Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

# Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C

Masayuki Kurosaki[1], Naoki Hiramatsu[2], Minoru Sakamoto[3], Yoshiyuki Suzuki[4], Manabu Iwasaki[5], Akihiro Tamori[6], Kentaro Matsuura[7], Sei Kakinuma[8], Fuminaka Sugauchi[9], Naoya Sakamoto[8], Mina Nakagawa[8], Namiki Izumi[1,*]

[1]*Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan;* [2]*Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka, Japan;* [3]*First Department of Internal Medicine, University of Yamanashi, Yamanashi, Japan;* [4]*Department of Hepatology, Toranomon Hospital, Tokyo, Japan;* [5]*Department of Computer and Information Science, Seikei University, Tokyo, Japan;* [6]*Department of Hepatology, Osaka City University Medical School, Osaka, Japan;* [7]*Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan;* [8]*Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan;* [9]*Department of Gastroenterology, Nagoya Koseiin Medical Welfare Center, Nagoya, Japan*

**Background & Aims:** Assessment of the risk of hepatocellular carcinoma (HCC) development is essential for formulating personalized surveillance or antiviral treatment plan for chronic hepatitis C. We aimed to build a simple model for the identification of patients at high risk of developing HCC.

**Methods:** Chronic hepatitis C patients followed for at least 5 years (n = 1003) were analyzed by data mining to build a predictive model for HCC development. The model was externally validated using a cohort of 1072 patients (472 with sustained virological response (SVR) and 600 with nonSVR to PEG-interferon plus ribavirin therapy).

**Results:** On the basis of factors such as age, platelet, albumin, and aspartate aminotransferase, the HCC risk prediction model identified subgroups with high-, intermediate-, and low-risk of HCC with a 5-year HCC development rate of 20.9%, 6.3–7.3%, and 0–1.5%, respectively. The reproducibility of the model was confirmed through external validation ($r^2 = 0.981$). The 10-year HCC development rate was also significantly higher in the high- and intermediate-risk group than in the low-risk group (24.5% *vs.* 4.8%; $p < 0.0001$). In the high-and intermediate-risk group, the incidence of HCC development was significantly reduced in patients with SVR compared to those with nonSVR (5-year rate, 9.5% *vs.* 4.5%; $p = 0.040$).

**Conclusions:** The HCC risk prediction model uses simple and readily available factors and identifies patients at a high risk of HCC development. The model allows physicians to identify patients requiring HCC surveillance and those who benefit from IFN therapy to prevent HCC.

Keywords: Decision tree; Prediction; Pegylated interferon; Ribavirin; Risk.
*Received 27 May 2011; received in revised form 8 August 2011; accepted 4 September 2011*

* Corresponding author. Address: Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino-shi, Tokyo 180-8610, Japan. Tel.: +81 422 32 3111; fax: +81 422 32 9551.
*E-mail address:* nizumi@musashino.jrc.or.jp (N. Izumi).



## Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide [1] and its incidence is increasing in many countries [2]. Chronic viral hepatitis is responsible for 80% of all HCC cases [2]. The need to conduct HCC surveillance should be determined according to the risk of HCC development because this surveillance is cost-effective only in populations with an annualized cancer development rate of ⩾1.5% [3]. The annualized rate of developing HCC from type C liver cirrhosis is 2–8% [4–6], indicating that this population with type C liver cirrhosis needs surveillance. However, the annualized rate of HCC development is <1.5% in patients with chronic hepatitis C but without cirrhosis and the benefit of surveillance for all patients with chronic hepatitis has not yet been established [3]. HCC surveillance may be needed for patients with advanced fibrosis because the risk of HCC development increases in parallel with the progression of liver fibrosis [7,8]. Liver biopsy is the most accurate means of diagnosing fibrosis, but a single liver biopsy cannot indicate long-term prognosis because liver fibrosis progresses over time. Serial liver biopsies are not feasible because of the procedure's invasiveness. Moreover, factors other than fibrosis, such as advanced age, obesity, sex, lower albumin, and low platelet counts, also contribute to the development of HCC from chronic hepatitis C [8–11]. Therefore, these factors must be considered while assessing the risk of HCC development.

A meta-analysis of controlled trials [12] has shown that interferon (IFN) therapy reduced the rate of HCC development in patients with type C liver cirrhosis. However, there was a marked heterogeneity in the magnitude of the prevention effect

Cancer



Please cite this article in press as: Kurosaki M et al. Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C. J Hepatol (2011), doi:10.1016/j.jhep.2011.09.011

of IFN on HCC development among the studies, probably due to the large differences in the baseline rate of HCC development among the different trials [12]. Whether the incidence of HCC development could be reduced in all patients with chronic hepatitis C, especially in those without liver cirrhosis, remains to be elucidated.

Data mining analysis, unlike conventional statistical analysis, is performed in an exploratory manner without considering a predefined hypothesis. Decision tree analysis, the major component of data mining analysis, is used to extract relevant factors from among various factors. These relevant factors are then combined in an orderly sequence to identify rules for predicting the incidence of the target outcome [13]. Data mining analysis has been used to define prognostic factors in various diseases [14–20]. In the field of hepatic diseases, data mining analysis has proven to be a useful tool for predicting early response [21], sustained virological response (SVR) [22–25], relapse [26], and adverse events [27] in patients with chronic hepatitis C treated with pegylated interferon (PEG-IFN) plus ribavirin (RBV). The findings of data mining analysis are expressed as flowcharts and are therefore easily understood [28] and readily available for clinical use, even by physicians without a detailed understanding of statistics.

In the present study, data mining analysis was used to identify risk factors for HCC development in a cohort of patients with chronic hepatitis C who had been followed for at least 5 years. An HCC risk prediction model was constructed on the basis of simple and generally available tests because the goal was to make the model easy to use in the clinic. The suitability, reproducibility, and generalizability of the results were validated using the data of an external cohort that was independent of the model derivation cohort.

Cancer

## Materials and methods

### *Patients*

The model derivation cohort consisted of 1003 chronic hepatitis C patients without cirrhosis who had a non-sustained virological response (nonSVR) to previous IFN administered at the Musashino Red Cross Hospital and were followed for at least 5 years. Patients who had SVR or those who were followed for less than 5 years were not included. An analytical database on age, body mass index, albumin, aspartate aminotransferase (AST) levels, alanine aminotransferase (ALT) levels, γ-glutamyltransferase (GGT) levels, total bilirubin levels, total cholesterol levels, hemoglobin levels, and platelet count at the start of the observation was created. Histological data such as fibrosis stage, activity grade, or degree of steatosis was not included in the database because the goal of the present study was to make the model on the basis of simple and generally available tests. The patients who developed HCC more than 5 years after the start of the observation were considered not to have developed HCC by the 5-year point because the model was intended to predict HCC development within 5 years. The 1072 chronic hepatitis C patients included in the external validation cohort were treated with PEG-IFN and RBV at the University of Yamanashi, Tokyo Medical and Dental University, Osaka University, Osaka City University, Nagoya City University, or Toranomon Hospital and followed for at least 5 years. Among them, 600 had nonSVR and 472 had SVR. Data from nonSVR patients in this external cohort were used for external validation of the HCC prediction model. To assess the preventive effect of PEG-IFN plus RBV therapy on HCC development, the cumulative HCC development rate was compared between SVR and nonSVR patients in the external validation cohort after stratification by the risk of HCC development as determined by data mining analysis. Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committees of all concerned hospitals.

### *HCC surveillance and diagnosis*

HCC surveillance was conducted by performing abdominal ultrasonography every 4–6 months. Contrast-enhanced computer tomography, magnetic resonance imaging, or angiography were performed when abdominal ultrasonography suggested a new lesion suspicious for HCC. Classical HCC was diagnosed for tumors showing vascular enhancement with washout on at least two types of diagnostic imaging. Tumor biopsy was used to diagnose tumors with non-classical imaging findings.

### *Statistical analysis*

The IBM-SPSS Modeler 13 (IBM SPSS Inc., Chicago, IL, USA) was used for decision tree analysis. The statistical methods used have been described previously [21,22,24–27]. In brief, the software searched the analytical database for the factor that most effectively predicted HCC development and for its cutoff value. The patients were divided into two groups according to that predictor. Each divided group was repeatedly assessed and divided according to this 2-choice branching method. Branching was stopped when the number of patients decreased to ⩽20 to avoid over fitting. Finally, an HCC risk prediction model was created through this analysis. The model classified patients into subgroups with different HCC development rates in a flowchart form. For model validation, nonSVR patients from an external cohort were individually fitted into the model and classified into the subgroups and the HCC development rates of those subgroups were then calculated. The suitability and reproducibility of the model were validated by comparing the subgroup HCC development rates of the model derivation group to those of the validation group.

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. A log-rank test for Kaplan–Meier analysis was used to statistically test HCC development rates over time. *p*-Values of <0.05 were considered significant. SPSS Statistics 18 (IBM SPSS Inc.) was used for these analyses.

## Results

### *Univariate and multivariate analysis of factors associated with HCC development*

The baseline characteristics of patients are shown in Table 1. The 5-year HCC development rate in the model derivation group was 6.2%, which did not differ significantly from the rate of 6.0% in the nonSVR group of the external cohort, but the rate of 2.0% in the SVR group of the external cohort was significantly lower than that in the model derivation group ($p = 0.0003$) and the nonSVR group of the external cohort ($p = 0.0012$). On univariate analysis, the factors found to be associated with HCC development in the model derivation cohort were age, AST levels, albumin levels, total cholesterol levels, and platelet count. On multivariate analysis, age (odds ratio 1.086), albumin levels (odds ratio 0.248), and platelet count (odds ratio 0.842) were significant predictors of HCC development (Table 2).

