

Serum *Wisteria floribunda* agglutinin-positive human Mac-2 binding protein may predict liver fibrosis and progression to hepatocellular carcinoma in patients with chronic hepatitis B virus infection

Short title: Predictive abilities of WFA⁺-M2BP in HBV

Yuki Ichikawa,^{1a} Satoru Joshita,^{1a} Takeji Umemura,^{1*} Yugo Shobugawa,²
Soichiro Shibata¹, Tomoo Yamazaki¹, Naoyuki Fujimori¹, Michiharu Komatsu¹,
Akihiro Matsumoto,¹ Eiji Tanaka¹

1: Department of Medicine, Division of Gastroenterology and Hepatology,
Shinshu University School of Medicine, Matsumoto, Japan

2: Division of International Health (Public Health), Niigata University Graduate
School of Medicine and Dental Sciences, Niigata, Japan

^a These authors contributed equally to this work

Grant Support: This work was supported in part by Grants-in-Aid for Scientific

Research from the Ministry of Education, Science, Sports, and Culture of Japan
(25460986) and from the Ministry of Health Labour and Welfare (GAX085).

Corresponding author:

Takeji Umemura, M.D., Ph.D.

Department of Medicine, Division of Gastroenterology and Hepatology, Shinshu

University School of Medicine

3-1-1 Asahi, Matsumoto 390-8621, Japan

Tel.: +81-263-37-2634

Fax: +81-263-32-9412

E-mail: tumemura@shinshu-u.ac.jp

ABSTRACT

Aim: Serum glycosylated *Wisteria floribunda* agglutinin-positive Mac-2 binding protein (WFA⁺-M2BP) is a reliable, non-invasive marker of liver fibrosis. This study assessed the ability of WFA⁺-M2BP to diagnose liver fibrosis in patients with chronic hepatitis B virus (HBV) infection and evaluated WFA⁺-M2BP as a predictor of hepatocellular carcinoma (HCC) development.

Methods: Serum WFA⁺-M2BP values were retrospectively evaluated in 112 treatment naïve patients with HBV-related chronic hepatitis and cirrhosis who had undergone liver biopsy at our hospital.

Results: Serum WFA⁺-M2BP levels were significantly related with liver fibrosis ($r=0.3725$, $p=0.001$). Fibrosis stage F2, F3, and F4 had a cutoff index of 0.94, 1.26, and 1.26, respectively. For diagnosing F \geq 2 fibrosis, the area under the receiver operating characteristic curve for WFA⁺-M2BP was 0.713 and comparable with those of other non-invasive fibrosis markers, such as hyaluronic acid, type IV collagen 7S, APRI, FIB-4, serum albumin, and platelet count. Multivariate analysis identified male, WFA⁺-M2BP ≥ 0.71 , ALT ≥ 80 IU/L, and platelet count $< 14.5 \times 10^9/L$ as independent risk factors for the development of HCC in patients with HBV infection.

Conclusions: Serum WFA⁺-M2BP values appear to be useful for assessing liver fibrosis stage and are independently associated with HCC development in patients with chronic HBV infection.

Keywords: hepatitis B virus, liver fibrosis, Serum *Wisteria floribunda* agglutinin-positive human Mac-2 binding protein, hepatocellular carcinoma

Introduction

Approximately 350 million individuals are chronically infected with the hepatitis B virus (HBV) worldwide.¹ HBV infection is a pressing global health concern since up to 25% of affected patients reportedly die as a result of disease progression to cirrhosis and hepatic failure with accompanying hepatocellular carcinoma (HCC).¹ As patients with significant fibrosis are particularly susceptible to these complications, it is important to accurately assess liver fibrosis and determine the appropriate therapeutic strategy for patients with HBV, including the optimal timing for antiviral therapy.

Although liver biopsy provides important information regarding the severity of necro-inflammatory activity and liver fibrosis, it is often limited by invasiveness and pain, sampling error, and inter-observer disparity.² Thus, simple and reliable non-invasive methods to estimate liver fibrosis and HBV progression are needed, such as hyaluronic acid (HA), type IV collagen 7S, aspartate aminotransferase (AST)-to-platelet ratio index (APRI), and FIB-4 index.^{3,4}

Recently, *Wisteria floribunda* agglutinin-positive human Mac-2 binding protein (WFA⁺-M2BP) has been reported as a novel marker to assess liver

fibrosis using the glycan “sugar chain”-based immunoassay.⁵ WFA⁺-M2BP was firstly found to predict liver fibrosis in chronic hepatitis C patients. Soon afterwards, Umemura et al. described that WFA⁺-M2BP levels were related to liver fibrosis stage and prognosis in patients with primary biliary cirrhosis (PBC).⁶ Later studies validated serum WFA⁺-M2BP as helpful for assessing the stage of liver fibrosis in patients with non-alcoholic fatty liver disease (NAFLD)⁷ and autoimmune hepatitis (AIH).⁸

