Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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LETTER TO THE EDITOR

Serum HBV RNA as a possible marker of HBV replication in the liver during nucleot(s)ide analogue therapy

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We read with interest the article by Tsuge et al. [1] published in the recent issue of the Journal of Gastroenterology. Treatment with nucleot(s)ide analogue (NUC) strongly suppresses the replication of hepatitis B virus (HBV) leading to a high rate of serum HBV DNA negativity. However, the incidence of relapse after the cessation of NUCs is high. Criterion for safe discontinuation of NUC therapy after long term therapy is not established to date. In HBe antigen positive patients, seroconversion, HBV DNA negativity and consolidation therapy of >6 months may be a consensus criteria but 30–50 % of patients fulfilling this criteria experience a relapse. In HBe antigen negative patients, NUC therapy is generally recommended until HBs antigen becomes undetected. Tsuge et al. [1] measured serum HBV RNA plus DNA by real time PCR and showed that the serum HBV DNA + RNA titer following 3 months of NUC treatment was a significant predictor of early (within 24 weeks) HBV DNA rebound after discontinuation of NUC. The serum HBV DNA + RNA titer was also associated with ALT rebound in HBe antigen positive patients. The results of the study by Tsuge et al. indicate that serum HBV DNA + RNA titer may serve as predictor of relapse after discontinuation of NUC.

The high rate of relapse after discontinuation of NUC is due to the persistence of HBV replication in the liver even during the NUC therapy. The replicative intermediate form of HBV, covalently closed circular DNA (cccDNA), may not be eliminated by NUC therapy and serves as a template for viral pre-genomic messenger RNA [2]. This concept was proved by a study showing that quantification of intrahepatic HBV cccDNA had a high accuracy of predicting sustained virological response after NUC discontinuation [3]. Still, we need a non-invasive and clinically usable marker for the assessment of HBV replication in the liver during NUC therapy. The measurement of HBV core related antigen may be an alternative [4]. The rationale of measuring HBV RNA in serum was that immature HBV particles including HBV RNA are released from hepatocytes during NUC treatment under the circumstances that pre-genomic HBV RNA are transcribed from HBV cccDNA, packaged into HBV core particles, but not reverse transcribed into plus-strand HBV DNA due to strong interference by NUC, and the excessive amounts of these immature particles are accumulated in hepatocytes [5, 6]. Tsuge et al. showed that serum HBV DNA + RNA titer following 3 months of NUC treatment was significantly lower in patients with no rebound of HBV DNA. By using a cut-off value of 4.8 log copies/mL, the cumulative incidence of HBV DNA rebound was significantly lower in patients with serum HBV DNA + RNA titer < 4.8 log at 3 months of NUC treatment. The same groups previously showed that HBV RNA levels at 3 months of lamivudine treatment were predictor of early emergence of resistant mutations [7]. Taken together, serum HBV DNA + RNA titer may be linked to the level of HBV replication in the liver during NUC therapy. Monitoring of serum HBV DNA + RNA response may be utilized in various decision makings in treatment of HBV patients with NUC therapy.

Based on these important findings, several questions may remain for future elucidation. Commercially available transcription-mediated amplification and hybridization assay

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(TMA) detects both HBV DNA and RNA. We do recognize that detection sensitivity of this assay is not sensitive but could this assay be used in alternative to real time PCR? In the present study, duration of therapy was 36 weeks in average. The question is whether serum HBV DNA + RNA decrease further by a longer duration of therapy and whether monitoring of serum HBV DNA + RNA (at the end of treatment) serve as a predictor of safe discontinuation after long term NUC therapy. Various protocols of sequential interferon therapy starting with NUC are reported in an attempt to enhance the antiviral activity or to achieve drug-free status [8]. However, their outcome varies considerably and negative HBe antigen at the start of interferon is the only predictor of response [9]. Since 26 out of 36 patients in the study by Tsuge et al. received sequential interferon therapy, serum HBV DNA + RNA titer may be an alternative predictor of favorable response to sequential interferon therapy. Further investigation may be necessary to solve these issues but readers of the journal may be interested if comments can be made by the authors.

Conflict of interest The authors declare that they have no conflict of interest.

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Original Article

Fibrosis score consisting of four serum markers successfully predicts pathological fibrotic stages of chronic hepatitis B

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Aim: In order to evaluate and judge a fibrotic stage of patients with chronic hepatitis B, multivariate regression analysis was performed using multiple fibrosis markers.

Method: A total of 227 patients from seven hepatology units and institutes were diagnosed by needle biopsy as having chronic liver disease caused by hepatitis B virus. Twenty-three variables and their natural logarithmic transformation were employed in the multivariate analysis. Multiple regression function was generated from data of 158 patients in one hospital, and validation was performed using the other data of 69 patients from six other hospitals.

Results: After stepwise variable selection, multivariate regression analysis finally obtained the following function: $z = 1.40 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.017 \times$ (platelet count) ($\times 1000^3/mm^3$) $+ 1.24 \times ln$ (tissue inhibitor of matrix metalloproteinase-2) (ng/mL) $+ 1.19 \times ln$ (α-2-macroglobulin) (mg/dL) $- 9.15$. Median values of fibrosis scores of F1 ($n = 73$), F2 ($n = 42$), F3 ($n = 31$) and F4 stages ($n = 12$) were calculated as 0.95, 2.07, 2.98 and 3.63, respectively. Multiple regression coefficient and coefficient of determination were 0.646 and 0.418, respectively. Validation with patient data from other institutions demonstrated good reproducibility of fibrosis score for hepatitis B (FSB), showing 1.33 in F1 ($n = 27$), 2.20 in F2 ($n = 20$), 3.11 in F3 ($n = 20$) and 5.30 in F4 ($n = 2$), respectively.

Conclusion: A concise multiple regression function using four laboratory parameters successfully predicted pathological fibrosis stage of patients with hepatitis B virus infection.

