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A proposed predictive model for advanced fibrosis in patients with chronic hepatitis B and its validation

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Abstract

We created a predictive model using serum-based biomarkers for advanced fibrosis (F3 or more) in patients with chronic hepatitis B (CHB) and to confirm the accuracy in an independent cohort.

A total of 249 CHB patients were analyzed. To achieve our study aim, a training group (n = 125) and a validation group (n = 124) were formed. In the training group, parameters related to the presence of advanced fibrosis in univariate and multivariate analyses were examined, and a formula for advanced fibrosis was created. Next, we verified the applicability of the predictive model in the validation group.

Multivariate analysis identified that gamma-glutamyl transpeptidase (GGT, $P = 0.0343$) and platelet count ($P = 0.0034$) were significant predictors of the presence of advanced fibrosis, while Wisteria floribunda agglutinin-positive Mac-2-binding protein (WFA^+-M2BP, $P = 0.0741$) and hyaluronic acid ($P = 0.0916$) tended to be significant factors. Using these 4 parameters, we created the following formula: GMPH score = $-0.755 - (0.015 \times \text{GGT}) - (0.268 \times \text{WFA}^+\text{-M2BP}) + (0.167 \times \text{platelet count}) + (0.003 \times \text{hyaluronic acid})$. In 8 analyzed variables (WFA^+-M2BP, aspartate aminotransferase-to-platelet ratio index, FIB-4 index, prothrombin time, platelet count, hyaluronic acid, Forns index, and GMPH score), GMPH score had the highest area under the receiver operating characteristic (AUROC) curve for advanced fibrosis with a value of 0.8064 in the training group and in the validation group, GMPH score also had the highest AUROC (0.7782). In all subgroup analyses of the hepatitis B virus (HBV) status (HB surface antigen quantification, HBV-DNA quantification, and HBe antigen seropositivity), GMPH score in F3 or F4 was significantly lower than that in F0 to F2. In the above mentioned 8 variables, differences between the liver fibrosis stages (F0 to F1 vs F2, F2 vs F3, F3 vs F4, F0 to F1 vs F3, F0 to F1 vs F4, and F2 vs F4) for the entire cohort (n = 249) were all significant only in GMPH score.

In conclusion, the GMPH scoring system may be helpful for detecting advanced liver fibrosis in patients with CHB.

Abbreviations: ALT = alanine aminotransferase, APRI = aspartate aminotransferase-to-platelet ratio index, AST = aspartate aminotransferase, AUROC = area under the receiver operating characteristic, CHB = chronic hepatitis B, CHC = chronic hepatitis C, COI = cutoff index, GGT = gamma-glutamyl transpeptidase, GPR = gamma-glutamyl transpeptidase-to-platelet ratio, HBs = HB surface, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, ID = identification, LC = liver cirrhosis, PT = prothrombin time, ROC = receiver operating characteristic, SD = standard deviation, WFA^+-M2BP = Wisteria floribunda agglutinin-positive Mac-2-binding protein.

Keywords: chronic hepatitis B, liver fibrosis, predictive model, serum biomarkers, validation

1. Introduction

Chronic hepatitis B (CHB) virus (HBV) infection is a major public health problem globally.[1,2] CHB patients have a high risk of progression to liver fibrosis, which may eventually result in liver cirrhosis (LC) and other serious complications such as hepatic failure and hepatocellular carcinoma (HCC).[1–4]

The risk of HCC incidence for patients with CHB is associated with the degree of liver fibrosis.[3,5,6] In patients with LC, the annual HCC incidence in Japan is reportedly high (7–8% per year).[5,6] Therefore, it is clinically important to identify patients with advanced liver fibrosis and to adequately manage such patients. In addition, identifying advanced liver fibrosis patients could help clinicians determine the suitability of patients and the optimal timing for antiviral treatment to obtain optimal treatment efficacy and to avoid excessive medication.[7] Because liver biopsy is invasive, alternative noninvasive methods for assessing liver fibrosis will be needed.

Both aspartate aminotransferase (AST)-to-platelet ratio index (APRI) and fibrosis index based on the 4 factors (FIB-4 index) are

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serum-based liver fibrosis markers, which are the most widely studied and validated noninvasive tools for evaluating liver fibrosis.[4,8,9] A recent meta-analysis demonstrated that APRI and FIB-4 index could identify CHB-related fibrosis with moderate sensitivity and accuracy (the summary of the area under the receiver operating characteristic [AUROC] curve values using APRI and FIB-4 for the diagnosis of advanced fibrosis: 0.7844 and 0.7268, respectively)[4]. On the other hand, recently in Japan, Wisteria floribunda agglutinin-positive Mac-2-binding protein (WFA^+-M2BP) has been established as a glycobiomarker associated with liver fibrosis especially in patients with chronic hepatitis C (CHC). WFA^+-M2BP is characterized by a fibrosis-related glycoalteration, and test results can be rapidly obtained with a bedside method.[10–14]

In the field of CHC, various serum-based predictive models such as Fibrotest, Forns index, enhanced liver fibrosis score, and Fibroindex for liver fibrosis have been proposed and validated to reduce the need for liver biopsy with the purpose of staging fibrosis and to overcome its limitations.[15–20] However, to the best of our knowledge, there are few well established predictive models using serum-based biomarkers for evaluating liver fibrosis stages in patients with CHB. The diagnostic accuracy of the above serum-based predictive models derived from investigations in CHC patients for CHB patients is under constant debate.[20,21] A predictive model using serum-based biomarkers in the field of CHB could be a point of focus. Thus, the goal of the present study was to create a predictive model using serum-based biomarkers for advanced fibrosis in patients with CHB and to verify the accuracy in an independent cohort.