WFA⁺-M2BP has also been found to predict the onset of HCC. Yamasaki et al. concluded that the cumulative incidence of HCC was associated with WFA⁺-M2BP levels,⁹ while Sasaki et al. reported that WFA⁺-M2BP could be a novel predictor of HCC development after a sustained virological response in patients with chronic hepatitis C treated with interferon (IFN).¹⁰

However, there are no published data to date regarding the usefulness of WFA⁺-M2BP as a liver fibrosis marker or predictor of HCC in individuals chronically infected with HBV. This study therefore evaluated the ability of serum WFA⁺-M2BP to detect significant histological fibrosis and analyzed the utility of WFA⁺-M2BP as a predictor of HCC onset in patients with chronic HBV infection.

Patients and Methods

A total of 157 patients chronically infected with HBV who had undergone liver biopsy at Shinshu University between 1998 and 2013 were recruited for this study. Individuals having a history of organ transplantation or concurrent use of immunomodulatory drugs or corticosteroids were excluded from the analysis, as were those coinfecting with the hepatitis C virus (HCV) or human immunodeficiency virus type 1 or who exhibited evidence of other liver disease, such as PBC, AIH, alcoholic liver disease, or NAFLD. Thirty-five patients were removed due to unavailability of histological findings or insufficient serum or laboratory data. Ten patients who had already received treatment with IFN or nucleos(t)ide analogues (NAs) at the time of liver biopsy were also excluded. Twenty-four (21%) patients received IFN monotherapy and 63 (56%) patients underwent NA treatment after enrollment. Ultimately, 112 treatment-naïve patients with chronic hepatitis B were enrolled to evaluate for associations of WFA⁺-M2BP with fibrosis stage and disease progression to HCC. The diagnosis of chronic HBV infection was based on the persistence of hepatitis B surface antigen (HBsAg) for more than six months.¹¹ Blood samples were obtained on the same day as the liver biopsy, and separated serum was

stored at -20°C. Subjects were followed at regular intervals throughout the observation period. The protocol of this study was approved by the ethics committee of Shinshu University School of Medicine in accordance with the principles of the 64th World Medical Association Declaration of Helsinki in 2013. All patients provided written informed consent.

Laboratory testing

HBsAg, hepatitis B e antigen (HBeAg), and hepatitis B e antibody (HBeAb), were assessed using commercially available enzyme immunoassay kits.¹² Serum levels of HBV DNA were quantified using the COBAS TaqMan HBV Test v2.0 (Roche Diagnostics, Tokyo, Japan) having a dynamic range of 2.1-9.0 log copies/mL. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other relevant biochemical evaluations were performed using standard methods.

Detection of serum WFA⁺-M2BP

WFA⁺-M2BP was quantified based on a lectin-antibody sandwich immunoassay using the fully automatic immunoanalyzer HISCL-2000i (Sysmex

Co., Hyogo, Japan).⁵ The measured values of WFA⁺-M2BP conjugated to WFA were then indexed with the obtained values using the equation:

Cutoff index (COI) = $([\text{WFA}^+\text{-M2BP}]_{\text{sample}} - [\text{WFA}^+\text{-M2BP}]_{\text{NC}}) / ([\text{WFA}^+\text{-M2BP}]_{\text{PC}} - [\text{WFA}^+\text{-M2BP}]_{\text{NC}})$, with WFA⁺-M2BP as the serum sample value, PC as the positive control, and NC as the negative control. The positive control was supplied as a calibration solution preliminarily standardized to yield a COI value of 1.0.¹³

Fibrosis markers

Serum HA and type IV collagen 7S levels were measured by commercially available enzyme-linked immunosorbent assays. APRI and FIB-4 index were calculated as: $(\text{AST} / \text{upper limit of normal; 40 IU/L}) \times (100 / \text{platelet count } [10^9/\text{L}])$ ¹⁴ and $(\text{age [years]} \times \text{AST [IU/L]}) / (\text{platelet count } [10^9/\text{L}] \times \text{ALT [IU/L]}^{1/2})$ ¹⁵, respectively.

Histological evaluation

Liver biopsies were performed on all patients by percutaneous sampling of the right lobe with a 14-gauge needle as reported previously.¹⁶ All

samples were 1.5 cm or more in length. Formalin-fixed, paraffin-embedded specimens were prepared and used for subsequent histopathological and immunohistochemical studies. Sections measuring 4 μ m were cut from each paraffin block and stained with hematoxylin and eosin, periodic acid-Schiff after diastase digestion, and Azan-Mallory staining. All liver biopsy samples were independently evaluated by two investigators who were blinded to the clinical data. Liver fibrosis stage was assessed according to the revised Inuyama classification of chronic hepatitis¹⁷ as: F0 = no fibrosis, F1 = portal fibrosis without bridging, F2 = portal fibrosis with rare bridging, F3 = abundant bridging without cirrhosis, and F4 = cirrhosis.

