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Prediction of a Null Response to Pegylated Interferon α-2b Plus Ribavirin in Patients with High Viral Load Genotype 1b Hepatitis C

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See editorial on page 335.

Background/Aims: The present study aimed to clarify whether virological response within 2 weeks after therapy initiation can predict a null response to pegylated interferon α-2b plus ribavirin therapy in patients with high viral load genotype 1b hepatitis C. **Methods:** The participants consisted of 72 patients with high viral load genotype 1b. The dynamics of viral load within 2 weeks were measured. **Results:** Significant differences between null responders and nonnull responders were noted for interleukin (IL)-28B genotype, amino acid 70 substitution, α-fetoprotein, low-density lipoprotein cholesterol, hyaluronic acid, and viral response. The area under the curve (AUC) for the receiver operating characteristic curve of the hepatitis C virus (HCV) RNA level decline at 2 weeks (AUC=0.993) was the highest among the factors predicting the null response. When the cutoff value for the HCV RNA level decline at 2 weeks was set at 0.80 log, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy in predicting a null response were 82%, 96%, 82%, 96%, and 94%, respectively. In comparison, values for the non-TT and mutant type of amino acid 70 substitution were similar to those for HCV RNA level decline at 2 weeks. **Conclusions:** Virological response at 2 weeks or the combination of IL-28B and amino acid 70 substitution are accurate predictors of a null response. **(Gut Liver 2014;8:421-427)**

Key Words: Hepatitis C virus; Peginterferon alfa-2b; Ribavirin; Single nucleotide polymorphisms; Amino acid substitution

INTRODUCTION

The aim of treatment for patients with hepatitis C virus (HCV) infection is to prevent progression to cirrhosis or hepatocellular carcinoma. Spontaneous viral elimination by interferon therapy can reduce the risk of carcinogenesis[1-3] and resolve histological fibrosis.[4] Although sustained virolgical response (SVR) rates improve with combining pegylated interferon (PEG-IFN) plus ribavirin, SVR rates in patients with high viral load genotype 1 HCV infection still range from 40% to 50%.[5]

Currently, some new direct antiviral agents (DAAs) against HCV have been developed. The first-generation protease inhibitor telaprevir became available for clinical use.[6] Triple therapy with telaprevir, PEG-IFN, and ribavirin have improved SVR rates to around 70%.[7] However, the triple therapy can produce some severe adverse reactions, including serious skin disorders and exacerbation of anemia, compared with PEG-IFN plus ribavirin therapy.[8] Furthermore, the safety of triple therapy including telaprevir in elderly or cirrhotic patients has not been established.[5] Accordingly, the standard PEG-IFN plus ribavirin combination therapy for patients with high viral load genotype 1 HCV infection is still necessary for patients at high risk for adverse reactions, such as elderly and/or cirrhotic patients.

In guidelines for the management for hepatitis C, response-guided therapy on the basis of prediction of therapeutic efficacy using response to treatment is recommended.[5,9] The current guidelines recommend that discontinuation of treatment should be considered in null responders, whose serum HCV RNA level does not decrease to <2 logIU/mL at week 12 after therapy initiation, because in such patients HCV clearance cannot be expected.[5,9,10] Therefore, if null responders can be accurately predicted before treatment or as soon as possible after treatment,

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unnecessary and expensive antiviral therapy can be avoided. Furthermore, as SVR rates are still low after triple therapy in patients who were nonresponders to previous PEG-IFN plus ribavirin combination therapy,[6] accurate prediction of null response to PEG-IFN plus ribavirin combination therapy is important in considering treatment strategies for elderly/cirrhotic patients.