2. Patients and methods

2.1. Patients

A total of 249 HBV-related chronic liver disease patients, for whom stored sera were available, were admitted to the Division of Hepatobiliary and Pancreatic disease, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan, between September 2005 and May 2015, and they were analyzed. All patients had HB surface (HBs) antigen positivity for at least 6 months. They had no evidence of drug-induced, autoimmune, or alcoholic liver disease, and no concurrent hepatitis C virus infection was found. All subjects underwent liver biopsy. Previous antiviral therapy was performed in 60 patients (24.1%). We included the following parameters into the analysis: age, gender, HBs antigen, HBV-DNA, HBe antigen, serum albumin, total bilirubin, AST, alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transpeptidase (GGT), total cholesterol, triglyceride, prothrombin time (PT), platelet count, fasting blood glucose, hyaluronic acid, and serum WFA^+-M2BP level.

To achieve our study aim, we divided the patients into a training group and a validation group. In the training group, the subjects (n = 125) had an odd number as the last digit of their identification (ID) number, and in the validation group subjects (n = 124), the last digit of their ID number was an even number. In the training group, we investigated variables related to advanced fibrosis using univariate and multivariate analyses, and created a formula for advanced fibrosis. Second, the applicability of the predictive model was confirmed in the validation group. We retrospectively examined clinical data in the training and validation groups. In both groups, we evaluated the diagnostic performance of the new predictive model for advanced fibrosis in comparison with other liver fibrosis markers such as WFA^+-M2BP level, APRI, FIB-4 index, PT, platelet count, Forns index, and hyaluronic acid.[8,9,16,22,23]

Ethical approval for the study protocol was obtained from the Ethics Committee of our hospital, and the present study protocol adhered to all provisions of the Declaration of Helsinki.

2.2. Liver histological findings, serological studies, and measurement of WFA^+-M2BP

Our protocol for liver biopsy was as previously described.[24] Liver fibrosis stages were graded as F0 to F4 by expert pathologists in our hospital. In the present study, we defined advanced fibrosis as F3 or more, and no patients had ascites as noted in imaging studies. Detection of HBs antigen and HBe antigen and measurement of HBV-DNA level were performed using commercial kits as formerly described.[24] We measured WFA^+-M2BP level using stored sera. The method for measurement of WFA^+-M2BP level was as described elsewhere.[22–24]

2.3. Calculation of Forns index, ARPI, and FIB-4 index

Forns index was calculated as formerly reported: $7.811 - 3.131 \times \ln(\text{platelet count}) + 0.781 \times \ln(\text{GGT}) + 3.467 \times \ln(\text{age}) - 0.014 \times (\text{total cholesterol})$.[16] APRI score was calculated as described elsewhere: (AST/upper limit of normal)/platelet count (expressed as platelets $\times 10^9$/L) $\times 100$.[8] The FIB-4 index was calculated as previously reported: age (years) $\times$ AST (IU/L)/platelet count ($\times 10^9$/L) $\times \sqrt{}$ALT (IU/L).[9]

2.4. Statistical analysis

First, as described earlier, we performed univariate analysis to detect candidate parameters for a new formula for detecting the presence of advanced liver fibrosis. Parameters with a *P* value less than 0.05 in the univariate analysis were included in the multivariate logistic regression analysis. Parameters with *P* value less than 0.10 in the multivariate analysis were chosen as components of the novel formula. Using these predictors in the multivariate analysis, we created a multiple fractional equation for the prediction of advanced fibrosis. A predictive model was created by modeling the values of parameters with *P* value less than 0.10 in the multivariate analysis and in their regression coefficients.[16] We conducted receiver operating characteristic (ROC) curve analysis in order to calculate the AUROC and to select the optimal cutoff value associated with maximal total value of sensitivity and specificity for the presence of advanced fibrosis in the training group. In the validation group, we examined the diagnostic accuracy of the formula that was derived from the training group.

For continuous variables, we compared the groups using Student *t* test or Mann–Whitney *U* test, as applicable. For categorical variables, we compared between groups using Fisher exact tests or Pearson χ^2 test, as applicable. We also represented the corresponding AUROC, sensitivity (%), specificity (%), positive predictive value (PPV) (%), negative predictive value (NPV) (%), and diagnostic accuracy (%), in addition to the ROC curve analysis. Data are shown as number or means ± standard deviation (SD) unless otherwise stated. We considered variables with *P* value less than 0.05 as statistically significant variables. We performed statistical analysis using JMP 11 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Patient baseline characteristics

The baseline characteristics for the training group (n = 125) and the validation group (n = 124) in this study are presented in Table 1. In the training group, there were 74 males and 51 females with a mean ± SD age of 45.9 ± 12.8 years. In the validation group, there were 81 males and 43 females with a mean ± SD age of 45.3 ± 12.4 years. In the training group, 25 patients (20.0%) had advanced fibrosis, while in the validation group, 35 patients (28.2%) had advanced fibrosis. No significant difference was found in baseline characteristics between the training group and the validation group (Table 1).

3.2. Univariate and multivariate analyses of parameters related to the presence of advanced liver fibrosis

Univariate analysis identified the following parameters as significantly related to the presence of advanced fibrosis for the training group: GGT ($P = 0.0118$), total bilirubin ($P = 0.0236$), PT ($P = 0.0015$), platelet count ($P < 0.0001$), hyaluronic acid ($P < 0.0001$), and WFA$^+$-M2BP ($P = 0.0002$) (Table 2). The odds ratio and 95% confidence intervals calculated in the multivariate analysis for the 6 factors with P value less than 0.05 in the univariate analysis are shown in Table 3. GGT ($P = 0.0343$) and platelet count ($P = 0.0034$) were revealed to be significant predictors of the presence of advanced fibrosis, while WFA$^+$-M2BP ($P = 0.0741$) and hyaluronic acid ($P = 0.0916$) tended to be significant predictors for the presence of advanced fibrosis.