Key words: chronic hepatitis, hepatitis B virus, liver cirrhosis, liver fibrosis, multiple regression analysis, stage

INTRODUCTION

WHEN HEPATITIS B virus (HBV)-related chronic liver disease is found by biochemical and virological examination, liver biopsy can establish the definitive diagnosis of chronic hepatitis and its fibrotic staging. Although these pathological procedures are reliable and informative both in diagnosis and treatment, they sometimes require medical invasion and financial costs, including the risk of bleeding from needle puncture, some pain experienced during the procedure and hospital stays of a few days. The pathological examination is, therefore, rarely performed repeatedly in a short period of time, unless disease activity is severe or progression of liver disease is highly suspected. Recently, many authors described the usefulness of ultrasonographic elastography and multiple resonance imaging technology in the estimation of staging of chronic hepatitis and cirrhosis.[1–5] These ways of estimation using the imaging apparatuses seem truly useful for current patients, but they cannot evaluate and compare with past fibrotic states of patients retrospectively. Moreover,

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the same apparatus for elastometry will not be available for repeated measurement for a follow-up examination, for example, several years later.

In spite of the accuracy of biopsy and convenience of elastography in chronic liver disease, clinical diagnosis based on biochemistry and hematology is still indispensable for the daily practice of many patients with HBV-related liver disease. Recently, several studies were published about estimation of hepatitis stages, using one or more serum biomarkers. Discriminant functions or multivariate analyses demonstrated that approximately 60–90% of patients with chronic hepatitis B were correctly classified as having mild hepatitis and severe hepatitis with advanced fibrosis.[2,6–13] Up to the present time, however, the usefulness of the discriminant functions are less valuable for a few reasons. First, these functions were made for the purpose of discrimination of severe hepatic fibrosis from mild fibrosis, and four histological classifications (F1–F4) were neglected in almost of the studies. Second, some studies analyzed both hepatitis B and hepatitis C virus infection, although the significance and actual values of each liver function test in the evaluation of the severity of liver disease were not similar among each viral hepatitis and alcoholic liver disease. Third, biochemical markers for liver fibrosis (e.g. hyaluronic acid, type IV collagen, procollagen III peptide)[14–16] were not always included in those previous studies.

We tried to generate a function estimating fibrotic stages of HBV-related chronic hepatitis, which were objectively diagnosed by liver biopsy. The purpose of this study is, therefore, to make a reliable multiple regression function and to obtain practical coefficients for significant variables also using fibrosis markers.

METHODS

Patients

A TOTAL OF 273 Japanese patients with chronic hepatitis B were recruited for the study from seven hospitals in Japan: Toranomon Hospital, Hiroshima University Hospital (K. Chayama, M.D.), Ehime University Hospital (M. Onji, M.D.), Musashino Red Cross Hospital (N. Izumi, MD), Shishu University Hospital (E. Tanaka, M.D.), Showa University Hospital (M. Imawari, M.D.) and Osaka University Hospital (T. Takehara, M.D.). Inclusion criteria for this study were: (i) positive hepatitis B surface antigen for more than 6 months; (ii) persistent or intermittent elevation in aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels; and (iii) liver biopsy showing chronic hepatitis (F1–F4). We excluded those patients with overt alcoholic liver disease or fatty liver, association of other types of liver disease (e.g. hepatitis C, primary biliary cirrhosis, autoimmune hepatitis), or those associated with hepatocellular carcinoma or other malignancy. Among the patients, 244 patients fulfilled the conditions for the study: complete demographic data, basic laboratory data of hematology and biochemistry, required liver biopsy specimens, and sufficient amount of frozen sera. Also, we excluded additional 17 patients with eventual histological diagnosis as F0 stage.

Finally, a total of 227 patients who were diagnosed as having chronic hepatitis or cirrhosis (F1–F4) were analyzed for the following hematological, biochemical and histopathological examination. There were 172 males and 55 females aged 16–70 years (median, 39 years).

All the patients presented written informed consent in individual hospitals and medical centers, and the study was approved in each ethical committee.

Hematological and biochemical examination

Hematological and standard biochemical evaluation had been performed in each medical institution: white blood cells, red blood cells, hemoglobin, platelets, total bilirubin, AST, ALT, AST/ALT ratio (AAR), γ-glutamyl transpeptidase (γ-GTP), total protein, albumin and γ-globulin.

Special biochemical examinations including "fibrosis markers" were carried out using stored frozen sera at −20°C or lower: α-2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipoprotein A1, hyaluronic acid, tissue inhibitor of matrix metalloproteinase (TIMP)-1, TIMP-2, procollagen III peptide and type IV collagen 7S.

Histological diagnosis of chronic hepatitis and cirrhosis

All the 227 cases fulfilled required standards of histological evaluation: sufficient length of specimen, hematoxylin–eosin staining, and at least one specimen with fiber staining. Four independent pathologists (Y. T., J. F., F. K. and T. F.), who were not informed of patients' background and laboratory features except for age and sex, evaluated the 227 specimens regarding the stages of fibrosis and activity. Pathological classification of chronic hepatitis staging was based on Desmet *et al.*[17]

Before judgment of histological staging of individual specimens, the pathologists discussed the objective and reproducible judgment of pathological diagnosis of

hepatitis. They made a panel about obvious criteria using typical microscopic pictures for each stage, and it was always referred to during the procedure of pathological judgment. When inconsistent results were found in the diagnosis of hepatitis stage among the pathologists, the final judgment accepted majority rule among them.

Statistical analysis

Non-parametric procedures were employed for the analysis of background characteristics and laboratory data among patients in each stage, including Mann–Whitney *U*-test, Kruskal–Wallis test and χ^2-test.

The normality of the distribution of the data was evaluated by a Kolmogorov–Smirnov one-sample test. Because certain variables partly did not conform to a normal distribution, natural logarithmic transformation of bilirubin, AST, ALT, γ-GTP, α-2-macroglobulin, hyaluronic acid, type IV collagen 7S and TIMP-2 were also analyzed in the following calculation. The natural logarithmic transformation of the results yielded a normal distribution or symmetrical distribution for all the analyzed factors. After the procedures, the following multiple regression analysis became rationally robust against deviations from normal distribution. In order to avoid introducing into the model any variables that were mutually correlated, we checked the interaction between all pairs of the variables by calculating variance inflation factors. Of the highly correlated variables, less significant factors were removed from the viewpoint of multicollinearity.

