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

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

Research Article

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 $\leqslant 20$ 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 ($\geqslant$60 years). The second predictor for younger patients (<60 years) was platelet count. The HCC development rate was 6.9% in patients with a lower platelet count ($<150 \times 10^9$/L) and 0.8% in patients with a higher count ($\geqslant 150 \times 10^9$/L). The second predictor for older patients ($\geqslant$60 years) was also platelet count. The HCC development rate was 13.1% in patients with a lower platelet count ($<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 ($\geq 150 \times 10^9$/L). The HCC development rate was 6.3% when albumin levels were lower (<3.75 g/dl) and 1.5% when levels were higher ($\geq$3.75 g/dl). The cutoff value for albumin levels was 4.0 g/dl in patients with a lower platelet count ($<150 \times 10^9$/L). The HCC development rate was 20.9% when albumin levels were lower (<4.0 g/dl) and 6.4% when levels were higher ($\geq$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 ($\geq$60 years) with a lower platelet count ($<150 \times 10^9$/L) and lower albumin levels (<4.0 g/dl) was 20.9% in the model derivation group and 17.9% in the external validation group. The intermediate-risk group or the patients with an HCC development rate of at least 5% consisted of the following three subgroups: (1) older age ($\geq$60 years), lower platelet count ($<150 \times 10^9$/L), higher albumin levels ($\geq$4.0 g/dl), and higher AST levels ($\geq$40 IU/L); (2) older age ($\geq$60 years), higher platelet count ($\geq 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$/L). In these intermediate-risk groups, the 5-year HCC development rate was 6.3–7.3% in the model derivation group and 5.3–7.9% in the external validation group. The low-risk group consisted of the following three subgroups: (1) younger age (<60 years) and higher platelet count ($\geq 150 \times 10^9$/L); (2) older age ($\geq$60 years), lower platelet count ($<150 \times 10^9$/L), higher albumin levels ($\geq$4.0 g/dl), and lower AST levels (<40 IU/L); and (3) older age ($\geq$60 years), higher platelet count ($\geq 150 \times 10^9$/L), and higher albumin levels ($\geq$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