### *HCC risk prediction model by data mining analysis*

The results of decision tree analysis are presented in Fig. 1. Age was selected as the first predictor. The 5-year HCC development rate was 3.4% in younger patients (<60 years) and 8.6% in older patients (⩾60 years). The second predictor for younger patients (<60 years) was platelet count. The HCC development rate was 6.9% in patients with a lower platelet count ($<150 \times 10^9$/L) and 0.8% in patients with a higher count ($\geqslant 150 \times 10^9$/L). The second predictor for older patients (⩾60 years) was also platelet count. The HCC development rate was 13.1% in patients with a lower platelet count ($<150 \times 10^9$/L) and 1.8% in patients with a higher count ($\geqslant 150 \times 10^9$/L). The third predictor was albumin levels,



Please cite this article in press as: Kurosaki M et al. Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C. J Hepatol (2011), doi:10.1016/j.jhep.2011.09.011

**Table 1. Baseline characteristics of patients for model deviation and external validation.**

| | Model derivation (n = 1003) | External cohort, non-SVR (n = 600) | External cohort, SVR (n = 472) |
|---|---|---|---|
| Sex: Male/Female* | 463 (46%)/540 (54%) | 306 (51%)/294 (49%) | 299 (63%)/173 (37%) |
| Age (yr) | 57.3 (11.1) | 55.9 (9.6) | 51.4 (10.6) |
| Body mass index (kg/m$^2$) | 23.5 (3.2) | 23.4 (3.3) | 23.3 (3.1) |
| Albumin (g/dl) | 4.1 (0.3) | 4.0 (0.4) | 4.0 (0.3) |
| AST (IU/L) | 64.2 (36.5) | 67.3 (43.8) | 62.5 (48.3) |
| ALT (IU/L) | 80.6 (55.1) | 81.2 (62.3) | 88.6 (82.1) |
| GGT (IU/L) | 59.3 (50.5) | 67.6 (65.1) | 55.7 (71.2) |
| Total cholesterol (mg/dl) | 172.1 (31.5) | 168.2 (31.0) | 174.3 (33.7) |
| Platelet ($10^9$/L) | 154.0 (53.0) | 153.7 (53.2) | 176.6 (49.7) |
| Hemoglobin (g/dl) | 13.3 (1.5) | 14.2 (1.5) | 14.4 (1.4) |
| HCC development within 5 years: n (%)* | 62 (6.2%) | 36 (6.0%) | 10 (2.0%) |

Data expressed as mean (standard deviation) unless otherwise indicated.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.
*Data expressed as number of patients (percentage).

whose cutoff value was 3.75 g/dl in patients with a higher platelet count ($\geqslant 150 \times 10^9$/L). The HCC development rate was 6.3% when albumin levels were lower (<3.75 g/dl) and 1.5% when levels were higher ($\geqslant$3.75 g/dl). The cutoff value for albumin levels was 4.0 g/dl in patients with a lower platelet count ($<150 \times 10^9$/L). The HCC development rate was 20.9% when albumin levels were lower (<4.0 g/dl) and 6.4% when levels were higher ($\geqslant$4.0 g/dl). The fourth and final predictor was AST levels. The HCC development rate was 7.3% when AST levels were at least 40 IU/L and 0% when the levels were <40 IU/L. On the basis of this analysis, seven subgroups with a 5-year HCC development rate of 0–20.9% were identified. The area under the receiver operating characteristic curve according to the HCC risk prediction model was 0.817.

*External validation of the HCC risk prediction model with an independent external cohort*

Six hundred nonSVR patients from an external cohort were fitted into the HCC risk prediction model and classified into the seven subgroups. The 5-year HCC development rate of these subgroups was 0–17.9%. The HCC development rate in the individual subgroups of the model derivation group was closely correlated to that in the corresponding subgroups of the external validation group (Fig. 2; correlation coefficient $r^2 = 0.981$). The HCC development rate in the subgroup of patients with the highest risk of HCC development (high-risk group) according to the model older age ($\geqslant$60 years) with a lower platelet count ($<150 \times 10^9$/L) and lower albumin levels (<4.0 g/dl) was 20.9% in the model derivation group and 17.9% in the external validation group. The intermediate-risk group or the patients with an HCC development rate of at least 5% consisted of the following three subgroups: (1) older age ($\geqslant$60 years), lower platelet count ($<150 \times 10^9$/L), higher albumin levels ($\geqslant$4.0 g/dl), and higher AST levels ($\geqslant$40 IU/L); (2) older age ($\geqslant$60 years), higher platelet count ($\geqslant 150 \times 10^9$/L), and lower albumin levels (<3.75 g/dl); and (3) younger age (<60 years) and lower platelet count ($<150 \times 10^9$/L). In these intermediate-risk groups, the 5-year HCC development rate was 6.3–7.3% in the model derivation group and 5.3–7.9% in the external validation group. The low-risk group consisted of the following three subgroups: (1) younger age (<60 years) and higher platelet count ($\geqslant 150 \times 10^9$/L); (2) older age ($\geqslant$60 years), lower platelet count ($<150 \times 10^9$/L), higher albumin levels ($\geqslant$4.0 g/dl), and lower AST levels (<40 IU/L); and (3) older age ($\geqslant$60 years), higher platelet count ($\geqslant 150 \times 10^9$/L), and higher albumin levels ($\geqslant$3.75 g/dl). In these low-risk groups, the 5-year HCC development rate was 0–1.5% in the model derivation group and 0–2.9% in the external validation group.

**Table 2. Multivariable analysis of factors associated with subsequent development of HCC within 5 years.**

| | Odds ratio | 95% CI | *p* value |
|---|---|---|---|
| Age | 1.086 | 1.029-1.146 | 0.003 |
| Albumin | 0.248 | 0.100-0.613 | 0.003 |
| Platelet | 0.842 | 0.769-0.921 | <0.0001 |

CI, confidence interval.

*Predictability of the HCC risk prediction model on HCC development rate beyond 5 years*

Cumulative HCC development rates in the high-, intermediate-, and low-risk groups were compared over time using the Kaplan–Meier method. The 10-year rates were 28.9% in the high-risk group, 22.9% in the intermediate-risk group, and 4.8% in the low-risk group (Fig. 3A). The high and intermediate-risk group created by pooling data from the high- and intermediate-risk groups had a significantly higher cumulative HCC development rate than the low-risk group beyond 5 years (Fig. 3B; 5-year rate, 11.6% *vs.* 1.0%; 10-year rate, 24.5% *vs.* 4.8%; $p < 0.0001$).

*Effect of response to PEG-IFN plus RBV therapy in the reduction of HCC development: analysis stratified by the HCC risk prediction model*

The 600 nonSVR patients and 472 SVR patients in the external cohort were fitted into the HCC risk prediction model and





Please cite this article in press as: Kurosaki M et al. Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C. J Hepatol (2011), doi:10.1016/j.jhep.2011.09.011

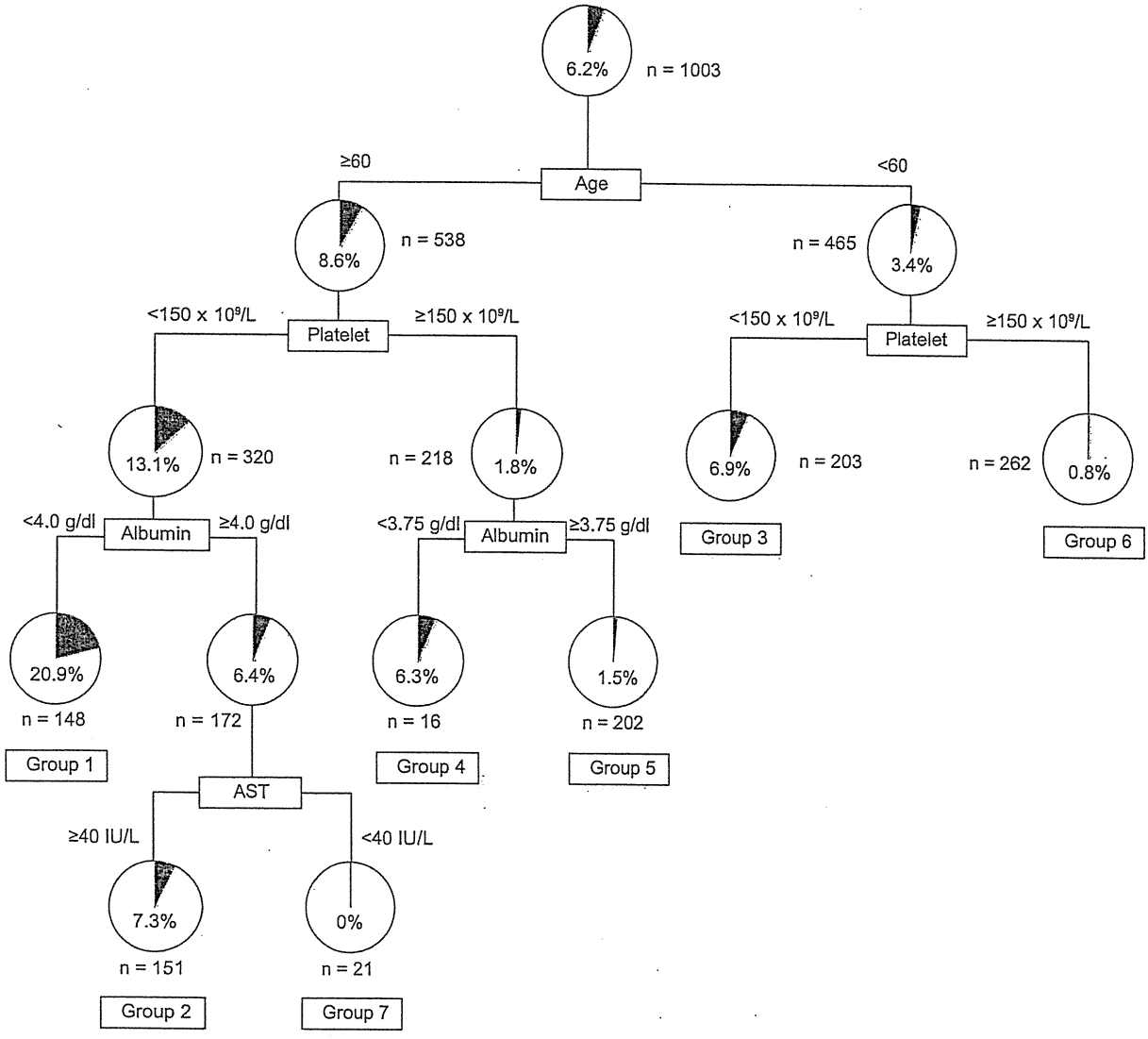



**Fig. 1. The decision tree model of HCC development within 5 years.** Boxes indicate the factors used to differentiate patients and the cutoff values for those different groups. Pie charts indicate the HCC development rate within 5 years for each group of patients after differentiation. Terminal groups of patients differentiated by analysis are numbered from 1 to 7.

classified into the high-and intermediate-risk group or the low-risk group, as defined above. The HCC development rate was significantly lower in SVR patients than in nonSVR patients in the high-and intermediate-risk group (5-year HCC rate, 9.5% *vs.* 4.5%; $p = 0.040$, log-rank test). In the low-risk group, the 5-year rate was 1.8% in nonSVR patients and 0.9% in SVR patients. Both rates were low and not significantly different ($p = 0.331$, log-rank test) (Fig. 4).