Multivariate regression analysis was performed using 158 patient data from Toranomon Hospital (training dataset) to generate a training data of predicting function. We used a stepwise method for selection of informative subsets of explanatory variables in the model. Multiple regression coefficient and coefficient of determination were also taken into account in the selection of variables. Next, we validated the obtained predictive function using the remaining 69 patient data from the other six liver institutions (validation dataset).

A *P*-value of less than 0.05 with two-tailed test was considered to be significant. Data analysis was performed using the computer program SPSS ver. 19.[18]

For evaluation of the efficiency and usefulness of obtained function for fibrosis estimation, we compared various fibrosis scores for hepatitis B and C, including AAR,[19] AST-to-platelet ratio index (APRI),[20] FIB-4,[21] FibroTest[22] and discrimination function of cirrhosis from hepatitis in Japanese patients.[23]

RESULTS

Pathological diagnosis

FOUR PATHOLOGISTS INDEPENDENTLY judged the fibrotic stages and inflammatory activity for 227 specimens of chronic hepatitis/cirrhosis caused by HBV. One hundred patients (44.1%) had a fibrosis stage of F1, 62 (27.3%) F2, 51 (22.5%) F3 and 14 (6.2%) F4. In the subgroup of the 158 patients in the training group, judgment as F1 was made in 73 cases, F2 in 42, F3 in 31 and F4 in 12. Of the 69 patients in the validation group, judgment as F1 was made in 27, F2 in 20, F3 in 20 and F4 in two.

According to hepatitis activity classification, A0 was found in five (2.2%), A1 in 100 (44.1%), A2 in 107 (47.1%) and A3 in 15 (6.6%).

Laboratory data of each hepatitis stage in the training group

There were 124 men and 34 women with a median age of 39 years ranged 16–70 years. Laboratory data of 158 patients in the training group are shown in Table 1. Although several individual items were well correlated with the severity of hepatic fibrosis, significant overlap values were noted among F1–F4 stages: platelet count, γ-globulin, α-2-macroglobulin, haptoglobin, hyaluronic acid, TIMP-2 and type IV collagen 7S.

Significant variables serving staging of hepatitis

Univariate analyses using trend analysis with the Cochran–Armitage method showed that the fibrotic stage of chronic hepatitis B (FSB) was significantly correlated with platelet count (Spearman: $r = -0.45$, $P < 0.001$), γ-GTP ($r = 0.19$, $P = 0.017$), γ-globulin ($r = 0.29$, $P < 0.001$), α-2-macroglobulin ($r = 0.32$, $P < 0.001$), hyaluronic acid ($r = 0.36$, $P < 0.001$), TIMP-2 ($r = 0.16$, $P = 0.043$), procollagen III peptide ($r = 0.30$, $P < 0.001$) and type IV collagen 7S ($r = 0.55$, $P < 0.001$).

Regression function generated from training patient group

After stepwise variable selection, multivariate regression analysis finally obtained the following function: $z = 1.40 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.017 \times$ (platelet count) ($\times 1000^3/mm^3$) $+ 1.24 \times ln$ (TIMP-2) (ng/mL) $+ 1.19 \times ln$ (α-2-macroglobulin) (mg/dL) $-$ 9.15. Median values of the fibrosis score of F1 ($n = 73$), F2 ($n = 42$), F3 ($n = 31$) and F4 stages ($n = 12$) were calculated as 0.95, 2.07, 2.98 and 3.63, respectively

Table 1 Demography and laboratory data of 158 patients in training group

	F1 ($n = 73$)	F2 ($n = 42$)	F3 ($n = 31$)	F4 ($n = 12$)
Demographics				
Men : women	58:15	33:9	23:8	10:2
Age (median, range)	36 (16–70)	39.5 (18–66)	39 (25–64)	43 (32–59)
Laboratory data (median, range)				
WBC (×1000/mm^3)	5.4 (2.5–10.6)	5.1 (2.4–8.7)	4.9 (3.0–8.7)	4.1 (3.7–6.6)
Hemoglobin (g/dL)	15.3 (10.3–18.8)	15.4 (12.5–17.9)	15.2 (11.5–17.2)	14.45 (12.1–18.2)
Platelet (×1000/mm^3)	204 (124–341)	173 (82–308)	155 (96–220)	130 (86–230)
Albumin (g/dL)	4.1 (3.2–4.9)	4.0 (3.2–5.1)	4.0 (3.3–4.9)	3.95 (3.4–4.6)
Bilirubin (mg/dL)	0.8 (0.2–1.7)	0.8 (0.3–2.3)	0.9 (0.4–5.4)	0.85 (0.6–2.3)
AST (IU/L)	48 (16–450)	55 (17–588)	54 (17–1446)	76.5 (27–396)
ALT (IU/L)	102 (10–839)	90 (12–886)	85 (19–2148)	89 (18–809)
γ-GTP (IU/L)	37 (7–247)	55 (8–687)	44 (14–564)	69 (33–262)
γ-Globulin (g/dL)	1.29 (0.78–2.11)	1.495 (0.62–3.20)	1.43 (0.90–2.30)	1.735 (0.92–2.47)
γ-Globulin (%)	17.3 (10.8–26.1)	19.3 (8.5–35.6)	19.9 (12.9–28.6)	22.55 (13.9–30.2)
α-2-Macroglobulin (mg/dL)	226 (116–446)	276 (148–495)	261 (202–565)	286.5 (166–425)
Haptoglobin (mg/dL)	77 (<5–318)	59 (<5–238)	61 (<5–151)	48.5 (<5–145)
Apolipoprotein A-I (mg/dL)	134 (89–212)	143 (78–250)	133 (87–189)	125 (73–169)
Hyaluronic acid (μg/L)	16 (<5–130)	32.5 (<5–204)	38 (<5–418)	49 (24–335)
TIMP-1 (ng/mL)	168 (93–271)	172 (116–314)	157 (119–365)	192 (145–365)
TIMP-2 (ng/mL)	80 (41–135)	80.5 (35–121)	92 (38–251)	85.5 (70–123)
Procollagen III peptide (U/mL)	0.75 (0.53–1.90)	0.835 (0.45–1.20)	0.89 (0.58–2.50)	1.05 (0.71–2.20)
Type IV collagen 7S (ng/ml)	4.0 (2.7–7.7)	4.6 (2.6–9.6)	5.6 (2.3–15.0)	7.2 (4.2–14.0)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; TIMP, tissue inhibitor of matrix metalloproteinase; WBC, white blood cells.