Statistical analysis

Statistical analyses and data visualization were carried out using IBM SPSS Statistics version 23.0 (IBM, Chicago, IL) and StatFlex version 6.0 (Artech Co., Ltd. Osaka, Japan) software. Data are presented as median \pm interquartile range for continuous variables. Groups were compared using the chi-square test for categorical variables. Correlations between fibrosis stage and serum WFA⁺-M2BP values were analyzed by means of Spearman's rank test.

Diagnostic accuracy was evaluated using the area under the receiver operating characteristic (ROC) curve (AUROC). Cutoff values were identified by the Youden index, and the nearest clinically applicable value to the cutoff was considered as the optimal cutoff value for clinical convenience. HCC incidence rates were estimated by the Kaplan-Meier method and assessed using log-rank tests. Multivariate analysis was performed using the Cox proportional hazards model with stepwise method. All statistical tests were two-sided and evaluated at the 0.05 level of significance.

Results

Baseline clinical characteristics

The baseline clinical characteristics of our cohort are summarized in Table 1. Of the 112 enrolled patients, 72 were male and 40 were female. Median age was 47 years. The average follow-up period was 173 weeks. During the observation period, 24 (21%) patients received IFN monotherapy and 63 (56%) underwent NA treatment. Fifteen (13%) cases became complicated with HCC during follow-up. Of these, 14 (93%) received NAs.

Based on histological findings, the number of patients with fibrosis

stage F0, F1, F2, F3, and F4 was 4, 36, 26, 24, and 22, respectively.

Liver fibrosis estimation by WFA-M2BP values

Figure 1 presents box plots of serum WFA⁺-M2BP values for each fibrosis stage. Median WFA⁺-M2BP in patients with fibrosis stage F0, F1, F2, F3, and F4 was 0.57, 0.75, 1.14, 1.03, and 1.64, respectively. We observed a significant association between WFA⁺-M2BP value and fibrosis stage ($r = 0.3725$, $p = 0.001$).

Correlation of liver fibrosis with other non-invasive markers

The correlation coefficients between WFA⁺-M2BP and other non-invasive markers (HA, type IV collagen 7S, APRI, FIB-4, serum albumin, and platelet count) are shown in Figure 2. Moderate but statistically significant correlations were found between WFA⁺-M2BP and HA ($r = 0.425$, $p < 0.0001$), type IV collagen 7S ($r = 0.377$, $p = 0.0001$), APRI ($r = 0.267$, $p < 0.0045$), FIB-4 ($r = 0.316$, $p = 0.0007$), and serum albumin ($r = -0.436$, $p < 0.0001$), but not platelet count.

Diagnostic ability of WFA⁺-M2BP for liver fibrosis stage

We next assessed the diagnostic ability of WFA⁺-M2BP to determine fibrosis stage using ROC analysis. The AUROC of WFA⁺-M2BP to diagnose F2-4, F3-4, and F4 was high at 0.713, 0.658, and 0.689, respectively (Figure 3).

The ROC curves of the other fibrosis markers are presented in Figure 4, whose AUROCs were 0.710 (HA), 0.747 (type IV collagen 7S), 0.680 (APRI), 0.699 (FIB-4), 0.759 (serum albumin), and 0.666 (platelet count). These were all comparable to that of WFA⁺-M2BP (0.713).

Prediction of HCC development in chronically infected HBV patients

Among the 112 patients, 15 (13%) developed the complication of HCC during the mean study period of 173 weeks. Based on our ROC results for WFA⁺-M2BP level to predict complicating HCC, a cutoff value of 0.71 yielded a sensitivity and specificity of 93% and 35%, respectively. We next performed univariate analysis using the chi-square test for categorical variables followed by multivariate analysis with the Cox proportional hazards model to identify factors associated with HCC in patients with HBV infection. WFA⁺-M2BP ≥ 0.71 , ALT ≥ 80 IU/L, and platelet count $< 14.5 \times 10^9/L$ were determined as independent predictive

factors for complicating HCC (Table 2). The cumulative incidence rate of HCC was significantly different among subjects with an independent risk factor of $\text{WFA}^+\text{-M2BP} \geq 0.71$ ($p = 0.020$, log-rank test) (Figure 5).