## Discussion

An awareness of the risk of HCC development in the context of routine care for chronic hepatitis C is essential for formulating an HCC surveillance plan personalized for individual patients. The risk of developing HCC from chronic hepatitis is lower than that from cirrhosis [7]; therefore, across-the-board surveillance for chronic hepatitis C is not recommended [3]. A method to easily determine this risk, without performing serial liver biopsies, would be extremely significant clinically. In the present study, an HCC risk prediction model that included the factors such as age, platelet count, albumin levels, and AST levels was constructed. The model was found to have excellent reproducibility when validated with an external cohort. This model could identify subgroups of chronic hepatitis C patients at high risk of HCC development; the 5-year HCC development rate for the high- and intermediate-risk groups was 11.6%, yielding an annual incidence of 2.3%. This HCC risk prediction model requires only



Please cite this article in press as: Kurosaki M et al. Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C. J Hepatol (2011), doi:10.1016/j.jhep.2011.09.011

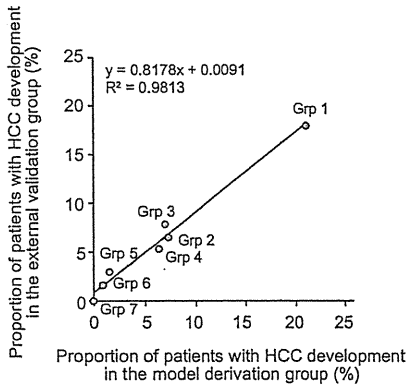



**Fig. 2. External validation of the decision tree model with an independent cohort.** Each patient in the external validation group was allocated to groups 1–7 following the flowchart of the decision tree. The HCC development rates were then calculated for each group and the graph plotted. The *x*-axis represents the HCC development rate in the model derivation group, and the *y*-axis represents the HCC development rate in the external validation group. The HCC development rates in each subgroup of patients are closely correlated between the model derivation group and the external validation group (correlation coefficient: $R^2 = 0.981$).

simple test values that are readily obtained in routine care and can therefore be easily used at the patient bedside. The model can be used to identify patients with a high risk of HCC development and therefore requiring surveillance, thereby allowing the formulation of surveillance plans personalized for individual patients.

Advanced fibrosis has been reported as independent risk factors for HCC development [7,8]. Platelet counts and albumin levels, which were factors selected for discrimination of the risk of HCC development, are closely related to the stage of fibrosis. Their correlation with the HCC risk has been repeatedly demonstrated [9–11,29–31]. The present study confirmed the impact of old age and advanced fibrosis, as reflected by low platelet counts and albumin levels. These results are consistent with our previous report [32]. What is unique to the present study was the study design to build a simple and reliable model for

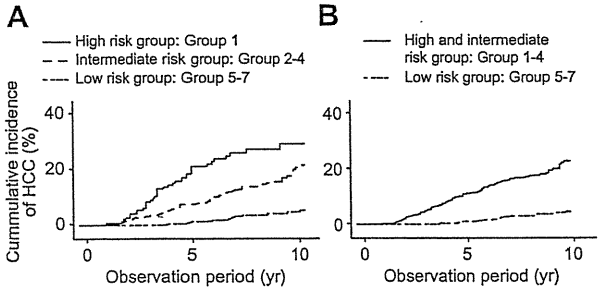



**Fig. 3. Cumulative incidence of HCC development beyond 5 years in subgroups of patients defined by the decision tree model.** Cumulative incidences of HCC in the groups classified by the decision tree model are compared. (A) The cumulative HCC development rate beyond 5 years is higher in the high- (group 1) and intermediate-risk (groups 2–4) groups compared to the low-risk group (groups 5–7). (B) The high and intermediate-risk group created by pooling data from the high- and intermediate-risk groups has a significantly higher cumulative HCC development rate than the low-risk group (5-year rate, 11.6% *vs.* 1.0%; 10-year rate, 24.5% *vs.* 4.8%; $p < 0.0001$).

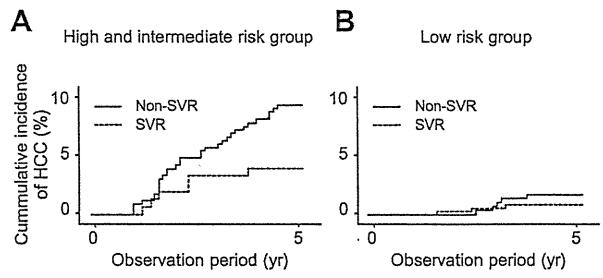



**Fig. 4. Sustained virological response to PEG-IFN plus RBV therapy reduces the incidence of HCC development after stratification by the HCC risk.** The 600 nonSVR patients and the 472 SVR patients in the external cohort were fitted into the HCC risk prediction model and classified into the high and intermediate-risk group or the low-risk group. The HCC development rate is significantly lower in SVR patients than in nonSVR patients in the high and intermediate-risk group (groups 1–4) (5-year HCC rate, 9.5% *vs.* 4.5%; $p = 0.040$). In the low-risk group (groups 5–7), the 5-year rate is 1.8% in nonSVR patients and 0.9% in SVR patients. Both rates are low and not significantly different ($p = 0.331$).

the prediction of HCC development that could be easily used in the clinic. For this purpose, a novel statistical method was used, histological factors were excluded in the analysis, the model derivation cohort was restricted to those who had nonSVR and had a long follow-up period duration (5 years), and the reproducibility of the model was independently validated by an external cohort. These are the major differences of the present study compared to our previous report. Many researchers have put a lot of efforts to formulate regression models for HCC prediction [9,10,33]. These prediction models are useful for identifying high-risk patients but are somewhat complicated to use at the bedside because they require calculations to be performed. Our prediction model is used simply by incorporating patients' data obtained through simple tests into the decision tree and following the flowchart. These prediction models based on factors easily accessible in routine clinical settings help physicians identify high-risk patients out of chronic hepatitis.

Viral eradication is the short-term goal of IFN therapy, but the ultimate goal is the prevention of HCC occurrence. Previous reports have shown that SVR to IFN therapy suppresses HCC occurrence in patients with type C liver cirrhosis and chronic hepatitis [7,12,30,34,35]. However, there is a marked heterogeneity in the magnitude of the treatment effect on the risk of HCC among studies, probably due to differences in the baseline risk of HCC among different trials [12]. Thus, the question remains whether the preventive effect of IFN therapy on HCC development could apply to all patients with chronic hepatitis C, especially those without liver cirrhosis. The result of the present study indicated that among high- and intermediate-risk patients, as assessed with our HCC risk prediction model, the cumulative HCC development rate was significantly reduced in SVR patients compared with nonSVR patients. This finding suggests that patients with chronic hepatitis, in whom disease has not yet progressed to hepatic cirrhosis but who are at a high risk of HCC development, benefit from antiviral treatment. The preventive effect of IFN on HCC development was not evident in low-risk patients within 5 years of observation. A longer observation term may be required to analyze the possible effect of antiviral therapy in these patients. Application of the present model on treatment decision may have limitations in that effect to prevent HCC development may differ in newer therapeutic agents such as protease





Please cite this article in press as: Kurosaki M et al. Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C. J Hepatol (2011), doi:10.1016/j.jhep.2011.09.011

inhibitors [36,37], and that low-risk patients may also benefit from therapy after a longer term observation period such as 15–20 years.

Patients with chronic hepatitis often have no subjective symptoms accompanying their disease and therefore have a low consciousness of the disease. The broad array of adverse reactions and the high cost of IFN therapy are frequent hurdles in motivating patients to undergo therapy. However, patients may be convinced to undergo therapy or remain motivated for continued therapy if they are made aware of their risk of HCC development and the preventive effect of IFN on HCC development.

In conclusion, a reproducible HCC risk prediction model, which includes the factors such as age, platelet count, albumin levels, and AST levels, was constructed to predict the 5-year HCC development rate in patients with chronic hepatitis C. The model requires only a combination of readily available test values and can therefore be easily used at the bedside. The information provided by the model allows the physician to identify patients requiring IFN therapy for the prevention of HCC and formulate plans for imaging HCC surveillance.

### Conflict of interest

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

### Financial support

This study was supported by a Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan (H20-kanen-006).

