Table 5. Risk factors associated with exceeding the Milan criteria in 246 patients with HCC after complete ablation by RFA

Risk factor	Univariate			Multivariate		
	RR	95% CI	p	RR	95% CI	p
Age <65 years	1.63	1.08–2.45	0.018	1.17	0.75–1.83	0.463
Sex, female	1.16	0.78–1.72	0.457			
Total bilirubin ≥2 mg/dl	2.94	1.05–8.33	0.039	3.57	1.25–10.0	0.017
Albumin ≤3.5 g/dl	0.97	0.64–1.47	0.857			
PT ≤70%	0.89	0.41–1.96	0.763			
AFP ≥100 ng/ml	2.17	1.38–3.44	0.0008	1.56	0.96–2.50	0.077
DCP ≥100 mAU/ml	2.32	1.42–3.70	0.0007	2.08	1.26–3.44	0.004
Tumor size >3.0 cm	1.03	0.61–1.72	0.914			
2 or 3 tumor nodules	2.98	1.91–4.64	<0.0001	3.05	1.91–4.88	<0.0001
K19 positive (>5%)	3.70	1.81–7.69	0.0003	2.47	1.19–5.18	0.016

RR = Risk ratio; CI = confidence interval; PT = prothrombin time.

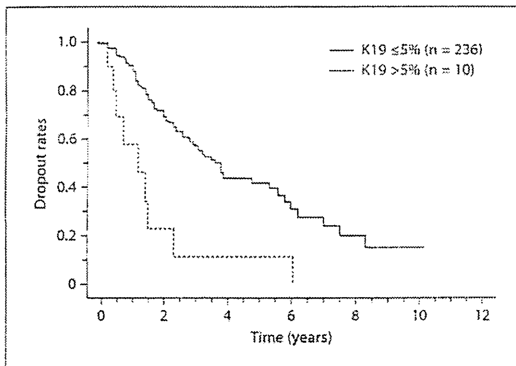

Fig. 5. The cumulative rate of exceeding the Milan criteria in patients with K19-positive HCC was significantly higher than that in patients with K19-negative HCC ($p < 0.0001$).

rate of recurrence and dropout from the Milan criteria were significantly higher in the patients with K19-positive (>1%) than in the patients with K19-negative HCC (data not shown).

Discussion

RFA therapy for HCC has been shown to achieve excellent results in appropriately selected patients [2–5]. However, recurrence of tumors is a serious impediment to improving the prognosis for patients treated with curative RFA. Therefore, several factors have been investigated as potential predictive markers for recurrence after curative RFA [7–9]. Recently, K19 was proposed as an independent prognostic factor for HCC [11–14]. However, these investigations were performed on surgically resected cases only and not on tumor biopsies. Although tumor biopsy is controversial because of potential complications such as tumor seeding [22], it would be beneficial to clinicians and patients to predict the individual tumor characteristics from a biopsy. Until now, the relationship between K19 expression and tumor recurrence after RFA treatment has not been assessed. Therefore, we have investigated the relationship between K19 expression in tumor biopsies and the clinicopathological findings in HCC. In this study, we investigated K19 expression in biopsy specimens taken just prior to the RFA session, and K19 expression (>5%) was demonstrated in 10 of 246 patients (4.1%). Because most of our patients were in early stage (within the Milan criteria) and 108 of 246 patients (43.9%) had well-differentiated HCC, the positive rate of K19 stain in our study was lower than that in surgical specimens.

We also analyzed another percentage of K19 stain (>1%) and the final results were the same for K19 positivity (>5 and >1%, respectively). K19 expression (>1%) was a statistically significant independent predictor for recurrence of HCC after RFA. Although the amount of tissue obtained by tumor biopsy is small compared to resected material, present data suggest that even biopsy can provide meaningful data on tumor recurrence irrespective of the percentage of K19 positivity (1 or 5%) (online sup-

plementary tables 1 and 2; for supplementary material see www.karger.com/doi/10.1159/000328448).

K19 positivity was not an independent predictor of the overall rate of survival, and serum AFP (≥100 ng/ml), total bilirubin (≥2 mg/dl) and female sex were significant independent predictors of survival. It is suggested that the level of total bilirubin affects the liver function of the patient, and liver function is one of the most important prognostic factors for survival of HCC patients.

The average age of our patients in this study was 68 ± 8 years, and no patients received liver transplantation in this study. However, liver transplantation is the most desirable treatment for HCC worldwide. Because of the prolonged waiting time for liver transplantation, RFA has been considered a safe and effective bridging therapy to liver transplantation. In addition, pretransplant RFA in patients with HCC has been considered for downstaging of HCC, thus improving the patient's survival [6, 7, 23]. In this study, K19 expression of HCC was a significant independent predictor for exceeding the Milan criteria ($p = 0.016$). In fact, 9 of 10 patients with K19-positive HCC exceeded the Milan criteria within 16.8 months. Therefore, if RFA is considered as a bridging therapy session prior to liver transplantation, it would be useful to obtain information on K19 expression in tumor tissue by performing a tumor biopsy before RFA. Therefore, careful observation for early detection of recurrence should be considered if K19-positive HCC patients are awaiting liver transplantation.

Compared to surgical specimens, biopsies taken prior to RFA may present some difficulties with regard to histological investigation. Needle biopsies of the nodules are less often indicated when typical vascular imaging of HCC is obtained, compared to hypovascular nodules. Needle tract seeding should also be considered. Needle biopsy has played an important role in making a diagnosis in the past. Recently, more reliance has been placed on the vascular imaging profile, because of its sensitivity and specificity without the risk of tumor dissemination. In addition, in comparison to recent advances in imaging, the information obtained from liver biopsy is lacking, as these only provide simple histological characterization, such as tumor differentiation [24]. Moreover, the positive predictive value of the vascular profile on dynamic imaging for diagnosis of HCC exceeds 95% [25]. Therefore, the current tendency is to consider needle biopsy as non-essential for diagnosis. However, in this study, K19-positive HCC showed exactly the same imaging findings as K19-negative HCC, suggesting that it is difficult to distinguish between these tumor types by imaging profile alone. In addition, K19-positive, moderately and/or poorly differentiated HCC showed similar cytological and structural abnormalities to K19-negative HCC, indicating that K19 positivity is unpredictable without staining. In figure 2, we present an impressive comparison of the features of K19-positive and -negative HCC, showing that, although the histology was similar, the prognosis for these patients was completely different. From these findings, it is clear that immunohistochemistry for K19 is the only way of demonstrating its positivity. Fortunately, staining for K19 on paraffin sections is common in diagnostic pathology, and it is not a problem to add this to routine hematoxylin and eosin (H&E) staining. Moreover, even for a general pathologist with no liver specialization, evaluating K19 expression should not be difficult, as long as care is taken not to count bile ducts, which may be associated with the remains of portal tracts. Taken together, these finding could indicate that it may be beneficial to check tumors for K19 positivity prior to RFA. Further research is warranted in larger groups to validate these findings and outweigh the potential additional clinical benefit compared to the potential risk of tract seeding during percutaneous biopsy.