(Fig. 1). The multiple regression coefficient and coefficient of determination were 0.646 ($P < 0.001$) and 0.418 ($P < 0.001$), respectively.

Because the generated regression function was obtained by multivariate analysis with stepwise variable selection, several variables were removed from the function due to multicollinearity among them. Mutual correlation among the fibrosis predictors are shown in Table 2.

A 28-year-old man of F1 fibrotic stage (Fig. 2a) had a serum type IV collagen concentration of 4.4 ng/mL, platelet 221×10^3 count/mm^3, TIMP-2 75 ng/mL and α-2-macroglobulin 226 mg/dL. The regression function provided a fibrosis score of 0.99. Another man aged 46 years had F3 fibrosis on histological examination (Fig. 2b). His type IV collagen was 5.3 ng/mL, platelet 137×10^3 count/mm^3, TIMP-2 92 ng/mL and α-2-macroglobulin 255, and the regression function calculated his fibrosis score as 3.10.

Validation of discriminant function

Validation data of 69 patients (Table 3) were collected from the other six institutions in Japan. When applying

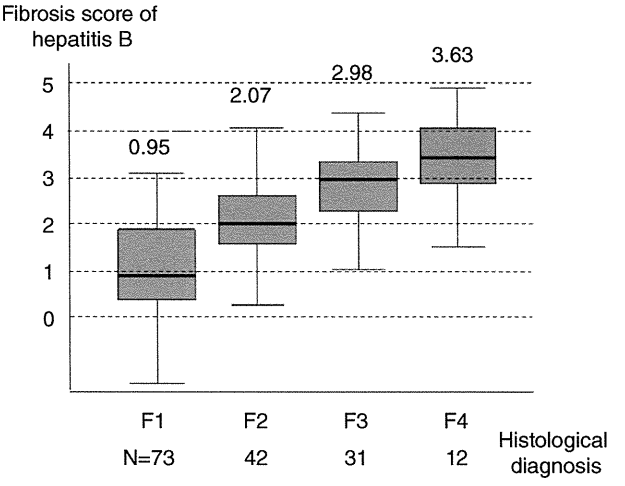

Figure 1 Box and whisker plots of fibrotic score of each histological fibrosis group in the training dataset. The fibrosis score of hepatitis B was generated by the function, $z = 1.40 \times ln$ (type IV collagen 7S) (ng/mL) − $0.017 \times$ (platelet count) (×1000^3/mm^3) + $1.24 \times ln$ (tissue inhibitor of matrix metalloproteinase-2) (ng/mL) + $1.19 \times ln$ (α-2-macroglobulin) (mg/dL) − 9.15.

Table 2 Correlation coefficients (Spearman's ρ) among fibrosis predictors used in multivariate analysis

	Platelet	gamma-globulin	*ln* (α-2-macroglobulin)	*ln* (hyaluronate)	*ln* (P-III-P)	*ln* (IV collagen)	*ln* (TIMP-2)
Platelet ($\times 10^3$/mm^3)	1.000	−0.214 ($P = 0.008$)	−0.260 ($P = 0.001$)	−0.384 ($P < 0.001$)	−0.045 ($P = 0.58$)	−0.297 ($P < 0.001$)	0.094 ($P = 0.24$)
γ-Globulin (g/dL)		1.000	0.276 ($P = 0.001$)	0.349 ($P < 0.001$)	0.342 ($P < 0.001$)	0.414 ($P < 0.001$)	0.268 ($P = 0.001$)
ln (α-2-macroglobulin) (mg/dL)			1.000	0.281 ($P < 0.001$)	0.141 ($P = 0.078$)	0.171 ($P = 0.032$)	−0.079 ($P = 0.32$)
ln (hyaluronic acid) (mg/L)				1.000	0.373 ($P < 0.001$)	0.493 ($P < 0.001$)	0.089 ($P = 0.27$)
ln (procollagen III peptide) (U/mL)					1.000	0.600 ($P < 0.001$)	0.145 ($P = 0.071$)
ln (type IV collagen) (mg/L)						1.000	0.358 ($P < 0.001$)
ln (TIMP-2) (mg/L)							1.000

TIMP, tissue inhibitor of matrix metalloproteinase.

the regression function for the validation set, the fibrosis score demonstrated good reproducibility, showing 1.33 in patients with chronic hepatitis of F1 ($n = 27$), 2.20 of F2 ($n = 20$), 3.11 of F3 ($n = 20$) and 5.30 of F4 ($n = 2$), respectively (Fig. 3). Although F4 fibrosis stage consisted of only two patients and the score 5.30 was regarded as of rather higher value, the scores of other stages of fibrosis were concordant with histological fibrosis.

Comparisons of efficacy with various fibrosis scores (Fig. 4)

In order to evaluate the efficacy and usefulness of the obtained FSB, we compared it with previously reported fibrosis scores using training data. AAR, APRI and FibroTest showed only slight correlation with actual histological stage. FIB-4 demonstrated an increasing trend of the score associated with histological fibrosis, but significant overlapping scores were found in F1–F4. Spearman's correlation coefficients of AAR, APRI, FIB-4 and FibroTest were 0.199 ($P = 0.012$), 0.265 ($P = 0.001$), 0.412 ($P < 0.001$) and 0.330 ($P < 0.001$), respectively. Our FSB showed a Spearman's correlation coefficient of 0.625 ($P < 0.001$), and was a much higher value than the others. The dichotomous discrimination function for cirrhosis and hepatitis C in Japanese patients[23] showed good differentiation also in patients with hepatitis B virus.