Discussion

$\text{WFA}^+\text{-M2BP}$ has been described as a superior non-invasive marker for estimating liver fibrosis in several chronic liver diseases. To the best of our knowledge, this is the first study demonstrating that $\text{WFA}^+\text{-M2BP}$ can also be a good indicator of fibrosis in patients with chronic HBV infection. Our results showed a significant correlation between $\text{WFA}^+\text{-M2BP}$ value and liver fibrosis that was comparable to other reported serum fibrosis markers, particularly HA, type IV collagen 7S, and serum albumin.

However, as compared with serum $\text{WFA}^+\text{-M2BP}$ values detected in patients with other chronic liver diseases, those in our HBV patients tended to be lower throughout the progression of liver fibrosis; median $\text{WFA}^+\text{-M2BP}$ for fibrosis stage F2 was 0.97 in HBV patients, versus 3.86 in HCV patients, 2.1 in AIH patients, 2.0 in PBC patients, and 1.07 in NASH patients.^{6-8,18} Artini et al. also found that total M2BP expression levels were higher in HCV-positive than in

HCV-negative patients throughout the progression of liver fibrosis.¹⁹ As WFA⁺-M2BP is a fraction of altered M2BP selected by lectin binding, there is a possibility that serum WFA⁺-M2BP may also be associated with HCV infection. Several other reports^{9,20} have mentioned differences in WFA⁺-M2BP levels between male and female patients, although WFA⁺-M2BP gender variability has not been precisely addressed. Since previous study cohorts of AIH and PBC were predominantly female at 82% and 81%, respectively, we cannot exclude the possibility of differences in WFA⁺-M2BP between male and female patients. In fact, we witnessed that WFA⁺-M2BP levels in women were significantly higher than those in men in our predominantly male test group (data not shown). These reasons may at least partly account for the comparatively lower median WFA⁺-M2BP value in this study.

Pretreatment levels of WFA⁺-M2BP were significantly associated with future complicating HCC in patients with chronic HBV. Specifically, multivariate analysis identified higher serum WFA⁺-M2BP, higher ALT, and lower platelet count as independent predictors of HCC development. These results indicate that the correlation between high WFA⁺-M2BP and HCC development remains significant even if HCC develops from a non-cirrhotic background. Moreover, no

patient whose WFA⁺-M2BP was under 0.50 developed HCC during the observation period (data not shown). Hence, the significance of WFA⁺-M2BP as an indicator of enhanced hepatocarcinogenesis suggests that this marker may predict HCC development in cancer-free liver tissue after treatment.

Lastly, Yamasaki et al.⁹ observed a greater incidence of HCC development in patients with chronic hepatitis C who exhibited higher serum WFA⁺-M2BP levels, even after stratification by liver fibrosis grade, for yet undetermined reasons. Total M2BP was firstly identified by Iacobelli et al. as a tumor-associated antigen.²¹ M2BP is a highly glycosylated secretory protein that principally mediates cell-to-cell proliferation and angiogenesis. Several proteomic studies uncovered that WFA⁺-M2BP also enhanced cell adhesion in the extracellular matrix to promote fibrosis.^{19,22,23} Therefore, WFA⁺-M2BP might possess additional characteristics as a tumor-associated antigen that is not associated with liver fibrosis.

The present study has several limitations. It was retrospective and single-center in nature, the liver biopsy for evaluating the degree of liver fibrosis was prone to sampling error, and the sample size was limited. Thus, larger prospective multicenter studies are warranted.

In conclusion, WFA⁺-M2BP may represent not only a non-invasive and reliable marker for assessing fibrosis stage, but also a predictor of complicating HCC, in patients chronically infected with HBV.

Acknowledgments

The authors thank Trevor Ralph for his English editorial assistance. The Sysmex Co. provided materials for WFA⁺-M2BP assay.