Although biopsy has an important role in understanding the biological characteristics of HCC [26], tumor seeding by needle biopsy should be avoided. In practice, this is a major concern with needle biopsy of tumors. A review of tumor seeding following therapeutic procedures in HCC indicated that seeding occurred in 0–12.5% of cases (median 0.95%, mean 2.5%) [22]. As the time between biopsy and the treatment procedure was not specified, it is difficult to identify the factors that could have caused seeding. In the present study, tumor biopsies were performed just before RFA, using a needle-guiding technique, and tumor seeding was not observed. The same puncture line was used for both tumor biopsy and RFA, allowing complete ablation of the tumor using the tumor biopsy route. This may be one of the reasons it was possible in this study to biopsy the tumors without dissemination or bleeding. After treatment by RFA, the tumor cannot be investigated for histological features and K19 expression; therefore, we recommend taking a biopsy just before RFA for predicting tumor behavior using K19 expression. This would be valuable to both the clinician and the patient.

The mechanism of K19-positive HCC remains unclear. The facts that K19-positive cells are present in HCCs and that these positive cells form a spectrum suggest that K19-positive HCC may have originated from hepatic progenitor cells. These hepatic progenitor cells,

which are liver-specific adult stem cells, have potential stem cell features such as proliferation and differentiation. Once a tumor takes on these phenotypes, K19-positive HCC can still preserve these stem cell phenotypes. Therefore, this could be a possible reason why K19-positive HCC shows aggressive behavior in comparison with K19-negative HCC. In fact, previous publications and our study confirm these features [27].

In conclusion, we successfully evaluated the positivity of K19 in biopsy specimens. K19-positive HCCs showed significantly more frequent recurrence after curative RFA than K19-negative tumors and positive staining of K19 in the cytoplasm of HCC is closely associated with early intrahepatic recurrence (<1 year) and dropout from the Milan criteria. On imaging, K19-positive HCC showed only typical HCC findings and it was difficult to distinguish between K19-positive and -negative HCC. Taken together, these findings could indicate that >5% K19 positivity in tumor biopsy tissue is important for predicting tumor recurrence, which is not possible by imaging. Because of the high risk of tumor recurrence in K19-positive HCC, close observation for early detection of recurrence should be required.

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References

1 Bruix J, Sherman M: Management of hepatocellular carcinoma. Hepatology 2005;42:1208–1236.

2 Tateishi R, Shiina S, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Yoshida H, Kawabe T, Omara M: Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. Cancer 2005;103:1201–1209.

3 Shiina S, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, Ishikawa T, Koike Y, Yoshida H, Kawabe T, Omata M: A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. Gastroenterology 2005;129:122–130.

4 Kudo M: Radiofrequency ablation for hepatocellular carcinoma: updated review in 2010. Oncology 2010;78:113–124.

5 Choi D, Lim HK, Rhim H, Kim YS, Lee WJ, Paik SW, Koh KC, Lee JH, Choi MS, Yoo BC: Percutaneous radiofrequency ablation for early-stage hepatocellular carcinoma as a first-line treatment: long-term results and prognostic factors in a large single-institution series. Eur Radiol 2007;17:684–692.

6 Lu DS, Yu NC, Raman SS, Lassman C, Tong MJ, Britten C, Durazo F, Saab S, Han S, Finn R, Hiatt JR, Busuttil RW: Percutaneous radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. Hepatology 2005;41:1130–1137.

7 Mazzaferro V, Battiston C, Perrone S, Pulvirenti A, Regalia E, Romito R, Sarli D, Schavo M, Garbagnati F, Marchiano A, Spreafico C, Camerini T, Mariani L, Miceli R, Andreola S: Radiofrequency ablation of small hepatocellular carcinoma in cirrhotic patients awaiting liver transplantation: a prospective study. Ann Surg 2004;240:900–909.

8 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.

9 Fernandes ML, Lin CC, Lin CJ, Chen WT, Lin SM: Risk of tumour progression in early-stage hepatocellular carcinoma after radiofrequency ablation. Br J Surg 2009;96:756–762.

10 Roskams T, De Vos R, Van Eyken P, Myazaki H, Van Damme B, Desmet V: Hepatic OV-6 expression in human liver disease and rat experiments: evidence for hepatic progenitor cells in man. J Hepatol 1998;29:455–463.

11 Yang XR, Xu Y, Shi GM, Fan J, Zhou J, Ji Y, Sun HC, Qiu SJ, Yu B, Gao Q, He YZ, Qin WZ, Chen RX, Yang GH, Wu B, Lu Q, Wu ZQ, Tang ZY: Cytokeratin 10 and cytokeratin 19: predictive markers for poor prognosis in hepatocellular carcinoma patients after curative resection. Clin Cancer Res 2008;14:3850–3859.

12 Zhuang PY, Zhang JB, Zhu XD, Zhang W, Wu WZ, Tan YS, Hou J, Tang ZY, Qin LX, Sun HC: Two pathologic types of hepatocellular carcinoma with lymph node metastasis with distinct prognosis on the basis of CK19 expression in tumor. Cancer 2008;112:2740–2748.

13 Uenishi T, Kubo S, Yamamoto T, Shuto T, Ogawa M, Tanaka H, Tanaka S, Kaneda K, Hirohashi K: Cytokeratin 19 expression in hepatocellular carcinoma predicts early postoperative recurrence. Cancer Sci 2003;94:851–857.

14 Durnez A, Verslype C, Nevens F, Fevery J, Aerts R, Pirenne J, Lesaffre E, Libbrecht L, Desmet V, Roskams T: The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. Histopathology 2006;49:138–151.

15 Sobin LH, Fleming ID: TNM Classification of Malignant Tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. Cancer 1997;80:1803–1804.

16 Kudo M, Okanoue T: Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. Oncology 2007;72(suppl 1):2–15.

17 Hirohashi S, Ishak K, Kojiro M, Wanless I, Theise N, Tsukuma H: Tumours of the liver and intrahepatic bile ducts; in Hamilton SR, Aaltonen LA (eds): *Pathology and Genetics of Tumours of the Digestive System*. Lyon, IARC Press, 2000, pp 157–202.