DISCUSSION

RECOGNITION OF SEVERITY of chronic hepatitis is essential in managing patients with chronic HBV infection: estimation of length of infection, existence of any previous hepatitis activity, presumption of current fibrotic stage, and prediction of future fibrosis progression and hepatocarcinogenesis. Differential diagnosis of cirrhosis from chronic hepatitis is especially important in the evaluation of chronic HBV infection. Identification of liver cirrhosis often leads to an important change in management of the patient: need for fiberscopic examination for esophageal varices, ultrasonographic exploration for the association of liver cancer, and prediction of hepatic decompensation. Guidelines published by the American Association of Study of Liver Disease[24] recommend liver biopsy for HBV carriers with aminotransferase elevation or for any candidates of antiviral therapy, because hepatic fibrosis sometimes shows unexpectedly far advancement to cirrhosis, and because it is very difficult to evaluate and translate the liver function tests or ultrasonographic findings compared to chronic hepatitis type C.

Table 3 Demography and laboratory data of 69 patients in training group

	F1 ($n = 27$)	F2 ($n = 20$)	F3 ($n = 20$)	F4 ($n = 2$)
Demographics				
Men : women	18:9	15:5	13:7	2:0
Age (median, range)	36 (13–64)	45 (14–64)	36.5 (24–59)	32 (25–39)
Laboratory data (median, range)				
WBC (×1000/mm^3)	5.0 (2.8–8.7)	5.8 (2.8–11.6)	5.3 (3.2–8.1)	3.85 (2.7–5.0)
Hemoglobin (g/dL)	14.8 (12.4–17.4)	15.0 (12.4–16.9)	14.4 (11.1–16.4)	14.4 (12.5–16.3)
Platelet (×1000/mm^3)	204 (86–322)	180 (90–275)	147 (90–276)	130 (67–183)
Albumin (g/dL)	4.4 (2.8–5.2)	4.2 (3.5–5.1)	4.3 (3.4–4.9)	4.45 (4.0–4.9)
Bilirubin (mg/dL)	0.9 (0.4–6.4)	0.8 (0.2–1.6)	0.75 (0.4–1.7)	1.15 (1.1–1.2)
AST (IU/L)	52 (17–575)	50.5 (21–272)	65 (22–284)	248.5 (51–446)
ALT (IU/L)	84 (16–1101)	101.5 (19–554)	86.5 (16–1113)	453.5 (74–833)
γ-GTP (IU/L)	42 (14–332)	54 (16–205)	52.5 (13–191)	193 (57–329)
γ-Globulin (g/dL)	1.30 (1.04–1.59)	1.35 (1.18–2.53)	1.62 (1.16–1.97)	1.545 (1.51–1.58)
γ-Globulin (%)	17.9 (14.3–22.1)	19.6 (15.5–30.8)	22.0 (16.5–24.6)	20.15 (19.3–21.0)
α-2-Macroglobulin (mg/dL)	287 (160–687)	270 (89–452)	272.5 (211–463)	389 (313–465)
Haptoglobin (mg/dL)	58 (<5–229)	74 (<5–154)	56.5 (<5–198)	<5 (<5–<5)
Apolipoprotein A-I (mg/dL)	146 (95–216)	137 (87–162)	120 (88–170)	100.5 (74–127)
Hyaluronic acid (μg/L)	27 (<5–113)	36 (10–1050)	59 (14–439)	331 (225–437)
TIMP-1 (ng/mL)	168.5 (83–302)	176 (127–408)	182 (104–303)	390.5 (283–498)
TIMP-2 (ng/mL)	76 (25–143)	86.5 (28–154)	77.5 (32–141)	100.5 (91–110)
Procollagen III peptide (U/mL)	0.71 (0.27–2.20)	0.88 (0.63–2.80)	0.995 (0.60–2.10)	1.75 (1.50–2.00)
Type IV collagen 7S (ng/ml)	3.6 (2.7–17.0)	5.25 (3.3–13.0)	5.7 (3.0–16.0)	15.5 (15.0–16.0)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; TIMP, tissue inhibitor of matrix metalloproteinase; WBC, white blood cells.

Recently, non-invasive estimation of severity of liver fibrosis has been reported in patients with HBV-related chronic hepatitis.[2,6–13] However, these studies were principally aimed at differentiation of advanced fibrotic stages of F3 or F4 from mild fibrotic stages of F1 or F2. Those discrimination functions were insufficient to recognize the stepwise progression of viral hepatitis from F1–F4. This dichotomy (mild or severe) of chronic hepatitis B seemed less valuable in the study of disease progression, disease control abilities of antiviral drugs and estimation of histological improvement after anti-inflammatory drugs. A histology-oriented, practical and reliable formula is therefore required for the diagnosis and investigation of chronic hepatitis B.

This study aimed to establish non-invasive evaluation and calculation of liver fibrosis for patients with chronic hepatitis B virus infection. Although it was retrospectively performed as a multicenter study of eight institutions, judgment of histological diagnosis was independently performed by four pathologists in another hospital, who were informed only of the patient's age, sex and positive HBV infection. Objective judgment of the histological staging and grading in sufficient biopsy specimens could be obtained.