References

- 1 Custer, B., Sullivan, S. D., Hazlet, T. K. *et al.* Global epidemiology of hepatitis B virus. *Journal of clinical gastroenterology* 2004; **38**: S158-68.
- 2 Bravo, A. A., Sheth, S. G. & Chopra, S. Liver biopsy. *The New England journal of medicine* 2001; **344**: 495-500.
- 3 Castera, L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. *Gastroenterology* 2012; **142**: 1293-302.e4.
- 4 Hui, A. Y., Chan, H. L., Wong, V. W. *et al.* Identification of chronic hepatitis B patients without significant liver fibrosis by a simple noninvasive predictive model. *The American journal of gastroenterology* 2005; **100**: 616-23.
- 5 Kuno, A., Ikehara, Y., Tanaka, Y. *et al.* A serum "sweet-doughnut" protein facilitates fibrosis evaluation and therapy assessment in patients with viral hepatitis. *Scientific reports* 2013; **3**: 1065.
- 6 Umemura, T., Joshita, S., Sekiguchi, T. *et al.* Serum Wisteria floribunda Agglutinin-Positive Mac-2-Binding Protein Level Predicts Liver Fibrosis and Prognosis in Primary Biliary Cirrhosis. *The American journal of gastroenterology* 2015; **110**: 857-64.
- 7 Abe, M., Miyake, T., Kuno, A. *et al.* Association between Wisteria floribunda agglutinin-positive Mac-2 binding protein and the fibrosis stage of non-alcoholic fatty liver disease. *Journal of gastroenterology* 2015; **50**: 776-84.
- 8 Nishikawa, H., Enomoto, H., Iwata, Y. *et al.* Clinical significance of serum Wisteria floribunda agglutinin positive Mac-2-binding protein level and high-sensitivity C-reactive protein concentration in autoimmune hepatitis. *Hepatology research : the official journal of the Japan Society of Hepatology* 2015.
- 9 Yamasaki, K., Tateyama, M., Abiru, S. *et al.* Elevated serum levels of Wisteria floribunda agglutinin-positive human Mac-2 binding protein predict the development of hepatocellular carcinoma in hepatitis C patients. *Hepatology (Baltimore, Md.)* 2014; **60**: 1563-70.
- 10 Sasaki, R., Yamasaki, K., Abiru, S. *et al.* Serum Wisteria Floribunda Agglutinin-Positive Mac-2 Binding Protein Values Predict the Development of Hepatocellular Carcinoma among Patients with Chronic Hepatitis C after Sustained Virological Response. *PloS one* 2015; **10**: e0129053.
- 11 <http://www.who.int/mediacentre/factsheets/fs204/en/>.
- 12 Umemura, T., Tanaka, E., Kiyosawa, K. & Kumada, H. Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2008; **47**: e52-6.

- 13 Kuno, A., Sato, T., Shimazaki, H. *et al.* Reconstruction of a robust glycodiagnostic agent supported by multiple lectin-assisted glycan profiling. *Proteomics. Clinical applications* 2013.
- 14 Wai, C. T., Greenson, J. K., Fontana, R. J. *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology (Baltimore, Md.)* 2003; **38**: 518-26.
- 15 Sterling, R. K., Lissen, E., Clumeck, N. *et al.* Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-25.
- 16 Umemura, T., Zen, Y., Hamano, H. *et al.* Immunoglobulin G4-hepatopathy: association of immunoglobulin G4-bearing plasma cells in liver with autoimmune pancreatitis. *Hepatology (Baltimore, Md.)* 2007; **46**: 463-71.
- 17 Ichida, F., Tsuji, T., Omata, M. *et al.* New Inuyama classification; new criteria for histological assessment of chronic hepatitis. *International Hepatology Communications* 1996; **6**: 112-19.
- 18 Ura, K., Furusyo, N., Ogawa, E. *et al.* Serum WFA(+)-M2BP is a non-invasive liver fibrosis marker that can predict the efficacy of direct-acting anti-viral-based triple therapy for chronic hepatitis C. *Alimentary pharmacology & therapeutics* 2016; **43**: 114-24.
- 19 Artini, M., Natoli, C., Tinari, N. *et al.* Elevated serum levels of 90K/MAC-2 BP predict unresponsiveness to alpha-interferon therapy in chronic HCV hepatitis patients. *Journal of hepatology* 1996; **25**: 212-7.
- 20 Fujiyoshi, M., Kuno, A., Gotoh, M. *et al.* Clinicopathological characteristics and diagnostic performance of Wisteria floribunda agglutinin positive Mac-2-binding protein as a preoperative serum marker of liver fibrosis in hepatocellular carcinoma. *Journal of gastroenterology* 2015; **50**: 1134-44.
- 21 Iacobelli, S., Sismondi, P., Giai, M. *et al.* Prognostic value of a novel circulating serum 90K antigen in breast cancer. *British journal of cancer* 1994; **69**: 172-6.
- 22 Iacovazzi, P. A., Cozzolongo, R., Lanzillotta, E. *et al.* Serum 90K/Mac-2 binding protein (Mac-2BP) as a response predictor to peginterferon and ribavirin combined treatment in HCV chronic patients. *Immunopharmacology and immunotoxicology* 2008; **30**: 687-700.
- 23 Sasaki, T., Brakebusch, C., Engel, J. & Timpl, R. Mac-2 binding protein is a cell-adhesive protein of the extracellular matrix which self-assembles into ring-like structures and binds beta1 integrins, collagens and fibronectin. *The EMBO journal* 1998; **17**: 1606-13.

