2 g/dl at week 2 was useful for predicting the probability of severe anemia, and has the potential to support clinical decisions regarding early dose reduction of ribavirin.

Keywords Data mining · Decision tree · Severe anemia · Chronic hepatitis C · Pegylated interferon · Ribavirin

Abbreviations

Hb	Hemoglobin
PEG-IFN	Pegylated interferon
Ccr	Creatinine clearance
SVR	Sustained virological response
AFP	Alpha-fetoprotein

Introduction

The current standard therapy for chronic hepatitis C is 48 weeks of pegylated interferon (PEG-IFN) plus ribavirin [1]. Sustained virological response (SVR), defined as negative hepatitis C virus (HCV) RNA for 24 weeks after cessation of therapy, can be achieved by the current treatment regimen, but this outcome can be attained in only less than 50% of patients infected with genotype 1 HCV [2, 3]. Hemolytic anemia is a common side effect of ribavirin and is the major reason for dose reduction. Age, gender, baseline platelet level, baseline hemoglobin (Hb) level [4, 5], haptoglobin phenotype [6], drug dose [7], plasma concentration of ribavirin [8], apparent clearance of ribavirin (CL/F) [9], and an early decline in Hb concentration [10, 11] have been reported to contribute to ribavirin-induced anemia. Predicting the possibility of severe anemia before therapy or at the early phase of therapy can help modify ribavirin dosage, decrease the discontinuance rate for ribavirin, and raise the SVR rate.

Data mining is a method of predictive analysis that explores data to discover hidden patterns and relationships in highly complex datasets and enables the development of predictive models. Decision-tree analysis is a core component of data mining and predictive modeling [12], and it is utilized by decision makers in various business fields. Recent publications concerning decision-tree analysis in the medical field indicate its usefulness for defining prognostic factors in various diseases such as prostate cancer [13], diabetes [14], melanoma [15, 16], colorectal carcinoma [17, 18], and liver failure [19]. The results of decision-tree analysis are presented in the form of a flow chart, which is easy to use in clinical practice [20]. This analysis was also used to predict early virological response (undetectable HCV RNA within 12 weeks of therapy) and SVR to PEG-IFN plus ribavirin combination therapy in chronic hepatitis C [21–24]. In the present study, we used decision-tree analysis to explore before- and during-treatment predictors of severe anemia during PEG-IFN alpha-2b/ribavirin combination therapy and used a prediction algorithm to try to identify chronic hepatitis C patients who are likely to develop severe anemia.

Materials and methods

Patients

This multicenter retrospective cohort study was supported by the Japanese Ministry of Health, Labour and Welfare. Data were collected from 1081 chronic hepatitis C patients who were treated with PEG-IFN alpha-2b plus ribavirin at Osaka University, Musashino Red Cross Hospital, Toranomon Hospital, Tokyo Medical and Dental University, Nagoya City University, Yamanashi University, and their related hospitals. The inclusion criteria applied in the present study were as follows: (1) infection by genotype 1b, (2) HCV RNA $\geq$ 100 KIU/ml by quantitative PCR (Cobas Amplicor HCV Monitor v 2.0, Roche Diagnostic Systems, CA, USA), (3) lack of co-infection with hepatitis B virus or human immunodeficiency virus, (4) lack of other causes of liver diseases such as autoimmune hepatitis and primary biliary cirrhosis, and (5) completion of at least 12 weeks of therapy. Patients received PEG-IFN alpha-2b (1.5 g/kg) subcutaneously every week and were administered a weight-adjusted dose of ribavirin (600 mg for <60 kg, 800 mg for 60–80 kg, and 1000 mg for >80 kg). The dosage of ribavirin was reduced from 1000 to 600 mg, 800 to 600 mg, or 600 to 400 mg when the Hb concentration decreased to less than 10 g/dl, and was discontinued when the Hb concentration decreased to less than 8.5 g/dl, based on the recommendations in the package inserts. No patient received erythropoietin or blood transfusion for the treatment of anemia. Anemia with Hb $<$ 8.5 g/dl was defined as severe anemia in this study.

For the analysis, patients were randomly assigned to either the model-building (n = 691) group or the internal validation (n = 390) group. 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 committee. The baseline characteristics and representative laboratory test results are listed in Table 1. There were no significant differences between the clinical backgrounds of the two groups.

Laboratory tests

Blood samples were obtained before therapy and at least once every month during therapy, and were used for hematological tests, blood chemistry analyses, and determination of HCV RNA. Pretreatment levels of HCV RNA

Table 1 Comparison of clinical parameters of model-building and internal validation groups

	All patients ($N = 1081$)	Model building ($N = 691$)	Internal validation ($N = 390$)
Age (years)	55.6 ± 10.5	55.6 ± 10.8	55.6 ± 10.4
Gender (male/female)	612/469	393/298	219/171
Body mass index (kg/m^2)	23.2 ± 3.3	23.4 ± 3.8	23.1 ± 3.0
Creatinine (mg/dl)	0.73 ± 0.16	0.74 ± 0.17	0.73 ± 0.16
AST (IU/l)	62.0 ± 44.8	63.2 ± 48.6	61.4 ± 42.5
ALT(IU/l)	74.6 ± 56.1	75.4 ± 60.5	74.2 ± 53.5
GGT (IU/l)	58.6 ± 57.0	59.5 ± 58.5	58.0 ± 56.2
Albumin	4.0 ± 0.3	4.0 ± 0.3	4.0 ± 0.4
Total cholesterol	171.8 ± 31.7	171.5 ± 32.3	172.2 ± 30.8
HDL cholesterol	50.9 ± 14.5	51.1 ± 14.3	50.5 ± 15.0
LDL cholesterol	95.5 ± 27.7	96.1 ± 27.9	94.1 ± 27.2
Triglyceride	108.8 ± 55.4	107.8 ± 57.3	110.9 ± 51.7
Glucose	111.2 ± 39.0	111.7 ± 39.8	110.3 ± 37.6
Alpha-fetoprotein	14.5 ± 43.9	13.3 ± 37.7	16.8 ± 54.1
White blood cell count (/μl)	4946 ± 1427	4851 ± 1355	4999 ± 1464
Hemoglobin (g/dl)	14.2 ± 1.4	14.2 ± 1.4	14.2 ± 1.4
Platelets (10^9/mm^3)	166.1 ± 51.4	165.6 ± 51.7	166.4 ± 51.2
Ccr (ml/min)	95.1 ± 26.5	94.8 ± 25.9	95.4 ± 26.9
HCV RNA (KIU/ml)	1978 ± 1442	1937 ± 1382	2001 ± 1476
Fibrosis stage (F0–2/F3–4/ND)	695/148/238	454/85/152	241/63/86
Activity (A0–1/2–3/ND)	457/383/241	295/241/154	162/141/87
PEG-IFN alpha-2b dosage (μg/kg/body weight)	1.48 ± 0.13	1.49 ± 0.13	1.48 ± 0.13
Ribavirin dosage (600/800/1000 mg)	581/457/43	370/298/23	211/159/20
Decline of Hb at week 1	−0.2 ± 0.8	−0.2 ± 0.8	−0.2 ± 0.8
Decline of Hb at week 2	−1.2 ± 1.2	−1.2 ± 1.3	−1.2 ± 1.1
Decline of Hb at week 4	−2.3 ± 1.5	−2.2 ± 1.4	−2.4 ± 1.5
Decline of Hb at week 8	−2.8 ± 1.4	−2.8 ± 1.4	−2.7 ± 1.4

Data are expressed as median ± standard deviation unless otherwise indicated

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *Ccr* creatinine clearance, *Hb* hemoglobin

were quantified by Cobas Amplicor (Roche Diagnostic Systems, CA, USA).

Database of variables and decision-tree analysis

A database of pretreatment variables was created containing 3 variables from hematological tests (Hb, white blood cells, and platelets), 11 variables from blood biochemical tests (creatinine, albumin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, fasting blood glucose, and alpha-fetoprotein), creatinine clearance (Ccr), serum level of HCV RNA, liver histology (activity, fibrosis), 3 variables from patient characteristics (age, gender, and body mass index), 2 variables from therapeutic factors (PEG-IFN alpha-2b dosage, ribavirin dosage), and the level of decline of Hb concentration (at the end of 1, 2, 4, and 8 weeks from the start of treatment). Ccr levels were calculated using the Cockcroft–Gault formula [25]. Variables with data deficiency of greater than 15% were not included in the decision-tree analysis. Data deficiency was 21% in liver histology (activity, fibrosis) and 16% in the level of decline of Hb concentration at the end of 1 week. Accordingly, these variables were excluded from the database.

On the basis of this database, we implemented the recursive partitioning analysis algorithm referred to as the decision-tree analysis algorithm [26] to define subgroups of patients with respect to the possibility of severe anemia. The data-mining software used was IBM SPSS Modeler 13 (IBM SPSS Inc, Chicago, IL, USA), as reported previously [21–24]. In brief, the software searched the patient population for the most significant variables and cutoffs to be used for dividing the total population into 2 subgroups, having different probabilities of severe anemia. Thereafter,

the analysis was repeated on all subgroups in the same manner until either no additional significant variable was detected or the sample size was less than 20.

For other statistical analyses, including multivariable analysis, IBM SPSS Statistics software v.15.0 (IBM SPSS Inc, Chicago, IL, USA) was used. Differences in proportions were tested by the chi-squared test. Differences in continuous variables were compared by Student's t test. For univariate and multivariate analyses, logistic regression analysis was used to predict ribavirin-induced severe anemia. A value of $P < 0.05$ (two-tailed) was considered to indicate significance.