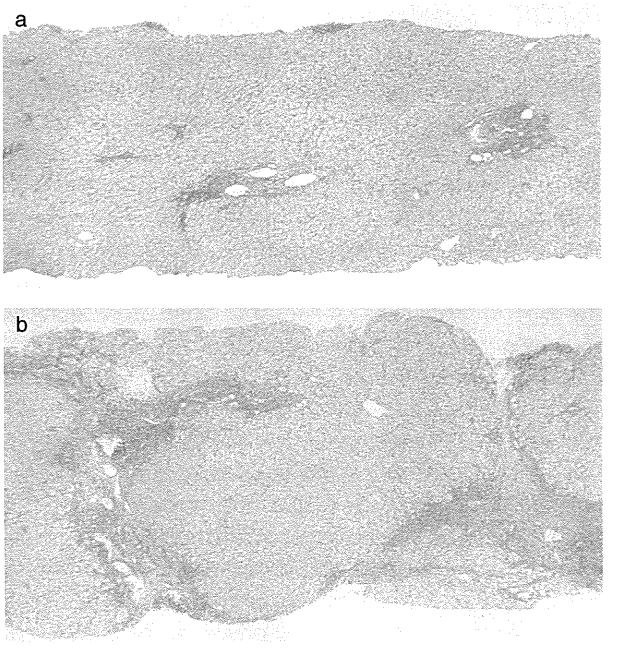

Figure 2 Case presentations of the training set. (a) A 28-year-old man with F1 fibrosis. Final regression function provided his fibrosis score as 0.99. (b) A 45-year-old man with F3 fibrosis. His regression coefficient was calculated as 3.10. Silver stain, ×40.

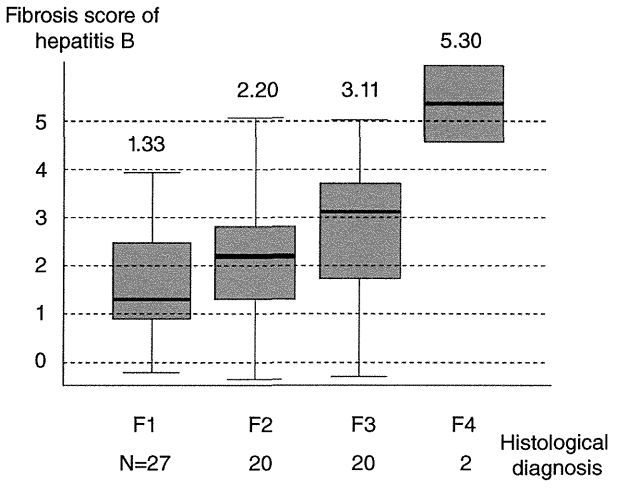

Figure 3 Box and whisker plots of fibrotic score of each group of histological fibrosis in the validation dataset. The fibrosis score of hepatitis B was generated by the function, $z = 1.40 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.017 \times$ (platelet count) ($\times 1000^3/mm^3$) $+ 1.24 \times ln$ (tissue inhibitor of matrix metalloproteinase-2) (ng/mL) $+ 1.19 \times ln$ (α-2-macroglobulin) (mg/dL) $- 9.15$.

As many as 227 patients with chronic hepatitis B were analyzed in this study, who had been diagnosed as having chronic hepatitis or cirrhosis by liver biopsy performed in experienced liver units in Japan. To obtain the most suitable equation approximating histological fibrotic stage, multivariate analysis was performed using two demographic parameters (age and sex) and 21 hematological and biochemical markers with or without logarithmic transformation. They included many kinds of fibrosis markers: α-2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipoprotein A1, hyaluronic acid, TIMP-1, TIMP-2, procollagen III peptide and type IV collagen 7S Multiple regression analysis finally generated a first-degree polynomial function consisting of four variables: type IV collagen 7S, platelet count, TIMP-2 and α2-macroglobulin. A constant numeral (−9.15) was finally adjusted in the regression equation in order to obtain fitted figures for a fibrotic stage of F1–F4. From the magnitude of the standardized partial regression coefficient of individual variable in the function, platelet count demonstrated the most potent contribution toward the prediction of liver fibrosis. Type IV collagen 7S and *ln* (TIMP-2) proved to be the second and third distinctive power in the model, respectively.

The FSB was sufficiently fitted to actual fibrotic stages with certain overlapping as is usually found in histological ambiguity judged by pathologists. Because judgment of fibrosis in chronic hepatitis often shows a transitional histological staging, pathological examination cannot always make a clear-cut diagnosis discriminating F1–F4. Considering the limitation of the pathological difficulty in differentiating the four continuous disease entities, the obtained regression function showed satisfactory high accuracy rates in the prediction of liver disease severity. The FSB can provide one or two decimal places (e.g. 3.2 or 3.24) and the utility of the score is possibly higher than the mere histological stage of F1–F4. The reproducibility was confirmed by the remaining 67 patients' data obtained from the other six hospitals. Although the validation data were collected from a different geographic area and different chronological situation, the FSB showed similar results in prediction of histological staging.

The FSB seemed a very useful quantitative marker in evaluating fibrotic severity of hepatitis B patients without invasive procedures and without any specialized ultrasonography or magnetic resonance imaging. The FSB also has an advantage of measurement, in which old blood samples are available for retrospective assessment of varied clinical settings: for example, old sera from 20 years prior to the time of initial liver biopsy, or paired sera before and after long-term antiviral therapy. These kinds of retrospective assessments of fibrotic staging will be valuable in estimating a long-term progression of liver disease, in evaluating efficacy of long-term medication or other medical intervention, or in making a political judgment from the viewpoints of socioeconomic efficacy.

The score can be calculated for any patients with chronic HBV infection. Although this multiple regression model dealt with appropriate logarithmic transformation for non-normal distribution parameters, the regression analysis was based on a linear regression model. Very slight fibrosis can be calculated as less than 1.00, which is commonly found to a slight degree in chronic hepatitis with tiny fibrotic change as F0. Very severe fibrosis might be calculated as more than 4.00, which is an imaginary and nonsense number in the scoring system of fibrosis. The FSB is, however, very useful and valuable in a real clinical setting: estimation of severity of liver fibrosis in an outpatient clinic, evaluation of the natural progression of a patient's fibrosis over 10 years and assessment of a long-term administration of interferon in patients with chronic hepatitis B from the viewpoint of fibrotic change. Recent development of new nucleoside/nucleotide analogs requires evaluation for long-term histological advantage, for aggravation of hepatitis stage during viral and biochemical breakthrough caused by HBV mutation, and even for