18 De Baere T, Rehim MA, Teriitheau C, Deschamps F, Lapeyre M, Dromain C, Boige V, Dromain C, Boige V, Ducreux M, Elias D: Usefulness of guiding needles for radiofrequency ablative treatment of liver tumors. Cardiovasc Intervent Radiol 2006;29:650–654.
19 Lorentzen T: A cooled needle electrode for radiofrequency tissue ablation: thermodynamic aspects of improved performance compared with conventional needle design. Acad Radiol 1996;3:556–563.
20 Goldberg SN, Gazelle GS, Solbiati L, Rittman WJ, Mueller PR: Radiofrequency tissue ablation: increased lesion diameter with a perfusion electrode. Acad Radiol 1996;3:636–644.
21 Komorizono Y, Oketani M, Sako K, Yamasaki N, Shibatou T, Maeda M, Kohara K, Shigenobu S, Ishibashi K, Arima T: Risk factors for local recurrence of small hepatocellular carcinoma tumors after a single session, single application of percutaneous radiofrequency ablation. Cancer 2003;97:1253–1262.
22 Stigliano R, Marelli L, Yu D, Davies N, Patch D, Burroughs AK: Seeding following percutaneous diagnostic and therapeutic approaches for hepatocellular carcinoma. What is the risk and the outcome? Seeding risk for percutaneous approach of HCC. Cancer Treat Rev 2007;33:437–447.
23 Brillet PY, Paradis V, Brancatelli G, Rangheard AS, Consigny Y, Plessier A, Durand F, Belghiti J, Sommacale D, Vilgrain V: Percutaneous radiofrequency ablation for hepatocellular carcinoma before liver transplantation: a prospective study with histopathologic comparison. AJR Am J Roentgenol 2006;186:S296–S305.
24 Pawlik TM, Gleisner AL, Anders RA, Assumpcao L, Maley W, Choti MA: Preoperative assessment of hepatocellular carcinoma tumor grade using needle biopsy: implications for transplant eligibility. Ann Surg 2007;245:435–442.
25 Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodes J, EASL Panel of Experts on HCC: Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001;35:421–430.
26 Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD: Liver biopsy. Hepatology 2009;49:1017–1044.
27 van Sprundel RG, van den Ingh TS, Spee B: Keratin 19 marks poor differentiation and a more aggressive behavior in canine and human hepatocellular tumours. Comp Hepatol 2010;9:4.

Research Article

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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.

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Introduction

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

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

Cancer

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* 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).

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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.

Materials and methods

Patients

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

HCC surveillance and diagnosis

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

Statistical analysis

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

On univariate analysis, Student's *t*-test was used for continuous variables and Fisher's exact test was used for categorical data. Logistic regression was used for multivariate analysis. A log-rank test for Kaplan–Meier analysis was used to statistically test HCC development rates over time. *p*-Values of <0.05 were considered significant. SPSS Statistics 18 (IBM SPSS Inc.) was used for these analyses.

Results

Univariate and multivariate analysis of factors associated with HCC development

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

HCC risk prediction model by data mining analysis

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

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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 (⩾150 × 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 (⩾3.75 g/dl). The cutoff value for albumin levels was 4.0 g/dl in patients with a lower platelet count (<150 × 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 (⩾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 (⩾60 years) with a lower platelet count (<150 × 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 (⩾60 years), lower platelet count (<150 × 10^9/L), higher albumin levels (⩾4.0 g/dl), and higher AST levels (⩾40 IU/L); (2) older age (⩾60 years), higher platelet count (⩾150 × 10^9/L), and lower albumin levels (<3.75 g/dl); and (3) younger age (<60 years) and lower platelet count (<150 × 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 (⩾150 × 10^9/L); (2) older age (⩾60 years), lower platelet count (<150 × 10^9/L), higher albumin levels (⩾4.0 g/dl), and lower AST levels (<40 IU/L); and (3) older age (⩾60 years), higher platelet count (⩾150 × 10^9/L), and higher albumin levels (⩾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

Cancer

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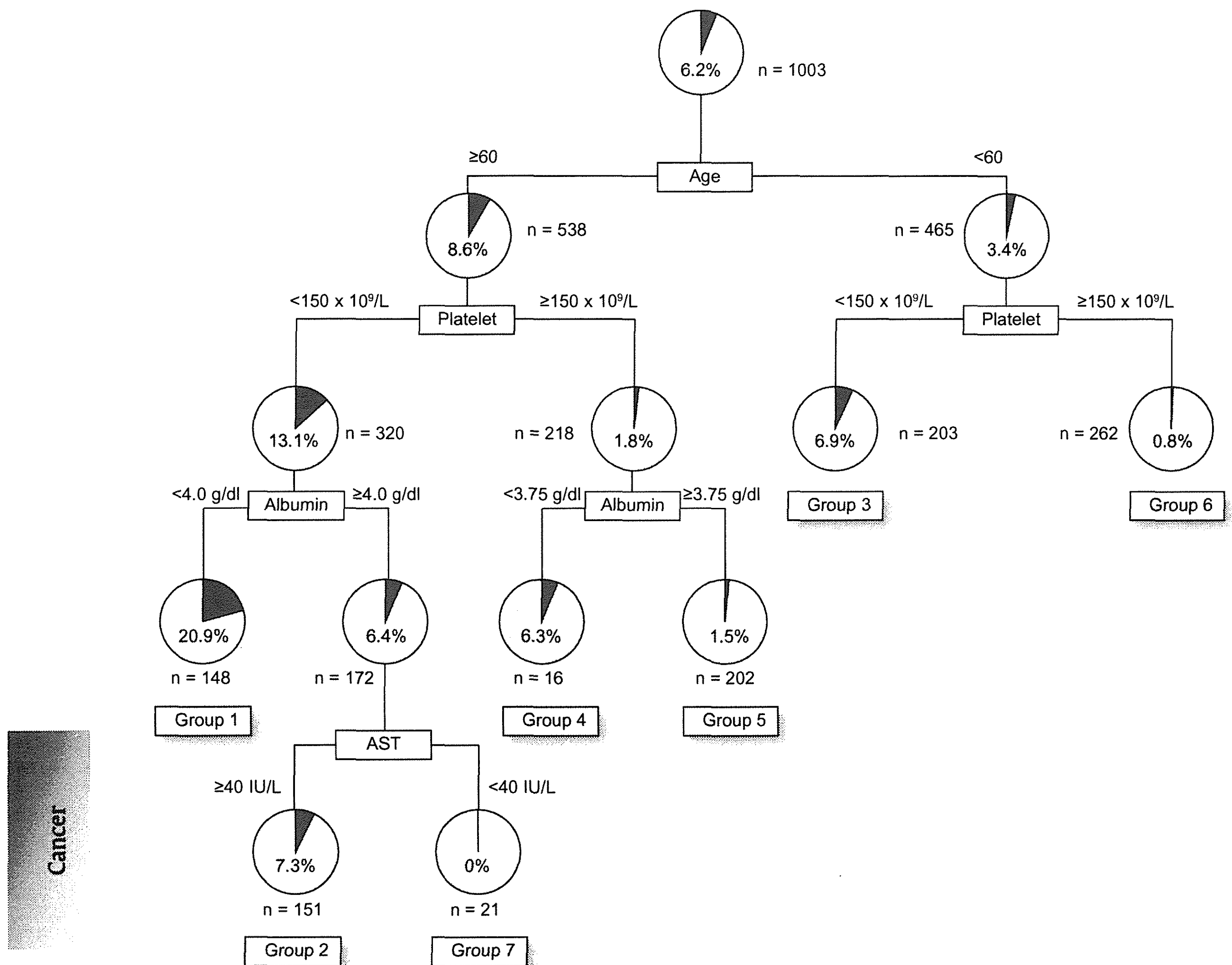