Recently, the combinations of interleukin (IL)-28B single nucleotide polymorphism (SNP), substitutions of amino acids in the HCV core region, and nonstructural 5A (NS5A) mutations have become recognized as pretreatment predictor of the therapeutic efficacy of PEG-IFN plus ribavirin combination therapy. The Japanese guidelines recommend that if IL-28B SNP minor alleles and amino acid 70 substitution in the HCV core region have been detected in elderly patients, careful follow-up with no antiviral therapy is an option until the next DAAs become available.[5] However, these tests, which are expensive, have not yet been approved by the national medical insurance in Japan. The present study sought to determine whether null responders can be predicted on the basis of virological response within 2 weeks of therapy initiation. Furthermore, the predictability of null response using viral response within 2 weeks was compared to that using IL-28B SNP, NS5A mutations, and amino acid substitution in the HCV core region.

MATERIALS AND METHODS

1. Patients

This was a prospective multicenter cohort study of PEG-IFN-α-2b plus ribavirin therapy for patients with high viral load HCV genotype 1 (more than 5.0 logIU/mL). A total of 72 chronic hepatitis C patients with high viral load genotype 1 were enrolled from March 2008 to December 2011 in the Wakayama Medical University Hospital, Naga Municipal Hospital, and Saiseikai Wakayama Hospital. Exclusion criteria were 1) pregnant women, women who may have been pregnant, lactating women, men whose partners were pregnant, or men whose partners hoped to become pregnant; 2) patients who used sho-saiko-to (a Kampo medicine); 3) intractable heart failure; 4) renal failure or renal dysfunction with creatinine clearance <50 mL/min; 5) patients with uncontrollable psychoneurotic disorders; 6) hemoglobin (Hb) level <12 g/dL; 7) platelet count <70,000/mm^3; 8) white blood cell count <1,500/mm^3; and 9) hepatic failure or cancer. All study protocols were approved by the ethics committees of the participating hospitals.

2. Treatment regimens

Standard doses of PEG-IFN-α-2b (Peg-Intron®; MSD, Tokyo, Japan) and ribavirin (Rebetol®; MSD) were used; 1.5 μg/kg PEG-IFN-α-2b was administered subcutaneously once a week, and ribavirin was given orally for 48 weeks (1,000 mg/day for patients weighing greater than 80 kg; 800 mg/day for patients weighing between 80 and 60 kg; and 600 mg/day for patients weighing less than 60 kg). When serum negativity of HCV RNA was achieved from 13 to 36 weeks, treatment duration was prolonged to 72 weeks following the Japanese guidelines for the management of HCV infection.[5]

The PEG-IFN-α-2b and ribavirin doses were reduced or discontinued based on the following criteria: 1) if the Hb fell below 10 g/dL, the ribavirin dose was reduced (to 800 from 1,000 mg/day, to 600 from 800 mg/day, or to 400 from 600 mg/day), and if the Hb fell below 8.5 g/dL, the ribavirin was discontinued; 2) if the granulocyte count fell below 750/mm^3 or the platelet count fell below 70,000/mm^3, the PEG-IFN dose was reduced to half of the initial dose; 3) if the granulocyte count fell below 500/mm^3 or the platelet count fell below 30,000/mm^3, the PEG-IFN was discontinued; and 4) PEG-IFN and ribavirin were discontinued if deemed necessary by the attending physician because of adverse events. The dose of PEG-IFN or ribavirin was increased back to the starting dose if the cytopenia improved. If there was no improvement in hematological parameters within 4 weeks, the therapy was discontinued.

