Table 2 Incidence of adverse events that occurred in two or more patients

	N = 15			
	Mild n (%)	Moderate n (%)	Severe n (%)	Total n (%)
Rash	5 (33.3)	3 (20.0)	0 (0.0)	8 (53.3)
Anaemia	7 (46.7)	0 (0.0)	0 (0.0)	7 (46.7)
Low-density lipoprotein increased	6 (40.0)	0 (0.0)	0 (0.0)	6 (40.0)
Blood uric acid increased	4 (26.7)	0 (0.0)	0 (0.0)	4 (26.7)
Pruritus	3 (20.0)	1 (6.7)	0 (0.0)	4 (26.7)
Anorexia	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Dysgeusia	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Headache	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Diarrhoea	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Pyrexia	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Thirst	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Nasopharyngitis	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Blood creatinine increased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Blood triglycerides increased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Platelet count decreased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Dizziness	1 (6.7)	1 (6.7)	0 (0.0)	2 (13.3)

MedDRA (Ver.12.0).

more than 25% of patients were rash (53.5%), anaemia (46.7%), low-density lipoprotein (LDL) increases (40.0%), blood uric acid increase (26.7%) and pruritus (26.7%). Two patients discontinued telaprevir treatment because of AEs (herpes zoster or rash pruritic). Except for the herpes zoster whose severity was judged as severe and serious, all the events were mild to moderate. Fifty of the 80 AEs were observed within the first 4 weeks.

In relation to skin AEs, rash, pruritus and rash pruritic were observed in 8, 4 and 1 patients, respectively. The onset day of these events is described in Fig. 2. The range of the onset day was Day 1 to Day 113, and the median was Day 15. Rash in three patients, pruritus in one patient and rash pruritic in one patient were moderate, and the others were mild. One patient discontinued telaprevir at Week 6 because of moderate rash pruritic. Most of the skin AEs were treated with oral antihistamines or topical steroids.

A decrease in haemoglobin levels was observed in all patients (Fig. 3a). Seven of 15 patients developed anaemia during and after the treatment. All anaemia events were mild and no patient needed discontinuation of telaprevir. Uric acid and LDL cholesterol increased during the treatment (Fig. 3b,c), but these changes were mild and no patient needed any medication for these AEs. There were no substantial increases in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (T-bil).

Sequence analysis at HCV NS3 protease domain

Amino acid substitutions in the NS3 protease domain were examined in 39 clones or more in each sample. Before Week 8, V36A/G, T54A and A156T/V as single substitutions, and T54A + R155K and A156T/V + V158I as multiple substitutions were observed. Among two patients who discontinued telaprevir within 2 weeks, all clones but three in one patient were wild-type variants after withdrawal of telaprevir. In three patients who discontinued at Weeks 5–7 because of viral breakthrough, predominant clones possessed A156V/T substitutions after the nadir of viral load. Predominant variants observed during and after telaprevir monotherapy in the eight patients who received telaprevir beyond 8 weeks are shown in Fig. 4 together with HCV RNA levels. In the two patients who showed the lowest HCV RNA level of on Week 4, the predominant clones detected after
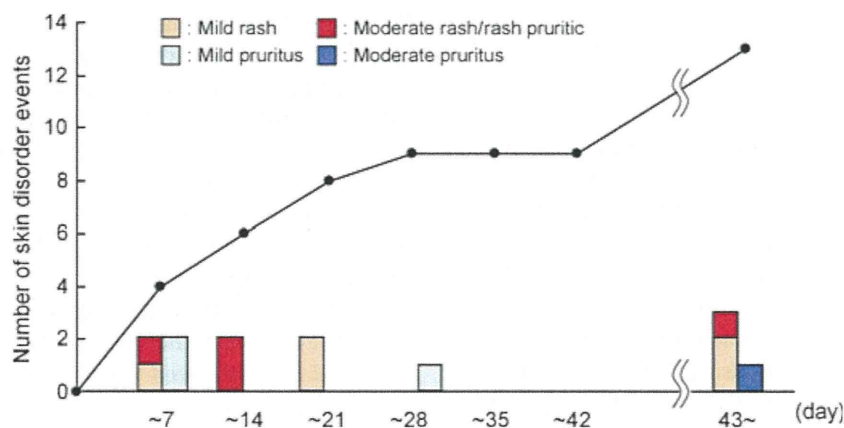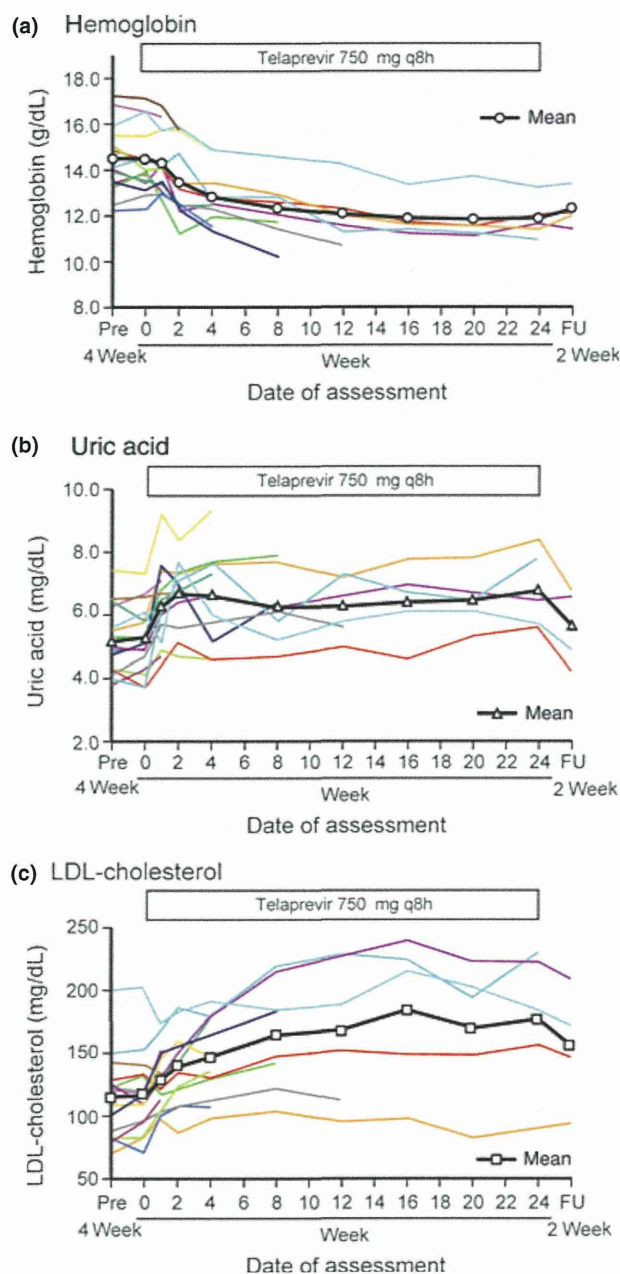

Fig. 2 Rash and pruritus occurrence.

Fig. 3 Changes in (a) hemoglobin, (b) uric acid, (c) LDL-cholesterol.

viral breakthrough were A156F and T54A. One other patient with nadir HCV RNA level on Week 8 had a predominant clone of T54A + I132L after viral breakthrough. Among the five patients who completed the telaprevir treatment for 24 weeks as scheduled, two patients were HCV RNA positive at the end of treatment. One of these two patients had an A156F substitution at the end of treatment, and a A156Y substitution was also detected on Week 1 of the follow-up period. In the two patients who relapsed during the follow-up period, the predominant clone was T54A which shifted to the wild-type variant in one patient.

DISCUSSION

Although higher SVR rates and shorter duration of treatment were achieved by telaprevir in combination with PEG-IFN and RBV in US, EU and Japan [2–6], the DAA combination regimens also increased the frequency and severity of side effects usually observed in the PEG-IFN and RBV therapy. As most patients in Japan are aged people, IFN-free regimens are in urgent need because these patients are intolerant to IFN-based therapies [12–14].