Fig. 1. The decision tree model of HCC development within 5 years. Boxes indicate the factors used to differentiate patients and the cutoff values for those different groups. Pie charts indicate the HCC development rate within 5 years for each group of patients after differentiation. Terminal groups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

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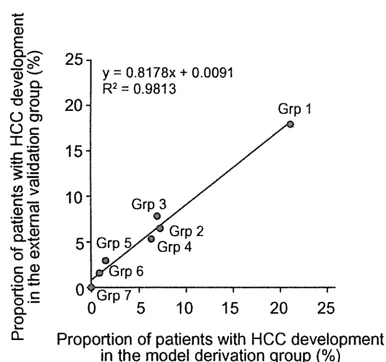

Fig. 2. External validation of the decision tree model with an independent cohort. Each patient in the external validation group was allocated to groups 1–7 following the flowchart of the decision tree. The HCC development rates were then calculated for each group and the graph plotted. The *x*-axis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

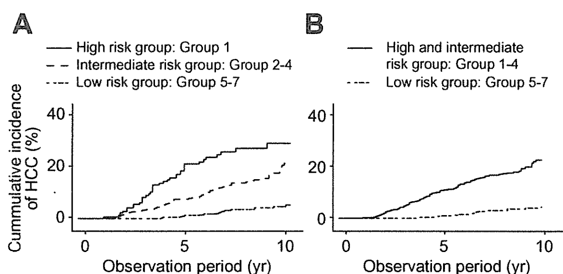

Fig. 3. Cumulative incidence of HCC development beyond 5 years in subgroups of patients defined by the decision tree model. Cumulative incidences of HCC in the groups classified by the decision tree model are compared. (A) The cumulative HCC development rate beyond 5 years is higher in the high- (group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

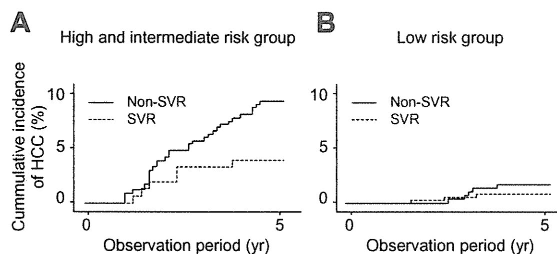

Fig. 4. Sustained virological response to PEG-IFN plus RBV therapy reduces the incidence of HCC development after stratification by the HCC risk. The 600 nonSVR patients and the 472 SVR patients in the external cohort were fitted into the HCC risk prediction model and classified into the high and intermediate-risk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

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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.

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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.

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[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.

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Hepatology Research

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Original Article

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

Masayuki Kurosaki,[1] Takanori Hosokawa,[1] Kotaro Matsunaga,[2] Itsuko Hirayama,[1] Tomohiro Tanaka,[1] Mitsuaki Sato,[1] Yutaka Yasui,[1] Nobuharu Tamaki,[1] Ken Ueda,[1] Kaoru Tsuchiya,[1] Teiji Kuzuya,[1] Hiroyuki Nakanishi,[1] June Itakura,[1] Yuka Takahashi,[1] Yasuhiro Asahina,[1] Nobuyuki Enomoto[3] and Namiki Izumi[1]

Divisions of [1]Gastroenterology and Hepatology, and [2]Pathology, Musashino Red Cross Hospital, Tokyo, and [3]First Department of Internal Medicine, University of Yamanashi, Yamanashi, Japan

Aim: Hepatic steatosis is linked to development of hepatocellular carcinoma (HCC) in non-viral liver disease such as non-alcoholic steatohepatitis. The present study aimed to assess whether hepatic steatosis is associated with the development of HCC in chronic hepatitis C.

Methods: We studied a retrospective cohort of 1279 patients with chronic hepatitis C who received interferon (IFN) therapy between 1994 and 2005 at a single regional hospital in Japan. Of these patients, 393 had a sustained virological response (SVR) and 886 had non-SVR to IFN therapy. After IFN therapy, these patients were screened for development of HCC every 6 months. The average period of observation was 4.5 years.

Results: HCC developed in 68 patients. The annual incidence of HCC was 2.73% for patients with a steatosis grade of 10% or greater and 0.69% for patients with a steatosis grade of 0–9%. On multivariate analysis, higher grade of steatosis was a significant risk factor for HCC independent of older age, male sex, higher body mass index (BMI), advanced fibrosis stage and non-SVR to IFN therapy. The adjusted risk ratio of hepatic steatosis was 3.04 (confidence interval 1.82–5.06, $P < 0.0001$), which was higher than that of older age (1.09), male sex (2.12), non-SVR to IFN (2.43) and higher BMI (1.69).

Conclusion: Hepatic steatosis is a significant risk factor for development of HCC in chronic hepatitis C independent of other known risk factors, which suggest the possibility that amelioration of hepatic steatosis may prevent hepatocarcinogenesis.

Key words: hepatocellular carcinoma, interferon, steatosis, virological response.

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers worldwide and its incidence has been increasing. This recent increase in HCC incidence may likely be attributed to the higher prevalence of non-alcoholic fatty liver disease (NAFLD) and hepatitis C virus (HCV) infection.[1]

Non-alcoholic fatty liver disease is characterized by hepatic steatosis with or without inflammation in the absence of excessive alcohol consumption. Several studies have indicated the etiological association between NAFLD and development of HCC.[2–4] Other studies have shown that obesity or diabetes, a common etiology of non-alcoholic hepatic steatosis, is associated with development of HCC.[5–7] Although the mechanism of carcinogenesis in NAFLD has not been determined, an animal model showed that obesity-related hepatic steatosis leads to the development of hepatic

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

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hyperplasia, suggesting the possibility that hepatic steatosis is a pre-malignant condition.[8]

Another important etiological agent for HCC is HCV infection. Because steatosis is a common pathological feature of HCV-infected patients,[9] the important question is whether steatosis influences the progression of liver disease in hepatitis C, by analogy with NAFLD. Several studies, including ours[10] indicated that hepatic steatosis promotes the progression of hepatic fibrosis.[11–15] The association between hepatic steatosis and the development of HCC in chronic hepatitis C has been proposed[16] and was confirmed in two studies[17,18] while another study failed to show such an association.[19] The present study was conducted to analyze the association between hepatic steatosis and development of HCC in a large cohort of chronic hepatitis C patients, which enabled to adjust for known risk factors for HCC.

