Table 2. Area Under the ROC Curves, Sensitivity, Specificity, and Negative as Well as Positive Predictive Values of Nonvirological Responses

Variables	AUC	95% CI	Cutoff	Sensitivity	Specificity	NPV	PPV
RIG-I (copies/int. control)	0.712	0.584-0.840	0.573	0.679	0.733	0.830	0.543
ISG15 (copies/int. control)	0.782	0.666-0.899	0.347	0.714	0.833	0.862	0.667
RIG-I/IPS-1 (copies/int. control)	0.732	0.611-0.852	0.651	0.679	0.750	0.833	0.559
IL28B genotype	0.662	0.537-0.787	TG*/CT†	0.607	0.717	0.796	0.500

AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.
*Genotype at rs8099917.
†Genotype at rs12979860.

associated with NVR (Table 3). Among these, multivariate analysis identified old age, HCV core double mutant, and higher hepatic expressions of *RIG-I* and *ISG15* as factors independently associated with NVR (Table 3).

IPS-1 and RIG-I Protein Expression in the Liver. Western blotting revealed that full-length and cleaved IPS-1 were variably present in all the samples from CH-C patients (Fig. 5A). Similar to mRNA

Table 3. Factors Associated with Nonvirological Response

	Univariate Analysis		Multivariate Analysis*	
Factors	Risk Ratio (95% CI)	*P*-value	Risk Ratio (95% CI)	*P*-value
Age (by every 10 year)	1.84 (1.10-3.14)	0.027	3.76 (1.19-11.7)	0.023
Sex				
Male	1			
Female	1.62 (0.59-4.42)	0.350		
BMI (by every 5 kg/m^2)	0.87 (0.46-1.65)	0.672		
Fibrosis stage				
F1/F2	1			
F3/F4	1.82 (0.69-4.85)	0.228		
Degree of steatosis				
<10%	1			
≥10%	1.46 (0.43-5.03)	0.544		
Albumin (by every 1 g/dL)	0.41 (0.11-1.56)	0.190		
AST (by every 40 IU/L)	0.89 (0.53-1.56)	0.681		
ALT (by every 40 IU/L)	0.85 (0.57-1.32)	0.481		
γ-GTP (by every 40 IU/L)	1.32 (0.82-2.07)	0.235		
Fasting blood sugar (by every 100 mg/dL)	1.35 (0.74-2.45)	0.340		
Hemoglobin (by every 1 g/dL)	0.93 (0.67-1.31)	0.683		
Platelet counts (by every 10^4/μL)	0.90 (0.82-0.99)	0.037	0.92 (0.78-1.08)	0.296
HCV load (by every 100 KIU/mL)	1.00 (1.00-1.00)	0.688		
Core 70 & 91 double mutation				
Wild	1		1	
Mutant	3.92 (1.14-13.5)	0.030	11.1 (1.40-88.7)	0.023
ISDR				
Nonwildtype	1			
Wildtype	1.38 (0.13-3.61)	0.513		
IL28B genotype				
Major allele†	1		1	
Minor allele‡	3.91 (1.52-10.0)	0.005	1.53 (0.20-11.9)	0.684
Hepatic gene expression (by every 0.1 copy/*int. control*)				
RIG-I	1.28 (1.10-1.50)	0.002	1.53 (1.07-2.22)	0.021
MDA5	1.53 (1.12-2.00)	0.001		
LGP2	1.34 (1.04-1.74)	0.026		
IPS-1	0.90 (0.78-1.04)	0.143		
RNF125	0.93 (0.83-1.04)	0.204		
ISG15	1.37 (1.16-1.62)	<0.001	1.28 (1.04-1.58)	0.021
USP18	1.67 (1.27-2.20)	<0.001		
IFNλ	1.02 (0.99-1.05)	0.170		
RIG-I/IPS-1 ratio (by every 0.1)	1.21 (1.07-1.36)	0.002		

Risk ratios for nonvirological response were calculated by the logistic regression analysis. BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus; ISDR, IFN sensitivity determining region.
*Multivariate analysis was performed with factors significantly associated with nonvirological response by univariate analysis except for *MDA5*, *LGP2*, *USP18*, and *RIG-I/IPS-1* ratio, which were significantly correlated with *RIG-I* and *ISG15*.
†rs8099917 TT and rs12979860 CC.
‡rs8099917 TG and rs12979860 CT.

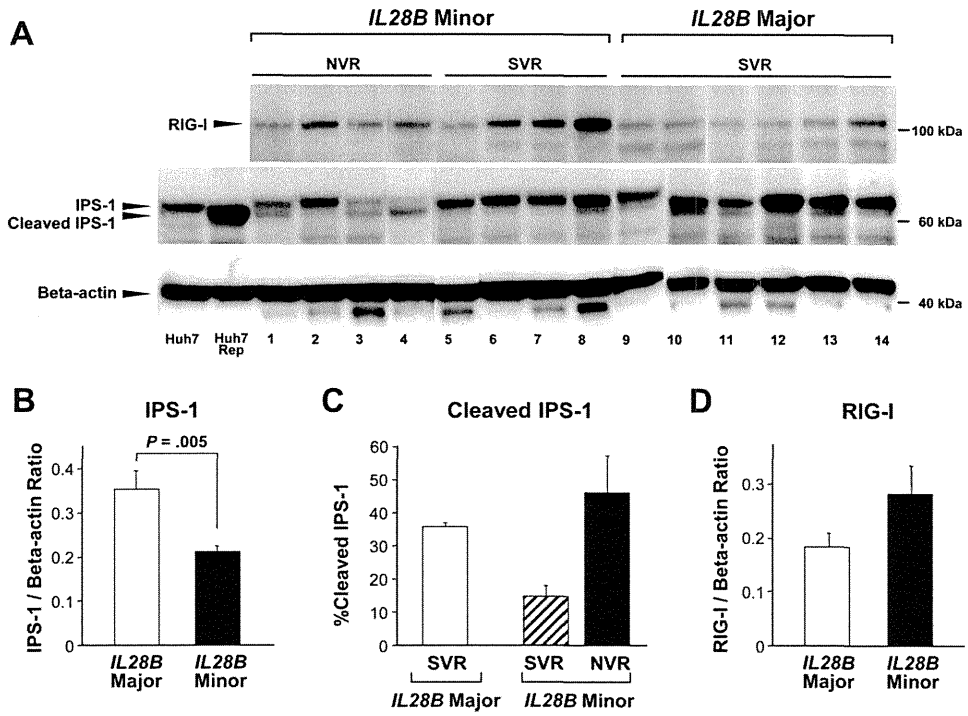

Fig. 5. (A) Western blotting for IPS-1 and RIG-I protein expression levels. Eight lanes contain samples from *IL28B* minor patients (lanes 1-8) and six lanes contain samples from *IL28B* major patients (lanes 9-14). Four lanes contain samples from nonvirological responders (NVR, lanes 1-4) and 10 lanes contain samples from sustained virological responders (SVR, lanes 5-14). Specific bands for RIG-I, full-length IPS-1, cleaved IPS-1, and β-actin are indicated by arrows. Naive Huh7 cells were used for a positive control for full-length IPS-1 (lane Huh7), and cells transfected with HCV-1b subgenomic replicon (Reference #20) were used for a positive control for cleaved IPS-1 (lane Huh7 Rep). (B) Total IPS-1 protein expression levels normalized to β-actin according to *IL28B* genotype. Error bars indicate standard error. *P*-value was determined by Mann-Whitney *U* test. (C) Percentage of cleaved IPS-1 products in total IPS-1 protein according to treatment responses stratified by *IL28B* genotype. Error bars indicate standard error. (D) RIG-I protein expression levels normalized to β-actin according to *IL28B* genotype. Error bars indicate standard error.

expression, total hepatic IPS-1 protein expression was significantly lower in *IL28B* minor patients than in *IL28B* major patients (Fig. 5B). With regard to *IL28B* minor patients, the percentage of cleaved IPS-1 protein in total IPS-1 in SVR was lower than that in NVR (Fig. 5C). In contrast to IPS-1 protein expression, hepatic RIG-I protein expression was higher in *IL28B* minor patients than that in *IL28B* major patients (Fig. 5D).