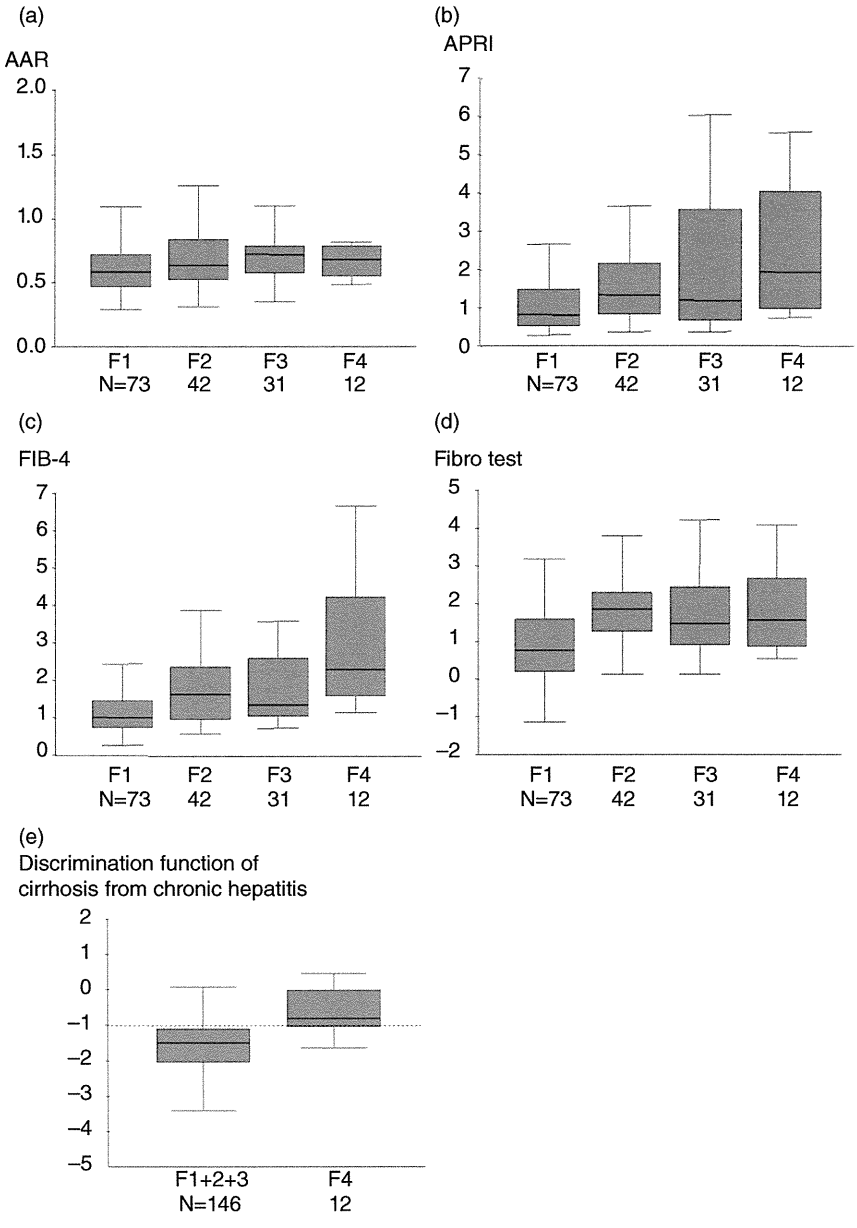

Figure 4 Previously published fibrosis scores. (a) Aspartate aminotransferase/alanine aminotransferase ratio (AAR),[19] (b) aspartate aminotransferase-to-platelet ratio index (APRI),[20] (c) FIB-4,[21] (d) FibroTest[22] and (e) discrimination function of cirrhosis from hepatitis in Japanese patients.[23]

the best management of patients with chronic hepatitis B. The FSB seems one of the ideal methods of approximating the fibrotic stage of chronic hepatitis B. Repeated measurement is quite suitable for patients with an unestablished treatment or trial, every 1 or 2 years, for example. Because the current regression function was generated from the data of HBV-related chronic liver disease, this equation would not be suitable for the recognition of hepatitis C virus-related chronic liver disease, alcoholic liver disease, and other congenital or autoimmune liver diseases. To recognize the latter diseases, other studies of individual diseases must be performed.

We compared the usefulness of the FSB with that of other fibrosis scores.[19–23] The more simple and less expensive AAR or APRI could not estimate fibrotic stages with poor correlation coefficients of 0.199 and 0.265, which are much lower than the coefficient of the FSB of 0.625. FibroTest, which contained three costly fibrosis markers (α-2-macroglobulin, haptoglobin and apolipo-

protein A1), also showed a low correlation coefficient of 0.330, suggesting that its usefulness was limited in HBV positive oriental patients. Although FIB-4 demonstrated the best coefficient of 0.412 among the fibrosis scores, significant overlaps were found between neighboring stages and obtained scores were not coordinated for real histological classification.

In conclusion, the FSB was a useful and reliable biomarker for prediction of liver fibrosis in patients with chronic HBV infection. The FSB is expected to be introduced and utilized in varied kinds of studies and trials. Its accuracy and reproducibility require further validation using higher numbers of patients in several countries other than Japan.

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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

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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.
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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

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 $\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,