METHODS

Patients

A TOTAL OF 1437 chronic hepatitis C patients were treated with interferon (IFN) at Musashino Red Cross Hospital between October 1994 and October 2005. Among them, 1279 patients who fulfilled the following inclusion criteria were enrolled in this study: (i) positive for HCV RNA by reverse-transcription polymerase chain reaction before IFN therapy; (ii) absence of other causes of liver disease, such as co-infection with hepatitis B virus, autoimmune hepatitis or primary biliary cirrhosis; (iii) had undergone liver biopsy within the 12 months prior to IFN treatment; (iv) were followed for more than 1 year after the completion of IFN therapy; and (v) absence of HCC during and within 1 year after the completion of therapy. A total of 158 patients were excluded: two patients who were positive for hepatitis B surface antigen, 97 patients lacking liver biopsy, 53 patients with less than 1 year's duration of follow up, and six patients who developed HCC within 1 year of the completion of IFN therapy. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committee.

Patients were followed up by regular visits to our hospital every 1–3 months. Six patients died of liver-unrelated disease (two patients with gastric cancer and one patient each with lung cancer, colon cancer, pancreatic cancer and leukemia). There were 122 patients who were lost to follow up because of relocation. We included their data in the analysis, censored at the time of their last visit. The start of follow up was defined as the date of completion of first IFN therapy and the end of follow up was defined as the date of diagnosis of HCC or the date of the last visit. The average period of follow up was 4.5 years.

Clinical characteristics and laboratory data were collected at the most recent time point before liver biopsy. Diabetes mellitus was diagnosed based on a fasting plasma glucose concentration that exceeded 126 mg/dL, a casual plasma glucose concentration that exceeded 200 mg/dL, or the need for insulin or oral antihyperglycemic drugs. Information regarding alcohol consumption was obtained through an interview. Body mass index (BMI) was calculated using the following formula: weight in kilograms/height in meters squared. The baseline clinical features of patients at enrollment are summarized in Table 1.

Histological examination

Liver biopsy specimens were obtained from all patients before therapy. The median length of liver biopsy specimens was 13 mm (range 10–42 mm) and median number of portal tracts was 11 (range 4–30). Histological findings were re-evaluated recently by three independent pathologists who were blinded to the clinical details to ensure consistency over time. Fibrosis and activity were scored according to the METAVIR scoring system.[20] Fibrosis was staged on a scale of 0–4: F0 (no fibrosis); F1 (mild fibrosis: portal fibrosis without septa); F2 (moderate fibrosis: few septa); F3 (severe fibrosis: numerous septa without cirrhosis); and F4 (cirrhosis). Activity of necroinflammation was graded on a scale of 0–3: A0 (no activity); A1 (mild activity); A2 (moderate activity); and A3 (severe activity). Percentage of steatosis was quantified by determining the average proportion of hepatocytes affected by steatosis and graded on a scale of 0%, 1–9%, 10–29% and 30% or greater as reported previously.[10] All three pathologists assigned the same scale in 85% of cases for fibrosis staging, 87% for inflammation grading and 95% for steatosis grading. If there was discordance, the scores assigned by two pathologists were used for the analysis.

Screening for HCC

At enrollment, no patient had HCC or any suspicious lesion on abdominal ultrasonography or computed tomography. Patients were examined for HCC by abdominal ultrasonography or computed tomography at least every 6 months. Suspicious lesions were examined further by a triphasic contrast-enhanced computerized tomography or magnetic resonance imaging,

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Table 1 Clinical characteristics of patients

Male, *n* (%)	643 (50%)
Age (years)	54.2 ± 11.9
BMI (kg/m²)	23.4 ± 3.1
Alcohol consumption ≥20 g/day, *n* (%)	44 (3%)
Diabetes Mellitus, *n* (%)	197 (15%)
AST level (IU/L)	68.9 ± 45.3
ALT level (IU/L)	92.9 ± 75.9
GGT level (IU/L)	41.2 ± 38.2
Platelet count (×10¹⁰/L)	16.4 ± 5.2
HCV genotype, *n* (%)	
1b	873 (68.2%)
2a	236 (18.4%)
2b	139 (10.9%)
3	2 (0.2%)
Not determined	29 (2.3%)
Histological findings	
Grade of activity, *n* (%)	
A0	154 (12%)
A1	574 (45%)
A2	441 (34%)
A3	110 (9%)
Stage of fibrosis, *n* (%)	
F0	24 (2%)
F1	591 (46%)
F2	378 (30%)
F3	242 (19%)
F4	44 (3%)
Grade of steatosis, *n* (%)	
0%	384 (30%)
1–9%	543 (42%)
10–29%	215 (17%)
≥30%	137 (11%)
SVR to interferon therapy, *n* (%)	393 (31%)
Development of HCC, *n* (%)	68 (5%)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyltransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.

angiography or tumor biopsy to confirm the diagnosis. Diagnostic criteria of HCC on radiological findings were hyper-vascularity at angiography or hyper-attenuation at triphasic contrast-enhanced computerized tomography or magnetic resonance imaging during the hepatic arterial phase.

Statistical analysis

The SPSS software package ver. 15.0 was used for statistical analysis. Categorical data were analyzed using Fisher's exact test. Continuous variables were compared with Student's *t*-test. The time for the development of HCC was defined as the time from the completion of IFN therapy to the time of diagnosis. Annual incidence of HCC was calculated using the person-years method. Effect of hepatic steatosis on time to development of HCC was analyzed by the Kaplan–Meier method and log-rank test, after stratification by age, sex, BMI, degree of fibrosis and response to IFN therapy, as well as multivariate analysis using Cox proportional hazards regression analysis. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Background factors for steatosis

PATIENTS WITH A steatosis grade of 10% or greater were older (53.6 ± 12.6 vs 56.0 ± 9.8, $P = 0.001$), had a higher BMI (23.0 ± 3.0 vs 24.6 ± 3.3, $P < 0.0001$), higher frequency of diabetes (12% vs 24%, $P < 0.0001$), higher serum levels of aspartate aminotransferase (AST) (66 ± 46 vs 75 ± 43, $P = 0.002$), γ-glutamyltransferase (GGT) (37 ± 52 vs 52 ± 33, $P < 0.0001$), total cholesterol (173 ± 32 vs 179 ± 33, $P = 0.005$), triglycerides (123 ± 56 vs 145 ± 68, $P < 0.0001$), and a lower serum level of albumin (4.2 ± 0.3 vs 4.1 ± 0.3, $P = 0.005$) and lower platelet counts (16.6 ± 5.2 vs 15.7 ± 5.1, $P = 0.007$). Histological grade of activity (A2–3: 39% vs 54%, $P < 0.0001$), and stage of fibrosis (F3–4: 18% vs 34%, $P < 0.0001$) were higher. The proportion of non-sustained virological response (SVR) to IFN also was higher (35% vs 19%, $P < 0.0001$). These results indicate that hepatic steatosis in hepatitis C is related to metabolic factors and associated with other risk factors for the development of HCC such as older age, advanced stage of fibrosis, and non-SVR to IFN therapy.