In this exploratory study, one of 15 patients on telaprevir monotherapy was able to achieve SVR. A low viral load of <4 $\log_{10}$ IU/mL in this patient probably contributed to the achievement of SVR, and Suzuki *et al.* [15] published this case report in detail. Although the SVR rate obtained in the study was not beneficial enough, the telaprevir monotherapy could decrease HCV RNA levels dramatically in all cases. The severity of skin-related AEs during telaprevir monotherapy was milder than those of cases developing in the co-administration with PEG-IFN and RBV [5,6,16–18]. All the events were mild to moderate and manageable with antihistamines or topical steroids. Similarly to the skin-related events, decreases in haemoglobin levels were mild, and the incidence of anaemia was 46.7%. As all the anaemia events were mild, there was no need for discontinuation of telaprevir or use of any medications. Severe skin rash and anaemia observed in the therapy with telaprevir in combination with PEG-IFN and RBV are probably ascribable to the synergistic effect of these three drugs. Although the mechanism of uric acid and LDL cholesterol elevation during treatment with telaprevir has been established, these changes disappeared at the end of telaprevir dosing. Telaprevir was generally well tolerated in all the patients.

Amino acid substitutions in the HCV NS3 protease domain were monitored during the study. The relationship between these substitutions and resistance to NS3-4A protease inhibitors has been well documented by *in vitro*, *in vivo* and clinical studies [19–22]. In the eight patients who received the telaprevir monotherapy beyond 8 weeks, the predominant breakthrough variants were T54A and A156F, which were not observed at the earlier time points (Fig. 4). Furthermore, in the clones accounting for more than 10% of each specimen, the secondary substitution of V158I and I132L was identified along with the primary resistant-associated substitution of A156T/V and T54A, respectively, and a novel substitution of A156Y was also observed. This study confirms the higher genetic barrier of HCV subtype 1b against the V36M ± R155K substitutions. Our results clearly indicate that the prolonged telaprevir monotherapy leads to the development of various variants. As the replication fitness of drug-resistant variants tends to be lower than that of wild type, the former are likely to be overtaken by the wild-type virus under drug-free conditions within 3–7 months [11,23,24]. As Ozeki *et al.* [25] reported that four patients with favourable IL28B SNP who failed to eradicate HCV with telaprevir monotherapy were
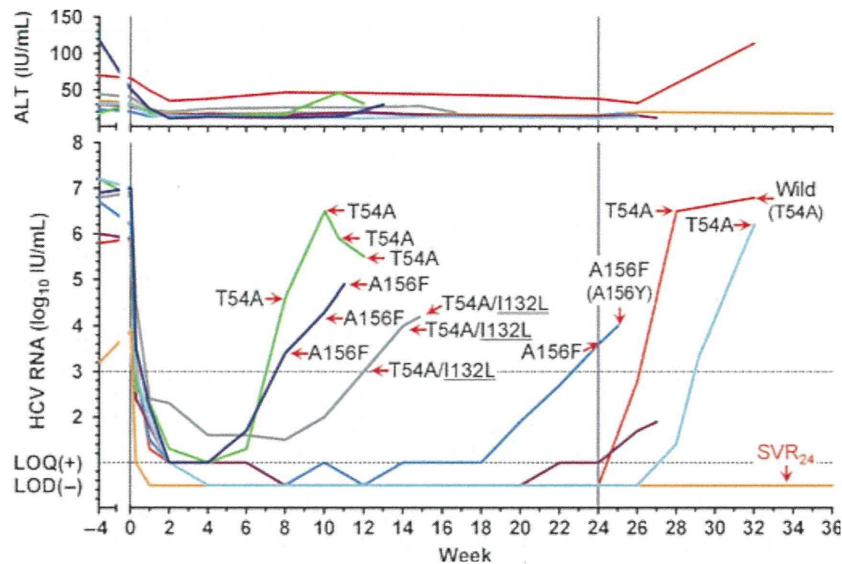
Fig. 4 Viral kinetics and predominant variants during and after telaprevir monotherapy beyond 8 weeks. Besides predominant clones, minority clones which account for 10% and more in a specimen are also summarized by brace notation. Putative secondary resistant-associated mutation is indicated by underline.

responsive to sequential therapy with PEG-IFN and RBV, the substitutions in the NS3 protease domain by the telaprevir treatment are not correlated with resistance to PEG-IFN and/or RBV directly as described previously [23,24]. Sequential therapy with PEG-IFN and RBV after relapse or viral breakthrough on telaprevir monotherapy might be a therapeutic option in some cases, including the case of low haemoglobin. By taking the error-prone nature of HCV replication into account, successful eradication with IFN-free DAA(s) regimens probably depends on how efficiently DAA can suppress various DAA-resistant variants that pre-exist and are selected under DAA pressure. The telaprevir-based combination therapy with other DAA(s) such as NS5A or NS5B polymerase inhibitors may be useful for successful treatment. Using a human chimeric liver mouse model for HCV infection, Ohara *et al.* [26] reported that the combination of telaprevir with a high-dose nucleoside analogue could successfully eradicate HCV infection. Recently, it was reported that the dual therapy with daclatasvir, an NS5A replication complex inhibitor, and asunaprevir, NS3-4A protease inhibitor, had high SVR rates in difficult-to-treat patients with subtype 1b and null responders [27,28]. These successful results are also

helpful for us to consider telaprevir-based IFN-free regimens in combination with other DAAs against HCV.

In conclusion, telaprevir monotherapy was well tolerated and provided potent but temporary antiviral activity in Japanese patients with subtype 1b HCV, with an SVR rate of 7%. Most AEs were mild to moderate and much milder than those recorded in patients on combinations with PEG-IFN and RBV. As the essential characteristics of DAAs including telaprevir are substantially masked in the co-administration with other antivirals, the knowledge obtained from the long-term telaprevir monotherapy is most likely to contribute to the future HCV treatment with DAA-based regimens.

ACKNOWLEDGEMENT AND DISCLOSURES

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

N. Tamaki, M. Kurosaki, K. Tanaka, Y. Suzuki, Y. Hoshioka, T. Kato, Y. Yasui, T. Hosokawa, K. Ueda, K. Tsuchiya, H. Nakanishi, J. Itakura, Y. Asahina and N. Izumi *Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan*

SUMMARY. The FIB-4 index is a simple formula to predict liver fibrosis based on the standard biochemical values (AST, ALT and platelet count) and age. We here investigated the utility of the index for noninvasive prediction of progression in liver fibrosis. The time-course alteration in the liver fibrosis stage between paired liver biopsies and the FIB-4 index was examined in 314 patients with chronic hepatitis C. The average interval between liver biopsies was 4.9 years. The cases that showed a time-course improvement in the fibrosis stage exhibited a decrease in the FIB-4 index, and those that showed deterioration in the fibrosis stage exhibited an increase in the FIB-4 index with a significant correlation ($P < 0.001$). Increase in the ΔFIB-4 index per year was an independent predictive factor for the progression in

liver fibrosis with an odds ratio of 3.90 ($P = 0.03$). The area under the receiver operating characteristic curve of the ΔFIB-4 index/year for the prediction of advancement to cirrhosis was 0.910. Using a cut-off value of the ΔFIB-4 index/year <0.4 or ≥0.4, the cumulative incidence of fibrosis progression to cirrhosis at 5 and 10 years was 34% and 59%, respectively in patients with the ΔFIB-4 index/year ≥0.4, whereas it was 0% and 3% in those with the ΔFIB-4 index/year <0.4 ($P < 0.001$). In conclusion, measurement of the time-course changes in the FIB-4 index is useful for the noninvasive and real-time estimation of the progression in liver fibrosis.