Research Article

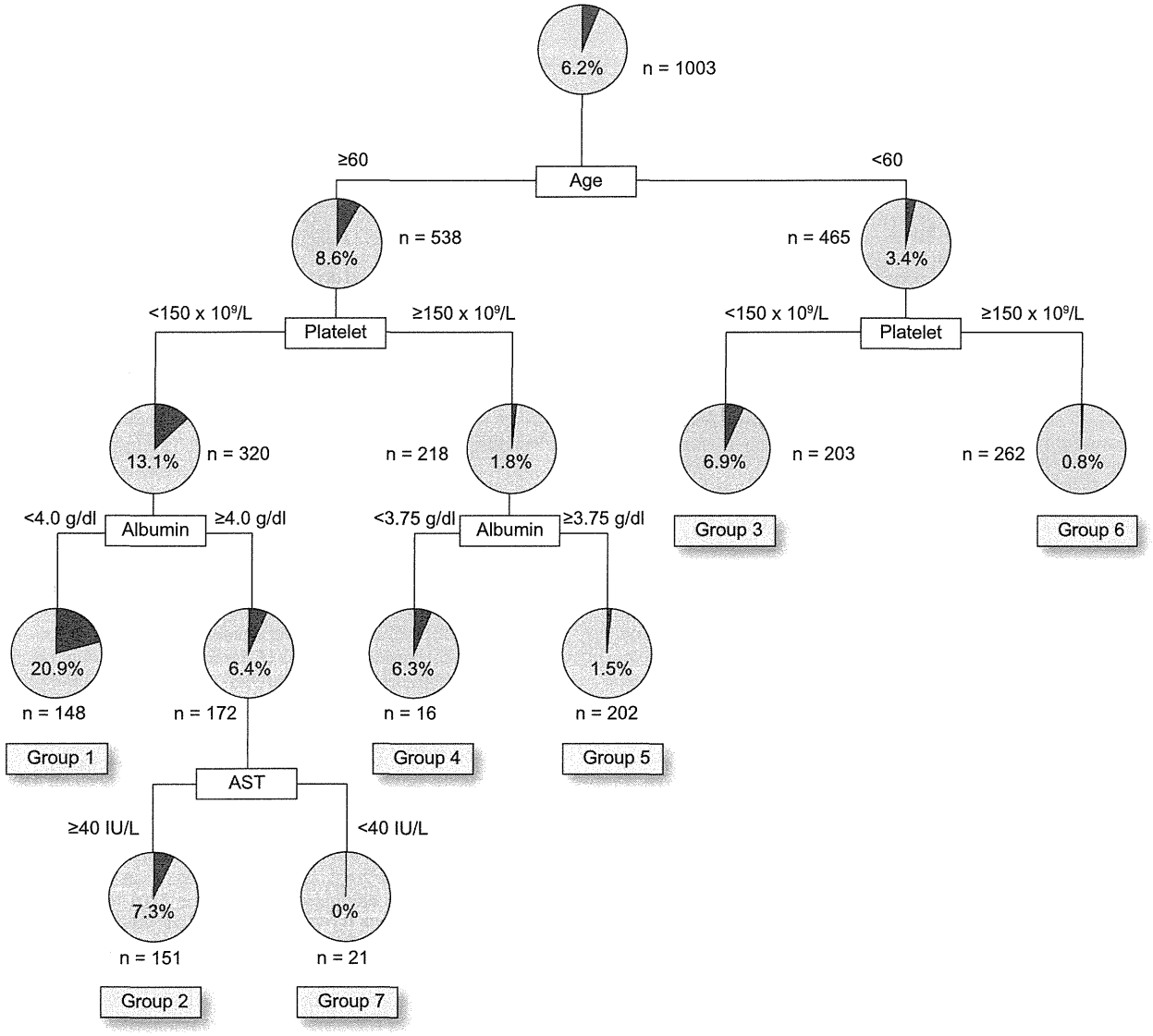


Fig. 1. The decision tree model of HCC development within 5 years. Boxes indicate the factors used to differentiate patients and the cutoff values for those different groups. Pie charts indicate the HCC development rate within 5 years for each group of patients after differentiation. Terminal groups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

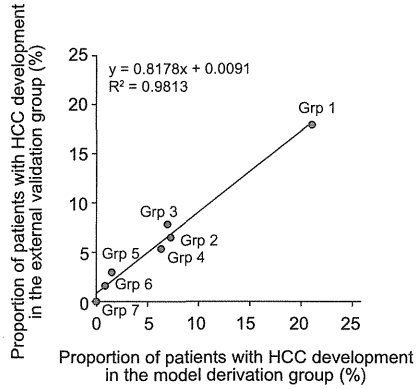


Fig. 2. External validation of the decision tree model with an independent cohort. Each patient in the external validation group was allocated to groups 1–7 following the flowchart of the decision tree. The HCC development rates were then calculated for each group and the graph plotted. The *x*-axis 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

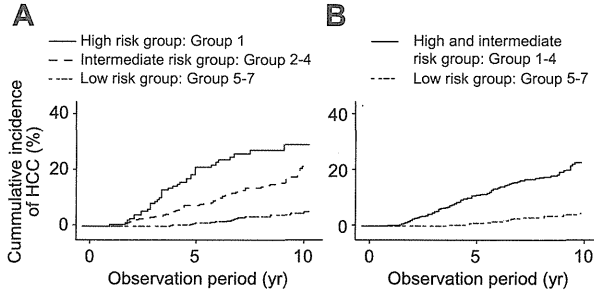


Fig. 3. Cumulative incidence of HCC development beyond 5 years in subgroups of patients defined by the decision tree model. Cumulative incidences of HCC in the groups classified by the decision tree model are compared. (A) The cumulative HCC development rate beyond 5 years is higher in the high- (group 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%; $p < 0.0001$).

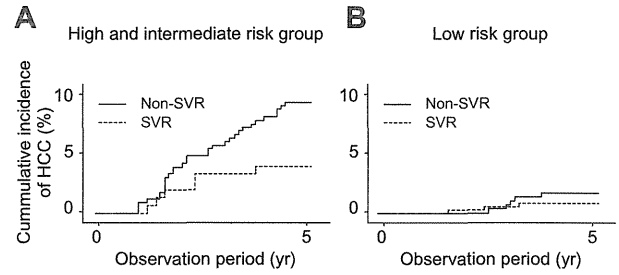


Fig. 4. Sustained virological response to PEG-IFN plus RBV therapy reduces the incidence of HCC development after stratification by the HCC risk. The 600 nonSVR patients and the 472 SVR patients in the external cohort were fitted into the HCC risk prediction model and classified into the high and intermediate-risk 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

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.249

World J Gastroenterol 2013 January 14; 19(2): 249-257
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

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

Takanori Hosokawa, Masayuki Kurosaki, Kaoru Tsuchiya, Shuya Matsuda, Masaru Muraoka, Yuichiro Suzuki, Nobuharu Tamaki, Yutaka Yasui, Toru Nakata, Takashi Nishimura, Shoko Suzuki, Ken Ueda, Hiroyuki Nakanishi, Jun Itakura, Yuka Takahashi, Namiki Izumi

Takanori Hosokawa, Masayuki Kurosaki, Kaoru Tsuchiya, Shuya Matsuda, Masaru Muraoka, Yuichiro Suzuki, Nobuharu Tamaki, Yutaka Yasui, Toru Nakata, Takashi Nishimura, Shoko Suzuki, Ken Ueda, Hiroyuki Nakanishi, Jun Itakura, Yuka Takahashi, Namiki Izumi, Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo 180-8610, Japan

Author contributions: Hosokawa T and Kurosaki M contributed equally to this work; Kurosaki M and Izumi N made substantial contributions to the conception and design of the study; 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 and Izumi N collected the clinical data; Hosokawa T and Kurosaki M contributed to the analysis and interpretation of the data; Hosokawa T wrote the draft of the manuscript; Kurosaki M and Izumi N made critical revisions of the manuscript; and Izumi N obtained a research fund.

Supported by A Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan

Correspondence to: Namiki Izumi, MD, Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino-shi, Tokyo 180-8610, Japan. nizumi@musashino.jrc.or.jp
Telephone: +81-422-323111 Fax: +81-422-329551

Received: June 17, 2012 Revised: September 6, 2012
Accepted: October 16, 2012
Published online: January 14, 2013

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 $\pm$ 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).