3.3. Diagnostic accuracies for advanced fibrosis

GGT, WFA$^+$-M2BP, platelet count, and hyaluronic acid were included in the final model to create the prediction formula for advanced fibrosis in the training group. The equation for the model (GMPH score) is GMPH score $= -0.755 - (0.015 \times$ GGT$) - (0.268 \times$ WFA$^+$-M2BP$) + (0.167 \times$ platelet count$) + (0.003 \times$ hyaluronic acid).

The AUROCs, optimal cutoff points, sensitivity (%), specificity (%), PPV (%), NPV (%), and diagnostic accuracy (%) for WFA$^+$-M2BP, APRI, FIB-4 index, PT, platelet count, hyaluronic acid, Forns index, and the GMPH score in the training group are shown in Table 4 and Fig. 1. In terms of ROC analysis of the GMPH score for advanced liver fibrosis, there were 2 optimal cutoff points associated with the maximal total value of sensitivity and specificity for the presence of advanced fibrosis in the training group (Fig. 1F). Of the 8 variables, the GMPH score yielded the highest AUROC (0.8064), followed by hyaluronic acid (AUROC = 0.7626). When optimal cutoff values in the training group in each variable were adapted to the validation group, the AUROCs, sensitivity (%), specificity (%), PPV (%), NPV (%), and diagnostic accuracy (%) for WFA$^+$-M2BP, APRI, FIB-4 index, PT, platelet count, hyaluronic acid, Forns index, and the GMPH score in the validation group are presented in Table 4 and Fig. 2. In the validation group, the GMPH score had the highest AUROC (0.7782) of the 8 variables, followed by Forns index (AUROC = 0.7780).

3.4. Comparison of GMPH score according to the degree of liver fibrosis in the entire cohort (n = 249), in patients with HBs antigen >2000 or ≤2000 IU/L, in patients with HBV-DNA ≥ 5 log copies/mL or <5 log copies/mL, and in patients with or without HBe antigen positivity

GMPH score ranged from −5.40644 to 5.09422 (median, 1.57044). As shown in Fig. 3A, as GMPH score elevated, the proportion of advanced fibrosis decreased. Boxplots of GMPH score according to the degree of liver fibrosis for the entire cohort (n = 249) is shown in Fig. 3B. The differences between the liver fibrosis stages (F0 to F1 vs F2, F2 vs F3, F3 vs F4, F0 to F1 vs F3, F2 vs F4, and F0 to F1 vs F4) were all significant.

Table 1

Baseline characteristics in the training and the validation group.

Variables	Training group (n = 125)	Validation group (n = 124)	P
Age, y	45.9 ± 12.8	45.3 ± 12.4	0.6978
Gender, male/female	74/51	81/43	0.3608
HBs antigen > 2000 IU/L, yes/no	89/36	79/45	0.2252
HBV-DNA ≥ 5 log copies/mL, yes/no	65/60	56/68	0.3113
HBe antigen seropositivity, yes/no	52/73	41/83	0.1906
AST, IU/L	47.7 ± 61.0	37.4 ± 22.4	0.5834
ALT, IU/L	64.8 ± 100.4	49.8 ± 47.5	0.6390
ALP, IU/L	226.7 ± 72.1	232.5 ± 93.6	0.8327
GGT, IU/L	39.5 ± 49.5	41.0 ± 38.4	0.2781
Serum albumin, g/dL	4.1 ± 0.5	4.1 ± 0.6	0.6037
Total bilirubin, mg/dL	0.8 ± 0.4	0.9 ± 0.4	0.1315
PT (%)	90.6 ± 10.8	90.8 ± 10.6	0.9219
Lymphocyte count, /μL	1603.1 ± 396.9	1695.7 ± 455.1	0.1453
Platelet count, $\times 10^4/mm^3$	18.5 ± 4.9	17.8 ± 5.0	0.2430
Total cholesterol, mg/dL	186.2 ± 36.4	183.4 ± 30.9	0.5132
Triglyceride, mg/dL	93.7 ± 46.5	101.6 ± 47.4	0.1865
Fasting blood glucose, mg/dL	95.5 ± 13.9	96.5 ± 15.9	0.6020
Hyaluronic acid, ng/mL	48.6 ± 81.5	44.6 ± 80.0	0.6949
WFA$^+$-M2BP (COI)	1.8 ± 2.0	1.4 ± 1.1	0.3569
Histological findings			
Fibrosis stage, 0/1/2/3/4	10/63/27/14/11	4/61/24/27/8	0.1191

Data are expressed as number or mean ± standard deviation. ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, COI = cutoff index, GGT = gamma-glutamyl transpeptidase, HBs = HB surface, HBV = hepatitis B virus, PT = prothrombin time, WFA$^+$-M2BP = Wisteria floribunda agglutinin-positive Mac-2-binding protein.

Table 2

Comparison of baseline characteristics between patients with advanced liver fibrosis (n = 25) and those without advanced fibrosis (n = 100) in the training group.

Variables	F3 or F4 (n = 25)	F0–2 (n = 100)	*P*
Age, y	47.5 ± 11.9	45.5 ± 13.1	0.5006
Gender, male/female	15/10	59/41	>0.999
HBs antigen > 2000 IU/L, yes/no	18/7	71/29	>0.999
HBV-DNA ≥ 5 log copies/mL, yes/no	17/8	48/52	0.0793
HBe antigen seropositivity, yes / no	12/13	40/60	0.5022
AST, IU/L	53.4 ± 53.0	46.3 ± 62.9	0.6019
ALT, IU/L	67.6 ± 83.2	64.1 ± 104.6	0.8789
ALP, IU/L	266.2 ± 101.6	216.8 ± 59.4	0.0619
GGT, IU/L	67.2 ± 85.1	32.6 ± 32.7	0.0118
Total bilirubin, mg/dL	1.0 ± 0.4	0.8 ± 0.3	0.0236
Serum albumin, g/dL	4.0 ± 0.4	4.1 ± 0.6	0.5322
Platelet count, $\times 10^4/mm^3$	14.5 ± 5.5	19.6 ± 4.2	<0.0001
Lymphocyte count, /μL	1669.8 ± 525.2	1586.4 ± 359.2	0.2003
PT (%)	84.6 ± 12.2	92.1 ± 9.9	0.0015
Total cholesterol, mg/dL	182.5 ± 36.2	187.1 ± 36.6	0.5712
Triglyceride, mg/dL	97.5 ± 48.6	92.8 ± 46.2	0.6538
Fasting blood glucose, mg/dL	98.8 ± 17.3	94.7 ± 12.9	0.5087
Hyaluronic acid, ng/mL	83.6 ± 107.1	39.9 ± 71.8	<0.0001
WFA^+-M2BP (COI)	3.1 ± 3.2	1.5 ± 1.5	0.0002