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

INTRODUCTION

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

A variety of noninvasive methods to diagnose liver fibrosis have been proposed. Recently, transient elastography [10–13] and real-time tissue elastography [14] using ultrasonography

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

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

PATIENTS AND METHODS

Patients

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

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

Correspondence: Namiki Izumi, MD, Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino-shi, Tokyo 180-8610, Japan. E-mail: nizumi@musashino.jrc.or.jp

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

Assessment of liver fibrosis stage

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

Calculation of FIB-4 index

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

Statistical analysis

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

RESULTS

Changes in liver fibrosis stage overtime

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

Comparison of FIB-4 index and liver fibrosis stage

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

Table 1 Clinical background of patients

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

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 2 Changes of fibrosis stage over time

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

(a)

FIB-4 index at first biopsy
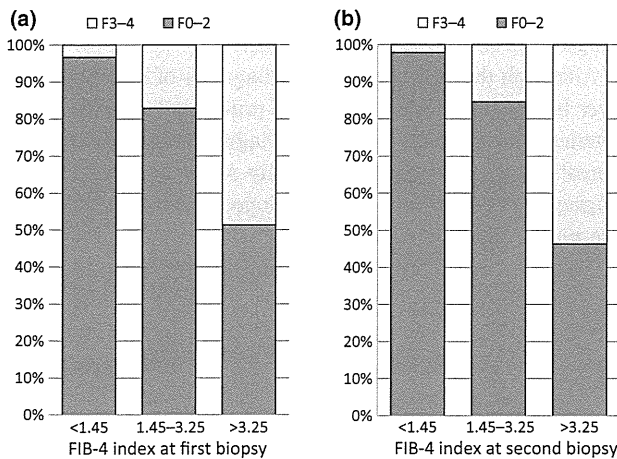
(b)

FIB-4 index at second biopsy

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

Predictive factors for the progression of fibrosis

Higher level of ΔAST/year, lower level of ΔALT/year, lower level of ΔPlatelet counts/year and higher level of the ΔFIB-4/year were significantly associated with the progression of fibrosis overtime (Table 3). Multivariate analysis demonstrated that only the ΔFIB-4 index/year was an independent

predictive factor for the progression of fibrosis stage (P = 0.03) with an odds ratio of 3.70 (95% CI:1.07–12.5).

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

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

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

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

DISCUSSION

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

Table 3 Factors associated with the progression of liver fibrosis

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

ΔAST/year: (AST at the second liver biopsy − AST at the first liver biopsy) /interval between paired biopsies (years); ΔALT/year: (ALT at the second liver biopsy − ALT at the first liver biopsy) /interval between paired biopsies (years); ΔPlatelet counts/year: (platelet counts at the second liver biopsy −platelet counts at the first liver biopsy) /interval between paired biopsies (years); ΔFIB-4 index /year: (the FIB-4 index at the second liver biopsy − the FIB-4 index at the first liver biopsy) /interval between paired biopsies (years).
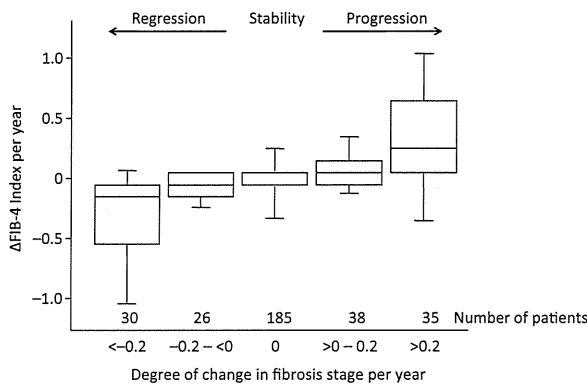
Fig. 2 Correlation between the degree of changes in the fibrosis stage and the ΔFIB-4 index per year. Boxplot of the ΔFIB-4 index/year is shown according to the degree of changes in the fibrosis stage per year. The bottom and top of each box represent the 25 and 75th percentiles, giving the interquartile range. The line through the box indicates the median value, and the error bar indicates the 5 and 95th percentiles.
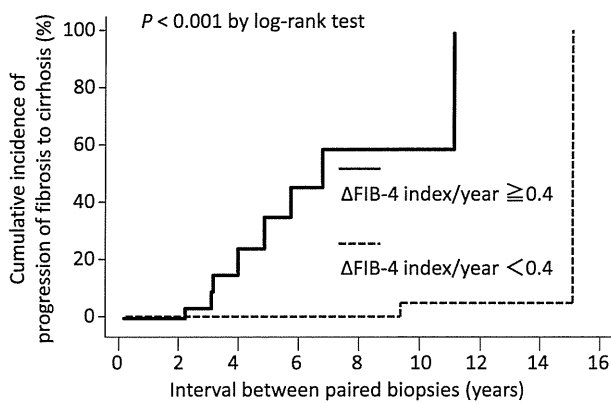

Fig. 3 Cumulative incidence of fibrosis progression to cirrhosis. Patients were categorized into two groups according to the ΔFIB-4 index/year using cut-off value of < 0.4 or ≥ 0.4.

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

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

be useful for the noninvasive and real-time estimation of the progression in liver fibrosis overtime.

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

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

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

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

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

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CONFLICT OF INTEREST

No conflicts of interest exist for all authors.

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Model Incorporating the *ITPA* Genotype Identifies Patients at High Risk of Anemia and Treatment Failure With Pegylated-Interferon Plus Ribavirin Therapy for Chronic Hepatitis C

Masayuki Kurosaki,[1] Yasuhito Tanaka,[2] Nao Nishida,[3] Naoya Sakamoto,[4] Nobuyuki Enomoto,[5] Kentaro Matsuura,[2] Yasuhiro Asahina,[6] Mina Nakagawa,[6] Mamoru Watanabe,[6] Minoru Sakamoto,[5] Shinya Maekawa,[5] Katsushi Tokunaga,[3] Masashi Mizokami,[7] and Namiki Izumi[1]*

[1]Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan
[2]Department of Virology, Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan
[3]Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[4]Department of Gastroenterology and Hematology, Hokkaido University, Sapporo, Japan
[5]First Department of Internal Medicine, University of Yamanashi, Yamanashi, Japan
[6]Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan
[7]Research Center for Hepatitis and Immunology, International Medical Center of Japan Konodai Hospital, Ichikawa, Japan

This study aimed to develop a model for predicting anemia using the inosine triphosphatase (*ITPA*) genotype and to evaluate its relationship with treatment outcome. Patients with genotype 1b chronic hepatitis C (n = 446) treated with peg-interferon alpha and ribavirin (RBV) for 48 weeks were genotyped for the *ITPA* (rs1127354) and *IL28B* (rs8099917) genes. Data mining analysis generated a predictive model for anemia (hemoglobin (Hb) concentration <10 g/dl); the CC genotype of *ITPA*, baseline Hb <14.0 g/dl, and low creatinine clearance (CLcr) were predictors of anemia. The incidence of anemia was highest in patients with Hb <14.0 g/dl and CLcr <90 ml/min (76%), followed by Hb <14.0 g/dl and *ITPA* CC (57%). Patients with Hb ≥14.0 g/dl and *ITPA* AA/CA had the lowest incidence of anemia (17%). Patients with two predictors (high-risk) had a higher incidence of anemia than the others (64% vs. 28%, *P* < 0.0001). At baseline, the *IL28B* genotype was a predictor of a sustained virological response [adjusted odds ratio 9.88 (95% confidence interval 5.01–19.48), *P* < 0.0001]. In patients who achieved an early virological response, the *IL28B* genotype was not associated with a sustained virological response, while a high risk of anemia was a significant negative predictor of a sustained virological response [0.47 (0.24–0.91), *P* = 0.026]. For high-risk patients with an early virological response, giving >80% of the planned RBV dose increased sustained virological responses by 24%. In conclusion, a predictive model incorporating the *ITPA* genotype could identify patients with a high risk of anemia and reduced probability of sustained virological response. *J. Med. Virol.* **85:449–458, 2013.**
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KEY WORDS: hemolytic anemia; ribavirin; creatinine clearance; antiviral therapy

