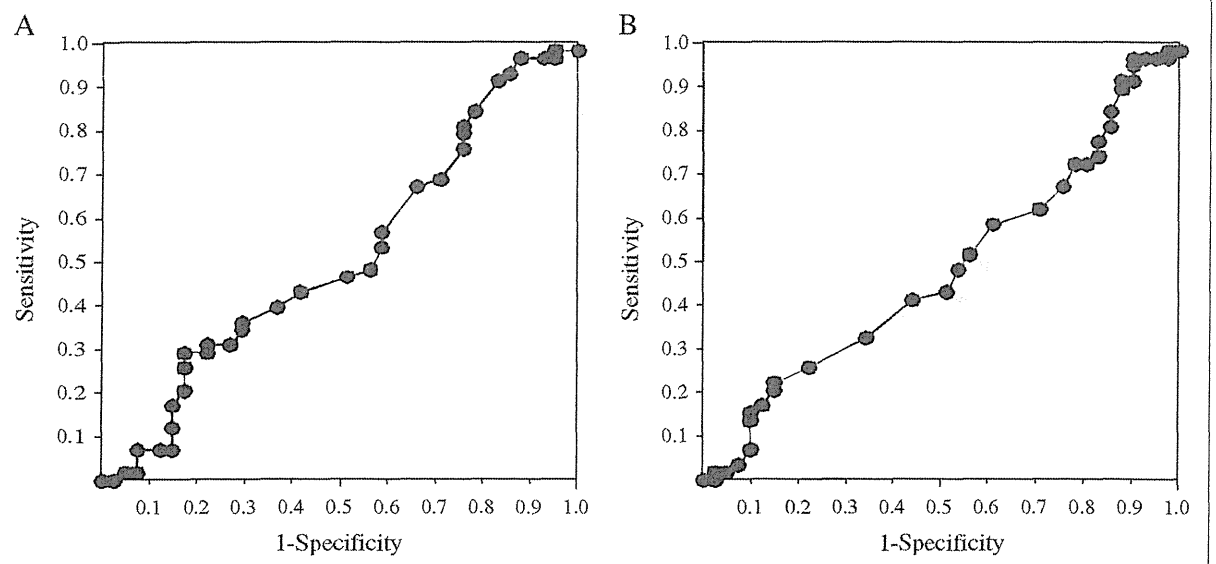

Figure 3 The receiver operating characteristics (ROC) analysis for the prediction of the sustained virologic response to combination therapy with peginterferon alpha-2b and ribavirin according to the reduction in serum HCV RNA levels in patients with delayed virologic response who underwent an extended 72-week regimen according to response-guided therapy. **A)** According to the reduction in serum HCV RNA levels 4 weeks after starting therapy. The area under the ROC curve was 0.516. **B)** According to the reduction in serum HCV RNA levels 12 weeks after starting therapy. The area under the ROC curve was 0.482.

sensitivity for predicting SVR. Previous studies from Asia evaluated the predictive value of the degree of reduction in serum HCV RNA levels 4 weeks after starting therapy, in addition to RVR [19-21]. However, the number of patients in these studies was small and the analyses were not sufficient to form reliable conclusions.

In the present study, we evaluated the ability of a decrease in serum HCV RNA levels 4 weeks after starting therapy to predict the likelihood of SVR as a final outcome in Japanese patients infected with HCV genotype 1b, based on the data from a large, multi-institution study. The ROC analyses showed that a reduction in serum HCV RNA levels 4 week after starting therapy was strongly associated with SVR, and its predictive value was higher than that of a reduction in serum HCV RNA levels 12 weeks after starting therapy, with higher area under the ROC curve and accuracy. Multivariate analyses including baseline factors that were associated with SVR revealed that the reductions of HCV RNA level at both 4 and 12 weeks after starting therapy were independent factors associated with SVR, and the reduction at 4 weeks had a second strongest impact for SVR, following genetic polymorphisms of rs8099917 near *IL28B* gene.

The important novelty from this study is that the reductions of HCV RNA level 4 and 12 weeks after starting therapy had no predictive value for SVR when focusing on patients who showed delayed virologic response and underwent the extended 72-week treatment regimen according to the response-guided therapy. This was in contrast to the prediction for SVR in all patients who underwent response-guided therapy. The impact of the reduction of HCV RNA level on the prediction of SVR would decline by the selection of patients based on the delayed virologic response. There were also no baseline factors that were associated with SVR in patients who underwent the extended 72-week treatment (data not shown). Prolonged treatment duration may relieve delayed virologic responders from unfavorable conditions. Further studies will be, therefore, needed to identify predictive factors for SVR in patients with delayed virologic response who underwent the 72-week treatment regimen.

