

RESEARCH ARTICLE

Wisteria floribunda agglutinin-positive Mac-2-binding protein in the prediction of disease severity in chronic hepatitis B patients

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Abstract

Background

Wisteria floribunda agglutinin-positive Mac-2-binding protein (WFA⁺-M2BP) was a novel marker of liver fibrosis. We aimed to investigate WFA⁺-M2BP level in assessing liver fibrosis in patients with chronic hepatitis B (CHB) infection.

Methods

A total of 160 CHB patients, who received a liver biopsy, were consecutively recruited. Serum WFA⁺-M2BP level was quantified at the time point of biopsy. The results were compared with histopathological manifestations and clinical characteristics of the patients.

Results

The median WFA⁺-M2BP level, aspartate aminotransferase-to-platelet ratio (APRI) and Fibrosis-4 (FIB-4) index were 1.20 COI, 1.19, and 1.63, respectively. Fifty-one (31.9%) patients had advanced fibrosis. There was a significant increase of WFA⁺-M2BP levels in parallel to necroinflammation/fibrosis stages. The areas under the receiver operating characteristic curve (AUROC) of WFA⁺-M2BP level for predicting fibrosis stages were 0.780 of F2, 0.785 of F3, and 0.769 of F4, respectively (all $p < 0.001$). The multivariate analysis identified age (Odds ratio [OR] 1.05, 95% confidence interval [CI]: 1.010–1.092, $p = 0.014$), platelet (OR: 0.99, 95%CI: 0.980–0.998, $p = 0.013$), and WFA⁺-M2BP level (OR: 1.97, 95% CI: 1.299–2.984, $p = 0.001$) as independent factors associated with advanced fibrosis. Combination of age, platelet and WFA⁺-M2BP level achieved a better diagnostic performance for advanced fibrosis (AUROC: 0.732, accuracy: 81.3%) than APRI (AUROC: 0.577, accuracy: 63.8%) or FIB-4 index (AUROC: 0.691, accuracy: 75.6%).

Competing interests: The authors have declared that no competing interests exist.

Conclusion

WFA⁺-M2BP had a good performance indistinguishing liver fibrosis in CHB patients. The combination of age, platelet, and WFA⁺-M2BP addressed more accuracy in identifying patients with advanced fibrosis.

Introduction

Hepatitis B virus (HBV) infection remained the leading cause of liver cirrhosis (LC) and hepatocellular carcinoma (HCC) worldwide, especially in the Asia-Pacific region.[1] HBV viral load, hepatitis B e antigen (HBeAg) and liver fibrosis severity were associated with the development of LC and HCC in patients with chronic HBV infection (CHB).[2,3] With long-term HBV antiviral therapy, nucleos(t)ide analogs, the risk of LC and HCC decreased and regression of fibrosis was also achieved.[4,5] The current regional guidelines recommended HBV antiviral therapy in patients with high HBV viral load with significant fibrosis (Metavir \geq F2), even the alanine aminotransferase (ALT) level was below the 2-fold upper limit of normal (ULN).[6,7] Therefore, disease severity assessment, namely fibrosis staging, is essential in the decision-making of antiviral therapy as well as complication evaluation in a clinical setting.

Fibrosis development is a subtle process during the natural course of CHB. Significant fibrosis development is an important checkpoint during fibrogenesis, and which implies that antiviral therapy should be implemented.[8] Liver biopsy is the current gold standard to assess the severity of necroinflammation and fibrosis.[9] However, liver biopsy was largely limited by the invasiveness and potential complications.[10] In addition, the sampling error, and inter-observer discrepancy were other limitations for the accuracy of liver biopsy. Recently, several non-invasive tools, including mechanical and serological methods, for liver fibrosis assessment have been vigorously developed.[11] Among the serological methods, aspartate aminotransferase (AST)-to-platelet ratio (APRI) index and Fibrosis-4 (FIB-4) index were commonly used and were easily assessed. However, the predictive value in CHB remained uncertain.[12,13] Wisteria floribunda agglutinin-positive Mac-2-binding protein (WFA⁺-M2BP), a product released during fibrogenesis process, was recently developed as a novel serological marker of liver fibrosis.[14,15] Recent studies demonstrated a significant association between serum WFA⁺-M2BP level and severity of liver fibrosis in subjects with hepatitis C virus infection and non-alcoholic steatohepatitis.[16–18] The comparison of WFA⁺-M2BP and directly fibrosis assessment, Fibroscan, had also been studied. In the recent study investigating a large scale of chronic hepatitis C patients, the authors demonstrated that serum WFA⁺-M2BP was superior to other non-invasive markers in the prediction of significant fibrosis and had the greatest specificity in the diagnosis of cirrhosis.[19] In addition, the serum WFA⁺-M2BP level could predict the development of liver-related events, including the occurrence of decompensation and the development of HCC.[16,20,21] However, application of WFA⁺-M2BP level in assessing liver fibrosis has rarely been investigated in CHB patients.

Consequently, we conducted the study aimed to investigate the performance of serum WFA⁺-M2BP level in the prediction of liver fibrosis in CHB patients. We also aimed to explore an accurate predictive model for significant fibrosis in CHB.

Materials and methods

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the ethics committee of Kaohsiung Medical University Hospital (KMUHIRB-E

(II)-20150130). Written informed consent was obtained from all of the patients. CHB patients who received a liver biopsy prior to antiviral therapy during 2000 and 2015, were recruited in the present study. The inclusion criteria included 1). Positive serum hepatitis B surface antigen (HBsAg) for more than 6 months; 2). The results of the liver biopsy consistent with the diagnosis of CHB based on the severity of necroinflammation and fibrosis. The exclusion criteria included the followings: hepatitis C virus or human immunodeficiency virus co-infection; evidence of other liver diseases such as α -1 anti-trypsin deficiency, hemochromatosis, Wilson's disease, etc.