Discussion

In the present study we found that the baseline expression levels of intrahepatic viral sensors and related regulatory molecules were significantly associated with the genetic variation of *IL28B* and final virological outcome in CH-C patients treated with PEG-IFNα/RBV combination therapy. Although the relationship between the *IL28B* minor allele and NVR in PEG-IFNα/RBV combination therapy is evident, mechanisms responsible for this association remain unknown. *In vitro* studies have suggested that cytoplasmic viral sensors, such as RIG-I and MDA5, play a pivotal role in the regulation of IFN production and augment IFN production through an amplification circuit.[7,8] Our results indicate that expressions of *RIG-I* and *MDA5* and a related amplification system may be up-regulated by endogenous IFN at a higher baseline level in *IL28B* minor patients. However, HCV elimination by subsequent exogenous IFN is insufficient in these patients, as reported,[19] suggesting that *IL28B* minor patients may have adopted a different equilibrium in their innate immune response to HCV. Our data are further supported by recent reports of an association between intrahepatic levels of IFN-stimulated gene expression and PEG-IFNα/RBV response as well as with *IL28B* genotype.[21-23]

In contrast to cytoplasmic viral sensor (*RIG-I, MDA5*, and *LGP2*) and modulator (*ISG15* and *USP18*) expression, the adaptor molecule (*IPS-1*) expression was significantly lower in *IL28B* minor patients. Moreover, western blotting further confirmed IPS-1 protein downregulation in *IL28B* minor patients by revealing decreased protein levels. Because IPS-1 is one of the main target molecules of HCV evasion,[9,18]

transcriptional and translational *IPS-1* expression are probably suppressed by HCV with resistant phenotype, which may be more adaptive in *IL28B* minor patients than in *IL28B* major patients. When we analyzed the proportion of full-length or cleaved IPS-1 to the total IPS-1 protein in a subgroup of *IL28B* minor patients, cleaved IPS-1 product was less dominant in SVR than in NVR, whereas uncleaved full-length IPS-1 protein was more dominant in SVR than in NVR. Therefore, the ability of HCV to evade host innate immunity by cleaving IPS-1 protein and/or host capability of protection from IPS-1 cleavage is probably responsible for the variable treatment responses in *IL28B* minor patients.

Our results indicated a close association between *IL28B* minor patients with higher γ-GTP level and higher frequency of HCV core double mutants, which are known factors for NVR. In contrast, no significant association was observed between *IL28B* genotype and age, gender, or liver fibrosis, which are also known to be unfavorable factors for virological response to PEG-IFNα/RBV. Therefore, certain factors other than the *IL28B* genotype may independently influence virological response. To elucidate whether gene expression involving innate immunity independently associates with a virological response from the *IL28B* genotype, we performed further analysis in a subgroup and conducted a multivariate regression and ROC analyses. Our multivariate and ROC analyses demonstrate that higher expressions of *RIG-I* and *ISG15* as well as a higher ratio of *RIG-I/IPS-1* are independently associated with NVR, and quantification of these values is more useful in predicting final virological response to PEG-IFNα/RBV than determination of *IL28B* genotype in each individual patients. However, the SVR rates in our patients were similar among *IL28B* genotypes, which suggests more SVR patients with the *IL28B* minor allele were included in the present study than those in the general CH-C population. Hence, our data did not necessarily exclude the possibility of the *IL28B* genotype in predicting NVR, although our multivariate analysis could not identify the *IL28B* minor allele as an independent factor for NVR. Interestingly, an association between *IL28B* genotype and expressions of *RIG-I* and *ISG15* as well as *RIG-I/IPS-1* expression ratio is still observed even in patients with the same subgroup of virological response (Fig. 3).

In the present study, although hepatic *IFNλ* expression was observed to be higher in *IL28B* minor and NVR patients, it was not statistically significant. Because *IL28B* shares 98.2% homology with *IL28A*, our primer could not distinguish the expression of *IL28B* from that of *IL28A*, and moreover, we could not specify which cell expresses *IFNλ* (i.e., hepatocytes or other immune cells that have infiltrated the liver). Therefore, the precise mechanisms underlying *IL28B* variation and expression of *IFNλ* in relation to treatment response need further clarification by specifying type of IFNλ and uncovering the producing cells.

In the present study we included genotype 1b patients because it is imperative to designate a virologically homogenous patient group to associate individual treatment responses with different gene expression profiles that direct innate immune responses. We have reported that the *RIG-I/IPS-1* ratio was significantly higher in NVR with HCV genotype 2.[19] However, our preliminary results indicated that baseline hepatic *RIG-I* and *ISG15* expression and the *RIG-I/IPS-1* expression ratio is not significantly different among *IL28B* genotypes in patients infected with genotype 2 (Supporting Figure). This may be related to the rarity of NVR with HCV genotype 2 and the lower effect of *IL28B* genotype on virological responses in patients infected with HCV genotype 2.[24] The association among treatment responses in all genotypes, the different status of innate immune responses, and *IL28B* genotype needs to be examined further.

Differences in allele frequency for *IL28B* SNPs among the population groups has been reported. The frequency of *IL28B* major allele among patients with Asian ancestry is higher than that among patients with European and African ancestry.[25] Because *IL28B* polymorphism strongly influences treatment responses within each population group,[5] our data obtained from Japanese patients can be applied to other population groups. However, the rate of SVR having African ancestry was lower than that having European ancestry within the same *IL28B* genotype.[5] Hence, further study is required to clarify whether this difference among the population groups with the same *IL28B* genotype could be explained by differences in expression of genes involved in innate immunity.

In a recent report, an SVR rate of telaprevir with PEG-IFNα/RBV was only 27.6% in *IL28B* minor patients.[26] Because new anti-HCV therapy should still contain PEG-IFNα/RBV as a platform for the therapy, our findings regarding innate immunity in addressing the mechanism of virological response and predicting NVR remain important in this new era of directly acting anti-HCV agents, such as telaprevir and boceprevir.

In conclusion, this clinical study in humans demonstrates the potential relevance of the molecules involved in innate immunity to the genetic variation

of *IL28B* and clinical response to PEG-IFNα/RBV. Both the *IL28B* minor allele and higher expressions of *RIG-I* and *ISG15* as well as higher *RIG-I/IPS-1* ratio are independently associated with NVR. Innate immune responses in *IL28B* minor patients may have adapted to a different equilibrium compared with that in *IL28B* major patients. Our data will advance both understanding of the pathogenesis of HCV resistance and the development of new antiviral therapy targeted toward the innate immune system.

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Early Decrease in α-Fetoprotein, but Not Des-γ-Carboxy Prothrombin, Predicts Sorafenib Efficacy in Patients with Advanced Hepatocellular Carcinoma

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Key Words

Antitumor response · Chemotherapy · Des-γ-carboxy prothrombin · α-Fetoprotein · Hepatocellular carcinoma · Sorafenib · Tumor markers

Abstract

Objectives: The aim of this study was to investigate the relationships between early changes in the tumor markers α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), and antitumor response in the early period following administration of sorafenib in patients with advanced hepatocellular carcinoma (HCC). ***Methods:*** Forty-eight advanced HCC patients were evaluated. AFP and DCP were measured at baseline, and after 2 and 4 weeks, and the antitumor responses were evaluated according to the RECIST criteria 4 weeks after starting sorafenib therapy. The ratios of each tumor marker were compared by stratifying the patients into the partial response (PR) + stable disease (SD) group or the progressive disease (PD) group. ***Results:*** Both 2 and 4 weeks after starting sorafenib therapy, the AFP ratio in the PR + SD group (n = 32) was significantly lower than in the PD group (n = 16; p = 0.002, p = 0.002). DCP was elevated in both the PR + SD group and the PD group 2 weeks and 4 weeks after starting sorafenib therapy. ***Conclusions:*** Evaluation of AFP ratios 2 and 4 weeks after starting sorafenib therapy may be useful for predicting antitumor response. On the other hand, early elevation of DCP does not necessarily suggest treatment failure by sorafenib, as DCP elevation can occur despite therapeutic efficacy.

Introduction

Sorafenib is a molecularly targeted multikinase inhibitor that suppresses both signal transduction of tumor growth and angiogenesis by inhibiting Raf kinase, and VEGF and PDGF receptor kinase [1]. The SHARP Study and the Asia-Pacific Study [2, 3], two large-scale, phase III, clinical studies, demonstrated that sorafenib significantly prolongs time to progression (TTP) and improves overall survival (OS) in patients with advanced hepatocellular carcinoma (HCC), and confirmed its efficacy in improving prognosis in these patients for the first time as a systemic chemotherapeutic agent. Accordingly,

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sorafenib has been recognized as the only standard systemic chemotherapeutic agent for patients with advanced HCC for whom resection and local therapy are not indicated [4–6].

α-Fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) are well-known and widely used serological tumor markers in the screening and diagnosis of HCC [7–11]. These tumor markers are also useful as indicators of the therapeutic effect by evaluating serial changes in these values before and after tumor resection and local ablation therapy. Although numerous studies have reported the relationships between the changes in tumor markers during treatment and antitumor response [12–19], there have been no comprehensive reports evaluating the relationship between prognosis and serial changes in AFP and DCP during treatment with sorafenib. Even in the SHARP Study and the Asia-Pacific Study, this relationship was not evaluated, despite the lack of systemic chemotherapeutic agents other than sorafenib that improve prognosis in advanced HCC.

