Original Article

Serum Wisteria floribunda agglutinin-positive Mac-2-binding protein for patients with chronic hepatitis B and C: a comparative study

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Summary
We compared Wisteria floribunda agglutinin-positive Mac-2-binding protein (WFA⁺-M2BP) levels between patients with chronic hepatitis B (n=249) and chronic hepatitis C (n=386) based on the degree of liver fibrosis. We examined WFA⁺-M2BP levels in patients with F4 (cirrhosis), F3 or more (advanced fibrosis) and F2 or more (significant fibrosis) in the two groups. We further examined the relationship between five fibrosis markers and the degree of fibrosis. The WFA⁺-M2BP values ranged from 0.25 cut-off index (COI) to 12.9 COI in patients with hepatitis B and 0.34–20.0 COI in patients with hepatitis C (P<.0001). The median WFA⁺-M2BP values in F4 in the two groups were 2.83 COI in patients with hepatitis B and 5.03 COI in patients with hepatitis C (P=.0046). The median WFA⁺-M2BP values in F3 or more in the two groups were 1.79 COI in patients with hepatitis B and 3.79 COI in patients with hepatitis C (P<.0001). The median WFA⁺-M2BP values in F2 or more in the two groups were 1.49 COI in the hepatitis B cohort and 3.19 COI in the hepatitis C group (P<.0001). Among five liver fibrosis markers, WFA⁺-M2BP had the highest correlation coefficient (rₛ=.629) in terms of correlation with the degree of fibrosis in the patients with hepatitis C and had the second highest rₛ value (.415) in the hepatitis B group. Although WFA⁺-M2BP could be a useful indicator of liver fibrosis, WFA⁺-M2BP levels in the two groups significantly differed even in the same degree of fibrosis. Individual cut-off values in each aetiology for the degree of fibrosis should be determined.

Keywords
chronic hepatitis, comparative study, liver fibrosis marker, Wisteria floribunda agglutinin-positive Mac-2-binding protein

1 | INTRODUCTION

Chronic hepatitis B (CHB) virus (HBV) infection and chronic hepatitis C (CHC) virus (HCV) infection are both associated with liver fibrosis.¹⁻⁶ HBV and HCV infection are the leading causes of liver cirrhosis (LC), liver disease-related events and development of hepatocellular carcinoma (HCC).¹⁻⁶ The exact diagnosis of liver fibrosis or cirrhosis plays an important role in the control of disease progression, determining treatment and evaluating prognosis in infected patients, although the mechanisms for progression of liver fibrosis in HBV and HCV infection can differ.⁷⁻⁹
The Wisteria floribunda agglutinin-positive Mac-2-binding protein (WFA+M2BP) was recently established as a novel liver fibrosis glyco-biomarker in patients with HCV-related chronic liver disease with a unique fibrosis-related glycoalteration and speedy point of care testing technology.10–12 Our recent research showed that WFA+M2BP levels are a simple and reliable noninvasive surrogate marker of liver fibrosis in patients with autoimmune hepatitis, primary biliary cirrhosis (PBC) or nonalcoholic steatohepatitis,13–15 and others showed that WF A+M2BP level was closely associated with clinical outcomes for patients with CHB, CHC, PBC, LC and HCC.10–12,16–21 Thus, WFA+M2BP has been found to be a useful predictor of liver fibrosis in different aetiologies of chronic liver diseases and different clinical stages of chronic liver diseases. However, to the best of our knowledge, there have been no studies of a direct comparison of WFA+M2BP level based on the degree of liver fibrosis in patients with CHB and CHC. As the mechanisms for liver fibrosis progression may differ among different aetiologies of chronic liver diseases,8,9 these investigations may be of importance.

In this study, we conducted a comparative study of WFA+M2BP levels on liver fibrosis between patients with CHB and CHC compared to other laboratory liver fibrosis markers.