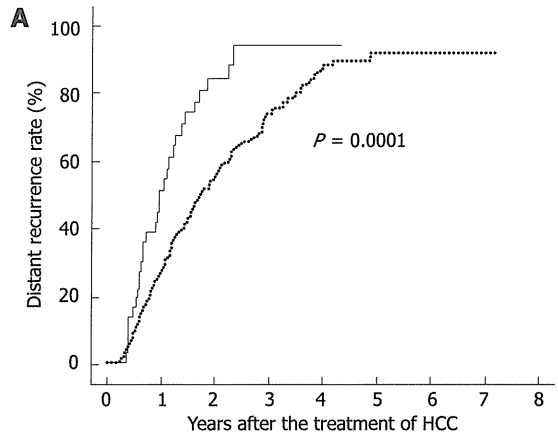
Comparing patients with diabetes ($n = 159$) and patients who did not have diabetes ($n = 185$), there was no significant difference in the recurrence rate ($P = 0.65$). Upon comparison of the three groups, i.e., the diabetes with inadequate maintenance of blood glucose group, the diabetes with adequate 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).

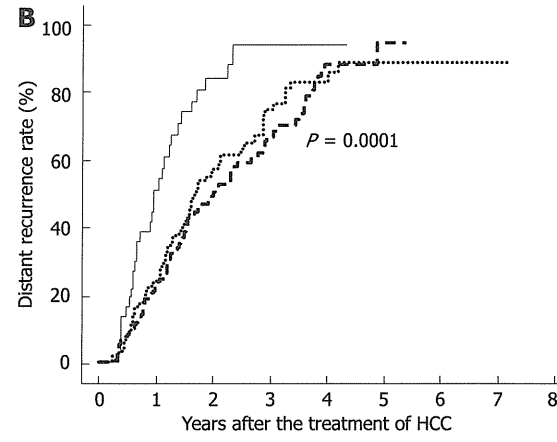
Independent risk factors for distant recurrence of HCC on multivariate analysis were inadequate maintenance of blood glucose in diabetic patients [adjusted relative risk, 1.97 (95%CI, 1.33-2.91), $P = 0.0007$], multiple HCC nodules [2.03 (1.51-2.73), $P < 0.0001$], and AFP ≥ 100 ng/mL [1.43 (1.04-1.97),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

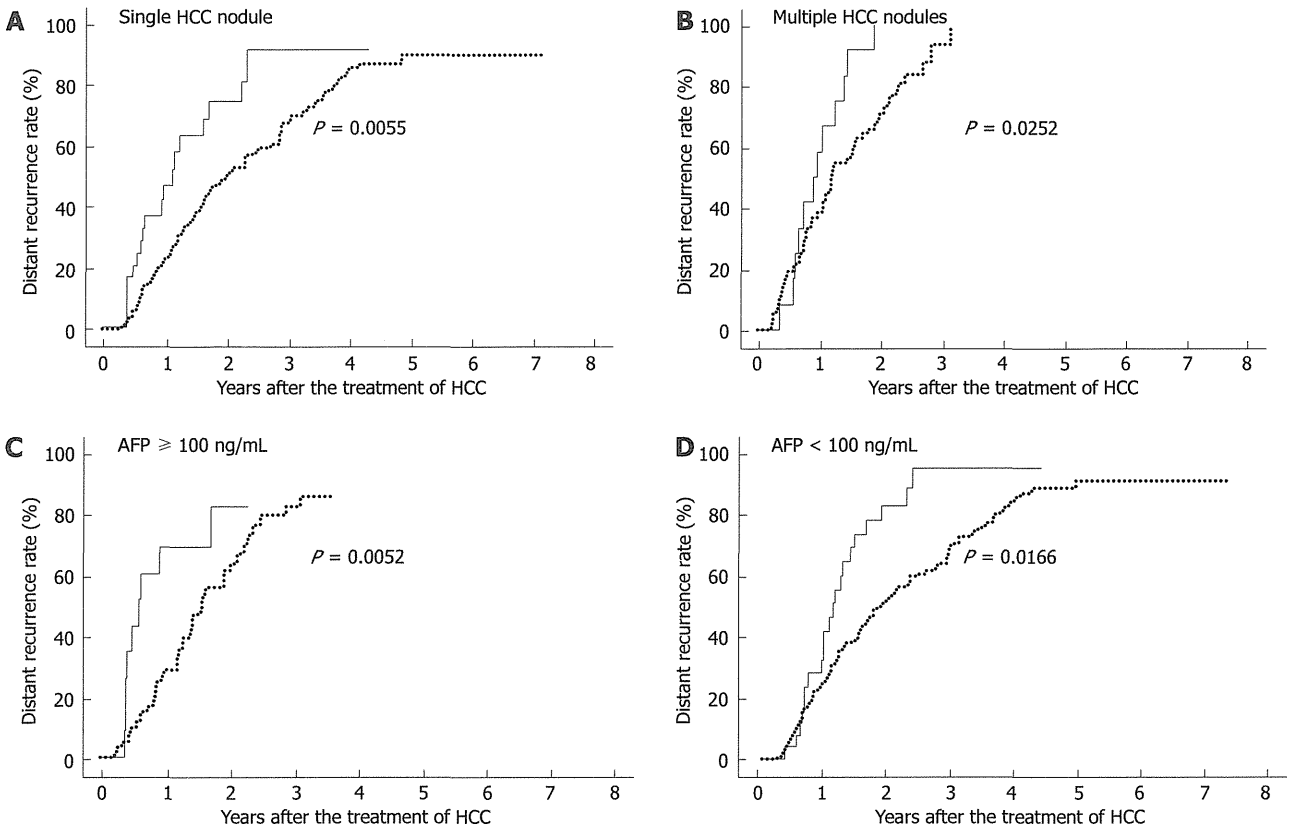


Figure 2 Diabetic patients with inadequate maintenance of blood glucose have higher rate of hepatocellular carcinoma recurrence after stratification by other risk factors. A: $P = 0.006$ for single hepatocellular carcinoma (HCC) nodule; B: $P = 0.025$ for multiple HCC nodules; C: $P = 0.005$ for AFP ≥ 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.