Accordingly, we investigated cumulative TTP and OS stratified by antitumor effects based on image analysis, and assessed the relationship between antitumor effects and changes in AFP and DCP in the early period of sorafenib administration in patients with advanced HCC.

Patients and Methods

Patient Eligibility

Between July 2009 and December 2010, a total of 52 patients with advanced HCC were consecutively started on sorafenib (Nexavar®; Bayer Health Care Pharmaceuticals, West Haven, Conn., USA) therapy at the Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital. Inclusion criteria for this study were as follows: HCC was diagnosed either by needle biopsy or by the combination of typical radiological findings on dynamic multidetector row computed tomography (MDCT) and elevated AFP serum levels, according to the American Association for the Study of Liver Diseases [20]; patients were classified as having advanced HCC if they were not eligible for or had disease progression after surgical or locoregional therapies; Eastern Cooperative Oncology Group performance status score of 0–1; Child-Pugh liver function class A or B (≤7); adequate hepatic function (albumin level >2.5 g/dl, total bilirubin level <3.0 mg/dl, and alanine and aspartate aminotransferase levels <5 times the upper limit of normal); dynamic MDCT was obtained at baseline and after 4 weeks of sorafenib treatment in order to assess the therapeutic effects.

Of 52 patients, 48 patients meeting the inclusion criteria were enrolled. HCC stage was diagnosed according to the criteria of the Liver Cancer Study Group of Japan [21]. This study was approved by the Ethics Committee of the Musashino Red Cross Hospital and was performed in compliance with the Helsinki Declaration.

Sorafenib Therapy

The starting dosage of sorafenib was 800 mg/day p.o. However, out of concern regarding the possibility of having to discontinue sorafenib treatment at an early stage due to adverse events, the initial dosage was set at 400 mg/day for patients aged ≥80 years, and those with a body weight ≤40 kg or a history of treatment for varices or ascites. Sorafenib therapy was continued until the occurrence of potentially fatal adverse events.

Image-Based Evaluation of Antitumor Effects

Dynamic MDCT images were taken at baseline and after 4 weeks of sorafenib treatment. Tumor responses were defined as the time point response [(in accordance with the Response Evaluation Criteria In Solid Tumors (RECIST; version 1.1)] [22] 4 weeks after sorafenib administration where the confirmation of response was not required. Patients in whom the effect was rated as partial response (PR) or stable disease (SD) were pooled in the PR + SD group, while patients showing progressive disease (PD) comprised the PD group. MDCT images were obtained every 2–6 weeks after the first MDCT image, which was obtained 4 weeks after the start of sorafenib administration.

Measurement and Evaluation of Serum AFP and DCP

The HCC tumor markers analyzed were serum AFP and DCP at baseline, and 2 and 4 weeks after starting sorafenib administration. Because DCP levels are influenced by vitamin K and warfarin, patients ingesting these agents were excluded from DCP analysis. For each patient, the baseline concentration of each tumor marker was assigned a value of 1, and the ratios for each tumor marker 2 and 4 weeks after the start of administration were calculated.

Statistics

Statistical analyses were performed using Stat View J software (version 5; SAS Institute, Cary, N.C., USA). TTP and OS after the start of sorafenib administration were analyzed by the Kaplan-Meier method, while comparisons between the two patient groups were performed by log-rank test. Tumor marker levels were analyzed by Wilcoxon signed-rank test, and comparisons of the ratios for the tumor markers between the two patient groups were performed by the Mann-Whitney U test. A value of $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient Baseline Characteristics

Table 1 shows baseline characteristics of the 48 HCC patients enrolled in this study. The study cohort consisted of 38 males and 10 females, with a mean age of 69.9 ± 10.0 years. Six patients had never been treated for HCC, while the remaining 42 patients had previously undergone therapy. None of these previous treatments had involved molecularly targeted therapy. The starting dosage of sorafenib in this study was 800 mg/day in 26 patients and 400 mg/day in 22 patients. Criteria for starting sorafenib at 400 mg/day were as follows: (a) age ≥80 years

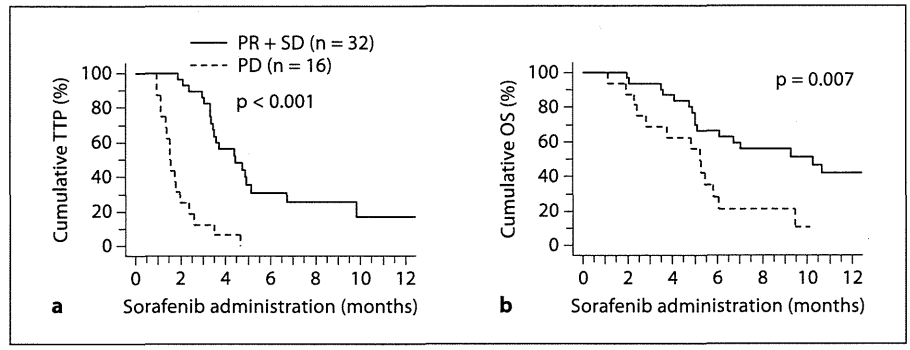

Fig. 1. Comparison of cumulative TTP (**a**) and OS (**b**) in the PR + SD and PD groups according to RECIST.

(n = 8); (b) body weight ≤40 kg (n = 2), and (c) history of treatment for varices or ascites (n = 12). The median baseline AFP level was 572 ng/ml (range, 2.3–148,000), and the median baseline DCP level was 424 mAU/ml (range, 15–305,000). The mean observation period was 7.2 ± 4.5 months.

Antitumor Responses 4 Weeks after the Start of Sorafenib Therapy

According to RECIST, 4 weeks after the start of sorafenib therapy, there were no complete responses, 2 PR, 30 SD, and 16 PD. The response rate was 4.2%, and the disease control rate was 66.7%.

Cumulative TTP and OS in the PR + SD and PD Groups

Cumulative TTP in the two groups according to RECIST is shown in figure 1a. The median observation period was 3.2 months. The median TTP was significantly longer in the PR + SD group than in the PD group (4.4 vs. 1.5 months; hazard ratio, 0.14; 95% CI, 0.06–0.29; $p < 0.001$).

Cumulative OS in the two groups according to RECIST is shown in figure 1b. The median observation period was 5.7 months. The median OS was significantly longer in the PR + SD group than in the PD group (10.3 vs. 5.2 months; hazard ratio, 0.36; 95% CI, 0.17–0.78; $p = 0.007$).

Comparison of Actual and Relative Levels of AFP at Baseline, and 2 and 4 Weeks after the Start of Sorafenib Therapy (Stratified by Antitumor Response)

AFP was not measured in 9 and 1 patients 2 and 4 weeks after starting sorafenib administration, respectively. Accordingly, AFP was analyzed in 39 and 47 patients 2 and 4 weeks after starting sorafenib administration, respectively.

Table 1. Baseline characteristics of the 48 HCC patients enrolled in this study

Mean age, years	69.9 ± 10.0
Male/female	38/10
HBV/HCV/NBNC	6/30/12
ECOG PS (0/1)	29/19
Child-Pugh score (5/6/7)	24/21/3
HCC stage (III/IVA/IVB)	11/18/19
Initial therapy/therapy for recurrence	6/42
Sorafenib starting dosage (800/400 mg)	26/22
Median serum AFP level, ng/ml	572
Range	2.3–148,000
Median serum DCP level, mAU/ml	424
Range	15–305,000
Mean observation period, months	7.2 ± 4.5

Numbers of patients are shown unless indicated otherwise. HBV/HCV = Hepatitis B/C virus; NBNC = non-HBV, non-HCV; ECOG = Eastern Cooperative Oncology Group; PS = performance status.

Data comparing actual AFP levels at baseline, and 2 and 4 weeks after starting sorafenib administration, both for the total patients and when stratified by antitumor response according to RECIST, are shown in table 2. Among the total number of patients, AFP showed no statistically significant differences between baseline and 2-week treatment levels, but in the PD group, AFP levels after 2 weeks of treatment were significantly elevated versus baseline levels ($p = 0.013$). Similarly, in the total number of patients, AFP showed no statistically significant differences between baseline and 4-week treatment levels, but in the PD group, AFP was significantly higher after 4 weeks of treatment compared with baseline levels ($p = 0.002$). In the PR + SD group, the median actual AFP level 4 weeks after starting sorafenib administration was higher than that at 2 weeks; however, there were no sig-

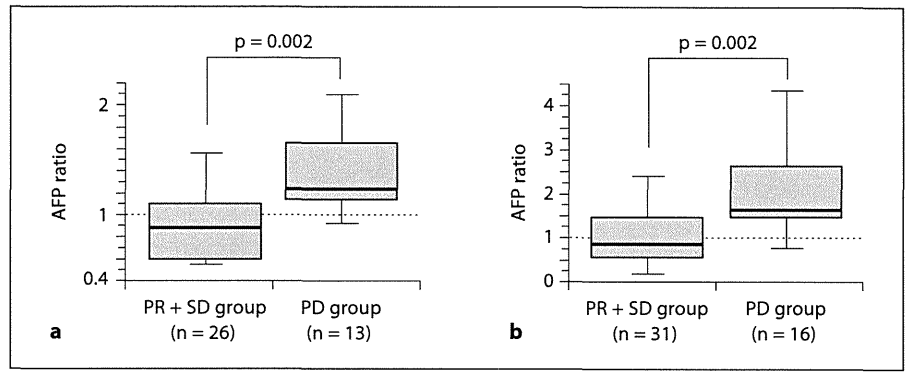

Fig. 2. AFP ratios 2 (**a**) and 4 weeks (**b**) after the start of sorafenib treatment in the PR + SD and PD groups according to RECIST.