3. Laboratory tests and ultrasounds

In all patients, laboratory tests and ultrasounds were performed before therapy. Fatty liver was defined as positive hepatorenal contrast on ultrasound. The amount of HCV RNA was measured using quantitative real-time polymerase chain reaction (RT-PCR) (COBAS® TaqMan® PCR assay; Roche Diagnostics, Branchburg, NJ, USA). A high viral load was defined as more than 5.0 logIU/mL using quantitative RT-PCR. Determination of HCV genotype was performed by Simmonds *et al*.[11]: the levels of HCV RNA and HCV core antigen (Ortho Clinical Diagnostics, Tokyo, Japan) were measured simultaneously at four time points (day of therapy initiation, the following day, and at weeks 1 and 2). NS5A mutations in the IFN sensitivity-determining region (ISDR)[12] and amino acid 70 and 91 substitutions in the HCV core regions[13] were also measured on the day of therapy initiation. ISDR was defined as wild type if no mutations were identified, and all others were defined as nonwild type. At the core 70 region, arginine was defined as the wild type, and glutamine or histidine as mutant types. At the core 91 region, leucine was defined as the wild type, and methionine as the mutant type. Serum ribavirin concentration was measured at week 2. After treatment, a SNP of the IL-28B host genotype (rs8099917), which has been reported as a pretreatment predictor for the efficacy of PEG-IFN plus ribavirin therapy in Japanese patients,[14] was also evaluated after written informed consent for genome analysis was obtained from each patient. Homozygosity for the major allele (T/T) was defined as the IL-28B major type, and heterozygosity (T/G) or homozygosity for the minor allele (G/G) was defined as the IL-28B minor type.

4. Liver histology

In all patients, core needle biopsy of the liver was performed

under ultrasound guidance using a 16-gauge core biopsy needle (Monopty®; Bard, Covington, GA, USA) within 3 months before the start of therapy. Histological findings were classified using the Metavir scoring system based on activity (grades A0, A1, A2, and A3) and fibrosis (stages F0, F1, F2, F3, and F4).[15]

5. Assessment of effectiveness

Null response was defined as serum HCV RNA decrease <2 logIU/mL at week 12 after initiation of therapy. SVR was defined as serum HCV RNA undetectable at 24 weeks after completion of treatment. Relapse was defined as reappearance of HCV RNA following treatment, having been undetectable during treatment. The virological response within 2 weeks after therapy initiation was assessed by viral level and viral depletion from baseline viral load at each time point.

6. Statistical analysis

Therapeutic effectiveness was evaluated using an intention-to-treat analysis. Predictive factors for null response were analyzed using a per protocol analysis that excluded patients who had discontinued therapy due to adverse events. The Mann-Whitney U-test was used to analyze continuous variables. Fisher exact test or the chi-square test was used to analyze categorical variables. Each optimal cutoff value for continuous variables of null response predicting factors was decided by the Youden Index method on the basis of the receiver operating characteristic curve. The predictability of significant null response contributing factors was evaluated by measuring the area under the curve (AUC). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy for null response according to significant predictive factors were calculated. Values of $p<0.05$ were considered statistically significant. SPSS statistical software version 20.0J for Windows (SPSS Inc., Tokyo, Japan) was used for data analysis.

RESULTS

1. Patients' baseline characteristics

The patients' baseline characteristics are summarized in Table 1. Their mean age was 54 years (range, 19 to 70 years); four patients (6%) were aged ≥65 years. Twenty-eight patients (39%) had advanced fibrosis (Metavir stages F3-F4). Sixteen patients (22%) had undergone prior interferon therapy. Five of those 16 patients had undergone interferon plus ribavirin therapy.

2. Treatment response and drug adherence

Of the 72 patients in the present study, six patients discontinued the therapy due to adverse effects. The reasons were depression (n=1), fundal hemorrhage with visual field defect (n=1), severe granulocytopenia (n=1), interstitial pneumonia (n=1), severe dermatitis (n=1), and hepatocellular carcinoma (n=1). SVR was achieved in 53% (38/72), and relapse occurred in 19% (14/72). Null response was identified in 15% (11/72) patients. The mean adherence rates (±SD) to both PEG-IFN-α-2b and ribavirin therapy among the 72 patients who were enrolled in the present study were 94%±17% and 92%±17%, respectively.