Table 1. Demographic and clinical characteristics of 112 patients with chronic

HBV infection

Baseline characteristic	Total n = 112	
	Number	(%)
Male/Female	72/40	(64/36)
Genotype A/B/C/ND	<u>1/8/69/34</u>	<u>(0.9/7.1/62/30)</u>
Pathological findings		
Fibrosis stage 0/1/2/3/4	4/36/26/24/22	(3.5/32/23/21.5/20)
Activity 0/1/2/3	5/32/57/18	(4.5/28.5/51/16)
IFN therapy	24	(21)
NA therapy	63	(56)
HCC development	15	(13)
Death	2	(2)
	Median	(First-third quartiles)
Age (years)	47	(36-57)
Albumin (g/dL)	4.2	(4.1-4.5)
Alanine aminotransferase (IU/L)	66.5	(40-132)
Total bilirubin (mg/dL)	0.86	(0.65-1.08)
Platelet count ($\times 10^9$ /L)	15.9	(11.9-20.95)
AFP (ng/ml)	5.5	(3.3-10.4)
HBeAg-positive	64	(57)
HBeAb-positive	49	(43)
HBV-DNA (log copies/ml)	6.1	(4.9-7.8)
WFA ⁺ -M2BP (COI)	0.97	(0.28-1.93)
HA (ng/mL)	17	(36-114)
Type IV collagen 7S (ng/ml)	6.20	(4.32-7.67)
APRI	0.65	(0.34-1.92)
FIB-4 index	1.1823	(0.5519-2.9406)

Data are expressed as number (%) or median (first-third quartiles).

ND, not determined; IFN, interferon; NA, nucleos(t)ide analogue; HCC,

hepatocellular carcinoma; AFP, α -fetoprotein; HBeAg, hepatitis B e antigen;

HBeAb, hepatitis B e antibody; HBV, hepatitis C virus; HA, hyaluronic acid;

WFA⁺-M2BP; *Wisteria floribunda* agglutinin-positive Mac-2 binding protein; COI,

cut off index; APRI, aspartate aminotransferase (AST)-to-platelet ratio index;

Table 2. Factors associated with HCC development in patients with chronic HBV infection as identified by univariate

and multivariate analyses

Factor	Univariate analysis		Multivariate analysis	
	p value	Chi-square score	p value	Hazard ratio (95% CI)
Age (years) ≥50 (vs. <50)	0.030	4.690	0.280	1.98 (0.57-6.88)
Male (vs. female)	0.172	1.860	0.040	4.14 (1.06-16.06)
Fibrosis stage ≥F2 (vs. <F2)	0.052	3.780		
WFA ⁺ -M2BP (COI) ≥0.71 (vs. <0.71)	0.030	4.600	0.047	8.32 (1.03-67.0)
ALT (IU/L) ≥80 (vs. <80)	0.013	6.160	0.018	0.079 (0.0096-0.65)
HA (ng/mL) ≥68 (vs. <68)	0.020	5.640		
Type IV collagen 7S (ng/ml) ≥5.6 (vs. <5.6)	0.050	3.700		
APRI ≥1.4 (vs. <1.4)	0.940	0.014		
FIB-4 ≥1.4 (vs. <1.4)	0.014	6.049		
Serum albumin (g/dL) ≥4.2 (vs. <4.2)	0.106	2.615		
Platelet count (10 ⁹ /L) <14.5 (vs. ≥14.5)	0.005	7.920	0.031	0.26 (0.075-0.88)
AFP(ng/ml) ≥7 (vs. <7)	0.003	8.664		

NA treatment (vs. others)	0.011	6.511
IFN treatment (vs. others)	0.118	2.439

WFA⁺-M2BP, *Wisteria floribunda* agglutinin-positive Mac-2 binding protein; HA, hyaluronic acid; APRI, aspartate aminotransferase (AST)-to-platelet ratio index; AFP, α -fetoprotein

Figure legends

Figure 1. Correlation between serum WFA⁺-M2BP level and liver fibrosis stage. The top and bottom of each box represent the first and third quartiles, respectively. The lines across the boxes indicate median values.

Figure 2. Correlation between WFA⁺-M2BP level and other surrogate fibrosis markers. Data were analyzed by Spearman's rank correlation coefficient test.

Figure 3. Diagnostic ability of serum WFA⁺-M2BP value to assess liver fibrosis stage. ROC curves and AUROCs for the diagnosis of fibrosis stage (a) F2-4, (b) F3-4, and (c) F4.

Figure 4. Diagnostic ability of other fibrosis markers for the diagnosis of F2-4 liver fibrosis stage. ROC curves and AUROCs for (a) HA, (b) type IV collagen 7S, (c) APRI, (d) FIB-4, (e) serum albumin, and (f) platelet count.

Figure 5. Cumulative incidence rate of HCC in patients with HBV infection. Patients were categorized into two groups according to WFA⁺-M2BP value. Cumulative incidences of HCC were analyzed using the Kaplan-Meier method.