Results

Decision-tree analysis

Decision-tree analysis was carried out on the data of the model-building group using 27 variables, as described above. The analysis automatically selected 3 predictive variables to produce a total of 5 patient subgroups to build the decision tree (Fig. 1). Baseline Hb was selected as the first splitting variable, with an optimal cutoff of 14 g/dl. The possibility of severe anemia was 6.5% for patients with Hb levels <14 g/dl compared to 1.0% for patients with Hb levels ≥14 g/dl. Among patients with Hb ≥ 14 g/dl, the level of decline of Hb at the end of 2 weeks from the start of treatment, with an optimal cutoff of 2 g/dl, was selected as the second splitting variable. Patients with lower decline levels had a lower probability of developing severe anemia [<2 g/dl (group A) 0.4% vs. ≥2 g/dl (group B) 2.5%]. Among patients whose Hb was less than 14 g/dl, Ccr was selected as the second splitting variable, with an optimal cutoff of 80 ml/min. Patients with higher Ccr levels had a lower probability of developing severe anemia [≥80 ml/min, 2.4% vs. <80 ml/min (group C) 11.8%]. Among patients with a Ccr ≥ 80 ml/min, the level of decline of Hb at the end of 2 weeks from the start of the treatment was selected as the third splitting variable, with an optimal cutoff of 2 g/dl. Patients with lower decline levels had a lower probability of developing severe anemia [<2 g/dl (group D) 1.4% vs. ≥2 g/dl (group E) 11.5%].

The probabilities of severe anemia for the 5 subgroups derived by this process were highly variable. The subgroup of patients with higher Hb levels (≥14 g/dl) (groups A and B) had a low probability of developing severe anemia (0.4–2.5%). Also, the subgroup of patients with lower Hb (<14 g/dl) but with a higher Ccr (≥80 ml/min) and lower Hb decline levels at the end of 2 weeks from the start of the treatment (<2 g/dl) (group D) showed a low probability of developing severe anemia (1.4%). On the other hand, the

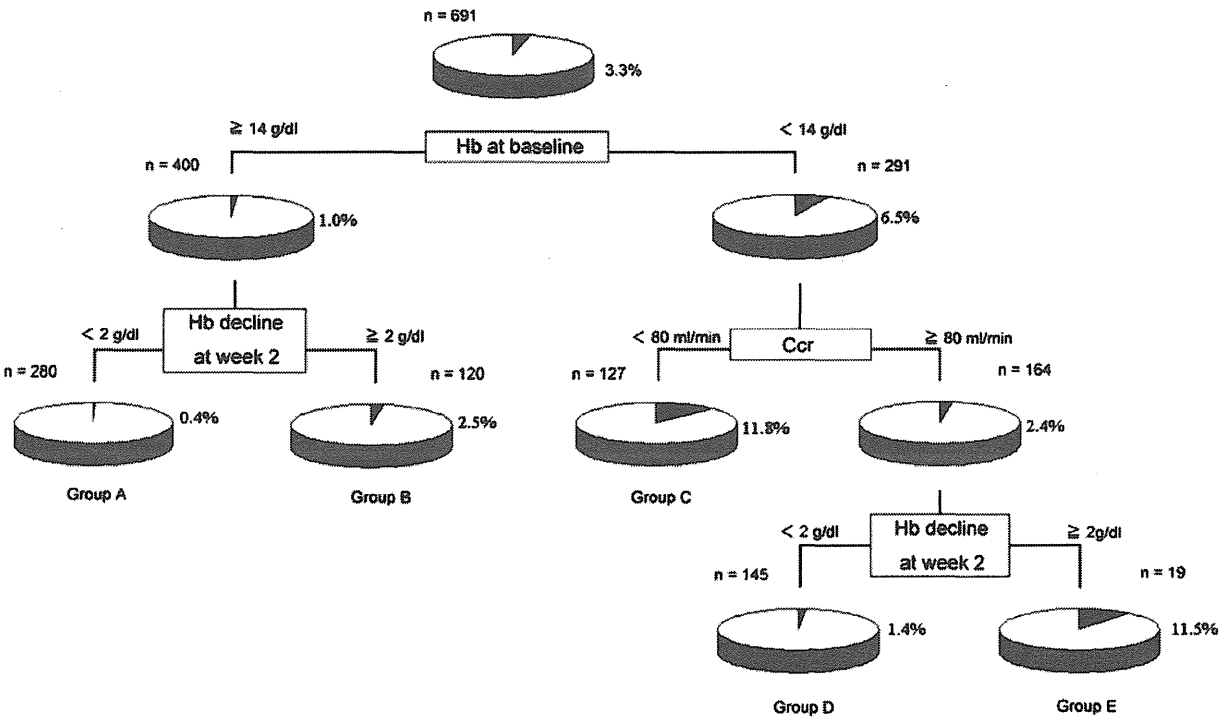

Fig. 1 Decision-tree analysis. *Boxes* indicate the splitting factors and the cutoff value for the split. *Pie charts* indicate the rate of severe anemia (Hb < 8.5 g/dl) for each group. Terminal groups classified by the analysis were labeled from A to E. *Hb* hemoglobin, *Ccr* creatine clearance

subgroup of patients with lower Hb (<14 g/dl) and lower Ccr (<80 ml/min) (group C) levels showed the highest probability of severe anemia (11.8%). Also, the subgroup of patients with lower Hb levels (<14 g/dl), higher Ccr (≥80 ml/min), and higher Hb decline levels at the end of 2 weeks from the start of treatment (≥2 g/dl) (group E) showed a high probability of developing severe anemia (11.5%).

Validation of the decision tree

The results of the decision tree were validated with the dataset of the internal validation group, which was independent of the model-building group dataset. Each patient in the validation group was allocated to groups A–E using the flow chart form of the decision tree. The rates of severe anemia (Hb < 8.5 g/dl) were 0.6% for group A, 3.0% for group B, 16.9% for group C, 2.3% for group D, and 11.0% for group E. The rates of severe anemia for each subgroup of patients were closely correlated between the model-building group and the internal validation group ($r^2 = 0.96$) (Fig. 2).

The efficiency and stability of the decision-tree model were validated using the discrimination efficiency curve (Fig. 3). The subgroups were sorted according to the order of incidence rate of severe anemia and validated using the correlation between the cumulative cases (%) and the cumulative incidence of severe anemia (%). The curve of the model-building group was located at the left upper part compared with the standard curve, indicating that the discrimination efficiency was high. Furthermore, the curve of the model-building group was extremely similar to the curve of the internal validation group, indicating that the stability was high.

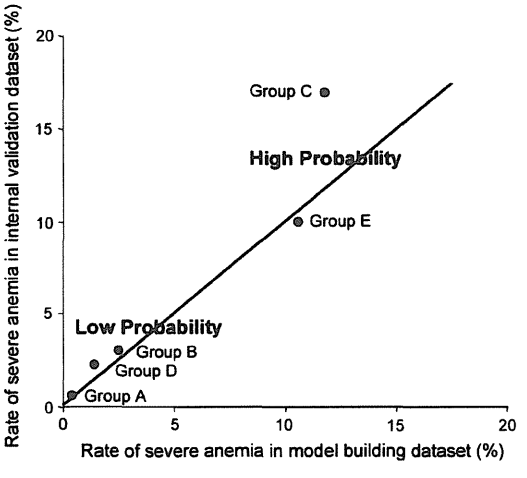

Fig. 2 Validation of decision-tree analysis with the internal validation dataset: subgroup-stratified comparison of the rate of severe anemia. The rate of severe anemia in each subgroup was plotted. The *X*-axis represents the model-building dataset and the *Y*-axis represents the internal validation dataset. There was a close correlation between the model-building and internal validation datasets ($r^2 = 0.96$)

Factors associated with severe anemia determined by multivariate logistic regression analysis

We also explored the factors associated with severe anemia using standard statistical analysis. By univariable analysis, age, creatinine, Hb, Ccr, fibrosis stage, and decline of Hb at 2, 4, and 8 weeks from the start of treatment were found to be associated with severe anemia (Table 2) and the odds ratio for these factors were 1.06, 9.61, 0.47, 0.95, 3.14, 0.76, 0.70, and 0.68, respectively, by univariable logistic regression analysis (Table 3). By multivariate analysis, Hb, Ccr, and decline of Hb at 2 weeks from the start of treatment were found to be independently associated with severe anemia (Table 3). Fibrosis was not included in the multivariable analysis because data were not available for 238 patients. Creatinine was not included in the multivariable analysis because creatinine and Ccr were confounding factors due to their close correlation. Decline of Hb at week 2, 4, and 8 were also closely correlated. We selected decline of Hb at week 2 in the multivariable analysis because we think that variables at earlier time points may be more useful in clinical use. As a result, decision-tree and multivariable logistic regression analyses identified the same factors for prediction of severe anemia.

Discussion

Hemolytic anemia, a major common side effect of ribavirin treatment, is one of the most important adverse effects of PEG-IFN and ribavirin combination treatment. Therefore, before- and during-treatment prediction of the likelihood of severe anemia can be very useful for physicians to support clinical decisions concerning the dose reduction of ribavirin. Reducing the dose of ribavirin has been shown to affect the HCV RNA negativity [27], and the discontinuation of ribavirin has been reported to lead to a marked decrease of SVR [9]. Therefore, averting ribavirin discontinuance, even if its dose must be reduced, can lead to an improvement in the SVR rate. It is important to identify patients prone to develop severe anemia leading to ribavirin dose reduction or discontinuance in the early phase of treatment.