INTRODUCTION

Hepatitis C virus (HCV) infection is a leading cause of cirrhosis and hepatocellular carcinoma worldwide [Kim, 2002]. The rate of eradication of HCV by pegylated interferon (PEG-IFN) plus ribavirin (RBV), defined as a sustained virological response, is around 50% in patients with HCV genotype 1 [Manns et al., 2001; Fried et al., 2002]. Failure of treatment is attributable to the lack of a virological response or relapse after completion of therapy. Genome-wide association studies and subsequent cohort studies

have shown that single nucleotide polymorphisms (SNPs) located near the *IL28B* gene are the most important determinant of virological response to PEG-IFN/RBV therapy [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Rauch et al., 2010]. On the other hand, among patients with a virological response, the probability of a sustained virological response decreases when the patients become intolerant to therapy because of RBV-induced hemolytic anemia and receive a reduced dose of RBV [McHutchison et al., 2002; Kurosaki et al., 2012]. Genome-wide association studies have shown that variants of the inosine triphosphatase (*ITPA*) gene protect against hemolytic anemia [Fellay et al., 2010; Tanaka et al., 2011]. These variants are associated with a reduced requirement for an anemia-related dose reduction of RBV [Sakamoto et al., 2010; Thompson et al., 2010a; Kurosaki et al., 2011d; Seto et al., 2011]. However, factors other than the *ITPA* gene also contribute to the risk of severe anemia or RBV dose reduction [Ochi et al., 2010; Kurosaki et al., 2011d] and the results of studies on the impact of the *ITPA* genotype on treatment outcome are inconsistent [Ochi et al., 2010; Sakamoto et al., 2010; Thompson et al., 2010a, 2011; Kurosaki et al., 2011d].

Data mining is a novel statistical method used to extract relevant factors from a plethora of factors and combine them to predict the incidence of the outcome of interest [Breiman et al., 1980]. Decision tree analysis, a primary component of data mining analysis, has found medical applications recently [Averbook et al., 2002; Miyaki et al., 2002; Baquerizo et al., 2003; Leiter et al., 2004; Garzotto et al., 2005; Zlobec et al., 2005; Valera et al., 2007] and has proven to be a useful tool for predicting therapeutic efficacy [Kurosaki et al., 2010, 2011a,b,c, 2012] and adverse events [Hiramatsu et al., 2011] in patients with chronic hepatitis C treated with PEG-IFN/RBV therapy. Because the results of data mining analysis are presented as a flowchart [LeBlanc and Crowley, 1995], they are easily understandable and usable by clinicians lacking a detailed knowledge of statistics.

For the general application of this genetic information in clinical practice, this study aimed to construct a predictive model of severe anemia using the *ITPA* genotype, together with other relevant factors. This study also aimed to analyze the impact of the risk of anemia on treatment outcome, after adjustment for the *IL28B* genotype. These analyses were carried out at baseline and during therapy, when the early virological response became evident.

MATERIALS AND METHODS

Patients

Data were collected from a total of 446 genotype 1b chronic hepatitis C patients who were treated with PEG-IFN alpha and RBV at five hospitals and universities throughout Japan. The inclusion criteria were: (1) infection by hepatitis C genotype 1b; (2) no

co-infection with hepatitis B virus or human immunodeficiency virus; (3) no other causes of liver disease such as autoimmune hepatitis and primary biliary cirrhosis; and (4) availability of DNA for the analysis of the genetic polymorphisms of *IL28B* and *ITPA*. Patients received PEG-IFN alpha-2a (180 µg) and 2b (1.5 µg/kg) subcutaneously every week and a daily weight-adjusted dose of RBV (600 mg for patients weighing <60 kg, 800 mg for patients weighing 60–80 kg, and 1,000 mg for patients weighing >80 kg) for 48 weeks. Dose reduction or discontinuation of PEG-IFN and RBV was primarily based on the recommendations on the package inserts and the discretion of the physicians at each university and hospital. The standard duration of therapy was set at 48 weeks. No patient received erythropoietin or other growth factors for the treatment of anemia. Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committees.

Laboratory Tests

Blood samples obtained before therapy were analyzed for hematologic data, blood chemistry, and HCV RNA. Genetic polymorphisms in SNPs of the *ITPA* gene (rs1127354) and the *IL28B* gene (rs8099917) were determined using ABI TaqMan Probes (Applied Biosystems, Carlsbad, CA) and the DigiTag2 assay, respectively. Baseline creatinine clearance (CLcr) levels were calculated using the formula of Cockcroft and Gault [1976]: for males, CLcr = [(140 − age in years) × body weight in kg] ÷ (72 × serum creatinine in mg/dl) and for females, CLcr = 0.85 × [(140 − age in years) × body weight in kg] ÷ (72 × serum creatinine in mg/dl). The stage of liver fibrosis was scored according to the METAVIR scoring system: F0 (no fibrosis), F1 (mild fibrosis: portal fibrosis without septa), F2 (moderate fibrosis: few septa), F3 (severe fibrosis: numerous septa without cirrhosis), and F4 (cirrhosis). A rapid virological response was defined as undetectable HCV RNA by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor, Roche Diagnostic Systems, Pleasanton, CA) at week 4 of therapy and a complete early virological response was defined as undetectable HCV RNA at week 12. A sustained virological response was defined as undetectable HCV RNA at 24 weeks after completion of therapy. Severe anemia was defined as hemoglobin (Hb) <10 g/dl.

Statistical Analysis

Database for analysis included the following variables: age, sex, body mass index, serum aspartate aminotransferase (AST) levels, alanine aminotransferase (ALT) levels, gamma-glutamyltransferase (GGT) levels, creatinine levels, CLcr, Hb, platelet count, serum levels of HCV RNA, and the stage of liver fibrosis

TABLE I. Patients' Baseline Characteristics

Age (years)	58.6	(9.6)
Gender: male (n, %)	185	(42%)
Body mass index (kg/m^2)	23.1	(3.7)
AST (IU/L)	59.9	(53.8)
ALT (IU/L)	69.8	(53.8)
GGT (IU/L)	48.5	(41.6)
Creatinine (mg/dl)	0.7	(0.2)
Creatinine clearance (ml/min)	89.5	(23.0)
Hemoglobin (g/dl)	14	(1.4)
Platelet count (10^9/L)	154.5	(52.1)
HCV RNA > 600,000 IU/ml (n, %)	354	(79%)
Liver fibrosis: F3-4 (n, %)	108	(24%)
Initial ribavirin dose (n, %)		
600 mg/day	300	(67%)
800 mg/day	138	(31%)
1,000 mg/day	9	(2%)
Pegylated interferon (n, %)		
alpha2a 180 mcg	58	(13%)
alpha2b 1.5 mcg/kg	388	(87%)
ITPA rs1127354: CC (n, %)	317	(71%)
IL28B rs809917: TT (n, %)	311	(70%)

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase.
Data expressed as mean (standard deviation) unless otherwise mentioned.

(Table I). Based on these data set, a model for predicting the risk of developing severe anemia was constructed by data mining analysis using the IBM-SPSS Modeler 13 as described previously [Kurosaki et al., 2010, 2011a,b,c; Hiramatsu et al., 2011]. Briefly, the software was used to explore the database automatically to search for optimal predictors that discriminated most efficiently patients with severe anemia from those without. The software also determined the optimal cutoff values of each predictor. Patients were divided into two groups according to the predictor and each of the two groups was repeatedly divided in the same way until no significant factor remained or 20 or fewer patients were in a group.

The incidence of severe anemia, the total dose of RBV, and treatment outcome were compared between groups with high and low risks of anemia. On univariate analysis, Student's *t*-test was used for continuous variables, and Fisher's exact test was used for categorical data. Logistic regression was used for multivariate analysis. *P* values of <0.05 were considered significant. SPSS Statistics 18 was used for these analyses.