2 | PATIENTS AND METHODS

2.1 | Patients

Between September 2005 and July 2015, a total of 635 patients diagnosed as CHB (n=249, the CHB group) or CHC (n=386, the CHC group) with available stored sera were admitted at the Division of Hepatobiliary and Pancreatic disease, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan, and were analysed in this study. For all subjects, percutaneous liver needle biopsy for assessing the degree of liver fibrosis was performed. In the CHB group, all patients had detection of HB surface (HBs) antigen for more than 6 months and there was no evidence of concurrent HCV infection, and no clear evidence of drug-induced or alcoholic liver disease. In the CHC group, all patients had detection of HCV antibody and there was no evidence of concurrent HBV infection, and no clear evidence of drug-induced or alcoholic liver disease. We examined the correlation between WFA+M2BP level and histological findings of liver fibrosis in the two groups, and we also evaluated the diagnostic accuracy of WFA+M2BP level for predicting the degree of liver fibrosis comparing with other laboratory markers, including aspartate aminotransferase (AST) to platelet ratio index (APRI), FIB-4 index, platelet count and hyaluronic acid as liver fibrosis markers in the two groups. Furthermore, we compared baseline characteristics in the two groups based on the degree of liver fibrosis and investigated variables associated with the degree of liver fibrosis using univariate and multivariate analyses in the two groups.

APRI score was calculated as reported previously: (aspartate aminotransferase (AST)/upper limit of normal)/platelet count (expressed as platelets × 10^9/L) × 100.22–25 The FIB-4 index was calculated as reported previously: age (years) × AST (IU/L)/platelet count (×10^9/L) × √alanine aminotransferase (ALT) (IU/L).7,24–26

The ethics committee of our hospital approved this study protocol, and this study protocol complied with all of the provisions of the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to performing liver biopsy.

2.2 | Measurement of WFA+M2BP

Serum WFA+M2BP level was tested as described previously using stored serum samples collected at baseline, and it was measured by a lectin-Ab sandwich immunoassay using a fully automatic immunoanalyzer, HISCL-2000i (Sysmex Co., Hyogo, Japan).10–12

2.3 | Serological studies and histological findings

HBs antigen level, HBe antigen positivity and HBV DNA level were tested as described previously.27 HCV RNA concentrations were measured as described previously.26 HCV genotype was determined using a HCV Genotype Primer Kit (Institute of Immunology, Tokyo, Japan).

Our liver biopsy protocols were explained in our previous study, and the degree of liver fibrosis and inflammation were determined as reported elsewhere.27,29 In this study, we defined advanced fibrosis as F3 or more and defined significant liver fibrosis as F2 or more.

2.4 | Statistical analysis

Receiver operating characteristic curve (ROC) analysis was performed for calculating the area under the ROC (AUROC) for serum WFA+M2BP level, platelet count, APRI, FIB-4 index and hyaluronic acid for selecting the optimal cut-off value that maximized the sum of sensitivity and specificity for the presence of liver cirrhosis (F4), advanced fibrosis (F3 or more) and significant fibrosis (F2 or more) in histological findings. In continuous variables, the statistical analysis among groups was performed using Student’s t-test, Mann–Whitney U-test or Spearman’s rank correlation coefficient r as applicable. Variables with a P value <.05 in univariate analysis were included into multivariate analysis using the logistic regression analysis. In multivariate analyses, cut-off value in each continuous variable was determined from the ROC curve analyses. Data are expressed as median value (range) unless otherwise stated. Variables of P<.05 were considered to be statistically significant. Statistical analysis was performed with the JMP 11 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

3.1 | Baseline characteristics

The baseline characteristics of the subjects (n=249 in the CHB group and n=386 in the CHC group) are shown in Table 1. In the CHB group, there were 155 males and 94 females with the median (range) age of 45 (18–78) years, while in the CHC group, there were 180 males and 206 females with the median (range) age of 62 (20–87) years (P value: P<.0001 for age and P=.0001 for gender). Previous antiviral therapy was completed in 60 patients (24.1%) in the CHB group and 287 patients (74.4%) in the CHC group. In terms of the degree of liver
fibrosis, F4 was observed in 19 patients, F3 in 41, F2 in 51, F1 in 124 and F0 in 14 in the CHB group and F4 was observed in 122 patients, F3 in 90, F2 in 63, F1 in 103 and F0 in 8 in the CHC group (P < .0001).

The WFA⁺-M2BP values in the current analysis ranged from 0.25 COI to 12.9 COI (median value, 1.14 COI) in the CHB group and 0.34 COI to 20.0 COI (median value, 2.12 COI) in the CHC group (P < .0001) (Fig. 1a).

3.2 | WFA⁺-M2BP levels in patients with F4, F3 or more and F2 or more in the CHB and CHC groups

The median WFA⁺-M2BP values (range) in patients with F4 in the CHB (n=19) and CHC (n=122) groups were 2.83 COI (0.73 COI–12.9 COI) in the CHB group and 5.03 COI (1.06–19.95) in the CHC group (P<.0001) (Fig. 1b).