Using decision-tree analysis, we constructed a simple model for predicting the incidence of severe anemia during therapy. The analysis highlighted 3 variables relevant to virological response: Hb, Ccr, and the decline of Hb concentration by 2 g/dl at the end of the 2 weeks from the start of treatment. Classification based on these variables identified subgroups of genotype 1b chronic hepatitis C patients with high probabilities of developing severe anemia. The

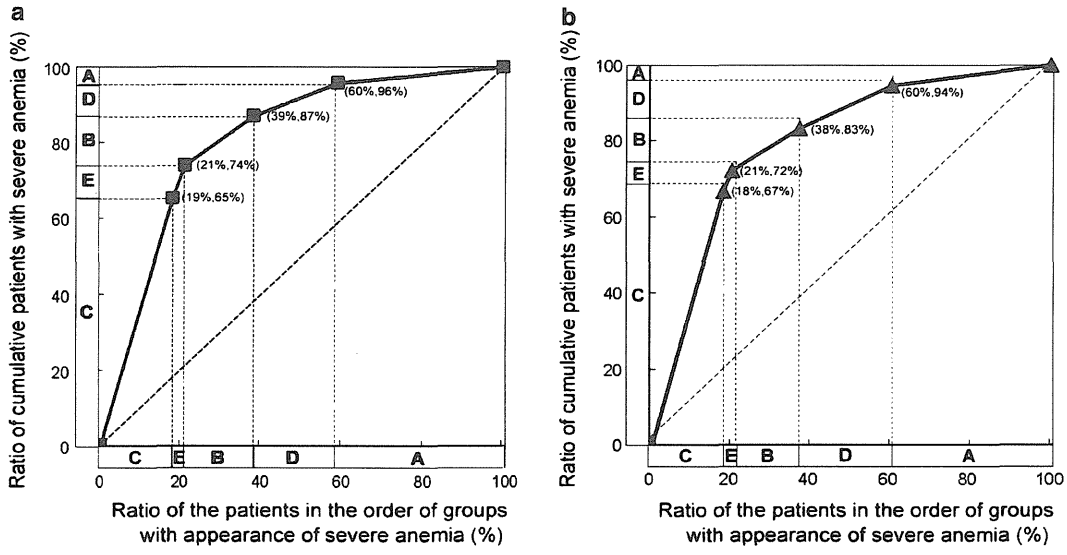

Fig. 3 Validation of the efficiency and stability by the discrimination efficiency curve. **a** Model-building group and **b** internal validation group. The groups were sorted in the order of incidence rate of severe anemia and validated using the correlation between cumulative cases (%) and the cumulative incidence of severe anemia (%). The *X*-axis represents the ratio of patients in the order of groups predicting the development of anemia and the *Y*-axis represents the cumulative patients suffering from severe anemia. The discrimination efficiency and stability of the curve of the model-building group were high

reproducibility of the model was confirmed with the internal validation dataset. An advantage of decision-tree analysis over traditional regression models is that the decision-tree model is user-intuitive and can be readily interpreted by medical professionals without the need for any specific knowledge of statistics. Patients can be allocated to specific subgroups based on a defined rate of severe anemia simply by following the flow chart format. Using this model, an estimate of the incidence of severe anemia can be obtained rapidly, which may facilitate clinical decision making for the reduction of ribavirin dosage. Thus, this model could be readily applicable for clinical practice.

According to the results of the decision tree, patients were categorized into 2 groups. The rates of severe anemia were 0.4–2.5% for the low probability group and 11.5–11.8% for the high probability group. For example, patients in the high probability group may be the most suitable candidates for dose reduction of ribavirin. Decision-tree analysis revealed that the high probability groups are patient groups with lower Hb (<14 g/dl) and lower Ccr (<80 ml/min) levels (group C) and patient groups with lower Hb (<14 g/dl), higher Ccr (≥80 ml/min), and higher Hb decline levels at 2 weeks from the start of treatment (≥2 g/dl) (group E). In particular, groups C and A were shown to be clinically significant in Fig. 3; group C includes the majority of patients suffering from severe anemia (65% in the model-building group and 67% in the internal validation group) and the very steep tilt angle of the group C slope means that group C patients have a very high probability of developing severe anemia. On the other hand, group A includes a large number of patients (40% in the model-building group and 40% in the internal validation group), and the very gentle tilt angle of the group A slope implies that group A patients have a very low probability of developing severe anemia.

Predicting the progression of anemia is necessary to decide whether medication can be continued while minimizing the disadvantages of anemia. The apparent clearance of ribavirin (CL/F), which reflects its plasma concentration at 4 weeks after the start of combination therapy, has been used as a predictive factor for developing ribavirin-induced hemolytic anemia before the start of treatment [9, 10]. However, the use of CL/F is not practical for general clinicians, because the calculation of CL/F is complicated. We revealed that a decline of Hb concentration by 2 g/dl at 2 weeks from the start of treatment ("2 by 2" standard) is both sensitive and convenient for identifying patients at high risk for severe anemia [10, 11]. The present study using decision-tree analysis revealed that Hb decline at week 2 was a significant and independent predictor of severe anemia. When considered along with other predictive factors, decision-tree analysis enables more exact identification of the patients prone to severe anemia.

Recently, a genome-wide association technique was used to show that ITPA polymorphism affects ribavirin-induced anemia. Polymorphisms (rs 1127354 and rs 7270101) that cause ITPase deficiency are strongly

Table 2 Comparison of clinical parameters of patients with and without severe anemia

	Anemia ($N = 41$)	No anemia ($N = 1040$)	P value
Age (years)	61.0 ± 7.6	55.4 ± 10.6	0.001
Gender (male/female)	18/23	594/446	0.109
Body mass index (kg/m^2)	22.4 ± 2.9	23.2 ± 3.3	0.119
Creatinine (mg/dl)	0.79 ± 0.24	0.7 ± 0.16	0.011
AST (IU/L)	74.2 ± 62.9	61.6 ± 43.9	0.075
ALT (IU/L)	79.6 ± 68.7	74.5 ± 55.6	0.565
GGT (IU/L)	40.7 ± 31.0	59.2 ± 57.6	0.071
Albumin	3.9 ± 0.3	4.0 ± 0.3	0.260
Total cholesterol	177.1 ± 23.1	171.6 ± 32.0	0.258
HDL cholesterol	50.8 ± 8.0	50.9 ± 14.7	0.986
LDL cholesterol	93.6 ± 22.0	95.5 ± 27.9	0.717
Triglyceride	109.1 ± 45.0	108.8 ± 55.8	0.974
Glucose	114.1 ± 32.9	111.1 ± 39.2	0.738
Alpha-fetoprotein	29.0 ± 71.4	13.9 ± 42.5	0.229
White blood cell count (/μl)	4632 ± 1828	4958 ± 1408	0.152
Hemoglobin (g/dl)	12.9 ± 1.3	14.2 ± 1.4	0.0001
Platelets (10^9/mm^3)	152.1 ± 51.7	166.6 ± 51.3	0.075
Ccr (ml/min)	75.4 ± 23.6	95.9 ± 26.4	0.0001
HCV RNA (KIU/ml)	1807 ± 1456	1985 ± 1442	0.438
Fibrosis stage (F0–2/F3–4/ND)	18/10/13	677/138/225	0.019
Activity (A0–1/2–3/ND)	14/14/13	443/369/228	0.701
PEG-IFN alpha-2b dosage	1.50 ± 0.13	1.48 ± 0.13	0.260
Ribavirin dosage (600/800/1000 mg)	28/13/0	553/444/43	0.105
Decline of hemoglobin at week 1	−0.2 ± 1.1	−0.2 ± 0.8	0.644
Decline of hemoglobin at week 2	−1.6 ± 1.9	−1.2 ± 1.2	0.022
Decline of hemoglobin at week 4	−3.0 ± 1.7	−2.2 ± 1.4	0.005
Decline of hemoglobin at week 8	−3.6 ± 1.6	−2.7 ± 1.4	0.003

Data are expressed as median ± standard deviation unless otherwise indicated

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *Ccr* creatinine clearance, *Hb* hemoglobin

Table 3 Univariable and multivariable logistic regression analysis of factors associated with severe anemia

	Univariable analysis			Multivariable analysis		
	Odds	95% CI	P value	Odds	95% CI	P value
Age (years)	1.06	1.03–1.11	<0.0001	1.02	0.96–1.08	0.984
Creatinine (mg/dl)	9.61	1.91–48.4	0.006	–	–	–
Hb (g/dl)	0.47	0.37–0.59	<0.0001	0.40	0.29–0.55	<0.0001
Ccr (ml/min)	0.95	0.94–0.97	<0.0001	0.97	0.95–0.99	0.012
Fibrosis (F3–4)	3.14	1.49–6.60	0.003	–	–	–
Decline of Hb at week 2	0.76	0.61–0.95	0.017	0.54	0.39–0.74	0.0001
Decline of Hb at week 4	0.70	0.57–0.87	0.001	–	–	–
Decline of Hb at week 8	0.68	0.55–0.85	0.001	–	–	–

Hb hemoglobin, *Ccr* creatinine clearance

associated with protection from ribavirin-induced hemolytic anemia and with a lesser need for ribavirin dose reduction [28–30]. These polymorphisms are very valuable, but the indication for treatment is determined not by them but by viral genotypes or by *IL28B* variations. The present decision tree, which involves a factor attained after initiation of PEG-IFN plus ribavirin therapy, i.e., Hb decline at week 2, is useful for selecting the best regimen, and can be easily used by general clinicians.