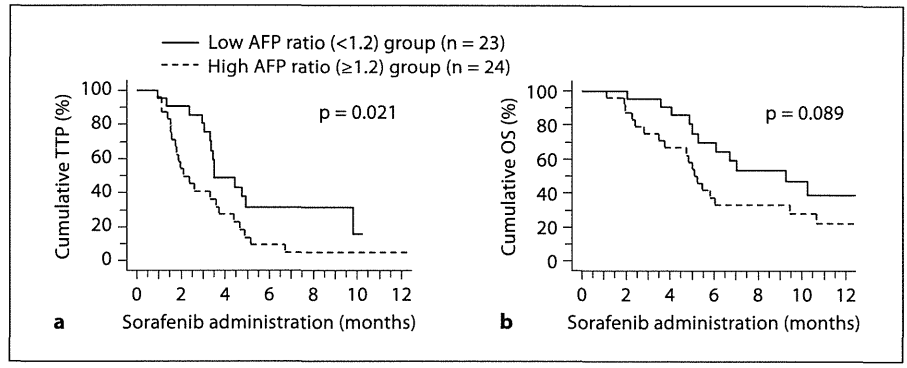

Fig. 3. Comparison of cumulative TTP (**a**) and OS (**b**) in the groups with low (<1.2) and high AFP ratio (≥1.2) 4 weeks after starting sorafenib therapy.

Table 2. Comparison of actual AFP levels (ng/ml) at baseline, and 2 and 4 weeks after the start of sorafenib therapy (stratified by antitumor response)

Groups	Baseline	After 2 weeks	p value	After 4 weeks	p value
Total	572 (2.3–148,000)	481 (2.2–163,300)	0.155	676 (1.1–281,700)	0.077
PR + SD	245.5 (2.3–148,000)	198 (2.2–163,300)	0.657	311 (1.1–281,700)	0.518
PD	2,321 (8.6–62,400)	3,303 (6.4–52,840)	0.013	6,258.5 (6.4–237,000)	0.002

nificant differences between AFP levels after 2 and 4 weeks (p = 0.423). On the other hand, in the PD group, the median actual AFP level 4 weeks after starting sorafenib administration was significantly higher than that after 2 weeks (p = 0.003).

Figure 2 compares the AFP ratios stratified by antitumor effects according to RECIST after 2 and 4 weeks of sorafenib treatment. AFP ratios 2 and 4 weeks after the start of sorafenib administration were 0.88 (range, 0.28–1.79) and 0.88 (range, 0.07–3.17) in the PR + SD group, and 1.24 (range, 0.74–2.12) and 1.63 (range, 0.64–7.35) in the PD group. At both time points, the ratio in the PR + SD group was significantly lower than in the PD group (p = 0.002, p = 0.002).

Cumulative TTP and OS in the Groups with Low and High AFP Ratio 4 Weeks after the Start of Sorafenib Therapy

The median AFP ratio 4 weeks after the start of sorafenib therapy was 1.2 (0.1–7.4).

Cumulative TTP (according to RECIST) in the groups with low (<1.2) and high AFP ratio (≥1.2) 4 weeks after the start of sorafenib therapy is shown in figure 3a. The median TTP was significantly longer in the low AFP (n = 23) ratio group than in the high AFP ratio group (n = 24; 3.5 vs. 2.1 months; hazard ratio, 0.46; 95% CI, 0.23–0.91; p = 0.021).

Cumulative OS in the low (n = 23) and high AFP ratio groups (n = 24) 4 weeks after the start of sorafenib therapy

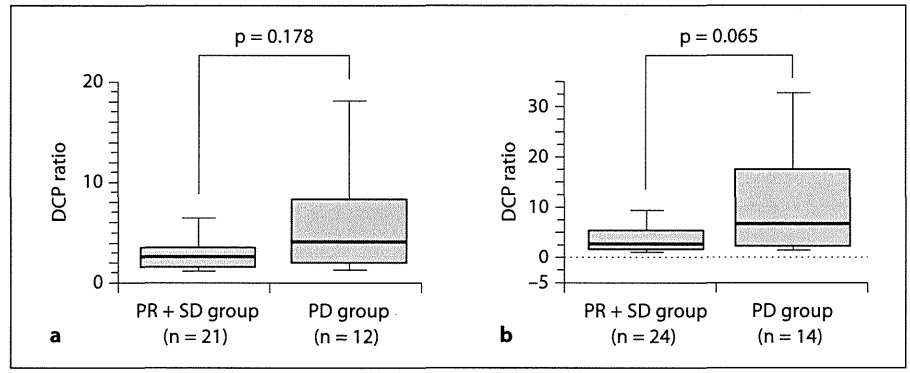

Fig. 4. DCP ratios 2 (**a**) and 4 weeks (**b**) after the start of sorafenib treatment in the PR + SD and PD groups according to RECIST.

Table 3. Comparison of actual DCP levels (mAU/ml) at baseline, and 2 and 4 weeks after the start of sorafenib therapy (stratified by antitumor response)

Patients	Baseline	After 2 weeks	p value	After 4 weeks	p value
Total	424.5 (15–305,000)	741 (26–798,000)	<0.001	2,025 (78–1,020,000)	<0.001
PR + SD	425.5 (15–216,000)	741 (30–323,000)	<0.001	1,715 (81–524,000)	<0.001
PD	575.5 (19–305,000)	6,186.5 (26–798,000)	0.002	20,550 (78–1,020,000)	0.001

is shown in figure 3b. The median OS tended to be higher in the low than in the high AFP ratio group (9.3 vs. 5.1 months; hazard ratio, 0.53; 95% CI, 0.25–1.12; p = 0.089).

Comparison of Actual and Relative Levels of DCP at Baseline, and 2 and 4 Weeks after the Start of Sorafenib Therapy (Stratified by Antitumor Response)

In the analysis of DCP, 7 patients who were taking vitamin K and 1 patient who was on warfarin were excluded. In addition, DCP was not determined in 7 and 2 patients 2 and 4 weeks after starting sorafenib administration, respectively. Accordingly, DCP was analyzed in 33 patients 2 weeks and in 38 patients 4 weeks after starting sorafenib administration.

Data comparing actual DCP levels at baseline, and 2 and 4 weeks after starting sorafenib administration, both for the total number of patients and patients stratified by antitumor response according to RECIST, are shown in table 3. Actual levels of DCP after 2 weeks of treatment were significantly higher than baseline levels in the total number of patients, the PR + SD group and the PD group. After 2 weeks of treatment, DCP was elevated in 97.0% (32/33) of the patients. Similarly, actual levels of DCP after 4 weeks of treatment were also significantly elevated from baseline levels in all patient groups; the total number of patients, the PR + SD group, and the PD group. After 4 weeks of treatment, DCP was elevated in 92.1% (35/38) of the patients.

Figure 4 compares the DCP ratios between the PR + SD and PD groups according to RECIST after 2 and 4 weeks of sorafenib therapy. The DCP ratios 2 and 4 weeks after the start of sorafenib administration were 2.57 (range, 0.87–10.02) and 2.72 (range, 0.30–13.46) in the PR + SD group, and 4.02 (range, 1.12–35.03) and 6.73 (range, 1.25–45.08) in the PD group. There were no significant differences between the PR + SD and PD groups at either time point (p = 0.178, p = 0.065).

Cumulative TTP and OS in the Low and High DCP Ratio Groups 4 Weeks after the Start of Sorafenib Therapy

The median DCP ratio 4 weeks after the start of sorafenib therapy was 4.0 (0.3–45.1).

Cumulative TTP (according to RECIST) in the low (<4) and the high DCP ratio (≥4) groups 4 weeks after the start of sorafenib therapy is shown in figure 5a. There were no significant differences in the median DCP ratio between the low (n = 19) and the high DCP ratio group (n = 19; 3.5 vs. 2.4 months; hazard ratio, 1.06; 95% CI, 0.51–2.19; p = 0.874).