Data are expressed as number or mean ± standard deviation. ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, COI = cutoff index, GGT = gamma-glutamyl transpeptidase, HBs = HB surface, HBV = hepatitis B virus, PT = prothrombin time, WFA^+-M2BP = Wisteria floribunda agglutinin-positive Mac-2-binding protein.

In patients with HBs antigen >2000 IU/L (n = 168, $P < 0.0001$) or ≤2000 IU/L (n = 81, $P < 0.0001$), in patients with HBV-DNA ≥ 5 log copies/mL (n = 121, $P < 0.0001$) or <5 log copies/mL (n = 128, $P < 0.0001$) and in patients with (n = 93, $P = 0.0001$) or without (n = 156, $P < 0.0001$) HBe antigen seropositivity, the GMPH scores in F3 or 4 were significantly lower than those in F0, 1, or 2 (Fig. 4A–F).

3.5. Comparison of WFA^+-M2BP, APRI, FIB-4 index, PT, platelet count, hyaluronic acid, and Forns index according to the degree of liver fibrosis in the entire cohort (n = 249)

We also compared the values of WFA^+-M2BP, APRI, FIB-4 index, PT, platelet count, hyaluronic acid, and Forns index according to the degree of liver fibrosis for the entire cohort. For WFA^+-M2BP, there were significant differences between the fibrosis stages except for the difference between F2 and F3 (Fig. 5A). For APRI, there were significant differences between the fibrosis stages except for the difference between F2 and F3 (Fig. 5B). In FIB-4 index, there were significant differences between the fibrosis stages except for the difference between F2 and F3 (Fig. 5C). For PT, there were significant differences between the fibrosis stages except for differences between F0 to F1 and F2, F2 and F3, and F0 to F1 and F3 (Fig. 6A). For platelet count, there were significant differences between the fibrosis stages except for differences between F0 to F1 and F2, and F3 and F4 (Fig. 6B). For hyaluronic acid, there were significant differences between the fibrosis stages except for the difference between F2 and F3 (Fig. 6C). In Forns index, there were significant differences between the fibrosis stages except for the difference between F0 to F1 and F2 (Fig. 6D).

Table 3

Multivariate analysis of factors contributing to the presence of advanced liver fibrosis in the training group.

	Multivariate analysis	
Variables	Odds ratio (95% CI)	*P**
Total bilirubin	0.944 (0.224–4.125)†	0.9376
GGT	0.989 (0.979–0.999)†	0.0343
Platelet count	1.235 (1.084–1.443)†	0.0034
Hyaluronic acid	1.007 (0.997–1.016)†	0.0916
PT	1.023 (0.967–1.085)†	0.4428
WFA^+-M2BP	0.715 (0.457–1.063)†	0.0741

CI = confidence interval, GGT = gamma-glutamyl transpeptidase, PT = prothrombin time, WFA^+-M2BP = Wisteria floribunda agglutinin-positive Mac-2-binding protein.

* Logistic regression analysis.

† Odds ratio for 1 unit in continuous variables.

3.6. Comparison of GMPH score according to liver fibrosis stages in the training and validation groups

In the training group, significant differences between the liver fibrosis stages were found except for the difference between F0 to F1 and F2 (Fig. 7A). Similarly, in the validation group, significant differences between the liver fibrosis stages were found except for the difference between F3 and F4 (Fig. 7B).

4. Discussion

As described above, since liver biopsy has several limitations including its invasive nature and sampling errors, a simple prediction model for advanced liver fibrosis using serum-based biomarkers will be ideal for avoiding unnecessary liver biopsy in daily clinical practice. The GMPH score is a new serum-based scoring system for the prediction of advanced liver fibrosis in patients with CHB.

From our data, in 8 analyzed parameters, the GMPH score had the highest AUROC (0.8064) in the training group and in the validation group, the GMPH score also yielded the highest AUROC (0.7782); and when optimal cutoff value of GMPH

Table 4

AUROC curve analysis in 7 fibrosis markers in the training and validation groups.

	AUROC	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Training group							
WFA^+-M2BP	0.7382	1.42	72.0	69.0	36.7	90.8	69.6
APRI	0.7260	0.626	72.0	70.0	37.5	90.9	70.4
FIB-4 index	0.7040	1.633	64.0	70.0	34.8	88.6	68.8
PT	0.7118	86.4	68.0	79.0	44.7	90.8	76.8
Platelet count	0.7560	14.7	56.0	90.0	58.3	89.1	83.2
Hyaluronic acid	0.7626	36	80.0	72.0	41.7	93.5	73.6
Forns index	0.7288	12.04	72.0	65.0	34.0	90.3	66.4
GMPH score	0.8064	1.17	72.0	80.0	47.4	92.0	78.4
	0.8064	1.33	76.0	76.0	44.2	92.7	76.0
Validation group							
WFA^+-M2BP	0.7157	1.42	62.9	69.7	44.9	82.7	68.5
APRI	0.7133	0.626	65.7	58.4	38.3	81.3	60.5
FIB-4 index	0.7519	1.633	54.3	74.2	45.2	80.5	68.5
PT	0.6071	86.4	42.9	66.3	33.3	74.7	59.7
Platelet count	0.7016	14.7	42.9	84.3	51.7	78.9	72.6
Hyaluronic acid	0.7689	36	54.3	84.3	57.6	82.4	75.8
Forns index	0.7780	12.04	74.29	60.67	42.6	85.7	64.5
GMPH score	0.7782	1.17	65.7	70.8	47.9	84.2	69.4
	0.7782	1.33	74.3	66.3	46.4	86.8	68.5