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

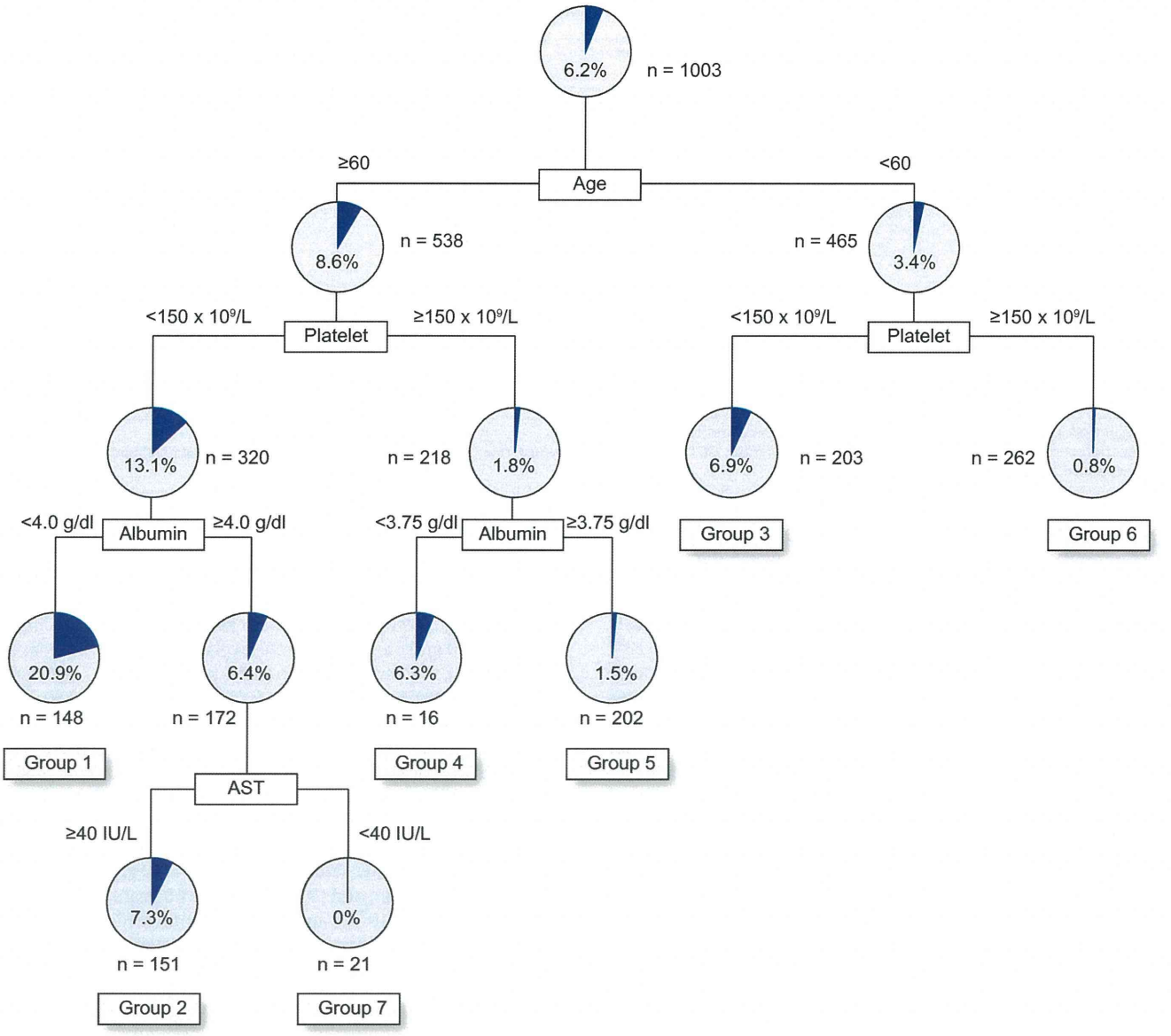

Fig. 1. The decision tree model of HCC development within 5 years. Boxes indicate the factors used to differentiate patients and the cutoff values for those different groups. Pie charts indicate the HCC development rate within 5 years for each group of patients after differentiation. Terminal groups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

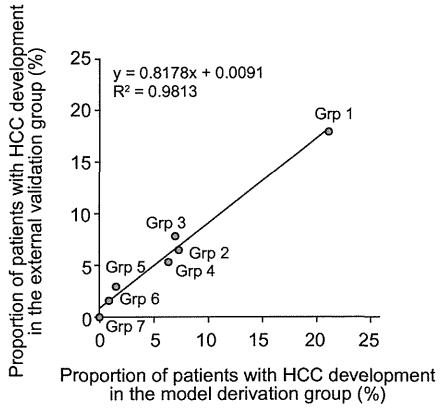

Fig. 2. External validation of the decision tree model with an independent cohort. Each patient in the external validation group was allocated to groups 1–7 following the flowchart of the decision tree. The HCC development rates were then calculated for each group and the graph plotted. The *x*-axis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

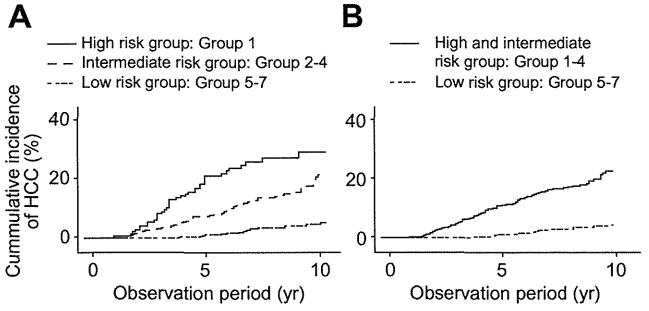

Fig. 3. Cumulative incidence of HCC development beyond 5 years in subgroups of patients defined by the decision tree model. Cumulative incidences of HCC in the groups classified by the decision tree model are compared. (A) The cumulative HCC development rate beyond 5 years is higher in the high- (group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

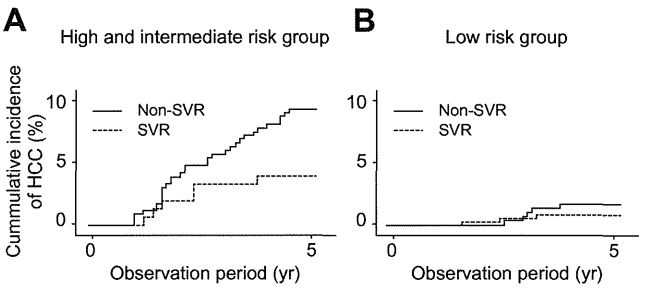

Fig. 4. Sustained virological response to PEG-IFN plus RBV therapy reduces the incidence of HCC development after stratification by the HCC risk. The 600 nonSVR patients and the 472 SVR patients in the external cohort were fitted into the HCC risk prediction model and classified into the high and intermediate-risk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

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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