The median WFA⁺-M2BP values (range) in patients with F3 or more in the CHB (n=60) and CHC (n=212) groups were 1.79 COI (0.49 COI–12.9 COI) in the CHB group and 3.79 COI (0.57–20.0) in the CHC group (P<.0001) (Fig. 1c).

The median WFA⁺-M2BP values (range) in patients with F2 or more in the CHB (n=111) and CHC (n=275) groups were 1.49 COI (0.49 COI–12.9 COI) in the CHB group and 3.19 COI (0.34–20.0) in the CHC group (P<.0001) (Fig. 1d).

The WFA⁺-M2BP value in patients with F3 in the CHC group was significantly higher than that in the CHB group (P<.001), while the WFA⁺-M2BP value in patients with F2 was not (P=.677) (Fig. 2a and b).

3.3 | WFA⁺-M2BP levels in patients with A3 and A2 or more in the CHB and CHC groups

The median WFA⁺-M2BP values (range) in patients with A3 in the CHB (n=15) and CHC (n=17) groups were 3.44 COI (0.51 COI–12.9 COI) in the CHB group and 9.57 COI (2.81 COI–20.0 COI) in the CHC group (P=.0127) (Fig. S1a). The median WFA⁺-M2BP values (range) in patients with A2 or more in the CHB (n=77) and CHC (n=225) groups were 1.80 COI (0.47 COI–12.9 COI) in the CHB group and 3.20 COI (0.46 COI–20.0 COI) in the CHC group (P<.0001) (Fig. S1b).

3.4 | Comparison of baseline characteristics in the CHB and CHC groups in patients with F4, F3 or more and F2 or more

We also compared baseline characteristics in the CHB group and the CHC group in patients with F4, F3 or more and F2 or more.

### Table 1: Baseline characteristics in the CHB group and the CHC group

| Variables                        | CHB group (n=249)     | CHC group (n=386)     | P value   |
|----------------------------------|----------------------|----------------------|-----------|
| Age (years)                      | 45.6±12.6            | 60.9±11.0            | <.0001    |
| Gender, male/female              | 155/94               | 180/206              | .0001     |
| AST (IU/L)                       | 42.6±46.2            | 49.4±33.0            | <.0001    |
| ALT (IU/L)                       | 57.3±78.8            | 52.8±42.7            | .027      |
| ALP (IU/L)                       | 229.6±83.4           | 271.2±124.8          | <.0001    |
| GGT (IU/L)                       | 40.3±44.2            | 53.1±71.6            | <.001     |
| Serum albumin (g/dL)             | 4.12±0.55            | 3.99±0.46            | .0001     |
| Total bilirubin (mg/dL)          | 0.88±0.39            | 0.84±0.33            | .483      |
| Prothrombin time (%)             | 90.7±10.7            | 89.2±12.6            | .253      |
| Platelets (×10⁹/mm³)             | 18.2±5.0             | 14.9±6.0             | <.0001    |
| Hyaluronic acid (ng/mL)          | 46.6±80.6            | 152.2±184.0          | <.0001    |
| Total cholesterol (mg/dL)        | 185.7±31.7           | 165.9±34.7           | <.0001    |
| Triglyceride (mg/dL)             | 98.1±46.6            | 104.3±53.5           | .250      |
| Fasting blood glucose (mg/dL)    | 96.0±14.9            | 104.9±30.2           | <.0001    |
| Previous antiviral therapy, yes/no| 60/189               | 287/99               | <.0001    |
| HBV DNA >5 log copies/mL, yes/no| 121/128              | 93/156               |           |
| HBe antigen positivity, yes/no   | 1/28                 | 17/155               |           |
| HCV genotype                     | 1b/2a/2b/others      | 288/66/23/9          |           |
| HCV RNA >5 log copies/mL, yes/no| 326/60               | 1/2                   |           |
| WFA⁺-M2BP (cut-off index, COI)    | 1.63±1.65            | 3.76±3.96            | <.0001    |
| Fibrosis stage, F4/3/2/1/0        | 19/41/51/124/14      | 122/90/63/103/8      | <.0001    |
| A stage, 0/1/2/3                  | 17/155/62/15         | 7/154/208/17         | <.0001    |