APRI = AST-to-platelet ratio index, AUROC = area under the receiver operating characteristic curve, PPV = positive predictive value, PT = prothrombin time, WFA^+-M2BP = Wisteria floribunda agglutinin-positive Mac-2-binding protein.

score in the training group (cutoff point = 1.33) was applied in the validation group, the NPV in GMPH score was the highest (86.8%). In addition, in 8 variables in the entire cohort (n = 249), all differences between the liver fibrosis stages were significant for GMPH score only. Overall, combinations of laboratory parameters still seem to have higher accuracy than single serum liver fibrosis markers in order to predict advanced fibrosis in patients with CHB. Furthermore, in all subgroup analyses for HBV status (i.e., HBs antigen quantification, HBV-DNA quantification, and HBe antigen seropositivity), GMPH score in F3 or F4 was significantly lower than that in F0 to F2. These results demonstrate that the GMPH scoring system can be helpful for detecting advanced fibrosis in patients with CHB. A major strength of our study is that our model was validated in the independent group, although the independent validation group was selected retrospectively. Another strength is that our training and validation groups were well balanced in baseline characteristics, although they were not randomized.

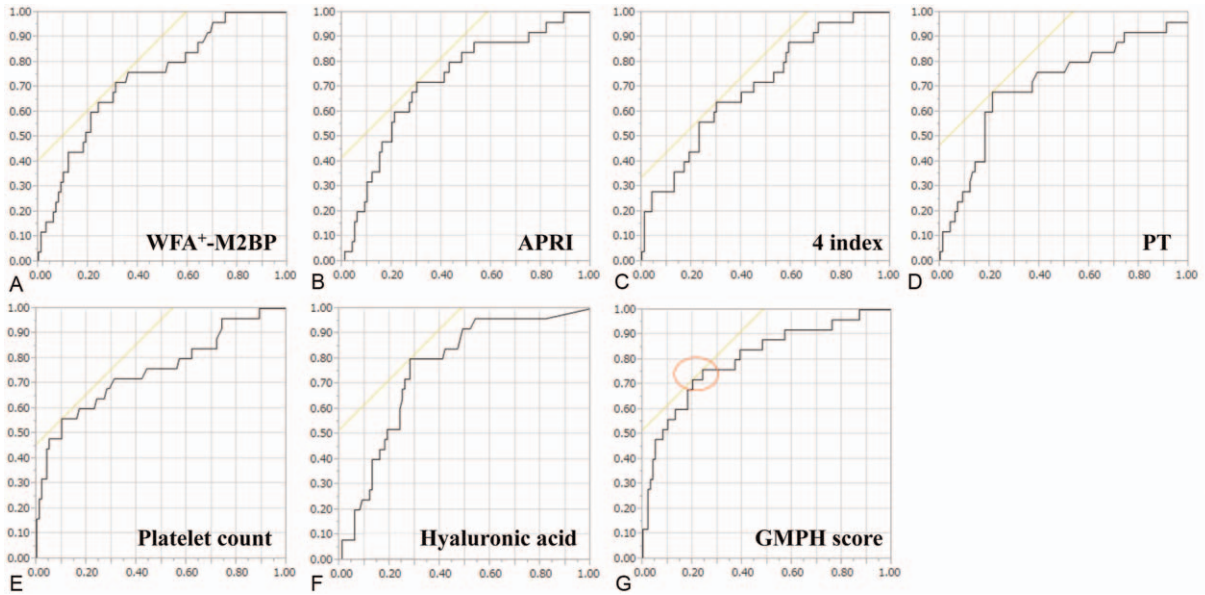

Figure 1. Receiver operating characteristic curves of Wisteria floribunda agglutinin-positive Mac-2-binding protein, aspartate aminotransferase-to-platelet ratio index, FIB-4 index, prothrombin time, platelet count, hyaluronic acid, and GMPH score for advanced fibrosis in the training group (n = 125). Vertical axis represents the sensitivity, and horizontal axis represents the 1-specificity. Red circle in GMPH score indicates that there were 2 optimal cutoff points associated with the maximal sum of sensitivity and specificity for the presence of advanced fibrosis in the training set.