Cumulative OS in the low (n = 19) and high DCP ratio groups (n = 19) 4 weeks after the start of sorafenib thera-

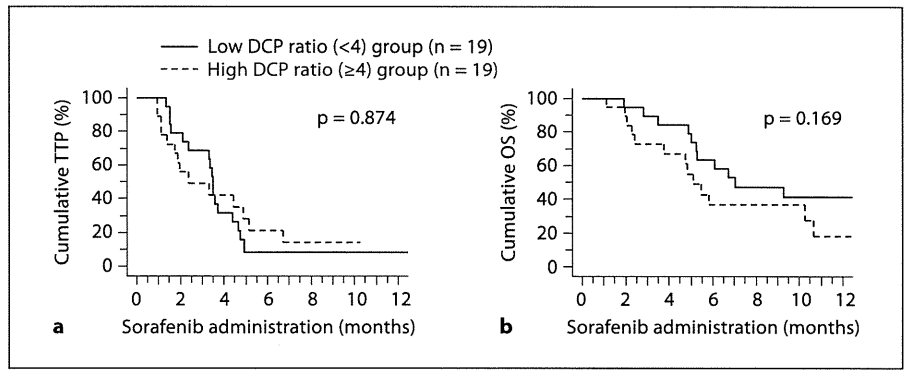

Fig. 5. Comparison of cumulative TTP (**a**) and OS (**b**) in the groups with low (<4) and high DCP ratio (≥4) 4 weeks after starting sorafenib therapy.

py is shown in figure 5b. There were no significant differences in median DCP ratio between the low and high DCP ratio groups (7.2 vs. 5.1 months; hazard ratio, 0.57; 95% CI, 0.25–2.55; p = 0.169).

Discussion

In the present study, we investigated the relationships between the changes in tumor markers, AFP and DCP, and antitumor responses in the early period following administration of sorafenib to patients with advanced HCC, and found that the relationship for AFP was different from that for DCP. With regard to AFP, both 2 and 4 weeks after starting sorafenib therapy, the ratio in the PR + SD group was significantly lower than in the PD group. On the other hand, DCP was elevated in both the PD group and the PR + SD group, and there were no statistically significant differences between the two groups either 2 or 4 weeks after starting sorafenib therapy. These results suggest that the time course changes for AFP in the early period after starting sorafenib administration are useful for predicting antitumor response assessed by image analysis.

Several studies have reported that the primary effect of sorafenib is inhibition of tumor growth rather than tumor shrinkage [2, 3, 23, 24]. In the SHARP Study, it was reported that the response rate in the sorafenib group based on RECIST criteria was only 2.3%, but both the cumulative TTP and cumulative survival duration were prolonged [2]. This can be considered an example of the limitations of using RECIST criteria, which focus on changes in the size of the entire tumor for evaluation of the therapeutic efficacy of molecularly targeted drugs. In the present study, cumulative TTP and OS were significantly better in the PR + SD group than in the PD group. In view of these findings, the primary clinical benefit of sorafenib is disease stabilization. Accordingly, it is important to evaluate treatment response in patients treated with sorafenib. In the present study, we analyzed the tumor marker response according to radiological response using the RECIST criteria. On the other hand, modified RECIST criteria were recently proposed as a method to assess arterial involvement [25]. Further investigation using these modified RECIST criteria is thus necessary.

In order to evaluate tumor responses, the formal recommendation of the panel of experts in HCC-Design Clinical Trials was to conduct imaging surveillance every 6–8 weeks using CT or MRI [4]. In our hospital, dynamic MDCT was obtained after 4 weeks of sorafenib treatment in order to assess early therapeutic effects. We found that antitumor responses 4 weeks after sorafenib administration correlated with both TTP and OS. Therefore, the present results indicate that it may be beneficial to evaluate the time point response 4 weeks after sorafenib administration in patients receiving sorafenib.

In the present study, AFP was significantly elevated in the PD group both 2 and 4 weeks after the start of administration compared with baseline. There has only been one report on AFP response after sorafenib therapy [26]. Shao et al. [26] reported the AFP responder group as patients whose AFP levels decreased to less than 0.8-fold of baseline levels within 1 month following sorafenib administration, while the non-responder group did not show this decrease. Consistent with our results, both the cumulative survival and TTP rates were significantly better in the AFP responder group than in the non-responder group. Hence, in the case of sorafenib therapy, changes in AFP levels may be correlated with the antitumor effects evaluated by image analysis, similarly to the course following other therapies for HCC, such as hepatic resection, radiofrequency ablation therapy, and transarterial chemoembolization. A comparison of the actual AFP levels 2 and 4

weeks after starting sorafenib administration in the PD group revealed that the median value after 4 weeks was significantly higher than that after 2 weeks. Even in the PR + SD group, the median value after 4 weeks was higher than that after 2 weeks. There were no significant differences between AFP levels after 2 and 4 weeks; thus, one of the reasons for this phenomenon was unevenness of AFP levels owing to the small sample size in this study.

With regard to DCP, there have been numerous reports that the time course change in DCP following treatment for HCC reflects therapeutic efficacy [17–19]. However, in the present study, we found that both the actual and relative levels of DCP were elevated in >90% of the patients, not only in the PD group but also in the PR + SD group, both 2 and 4 weeks after starting sorafenib therapy. To our knowledge, there have been no comprehensive clinical reports regarding the time course changes in DCP following sorafenib treatment. In a case report by Nakazawa et al. [27], DCP levels were markedly increased following treatment, even in patients who achieved a complete response on the basis of image analysis. From basic research, Murata et al. [28] reported that culturing a liver cancer cell line (HepG2) under hypoxic conditions resulted in increased DCP production by the cells. One possible mechanism for the increased DCP levels following sorafenib administration is that sorafenib-mediated inhibition of angiogenesis places tumor cells under hypoxic conditions, subsequently leading to increased DCP production. Thus, the increase in DCP levels following sorafenib administration may reflect HCC cell ischemia. Based on our results, increases in DCP soon after the start of sorafenib administration, regardless of antitumor effect, are not useful for assessing the antitumor responses, as DCP may increase in response to the ischemia caused by sorafenib.

Assessment by image analysis is the gold standard for evaluating antitumor responses of anticancer drugs [4, 22, 23]. However, such image analysis can be difficult in patients with multiple HCC lesions, vascular invasion, extrahepatic metastases, or ischemic tumors. In particular, patients in whom therapy using sorafenib is indicated are often in advanced stages of disease. There are limitations in using only radiological criteria to evaluate sorafenib treatment.

Our results suggest that the determination of early changes in AFP is useful for evaluating both antitumor response and prognostic efficacy of sorafenib, as assessed by TTP and OS, in patients with advanced HCC. In patients with advanced HCC treated with sorafenib, it is important to evaluate therapeutic efficacy as early as possible, as appropriate and early evaluation of sorafenib therapy can avoid unnecessary adverse events and allow second-line therapy when sorafenib therapy is not effective. In addition, determination of early changes in AFP is useful for evaluating the efficacy of new molecularly targeted agents currently under development. At present, there is no effective second-line treatment and we could not confirm whether continuing sorafenib administration would prolong the survival of patients with elevated AFP. Therefore, we cannot conclude that sorafenib therapy should be stopped in the case of elevated AFP ratio after 2 or 4 weeks of treatment. However, when an effective second-line treatment becomes available, an elevated AFP ratio may be a good indicator for switching to second-line therapy.

On the other hand, with regard to early changes in DCP, caution is required when assessing the antitumor response of sorafenib, as DCP elevation can occur irrespective of therapeutic effects.

In conclusion, our results suggest that early evaluation of AFP after starting sorafenib therapy is useful for predicting antitumor response. In contrast, early elevation of DCP does not necessarily suggest treatment failure of sorafenib. Appropriate and early evaluation of efficacy of sorafenib by AFP determination can provide valuable information that may influence subsequent decisions regarding patient management, thus avoiding unnecessary adverse events and allowing the opportunity for second-line therapy.

Acknowledgment

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Disclosure Statement

The authors declare that they have no financial conflicts of interest.

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Original Article

Changes in hepatitis C viral load during first 14 days can predict the undetectable time point of serum viral load by pegylated interferon and ribavirin therapy

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Aim: In the treatment of chronic hepatitis C, pegylated interferon (PEG-IFN) and ribavirin combination therapy must be continued for an adequate duration to improve the rate of sustained virological response. We attempted to predict the time point at which serum hepatitis C virus (HCV) RNA are undetectable during combination therapy.

Methods: Patients with HCV genotype 1b were enrolled in a model preparation ($n = 35$) and a validation group ($n = 70$). All patients received PEG-IFN-α-2b/ribavirin combination therapy for at least 48 weeks, and serological samples were screened a minimum of 17 times during the therapy. Serum HCV RNA were measured by the Abbott RealTime HCV assay. Using the HCV dynamics model described by Neumann *et al.*, we used multiple linear regression analysis to select factors that affected the undetectable time point.