RESULTS

Predictive Model of Severe Anemia

The incidence of severe anemia in the whole cohort was 49% (Fig. 1). The best predictor of severe anemia was the baseline Hb concentration. Patients with a low baseline Hb concentration (<14 g/dl) were more likely to develop severe anemia (67%) than those with a higher Hb (>14 g/dl) (34%). The second best predictor for those patients with a baseline Hb <14.0 g/dl was CLcr. Patients with a CLcr below 90 ml/min had

the highest incidence of severe anemia (76%). In those with a CLcr above >90 ml/min the incidence of severe anemia was 57% in patients with the CC allele of the *ITPA* gene while it was 37% in patients with the CA or AA allele. On the other hand, the second best predictor for those patients with a baseline Hb concentration above 14 g/dl was the *ITPA* genotype. Patients with the AA or AC allele had the lowest incidence of anemia (17%). For those with the *ITPA* CC allele, CLcr was the third best predictor; the optimal cutoff value was 85 ml/min for this group. The incidence of severe anemia was 49% in patients with a CLcr below 85 ml/min while it was 32% in those with a CLcr above 85 ml/min.

Following this analysis, the patients were divided into six groups, with the incidence of severe anemia ranging from 17% to 76%. Three groups with two predictors, having an incidence of anemia >40%, were defined as the high-risk group and the remainder were defined as the low-risk group. The incidence of severe anemia was higher in the high-risk group than the low-risk group (65% vs. 28%, *P* = 0.029) (Fig. 2). Comparison of the *ITPA* genotype and the predictive model showed that the sensitivity for the prediction of severe anemia was similar (75.9% vs. 76.4%) but the specificity of the predictive model was greater (33.6% vs. 59.3%).

The Risk of Anemia Impacts on Sustained Virological Responses by Patients Who Achieved an Early Virological Response

The impact of *IL28B* genotype, *ITPA* genotype, and risk group of anemia on the rate of sustained virological response was studied at baseline and week 12. At baseline, patients with the TT allele of the *IL28B* gene had a significantly higher rate of sustained virological response than those with the TG or GG allele (43% vs. 10%, *P* < 0.0001), the high-risk group for anemia had a significantly lower rate of sustained virological response than the low-risk group (28% vs. 40%, *P* = 0.011), and the *ITPA* genotype was not associated with a sustained virological response (Fig. 3A–C). At week 4, patients with rapid virological response had a high rate of sustained virological response, irrespective of the *IL28B* genotype (TT vs. TG/GG; 97% vs. 100%, *P* = 1.000), the *ITPA* genotype (CC vs. CA/AA; 95% vs. 100%, *P* = 1.000), and the risk of anemia (high vs. low; 95% vs. 100%, *P* = 1.000). Among the patients who did not achieve a rapid virological response, those with the *IL28B* TT allele had a significantly higher rate of sustained virological response than those with the TG or GG allele (38% vs. 8%, *P* < 0.0001), and the high-risk group for anemia had a significantly lower rate of sustained virological response than the low-risk group (24% vs. 35%, *P* = 0.015). At week 12, in patients who achieved a complete early virological response, the *IL28B* genotype was not associated with a sustained virological response, while the high-risk group for anemia had a
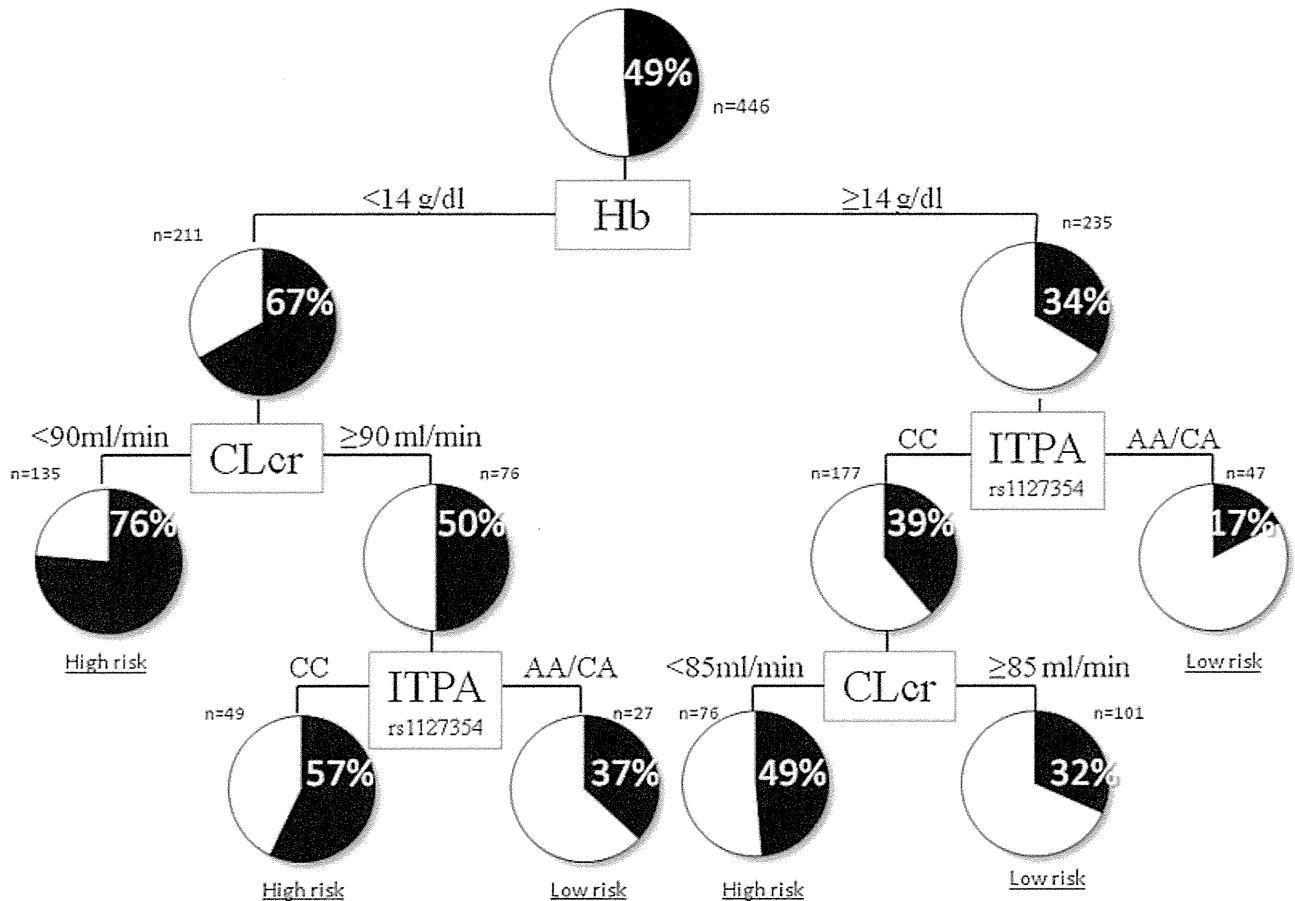
Fig. 1. The predictive model for severe anemia. The boxes indicate the factors used to differentiate patients and the cutoff values for the different groups. The pie charts indicate the rate of severe anemia (Hb <10.0 g/dl) for each group of patients, after differentiation. Terminal groups of patients differentiated by analysis are classified as at high risk if the rate is >40% and low risk if the rate is <40%. *ITPA*, inosine triphosphatase; CLcr, creatinine clearance; Hb, hemoglobin.

significantly lower rate of sustained virological response than the low-risk group (59% vs. 76%, $P = 0.013$) (Fig. 3D–F). In patients who did not achieve a complete early virological response, the *IL28B* genotype was a significant predictor of a sustained virological response (TT vs. TG/GG; 14% vs. 2%, $P < 0.0001$) but a high risk for anemia was not (high vs. low; 10% vs. 6%, $P = 0.361$).