There are several limitations to this study. The data were based on Japanese patients infected with HCV genotype 1b. Therefore, these results should be confirmed in patients of other ethnicities and patients infected with HCV genotype 1a. In addition, the value of the reduction in HCV RNA levels 4 and 12 weeks after starting therapy as predictors of SVR should be evaluated in patients who underwent therapy with PEG-IFN alpha 2a and ribavirin to determine the best cut-off levels with that regimen. Statistically, there were many missing data. We performed complete case analysis without the imputation of missing data for multivariate analysis. Although comparison between cases with and without missing data did not show statistically significant differences for cases characteristics, we cannot rule

out that the condition of data missing completely at random does not hold. Furthermore, this resulted in the decrease in the number of patients analyzed in multivariate analysis and might have substantially caused the reduction of statistical power, altering the value of non-significant results. In addition, the study did not perform internal validation. The use of hold-out method or split-group validation was difficult because of the number of study patients. Therefore, the validation in another larger study patients will be required in the future for confirming the results of this study.

Conclusions

A reduction in HCV RNA levels 4 and 12 weeks after starting therapy indicated likelihoods that patients will achieve SVR as a final outcome of combination therapy for HCV infection when patients underwent the response-guided therapy according to the AASLD guidelines. These reductions in serum HCV RNA levels were not predictive for SVR when focusing on patients who showed delayed virologic response and underwent the extended 72-week regimen.

Abbreviations

HCV: Hepatitis C virus; PEG-IFN: Peginterferon; SVR: Sustained virologic response; ROC: Receiver operating characteristics; ISDR: Interferon sensitivity-determining region; RVR: Rapid virologic response; EVR: Early virologic response; AASLD: American Association for the Study of the Liver Diseases; BMI: Body mass index; PPV: Positive predictive value; NPV: Negative predictive value.

Competing interests

The authors declare the following matters.
The authors have not received reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, neither now nor in the future.
The authors have no stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, neither now nor in the future.
The authors are currently applying no patents relating to the content of the manuscript. We have not received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript.
The authors do not have any other financial competing interests.
There are no non-financial competing interests to declare in relation to this manuscript.
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Authors' contributions

Study design: HT, TK, NS, KT, TI, MS, HG, KM, and NI. Treatment of patients and data acquisition: HT, TK, NS, KT, TI, MS, and NI. Data analyses: HG and KM. Manuscript preparation: HT. Read and approval of the final manuscript: HT, TK, NS, KT, TI, MS, HG, KM, and NI. All authors read and approved the final manuscript.

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References

1. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB: Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009, 461:399–401.
2. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J: IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009, 41:1100–1104.
3. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M: Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009, 41:1105–1109.
4. McCarthy JJ, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, Tillmann HL, Muir AJ, McHutchison JG: Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. *Gastroenterology* 2010, 138:2307–2314.
5. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY, Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study: Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010, 138:1338–1345.
6. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996, 334:77–81.
7. Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Association of amino acid substitution pattern in core protein of hepatitis C virus genotype1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005, 48:372–380.
8. Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, Belle SH, Di Bisceglie AM, Aurora R, Tavis JE: Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy. *J Virol* 2007, 81:8211–8224.
9. Maekawa S, Enomoto N: Viral factors influencing the response to the combination therapy of peginterferon plus ribavirin in chronic hepatitis C. *J Gastroenterol* 2009, 44:1009–1015.
10. Hayes NC, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, Miki D, Imamura M, Ochi H, Kamatani N, Nakamura Y, Chayama K: HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy. *Gut* 2011, 60:261–267.
11. Zeuzem S, Herrmann E, Lee JH, Fricke J, Neumann AU, Modi M, Colucci G, Roth WK: Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon alpha2a. *Gastroenterology* 2001, 120:1438–1447.
12. Buti M, Sanchez-Avila F, Lurie Y, Stalgis C, Valdes A, Martell M, Esteban R: Viral kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon alfa-2b plus ribavirin. *Hepatology* 2002, 35:930–936.
13. Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R, Wiedenmann B, Hopf U, Zeuzem S: Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. *Hepatology* 2003, 37:600–609.
14. Lee SS, Ferenci P: Optimizing outcomes in patients with hepatitis C virus genotype 1 or 4. *Antiviral Res* 2008, 13:S9–S16.
15. Martinez-Bauer E, Crespo J, Romero-Gomez M, Moreno-Otero R, Sola R, Tesei N, Pons F, Forns X, Sanchez-Tapias JM: Development and validation

of two models for early prediction of response to therapy in genotype 1 chronic hepatitis C. *Hepatology* 2006, 43:72–80.