Data are expressed as number or mean±standard deviation. AST; aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase, GGT; gamma glutamyl transpeptidase, CHB; chronic hepatitis B, CHC; chronic hepatitis C, WFA⁺-M2BP; Wisteria floribunda agglutinin-positive Mac-2-binding protein.
In patients with F4, the differences in the two groups reached significance in terms of age ($P = .0004$), total cholesterol ($P = .0049$), hyaluronic acid ($P = .0041$), WFA$^+$$-$$M2BP$ ($P = .0046$) and the degree of inflammation activity ($P = .018$) (Table 2). In patients with F3 or more, the differences in the two groups reached significance in terms of age ($P < .001$), gender ($P = .0082$), AST ($P = .0065$), serum albumin ($P < .001$), platelet count ($P < .001$), total cholesterol ($P < .001$), hyaluronic acid ($P < .001$), WFA$^+$$-$$M2BP$ ($P < .001$) and the degree of inflammation activity ($P = .033$) (Table S1). In patients with F2 or more, there were significant differences in the two groups in terms of age ($P < .001$), gender ($P = .0001$), ALT ($P = .011$), alkaline phosphatase (ALP) ($P = .021$), serum albumin ($P < .001$), platelet count ($P < .001$), total cholesterol ($P < .001$), fasting blood glucose (FBG) ($P = .022$), hyaluronic acid ($P < .001$), WFA$^+$$-$$M2BP$ ($P < .001$) and the degree of inflammation activity ($P < .001$) (Table S2).

### 3.5 ROC analyses in the CHB and CHC groups

In the CHB group, for predicting F4, F3 or more and F2 or more, hyaluronic acid level yielded the highest AUROCs with levels of 0.811 (F4), 0.766 (F3 or more) and 0.774 (F2 or more) among five liver fibrosis markers. ROC curves, AUROCs, optimal cut-off points, sensitivity and specificity in each fibrotic marker are shown in Figure S2 and Table S3.

In the CHC group, for predicting F4 and F3 or more, WFA$^+$$-$$M2BP$ had the highest AUROCs with levels of 0.844 (F4) and 0.830 (F3 or more) among five liver fibrosis markers, while for predicting F2 or more, hyaluronic acid level had the highest AUROC with a level of 0.827 among five markers. ROC curves, AUROCs, optimal cut-off points, sensitivity and specificity in each fibrotic marker are demonstrated in Figure S3 and Table S3.

### 3.6 Correlation between the degree of liver fibrosis and levels of liver fibrotic markers

In the CHB group, the dot plots according to different fibrosis stages in each liver fibrosis marker are shown in Fig. 3. Among five markers, hyaluronic acid had the highest correlation coefficient ($r = .497$), followed by WFA$^+$$-$$M2BP$ ($r = .415$). Similarly, in the CHC group, the dot...
3.7 | Univariate and multivariate analyses of factors contributing to F4, F3 or more and F2 or more in the CHB group

Significant variables linked to F4, F3 or more and F2 or more in the CHB group in univariate analyses are presented in Table S4. In multivariate analysis of factors contributing to F4, no significant variables were found. In multivariate analysis of factors contributing to F3 or more, FBG (P=.024), platelet count (P=.001), hyaluronic acid (P=.023) and WFA-M2BP (P=.034) were found to be significant factors. In multivariate analysis of factors contributing to F2 or more, hyaluronic acid (P<.0001), APRI (P=.039) and WFA-M2BP (P=.0073) were found to be significant factors. The hazard ratios (HRs) and 95% confidence intervals (CIs) of above significant factors are shown in Table S4.

3.8 | Univariate and multivariate analyses of factors contributing to F4, F3 or more and F2 or more in the CHC group

Significant variables linked to F4, F3 or more and F2 or more in the CHC group in univariate analyses are presented in Table S5. In multivariate analysis of factors contributing to F4, prothrombin time (PT) (P=.0014), hyaluronic acid (P=.020) and WFA-M2BP (P<.001) revealed to be significant factors. In multivariate analysis of factors contributing to F3 or more, PT (P<.001), hyaluronic acid (P=.0017) and WFA-M2BP (P<.001) revealed to be significant factors. In multivariate analysis of factors contributing to F2 or more, hyaluronic acid (P<.001) and WFA-M2BP (P<.001) revealed to be significant factors. The HRs and 95% CIs of above significant factors are demonstrated in Table S5.