Factors associated with the development of HCC

Hepatocellular carcinoma developed in 68 patients during follow up. An overall annual incidence of HCC development was 1.19% by person-years. The annual incidence of HCC development by person-years was higher in patients with higher grade of steatosis: 0.45% for patients without steatosis, 0.78% for patients with 1–9% of steatosis, 2.30% for patients with 10–29% of steatosis, and 3.56% for patients with 30% of steatosis. The relative risk of hepatic steatosis (grade of ≥10%) for HCC development was 4.39 (95% confidence interval 2.66–7.26, $P < 0.0001$). The difference remained significant, even after stratification for other risk factors such as IFN therapy, stage of fibrosis, age, sex and BMI (Fig. 1). When analyzed by the multivariate Cox proportional hazards regression method, a higher grade of steatosis,

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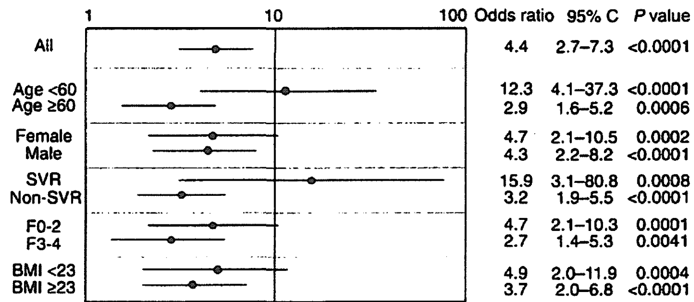

Figure 1 Relative risk differences of hepatocellular carcinoma (HCC) among patients with and without steatosis. The relative risk of hepatic steatosis (grade ≥10%) for HCC development was analyzed, after stratification for other risk factors such as interferon (IFN) therapy, stage of fibrosis, age, sex and body mass index (BMI). SVR, sustained virological response.

older age, male sex, higher BMI, an advanced stage of fibrosis and non-SVR to IFN therapy were independent risk factors associated with the development of HCC (Table 2). The adjusted risk ratio of hepatic steatosis was 3.04 (95% confidence interval 1.82–5.06, $P < 0.0001$). The presence of diabetes and consumption of ethanol were not significant. Figure 2(a) shows the Kaplan–Meier curve of the time to development of HCC in the entire cohort. The cumulative incidence of HCC was significantly higher with hepatic steatosis of 10% or greater. To adjust for other risk factors, patients were stratified according to response to IFN therapy, stage of fibrosis, age, sex and BMI. The difference remained significant, even after stratification for these confounding factors (Fig. 2b–f). Three patients died after the development of HCC. All were over 60 years old, and had significant steatosis. The impact of hepatic steatosis on the survival rate could not be analyzed due to the small number of death.

DISCUSSION

IN THIS STUDY, we have shown that the presence of significant steatosis is an independent risk factor for the development of HCC in chronic hepatitis C. Our study involved the largest number of patients, compared to previous reports, and this enabled us to adjust for other known risk factors for HCC. The impact of steatosis on HCC development remained significant even after adjusting for other risk factors such as older age, male sex, higher BMI, advanced fibrosis and non-SVR to IFN therapy. These findings indicate the need of intensive surveillance for HCC in patients with significant steatosis and provide an argument for therapeutic interventions aimed at reducing steatosis, in order to reduce the risk of HCC.

The association between hepatic steatosis and the development of HCC in chronic hepatitis C has been proposed and the possible mechanism has been discussed.[16] There are several cohort studies on this topic but their results are conflicting. The first report included 20 patients with SVR to IFN, 51 patients with non-SVR to IFN and 90 patients who did not receive IFN therapy.[17] In this cohort of 161 patients, older age, absence of IFN therapy, cirrhosis and steatosis were associated with HCC development. Another study involved 25 patients with HCC and an equal number of patients who did not develop HCC, matched for

Table 2 Multivariate analysis of risk factors for hepatocellular carcinoma

Predictor		Odds ratio (95% CI)	P-value
Age	By every 10 years	1.09 (1.05–1.13)	<0.0001
Sex	Male vs female	2.12 (1.28–3.51)	0.004
Stage of fibrosis	F3-4 vs F0-2	4.30 (2.59–7.14)	<0.0001
Grade of steatosis	≥10% vs <10%	3.04 (1.82–5.06)	<0.0001
Response to IFN	Non-SVR vs SVR	2.43 (1.13–5.23)	0.023
Diabetes	Present vs absent	0.75 (0.42–1.33)	0.319
Ethanol consumption (g/day)	≥20 vs <20	0.50 (0.07–3.60)	0.478
BMI (kg/m2)	≥23 vs <23	1.69 (1.02–2.86)	0.043

BMI, body mass index; CI, confidence interval; IFN, interferon; SVR, sustained virological response.

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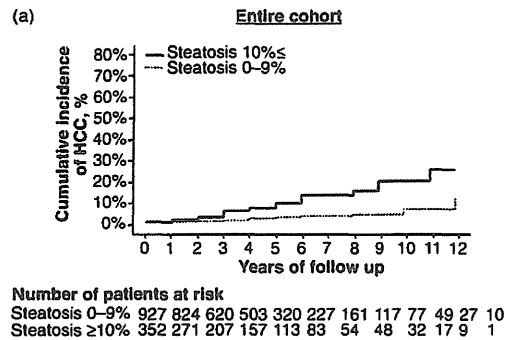