3. Contributing factors for null response and predictability of null response

Of the 66 patients who did not discontinue therapy due to adverse effects, 11 null responders and 55 nonnull responders were identified. The comparison of the factors between the patients with and without null response is shown in Table 2. Significant differences were noted between the null responders and the nonnull responders with respect to α-fetoprotein (AFP), hyaluronic acid, low-density lipoprotein (LDL)-cholesterol, IL-28B host genotype, and amino acid 70 substitution in the core region. Significant differences between the two groups were also seen in the mean HCV core antigen and HCV RNA levels during the two weeks after therapy initiation. The kinetics of the HCV core antigen and the HCV RNA levels according to the response within 2 weeks after therapy initiation are shown in Fig. 1.

The AUCs according to significant contributing factors for null response are summarized in Table 3. The highest AUC for

Table 1. Patient Characteristics (n=72)

Characteristic	Value
Age, yr	54±11
Sex, male/female	42/30
Body weight, kg	60.5±10.9
Body mass index, kg/m^2	22.4±3.1
Prior interferon therapy	16 (22)
Genotype, 1a/1b	0/71
HCV RNA, logIU/mL	6.4±0.6
White blood cell count, /mm^3	5,325±1,400
Hemoglobin, g/dL	14.1±1.4
Platelets, ×10^4/mm^3	18.5±4.3
ALT, IU/L	76±69
γ-GTP, IU/L	55±74
LDL-cholesterol, mg/dL	103.6±23.1
α-Fetoprotein, ng/mL	9.1±15.1
Ferritin, ng/mL	179±227
Type IV collagen 7S, ng/mL	4.7±2.3
Hyaluronic acid, ng/mL	95.1±119.6
HOMA-IR	2.0±1.5
Fatty liver	18 (25)
Activity grade, A0/A1/A2/A3	6/31/30/5
Fibrosis stage, F0/F1/F2/F3/F4	7/18/19/17/11

Data are presented as mean±SD or number (%).
HCV, hepatitis C virus; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltransferase; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance.

Table 2. Comparison of Factors between Patients with or without a Null Response

Factor	Null (n=11)	Nonnull (n=55)	p-value
Age, yr	57.4±5.9	52.5±11.6	0.282
Sex, male/female	5/6	33/22	0.507
Prior interferon therapy	4	11	0.254
Body weight, kg	58.1±11.2	61.2±10.9	0.399
Body mass index, kg/m^2	21.8±3.4	22.6±3.2	0.530
White blood cell count, /mm^3	5,266±1,375	5,341±1,382	0.770
Hemoglobin, g/dL	13.8±1.3	14.2±1.3	0.344
Platelets, ×10^4/mm^3	17.0±3.8	18.8±4.2	0.294
ALT, IU/L	77±60	76±73	0.699
γ-GTP, IU/L	67±63	53±79	0.117
LDL-cholesterol, mg/dL	91.6±14.4	106.2±24.0	0.036
α-Fetoprotein, ng/mL	25.7±34.3	6.9±8.2	0.002
Ferritin, ng/mL	127.6±108.6	189.3±243.6	0.806
Type IV collagen 7S, ng/mL	5.6±2.6	4.4±2.1	0.102
Hyaluronic acid, ng/mL	149.0±144.2	79.8±110.0	0.031
HOMA-IR	2.2±1.6	2.1±1.5	0.908
Fatty liver, –/+	9/2	41/14	1.000
Activity grade, A0-1/A2-3	5/6	30/25	0.743
Fibrosis stage, F0-2/F3-4	5/6	36/19	0.308
IL-28B, major/minor	1/10	44/11	<0.001
Core 70 substitution, mutant/wild	10/1	12/43	<0.001
Core 91 substitution, mutant/wild	3/8	21/34	0.733
NS5A mutation, wild/nonwild	10/1	47/8	1.000
Baseline HCV RNA, logIU/mL	6.4±0.5	6.4±0.6	0.993
Baseline HCV core Ag, fmol/L	5352±4194	6515±7139	0.966
Ribavirin at week 2, ng/mL	2,255.0±520.5	2,051.6±680.9	0.282
PEG-IFN adherence, %	98.7±4.2	97.2±8.6	0.415
Ribavirin adherence, %	96.5±6.9	94.7±11.6	0.991

Data are presented as mean±SD.
ALT, alanine aminotransferase; γ-GTP, γ-glutamyltransferase; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; IL-28B, interleukin 28B; HCV, hepatitis C virus; Ag, antigen; PEG-IFN, pegylated interferon.