### References

[1] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
[2] Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology 2004;127:S5–S16.
[3] Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology 2005;42:1208–1236.
[4] Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology 1997;112:463–472.
[5] Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28:1687–1695.
[6] Degos F, Christidis C, Ganne-Carrie N, Farmachidi JP, Degott C, Guettier C, et al. Hepatitis C virus related cirrhosis: time to occurrence of hepatocellular carcinoma and death. Gut 2000;47:131–136.
[7] Yoshida H, Tateishi R, Arakawa Y, Sata M, Fujiyama S, Nishiguchi S, et al. Benefit of interferon therapy in hepatocellular carcinoma prevention for individual patients with chronic hepatitis C. Gut 2004;53:425–430.
[8] Kurosaki M, Hosokawa T, Matsunaga K, Hirayama I, Tanaka T, Sato M, et al. Hepatic steatosis in chronic hepatitis C is a significant risk factor for developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage and response to interferon therapy. Hepatol Res 2010;40: 870–877.
[9] Velazquez RF, Rodriguez M, Navascues CA, Linares A, Perez R, Sotorrios NG, et al. Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. Hepatology 2003;37:520–527.
[10] Lok AS, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, et al. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. Gastroenterology 2009;136:138–148.
[11] Cardoso AC, Moucari R, Figueiredo-Mendes C, Ripault MP, Giuily N, Castelnau C, et al. Impact of peginterferon and ribavirin therapy on hepatocellular carcinoma: incidence and survival in hepatitis C patients with advanced fibrosis. J Hepatol 2010;52:652–657.
[12] Craxi A, Camma C. Prevention of hepatocellular carcinoma. Clin Liver Dis 2005;9:329–346, viii.
[13] Breiman LJH, Friedman RA, Olshen CJ, Stone CM. Classification and regression trees. Calif: Wadsworth; 1980.
[14] Garzotto M, Park Y, Mongoue-Tchokote S, Bledsoe J, Peters L, Blank BH, et al. Recursive partitioning for risk stratification in men undergoing repeat prostate biopsies. Cancer 2005;104:1911–1917.
[15] Miyaki K, Takei I, Watanabe K, Nakashima H, Omae K. Novel statistical classification model of type 2 diabetes mellitus patients for tailor-made prevention using data mining algorithm. J Epidemiol 2002;12:243–248.
[16] Averbook BJ, Fu P, Rao JS, Mansour EG. A long-term analysis of 1018 patients with melanoma by classic Cox regression and tree-structured survival analysis at a major referral center: implications on the future of cancer staging. Surgery 2002;132:589–602.
[17] Leiter U, Buettner PG, Eigentler TK, Garbe C. Prognostic factors of thin cutaneous melanoma: an analysis of the central malignant melanoma registry of the german dermatological society. J Clin Oncol 2004;22: 3660–3667.
[18] Valera VA, Walter BA, Yokoyama N, Koyama Y, Iiai T, Okamoto H, et al. Prognostic groups in colorectal carcinoma patients based on tumor cell proliferation and classification and regression tree (CART) survival analysis. Ann Surg Oncol 2007;14:34–40.
[19] Zlobec I, Steele R, Nigam N, Compton CC. A predictive model of rectal tumor response to preoperative radiotherapy using classification and regression tree methods. Clin Cancer Res 2005;11:5440–5443.
[20] Baquerizo A, Anselmo D, Shackleton C, Chen TW, Cao C, Weaver M, et al. Phosphorus as an early predictive factor in patients with acute liver failure. Transplantation 2003;75:2007–2014.
[21] Kurosaki M, Matsunaga K, Hirayama I, Tanaka T, Sato M, Yasui Y, et al. A predictive model of response to peginterferon ribavirin in chronic hepatitis c using classification and regression tree analysis. Hepatol Res 2010;40: 251–260.
[22] Kurosaki M, Sakamoto N, Iwasaki M, Sakamoto M, Suzuki Y, Hiramatsu N, et al. Sequences in the interferon sensitivity-determining region and core region of hepatitis C virus impact pretreatment prediction of response to PEG-interferon plus ribavirin: data mining analysis. J Med Virol 2011;83: 445–452.
[23] Izumi N, Asahina Y, Kurosaki M. Predictors of virological response to a combination therapy with pegylated interferon plus ribavirin including virus and host factors. Hepat Res Treat 2010;2010:703602.
[24] Kurosaki M, Sakamoto N, Iwasaki M, Sakamoto M, Suzuki Y, Hiramatsu N, et al. Pretreatment prediction of response to peginterferon plus ribavirin therapy in genotype 1 chronic hepatitis C using data mining analysis. J Gastroenterol 2011;46:401–409.
[25] Kurosaki M, Tanaka Y, Nishida N, Sakamoto N, Enomoto N, Honda M, et al. Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in IL28B and viral factors. J Hepatol 2011;54:439–448.
[26] Kurosaki M, Hiramatsu N, Sakamoto M, Suzuki Y, Iwasaki M, Tamori A, et al. Age and total ribavirin dose is an independent predictor of relapse among early virological responders to peg-interferon plus ribavirin therapy in chronic hepatitis C revealed by data mining analysis. Antivir Ther, in press.
[27] Hiramatsu N, Kurosaki M, Sakamoto N, Iwasaki M, Sakamoto M, Suzuki Y, et al. Pretreatment prediction of anemia progression by pegylated interferon alpha-2b plus ribavirin combination therapy in chronic hepatitis C infection: decision-tree analysis. J Gastroenterol 2011;46:1111–1119.
[28] LeBlanc M, Crowley J. A review of tree-based prognostic models. Cancer Treat Res 1995;75:113–124.
[29] Lu SN, Wang JH, Liu SL, Hung CH, Chen CH, Tung HD, et al. Thrombocytopenia as a surrogate for cirrhosis and a marker for the identification of patients at high-risk for hepatocellular carcinoma. Cancer 2006;107: 2212–2222.
[30] Bruno S, Stroffolini T, Colombo M, Bollani S, Benvegnu L, Mazzella G, et al. Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. Hepatology 2007;45:579–587.
[31] Bonis PA, Tong MJ, Blatt LM, Conrad A, Griffith JL. A predictive model for the development of hepatocellular carcinoma, liver failure, or liver transplantation for patients presenting to clinic with chronic hepatitis C. Am J Gastroenterol 1999;94:1605–1612.





Please cite this article in press as: Kurosaki M et al. Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C. J Hepatol (2011), doi:10.1016/j.jhep.2011.09.011

[32] Asahina Y, Tsuchiya K, Tamaki N, Hirayama I, Tanaka T, Sato M, et al. Effect of aging on risk for hepatocellular carcinoma in chronic hepatitis C virus infection. Hepatology 2010;52:518–527.
[33] Ganne-Carrie N, Chastang C, Chapel F, Munz C, Pateron D, Sibony M, et al. Predictive score for the development of hepatocellular carcinoma and additional value of liver large cell dysplasia in Western patients with cirrhosis. Hepatology 1996;23:1112–1118.
[34] Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995;346:1051–1055.
[35] Shiratori Y, Ito Y, Yokosuka O, Imazeki F, Nakata R, Tanaka N, et al. Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. Ann Intern Med 2005;142:105–114.
[36] Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839–1850.
[37] McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360:1827–1838.





Please cite this article in press as: Kurosaki M et al. Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C. J Hepatol (2011), doi:10.1016/j.jhep.2011.09.011

Antiviral Therapy 2011; 16:685–694 (doi: 10.3851/IMP1796)

# Original article

# Relationship between polymorphisms of the inosine triphosphatase gene and anaemia or outcome after treatment with pegylated interferon and ribavirin

*Masayuki Kurosaki[1], Yasuhito Tanaka[2], Keisuke Tanaka[1], Yuichiro Suzuki[1,3], Yoshihide Hoshioka[1], Nobuharu Tamaki[1], Tomoji Kato[1], Yutaka Yasui[1], Takanori Hosokawa[1], Ken Ueda[1], Kaoru Tsuchiya[1], Teiji Kuzuya[1], Hiroyuki Nakanishi[1], Jun Itakura[1], Yuka Takahashi[1], Yasuhiro Asahina[1], Kentaro Matsuura[2], Fuminaka Sugauchi[2], Nobuyuki Enomoto[3], Nao Nishida[4], Katsushi Tokunaga[4], Masashi Mizokami[5], Namiki Izumi[1]**

[1]Division 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]First Department of Internal Medicine, University of Yamanashi, Yamanashi, Japan
[4]Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[5]Research Center for Hepatitis and Immunology, International Medical Center of Japan Konodai Hospital, Ichikawa, Japan

*Corresponding author e-mail: nizumi@musashino.jrc.or.jp

Background: A genome-wide association study revealed an association between variants of the inosine triphosphatase (*ITPA*) gene and ribavirin (RBV)-induced anaemia. The aim of this study was to replicate this finding in an independent Japanese cohort and to define a method to allow pretreatment prediction of anaemia in combination with other factors.

Methods: Genotype 1b chronic hepatitis C patients ($n$=132) treated with pegylated interferon (PEG-IFN)-$\alpha$ and RBV for 48 weeks were genotyped for *ITPA* rs1127354 and examined for anaemia and treatment outcome.

Results: Variants of the *ITPA* gene protected against severe anaemia throughout the 48-week treatment period and were associated with lower incidence of anaemia-related RBV dose reduction. A combination of the *ITPA* genotype with baseline haemoglobin (Hb) and creatinine clearance (CLcr) levels predicted severe anaemia with high accuracy (90% sensitivity and 62% specificity). Among a subset of patients with the *IL28B* genotype of TT at rs8099917, patients with variants of the *ITPA* gene were associated with a higher rate of receiving >80% of the expected RBV dose, a higher rate of sustained virological response (SVR), and a lower rate of relapse.

Conclusions: The variants of the *ITPA* gene, which could protect against haemolytic anaemia and RBV dose reduction, were associated with a high rate of SVR by standard PEG-IFN and RBV therapy in a subset of Japanese patients with the favourable TT genotype at rs8099917 of *IL28B*. A combination of *ITPA* genetic polymorphisms with baseline Hb and CLcr levels further improves the predictive accuracy of severe anaemia.

## Introduction

Treatment with pegylated interferon (PEG-IFN) combined with ribavirin (RBV) is the most effective standard treatment for chronic HCV infection. Successful eradication of HCV is associated with a reduced risk of developing hepatocellular carcinoma. However, the rate of sustained virological response (SVR) is approximately 50% in patients with HCV genotype 1 [1,2]. The probability of SVR decreases when the patients become intolerant to therapy and receive <80% of the planned dose of PEG-IFN and/or RBV [3]. One of the major reasons for intolerance to therapy is severe haemolytic anaemia induced by RBV [1]. The degree of haemolytic anaemia caused by RBV varies among individuals, and no reliable baseline predictors exist for this severe anaemia.

Recently, a genome-wide association study revealed that a single nucleotide polymorphism (SNP) at rs6051702 is strongly associated with RBV-induced haemolytic anaemia at week 4 of treatment [4]. This SNP was linked to two functional SNPs (rs1127354 and rs7270101) in the inosine triphosphatase (*ITPA*)

gene on chromosome 20, which had previously been well-characterized in studies of patients with ITPase deficiency [5–8]. Subsequent studies confirmed independently that variants of the *ITPA* gene are protective against haemolytic anaemia during the early weeks of treatment [9,10]. Furthermore, Thompson *et al.* [9] showed that the variants are protective against anaemia over the entire 48-week course of therapy and are associated with reduced requirement for an anaemia-related dose reduction of RBV. Notably, despite these protective effects, variants in the *ITPA* gene were not associated with treatment outcome [4,9] or showed only a marginal association [10].

In the present study, we aimed to replicate the association between *ITPA* genetic polymorphisms and RBV-induced anaemia in the early weeks, as well as throughout the entire course, of therapy in an independent Japanese cohort. In addition, for the general application of these genetic associations in clinical practice, we aimed to define a pretreatment prediction for severe anaemia in combination with other clinical covariates.