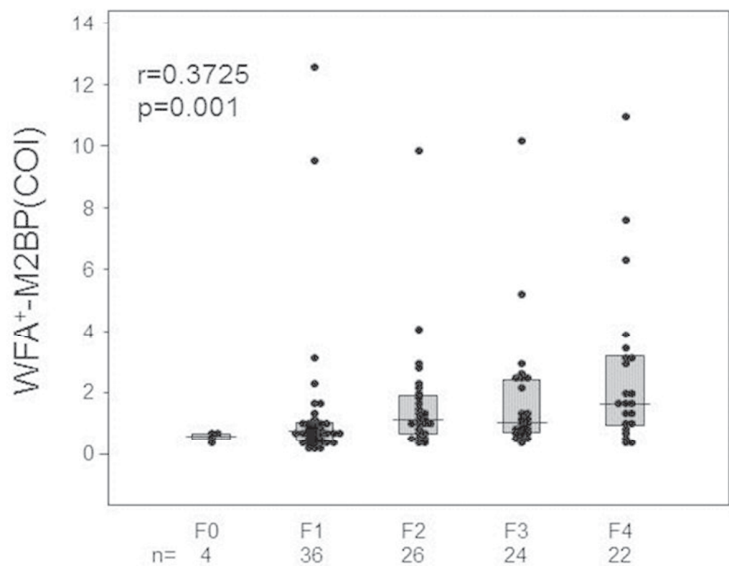


Figure 1. Correlation between serum WFA+-M2BP level and liver fibrosis stage. The top and bottom of each box represent the first and third quartiles, respectively. The lines across the boxes indicate median values.

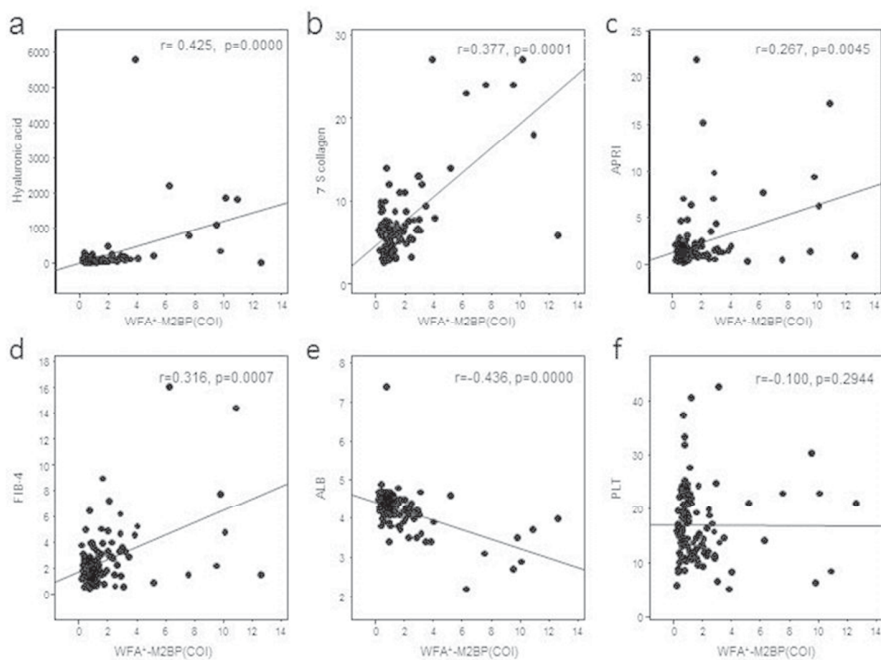


Figure 2. Correlation between WFA+-M2BP level and other surrogate fibrosis markers. Data were analyzed by Spearman's rank correlation coefficient test.

196x143mm (124 x 128 DPI)

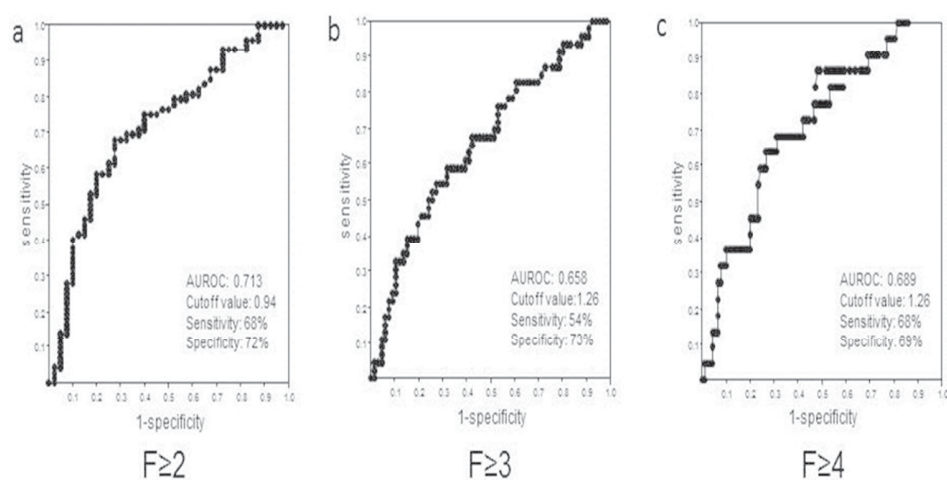


Figure 3. Diagnostic ability of serum WFA+-M2BP value to assess liver fibrosis stage. ROC curves and AUROCs for the diagnosis of fibrosis stage (a) F2-4, (b) F3-4, and (c) F4.
239x112mm (102 x 150 DPI)