16. Poordad F, Reddy KR, Martin P: Rapid virologic response: a new milestone in the management of chronic hepatitis C. *Clin Infect Dis* 2008, 46:78–84.
17. Martinot-Peignoux M, Maylin S, Moucari R, Ripault M-P, Boyer N, Cardoso A-C, Giuily M, Castelnau C, Pouteau M, Stern C, Auperin A, Bedossa P, Asselah T, Marcellin P: Virological response at 4 weeks to predict outcome of hepatitis C treatment with pegylated interferon and ribavirin. *Antivir Ther* 2009, 14:501–511.
18. de Segadas-Soares JA, Villela-Nogueira CA, Perez RM, Nabuco LC, Brandao-Mello CE, Coelho HSM: Is the rapid virologic response a positive predictive factor of sustained virologic response in all pretreatment status genotype 1 hepatitis C patients treated with peginterferon-α2b and ribavirin? *J Clin Gastroenterol* 2009, 43:362–366.
19. Yu JW, Wang GQ, Sun LJ, Li XG, Li SC: Predictive value of rapid virological response and early virological response on sustained viroogical response in HCV patients treated with pegylated interferon α-2a and ribavirin. *J Gastroenterol Hepatol* 2007, 22:832–836.
20. Huang C-F, Yang J-F, Huang J-F, Dai C-Y, Chiu C-F, Hou N-J, Hsieh M-Y, Lin Z-Y, Chen S-C, Hsieh M-Y, Wang L-Y, Chang W-Y, Chuang W-L, Yu M-L: Early identification of achieving a sustained virological response in chronic hepatitis C patients without a rapid virological response. *J Gastroenterol Hepatol* 2010, 25:758–765.
21. Toyoda H, Kumada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Tada T, Arakawa T, Fujimori M, Niinomi T, Ando N, Yasuda S, Sakai K, Kimura J: High ability to predict the treatment outcome of peginterferon and ribavirin combination therapy based on the reduction in HCV RNA levels at 4 weeks after starting therapy and amino acid substitutions in hepatitis C virus in patients infected with HCV genotype 1b. *J Gastroenterol* 2011, 46:501–509.
22. Marcellin P, Reau N, Ferenci P, Hadziyannis S, Messinger D, Tatsch F, Jensen D: Refined prediction of week 12 response and SVR based on week 4 response in HCV genotype 1 patients treated with peginterferon alfa-2a (40KD) and ribavirin. *J Hepatol* 2012, 56:1276–1282.
23. Toyoda H, Kumada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Tada T, Hosokawa T, Arakawa T, Fujimori M: Outcome in partial early virologic responders to combination therapy with peginterferon and ribavirin in patients infected with hepatitis C virus genotype 1b. *J Med Virol* 2011, 83:101–107.
24. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S, ADVANCE Study Team: Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011, 364:2405–2416.
25. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M, REALIZE Study Team: Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011, 364:2417–2428.
26. Poordad F, McCone J Jr, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP: SPRINT-2 Investigators. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011, 364:1195–1206.
27. Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R: HCV RESPOND-2 Investigators. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011, 364:1207–1217.
28. Lok AS, Gardiner DF, Lawitz E, Martorell C, Everson GT, Ghalib R, Reindollar R, Rustgi V, McPhee F, Wind-Rotolo M, Persson A, Zhu K, Dimitrova DI, Eley T, Guo T, Grasela DM, Pasquinelli C: Preliminary study of two antiviral agents for hepatitis C genotype 1. *N Engl J Med* 2012, 366:216–224.
29. Colucci G, Ferguson J, Harkleroad C, Lee S, Romo D, Soviero S, Thompson J, Velez M, Wang A, Miyahara Y, Young S, Sarrazin C: Improved COBAS TaqMan hepatitis C virus test (version 2.0) for use with the High Pure system: enhanced genotype inclusivity and performance characteristics in a multisite study. *J Clin Microbiol* 2007, 45:3595–3600.
30. Pittaluga F, Allice T, Abate ML, Ciancio A, Cerutti F, Varetto S, Colucci G, Smedile A, Ghisetti V: Clinical evaluation of the COBAS Ampliprep/COBAS TaqMan for HCV RNA quantitation in comparison with the branch-DNA assay. *J Med Virol* 2008, 80:254–260.
31. Ghany MG, Strader DB, Thomas DL, Seeff LB: Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009, 49:1335–1374.
32. Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K: Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. *Proc Natl Acad Sci USA* 1990, 87:9524–9528.
33. Tanaka Y, Nishida N, Sugiyama M, Tokunaga K, Mizokami M: λ-interferons and the single nucleotide polymorphisms: a milestone to tailor-made therapy for chronic hepatitis C. *Hepatol Res* 2010, 40:449–460.

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