## Methods

### Patients

Data were collected retrospectively from a total of 132 genotype 1b chronic hepatitis C patients who were treated with PEG-IFN-α and RBV at Musashino Red Cross Hospital (Tokyo, Japan) and at Nagoya City University Graduate School of Medical Sciences (Nagoya, Japan). The inclusion criteria were: genotype 1b, HCV RNA titre >100 KIU/ml by quantitative PCR (Cobas Amplicor HCV Monitor version 2.0; Roche Diagnostic Systems, Indianapolis, IN, USA), no coinfection with HBV or HIV, no other causes of liver disease such as autoimmune hepatitis and primary biliary cirrhosis, and availability of DNA for the analysis of the genetic polymorphism of *ITPA*. Patients received PEG-IFN-α2a (180 μg) and -α2b (1.5 μg/kg) subcutaneously every week and were administered 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 of RBV was considered by physicians based on the clinical conditions of the individual patients or the recommendations on the package inserts: dose reduction from 800 mg and 1,000 mg to 600 mg or from 600 mg to 400 mg for haemoglobin levels <10 g/dl and drug discontinuation when haemoglobin levels drop to <8.5 g/dl. No patient received erythropoietin or other growth factors for the treatment of anaemia. PEG-IFN and RBV was stopped prematurely in 22 patients: in 15 patients due to non-virological response and in 7 patients due to adverse events. 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 and histological tests

Blood samples were obtained before therapy and at 1, 2, 4, 6, 8, 12, 16, 20, 24, 36 and 48 weeks after the start of therapy, and were analysed for haematological tests, blood chemistry and HCV RNA. Genetic polymorphisms in an SNP located in exon 2 (rs1127354) and in intron 2 (rs7270101) of the *ITPA* gene were determined using ABI TaqMan Probes (Applied Biosystems, Carlsbad, CA, USA) [4]. Since a recent paper studying Japanese patients showed no variants in rs7270101 [10] and our preliminary genotyping data for 100 Japanese patients also showed no variations in rs7270101, rs1127354 was used for further analysis (major allele =C and minor allele =A). Genetic polymorphisms in the *IL28B* gene (rs8099917), an SNP recently identified to be associated with hepatitis C treatment response [11–14], was also determined by a DigiTag2 assay [15]. Viral factors affecting therapeutic efficacy was determined. A stretch of 40 amino acids in the NS5A region of HCV, designated as the interferon sensitivity-determining region (ISDR) [16,17] and amino acid substitutions at positions 70 of the core region (Core70) [18] were determined by direct sequencing after amplification by reverse transcription and PCR as reported previously. Arginine at Core70 was defined as the wild type, and glutamine or histidine was defined as the mutant type. Baseline creatinine clearance (CLcr) levels were calculated using the formula of Cockcroft and Gault [19]: 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). Fibrosis was evaluated on a scale of 0–4: F0 indicates no fibrosis, F1 indicates mild fibrosis, F2 indicates moderate fibrosis, F3 indicates severe fibrosis and F4 indicates cirrhosis according to the Metavir scoring system [20]. The end of treatment response was defined as an undetectable HCV RNA level by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor; Roche Diagnostic Systems) at the end of therapy. SVR was defined as an undetectable HCV RNA level 24 weeks after the completion of therapy. A relapse was defined as the reappearance of HCV RNA after the completion of therapy.

### Statistical analysis

We analysed the association between an SNP of the *ITPA* gene (rs1127354) and the following: the incidence of haemoglobin (Hb) reduction of >3.0 g/dl at week 4 and the incidence of severe anaemia (Hb<10 g/dl) at week 4 or at any time point during the therapy; the time-dependent decrease in Hb levels throughout

the treatment period; the time-dependent requirement for RBV dose reduction throughout the treatment period; and the rate of virological response or relapse. Associations between pretreatment variables and anaemia were analysed by multivariable regression. The association between the *ITPA* polymorphisms and anaemia or treatment outcome was analysed by Fisher's exact test. The association between the *ITPA* polymorphisms and the time-dependent reduction in Hb levels or the requirement for RBV dose reduction was analysed by Kaplan–Meier survival analysis. SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA) was used for these analyses.

Table 1. Clinical characteristics of the study population

| Characteristic | Value |
|---|---|
| Age, years | 57.5 (±9.5) |
| Sex, male/female | 50/82 |
| Baseline platelet count, $10^9$/l | 150.4 (±55.8) |
| Baseline Hb, g/dl | 14.0 (±1.5) |
| Baseline creatinine clearance, ml/min | 94.8 (±24.1) |
| Baseline liver fibrosis, F0–2/F3–4 | 102/30 |
| Initial ribavirin dose | |
| 600 mg/day, *n* (%) | 91 (69) |
| 800 mg/day, *n* (%) | 38 (29) |
| 1,000 mg/day, *n* (%) | 3 (2) |
| Dose reduction of ribavirin, *n* (%) | 58 (43) |
| Hb reduction at week 4, g/dl | 2.2 (±1.4) |
| Hb reduction >3.0 g/dl at week 4, *n* (%) | 37 (28) |
| Severe anaemia at week 4, *n* (%)[a] | 21 (16) |
| Severe anaemia at any time point, *n* (%)[a] | 57 (43) |
| *ITPA* rs1127354, AA/CA/CC | 4/33/95 |
| ISDR mutation ≤1, *n*/total *n* (%) | 96/114 (84) |
| Core70 mutant type, *n*/total *n* (%) | 42/105 (40) |

Continuous variables were described as mean (±SD) and categorical variables were described as frequency and percentage. [a]Severe anaemia defined as haemoglobin (Hb)<10 g/dl. Core70, amino acid substitutions at position 70 of the core region; ISDR, interferon sensitivity-determining region; *ITPA*, inosine triphosphatase gene.

## Results

### *ITPA* rs1127354 minor genotype alleles AA and CA were protective for anaemia during drug therapy

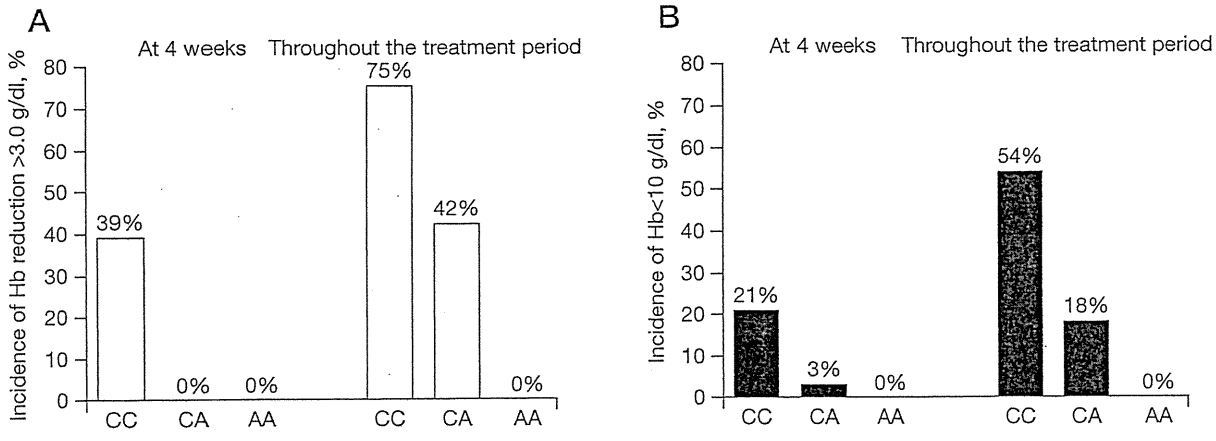
The baseline characteristics are listed in Table 1. Genotyping of rs1127354 revealed that 4 patients were homozygous for the minor allele (AA), 95 were homozygous for the major allele (CC) and 33 were heterozygous (CA). The frequency of the minor allele A was 0.16. The *ITPA* genotype was not associated with any baseline factors including age, gender, Hb levels, CLcr, platelet counts, liver fibrosis, mutations in the ISDR and Core70 (Table 2). The mean value of Hb reduction at week 4 was 2.2 g/dl and a reduction of >3.0 g/dl developed in 37 patients (28%) at week 4. Severe anaemia (Hb<10 g/dl) developed in 21 (16%) patients at week 4 of therapy and in 57 (43%) patients at any time point during the entire 48 weeks of therapy. Figure 1A and 1B shows the percentages of patients with anaemia according to the rs1127354 genotypes. At week 4, Hb reduction of >3.0 g/dl developed in 37 patients (39%) with the CC genotype, which is in contrast to 0 patients with the CA or AA genotypes (Figure 1A). Severe anaemia developed in 20 (21%) patients with the CC genotype, which is in contrast to only 1 (3%) patient with the CA genotype and 0 patients with the AA genotype (CC versus AA/CA, $P$=0.008; Figure 1B). Throughout the course of the 48-week therapy, Hb reduction of >3.0 g/dl developed in 71 (75%) patients with the CC genotype in contrast to 14 (42%) patients with the CA genotype and 0 patients with the AA genotype (CC versus AA/CA, $P$=0.0001). Severe anaemia was observed in 51 (54%) patients with the CC genotype, which is in contrast to 6 (18%) patients with the CA genotype and 0 patients with the AA genotype (CC versus AA/CA, $P$<0.0001). The mean reduction of Hb levels and the time course of therapy are shown in Figure 2. Patients with genotypes AA and CA showed less Hb reduction at weeks 2, 4, 6, 8 and 12 during drug therapy compared to those with the

Table 2. Clinical characteristics of patients according to *ITPA* genotype

| Characteristic | rs1127354 | | *P*-value |
|---|---|---|---|
| | AA/CA | CC | |
| Age, *n* (%) | 56.0 (10.9) | 58.1 (8.8) | 0.316 |
| Sex, male/female | 17/20 | 33/62 | 0.239 |
| Baseline platelet count, $10^9$/l | 153.3 (±48.5) | 149.2 (±58.5) | 0.711 |
| Baseline Hb, g/dl | 14.3 (±1.4) | 13.8 (±1.5) | 0.132 |
| Baseline creatinine clearance, ml/min | 93.4 (±23.3) | 95.3 (±24.5) | 0.692 |
| Baseline liver fibrosis, F0–2/F3–4 | 33/4 | 69/26 | 0.063 |
| ISDR mutation ≤1, *n*/total *n* (%) | 26/30 (87) | 70/84 (83) | 0.777 |
| Core70 mutant type, *n*/total *n* (%) | 11/27 (41) | 31/78 (40) | 1.000 |

Continuous variables were described as mean (±SD) and categorical variables were described as frequency and percentage. Core70, amino acid substitutions at position 70 of the core region; Hb, haemoglobin; ISDR, interferon sensitivity-determining region.