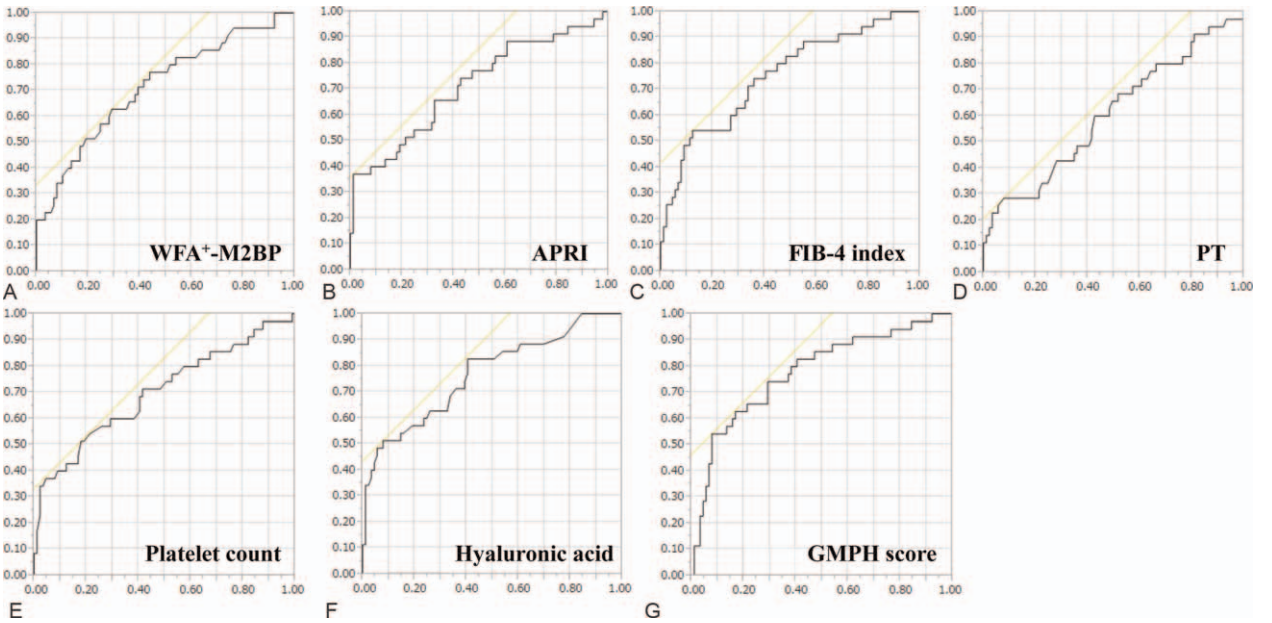

Figure 2. Receiver operating characteristic curves of Wisteria floribunda agglutinin-positive Mac-2-binding protein, aspartate aminotransferase-to-platelet ratio index, FIB-4 index, prothrombin time, platelet count, hyaluronic acid, and our proposed GMPH score for advanced fibrosis in the validation group (n = 124). Vertical axis represents the sensitivity, and horizontal axis represents the 1-specificity.

Transient elastography (Fibroscan®, ECOSENS Co., France), which is a rapid and user-friendly equipment for evaluating the degree of liver fibrosis, can easily be utilized both at the bedside and in the outpatient clinic with good reproducibility and immediate results.[25–27] A recent meta-analysis demonstrated that AUROC for advanced fibrosis in transient elastography ranged from 0.72 to 0.97, whereas in our present data, AUROCs for GMPH score for advanced fibrosis was 0.8064 in the training group and 0.7782 in the validation group.[28] Thus, diagnostic performance of GMPH score for advanced fibrosis may be similar to that of transient elastography.

It is notable that GGT and platelet count were independent predictors for advanced fibrosis in our multivariate analysis. In particular, GGT has been reported to be an independent predictor linked to liver fibrosis in several studies.[29–31] On the other hand, Lemoine et al[29] showed that GGT and platelet count were independent predictors of significant fibrosis (F2 or more) in their multivariate analysis of 135 CHB patients, which is similar to our results. In addition, they demonstrated that their proposed prediction model for liver fibrosis (GGT-to-platelet ratio [GPR]) was a simple and more accurate routine laboratory parameter than APRI and FIB-4 index to stage liver fibrosis in patients with CHB in west Africa.[29] In our case, AUROCs of GPR for advanced fibrosis in the training and validation groups were 0.7594 and 0.76501, respectively, which were higher than those of APRI and FIB-4 index in the training (0.726 and 0.704,

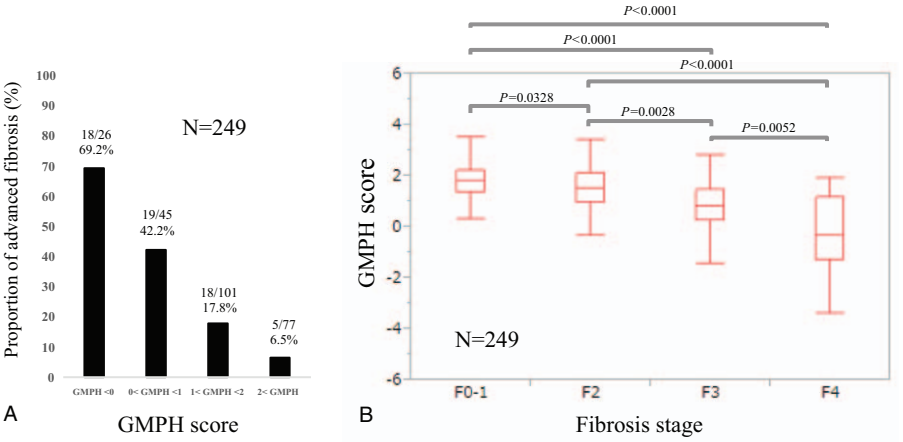

Figure 3. (A) The proportion of advanced fibrosis based on GMPH score. As GMPH score increased, the proportion of advanced fibrosis decreased. (B) Boxplots of GMPH score according to the degree of liver fibrosis for the entire cohort (n = 249).