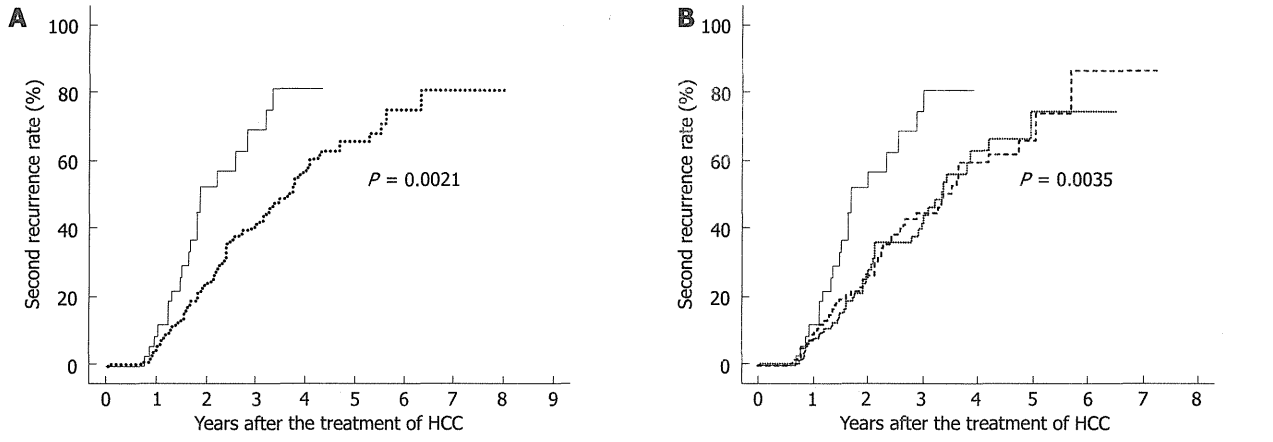
($P = 0.11$). of the survival rate was compared among the three groups, i.e., the diabetes with inadequate maintenance of blood glucose group, the diabetes with adequate maintenance of blood glucose group, and the non-diabetes group. The survival rate was significantly poorer in the diabetes with inadequate 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%

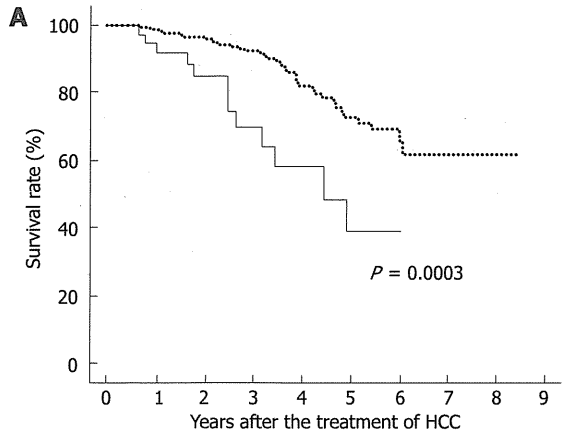
Second recurrence rate at	1-year	2-year	3-year
Diabetes with inadequate maintenance of blood glucose	9.0%	53.1%	69.6%
Diabetes with adequate maintenance of blood glucose	5.9%	22.1%	43.7%
Non-diabetes	5.6%	21.5%	36.5%

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.

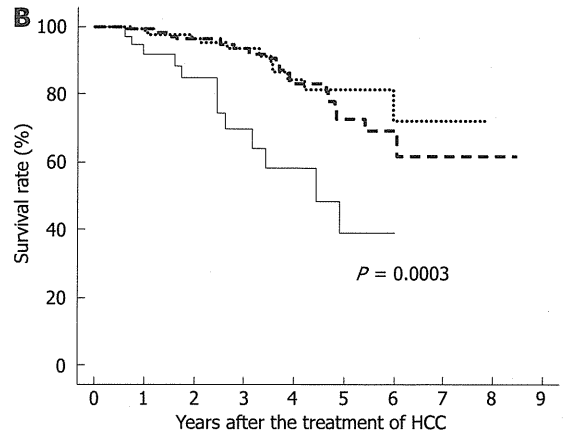
the blood glucose was adequately maintained, the HCC recurrence rate and survival did not differ significantly compared with those in non-diabetic patients. These results indicate the possibility that adequate management of hyperglycemia may lead to reduction in the risk of HCC recurrence and improvement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

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]

2 **Bosch FX**, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; **127**: S5-S16 [PMID: 15508102 DOI: 10.1053/j.gastro.2004.09.011]

3 **Arii S**, Sata M, Sakamoto M, Shimada M, Kumada T, Shiina S, Yamashita T, Kokudo N, Tanaka M, Takayama T, Kudo M.