Results: Difference in viral load between weeks 1 and 2 was the only predictive factor for the undetectable time point of serum HCV RNA ($r^2 = 0.67$, $P < 0.0005$), and we derived the following prediction equation: undetectable time point (week) = 13.495 × (viral load at day 14 [log IU/mL] – viral load at day 7 [log IU/mL]) + 25.456. The equation was applicable to the validation group.

Conclusion: We created a formula for predicting the undetectable time point from viral load measurements early in PEG-IFN-α-2b/ribavirin combination therapy. An early response reflects sensitivity to therapy, and the estimation of an undetectable time point would be useful for determining the optimal duration of treatment for chronic hepatitis C patients.

Key words: hepatitis C, interferon, kinetics, real-time polymerase chain reaction, undetectable time point

INTRODUCTION

INTERFERON (IFN)-BASED therapy is the main form of therapy for chronic hepatitis C, but it requires a long-term period to complete, typically lasting at least 48 weeks for hepatitis C virus (HCV) genotypes 1 and 4. The final therapeutic effect is eradication of HCV, which is referred to as a sustained virological response (SVR). Although combination therapy with pegylated (PEG)-IFN-α and ribavirin is now established as the standard treatment for chronic HCV infection genotype 1b, the SVR rate in these patients is still approximately 50%.[1–3] Moreover, it is difficult to know the treatment outcomes during treatment and follow-up period.

Various factors have been investigated to predict the treatment efficacy before initiation of therapy, including pretreatment viral load,[4] viral genotype,[5] and gene sequences, such as IFN sensitivity determining region,[6] and host factors, including sex, age, fibrosis stage and race.[7,8] These factors cannot be modified by therapy and are unfortunately not completely reliable for predicting therapeutic response. However, other studies have documented the importance of the period when HCV is cleared from the serum (we define this as the

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"undetectable time point").[9–13] When an undetectable time point is achieved within 4 weeks of therapy initiation, the SVR rate is high. In contrast, the later the undetectable time point, the lower the SVR rate. One disadvantage with this prediction method during therapy is that SVR cannot be predicted until serum viral clearance. If one can predict the undetectable time point early during the treatment, physicians can modify and optimize the ongoing treatment.

There are various patterns of patient response to IFN therapy. In clinical settings, the following three response patterns are observed: (i) SVR; (ii) non-virological response (NVR), in which viral loads continue to be detected during therapy; and (iii) relapse, in which viral loads transiently drop below the detection limit but become detectable again after the end of therapy.[8] Mathematical models have been developed for analyzing therapy-induced changes in HCV viral load. Neumann *et al.*[14] introduced a model for IFN monotherapy in 1998, and a pharmacokinetic model for PEG-IFN has been developed by Powers *et al.*[15] These models are very useful for understanding the therapeutic effects of IFN on HCV.

In recent years, techniques to quantify serum viral RNA levels have advanced. The detection limit and the dynamic range of the quantitative real-time polymerase chain reaction (PCR) assay are lower and wider than those of Amplicor PCR assay.[16,17] As a result, the real-time PCR assay can show us the more accurate viral dynamics. In the present study, we used the model of Powers *et al.*[15] and real-time PCR to measure serum viral loads. Our aim was to ascertain whether it is possible to predict the undetectable time point during the early stage of PEG-IFN-α-2b/ribavirin combination therapy for genotype 1b patients with a high viral load, which is the most difficult-to-treat phenotype of HCV.

METHODS

Patients

THE MODEL PREPARATION group comprised 35 patients with biopsy-proven chronic hepatitis C who were treated at the Musashino Red Cross Hospital from 2000–2001. All patients had HCV genotype 1b and a high viral load (>100 000 IU/mL) as determined by the Amplicor-HCV Monitor Assay (Roche Diagnostics, Tokyo, Japan). Patients with other liver disease, such as liver cirrhosis, autoimmune hepatitis or alcoholic liver injury, were excluded. None of the patients had hepatitis B virus-related antigens, antibodies or anti-HIV antibodies. At the time of enrollment, it was confirmed that none of the patients were taking drugs that could affect their immune system. The dosage of ursodeoxycholic acid and glycyrrhizin was not changed during therapy.

The model validation group comprised 70 patients with biopsy-proven chronic hepatitis C who were treated at the Musashino Red Cross Hospital from 2004–2006. As with the model preparation group, all patients had HCV genotype 1b and a high viral load, and patients with liver cirrhosis or alcoholic liver injury were excluded. None of the patients had hepatitis B virus-related antigens, antibodies or anti-HIV antibodies.

Informed consent was obtained from all patients in writing. The present study was approved by the Ethics Review Board of Musashino Red Cross Hospital in accordance with the Declaration of Helsinki.

Treatment protocol

All patients received at least 48 weeks of PEG-IFN-α-2b (PegIntron; Schering-Plough, Kenilworth, NJ, USA) and ribavirin (Rebetol; Schering-Plough) combination therapy. In the model validation group, if viral clearance was not achieved by week 12, combination therapy was prolonged to 72 weeks. PEG-IFN-α-2b (1.5 μg/kg per week) was administrated s.c. Ribavirin was administrated p.o. at 600 mg/day twice daily to patients weighing less than 60 kg, and 800 mg/day was given to patients weighing between 60 and 80 kg. The dosage of PEG-IFN-α-2b was reduced to 0.75 μg/kg per week when white blood cells, neutrophils or platelets dropped below 1500, 750 or $80 \times 10^3/mm^3$, respectively. When hemoglobin concentration dropped below 10 g/dL, the dosage of ribavirin was reduced from 600 to 400 mg/day for patients weighing less than 60 kg, and from 800 to 600 mg/day for patients weighing between 60 and 80 kg. Both drugs were discontinued when white blood cells, neutrophils, platelets or hemoglobin levels dropped below $1000/mm^3$, $500/mm^3$, $50 \times 10^3/mm^3$ or 8.5 g/dL, respectively.

HCV dynamics in serum

To analyze viral dynamics, serum samples were collected from each patient according to the following schedule with respect to the start of PEG-IFN-α2b/ribavirin combination therapy: immediately before and at 4, 8 h, and 1, 2, 4, 7, 8, 14 and 28 days after the therapy was started; and then at 4-week intervals until completion of the therapy. HCV viral loads were measured in all serum samples using the Abbott RealTime HCV assay (Abbott Molecular, Des Plaines, IL, USA) at an Abbott laboratory in the USA.[16] The dynamic range

was 1.08–8 $\log_{10}$ IU/mL. The assay is standardized to the 2nd World Health Organization (WHO) International Standard for HCV RNA (National Institute for Biological Standards and Control code 96/798). Nucleic acid extraction was performed on 0.5-mL samples using an Abbott *m2000sp* (Abbott Molecular). The Abbott *m2000rt* (Abbott Molecular) was used for reverse transcription, PCR amplification and detection/quantification. A single-stranded linear probe was used as the HCV probe.

Definitions of response to therapy

The undetectable time point was defined as the first time the viral load dropped below the detection limit (1.08 $\log_{10}$ IU/mL) during therapy. Patients with SVR had no detectable viral load 6 months after the end of PEG-IFN-α-2b/ribavirin combination therapy. Patients in relapse had no detectable viral load at the end of therapy but had a detectable viral load 6 months after the end of therapy. Patients with NVR had a detectable viral load throughout the treatment period.

Calculation of the HCV dynamic parameters

Hepatitis C virus dynamic parameters (c, δ, ε, T_0 and V_0) were calculated from viral loads with equations for HCV dynamics.[15] The parameter c is the constant viral death rate, δ is the death rate of infected cells, ε is the effect of PEG-IFN on blocking production of virus from infected cells, and T_0 and V_0 are the numbers of uninfected cells and virus at the start of therapy, respectively.

Statistical analysis

SAS ver. 9.13 was used for the statistical analysis. *P*-values of less than 0.05 were considered significant.