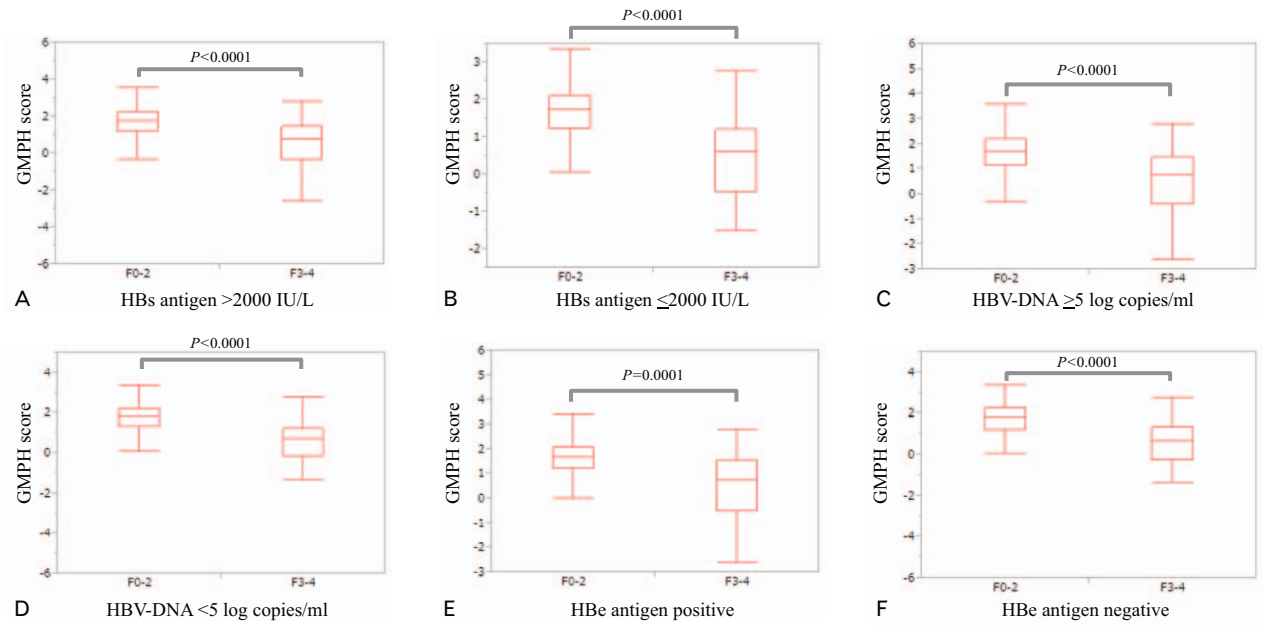

Figure 4. Boxplots of the GMPH score in each subgroup stratified by hepatitis B virus (HBV) status according to the degree of liver fibrosis. (A) Data for patients with HB surface (HBs) antigen > 2000 IU/L (n = 168). (B) Data for patients with HBs antigen ≤2000 IU/L (n = 81). (C) Data for patients with HBV-DNA ≥ 5 log copies/mL (n = 121). (D) Data for patients with HBV-DNA < 5 log copies/mL (n = 128). (E) Data for patients with HBe antigen positivity (n = 93). (F) Data for patients without HBe antigen positivity (n = 156).

respectively) and validation (0.7133 and 0.7519) groups, but were lower than those of GMPH score in the training (0.8064) and validation (0.7782) groups. These results suggest that the GMPH score may be superior to GPR for predicting advanced fibrosis in CHB patients. Our results may be attributed to the combination of 4 variables (i.e., GGT, WFA^+-M2BP, platelet count, and hyaluronic acid) in our model.

The GMPH scoring system involves WFA^+-M2BP, which has been established as a liver fibrosis marker in Japan, and it is characteristic in our predictive model.[10–14] Although the diagnostic accuracy of WFA^+-M2BP for liver fibrosis has not yet been validated outside Japan, previous Japanese studies demonstrated that it is useful for grading liver fibrosis and can be a useful predictor associated with clinical outcomes.[10–14,32,33] Thus, in the near future, this novel liver fibrosis biomarker should attract much attention in Western countries.

Patients with higher GMPH scores are expected to have less-advanced liver fibrosis. Indeed, in patients with GMPH score >2 (n = 77), 72 (93.5%) did not have advanced fibrosis. As demonstrated in Table 4, GMPH score is characterized by higher NPV values. Thus, unneeded liver biopsy should be avoided in such patients. However, 5 patients with GMPH score >2 (6.5%) were determined as having advanced fibrosis. All these patients had platelet counts more than $20.0 \times 10^4/mm^3$, indicating clinically less-advanced fibrosis. Higher platelet counts may result in higher GMPH scores. Interpretation of liver biopsy specimens may account for these discrepancies.

This study has several limitations. First, since our study was retrospective, our data should be cautiously interpreted. Second, since we performed internal validation alone, our results need prospective external confirmation. Third, all samples were recruited from Japanese CHB patients. Additional research is

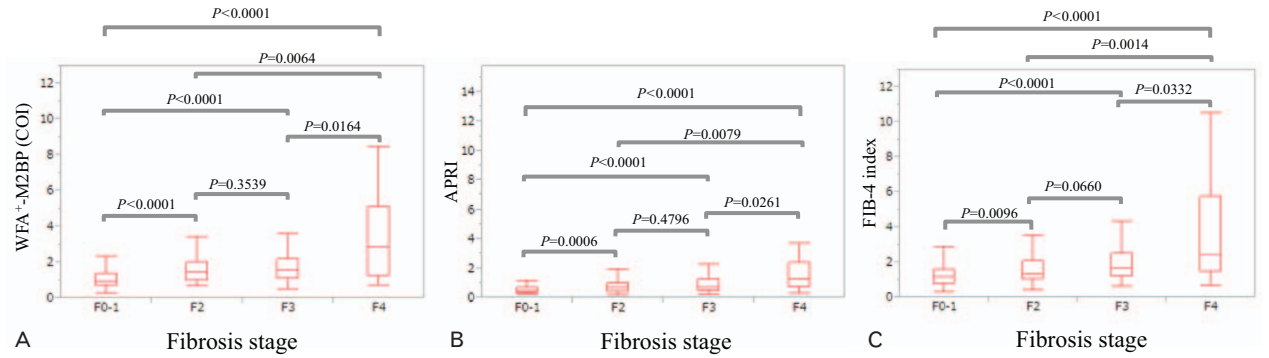

Figure 5. Boxplots of serum markers according to the degree of liver fibrosis for the entire cohort (n = 249). (A) Wisteria floribunda agglutinin-positive Mac-2-binding protein. (B) Aspartate aminotransferase-to-platelet ratio index. (C) FIB-4 index.