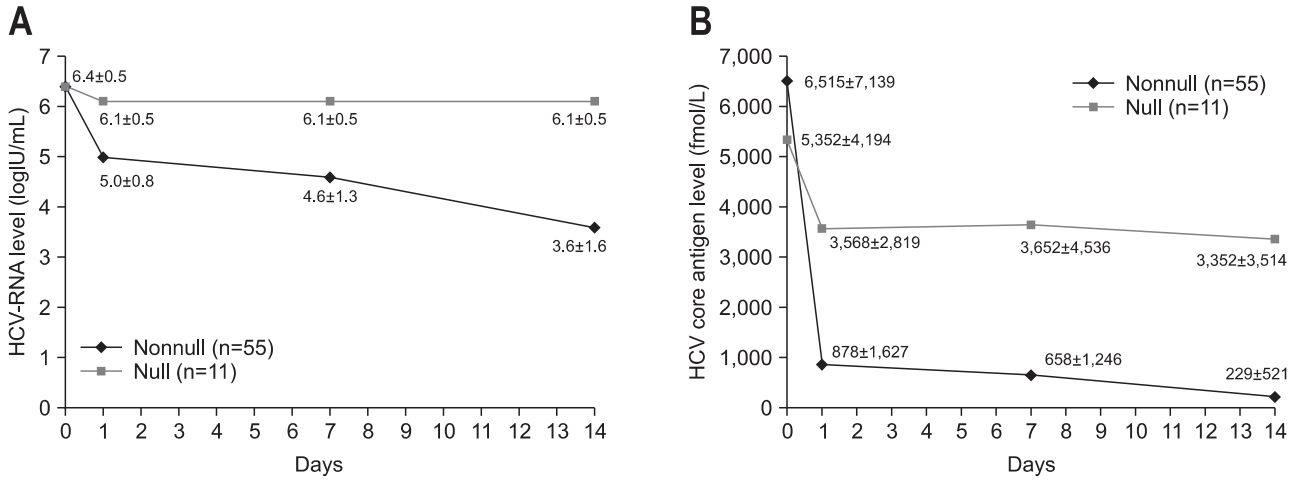

Fig. 1. The kinetics of the hepatitis C virus (HCV) RNA (A) and HCV core antigen (B) levels are shown by line graphs according to the response to therapy. Significant differences were observed in the virological response of HCV RNA and HCV core antigen levels at all time points within 2 weeks of therapy initiation between the null and nonnull responders (p<0.001).

Table 3. Areas Under the Receiver Operating Characteristic Curve according to Significant Contributing Factors to a Null Response

Factor	AUC	95% CI	p-value
LDL-cholesterol	0.710	0.583–0.837	0.036
α-Fetoprotein	0.796	0.681–0.910	0.002
Hyaluronic acid	0.707	0.539–0.876	0.031
HCV RNA			
At day 1	0.879	0.763–0.995	<0.001
At week 1	0.878	0.791–0.964	<0.001
At week 2	0.951	0.902–1.000	<0.001
At week 4	0.978	0.948–1.000	<0.001
HCV core Ag			
At day 1	0.856	0.721–0.992	<0.001
At week 1	0.888	0.808–0.968	<0.001
At week 2	0.959	0.914–1.000	<0.001
Depletion of HCV RNA			
At day 1	0.951	0.887–1.000	<0.001
At week 1	0.921	0.854–0.989	<0.001
At week 2	0.983	0.956–1.000	<0.001
At week 4	0.978	0.943–1.000	<0.001
Depletion of HCV core Ag (log)			
At day 1	0.924	0.814–1.000	<0.001
At week 1	0.939	0.882–0.996	<0.001
At week 2	0.979	0.949–1.000	<0.001
IL-28B	0.855	0.736–0.973	<0.001
Core 70 substitution	0.845	0.725–0.966	<0.001
IL-28B and core 70 substitution	0.891	0.753–1.000	<0.001

AUC, area under the curve; CI, confidence interval; LDL, low-density lipoprotein; HCV, hepatitis C virus; Ag, antigen; IL-28B, interleukin 28B.