Management of hepatocellular carcinoma: Report of Consensus Meeting in the 45th Annual Meeting of the Japan Society of Hepatology (2009). *Hepatol Res* 2010; **40**: 667-685 [PMID: 20633193 DOI: 10.1111/j.1872-034X.2010.00673.x]

4 **Chen MS**, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243**: 321-328 [PMID: 16495695 DOI: 10.1097/01.sla.0000201480.65519.b8]

5 **Tateishi R**, Shiina S, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Yoshida H, Kawabe T, Omata M. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. *Cancer* 2005; **103**: 1201-1209 [PMID: 15690326 DOI: 10.1002/cncr.20892]

6 **Izumi N**, Asahina Y, Noguchi O, Uchihara M, Kanazawa N, Itakura J, Himeno Y, Miyake S, Sakai T, Enomoto N. Risk factors for distant recurrence of hepatocellular carcinoma in the liver after complete coagulation by microwave or radiofrequency ablation. *Cancer* 2001; **91**: 949-956 [PMID: 11251946]

7 **Curley SA**, Izzo F, Ellis LM, Nicolas Vauthey J, Vallone P. Radiofrequency ablation of hepatocellular cancer in 110 patients with cirrhosis. *Ann Surg* 2000; **232**: 381-391 [PMID: 10973388 DOI: 10.1097/00000658-200009000-00010]

8 **Poon RT**, Fan ST, Lo CM, Liu CL, Wong J. Intrahepatic recurrence after curative resection of hepatocellular carcinoma: long-term results of treatment and prognostic factors. *Ann Surg* 1999; **229**: 216-222 [PMID: 10024103 DOI: 10.1097/00000658-199902000-00009]

9 **Tung-Ping Poon R**, Fan ST, Wong J. Risk factors, prevention, and management of postoperative recurrence after resection of hepatocellular carcinoma. *Ann Surg* 2000; **232**: 10-24 [PMID: 10862190 DOI: 10.1097/00000658-200007000-00003]

10 **Kudo M**, Chung H, Haji S, Osaki Y, Oka H, Seki T, Kasugai H, Sasaki Y, Matsunaga T. Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. *Hepatology* 2004; **40**: 1396-1405 [PMID: 15565571 DOI: 10.1002/hep.20486]

11 **Fuke H**, Sugimoto K, Shiraki K, Tanaka J, Beppu T, Yoneda K, Yamamoto N, Ito K, Takaki H, Nakatsuka A, Yamakado K, Takeda K, Takei Y. Predictive factors for distant recurrence of HCV-related hepatocellular carcinoma after radiofrequency ablation combined with chemoembolization. *Aliment Pharmacol Ther* 2008; **27**: 1253-1260 [PMID: 18221404 DOI: 10.1111/j.1365-2036.2008.03627.x]

12 **Hagihara H**, Nouso K, Kobayashi Y, Iwasaki Y, Nakamura S, Kuwaki K, Toshimori J, Miyatake H, Ohnishi H, Shiraha H, Yamamoto K. Effect of pegylated interferon therapy on intrahepatic recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *Int J Clin Oncol* 2011; **16**: 210-220 [PMID: 21152943 DOI: 10.1007/s10147-010-0150-x]

13 **Miyake Y**, Takaki A, Iwasaki Y, Yamamoto K. Meta-analysis: interferon-alpha prevents the recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *J Viral Hepat* 2010; **17**: 287-292 [PMID: 19732321 DOI: 10.1111/j.1365-2893.2009.01181.x]

14 **Wong JS**, Wong GL, Tsoi KK, Wong VW, Cheung SY, Chong CN, Wong J, Lee KF, Lai PB, Chan HL. Meta-analysis: the efficacy of anti-viral therapy in prevention of recurrence after curative treatment of chronic hepatitis B-related hepatocellular carcinoma. *Aliment Pharmacol Ther* 2011; **33**: 1104-1112 [PMID: 21488914 DOI: 10.1111/j.1365-2036.2011.04634.x]

15 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140 [PMID: 12105842 DOI: 10.1016/S1590-8658(02)90208-2]

16 **Calle EE**, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625-1638 [PMID: 12711737 DOI: 10.1056/NEJMoa021423]

17 **Renehan AG**, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008; **371**: 569-578 [PMID: 18280327 DOI: 10.1016/S0140-6736(08)60269-X]

18 **Inoue M**, Iwasaki M, Otani T, Sasazuki S, Noda M, Tsugane S. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. *Arch Intern Med* 2006; **166**: 1871-1877 [PMID: 17000944]

19 **Hu FB**, Manson JE, Liu S, Hunter D, Colditz GA, Michels KB, Speizer FE, Giovannucci E. Prospective study of adult onset diabetes mellitus (type 2) and risk of colorectal cancer in women. *J Natl Cancer Inst* 1999; **91**: 542-547 [PMID: 10088625 DOI: 10.1093/jnci/91.6.542]

20 **Toyoda H**, Kumada T, Nakano S, Takeda I, Sugiyama K, Kiriyama S, Tanikawa M, Sone Y, Hisanaga Y. Impact of diabetes mellitus on the prognosis of patients with hepatocellular carcinoma. *Cancer* 2001; **91**: 957-963 [PMID: 11251947]