What is unique to the present study is the visualization of the probability of severe anemia by combining factors and its high reproducibility, as revealed by high-quality validation of the internal validation dataset that was completely independent of the model-building dataset. The

factors used in the decision-tree model were clinical parameters that are readily available through the usual work-up of patients. This model can be immediately applied to clinical practice without imposing any cost for additional examinations.

A potential limitation of the present study is that data-mining analysis has an intrinsic risk of showing relationships that are relevant to the original dataset but are not reproducible across different populations. Although internal validation showed that our model had high reproducibility, we recognize that further validation using a larger external validation cohort, especially in populations other than Japanese, is necessary to verify the reliability of our model.

In conclusion, we built the decision-tree model for predicting severe anemia caused by PEG-IFN alpha-2b plus ribavirin combination therapy in chronic hepatitis C with genotype 1b and high viral load. Because this decision-tree model was composed of simple variables, it can be easily applied to clinical practice. This model may have the potential to support decisions concerning ribavirin dose reduction during PEG-IFN alpha-2b plus ribavirin combination therapy and contribute to increasing the rate of SVR.

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Conflict of interest All authors have no financial relationship relevant to this study to disclose.

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BRIEF ARTICLE

Hyperglycemia is a significant prognostic factor of hepatocellular carcinoma after curative therapy

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Abstract

AIM: To evaluate whether metabolic factors are related to distant recurrence of hepatocellular carcinoma (HCC) and survival after curative treatment.

METHODS: This retrospective study included 344 patients whose HCC was treated curatively by radiofrequency ablation (RFA) therapy. The mean age was 67.6 years and the mean observation period was 4.04 years. The etiological background of liver disease was hepatitis B virus infection in 30, hepatitis C virus infection in 278, excessive alcohol drinking in 9, and other in 27 patients. The Child-Pugh classification grade was A (n = 307) or B (n = 37). The number of HCC nodules was one in 260, two in 61, and three in 23 patients. For surveillance of HCC recurrence after curative therapy with RFA, patients were radiologically evaluated every 3 mo. Factors associated with distant recurrence of HCC or survival were studied.

RESULTS: Inadequate maintenance of blood glucose in diabetic patients was associated with higher incidence of distant recurrence. The 1-, 2-, and 3-year recurrence rates were significantly higher in diabetic patients with inadequate maintenance of blood glucose compared with the others: 50.6% *vs* 26.8%, 83.5% *vs* 54.4%, and 93.8% *vs* 73.0%, respectively (P = 0.0001). Inadequate maintenance of blood glucose was an independent predictor of distant recurrence [adjusted relative risk 1.97 (95%CI, 1.33-2.91), (P = 0.0007)] after adjustment for other risk factors, such as number of HCC nodules [2.03 (95%CI, 1.51-2.73), P < 0.0001] and initial level of serum alpha fetoprotein (AFP) [1.43 (95%CI, 1.04-1.97), P = 0.028]. Obesity was not an independent predictor of recurrence. The incidence of distant recurrence did not differ between diabetic patients with adequate maintenance of blood glucose and non-diabetic patients. Among 232 patients who had HCC recurrence, 138 had a second recurrence. The 1-, 2-, and 3-year rates of second recurrence were significantly higher in diabetic patients with inadequate maintenance of blood glucose than in the others: 9.0% *vs* 5.9%, 53.1% *vs* 24.3%, and 69.6% *vs* 42.3%, respectively (P = 0.0021). Inadequate maintenance of blood glucose in diabetic patients [1.99 (95%CI, 1.23-3.22), P = 0.0049] and presence of multiple HCC nodules [1.53 (95%CI, 1.06-2.22), P = 0.024] were again significantly associated with second HCC recurrence. Inadequate maintenance of blood glucose in diabetic

patients was also a significant predictor of poor survival [2.77 (95%CI, 1.38-5.57), P = 0.0046] independent of excessive alcohol drinking [6.34 (95%CI, 1.35-29.7), P = 0.019], initial level of serum AFP [3.40 (95%CI, 1.88-6.18), P < 0.0001] and Child-Pugh classification grade B [2.24 (95%CI, 1.12-4.46), P = 0.022]. Comparing diabetic patients with inadequate maintenance of blood glucose *vs* the others, the 1-, 2-, and 3-year survival rates were significantly lower in diabetic patients with inadequate maintenance of blood glucose: 92% *vs* 99%, 85% *vs* 96%, and 70% *vs* 92%, respectively (P = 0.0003).

CONCLUSION: Inadequate maintenance of blood glucose in diabetic patients is a significant risk factor for recurrence of HCC and for poor survival after curative RFA therapy.

Key words: Hyperglycemia; Hepatocellular carcinoma; Recurrence; Radio frequency ablation; Survival

Hosokawa T, Kurosaki M, Tsuchiya K, Matsuda S, Muraoka M, Suzuki Y, Tamaki N, Yasui Y, Nakata T, Nishimura T, Suzuki S, Ueda K, Nakanishi H, Itakura J, Takahashi Y, Izumi N. Hyperglycemia is a significant prognostic factor of hepatocellular carcinoma after curative therapy. *World J Gastroenterol* 2013; 19(2): 249-257 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/249.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.249

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide[1] and its incidence has been increasing in many countries[2]. Surgical resection, liver transplantation, and local ablation therapy, such as radiofrequency ablation (RFA) therapy, have been considered as efficient curative therapies for HCC. RFA therapy is now widely performed in patients with small HCC[3] and a randomized controlled study demonstrated that the survival rates were similar in patients with small HCC receiving RFA or surgical resection[4]. A characteristic of HCC is its high rate of recurrence after curative resection or local ablation therapy, reaching approximately 80% within 5 years[5-7]. Identification of factors related to recurrence of HCC and therapeutic intervention targeting these factors may lead to prevention of frequent recurrence of HCC and improved survival.

Tumor factors, such as the number of HCC nodules and their size, are associated with the recurrence of HCC and survival prognosis[8-10]. Another factor that is associated with the recurrence of HCC and survival is the hepatic reserve function at the time of HCC therapy[8,10,11]. Hepatitis C virus (HCV) and hepatitis B virus (HBV) infection are the major causes responsible for 80% of HCC cases[2] and antiviral therapy targeting HCV[12,13] or HBV[14] has been shown to decrease HCC recurrence, and improve hepatic reserve function and survival. Non-alcoholic steatohepatitis (NASH) has also received attention as a cause of HCC[15]. Metabolic factors, such as obesity and diabetes, are closely linked to the etiology of NASH. These metabolic factors have also been identified as risk factors for several other types of cancer. Obesity is associated with increased mortality rates of several cancers[16,17] and diabetes is also reported as a risk factor for liver, pancreatic, renal, and colon cancers[18,19]. If these metabolic factors are related to the recurrence of HCC, therapeutic intervention targeting these factors may lead to prevention of frequent recurrence of HCC and improved survival. The impact of diabetes on the recurrence of HCC after treatment has been discussed, but with conflicting results[20-23].

In this study, factors contributing to the recurrence and prognosis of HCC after curative treatment were analyzed. We found that inadequate maintenance of blood glucose was related to the high rate of HCC recurrence and poor survival.

MATERIALS AND METHODS

Patients whose HCC was treated by RFA at the Musashino Red Cross Hospital were studied retrospectively for factors associated with recurrence of HCC and survival. The inclusion criteria were as follows: (1) HCC treated curatively with RFA at the Musashino Red Cross Hospital between 1999 and 2007; (2) maximum diameter of HCC nodule $\leqslant$ 3 cm; (3) number of HCC nodules $\leqslant$ 3; (4) no previous history of treatment for HCC; and (5) follow-up observation for at least 6 mo after RFA therapy. 344 patients met these criteria, including 140 women and 204 men, with a mean age of 67.6 years and mean observation time of 4.04 years. The clinical characteristics of the patients are summarized in Table 1. The etiological background of liver disease was HBV infection in 30, HCV infection in 278, excessive alcohol drinking (intake of ethanol $\geqslant$ 60 g/d for $\geqslant$ 5 years continuously) in 9, and non-B non-C non-alcoholic etiology in 27 patients. The Child-Pugh classification grade was either A (n = 307) or B (n = 37). The number of HCC nodules was one in 260, two in 61, and three in 23 patients. Thus, 260 patients had a single lesion, and 84 had multiple lesions. The maximum diameter of HCC nodules was 19.9 ± 0.3 mm.