4 | DISCUSSION

To the best of our knowledge, this is the first report for direct comparison of levels of serum liver fibrosis markers including WFA-M2BP, which has recently been established as a novel liver fibrosis marker, in hepatitis B and C according to the degree of liver fibrosis. The mechanism of liver fibrosis may differ between patients with different aetiologies of chronic liver diseases.8,9 Thus, comparing the effect of fibrosis markers on liver fibrosis in different aetiologies of chronic liver diseases is clinically of importance. It is imperative for clarifying these issues. We therefore conducted current analyses.

In our data, the median values and optimal cut-off points of WFA-M2BP level in F4, F3 or more and F2 or more in the CHB and CHC groups significantly differed. In addition, as for other liver fibrosis markers, similar results were obtained. The median values of WFA-M2BP level in F4, F3 or more and F2 or more in the CHB and CHC groups also significantly differed. These results indicate that different mechanisms for liver fibrosis or liver inflammation are underlying in the two groups as reported previously.8,9 In comparison with pattern of liver fibrosis and regenerative nodules between hepatitis B- and C-related LC, HBV-related LC showed large regenerative nodules with thin fibrous septa, while HCV-related LC showed small regenerative nodules with thick fibrous septa. Thus, total amount of fibrous tissue may differ in hepatis B- and C-related LC, although we did not calculate it in the present study.8,9 Our observations that the differences of WFA-M2BP level and hyaluronic acid in the two groups in patients with F4, F3 or more and F2 or more reached significance may be linked to these facts.8,9 On the other hand, in the liver, hyaluronic acid is synthesized by Ito cells and finally degraded by sinusoidal endothelial cells and the serum hyaluronic acid level in chronic liver disease is considered to increase due to the decreased clearance of hyaluronic acid related to the destruction of hepatocytes.30,31 Our current results also may reflect the difference of the degree of destruction of hepatocytes in the two groups. The baseline significant differences of AST value (median value: 29 IU/L in the CHB group and 41 IU/L in the CHC group, P=.0001) and ALT value (median value: 34 IU/L in the CHB group and 41.5 IU/L in the CHC group, P=.027) in the two groups support this hypothesis. In cases with CHB infection, most cases with advanced fibrosis had seroconversion from HBe antigen to HBe antibody and inflammation activity became quiescent, resulting in termination of progression of liver fibrosis.32,33 On the other hand, in cases with CHC infection, HCV could replicate continuously even after progression to LC, resulting in

| TABLE 2 | Comparison of baseline characteristics in the CHB group and the CHC group in patients with F4 |
|---|---|---|
| Variables | CHB group (n=19) | CHC group (n=122) | P value |
| Age (years) | 53.2±12.5 | 63.8±9.6 | <.001 |
| Gender, male/female | 11/8 | 60/62 | .628 |
| AST (IU/L) | 63.8±58.2 | 63.4±41.5 | .201 |
| ALT (IU/L) | 73.8±86.0 | 60.3±48.7 | .930 |
| ALP (IU/L) | 335.0±121.6 | 317.1±147.1 | .295 |
| GGT (IU/L) | 79.4±9.4 | 81.1±11.9 | .463 |
| Total bilirubin (mg/dL) | 0.98±0.7 | 0.92±0.38 | .506 |
| Serum albumin (g/dL) | 3.81±0.49 | 3.73±0.52 | .543 |
| Total bilirubin (mg/dL) | 171.2±30.0 | 150.9±30.9 | .0049 |
| Platelet count (×10³/mm³) | 13.4±5.4 | 12.4±6.4 | .265 |
| Triglyceride (mg/dL) | 87.2±40.7 | 102.1±52.1 | .328 |
| Fasting blood glucose (mg/dL) | 102.4±19.4 | 108.7±35.9 | .587 |
| Hyaluronic acid (ng/mL) | 163.5±194.9 | 256.2±204.2 | .0041 |
| WFA-M2BP (cut-off index) | 3.74±3.09 | 6.67±4.73 | .0046 |
| A stage, 0/1/2/3 | 1/6/8/4 | 0/2/85/13 | .018 |

Data are expressed as number or mean±standard deviation. CHB; chronic hepatitis B, CHC; chronic hepatitis C, AST; aspartate ami, ALT; alanine ami-notransferase, ALP; alkaline phosphatase, GGT; gamma glutamyl transpeptidase, WFA-M2BP; Wisteria floribunda agglutinin-positive Mac-2-binding protein.
persistent inflammatory responses and fibrogenesis throughout its clinical course. This is also associated with our current results.