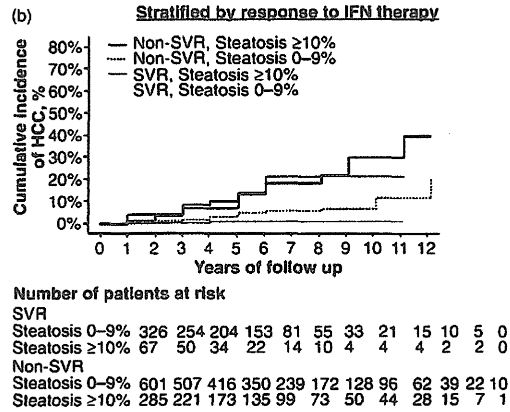

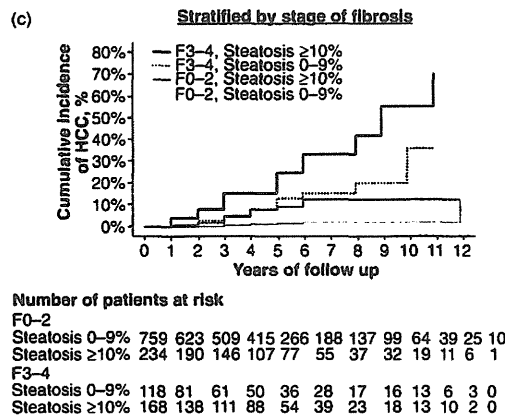

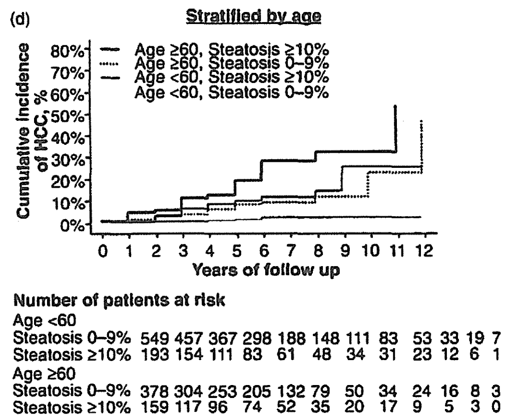

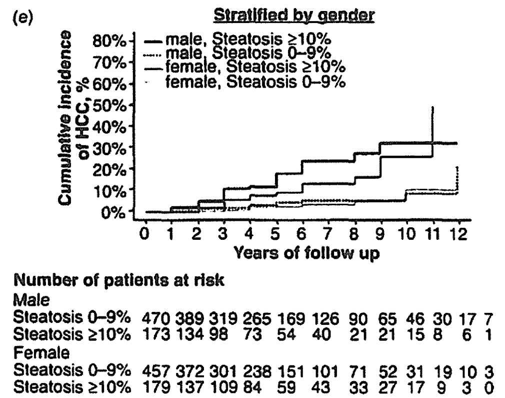

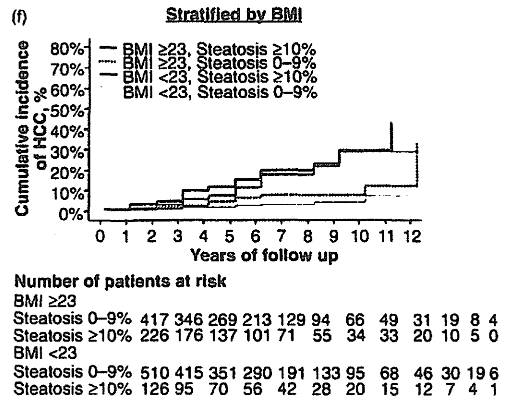

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Figure 2 Cumulative incidence of hepatocellular carcinoma (HCC) among patients with steatosis (solid line) and without steatosis (dotted line), stratified by other risk factors. The cumulative incidence of HCC was (a) significantly higher in patients with a steatosis grade of 10% or greater ($P < 0.0001$ by the log-rank test), even after (b) stratification by the response to interferon therapy ($P < 0.0001$ for sustained virological response [SVR] and non-SVR by the log-rank test), (c) stratification by the stage of fibrosis ($P < 0.0001$ for F0–2 and $P = 0.0036$ for F3–4 by the log-rank test), (d) stratification by age ($P = 0.0001$ for age ≥60 and $P < 0.0001$ for age <60 by the log-rank test), (e) stratification by sex ($P < 0.0001$ for men and women by the log-rank test), and (f) stratification by body mass index (BMI) ($P < 0.0001$ for BMI ≥23 kg/m^2 and <23 kg/m^2 by the log-rank test). The number of patients at risk is shown below each graph.

age, sex, HCV genotype and stage of fibrosis.[19] In this study, only ALT and albumin were identified as predictors of HCC and steatosis was not. The authors acknowledged the small size of the cohort as a limitation and emphasized the need for larger cohort studies. The third study analyzed explanted liver from cirrhotic patients who underwent liver transplantation and included 32 patients with HCC and 62 patients without HCC.[18] The authors found that older age, higher α-fetoprotein levels and steatosis were significantly associated with HCC. The major advantage of this study was the standardization of fibrosis stage to cirrhosis. On the other hand, a limitation was the retrospective nature of the study; steatosis was evaluated after the diagnosis of HCC, when cirrhosis already was present (fibrosis stage F4). Because steatosis has been reported to decrease once cirrhosis has developed, this study may have underestimated the grade of steatosis present prior to the development of HCC. Thus, we cannot simply apply their findings to a clinical setting where biopsies are usually obtained before the development of cirrhosis and years before the development of HCC. Based on that background, the principal aim of this study was to analyze the association between hepatic steatosis and the development of HCC in chronic hepatitis C patients, adjusting for known risk factors. We found that steatosis was an independent risk factor by the multivariate Cox proportional hazards regression analysis and by the Kaplan–Meier method and log-rank test after stratification by other risk factors. To our surprise, the adjusted risk ratio of hepatic steatosis was higher than that of older age, male sex, non-SVR to IFN and higher BMI.

How steatosis contributes to the development of HCC remains unclear. Several studies including ours,[10] indicated that hepatic steatosis promotes the progression of hepatic fibrosis,[11–15] which potentiates the risk of HCC indirectly. On the other hand, the ob/ob mouse model of NAFLD showed that hepatic neoplasia developed in the absence of advanced fibrosis, supporting the concept that metabolic abnormalities related to obesity initiate the neoplastic process.[8] Leptin, an adipocytokine related to steatosis in chronic hepatitis C,[21] was shown recently to be mitogenic in human liver[22] and thus may be a link between steatosis and HCC development. Otherwise, steatosis may be responsible for increased lipid peroxidation and reactive oxygen species which induce genetic damage.[23–25] Another study showed that mice transgenic for the HCV core gene developed hepatic steatosis early in life and thereafter HCC which indicates that the HCV core protein has a chief role in the development of both steatosis and HCC development.[26] The precise mechanism of the association between steatosis and carcinogenesis needs further investigation.

The higher incidence of HCC in patients with significant steatosis has important clinical implications. The most important question is whether therapeutic interventions aimed at reducing steatosis could reduce the risk of HCC in chronic hepatitis C. Because the adjusted risk ratio of hepatic steatosis was higher than that of older age, male sex, non-SVR to IFN and higher BMI, we hypothesize that modification of lifestyle and the amelioration of hepatic steatosis may efficiently prevent hepatocarcinogenesis in patients having concomitant risk factors. Apparently, further prospective studies focusing on this point are necessary. Weight reduction may provide an important treatment strategy because one study indicated that weight reduction in chronic hepatitis C leads to a reduction in steatosis and an improvement in fibrosis despite the persistence of HCV infection.[27] Alternatively, insulin resistance may be another target of therapy because a study showed that the administration of pioglitazone led to metabolic and histological improvement in subjects with non-alcoholic steatohepatitis.[28] A limitation of the present study was that data for the plasma insulin concentration was not available and thus insulin resistance could not be assessed. Whether insulin resistance plays a role in hepatocarcinogenesis or its amelioration could improve steatosis and ultimately prevent development of HCC in chronic hepatitis C awaits future investigation.

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Another important finding of the present study was that steatosis was a significant risk factor for the development of HCC in patients with SVR to IFN therapy. Thus, steatosis may play a role in carcinogenesis in patients who have cleared HCV. Several studies have shown that the incidence of HCC is reduced but not eliminated in those with SVR to IFN.[29–31] Because the predictors of HCC development in SVR patients have not been established to date, steatosis may be used to identify patients who need intensive surveillance and long-term follow up, even after the clearance of HCV. In conclusion, we showed that hepatic steatosis is significantly associated with the development of HCC in chronic hepatitis C independent of age, sex, BMI, degree of fibrosis and response to previous IFN therapy. Steatosis may be a useful marker for identifying patients at higher risk for HCC. Further studies are needed to evaluate the hypothesis that therapeutic interventions aimed at reducing steatosis may prevent hepatocarcinogenesis.