Figure 1. *ITPA* rs1127354 genotypes and anaemia during drug therapy




The percentage of patients with (A) haemoglobin (Hb) reduction of >3.0 g/dl or (B) Hb concentrations of <10 g/dl at week 4 and at any time point throughout the treatment period is shown for rs1127354 genotypes. Severe anaemia was less frequent in patients with the rs1127354 genotypes AA and CA (Hb reduction >3.0 g/dl at any time point: CC versus AA/CA, $P$=0.0001; Hb concentrations <10 g/dl at week 4: CC versus AA/CA, $P$=0.008; and Hb concentrations <10 g/dl at any time point: CC versus AA/CA, $P$<0.0001). *ITPA*, inosine triphosphatase gene.

CC genotype ($P$<0.0001 for weeks 2, 4 and 6; $P$=0.02 for weeks 8 and 12). These results show that the AA and CA genotypes are significantly associated with less absolute reduction in Hb levels, especially during the early weeks of therapy, and are protective against the development of severe anaemia. The sensitivity and specificity of the *ITPA* genotype for the prediction of severe anaemia (Hb<10 g/dl) throughout the course of treatment was 89% (51/57) and 41% (31/75), respectively.

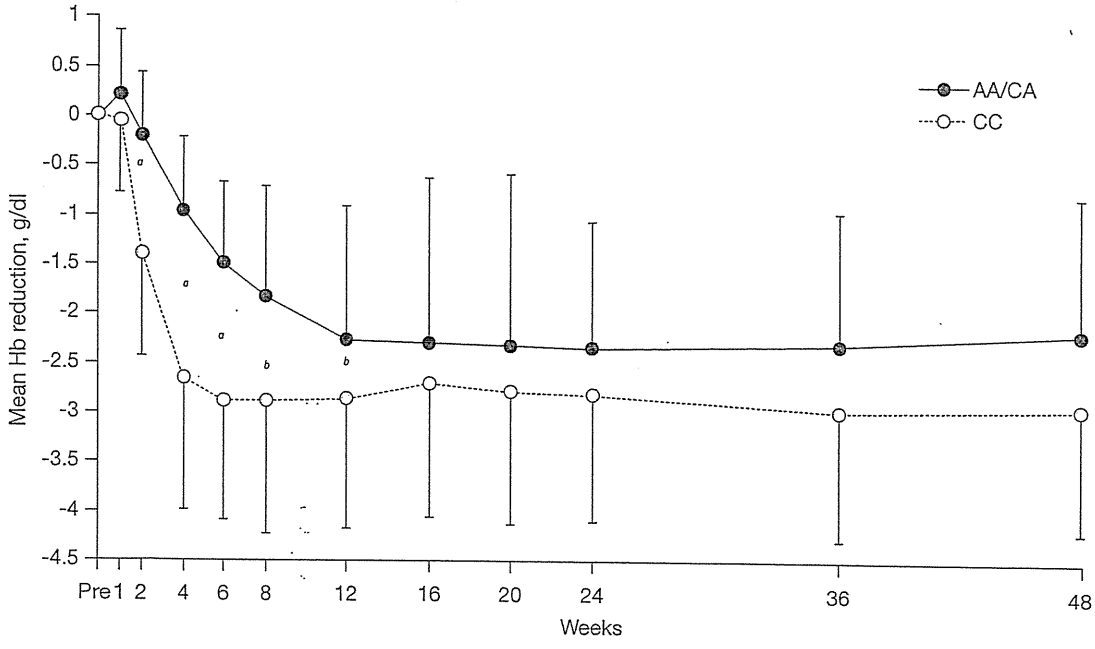
### *ITPA* rs1127354 minor genotypes AA and CA were protective against the requirement for RBV dose reduction

The dose of RBV was reduced in 58 (43%) patients. Severe anaemia was the indication for dose reduction in 45 of the 58 (78%) patients. In the remaining 13 patients, the RBV dose was reduced because of other adverse events such as fatigue, skin eruption or loss of appetite. Figure 3 shows the time to the first RBV dose reduction during the 48 weeks of therapy. A dose reduction of RBV for any reason was less frequent and delayed in patients with the AA and CA genotypes compared to those with the CC genotype (Figure 3A; $P$=0.048). The difference was more significant for anaemia-related RBV dose reduction (Figure 3B; $P$=0.004).

### Other factors associated with severe anaemia during therapy

Since 18% of the patients with the protective *ITPA* genotype of CA developed severe anaemia, we analysed the patients for other predictive factors of severe anaemia. By univariable analysis, the rs1127354 CC genotype, female gender, older age, and lower baseline Hb levels, platelet counts and CLcr levels were associated with severe anaemia. Next, multivariable regression models with backward selection were used to identify the independent predictors of severe anaemia. Covariates included age, sex, fibrosis stage, baseline Hb levels, CLcr levels and platelet counts, and the rs1127354 genotype. The multivariable regression analysis showed that the rs1127354 CC genotype, a baseline Hb of <14 g/dl and a baseline CLcr of ≤95 ml/min were independent predictors of severe anaemia at week 4 and at any time point during the 48 weeks of therapy (Table 3). Figure 4 shows the percentage of patients with Hb concentrations of <10 g/dl at any time point during therapy for the subgroups of patients stratified by rs1127354 genotype, baseline Hb levels and baseline CLcr levels. Among patients with the rs1127354 CC genotype, the risk of developing severe anaemia was more prominent in those with a baseline Hb<14 g/dl and a baseline CLcr≤95 ml/min (88%) compared to those with a baseline Hb≥14 g/dl and a baseline CLcr >95 ml/min ($P$<0.0001) or those with a baseline Hb<14 g/dl or a baseline CLcr≤95 ml/min ($P$=0.0036). Notably, the incidence of severe anaemia was only 12% in patients with the rs1127354 CC genotype if the baseline Hb was ≥14 g/dl and the CLcr was >95 ml/min. By contrast, there was a moderate risk of severe anaemia (33%) even in patients with the rs1127354 protective genotypes AA or CA when the baseline Hb was <14 g/dl and the baseline CLcr was ≤95 ml/min. Thus, patients who have >30%

Figure 2. *ITPA* rs1127354 genotypes and the quantitative Hb reduction from baseline




The mean reduction of haemoglobin (Hb) levels along the time points of treatment is shown for the rs1127354 genotypes. Solid and dotted lines indicate patients with the AA/CA and CC genotypes, respectively. The error bars indicate standard deviation. The AA/CA genotype had less of a reduction in the mean Hb levels at weeks 2–12 during therapy compared to the CC genotype. [a]$P<0.001$; [b]$P=0.02$. *ITPA*, inosine triphosphatase gene; Pre, pretreatment.

risk of severe anaemia had the following characteristics: rs1127354 CC genotype, baseline Hb<14 g/dl and CLcr≤95 ml/min; rs1127354 CC genotype and baseline Hb<14 g/dl or CLcr≤95 ml/min; and rs1127354 AA or CA genotype, baseline Hb<14 g/dl and CLcr≤95 ml/min. The sensitivity and specificity of the combination of these three factors for the prediction of severe anaemia (Hb<10 g/dl) throughout the course of treatment was 89% (51/57) and 64% (48/75). Compared to the *ITPA* genotype alone, specificity improved from 41% to 64% with the same sensitivity (89%), indicating that the combination of the *ITPA* genotype, baseline Hb levels and baseline CLcr levels could improve the prediction accuracy. The AA/CA genotypes of rs1127354 were protective against the requirement for RBV dose reduction even after standardization by baseline Hb and CLcr (Figure 3C). The predictive model for anaemia and recommendations for monitoring and treatment were made for clinical practice application (Table 4).

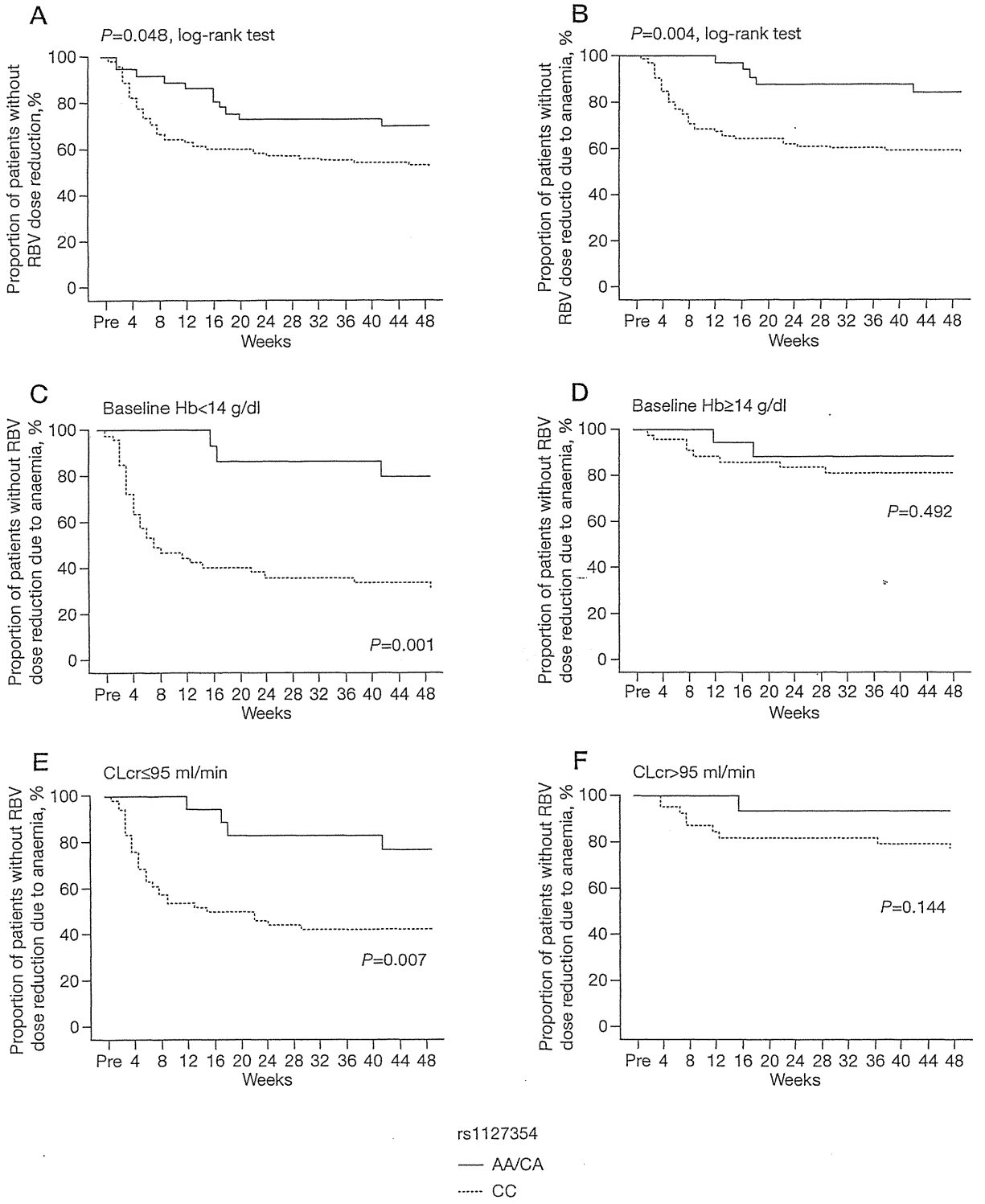
### ITPA rs1127354 minor genotypes AA and CA were associated with higher adherence to RBV, higher rate of SVR and lower rate of relapse