HCV RNA and HCV core antigen levels each occurred at week 2 among all-time points within 2 weeks. Furthermore, the AUCs for depletion of HCV RNA level and HCV RNA level at week 4 were equivalent to those at week 2. Among all significant factors including viral response at week 4, the AUC for depletion of HCV RNA level at week 2 was the highest. The sensitivity, specificity, PPV, NPV, and accuracy for predicting null response according to viral response within 2 weeks after therapy initiation, IL-28B and/or amino acid 70 substitution in the core region are summarized in Table 4. Among the viral response factors for predicting null response, the accuracy of the depletion of HCV RNA level at week 2 was the highest; its accuracy was similar to that of the combination of IL-28B SNP and amino acid 70 substitution in the core region.

DISCUSSION

Adherence to drug therapy is one of the major factors associated with treatment success in HCV genotype-1-infected patients who can be maintained on >80% of the recommended PEG-IFN and ribavirin dosage show enhanced SVR rates.[16] In the present study, however, no significant differences were seen in drug adherence between null responders and nonnull responders, and the drug adherence in both groups was >80%. Accordingly, our results indicated that drug adherence was not associated with null response.

The clinical characteristics of null responders to PEG-IFN plus ribavirin therapy have not yet been clarified. However, the host factors related to SVR were IL-28B SNP, body mass index, age, insulin resistance, gender, and the characteristics of the patient's liver disease, including levels of alanine aminotransferase and γ-glutamyltransferase, and the stage of fibrosis.[9] In the present study, significant baseline factors related to null response were LDL-cholesterol level, AFP level, hyaluronic acid level, amino acid 70 substitution in the core region, and IL-28B SNP. Although age and gender are well-known independent factors influencing SVR, no significant differences were noted in age and gender between null responders and nonnull responders in the present study. This discrepancy might be attributed to the small number of patients and few elderly patients in the present study. Amino acid 70 substitution in the core region and LDL-cholesterol level have already been shown to be predictive factors of SVR to PEG-IFN plus ribavirin therapy.[17] In addition, it has been reported that a high serum level of LDL-cholesterol is linked to the IL-28B major allele.[18,19] Regarding AFP, it has been reported that AFP level is an independent factor for response to triple therapy of telaprevir, PEG-IFN, and ribavirin in previous nonresponders to PEG-IFN plus ribavirin therapy.[20] Serum AFP levels of patients without hepatocellular carcinoma elevate nonspecifically with active hepatitis, and a high level of AFP is associated with advanced fibrosis.[21] Therefore, AFP level can be considered as an index of both liver cell renewal and fibrosis grade. Both the activity of hepatitis and the fibrosis grade might be contributing factors to null response. Serum hyaluronic acid has been more strongly associated with null response than other fibrosis indices such as type IV collagen, platelet count, or fibrosis stage in the present study. Accordingly, assessment of serum levels of hyaluronic acid may become more useful in predicting therapeutic response than other fibrosis indices. However, the predictability for null response by LDL-cholesterol, AFP and hyaluronic acid were not very high in the present study. Therefore, these factors should not be used to deny therapy. The IL-28B SNP had a higher predictability for null response than these other factors in the present study; however, only the IL-28B SNP is insufficient for deciding treatment strategy, because its PPV is low.