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REFERENCES

1 El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; 132: 2557–76.
2 Shimada M, Hashimoto E, Taniai M *et al.* Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. *J Hepatol* 2002; 37: 154–60.
3 Bugianesi E, Leone N, Vanni E *et al.* Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; 123: 134–40.
4 Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology* 2002; 36: 1349–54.
5 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: 1625–38.
6 El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; 126: 460–8.
7 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: 533–9.
8 Yang S, Lin HZ, Hwang J, Chacko VP, Diehl AM. Hepatic hyperplasia in noncirrhotic fatty livers: is obesity-related hepatic steatosis a premalignant condition? *Cancer Res* 2001; 61: 5016–23.
9 Lefkowitch JH, Schiff ER, Davis GL *et al.* Pathological diagnosis of chronic hepatitis C: a multicenter comparative study with chronic hepatitis B. The Hepatitis Interventional Therapy Group. *Gastroenterology* 1993; 104: 595–603.
10 Kurosaki M, Matsunaga K, Hirayama I *et al.* The presence of steatosis and elevation of alanine aminotransferase levels are associated with fibrosis progression in chronic hepatitis C with non-response to interferon therapy. *J Hepatol* 2008; 48: 736–42.
11 Hourigan LF, Macdonald GA, Purdie D *et al.* Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 1999; 29: 1215–19.
12 Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; 33: 1358–64.
13 Westin J, Nordlinder H, Lagging M, Norkrans G, Wejstal R. Steatosis accelerates fibrosis development over time in hepatitis C virus genotype 3 infected patients. *J Hepatol* 2002; 37: 837–42.
14 Fartoux L, Chazouilleres O, Wendum D, Poupon R, Serfaty L. Impact of steatosis on progression of fibrosis in patients with mild hepatitis C. *Hepatology* 2005; 41: 82–7.
15 Leandro G, Mangia A, Hui J *et al.* Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. *Gastroenterology* 2006; 130: 1636–42.
16 Koike K. Hepatitis C virus contributes to hepatocarcinogenesis by modulating metabolic and intracellular signaling pathways. *J Gastroenterol Hepatol* 2007; 22 (Suppl 1): S108–11.
17 Ohata K, Hamasaki K, Toriyama K *et al.* Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Cancer* 2003; 97: 3036–43.
18 Pekow JR, Bhan AK, Zheng H, Chung RT. Hepatic steatosis is associated with increased frequency of hepatocellular carcinoma in patients with hepatitis C-related cirrhosis. *Cancer* 2007; 109: 2490–6.
19 Kumar D, Farrell GC, Kench J, George J. Hepatic steatosis and the risk of hepatocellular carcinoma in chronic hepatitis C. *J Gastroenterol Hepatol* 2005; 20: 1395–400.
20 Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; 24: 289–93.
21 Romero-Gomez M, Castellano-Megias VM, Grande L *et al.* Serum leptin levels correlate with hepatic steatosis in chronic hepatitis C. *Am J Gastroenterol* 2003; 98: 1135–41.
22 Ramani K, Yang H, Xia M, Ara AI, Mato JM, Lu SC. Leptin's mitogenic effect in human liver cancer cells requires induc-

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tion of both methionine adenosyltransferase 2A and 2beta. *Hepatology* 2008; 47: 521–31.

23 Okuda M, Li K, Beard MR *et al.* Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002; 122: 366–75.

24 Cai D, Yuan M, Frantz DF *et al.* Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 2005; 11: 183–90.

25 Arkan MC, Hevener AL, Greten FR *et al.* IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 2005; 11: 191–8.

26 Moriya K, Fujie H, Shintani Y *et al.* The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nat Med* 1998; 4: 1065–7.

27 Hickman IJ, Clouston AD, Macdonald GA *et al.* Effect of weight reduction on liver histology and biochemistry in patients with chronic hepatitis C. *Gut* 2002; 51: 89–94.

28 Belfort R, Harrison SA, Brown K *et al.* A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; 355: 2297–307.

29 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; 131: 174–81.

30 Nishiguchi S, Shiomi S, Nakatani S *et al.* Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. *Lancet* 2001; 357: 196–7.

31 Shiratori Y, Ito Y, Yokosuka O *et al.* Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. *Ann Intern Med* 2005; 142: 105–14.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Pretreatment prediction of anemia progression by pegylated interferon alpha-2b plus ribavirin combination therapy in chronic hepatitis C infection: decision-tree analysis

Naoki Hiramatsu · Masayuki Kurosaki · Naoya Sakamoto · Manabu Iwasaki · Minoru Sakamoto · Yoshiyuki Suzuki · Fuminaka Sugauchi · Akihiro Tamori · Sei Kakinnuma · Kentaro Matsuura · Namiki Izumi

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Abstract
Background This study aimed to develop a model to predict the development of severe anemia during pegylated interferon alpha-2b plus ribavirin combination therapy.
Methods Data were collected from 1081 genotype 1b chronic hepatitis C patients who were treated at 6 hospitals in Japan. These patients were randomly assigned to a model-building group ($n = 691$) or an internal validation group ($n = 390$). Factors predictive of severe anemia (hemoglobin, Hb < 8.5 g/dl) were explored using data-mining analysis.
Results Hb values at baseline, creatinine clearance (Ccr), and an Hb concentration decline by 2 g/dl at week 2 were used to build a decision-tree model, in which the patients were divided into 5 subgroups based on variable rates of severe anemia ranging from 0.4 to 11.8%. The reproducibility of the model was confirmed by the internal validation group ($r^2 = 0.96$). The probability of severe anemia was high in patients whose Hb value was <14 g/dl before treatment (6.5%), especially (a) in those whose Ccr was <80 ml/min (11.8%) and (b) those whose Ccr was ≥80 ml/min but whose Hb concentration decline at week 2 was ≥2 g/dl (11.5%). The probability of severe anemia was low in the other patients (0.4–2.5%).
Conclusions The decision-tree model that included Hb values at baseline, Ccr, and an Hb concentration decline by

N. Hiramatsu and M. Kurosaki contributed equally to this work.

N. Hiramatsu
Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka, Japan

M. Kurosaki · N. Izumi (✉)
Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino, Tokyo 180-8610, Japan
e-mail: nizumi@musashino.jrc.or.jp

N. Sakamoto · S. Kakinnuma
Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan

M. Iwasaki
Department of Computer and Information Science, Seikei University, Tokyo, Japan

M. Sakamoto
First Department of Internal Medicine, University of Yamanashi, Yamanashi, Japan

Y. Suzuki
Department of Hepatology, Toranomon Hospital, Tokyo, Japan

F. Sugauchi
Department of Gastroenterology, Nagoya Koseiin Medical Welfare Center, Nagoya, Japan

A. Tamori
Department of Hepatology, Osaka City University Medical School, Osaka, Japan

K. Matsuura
Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

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