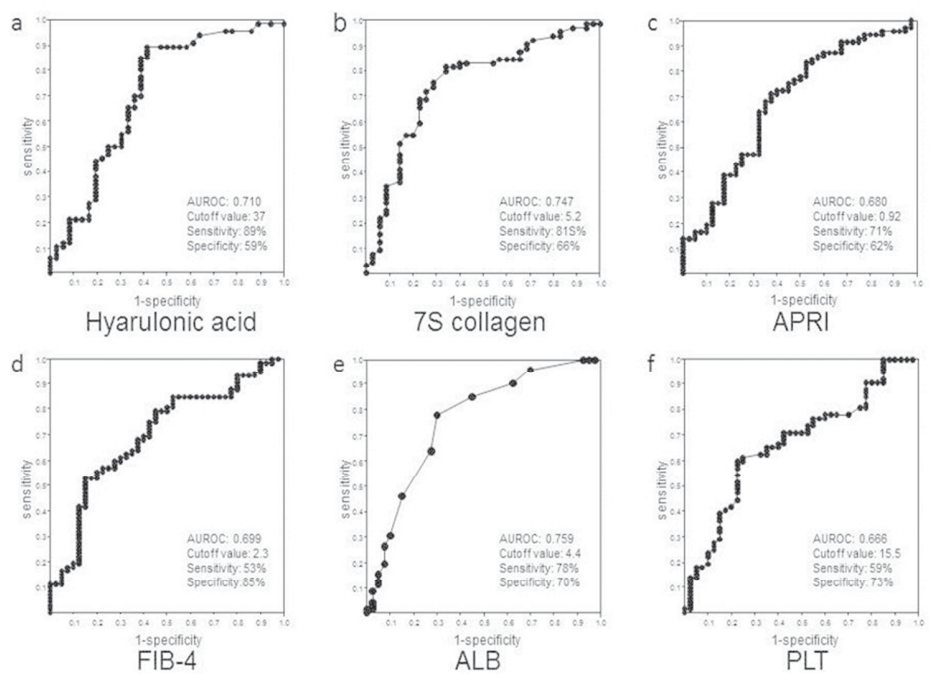


Figure 4. Diagnostic ability of other fibrosis markers for the diagnosis of F2-4 liver fibrosis stage. ROC curves and AUROCs for (a) HA, (b) type IV collagen 7S, (c) APRI, (d) FIB-4, (e) serum albumin, and (f) platelet count.
245x172mm (99 x 106 DPI)

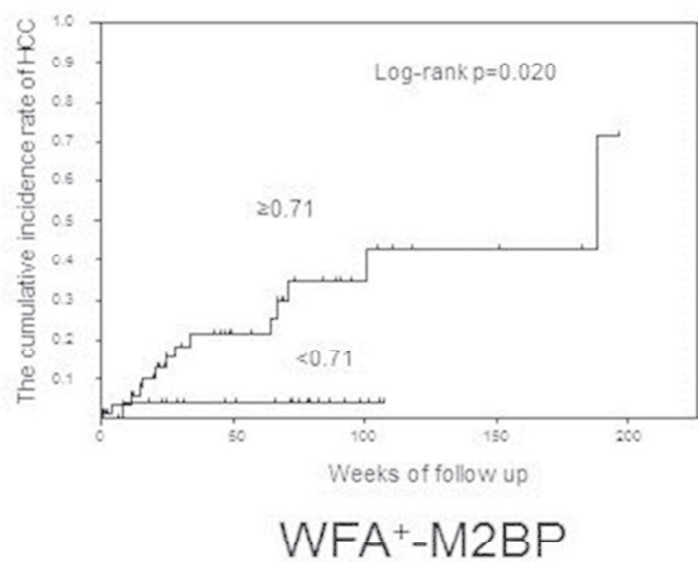


Figure 5. Cumulative incidence rate of HCC in patients with HBV infection. Patients were categorized into two groups according to WFA+-M2BP value. Cumulative incidences of HCC were analyzed using the Kaplan-Meier method.
118x92mm (150 x 150 DPI)