From multivariate analysis (Table II), the *IL28B* genotype was the most important predictor of a sustained virological response at baseline [adjusted odds ratio 9.88 (95% confidence interval 5.01–19.48), $P < 0.0001$], along with female sex [0.42 (0.26–0.68), $P < 0.0001$], platelet count [1.09 (1.04–1.15), $P < 0.0001$], advanced fibrosis [0.49 (0.27–0.91), $P = 0.024$], and baseline HCV RNA load [4.14 (2.27–7.55), $P < 0.0001$]. At week 4, in patients without a rapid virological response, the *IL28B* genotype remained the most important predictor of a sustained virological response [7.16 (3.60–14.25), $P < 0.0001$], along with female sex and platelet count. At week 12, in patients with a complete early virological response, the risk of anemia was an independent and significant

predictor of a sustained virological response [0.47 (0.24–0.91), $P = 0.026$], together with the platelet count and HCV RNA load, but the *IL28B* genotype was not associated with a sustained virological response. In patients without a complete early virological response, the *IL28B* genotype was a predictor of a sustained virological response [9.13 (2.02–41.3), $P = 0.004$] along with the platelet count. Thus, *IL28B* was a significant predictor of a sustained virological response at baseline and among virological non-responders at weeks 4 and 12. On the other hand, once a complete early virological response was achieved, the *IL28B* genotype was no longer associated with a sustained virological response but the risk of anemia was an independent predictor of a sustained virological response.

The Risk of Anemia, RBV Dose, and Treatment Outcome in Patients With a Complete Early Virological Response

Patients who achieved a complete early virological response were stratified according to adherence to
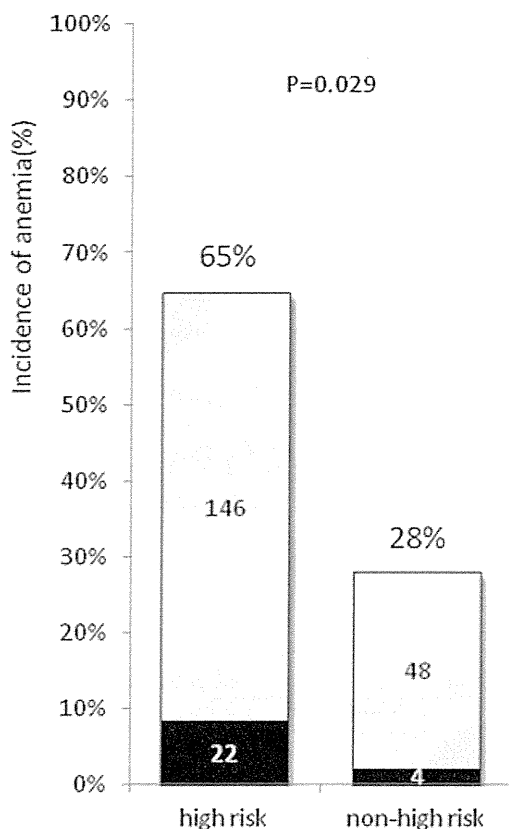
Fig. 2. The incidence of severe anemia stratified by risk of anemia. The incidence of anemia during therapy is shown for each group of patients at high and low risk of anemia. The black and white bars represent the percentages of patients with Hb concentrations below 8.5 g/dl and above 10 g/dl, respectively.

RBV ($\leq$40%, 41–60%, 61–80%, and >80%), which showed that patients with a high risk of anemia were predominantly in subgroups with a lower adherence to RBV ($\leq$40%, 41–60%, and 61–80%), whereas patients with a low risk of anemia were predominantly in subgroups with a higher adherence to RBV (>80%) (Fig. 4, upper panel). The percentage of patients who received >80% of the planned dose of RBV was significantly higher in the low-risk group for anemia than in the high-risk group (74% vs. 55%, $P < 0.0001$).

Within the groups with high and low risks of anemia, there was a stepwise increase in the rate of sustained virological response according to the increase in adherence to RBV (Fig. 4, lower panel). The rate of sustained virological response was higher in patients who received >80% of the planned dose of RBV than those who received less, for both high-risk patients (71% vs. 47%, $P = 0.016$) and low-risk patients (81% vs. 60%, $P = 0.072$). Within the same subgroup of RBV adherence, however, the rate of sustained virological response did not differ between patients with a high risk and a low risk of anemia. Taken together, these results suggest that patients with a high risk of anemia have a disadvantage because they are likely

to be intolerant to RBV, leading to reduced adherence to RBV throughout the 48 weeks of therapy and a reduced rate of sustained virological response. However, if >80% adherence to RBV could be obtained, the rate of sustained virological response would increase by 24%.

DISCUSSION

This study confirmed previous reports that the *IL28B* genotype is the most significant predictor of a sustained virological response to PEG-IFN plus RBV therapy in chronic hepatitis C patients at baseline [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Rauch et al., 2010; Kurosaki et al., 2011c] and at week 4 [Thompson et al., 2010b], but it had no impact on the rate of sustained virological response among those patients who achieved a complete early virological response [Thompson et al., 2010b; Kurosaki et al., 2011c]. In contrast, the risk of anemia, assessed by the combination of the *ITPA* genotype, baseline Hb concentration, and baseline CLcr, was found to be associated with a sustained virological response in patients who achieved a complete early virological response. Generally, a complete early virological response is the hallmark of a high probability of a sustained virological response, but the rate of sustained virological responses in patients who achieved a complete early virological response and had a high risk of anemia was as low as 59%. This reduced rate of sustained virological response in these patients was attributable to poor adherence to RBV throughout the 48 weeks of therapy. Because administration of >80% of the planned RBV dose increased the rate of sustained virological response by 24%, it may be postulated that personalizing the treatment schedule to achieve a sufficient dose of RBV, such as extension of treatment duration, may improve sustained virological response rates in these patients. Clearly, this postulate needs to be confirmed in future study. Thus, the findings presented here may have the potential to support selection of the optimum, personalized treatment strategy for an individual patient, based on the risk of anemia.