Obesity was defined as a body mass index > 25 kg/m^2 according to the definition of the Japan Society for the Study of Obesity[24]. Blood glucose was measured monthly for 6 mo after HCC treatment and the average value was determined. Inadequate maintenance of blood glucose was defined as an average value of blood glucose $\geqslant$ 200 mg/dL. The level of hemoglobin A1c (HbA1c) was not used in the present study because the lifespan of erythrocytes is shortened due to hypersplenism in patients with chronic hepatitis or cirrhosis, leading to lower HbA1c levels relative to the blood glucose level[25]. Diagnosis of type 2 diabetes was made according to the

Table 1 Characteristics of patients undergoing curative radiofrequency ablation for hepatocellular carcinoma *n* (%)

Variable	Value
Sex (male/female)	204/140
Age(yr)	67.6 ± 8.4
Etiology of liver disease: HBV/HCV/NBNC	30/278/36
AST (IU/L)	84.0 ± 34.5
ALT (IU/L)	73.2 ± 36.5
GGT (IU/L)	82.9 ± 96.8
T-Chol (mg/dL)	157.8 ± 32.0
TG (mg/dL)	112.3 ± 55.7
Mean blood sugar (mg/dL)	139.3 ± 44.0
Diabetes mellitus	159 (48)
BMI > 25 kg/m^2	86 (25)
Maximum diameter of HCC nodule (mm)	19.9 ± 0.3
Number of HCC nodules: single/2 or 3	260/84
AFP (ng/mL)	214 ± 1025
Alcohol drinking > 60 g/d	9 (2.6)
Child-Pugh grade: A/B	307/37

HBV: Hepatitis B virus; HCV: Hepatitis C virus; NBNV: Neither HBV nor HCV; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyltransferase; T-Chol: Total cholesterol; TG: Triglyceride; BMI: Body mass index; AFP: α-fetoprotein; HCC: Hepatocellular carcinoma.

American Diabetes Association criteria of a fasting blood glucose level ≥ 126 mg/dL (≥ 7.0 mmol/L) and/or HbA1c level ≥ 6.5[26]. After initial treatment of HCC by RFA, the ablated area was confirmed by contrast-enhanced computed tomography (CT) within one week. If the ablated area was not sufficient, then RFA therapy was repeated until the HCC nodule was completely ablated.

HCC surveillance and diagnosis of recurrence

Diagnosis of HCC was based on abdominal ultrasonography, contrast-enhanced CT, magnetic resonance imaging (MRI), or angiography. 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.

For surveillance of HCC recurrence after curative therapy with RFA, patients were evaluated by abdominal ultrasonography, contrast-enhanced CT, or contrast-enhanced MRI every three months. Recurrence of HCC was diagnosed based on a new lesion detected by ultrasonography showing vascular enhancement with washout on CT or MRI. If the tumor was not hypervascular, a tumor biopsy was performed to confirm the diagnosis.

Statistical analysis

For analysis of survival and recurrence, the time of initial RFA treatment was defined as day zero. Survival rate and recurrence rate were analyzed by the Kaplan-Meier method and log rank test. Multivariate analysis was performed using a Cox proportional hazard model. Data were analyzed using StatView Version 5.0 (SAS Institute Inc, Cary, North Carolina, United States) and IBM-SPSS statistics version 18 (IBM SPSS Inc, Chicago, IL, United States). Statistical significance was set at $P < 0.05$.

RESULTS

Factors associated with HCC recurrence

Of the 344 patients whose HCC was curatively treated by RFA, 232 had HCC recurrence. The 1-, 2-, and 3-year recurrence rates were 29.3%, 57.5%, and 75.2%, respectively. On univariate analysis, inadequate maintenance of blood glucose, higher initial level of serum AFP and multiple HCC nodules were significantly associated with HCC recurrence. Obesity ($P = 0.06$) and diabetes ($P = 0.65$) were not significantly associated with HCC recurrence.

Thirty-seven patients had diabetes with inadequate maintenance of blood glucose, 122 patients had diabetes with adequate maintenance of blood glucose, and 185 patients did not have diabetes. The HCC recurrence rate was significantly higher in diabetic patients with inadequate maintenance of blood glucose than in the others ($P = 0.0001$) (Figure 1A).

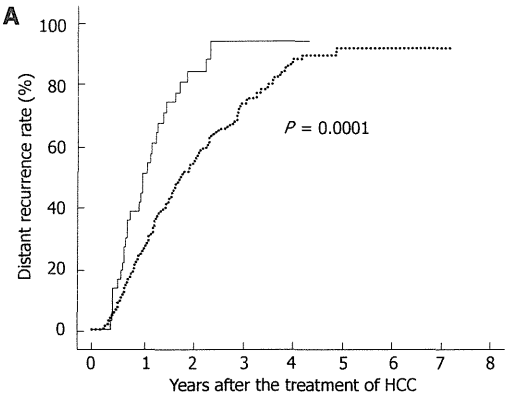
Comparing patients with diabetes ($n = 159$) and patients who did not have diabetes ($n = 185$), there was no significant difference in the recurrence rate ($P = 0.65$). Upon comparison of the three groups, i.e., the diabetes with inadequate maintenance of blood glucose group, the diabetes with adequate maintenance of blood glucose group, and the non-diabetes group, the recurrence rate was significantly higher in the diabetes with inadequate maintenance of blood glucose group than in the other two groups ($P = 0.0001$) (Figure 1B). On the other hand, there was no significant difference in the HCC recurrence rate between the diabetes patients with adequate maintenance of blood glucose group and the non-diabetes group.

In terms of the number of HCC nodules, namely, single ($n = 260$) *vs* multiple ($n = 84$), the recurrence rate was significantly higher in patients with multiple HCC nodules ($P = 0.0001$). Within each subgroup of patients with single and multiple HCC nodules, diabetes with inadequate maintenance of blood glucose was significantly associated with recurrence of HCC (single, $P = 0.006$; multiple, $P = 0.025$) (Figure 2A, B). In terms of the initial level of serum AFP ≥100 ng/mL ($n = 70$) *vs* < 100 ng/mL ($n = 274$), the recurrence rate was significantly higher in patients with AFP ≥ 100 g/mL ($P = 0.018$). Within each subgroup of patients with AFP ≥ 100 ng/mL and < 100 ng/mL, diabetes with inadequate maintenance of blood glucose was associated with a higher rate of recurrence (AFP ≥ 100 ng/mL, $P = 0.005$; AFP < 100 ng/mL, $P = 0.017$) (Figure 2C, D).

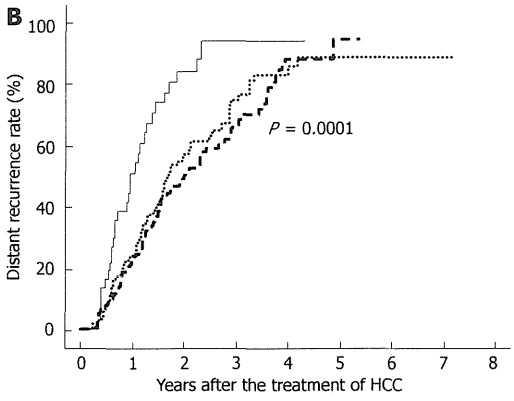
Independent risk factors for distant recurrence of HCC on multivariate analysis were inadequate maintenance of blood glucose in diabetic patients [adjusted relative risk, 1.97 (95%CI, 1.33-2.91), $P = 0.0007$], multiple HCC nodules [2.03 (1.51-2.73), $P < 0.0001$], and AFP ≥ 100 ng/mL [1.43 (1.04-1.97), $P = 0.028$] (Table 2).

Factors associated with second recurrence

Among the 232 patients who had HCC recurrence, 138 had a second recurrence. Regarding second recurrence, inadequate maintenance of blood glucose in diabetic pa-



Recurrence rate at	1-year	2-year	3-year
Inadequate maintenance of blood glucose	50.6%	83.5%	93.8%
Others	26.8%	54.4%	73.0%



Recurrence rate at	1-year	2-year	3-year
Diabetes with inadequate maintenance of blood glucose	50.6%	83.5%	93.8%
Diabetes with adequate maintenance of blood glucose	21.9%	48.7%	66.5%
Non-diabetes	23.6%	55.5%	74.4%

Figure 1 Kaplan-Meier curves showing a higher rate of hepatocellular carcinoma recurrence in diabetic patients with hyperglycemia. A: The cumulative incidence of the recurrence of hepatocellular carcinoma (HCC) was significantly higher in diabetic patients with inadequate maintenance of blood glucose (blood glucose ≥ 200 mg/dL solid line) than in the others (dotted line) (P = 0.0001); B: The HCC recurrence rate was significantly higher in diabetic patients with inadequate maintenance of blood glucose (solid line) than in diabetic patients with adequate maintenance of blood glucose (blood glucose < 200 mg/dL, broken line) or non-diabetic patients (dotted line) (P = 0.0001). There was no significant difference in HCC recurrence rate between diabetic patients with adequate maintenance of blood glucose and non-diabetic patients.

Table 2 Multivariate analysis of factors associated with recurrence of hepatocellular carcinoma

Factors	Odds ratio (95%CI)	*P*-value
First recurrence		
Inadequate maintenance of blood glucose	1.97 (1.33-2.91)	0.0007
Multiple HCC nodules	2.03 (1.51-2.73)	< 0.0001
AFP ≥ 100 ng/mL	1.43 (1.04-1.97)	0.028
Second recurrence		
Inadequate maintenance of blood glucose (mg/dL)	1.99 (1.23-3.22)	0.0049
Multiple HCC nodules	1.53 (1.06-2.22)	0.024

Inadequate maintenance of blood glucose was defined as an average of casual blood glucose of ≥ 200 mg/dL. HCC: Hepatocellular carcinoma; AFP: α-fetoprotein.

tients and multiple HCC nodules were again significantly associated with HCC recurrence. Obesity (P = 0.18), diabetes (P = 0.31) and initial level of serum AFP (P = 0.08) were not associated with second recurrence. In terms of the number of HCC nodules, namely, single *vs* multiple, the 1-, 2-, and 3-year recurrence rates were significantly higher in patients with multiple lesions (6.4% *vs* 6.1%, 39.3% *vs* 23.1%, and 52.5% *vs* 42.3%, respectively, P = 0.013). Upon comparing diabetic patients with inadequate maintenance of blood glucose *vs* the others, the rate of second recurrence was significantly higher in diabetic patients with inadequate maintenance of blood glucose (P = 0.0021) (Figure 3A). Upon comparing patients with diabetes *vs* patients who did not have diabetes, the rates of second recurrence were not significantly different (P = 0.31). Upon comparison of the three groups, i.e., the diabetes with inadequate maintenance of blood glucose group, the diabetes with adequate maintenance of blood glucose group, and the non-diabetes group, the second recurrence rate was again significantly higher in the diabetes with inadequate maintenance of blood glucose group than in the other two groups (P = 0.0035) (Figure 3B). On the other hand, there was no significant difference in the second recurrence rate between the diabetes with adequate maintenance of blood glucose group and the non-diabetes group.