21 **Huo TI**, Wu JC, Lui WY, Huang YH, Lee PC, Chiang JH, Chang FY, Lee SD. Differential mechanism and prognostic impact of diabetes mellitus on patients with hepatocellular carcinoma undergoing surgical and nonsurgical treatment. *Am J Gastroenterol* 2004; **99**: 1479-1487 [PMID: 15307864 DOI: 10.1111/j.1572-0241.2004.30024.x]

22 **Komura T**, Mizukoshi E, Kita Y, Sakurai M, Takata Y, Arai K, Yamashita T, Ohta T, Shimizu K, Nakamoto Y, Honda M, Takamura T, Kaneko S. Impact of diabetes on recurrence of hepatocellular carcinoma after surgical treatment in patients with viral hepatitis. *Am J Gastroenterol* 2007; **102**: 1939-1946 [PMID: 17573788 DOI: 10.1111/j.1572-0241.2007.01354.x]

23 **Chen WT**, Macatula TC, Lin CC, Lin CJ, Lin SM. Diabetes may not affect outcomes in hepatocellular carcinoma after radio-frequency ablation. *Hepatogastroenterology* 2011; **58**: 551-557 [PMID: 21661430]

24 **Youkou A**, Hasegawa T, Suzuki K, Koya T, Sakagami T, Toyabe S, Arakawa M, Gejyo F, Narita I, Suzuki E. Influence of obesity on control in asthmatic Japanese patients defined by the Japanese definition of obesity. *Intern Med* 2011; **50**: 1911-1916 [PMID: 21921368 DOI: 10.2169/internalmedicine.50.5474]

25 **Aizawa N**, Enomoto H, Imanishi H, Saito M, Iwata Y, Tanaka H, Ikeda N, Sakai Y, Takashima T, Iwai T, Moriwaki E, Shimomura S, Iijima H, Nakamura H, Nishiguchi S. Elevation of the glycated albumin to glycated hemoglobin ratio during the progression of hepatitis C virus related liver fibrosis. *World J Hepatol* 2012; **4**: 11-17 [PMID: 22312451 DOI: 10.5754/hge12064]

26 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; **33** Suppl 1: S62-S69 [PMID: 20042775]

27 **Davila JA**, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut* 2005; **54**: 533-539 [PMID: 15753540 DOI: 10.1136/gut.2004.052167]

28 **Lai MS**, Hsieh MS, Chiu YH, Chen TH. Type 2 diabetes and hepatocellular carcinoma: A cohort study in high prevalence area of hepatitis virus infection. *Hepatology* 2006; **43**: 1295-1302 [PMID: 16729295 DOI: 10.1002/hep.21208]

29 **El-Serag HB**, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006; **4**: 369-380 [PMID: 16527702 DOI: 10.1016/j.cgh.2005.12.007]

30 **Hassan MM**, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, Beasley P, Patt YZ. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis

and diabetes mellitus. *Hepatology* 2002; **36**: 1206-1213 [PMID: 12395331 DOI: 10.1053/jhep.2002.36780]

31 **Liu H**, Ma Q, Li J. High glucose promotes cell proliferation and enhances GDNF and RET expression in pancreatic cancer cells. *Mol Cell Biochem* 2011; **347**: 95-101 [PMID: 20960036 DOI: 10.1007/s11010-010-0617-0]

32 **Okumura M**, Yamamoto M, Sakuma H, Kojima T, Maruyama T, Jamali M, Cooper DR, Yasuda K. Leptin and high glucose stimulate cell proliferation in MCF-7 human breast cancer cells: reciprocal involvement of PKC-alpha and PPAR expression. *Biochim Biophys Acta* 2002; **1592**: 107-116 [PMID: 12379472 DOI: 10.1016/S0167-4889(02)00276-8]

33 **Yamamoto M**, Patel NA, Taggart J, Sridhar R, Cooper DR. A shift from normal to high glucose levels stimulates cell proliferation in drug sensitive MCF-7 human breast cancer cells but not in multidrug resistant MCF-7/ADR cells which overproduce PKC-betaII. *Int J Cancer* 1999; **83**: 98-106 [PMID: 10449615]

34 **Nishikawa T**, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000; **404**: 787-790 [PMID: 10783895 DOI: 10.1038/35008121]

35 **Takahashi H**, Mizuta T, Eguchi Y, Kawaguchi Y, Kuwashiro T, Oeda S, Isoda H, Oza N, Iwane S, Izumi K, Anzai K, Ozaki I, Fujimoto K. Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C. *J Gastroenterol* 2011; **46**: 790-798 [PMID: 21331763 DOI: 10.1007/s00535-011-0381-2]

36 **Monnier L**, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 2006; **295**: 1681-1687 [PMID: 16609090 DOI: 10.1001/jama.295.14.1681]

37 **Fisher WE**, Boros LG, Schirmer WJ. Insulin promotes pancreatic cancer: evidence for endocrine influence on exocrine pancreatic tumors. *J Surg Res* 1996; **63**: 310-313 [PMID: 8661216 DOI: 10.1006/jsre.1996.0266]

38 **Renehan A**, Smith U, Kirkman MS. Linking diabetes and cancer: a consensus on complexity. *Lancet* 2010; **375**: 2201-2202 [PMID: 20609959 DOI: 10.1016/S0140-6736(10)60706-4]

39 **Giovannucci E**, Michaud D. The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. *Gastroenterology* 2007; **132**: 2208-2225 [PMID: 17498513 DOI: 10.1053/j.gastro.2007.03.050]