The degree of hemolytic anemia caused by RBV varies among individuals. A reduction of the Hb concentration early during therapy predicts the likely development of severe anemia [Hiramatsu et al., 2008, 2011] but there are no reliable predictors at baseline. A breakthrough came from the results of a genome-wide association study that revealed that variants of the *ITPA* gene are protective against hemolytic anemia [Fellay et al., 2010]. The *ITPA* genotype has been shown repeatedly to be associated with the degree of hemolytic anemia and dose reduction of RBV [Fellay et al., 2010; Sakamoto et al., 2010; Thompson et al., 2010a; Seto et al., 2011; Tanaka et al., 2011; Kurosaki et al., 2011d]. However, factors other than the *ITPA* gene, such as baseline Hb concentrations [Ochi et al., 2010; Kurosaki et al., 2011d], platelet counts [Ochi
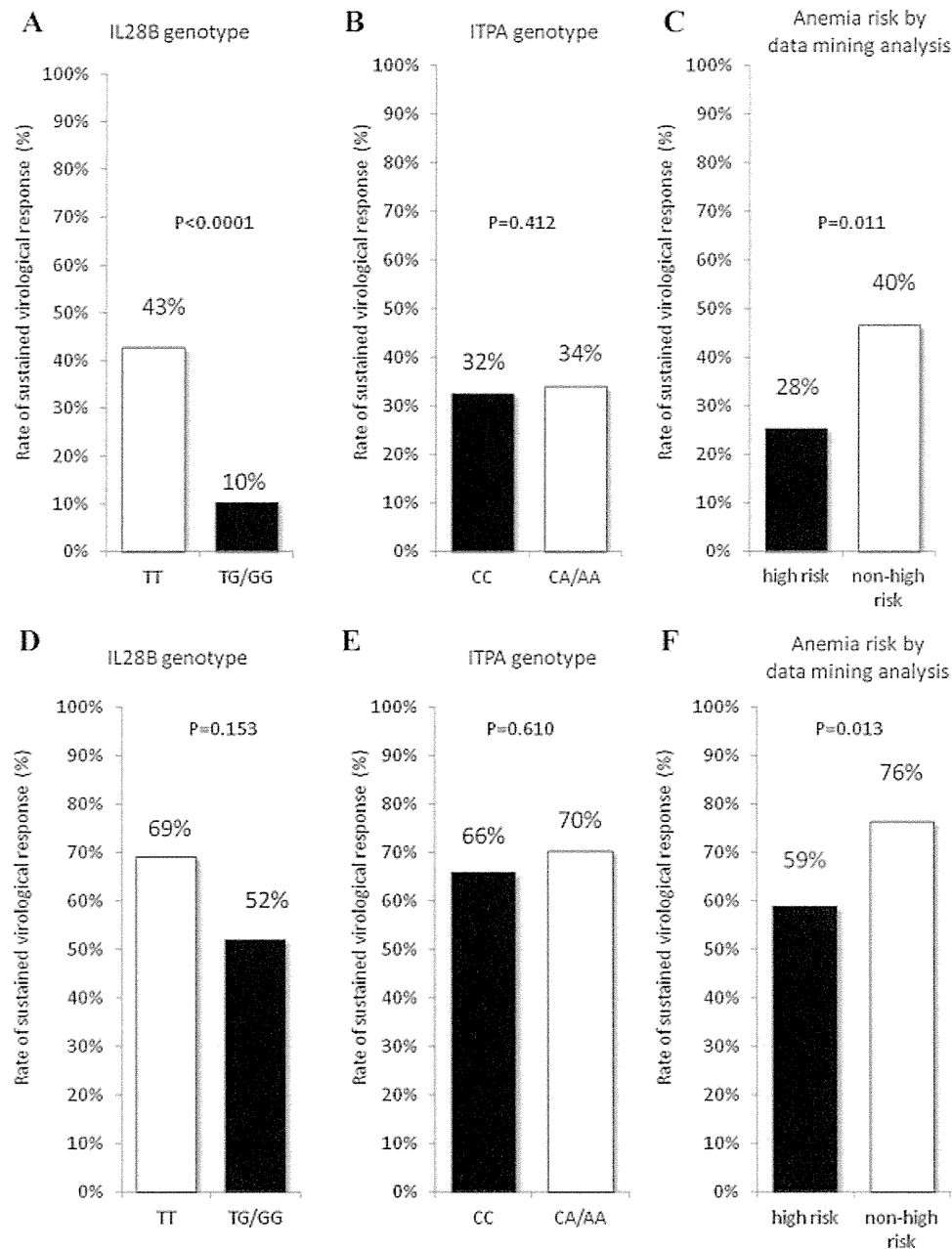
Fig. 3. Rates of sustained virological responses at baseline and among those with a virological re-
sponse at week 12. The impacts of *IL28B* genotype, *ITPA* genotype, and risk group of anemia on the
rate of sustained virological response were studied at baseline (A–C) and among those with complete
early virological responses (defined as undetectable HCV RNA at week 12) (D–F). At baseline, those
with the TT allele of the *IL28B* gene had a significantly higher rate of sustained virological response
than those with the TG or GG allele and the group at high-risk of anemia had a significantly lower
rate of sustained virological response than the low-risk group. Among patients with complete early
virological responses, the *IL28B* genotype was not associated with a sustained virological response,
while the group at high-risk of anemia had a significantly lower rate of sustained virological response
than the low-risk group.

et al., 2010], and CLcr [Kurosaki et al., 2011d], also
contribute to the risk of severe anemia or RBV dose
reduction. In the present study, the predictive model
of anemia based on the data mining analysis selected
the *ITPA* genotype, baseline Hb concentration, and

baseline CLcr as predictive factors and identified six
subgroups of patients with a variable rate of severe
anemia, ranging from 17% to 76%. The specificity of
the prediction of severe anemia was improved by
25.7% in the predictive model, compared to *ITPA*

TABLE II. Logistic Regression Analysis for Factors
Associated With Sustained Virological Response at
Baseline, Week 4 and Week 12

	Multi-variable		
	Odds	95% CI	P-value
Pre-treatment			
Sex: female	0.42	0.26–0.68	<0.0001
Platelet (10^9/L)	1.09	1.04–1.15	<0.0001
Fibrosis: F3-4	0.49	0.27–0.91	0.024
HCV RNA: <600,000 IU/L	4.14	2.27–7.55	<0.0001
IL28B rs8099917: TT	9.88	5.01–19.48	<0.0001
At week 4			
Non-RVR patients			
Sex: female	0.45	0.28–0.72	0.001
Platelet (10^9/L)	1.10	1.05–1.16	0.000
IL28B rs8099917: TT	7.16	3.60–14.25	<0.0001
At week 12			
cEVR patients			
Platelet (10^9/L)	1.09	1.02–1.17	0.015
HCV RNA: <600,000 IU/L	3.21	1.39–7.55	0.007
High-risk of anemia[a]	0.47	0.24–0.91	0.026
At week 12			
Non-cEVR patients			
Platelet (10^9/L)	1.11	1.02–1.21	0.017
IL28B rs8099917: TT	9.13	2.02–41.3	0.004

RVR: rapid virological response, defined as undetectable HCV RNA at week 4.
cEVR: complete early virological response, defined as undetectable HCV RNA at week 12.
[a]High-risk of anemia defined by decision tree analysis includes the following groups: (1) baseline hemoglobin <14.0 g/dl and creatinine clearance <90 ml/min, (2) baseline hemoglobin <14.0 g/dl, creatinine clearance ≥90 ml/min and ITPA rs1127354 genotype CC, and (3) baseline hemoglobin ≥14.0 g/dl, ITPA rs1127354 genotype CC, and creatinine clearance <85 ml/min.

genotyping alone. Because hemolytic anemia induced by RBV is one of the major adverse events leading to premature termination of therapy [Fried et al., 2002], a method to predict the risk of severe anemia before treatment is important clinically. A predictive model of anemia may have the potential to support individualized treatment strategies; patients at high risk of anemia may be tested intensively for anemia or may be candidates for erythropoietin therapy, whereas those with a low risk of anemia may be treated with a higher dose of RBV. Prediction of anemia will remain important in the era of direct antiviral agents for chronic hepatitis C, because these newer therapies still require RBV and PEG-IFN in combination, and the degree of anemia complicating these therapies may be even greater than with the current combination therapy [McHutchison et al., 2009; Kwo et al., 2010].

Studies of the impact of the ITPA genotype on treatment outcome have produced conflicting results. Previous studies of American [Thompson et al., 2010a] and Italian [Thompson et al., 2011] cohorts did not find any association between the ITPA genotype and treatment outcome, whereas a marginal difference was observed in a report from Japan [Ochi et al., 2010]. Moreover, with a subgroup analysis of Japanese patients, the variant of the ITPA gene was

associated with a sustained virological response in patients with the IL28B major genotype [Kurosaki et al., 2011d], in patients infected with HCV other than genotype 1[Sakamoto et al., 2010], and in patients with pre-treatment Hb concentrations between 13.5 and 15 g/dl [Azakami et al., 2011]. These inconsistent results may be because the impact of anemia may be greater on a cohort of aged patients, such as in Japan. Another reason may be that the ITPA genotype is not the sole determinant of anemia; the ITPA genotype alone was not associated with treatment outcome in the present study but a high-risk of anemia, defined by the combination of the ITPA genotype, baseline Hb concentration, and baseline CLcr, was associated with sustained virological responses by patients with complete early virological responses, even after adjustment for the IL28B genotype and other relevant factors. This is in contrast to the finding that the IL28B genotype is an independent and significant predictor at baseline of a sustained virological response by patients without a rapid virological response and those without a complete early virological response, but not those with a complete early virological response. These results indicate that the IL28B genotype could be used to predict a sustained virological response at baseline or during therapy in patients in whom HCV RNA has not yet become undetectable, but it has no predictive value in patients in whom HCV RNA has become undetectable. The risk of anemia may be used to predict sustained virological responses in a selected subgroup of patients who achieve a complete early virological response.