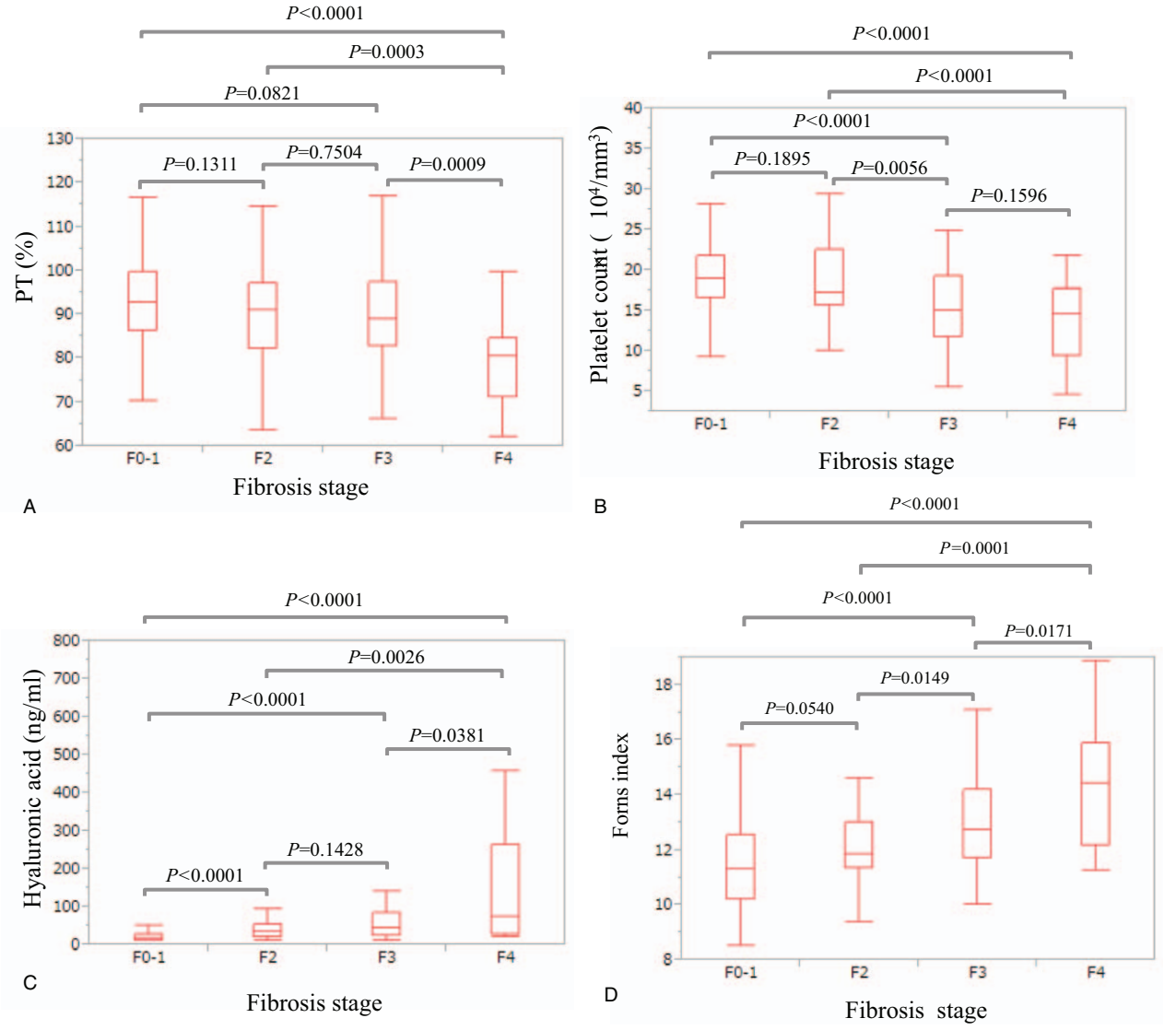

Figure 6. Boxplots of serum markers according to the degree of liver fibrosis for the entire cohort (n = 249). (A) Prothrombin time. (B) Platelet count. (C) Hyaluronic acid. (D) Forns index.

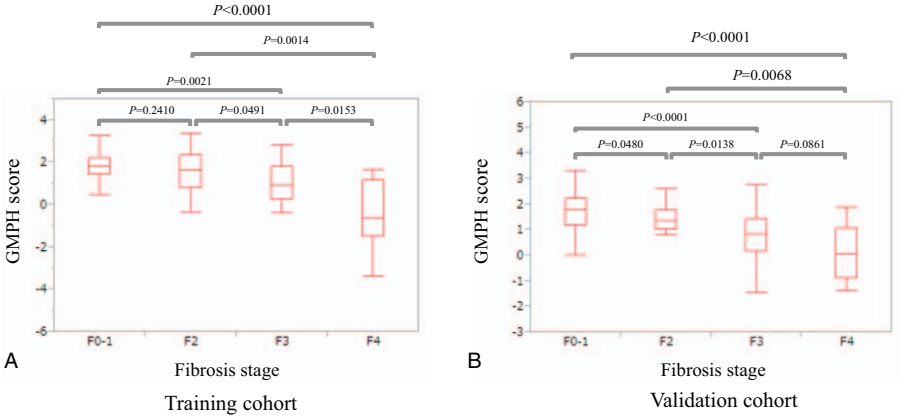

Figure 7. Boxplots of GMPH score according to the degree of liver fibrosis in the training group (A) and the validation group (B).

required to ascertain whether our current results can be extrapolated to CHB patients of different ethnicities. The validity of GMPH score should, thus, be confirmed in non-Japanese CHB patients. Finally, liver biopsy has a drawback of being prone to sampling errors in order to assess the degree of liver fibrosis, potentially leading to bias. However, our results suggest that the GMPH score was useful as a screening method for identifying CHB patients with advanced fibrosis. In conclusion, our proposed GMPH scoring system can become useful for detecting advanced liver fibrosis in patients with CHB.

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