40 **Giovannucci E**, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report. *Diabetes Care* 2010; **33**: 1674-1685 [PMID: 20587728 DOI: 10.3322/caac.20078]

41 **Kawaguchi T**, Taniguchi E, Morita Y, Shirachi M, Tateishi I, Nagata E, Sata M. Association of exogenous insulin or sulphonylurea treatment with an increased incidence of hepatoma in patients with hepatitis C virus infection. *Liver Int* 2010; **30**: 479-486 [PMID: 20040053 DOI: 10.1111/j.1478-3231.2009.02191.x]

42 **Currie CJ**, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* 2009; **52**: 1766-1777 [PMID: 19572116 DOI: 10.1007/s00125-009-1440-6]

P- Reviewer Rana SV **S- Editor** Gou SX
L- Editor Stewart GJ **E- Editor** Zhang DN

J Gastroenterol
DOI 10.1007/s00535-012-0641-9

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

Namiki Izumi · Yasuhiro Asahina · Masayuki Kurosaki · Gotaro Yamada · Tsutomu Kawai · Eiji Kajiwara · Yukishige Okamura · Takayuki Takeuchi · Osamu Yokosuka · Kazuya Kariyama · Joji Toyoda · Mie Inao · Eiji Tanaka · Hisataka Moriwaki · Hiroshi Adachi · Shinji Katsushima · Masatoshi Kudo · Kouichi Takaguchi · Yoichi Hiasa · Kazuaki Chayama · Hiroshi Yatsuhashi · Makoto Oketani · Hiromitsu Kumada

Received: 23 April 2012 / Accepted: 25 June 2012

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

N. Izumi (✉) · Y. Asahina · M. Kurosaki
Department of Gastroenterology and Hepatology, Musashino Red-Cross Hospital, Musashino, Japan
e-mail: nizumi@musashino.jrc.or.jp

G. Yamada
Department of Internal Medicine, Kawasaki Hospital of Kawasaki Medical University, Okayama, Japan

T. Kawai
Department of Gastroenterology, Kanbara General Hospital, Fuji, Japan

E. Kajiwara
Department of Gastroenterology, Shinnittetsu Yahata Memorial Hospital, Kitakyushu, Japan

Y. Okamura
Department of Gastroenterology, Sano Kousei Hospital, Kitakyushu, Japan

T. Takeuchi
Department of Gastroenterology, Notogawa Hospital, Higashioumi, Japan

O. Yokosuka
Department of Gastroenterology and Hepatology, Chiba University, Chiba, Japan

K. Kariyama
Department of Hepatology, Okayama Citizens' Hospital, Okayama, Japan

J. Toyoda
Department of Gastroenterology and Hepatology, Sapporo Kousei Hospital, Sapporo, Japan

M. Inao
Department of Gastroenterology and Hepatology, Saitama Medical University, Moroyama, Japan

E. Tanaka
Second Department of Internal Medicine, Shinshu University, Matsumoto, Japan

H. Moriwaki
Department of Gastroenterology and Hepatology, Gifu University, Gifu, Japan

H. Adachi
Department of Hepatology, Tonami General Hospital, Tonami, Japan

S. Katsushima
Department of Gastroenterology, Kyoto Medical Center, Kyoto, Japan

M. Kudo
Department of Gastroenterology and Hepatology, Kinki University, Higashiosaka, Japan

K. Takaguchi
Department of Gastroenterology, Kagawa Central Hospital, Takamatsu, Japan

Published online: 09 August 2012

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

Y. Hiasa
Department of Gastroenterology and Hepatology, Ehime University, Matsuyama, Japan

K. Chayama
Department of Gastroenterology and Hepatology, Hiroshima University, Hiroshima, Japan

H. Yatsuhashi
Department of Gastroenterology and Hepatology, Nagasaki Medical Center, Nagasaki, Japan

M. Oketani
Department of Gastroenterology and Hepatology, Kagoshima University, Kagoshima, Japan

H. Kumada
Department of Hepatology, Toranomon Hospital, Tokyo, Japan

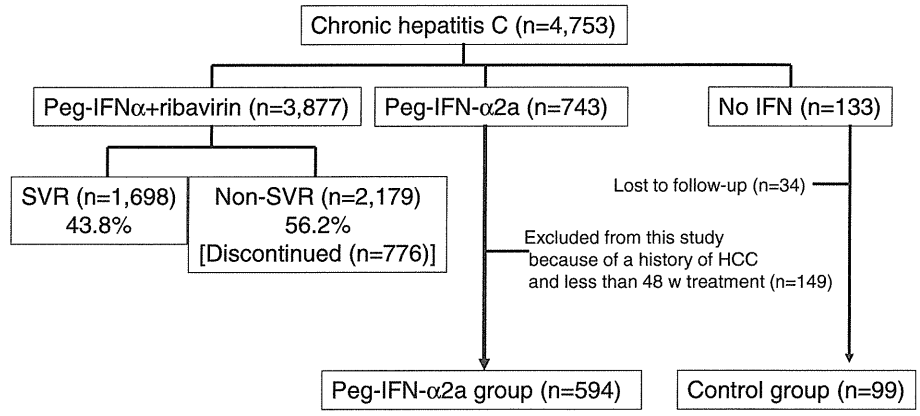


Fig. 1 Flow diagram of the patients' enrollment in the study. *Peg-IFNα* pegylated interferon α, *SVR* sustained viral response, *HCC* hepatocellular carcinoma, *w* week