The association of the rs1127354 genotype with the adherence to RBV or treatment outcome was analysed. When analysed in the entire population, the percentage of patients receiving >80% of the expected RBV dose, which was reported to be a threshold for an enhanced response to therapy [3], was not significantly different among the rs1127354 genotypes. Treatment outcomes such as the end-of-treatment response, SVR and relapse were also not different among the rs1127354 genotypes (Table 5). By contrast, SVR was closely associated with the *IL28B* genotype [11–14,21]: the rate of SVR was 0% (0/51) for *IL28B* minor type (TG/GG genotype at rs8099917) and 48% (39/81) for *IL28B* major type (TT genotype at rs8099917). This finding confirms that *IL28B* genotype is a significant factor for the prediction of SVR. Thus, we performed a subset analysis on subgroup of patients with the favourable *IL28B* genotype (TT at rs8099917). As a result, patients with the rs8099917 TT genotype and the rs1127354 AA or CA genotypes had a significantly higher rate of receiving >80% of the expected RBV dose ($P=0.016$), a higher rate of SVR ($P=0.031$), as well as a lower rate of relapse ($P=0.046$) compared to patients with the rs8099918 TT and rs1127354 CC genotype (Table 5).

## Discussion

In the present study, we confirmed that variants of the *ITPA* gene protect against severe haemolytic anaemia not

Figure 3. *ITPA* rs1127354 genotypes and the time-dependent incidence of RBV dose reduction




The time to the first reduction of the ribavirin (RBV) dose (A) due to any reason or (B) due to anaemia is shown stratified by the rs1127354 genotypes. Solid and broken lines indicate patients with the AA/CA and CC genotypes, respectively. The AA/CA genotype protected against the requirement for RBV dose reduction. (C–F) Patients were standardized according to the baseline haemoglobin (Hb) and creatinine clearance (CLcr). Even after standardization by baseline Hb and CLcr, the AA/CA genotype protected against the requirement for RBV dose reduction. *ITPA*, inosine triphosphatase gene; Pre, pretreatment.

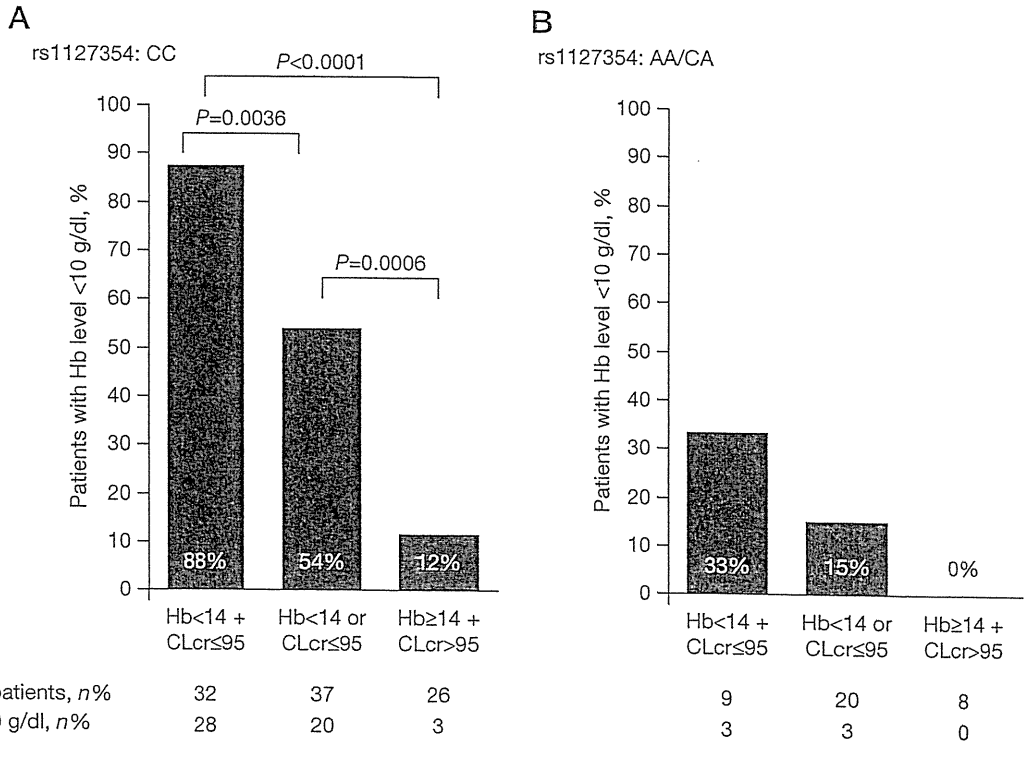
only at the early stage of treatment, but also throughout the 48-week course of treatment in a Japanese cohort of genotype 1b chronic hepatitis C patients treated with PEG-IFN and RBV. We also replicated a previous study [9] that showed that the *ITPA* genotype is significantly associated with a time-dependent reduction of the RBV dose. Furthermore, we found that a combination of the *ITPA* genotype and the baseline Hb and CLcr levels improve the accuracy of predicting RBV-induced severe anaemia. Previous reports on the IDEAL [4] or Vira-Hep-C [9] studies did not find any association between the *ITPA* genotype and treatment outcome; however, we were able to demonstrate the association of the *ITPA* genotype with a higher adherence to RBV, a higher rate

Table 3. Multivariable regression analysis of factors associated with severe anaemia during therapy[a]

| Predictor | OR | 95% CI | *P*-value |
|---|---|---|---|
| At week 4 | | | |
| Baseline Hb<14 g/dl | 7.18 | 1.90–27.09 | 0.004 |
| Baseline creatinine clearance ≤95 ml/min | 5.30 | 1.39–20.26 | 0.015 |
| *ITPA* rs1127354: CC | 10.17 | 1.25–82.85 | 0.030 |
| At any time point | | | |
| Baseline Hb<14 g/dl | 7.67 | 3.07–19.12 | <0.0001 |
| Baseline creatinine clearance ≤95 ml/min | 5.51 | 2.21–13.73 | <0.0001 |
| *ITPA* rs1127354: CC | 9.66 | 3.11–29.95 | <0.0001 |

[a]Severe anaemia was defined as haemoglobin (Hb)<10 g/dl. *ITPA*, inosine triphosphatase gene.

Figure 4. Combination of the *ITPA* rs1127354 genotype, baseline Hb level and baseline CLcr level is predictive of severe anaemia during the therapy




Patients with rs1127354 genotype (A) CC and (B) AA/CA were further stratified by the baseline haemoglobin (Hb) and creatinine clearance (CLcr) levels. The percentage of patients with Hb concentrations of <10 g/dl (severe anaemia) at any time point during therapy is shown for the subgroups of patients. Patients with baseline Hb levels of <14 g/dl and CLcr levels of <95 ml/min had a higher incidence of severe anaemia among patients with the rs1127354 genotype CC (Hb<14 g/dl and CLcr≤95 ml/min versus Hb≥14 g/dl and CLcr>95 ml/min, *P*<0.0001; Hb<14 g/dl and CLcr≤95 ml/min versus Hb<14 g/dl or CLcr≤95 ml/min, *P*=0.0036). *ITPA*, inosine triphosphatase gene.

Table 4. Prediction model for severe anaemia and recommendation for monitoring and treatment

| *ITPA* genotype (rs1127354) | Baseline Hb and CLcr | Risk of anaemia | Recommendation | |
|---|---|---|---|---|
| | | | Monitoring | Treatment option |
| CC | Hb<14 g/dl and CLcr≤95 ml/min | High | Intensive | Consider erythropoietin |
| | Hb<14 g/dl or CLcr≤95 ml/min | Intermediate | Intensive | Early dose reduction of RBV |
| | Hb≥14 g/dl and CLcr>95 ml/min | Low | As usual | – |
| AA/CA | Hb<14 g/dl and CLcr≤95 ml/min | Intermediate | Intensive | Early dose reduction of RBV |
| | Hb<14 g/dl or CLcr≤95 ml/min | Low | As usual | – |
| | Hb≥14 g/dl and CLcr>95 ml/min | Absent | As usual | May consider higher RBV dose |

CLcr, creatinine clearance; Hb, haemoglobin; *ITPA*, inosine triphosphatase gene; RBV, ribavirin.

Table 5. Treatment response and ribavirin adherence in terms of *ITPA* rs1127354 genotype

| Response | rs1127354 | | *P*-value |
|---|---|---|---|
| | AA/CA, *n*/total *n* (%) | CC, *n*/total *n* (%) | |
| All patients | | | |
| Ribavirin adherence >80% | 19/37 (51) | 40/95 (42) | 0.436 |
| End-of-treatment response | 19/37 (51) | 58/95 (61) | 0.332 |
| Sustained virological response | 13/37 (35) | 26/95 (27) | 0.401 |
| Relapse | 6/19 (32) | 32/58 (55) | 0.112 |
| Subgroup of patients with IL28B rs8099917 TT | | | |
| Ribavirin adherence >80% | 14/18 (78) | 28/63 (49) | 0.016 |
| End of treatment response | 16/18 (89) | 50/63 (79) | 0.501 |
| Sustained virological response | 13/18 (79) | 26/63 (41) | 0.031 |
| Relapse | 3/16 (19) | 24/50 (48) | 0.046 |

*ITPA*, inosine triphosphatase gene.

of SVR and a lower rate of relapse among a subset of Japanese patients with the favourable *IL28B* genotype (TT at rs8099917).