Independent risk factors for second recurrence of HCC on multivariate analysis were inadequate maintenance of blood glucose [1.99 (95%CI, 1.23-3.22), P = 0.0049] and multiple HCC nodules [1.53 (95%CI, 1.06-2.22), P = 0.024] (Table 2).

Factors associated with survival

There were 52 HCC-related or hepatic failure deaths. On univariate analysis, inadequate maintenance of blood glucose, excessive alcohol drinking, higher initial level of serum AFP and Child-Pugh classification grade B were significantly associated with survival. Obesity (P = 0.81) and diabetes (P = 0.11) were not significantly associated with survival.

Upon comparing diabetic patients with inadequate maintenance of blood glucose *vs* the others, the survival rate was significantly lower in patients with inadequate maintenance of blood glucose (P = 0.0003) (Figure 4A). Upon comparing diabetic patients *vs* non-diabetic patients, the survival rates were not significantly different

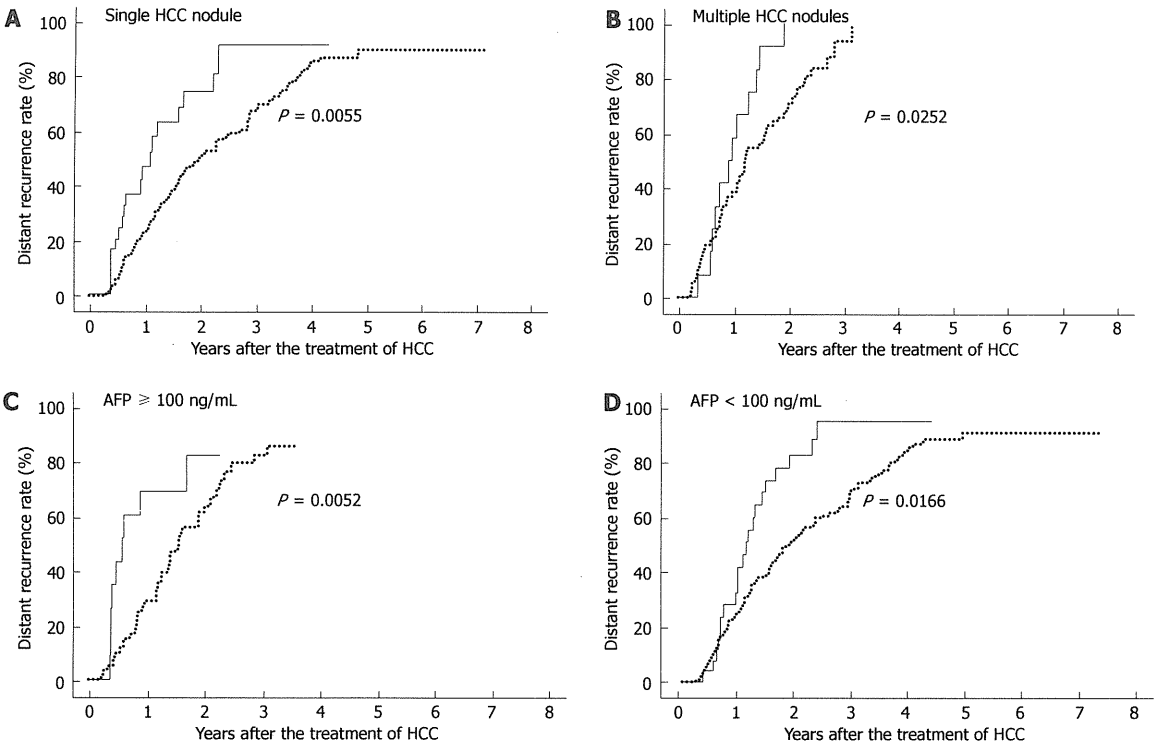


Figure 2 Diabetic patients with inadequate maintenance of blood glucose have higher rate of hepatocellular carcinoma recurrence after stratification by other risk factors. A: $P = 0.006$ for single hepatocellular carcinoma (HCC) nodule; B: $P = 0.025$ for multiple HCC nodules; C: $P = 0.005$ for AFP ≥ 100 ng/mL; D: $P = 0.017$ for α-fetoprotein (AFP) < 100 ng/mL. The cumulative incidence of the recurrence of HCC was significantly higher in diabetic patients with inadequate maintenance of blood glucose (solid line) than in the others (dotted line). after stratification by number of HCC nodules and by initial level of AFP.

Table 3 Multivariable analysis of factors associated with survival

Factors	Odds ratio (95%CI)	*P*-value
Inadequate maintenance of blood glucose	2.77 (1.38-5.57)	0.0046
Alcohol drinking ≥ 60 g/d	6.34 (1.35-29.7)	0.019
Child Pugh grade B	2.24 (1.12-4.46)	0.022
AFP ≥ 100 ng/mL	3.40 (1.88-6.18)	< 0.0001

Inadequate maintenance of blood glucose was defined as an average of casual blood glucose of ≥ 200 mg/dL. AFP: α-fetoprotein.

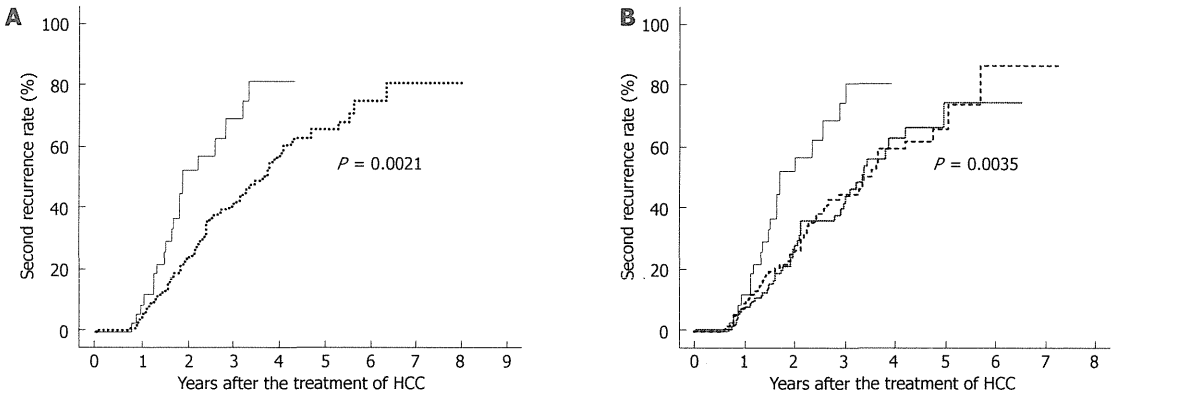
($P = 0.11$). of the survival rate was compared among the three groups, i.e., the diabetes with inadequate maintenance of blood glucose group, the diabetes with adequate maintenance of blood glucose group, and the non-diabetes group. The survival rate was significantly poorer in the diabetes with inadequate maintenance of blood glucose group than in the other two groups ($P = 0.0003$) (Figure 4B), while it did not differ between the diabetes with adequate maintenance of blood glucose group and the non-diabetes group.

The number of HCC nodules, which was a significant factor for HCC recurrence, was not related to survival ($P = 0.34$). Patients with excessive alcohol drinking had poor survival prognosis compared to those with non-excessive or no alcohol drinking ($P = 0.046$). Survival was better in patients in Child-Pugh A class than in patients in Child-Pugh B class ($P = 0.0082$). AFP ≥ 100 ng/mL was associated with poor survival compared with AFP < 100 ng/mL ($P < 0.0001$).

On multivariate analysis, inadequate maintenance of blood glucose was a significant predictor of poor survival [2.77 (95%CI, 1.38-5.57), $P = 0.0046$] independent of excessive alcohol drinking [6.34 (95%CI, 1.35-29.7), $P = 0.019$], initial level of serum AFP ≥ 100 ng/mL [3.40 (95%CI, 1.88-6.18), $P < 0.0001$] and Child-Pugh classification grade B [2.24 (95%CI, 1.12-4.46), $P = 0.022$] (Table 3).