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* asymtomatic 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

incidence of HCC was analyzed according to the ALT, AFP, and hepatitis C virus (HCV) RNA levels 24 weeks after the start of PegIFNα-2a administration by using the Kaplan–Meier method. The risk of HCC was analyzed, using the Kaplan–Meier method, only in the non-responders with detectable HCV RNA during PegIFNα-2a administration by dividing them according to the ALT and AFP levels 24 weeks after the start of therapy. The incidence of HCC was compared between the patients with ALT levels of <41 IU/L and those with levels of ≥41 IU/L, and between patients with serum AFP levels of <10 ng/L and those with levels of ≥10 ng/mL at 24 weeks after starting treatment, because at most of the centers participating in the this study, the upper normal range of serum ALT is set at 40 IU/L, and the most significant difference in the incidence of HCC was observed between the PegIFNα-2a and control group with the cut-off serum ALT set at 41 IU/L and cutoff serum AFP set at 10 ng/mL, 24 weeks after starting treatment. The HCV RNA level was measured using the Amplicor Monitor method with a lower detection limit of 50 IU/L (Roche Diagnostics, Tokyo, Japan). A history of excess alcohol consumption was determined as >60 g alcohol per day in order to exclude alcoholic liver disease.

An asymptomatic carrier was defined as a patient with a serum ALT level within the normal range and minimal inflammation or fibrosis in the biopsied tissues of the liver. Chronic hepatitis was defined as mild-to-severe fibrosis of the liver according to liver biopsy [18]. The diagnosis of liver cirrhosis was based on the results of histological examination of the biopsied liver tissues.

Study 2: incidence of HCC in the PegIFNα-2a therapy and non-administration (control) groups in comparison with propensity-matched controls

Ninety-nine of the 133 chronic hepatitis C patients who had not received IFN were examined as controls; patients in this group received liver-protective agents such as glycyrrhizin or were untreated, and the group was observed for more than 1 year. None of the individuals in the control groups had received IFN alone or PegIFNα and ribavirin combination treatment. They were treated for a median of 1,395 days (range 75–6,556 days). Fifty-nine of these patients underwent liver biopsy before the treatment and were considered the control group for the propensity-matched study. For the propensity-matched study, 59 patients were selected from the PegIFNα-2a group according to their age, sex, platelet count, and total bilirubin levels, which had been identified as independent pretreatment risk factors for the development of HCC in Study 1. The rates of HCC were analyzed using the Kaplan–Meier method, and the risk of HCC was analyzed particularly in patients with advanced fibrosis of the liver (F3 and F4).

Table 2 Comparison of HCC and non-HCC patients with long-term PegIFNα-2a administration ($n = 594$)

	Patients with or without development of HCC		p value
	With HCC ($n = 49$)	Without HCC ($n = 545$)	
Pretreatment parameters			
Age (years)	63.8 ± 1.7	61.3 ± 0.5	<0.05
Sex (male/female)	32/17	226/319	<0.01
BMI	24.0 ± 0.5	23.1 ± 0.2	n.s.
Genotype (1/2)	47/6	397/148	n.s.
History of excess alcohol consumption (≥60 g/day; yes/no)	11/38	107/338	n.s.
Fibrosis (F0, 1, 2/F3, 4)	25/24	418/127	<0.001
Inflammatory activity (A0, 1/A2, 3)	7/42	462/83	<0.001
Diabetes mellitus (no/yes)	38/11	461/84	n.s.
LDL cholesterol (mg/dL)	88.2 ± 9.0	94.7 ± 2.6	n.s.
White blood cell count (/mm^3)	4,355 ± 210	4,360 ± 64	n.s.
Red blood cell count (×10^6/μL)	420.8 ± 8.1	424.1 ± 2.6	n.s.
Hemoglobin (g/dL)	13.6 ± 0.3	13.3 ± 0.1	n.s.
Platelet count (×10^3/μL)	106 ± 8	140 ± 2	<0.001
Albumin (g/dL)	3.8 ± 0.1	4.0 ± 0.1	<0.001
Total bilirubin (mg/dL)	1.2 ± 0.1	0.8 ± 0.1	<0.001
AST (IU/L)	78.1 ± 6.8	64.6 ± 2.1	n.s.
ALT (IU/L)	72.8 ± 9.7	72.0 ± 2.9	n.s.
Gamma-GTP (IU/L)	68.7 ± 7.5	53.9 ± 2.3	n.s.
Alpha fetoprotein (ng/L)	17.1 (4.4–36.8)	16.7 (4.1–23.1)	n.s.
Esophageal varices	29.0 % (9/31)	6.4 % (22/344)	<0.01
On-treatment parameters			
ALT (IU/L)	59.4 ± 5.7	44.6 ± 1.8	<0.05
Alpha fetoprotein (ng/L)	9.8 (4.6–17.4)	5.5 (3.7–11.1)	<0.01
HCV RNA level (KIU/mL)	236 (<0.5–2,210)	21 (<0.5–1,780)	<0.05

n.s. not significant

Statistical analysis

Categorical data were compared using the χ^2 test or Fisher's exact test. The distributions of continuous variables were analyzed using Student's t-test and the Mann–Whitney U-test for two groups. Multivariate analysis was