With respect to baseline viral factors contributing to null response, amino acid 70 substitution in the core region was the only significant viral factor. The predictability of null response by amino acid 70 substitution in the core region was equiva-

Table 4. Predictive Values for a Null Response according to Viral Response, Interleukin 28B, and/or Amino Acid 70 Substitution in the Core Region

Significant predictive factor	Sensitivity	Specificity	PPV	NPV	Accuracy
HCV RNA, logIU/mL					
At day 1 >5.85	82 (9/11)	93 (51/55)	69 (9/13)	96 (51/53)	91 (60/66)
At week 1 >5.45	100 (11/11)	73 (40/55)	42 (11/26)	100 (40/40)	77 (51/66)
At week 2 >5.15	100 (11/11)	84 (46/55)	55 (11/20)	65 (46/46)	86 (57/66)
At week 4 >4.80	82 (9/11)	91 (50/55)	64 (9/14)	96 (50/52)	89 (59/66)
HCV core Ag, fmol/mL					
At day 1 <1,895	82 (9/11)	91 (50/55)	64 (9/14)	96 (50/52)	89 (59/66)
At week 1 >533	100 (11/11)	73 (40/55)	42 (11/26)	100 (40/40)	77 (51/66)
At week 2 >332	100 (11/11)	87 (48/55)	61 (11/18)	100 (48/48)	89 (59/66)
Depletion of HCV RNA, log					
At day 1 <0.75	91 (10/11)	89 (49/55)	63 (10/16)	98 (49/50)	89 (59/66)
At week 1 <0.75	91 (10/11)	87 (48/55)	59 (10/17)	98 (48/49)	88 (58/66)
At week 2 <0.80	82 (9/11)	96 (53/55)	82 (9/11)	96 (53/55)	94 (62/66)
At week 4 <1.55	100 (11/11)	91 (50/55)	69 (11/16)	100 (50/50)	92 (61/66)
Depletion of HCV core Ag, log					
At day 1 <0.65	91 (10/11)	87 (48/55)	59 (10/17)	98 (48/49)	88 (58/66)
At week 1 <0.86	100 (11/11)	82 (45/55)	52 (11/21)	100 (45/45)	85 (56/66)
At week 2 <1.00	100 (11/11)	89 (49/55)	65 (11/17)	100 (49/49)	91 (60/66)
IL-28B minor	91 (10/11)	80 (44/55)	48 (10/21)	98 (44/45)	82 (54/66)
Core 70 substitution mutant	91 (10/11)	78 (43/55)	45 (10/22)	98 (43/44)	80 (53/66)
IL-28B minor and core 70 substitution mutant	82 (9/11)	96 (53/55)	82 (9/11)	96 (53/55)	94 (62/66)

Data are presented as percentage (number).
PPV, positive predictive value; NPV, negative predictive value; HCV, hepatitis C virus; Ag, antigen; IL-28B, interleukin 28B.

lent to that by IL-28B SNP, and its PPV was also not very high. However, the combination of IL-28B SNP and amino acid 70 substitution in the core region could improve the PPV of null response. The Japanese guidelines do not recommend triple therapy for patients with IL-28B minor alleles and the mutant type of amino acid 70 substitutions in the core region[5]. Our results agree with this recommendation.

Suzuki *et al*.[22] have reported that there were no differences in the clinical characteristics between the responders and null-responders except for the titer and declining rates of HCV RNA at 1 week and 4 weeks. The present study revealed that super rapid virological response within 2 weeks was strongly associated with null response. As the predictability of null response by viral response at week 4 was equivalent to that at week 2, there would be no benefit of waiting for the assessment of viral response until week 4 in order to predict null response. Therefore, the best time point for prediction of null response would be treatment week 2. Furthermore, the predictability of null response by viral decline at week 2 was equivalent to that of the combination of IL28B SNP and amino acid 70 substitution in the HCV core region.

Analysis of HCV core antigen level is better than the HCV RNA assay in terms of lower cost, convenience, and rapid results.[23] In the present study, the predictability of null-responder by HCV core antigen decline at week 2 was equivalent to that of quantitative HCV RNA. These results indicated that as a substitute for HCV RNA, the virological response of HCV core antigen level at week 2 might be able to be used as a sensitive test for predicting outcome and deciding on treatment strategy. However, the p-value of HCV core antigen level at week 2 was almost similar to that of the other various factors. In order to validate the present results, further large-scale prospective studies are necessary.