Haemolytic anaemia induced by RBV is one of the major adverse events of PEG-IFN and RBV therapy leading to dose reduction of RBV or premature termination of therapy [1]. RBV is essential for improving SVR by prevention of relapses and a breakthrough [22], and a reduction of the RBV dose can lower the response rates considerably. It was reported that the maintenance of >80% of the expected RBV dose is associated with an increased SVR [23]. Thus, the prediction and prevention of RBV-induced haemolytic anaemia is clinically important. Previously, no reliable means were available to predict RBV-induced anaemia before therapy, but a recent genome-wide association study identified a strong association between two functional SNPs (rs1127354 and rs7270101) in the *ITPA* gene on chromosome 20 [4] and severe anaemia at week 4 of treatment. This genetic association has been replicated recently by two studies [9,10]. However, the effect of these variants on the long-term development of anaemia or on the requirement for RBV dose reduction has been reported by only one study to date [9]. Therefore, validation of these results by an independent cohort with respect to different geographical areas, age, gender or race is needed. Although the clinical background of our cohort was different from that of the US cohort [9], such as their race, older age (mean age of 57.5 years versus the median age of 48.5 years), and higher predominance of females (62% versus 35%), we were still able to replicate the results that the rs1127354 genotypes AA and CA are protective against anaemia throughout the 48-week course of treatment, especially within the 12 weeks following the initial treatment. We also replicated the association of this genotype with less requirement for RBV dose reduction. These results indicate that the *ITPA* genotype is universally an important determinant of RBV-induced haemolytic anaemia.

For the general application of these genetic associations in clinical practice, we aimed to further improve the accuracy of prediction by combining other clinical covariates. Among the patients with the rs1127354 CC genotype, the risk of developing severe anaemia was as high as 88% in those with baseline Hb levels of <14 g/dl and baseline CLcr levels of ≤95 ml/min, which is in contrast to only 12% in patients with Hb levels of ≥14 g/dl and CLcr levels of >95 ml/min. The rs1127354 AA and CA genotypes were protective against anaemia, but an exception occurred when patients (33%) with a baseline Hb level of <14 g/dl and a CLcr level of ≤95 ml/min developed severe

anaemia. The combination of these three factors may therefore be useful in clinical practice, since it improved the specificity of prediction from 41% to 64% with the same sensitivity (89%) compared to examining just the *ITPA* genotype. These findings may have the potential to support individualized treatment strategies. Patients with the rs1127354 CC genotype, especially those with a baseline Hb level of <14 g/dl and a baseline CLcr level of ≤95 ml/min, require intensive monitoring for anaemia during therapy, and an early dose reduction of RBV or support by erythropoietin may be indicated for safety. By contrast, patients with the AA and CA genotypes, excluding those with a baseline Hb level of <14 g/dl and a baseline CLcr level of ≤95 ml/min, may be candidates for therapy with a higher RBV dose, which may lead to higher rates of SVR. The prediction of RBV-induced anaemia will remain an important issue even in the near future, since direct antiviral agents require RBV and PEG-IFN in combination in order to achieve higher SVR rates for genotype 1 [24,25] and this combination will remain a standard therapy for other genotypes.

In a previous study, there was no clear association between ITPase deficiency and treatment outcome [4,9,10], even after a detailed subset analysis that excluded patients in whom RBV had been reduced for indications other than anaemia or after stratification by the *IL28B* genotype [9]. Thompson *et al.* [9] speculated that the lack of association may derive from several reasons such as an underpowered error due to the small number of patients, a high incidence of RBV dose reduction unrelated to anaemia, and the possibility that the ITPase deficiency may reduce antiviral efficacy. In the present study, we also failed to show associations between the *ITPA* genotype and treatment outcomes among the entire cohort. However, when patients were stratified by the *IL28B* genotype, which is now recognized as the major determinant of treatment outcome [11–14,21], the AA and CA genotypes at rs1127354 were linked to a higher adherence to RBV, a lower rate of relapse and a significantly higher rate of SVR. One of the reasons for this discrepancy may be the lower incidence of anaemia-unrelated RBV dose reduction in our study compared to the participants of the Vira-Hep-C study (22% versus 48%) [9]. The effect of the *ITPA* genotype on RBV adherence and treatment outcome may be less apparent in patients who reduced their RBV dose in the absence of anaemia. Another possibility is that the difference in mean age may have some effect on this association between the *ITPA* genotype and treatment outcome since older age has been reported to compromise drug adherence or treatment outcomes [26,27]. Our results indicated that, although IL28B genotype is the major determinant of SVR, the *ITPA* genotype may be used supplementary to predict the treatment outcome in patients with a favourable *IL28B* genotype (TT at rs8099917), as long as the RBV dose is not reduced in the absence of anaemia. Further studies involving larger populations in different geographical areas or races may be necessary to confirm this speculation.

In conclusion, variants of the *ITPA* gene, which could protect against haemolytic anaemia and RBV dose reduction, were associated with a high rate of SVR by standard PEG-IFN and RBV therapy in a subset of Japanese patients with the favourable *IL28B* genotype. A combination of the *ITPA* genetic polymorphism with baseline Hb and CLcr levels further improved the predictive accuracy of severe anaemia. These findings may have the potential to support selection of the optimum and personalized treatment strategy for individual patients.

## Acknowledgements

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

## Disclosure statement

The authors declare no competing interests.

## References

1. Fried MW, Shiffman ML, Reddy KR, *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347:975–982.
2. Manns MP, McHutchison JG, Gordon SC, *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358:958–965.
3. McHutchison JG, Manns M, Patel K, *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; 123:1061–1069.
4. Fellay J, Thompson AJ, Ge D, *et al. ITPA* gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature* 2010; 464:405–408.
5. Sumi S, Marinaki AM, Arenas M, *et al.* Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. *Hum Genet* 2002; 111:360–367.
6. Maeda T, Sumi S, Ueta A, *et al.* Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency in the Japanese population. *Mol Genet Metab* 2005; 85:271–279.
7. Shipkova M, Lorenz K, Oellerich M, Wieland E, von Ahsen N. Measurement of erythrocyte inosine triphosphate pyrophosphohydrolase (*ITPA*) activity by HPLC and correlation of *ITPA* genotype-phenotype in a Caucasian population. *Clin Chem* 2006; 52:240–247
8. Atanasova S, Shipkova M, Svinarov D, *et al.* Analysis of *ITPA* phenotype-genotype correlation in the Bulgarian population revealed a novel gene variant in exon 6. *Ther Drug Monit* 2007; 29:6–10.
9. Thompson AJ, Fellay J, Patel K, *et al.* Variants in the *ITPA* gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. *Gastroenterology* 2010; 139:1181–1189.
10. Ochi H, Maekawa T, Abe H, *et al.* Inosine triphosphate pyrophosphatase polymorphism affects ribavirin-induced anemia and outcomes of therapy – a genome-wide study of Japanese hepatitis C virus patients. *Gastroenterology* 2010; 139:1190–1197.

11. Ge D, Fellay J, Thompson AJ, *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461:399–401.
12. Tanaka Y, Nishida N, Sugiyama M, *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; 41:1105–1109.
13. Suppiah V, Moldovan M, Ahlenstiel G, *et al. IL28B* is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; 41:1100–1104.
14. Rauch A, Kutalik Z, Descombes P, *et al.* Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010; 138:1338–1345.
15. Nishida N, Tanabe T, Takasu M, Suyama A, Tokunaga K. Further development of multiplex single nucleotide polymorphism typing method, the DigiTag2 assay. *Anal Biochem* 2007; 364:78–85.
16. Enomoto N, Sakuma I, Asahina Y, *et al.* Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; 334:77–82.
17. Kurosaki M, Enomoto N, Murakami T, *et al.* Analysis of genotypes and amino acid residues 2209 to 2248 of the NS5A region of hepatitis C virus in relation to the response to interferon-beta therapy. *Hepatology* 1997; 25:750–753.
18. Akuta N, Suzuki F, Sezaki H, *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; 48:372–380.
19. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16:31–41.
20. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; 24:289–293.
21. Tanaka Y, Nishida N, Sugiyama M, Tokunaga K, Mizokami M. λ-Interferons and the single nucleotide polymorphisms: a milestone to tailor-made therapy for chronic hepatitis C. *Hepatol Res* 2010; 40:449–460.
22. Bronowicki JP, Ouzan D, Asselah T, *et al.* Effect of ribavirin in genotype 1 patients with hepatitis C responding to pegylated interferon alfa-2a plus ribavirin. *Gastroenterology* 2006; 131:1040–1048.
23. McHutchison JG, Lawitz EJ, Shiffman ML, *et al.* Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; 361:580–593.
24. McHutchison JG, Everson GT, Gordon SC, *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; 360:1827–1838.
25. Kwo PY, Lawitz EJ, McCone J, *et al.* Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre Phase 2 trial. *Lancet* 2010; 376:705–716.
26. Hiramatsu N, Oze T, Tsuda N, *et al.* Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy? *Hepatol Res* 2006; 35:185–189.
27. Iwasaki Y, Ikeda H, Araki Y, *et al.* Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* 2006; 43:54–63.


Accepted 28 November 2010; published online 18 May 2011

Hepatology Research

*Hepatology Research* 2011; 41: 217–224 doi: 10.1111/j.1872-034X.2010.00768.x

*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

Jun Itakura,[1] Yasuhiro Asahina,[1] Nobuharu Tamaki,[1] Itsuko Hirayama,[1] Yutaka Yasui,[1] Tomohiro Tanaka,[1] Mitsuaki Sato,[1] Ken Ueda,[1] Teiji Kuzuya,[1] Kaoru Tsuchiya,[1] Hiroyuki Nakanishi,[1] Masayuki Kurosaki,[1] Gretchen S. Gabriel,[2] George J. Schneider[2] and Namiki Izumi[1]

[1]*Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan; and* [2]*Abbott Molecular, Des Plaines, Illinois, USA*

*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

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

There are no conflicts of interests regarding this study.

*Received 13 November 2009; revised 18 November 2010; accepted 18 December 2010.*

"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 ($c$, $\delta$, $\varepsilon$, $T_0$ and $V_0$) were calculated from viral loads with equations for HCV dynamics.[15] The parameter $c$ is the constant viral death rate, $\delta$ is the death rate of infected cells, $\varepsilon$ 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 ($\times 10^3/\mu L$) | 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.