RESULTS

Baseline patient characteristics

TABLE 1 SHOWS the baseline characteristics of the patients. The SVR rate was 60% and 27 patients accomplished undetectable serum HCV until 24 weeks after the therapy was started. The therapy was discontinued in three of the 35 patients because of a reduction in

Table 1 Patient characteristics at baseline

	Model preparation group ($n = 35$)	Model verification group ($n = 70$)
Age (years)	52.1 ± 9.9	57.8 ± 11
Sex (male/female)	24/11	36/34
BMI	23.7 ± 2.9	23.9 ± 3.7
Hemoglobin (g/dL)	14.7 ± 1.2	14.2 ± 1.6
Platelet count (×10³/μL)	17.9 ± 4.8	15.5 ± 5.2
Albumin (g/dL)	4.2 ± 0.33	3.92 ± 0.048
ALT (U/L)	91.7 ± 64	80.0 ± 7.4
Liver histology (Metavir score)		
A (0/1/2/3/4/not measured)	0/17/13/5/0/0	0/40/26/2/0/2
F (0/1/2/3/4/not measured)	0/17/15/3/0/0	2/23/25/18/0/2
Viral load (log IU/mL)		
At pretreatment	5.49 ± 0.52	5.54 ± 0.92
At 7th day of treatment	4.05 ± 0.98	4.75 ± 1.05
at 14th day of treatment	3.23 ± 1.41	4.23 ± 1.29
Durations of therapy		
(48 weeks/72 weeks/dropout)	32/0/3	45/7/18
Drug adherence†		
(PEG-IFN/ribavirin/both/non-)	7/5/2/21	6/21/30/13
Outcome (SVR/relapse/NVR)	21/6/8	20/26/24
Actual undetectable time point‡		
(14/28 days/8/12/16/20/24/28/32 weeks/therapy end)	3/7/8/4/1/2/2/0/0	2/2/12/14/4/4/2/2/4

†Patients numbers with dose reduction during the therapy.
‡NVR cases were excluded.
BMI, body mass index; ALT, alanine aminotransferase; PEG-IFN, pegylated interferon; SVR, sustained virological response; NVR, non-virological response.

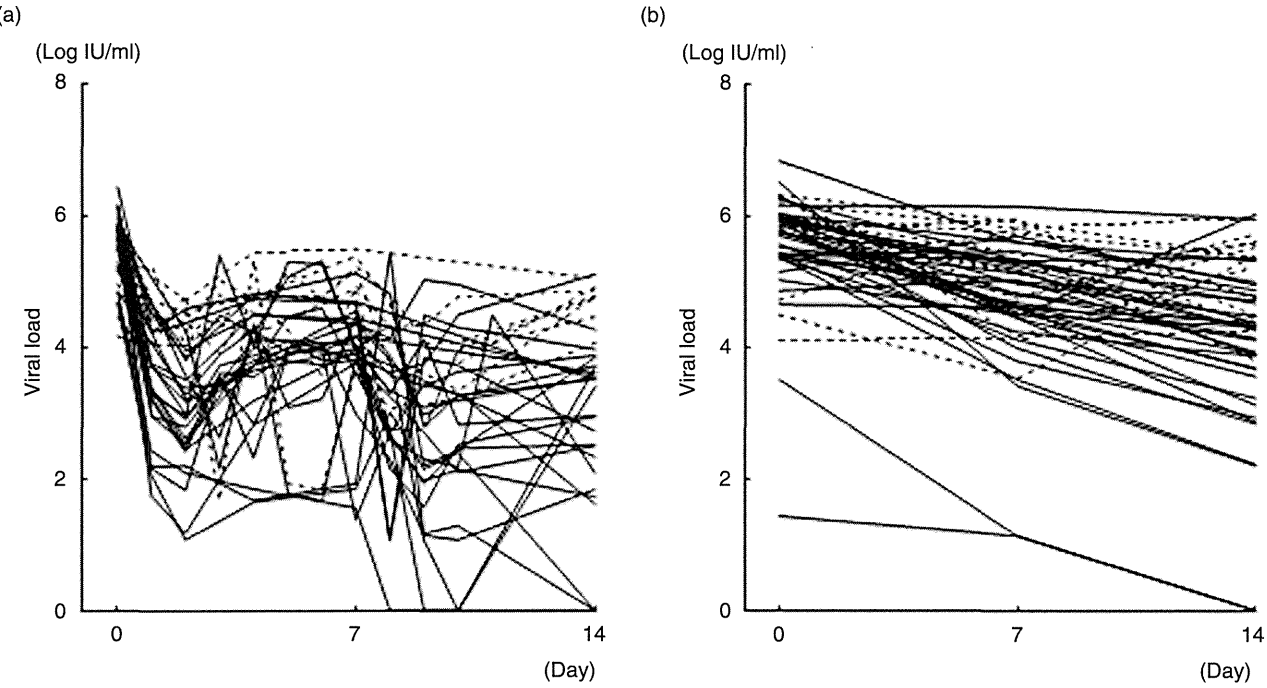

Figure 1 Early hepatitis C virus (HCV) dynamics of model preparation group (a) and of model validation group (b). The patients with incomplete blood collection were excluded from the figure of the model validation group. Solid line, dynamics of those who accomplished undetectable serum HCV until the therapy ended; dotted line, of those in whom serum HCV was detected through the whole therapy.

the hemoglobin concentration, a reduction in the neutrophil count and a worsening of depressive symptoms. In comparison to the model preparation group, there were more NVR patients, and the SVR rate was 29% in the model validation group. There were six patients who accomplished undetectable serum HCV after 24 weeks, and the latest patients achieved it 40 weeks after the therapy started. More patients had advanced hepatic fibrosis in the model validation group than in the model preparation group. Eighteen patients discontinued the combination therapy for various reasons, for example, decreased neutrophil count. The early HCV dynamics of both group are shown in Figure 1.

Undetectable time point prediction

From the model preparation group, 29 patients were analyzed and six patients were excluded for the following reasons: therapy was discontinued before viral clearance in one patient, PEG-IFN dosage was decreased before viral clearance in three patients, viral load increased during therapy in one patient, and an incomplete series of samples were obtained from one patient.

First, we hypothesized that the HCV dynamic parameters have a possibility to predict the undetectable time point. HCV dynamic parameters were calculated with three dataset patterns of viral loads, as follows: (i) immediately before and at 4, 8 h, and 1, 2, 4, 7 and 8 days; (ii) before and at 8 h, and 1, 2, 4 and 7 days; and (iii) before and at 4, 8 h, and 1, 2, 4 and 7 days after the therapy was started. Unfortunately, no significant factors for prediction of the undetectable time points were detected in these HCV dynamic parameters (Table 2), even when adding parameters of age and sex.

Next, we investigated the possibility using early-stage treatment dynamics. Multiple linear regression analysis was conducted for viral load, and changes in viral load up to day 14 as the explanatory variables and undetectable time points as the objective variables. Among various factors which became significant alone, the decrease in viral load from day 7 to 14 was found to be the best predictor for the undetectable time points by multiple linear regression analysis ($r^2 = 0.67$, Table 3). Then, whole datasets were analyzed again including HCV dynamic parameters, sex, age, viral loads and viral

Table 2 Calculated HCV-dynamic parameters of model preparation group

Dataset	Dataset 1† median (range)	*P*	Dataset 2‡ median (range)	*P*	Dataset 3§ median (range)	*P*
c	0.77 (0.032–5.21)	0.73	1.54 (0.0515–7.58)	0.37	2.75 (0.040–6.19)	0.85
δ	0.0033 (0–0.69)	0.76	0.013 (0–0.99)	0.094	0.053 (0–0.70)	0.91
ε	0.28 (0.023–0.84)	0.30	0.067 (0.0083–0.72)	0.038	0.28 (0.023–0.71)	0.18
T_0	0.36 (0.0001–0.95)	0.63	0.415 (0.0049–0.98)	0.23	0.36 (0.007–0.90)	0.21
V_0	5.49 (4.40–6.69)	0.53	4.99 (4.10–6.48)	0.090	5.29 (4.30–6.69)	0.29
R^2	0.012		0.090		0.056	

†Dataset 1: serum hepatitis C virus (HCV) load immediately before and at 4, 8 h, and 1, 2, 4, 7, 8 days after the therapy was started.
‡Dataset 2: serum HCV load before and at 8 h, and 1, 2, 4, 7 days after the therapy was started.
§Dataset 3: serum HCV load before and at 4, 8 h, and 1, 2, 4, 7 days after the therapy was started.

load changes. The results showed that only the change in viral load from day 7 to 14 was associated with the prediction of the undetectable time point ($r^2 = 0.67$). Finally, prediction in each patient was valid (Cook's D = 0.046, mean, data not shown), and we derived the following prediction formula:

Undetectable time point (week) = 13.495 × (viral load at day 14 [log IU/mL] − viral load at day 7 [log IU/mL]) + 25.456.