The median age in the CHC group was significantly higher than that in the CHB group ($P < 0.0001$), and the proportions of patients with LC or advanced fibrosis in the CHC group was significantly higher than those in the CHB group ($P < 0.0001$) in this analysis. This may be attributed to the fact that in cases with CHC infection, the evolution from chronic hepatitis with early stage to advanced fibrosis or LC occurred more frequently in patients aged 50 years or more. $^{9,34,35}$ On the other hand, prevalence of A2 or more in the CHC group was significantly higher than that in the CHB group. Previous studies emphasized the effect of ageing on the inflammation-related immune reaction in the liver, and this may explain the reasons for our current results. $^{9,34}$ A previous study demonstrated that three features more likely can be found in CHC than in CHB: bile duct damage, lymphoid follicles and large-droplet fat. $^{36}$ These differences of pathological features in CHB
and CHC may partly explain the reasons of significant differences of our baseline characteristics in the two groups in terms of ALP or gamma glutamyl transpeptidase.

In our multivariate analyses, WFA\textsuperscript{+}-M2BP revealed to be an independent predictor for predicting F3 or more and F2 or more in the CHB group and for predicting F4, F3 or more and F2 or more in the CHC group. Furthermore, among five liver fibrosis markers, WFA\textsuperscript{+}-M2BP had the highest $r_s$ value in terms of correlation with the degree of liver fibrosis in the CHC group and had the second highest $r_s$ value in the CHB group. In ROC analyses, WFA\textsuperscript{+}-M2BP had favourable ROC values of 0.7 or more for predicting F4, F3 or more and F2 or more in the two groups. These results suggest that WFA\textsuperscript{+}-M2BP could be a useful indicator for predicting liver fibrosis regardless of aetologies of viral hepatitis, which are in accordance with previous reports.\textsuperscript{10–18}

On the other hand, Toshima et al.\textsuperscript{10} reported that AUROC of serum WFA\textsuperscript{+}-M2BP values for predicting F4 in patients with HCV-related disease was 0.795 (cut-off value, 3.67), whereas in our data, AUROC for F4 was 0.844 (cut-off value, 2.42). The reason for this discrepancy remains unclear, and further validation will be needed in the future. In the CHB group, hyaluronic acid had the highest $r_s$ value and AUROCs for predicting F4, F3 or more and F2 or more, indicating that WFA\textsuperscript{+}-M2BP was inferior to hyaluronic acid for predicting liver fibrosis in patients with CHB. Although WFA\textsuperscript{+}-M2BP appears to be a useful liver fibrosis marker, caution should be exercised for assessing the degree of liver fibrosis especially in patients with CHB.

There are several limitations to the present study. First, although our study had large sample size, this is a retrospective comparative study. Second, liver biopsy can lead to sampling errors for assessing the degree of liver fibrosis. Third, the number of subjects with F4 in the CHB group was small for analysis as compared with that in the CHC group, potentially leading to bias. Our results therefore need to be interpreted with caution. Finally, data for elastography were not included in this study. As elastography is a well-established alternative measurement method for liver fibrosis other than liver biopsy, direct comparisons of predictive accuracy of the degree of liver fibrosis between WFA\textsuperscript{+}-M2BP levels and data for elastography will be required in the future.\textsuperscript{17} However, in the current analysis, we demonstrated that levels of serum liver fibrosis markers in the CHB and CHC groups significantly differed even in the same degree of liver fibrosis. Individual cut-off values of serum markers in each aetiology of chronic liver diseases for the degree of liver fibrosis should thus be determined.

**CONFLICT OF INTEREST**

In the past year, Shuhei Nishiguchi received financial support from Chugai Pharmaceutical, MSD, Dainippon Sumitomo Pharma, Ajinomoto Pharma and Otsuka Pharmaceutical. The remaining authors declare that they have no conflict of interests.

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**ABBREVIATIONS**

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate ami; CHB, chronic hepatitis B; CHC, chronic hepatitis C; FBG, fasting blood glucose; GGT, gamma glutamyl transpeptidase; WFA\textsuperscript{+}-M2BP, Wisteria floribunda agglutinin-positive Mac-2-binding protein.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.