Patients who received more than 80% of the planned dose of PEG-IFN or RBV had a higher rate of sustained virological responses than those who received a lower cumulative dose [McHutchison et al., 2002; Davis et al., 2003]. Patients who achieve a complete early virological response usually have a good chance of a sustained virological response and the treatment duration is not extended beyond 48 weeks. However, reduced adherence to drugs in these patients was related to relapse after the completion of 48 weeks of therapy [Hiramatsu et al., 2009; Kurosaki et al., 2012]. In the present study, the rate of sustained virological response was 59% in patients who achieved a complete early virological response but had a high risk of anemia, 17% lower than in patients with a low risk of anemia. However, there was a stepwise increase in the rate of sustained virological response according to the increase in adherence to RBV, and the rate of sustained virological response was higher in high-risk patients who received >80% of the planned dose of RBV (71% vs. 47%). This 24% increase in sustained virological response was observed among the patients in the present study who received 48 weeks of treatment. These findings suggest that receiving a sufficient RBV dose is essential for patients with a complete early virological response to attain a sustained virological response and that the treatment strategy should be personalized for patients with a
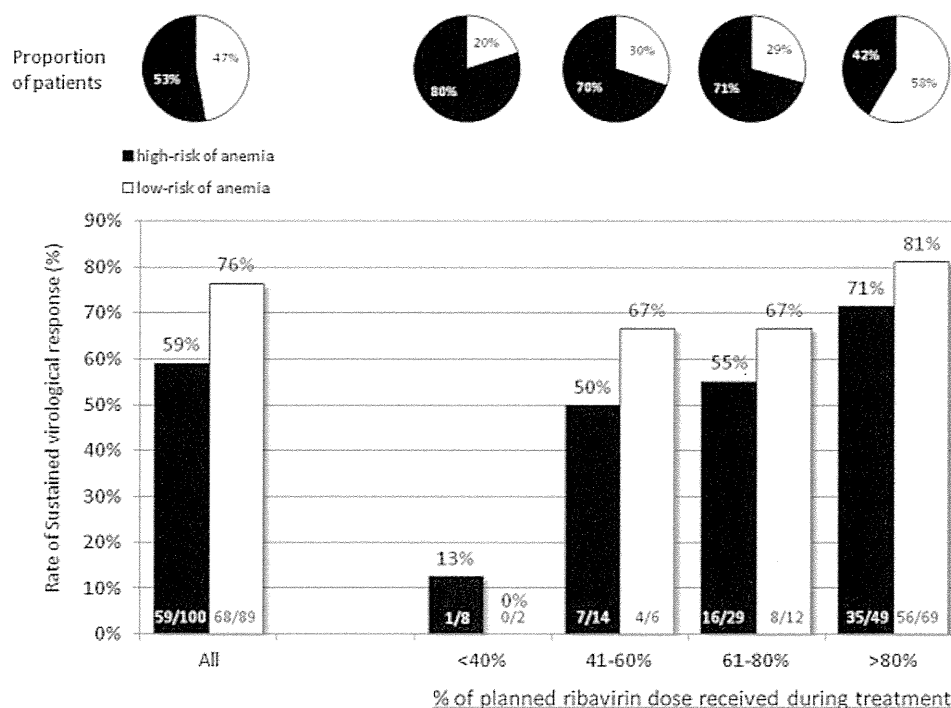
Fig. 4. The impact of risk of anemia and RBV dose on treatment outcome after a complete early virological response. Patients with complete early virological responses were divided into subgroups according to their adherence to RBV: ≤40%, 41–60%, 61–80%, and >80%. For each subgroup, the proportion of patients with a high risk and a low risk of anemia is shown in the upper panel by pie charts, and the rates of sustained virological responses, stratified by high risk and low risk of anemia, are shown in the lower panel by bar graphs. The black and white bars or charts represent patients with high and low risks of anemia, respectively.

high risk of anemia to extend the duration of treatment, even those patients with a complete early virological response, to obtain >80% adherence to RBV.

In conclusion, the combination of the *ITPA* genotype, baseline Hb concentration, and baseline CLcr could be used as a pre-treatment predictor of anemia. The risk of anemia thus identified is associated with adherence to RBV and impacts on the treatment outcome of patients who achieve a complete early virological response. This is in contrast to the major role of the *IL28B* genotype in the prediction of sustained virological responses at baseline and among non-responders at weeks 4 and 12. Patients who achieve a complete early virological response generally have a high probability of a sustained virological response but those who have a high risk of anemia have a high rate of relapse because of reduced adherence to RBV. To improve the rate of sustained virological responses in these patients, it may be postulated that the treatment schedule may be personalized to obtain >80% adherence to RBV. Clearly, this postulate needs to be confirmed in a future study.

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Original Article

Impaired brain activity in cirrhotic patients with minimal hepatic encephalopathy: Evaluation by near-infrared spectroscopy

Hiroyuki Nakanishi,[1] Masayuki Kurosaki,[1] Kaoru Nakanishi,[2] Kaoru Tsuchiya,[1] Takamasa Noda,[3] Nobuharu Tamaki,[1] Yutaka Yasui,[1] Takanori Hosokawa,[1] Ken Ueda,[1] Jun Itakura,[1] Kimitaka Anami,[4] Yasuhiro Asahina,[5] Nobuyuki Enomoto,[6] Teruhiko Higuchi[3] and Namiki Izumi[1]

[1]Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Musashino, [2]Department of Psychiatry, Seiwa Hospital, Shinjuku, [3]Department of Psychiatry, National Center of Neurology and Psychiatry, Kodaira, [4]Oomiyamusashino Clinic, Saitama, [5]Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, and [6]First Department of Internal Medicine, Faculty of Medicine, University of Yamanashi, Chuo, Japan

Aim: Near-infrared spectroscopy (NIRS) is a tool that could non-invasively measure the regional cerebral oxygenated hemoglobin (oxy-Hb) concentration with high time resolution. The aim of the present study is to reveal the time-dependent regional cerebral oxy-Hb concentration change coupled with brain activity during task performance in patients with minimal hepatic encephalopathy (MHE).

Methods: Cerebral oxy-Hb concentration was measured by using NIRS in 29 cirrhotic patients without overt hepatic encephalopathy (HE). Of those, 16 patients who had abnormal electroencephalography findings were defined as having MHE. Responsive increase in oxy-Hb during a word-fluency task was compared between MHE and non-MHE patients.

Results: There was no difference in the maximum value of oxy-Hb increase between patients with and without MHE (0.26 ± 0.12 vs 0.32 ± 0.22 mM·mm, *P* = 0.37). However, the pattern of the time course changes of oxy-Hb was different between the two groups. The MHE group was characterized by a gradual increase of oxy-Hb throughout the task compared to steep and repetitive increase in the non-MHE group. Increase in oxy-Hb concentration at 5 s after starting the task was significantly small in the MHE group compared to the non-MHE (0.03 ± 0.05 vs 0.11 ± 0.09 mM·mm, *P* = 0.006).

Conclusion: The cerebral oxygen concentration is poorly reactive in response to tasks among cirrhotic patients without overt HE but having abnormal electroencephalography findings. These impaired responses in regional cerebral oxy-Hb concentration may be related to the latent impairment of brain activity seen in MHE.

Key words: hepatic encephalopathy, near-infrared spectroscopy

INTRODUCTION

HEPATIC ENCEPHALOPATHY (HE) is a major complication of liver cirrhosis. Apart from

clinically overt HE (OHE), minimal HE (MHE) is troublesome because it is associated with reduced quality of life (QOL), reduced cognitive function, lowered work efficiency, higher risk of progression to OHE and may be a cause of traffic accidents.[1–3] MHE treatment can improve QOL, driving capability and progression of OHE.[4–6] Adequate diagnosis of MHE and early therapeutic intervention are precluded by the lack of reliable diagnostic standards, and HE is usually diagnosed only after the presentation of overt symptoms. For the diagnosis of MHE, neuropsychological function tests, such as number connection test, light/sound reaction time, inhibitory control test, Wechsler adult intelligence scale (WAIS) or electro-psychological tests

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