The degree of decrease in viral load from day 7 to 14 for the model preparation group and the actual

Table 3 Early viral dynamics of model preparation group, correlation to undetectable time point and the result of multiple linear regression analysis

	Viral load (log IU/mL)	Spearman's rank correlation test coefficient (*P*-value)	Multiple linear regression analysis r^2 (*P*-value)
Pretreatment (0 days)	5.48 ± 0.30	0.27 (0.28)	Excluded
4 h	5.66 ± 0.22	0.045 (0.82)	Excluded
8 h	5.55 ± 0.19	0.026 (0.89)	Excluded
1 day	3.74 ± 0.75	0.68 (<0.001)	Excluded
2 days	3.20 ± 0.76	0.66 (<0.001)	Excluded
4 days	4.01 ± 0.74	0.56 (0.002)	Excluded
7 days	4.05 ± 0.75	0.77 (<0.001)	Excluded
8 days	3.34 ± 0.80	0.67 (<0.001)	Excluded
14 days	3.52 ± 0.95	0.87 (<0.001)	Excluded
Subtracted values of viral load (log scale)			
1 day – 0 days	−1.78 ± 0.88	0.59 (0.001)	Excluded
2 days – 0 days	−2.18 ± 0.79	0.53 (0.003)	Excluded
4 days – 0 days	−1.46 ± 0.65	0.72 (0.000)	Excluded
7 day – 0 days	−1.38 ± 0.80	0.38 (0.049)	Excluded
14 days – 0 days	−2.24 ± 1.17	0.83 (0.000)	Excluded
2 days – 1 day	−0.55 ± 0.13	0.085 (0.67)	Excluded
4 days – 1 day	0.17 ± 0.25	0.22 (0.27)	Excluded
7 days – 1 day	0.44 ± 0.46	0.27 (0.19)	Excluded
14 days – 1 day	−0.42 ± 0.46	0.76 (<0.001)	Excluded
4 days – 2 days	0.61 ± 0.23	0.12 (0.54)	Excluded
7 days – 2 days	0.86 ± 0.50	0.12 (0.56)	Excluded
14 days – 2 days	0.11 ± 0.44	0.76 (<0.001)	Excluded
7 days – 4 days	−0.11 ± 0.17	0.047 (0.82)	Excluded
14 days – 4 days	−0.7 ± 0.37	0.78 (<0.001)	Excluded
14 days – 7 days	−0.86 ± 0.50	0.76 (<0.001)	0.667 (<0.0005)

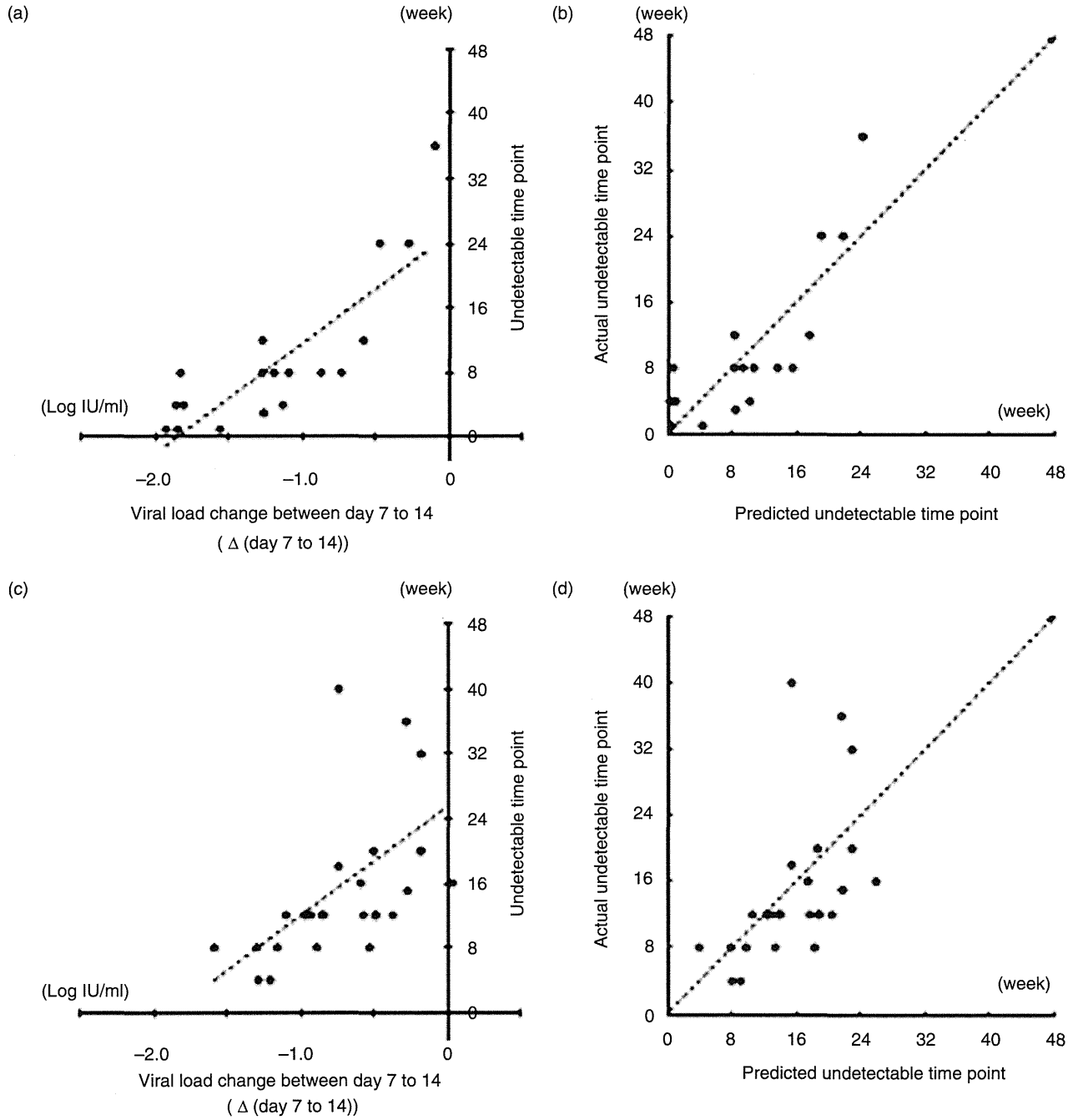

Figure 2 Correlation between the undetectable time point and the decrease in viral load from day 7 to 14 (a,b) and correlation between the actual and predicted undetectable time points (c,d). (a,c) Results of analyses for the model preparation group; and (b,d) analyses for the model validation group. Black circles, actual cases; dotted line, (a,c) estimate obtained from the prediction formula; (b,d) equal values of actual and predicted undetectable time points.

undetectable time point are plotted in Figure 2(a), which shows a very strong and a significant correlation ($r^2 = 0.67$, $P < 0.0005$).

The validity of the prediction formula was investigated in the validation group. Analysis was possible in 32 patients, as the other patients were excluded from the analysis due to the following reasons: therapy was discontinued before viral clearance in eight patients, PEG-IFN dosage was reduced before viral clearance in nine patients and viral clearance was achieved before day 14 in two patients. There were six cases of NVR, and incomplete blood collections from 13 patients on day 7 and/or 14. A strong and a significant correlation was demonstrated between the undetectable time points that were predicted using this formula and the actual undetectable time points (Fig. 2c, $r = 0.53$, $P = 0.005$).

Although only one case was predicted to achieve a rapid virological response (undetectable viral load at week 4)[13] in the model validation group, the actual undetectable time point of this patient was week 8 (Fig. 2d). In contrast, all nine cases who were predicted to achieve a complete early virological response (undetected viral load until week 12),[13] the actual undetectable time points of these patients were within week 12. Because the prediction formula was derived by the least squares method, half of the patients, who were predicted not to achieve the complete early virological response, actually achieved it.

DISCUSSION

NUMEROUS STUDIES HAVE documented that the undetectable time point is related to therapeutic responses, and its usefulness in predicting therapeutic efficacy is clear.[9–13] In the present study, we were able to derive a formula for predicting the undetectable time point for patients with HCV genotype 1b and high serum viral loads during PEG-IFN-α-2b/ribavirin combination therapy. Though the various parameters for the HCV dynamics were investigated, the change in viral load from day 7 to 14 was the only parameter that was useful for predicting the undetectable time point.

The standard length of PEG-IFN/ribavirin combination therapy is 48 weeks for patients with HCV genotype 1b and high serum viral loads; however, a 72-week administration is recommended to improve therapeutic response.[3,13,18] Therefore, when undetectable time points are predicted as from weeks 13–24 by our formula, the SVR rates could be improved by continuing the IFN therapy for longer periods. By prediction of the undetectable time point early during the treatment using our formula, the physician can make early modification and optimization of currently ongoing therapy.

Another important issue of PEG-IFN/ribavirin treatment is adherence to treatment. Because dose reductions may delay the time until serum viral clearance, patients in whom the dosage of IFN and ribavirin was reduced during therapy were excluded in the present study. However, there are many patients in whom the dosage of drugs has to be reduced during therapy for a wide variety of clinical reasons. If reducing dosage before the predicted undetectable time point, administration of IFN for longer periods should be considered.

In conclusion, we created a formula for predicting the undetectable time point in patients treated with PEG-IFN-α-2b/ribavirin combination therapy. Viral eradication is the ultimate objective of IFN-based therapy, but many patients failed to achieve viral eradication for some reason. Because our prediction formula for the undetectable time point was made with a small population, it is necessary to correct it by further analysis with a larger population. However, an early viral response reflects efficacy of the therapy, and the estimation of an undetectable time point by our formula would be useful for determining the optimal duration of treatment in the early period of the therapy for each chronic hepatitis C patient.

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