DISCUSSION

The impact of metabolic factors, such as hyperglycemia, diabetes and obesity, on distant recurrence and survival after curative RFA therapy for HCC was analyzed retrospectively. We identified that inadequate maintenance of blood glucose in diabetic patients was a significant and independent risk factor for early recurrence of HCC and a risk factor for poor survival, whereas obesity and diabetes were not. Diabetic patients with inadequate maintenance of blood glucose had a higher rate of HCC recurrence and poorer survival compared with diabetic patients with adequate maintenance of blood glucose and non-diabetic patients. In other words, even in patients with diabetes, if



Second recurrence rate at	1-year	2-year	3-year
Inadequate maintenance of blood glucose	9.0%	53.1%	69.6%
Others	5.9%	24.3%	42.3%

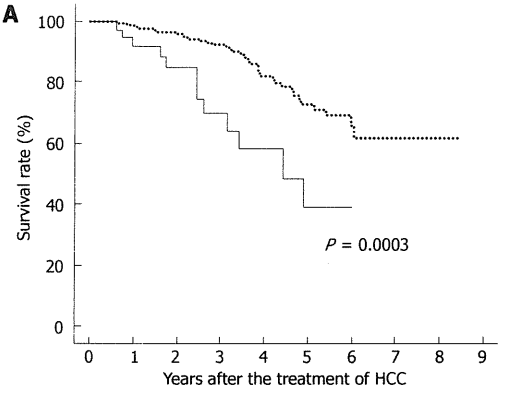
Second recurrence rate at	1-year	2-year	3-year
Diabetes with inadequate maintenance of blood glucose	9.0%	53.1%	69.6%
Diabetes with adequate maintenance of blood glucose	5.9%	22.1%	43.7%
Non-diabetes	5.6%	21.5%	36.5%

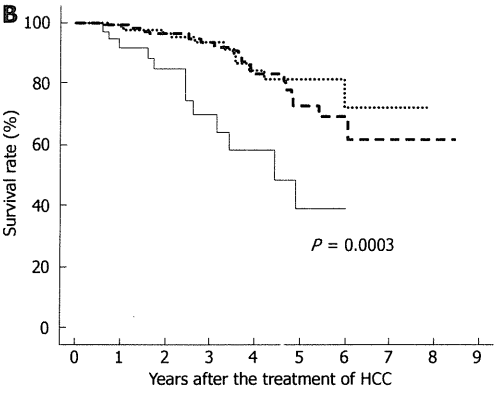
Figure 3 Kaplan-Meier curves showing a higher rate of second recurrence of hepatocellular carcinoma in diabetic patients with inadequate maintenance of blood glucose. A: The cumulative incidence of the second recurrence of hepatocellular carcinoma (HCC) was significantly higher in diabetic patients with inadequate maintenance of blood glucose (blood glucose ≥ 200 mg/dL solid line) than in the others (dotted line) (P = 0.002); B: The rate of second recurrence of HCC was significantly higher in diabetic patients with inadequate maintenance of blood glucose (solid line) than in diabetic patients with adequate maintenance of blood glucose (blood glucose < 200 mg/dL, broken line) or non-diabetic patients (dotted line) (P = 0.004). There was no significant difference in the rate of second recurrence of HCC between diabetic patients with adequate maintenance of blood glucose and non-diabetic patients.

the blood glucose was adequately maintained, the HCC recurrence rate and survival did not differ significantly compared with those in non-diabetic patients. These results indicate the possibility that adequate management of hyperglycemia may lead to reduction in the risk of HCC recurrence and improvement of overall survival.

The contribution of diabetes to the development of HCC has been confirmed in several reports[27-30]. The impact of diabetes on the recurrence of HCC after treatment has also been discussed, but with conflicting results[20-22]. A recent study from Taiwan demonstrated that diabetes may not affect the intra-hepatic HCC recurrence and survival after RFA[23]. The results of the present study also indicated that diabetes itself is not a significant risk factor if the level of blood glucose is adequately managed. Rather, hyperglycemia was a significant risk factor for the recurrence of HCC. There may be several mechanisms involved in the relationship between hyperglycemia and HCC recurrence. Hyperglycemia promotes cancer cell proliferation in pancreatic cancer cells and breast cancer cells[31-33] through accelerated cell cycle progression or through the production of reactive oxygen species, leading to activation of protein kinase C and increased DNA synthesis in cancer cells[34]. A previous study in hepatitis C patients indicated that hyperglycemia after challenge with 75-g oral glucose tolerance test was associated with the risk for HCC while hyperglycemia at fasting was not[35]. A possible reason for this result may be that patients with post-challenge hyperglycemia may have higher fluctuations in daily glucose levels that lead to oxidative stress[35], because it was reported that acute fluctuations in blood glucose levels cause greater oxidative stress than sustained chronic hyperglycemia[36]. Taken together, a possible mechanism for the relationship between higher level of casual blood glucose and development of HCC in the present study may be that daily fluctuations in serum glucose levels caused greater oxidative stress. Alternatively, hyper-insulinemia or increased level of insulin-like growth factor, which are caused by hyperglycemia, may be related to carcinogenesis[37-39]. Insulin levels were not measured in our study; therefore, the effects of insulin could not be identified.

Discussions are now taking place on methods of treating diabetes from the standpoint of cancer prevention. Control of hyperglycemia could reduce cancer incidence, which means that hyperglycemia could directly contribute to the development of cancer[39]. The results of our study also showed that adequate management of hyperglycemia may lead to reduction in the risk of HCC recurrence and improvement of overall survival. Improvement in insulin resistance is undoubtedly the most important factor for the treatment of diabetes, but glycemic control is often difficult to achieve with dietary therapy, exercise, or insulin resistance-improving drugs alone. It was reported that metformin may be associated with a lower risk of cancer[38] and there is a theoretical concern that exogenous insulin may be associated with an increased risk of cancer[40]. In fact, a recent study reported that insulin therapy in patients with HCV infection is linked with the development of HCC[41]. On the other hand, with insulin treatment, concomitant use of metformin has been reported to offset the carcinogenic risk of insulin[42]. Whether glycemic control should be a priority, or whether avoiding hyper-insulinemia because





Second recurrence rate at	1-year	2-year	3-year
Inadequate maintenance of blood glucose	91.7%	84.6%	69.7%
Others	98.7%	95.9%	92.4%

Second recurrence rate at	1-year	2-year	3-year
Diabetes with inadequate maintenance of blood glucose	91.7%	84.6%	69.7%
Diabetes with adequate maintenance of blood glucose	99.2%	96.3%	93.7%
Non-diabetes	99.2%	96.4%	93.3%

Figure 4 Patients with inadequate maintenance of blood glucose have a lower survival rate. A: The survival rate after curative local ablation therapy for hepatocellular carcinoma (HCC) was significantly lower in diabetic patients with inadequate maintenance of blood glucose (blood glucose ≥ 200 mg/dL solid line) than in the others (dotted line) (P = 0.0003); B: The survival rate was significantly lower in diabetic patients with inadequate maintenance of blood glucose (solid line) than in diabetic patients with adequate maintenance of blood glucose (blood glucose < 200 mg/dL, broken line) or non-diabetic patients (dotted line) (P = 0.0003). There was no significant difference in survival rate between diabetic patients with adequate maintenance of blood glucose and non-diabetic patients.

of therapy should be a priority, is an issue for future investigation.

In terms of survival of HCC patients, associations with liver function and tumor factors have been reported[10], but conflicting results have been reported for the relationship with diabetes[20,21]. These two studies involved heterogeneous groups of HCC patients treated with various therapies, including surgery, local ablation therapy and transcatheter arterial embolization. This heterogeneity may have led to the conflicting results, because the survival of HCC patients may be strongly affected by the initial treatment. Our study involved a homogeneous patient population, i.e., all patients were initially treated curatively by RFA. The results of our study suggest that glycemic control in diabetic patients, more so than diabetes itself, plays a role in survival. The mechanism by which glycemic control and survival are related is unknown, but frequent recurrence of HCC in hyperglycemic patients and the accumulation of damage in liver function because of repeated treatment intervention for HCC may lead to worsening survival.

In conclusion, inadequate maintenance of blood glucose in diabetic patients was a significant and independent risk factor for early recurrence of HCC and for poor survival. Adequate management of hyperglycemia in diabetic patients may lead to reduction in the risk of HCC recurrence and improvement in overall survival.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Radiofrequency ablation (RFA) therapy is an efficient curative therapy for HCC, but long-term survival is limited because of the high rate of distant recurrence of approximately 80% within 5 years. Identification of factors related to recurrence of HCC and therapeutic intervention targeting these factors may lead to prevention of frequent recurrence of HCC and improved survival.

Research frontiers

Metabolic factors, such as obesity and diabetes, have been identified as risk factors for several types of cancer, such as cancer of the liver, pancreas, kidney, and colon. These metabolic factors may be related to recurrence of HCC. The impact of diabetes on the recurrence of HCC after treatment has been discussed, but with conflicting results.

Innovations and breakthroughs

The authors identified that inadequate maintenance of blood glucose in diabetic patients was a significant and independent risk factor for early recurrence of HCC and a risk factor for poor survival, whereas diabetes was not. In other words, even in patients with diabetes, if the blood glucose was adequately maintained, then the HCC recurrence rate and survival did not differ significantly from those in non-diabetic patients.

Applications

The results of the present study indicate the possibility that adequate management of hyperglycemia in diabetic patients may lead to reduction in the risk of HCC recurrence and improvement of overall survival.