In conclusion, from the analyses of viral dynamics within 2 weeks of therapy initiation, IL-28B SNP and viral mutations, it was demonstrated that the HCV viral response at 2 weeks is the most useful factor in predicting which patients would be null-responders, and its predictability for null-responders was equivalent to that of the combination of IL-28B SNP and amino acid 70 substitution in the HCV core region. Therefore, viral decline at week 2 has the potential to be used as an alternative predictor instead of these tests.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was

reported.

REFERENCES

1. Ikeda K, Saitoh S, Arase Y, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999;29:1124-1130.
2. Cardoso AC, Moucari R, Figueiredo-Mendes C, et al. Impact of peginterferon and ribavirin therapy on hepatocellular carcinoma: incidence and survival in hepatitis C patients with advanced fibrosis. J Hepatol 2010;52:652-657.
3. Singal AK, Singh A, Jaganmohan S, et al. Antiviral therapy reduces risk of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis. Clin Gastroenterol Hepatol 2010;8:192-199.
4. Pockros PJ, Hamzeh FM, Martin P, et al. Histologic outcomes in hepatitis C-infected patients with varying degrees of virologic response to interferon-based treatments. Hepatology 2010;52:1193-1200.
5. Editors of the Drafting Committee for Hepatitis Management Guidelines, The Japan Society of Hepatology. Guidelines for the management of hepatitis C virus infection: first edition, May 2012, The Japan Society of Hepatology. Hepatol Res 2013;43:1-34.
6. Hayashi N, Okanoue T, Tsubouchi H, Toyota J, Chayama K, Kumada H. Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C. J Viral Hepat 2012;19:e134-e142.
7. Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N. Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. J Hepatol 2012;56:78-84.
8. Hézode C, Forestier N, Dusheiko G, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839-1850.
9. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. J Hepatol 2011;55:245-264.
10. 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;49:1335-1374.
11. Simmonds P, Holmes EC, Cha TA, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J Gen Virol 1993;74(Pt 11):2391-2399.
12. 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.
13. 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;48:372-380.
14. 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;41:1105-1109.
15. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 1996;24:289-293.
16. McHutchison JG, Manns M, Patel K, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002;123:1061-1069.
17. Akuta N, Suzuki F, Kawamura Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46:403-410.
18. Li JH, Lao XQ, Tillmann HL, et al. Interferon-lambda genotype and low serum low-density lipoprotein cholesterol levels in patients with chronic hepatitis C infection. Hepatology 2010;51:1904-1911.
19. Clark PJ, Thompson AJ, Zhu M, et al. Interleukin 28B polymorphisms are the only common genetic variants associated with low-density lipoprotein cholesterol (LDL-C) in genotype-1 chronic hepatitis C and determine the association between LDL-C and treatment response. J Viral Hepat 2012;19:332-340.
20. Akuta N, Suzuki F, Seko Y, et al. Determinants of response to triple therapy of telaprevir, peginterferon, and ribavirin in previous non-responders infected with HCV genotype 1. J Med Virol 2012;84:1097-1105.
21. Hu KQ, Kyulo NL, Lim N, Elhazin B, Hillebrand DJ, Bock T. Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C, but not hepatocellular carcinoma. Am J Gastroenterol 2004;99:860-865.
22. Suzuki H, Kakizaki S, Horiguchi N, et al. Clinical characteristics of null responders to Peg-IFNalpha2b/ribavirin therapy for chronic hepatitis C. World J Hepatol 2010;2:401-405.
23. Hayashi K, Hasuike S, Kusumoto K, et al. Usefulness of a new immuno-radiometric assay to detect hepatitis C core antigen in a community-based population. J Viral Hepat 2005;12:106-110.