Peer review

This is an important study in which the effect of inadequate maintenance of blood glucose in diabetes has been shown as a significant risk factor for distant recurrence of hepatocellular carcinoma and poor survival after curative radiofrequency ablation therapy.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Inhibition of hepatocellular carcinoma by PegIFNα-2a in patients with chronic hepatitis C: a nationwide multicenter cooperative study

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Abstract
Background We investigated whether the administration of maintenance doses of interferon prevented hepatocellular carcinoma (HCC) in patients with chronic hepatitis C.
Methods Study 1: A multicenter, retrospective, cooperative study was carried out to determine whether long-term administration of low-dose peginterferon alpha-2a (PegIFNα-2a) prevented HCC development in patients with chronic hepatitis C. In total, 594 chronic hepatitis C patients without a history of HCC were enrolled and treated with 90 μg PegIFNα-2a administered weekly or bi-weekly for at least 1 year. Study 2: HCC developed in 16 of 99 additional patients without PegIFNα-2a treatment during 3.8 years of observation. A propensity-matched control study was then carried out to compare the incidence of

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HCC between the 59 patients who received low-dose PegIFNα-2a (PegIFNα-2a group) and 59 patients who did not receive PegIFNα-2a treatment (control group), matched for sex, age, platelet count, and total bilirubin levels.

Results Study 1: HCC developed in 49 patients. The risk of HCC was lower in patients with undetectable hepatitis C virus RNA, ≤40 IU/L alanine aminotransferase (ALT), or ≤10 ng/L alpha-fetoprotein (AFP) 24 weeks after the start of therapy. Study 2: The incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group.

Conclusions Low-dose and long-term maintenance administration of PegIFNα-2a decreased the incidence of HCC in patients with normalized ALT and AFP levels at 24 weeks compared with patients without normal ALT and AFP levels.

Keywords Chronic hepatitis C · Hepatocellular carcinoma · Peginterferon

Introduction

Hepatocellular carcinoma (HCC), the sixth most common cancer worldwide, often develops because of long-term hepatitis B or C virus infection [1, 2]. In particular, chronic hepatitis C and hepatic cirrhosis increase the risk of HCC; the annual incidence of tumor development in such patients may be as high as 2–4 % [3–5]. The incidence of HCC decreases in patients who achieve a sustained virological response (SVR) to interferon (IFN) treatment, although the incidence remains high in non-SVR patients [6–9]. A detailed analysis of HCC development revealed that chronic hepatitis C patients aged 65 years or more, especially those with advanced fibrosis of the liver, were at an increased risk of developing HCC [10]. For patients 65 years or older with advanced liver fibrosis, the dose of ribavirin is often reduced or the agent is discontinued, resulting in lower SVR rates in those with discontinuation of ribavirin. Establishing an effective treatment strategy for preventing the development of HCC is important for these high-risk patients.

Factors related to the development of HCC have been analyzed in patients who did not achieve an SVR even after IFN treatment; advanced fibrosis of the liver and high levels of serum alanine aminotransferase (ALT), and alpha-fetoprotein (AFP) are risk factors for HCC development [11, 12]. A randomized controlled trial was conducted in Western countries to determine whether combined peginterferon and ribavirin treatment with weekly administration of 90 μg peginterferon alpha-2a (PegIFNα-2a) could prevent HCC in non-responders. A 3.5-year follow up showed that administration of a maintenance dose of PegIFNα-2a did not reduce tumor incidence in these patients [13]. However, after 8.5 years of observation, the incidence of HCC was decreased among those in the PegIFNα-2a group with cirrhosis [14]. Meanwhile, Bruix et al. [15] reported that maintenance therapy with PegIFNα-2b did not prevent HCC in chronic hepatitis C patients with cirrhosis. In Japan, long-term low-dose administration of natural IFN has been reported to decrease the incidence of HCC [16]. In light of these conflicting results, investigations should be carried out in a large number of patients with chronic hepatitis C to resolve the question of whether IFN treatment prevents the development of HCC.

We carried out a multicenter retrospective cooperative study of patients with chronic hepatitis C to determine whether those treated with 90 μg PegIFNα-2a without ribavirin had a reduced incidence of HCC compared with those not treated with IFN.

Patients and methods

Study 1: analysis of risk factors for HCC in patients treated with long-term low-dose-PegIFNα-2a

In total, at 21 hepatitis centers throughout Japan, 743 patients with hepatitis C who had received 90 μg of PegIFNα-2a therapy weekly or bi-weekly for 1 year or more without having received the full dose (180 μg) since December 2003 were examined retrospectively for the development of HCC. The end of enrollment in this study was the end of December 2008 and the end of follow up was the end of December 2010. Patients with a history of HCC before the start of therapy and those with a therapy period of less than 48 weeks were excluded, leaving 594 patients who had undergone long-term administration of PegIFNα-2a for analysis. At the 21 centers involved in this

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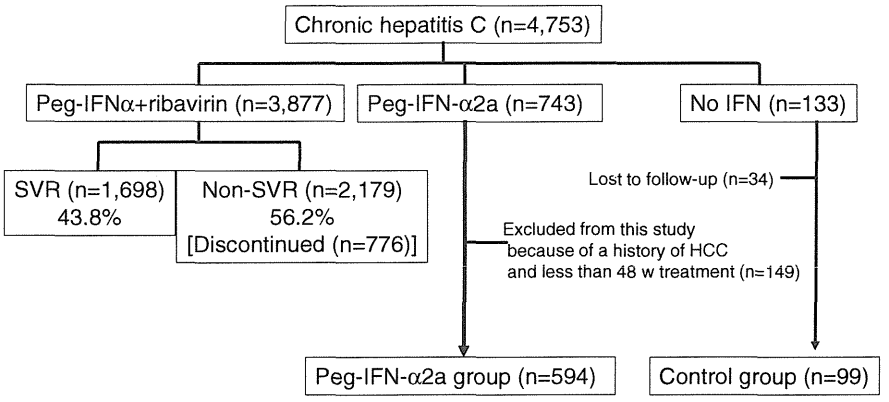

Fig. 1 Flow diagram of the patients' enrollment in the study. *Peg-IFNα* pegylated interferon α, *SVR* sustained viral response, *HCC* hepatocellular carcinoma, *w* week

study, 4,753 patients with chronic hepatitis C had been treated; Peg-IFN and ribavirin combination treatment had been administered to 3,877 patients, 743 patients had received Peg-IFN alone, and 133 patients had not agreed to receive IFN (a flow diagram of the enrollment of patients in this study is shown in Fig. 1). In the patients with Peg-IFN and ribavirin combination treatment, the SVR rate was 43.8 %; SVR was not achieved in 2,179 patients, and in 776 of these patients, the combination therapy was discontinued owing to adverse events or the patient's choice. Patients who failed to achieve an SVR were not included in this study, because the incidence of HCC is known to be reduced even in non-responders to IFN [17].

The backgrounds of the 594 patients studied are shown in Table 1. Findings from the liver biopsies of the patients were classified according to international standards [18]. Long-term PegIFNα-2a treatment is approved by the Japanese Medical Insurance system. Written informed consent was obtained from all patients prior to participation in this study. The study design was approved by the regional ethics committees of the 21 centers involved in this study, including the Musashino Red Cross Hospital, in accordance with the Helsinki Declaration. The 743 patients treated with PegIFNα-2a alone were not indicated for Peg-IFNα and ribavirin combination therapy because of anemia or heart disease. The 133 patients who did not agree to receive IFN served as the control group (see Fig. 1). A large proportion of the 594 study patients had advanced fibrosis of the liver and active inflammation. A dose of 90 μg PegIFNα-2a was administered to 512 and 82 patients weekly and biweekly, respectively, according to the patients' wishes. There were no significant differences between the weekly and biweekly groups in the patients' background data (data not shown).

The median duration of follow up in the PegIFNα-2a group was 1,273 days (range 228–2,768 days) and HCC was observed in 49 of the 594 patients (Table 1). Pretreatment and on-treatment factors associated with the development of HCC were analyzed by Student's *t*-test, the Mann–Whitney *U*-test, and the χ^2 test (Table 2). Independent factors for the development of HCC were assessed by multivariate analysis using logistic regression. The

Table 1 Background data of patients treated with PegIFNα-2a ($n = 594$)

	$n = 594$
Age (years)	61.7 ± 11.7
Sex (male/female)	258/336
BMI	23.2 ± 3.3
Genotype (1/2)	443/151
Diagnosis (ASC/CH/LC)	4/460/130
History of excess alcohol consumption (≥60 g/day; yes/no)	118/376
Fibrosis (F0, 1, 2/F3, 4)	443/151
Inflammatory activity (A0, 1/A2, 3)	469/125
Diabetes mellitus (no/yes)	499/95
LDL cholesterol (mg/dL)	94.2 ± 31.1
Fasting blood sugar (mg/dL)	106.3 ± 28.5
White blood cell count (/mm^3)	4,360 ± 1,470
Red blood cell count (×10^6/μL)	423.8 ± 56.4
Hemoglobin (g/dL)	13.3 ± 1.8
Platelet count (×10^3/μL)	137 ± 56
Albumin (g/dL)	4.0 ± 0.5
Total bilirubin (mg/dL)	0.8 ± 0.6
AST (IU/L)	65.8 ± 47.8
ALT (IU/L)	72.1 ± 68.0
Gamma-GTP (IU/L)	55.2 ± 51.3
Esophageal varices (no/yes)	344/31
Alpha fetoprotein (ng/L)	6.9 (4.2–13.8)
Once weekly or biweekly PegIFNα-2a	512:82
Baseline HCV RNA (KIU/mL)	1,024 (73–2,130)
Development of HCC (no/yes)	545/49

PegIFN pegylated interferon, *BMI* body mass index, *ASC* asymtotomatic carrier, *CH* chronic hepatitis, *LC* liver cirrhosis, *LDL* low-density lipoprotein, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GTP* guanosine triphosphate, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma

Values are means ± SD, with ranges in parentheses