The liver biochemistry, HBV serological markers, and HBV DNA level were tested at enrollment. HBsAg and HBeAg were detected by commercially available enzyme-linked immunosorbent assay kits (Abbott Laboratories, North Chicago, IL, USA). The serum HBV DNA levels were determined using a Cobas AmpliPrep/Cobas TaqMan HBV assay (CAP/CTM, version 2.0, Roche Diagnostics, Indianapolis, IN, USA; the dynamic range was 20 IU/mL–1.7x10⁸ IU/mL). APRI and FIB-4 index were calculated using the formula: APRI = AST [U/LN] x 100/platelet count (x10⁹/L)[22]; FIB-4 = age (years) x AST [U/L]/(platelet count [10⁹/L] x (ALT [U/L])^{1/2}). [23]

Measurement of WFA⁺-M2BP

Stored serum obtained at the same time of liver biopsy was used for WFA⁺-M2BP measurement. Serum WFA⁺-M2BP level was quantified by lectin-Ab sandwich immunoassay, using an automated immunoanalyzer (HISCL-800; Sysmex Co, Hyogo, Japan).[15] The measured WFA⁺-M2BP levels were further indexed using the following equation:

$$\text{Cutoff index (COI)} = ([\text{WFA}^+\text{-M2BP}]_{\text{sample}} - [\text{WFA}^+\text{-M2BP}]_{\text{NC}}) / ([\text{WFA}^+\text{-M2BP}]_{\text{PC}} - [\text{WFA}^+\text{-M2BP}]_{\text{NC}})$$

Where [WFA⁺-M2BP]_{sample} means WFA⁺-M2BP level of the serum sample, NC means negative control, and PC means positive control.

Histopathology

All enrolled patients received ultrasound-guided liver core biopsy using a 16- or 18-gauge biopsy needle. Two experienced hepato-pathologists reviewed and determined the grade of necroinflammation (A0–3) and stage of fibrosis (F0–4) for each specimen according to the METAVIR scoring system.[24] The significant fibrosis was defined as \geq F2 and advanced fibrosis was \geq F3.

Statistical analysis

Descriptive statistics were computed for all of the variables, including the median and 25th, 75th percentile for continuous variables. For the categorical variables, the frequencies and percentages were estimated. The Mann-Whitney U test was used to compare continuous variables and the chi-squared and Fisher's exact tests were used to compare categorical variables. Binary logistic regression analysis was used to identify the independent factors associated with significant fibrosis. Kruskal-Wallis H test was used to compare serum WFA⁺-M2BP level between different grades of necroinflammation and stages of fibrosis. The Spearman rank correlation coefficient test was used for the correlation between continuous variables and WFA⁺-M2BP level. Area under the receiver operating characteristics curve (AUROC) analysis was done to evaluate the diagnostic accuracy of WFA⁺-M2BP, APRI and FIB-4 index for liver fibrosis. The optimal cutoff value for fibrosis stage was determined by the Youden's index. All tests were two-sided, and $p < 0.05$ was considered significant. All analyses were performed using the SPSS 19.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Patients' characteristics

A total of 160 CHB patients (123 males, median age 40 years) were consecutively recruited. Table 1 showed the demographic characteristics of the patients. Fifty-six (35.0%) of the patients were HBeAg positive. The mean HBV DNA level was 5.9 log₁₀ IU/ml. The median platelet, AST, and ALT levels were 182.0 × 10³/μL, 77.5, and 133.0 U/L, respectively. The mean serum WFA⁺-M2BP level, APRI, and FIB-4 index were 1.20, 1.19, and 1.63, respectively. The histopathological results revealed 51.9% and 55.0% of patients were of significant necroinflammation and significant fibrosis, respectively.

Correlation between WFA⁺-M2BP level and laboratory parameters

The serum WFA⁺-M2BP level was significantly correlated with age ($r = 0.354$, $p < 0.001$, 95% CI 0.033–0.080), AST ($r = 0.179$, $p = 0.023$, 95% CI 0.000–0.003), APRI ($r = 0.273$, $p < 0.001$, 95% CI 0.052–0.180), FIB-4 index ($r = 0.484$, $p < 0.001$, 95% CI 0.214–0.383) and inversely correlated with platelet count ($r = 0.433$, $p < 0.001$, 95% CI -0.019 – -0.010). FIB-4 index showed the highest coefficient correlation level, followed by platelet count. There were no significant correlations between ALT, HBV DNA levels and WFA⁺-M2BP.

Correlation between WFA⁺-M2BP level and histopathology

The correlations between serum WFA⁺-M2BP level and necroinflammation grade and fibrosis stage were shown in Fig 1. There was a significant increase in serum WFA⁺-M2BP level

Table 1. Characteristics of all the 160 patients with chronic HBV infection.

	All (N = 160)
Age, years	40.0 (30.3, 52.0)
Male gender	123 (76.9)
platelet, ×10 ³ /μl	182.0 (140.3, 225.8)
AST, U/L	77.5 (54.0, 144.5)
ALT, U/L	133.0 (83.0, 243.8)
Positive HBeAg	56 (35.0)
HBV DNA, log ₁₀ IU/ml	5.9 (4.1, 7.4)
WFA ⁺ -M2BP, COI	1.20 (0.54, 1.99)
APRI	1.19 (0.67, 2.31)
FIB-4	1.63 (0.90, 3.33)
Necro-inflammation grade	
A0	48 (30.0)
A1	29 (18.1)
A2	54 (33.8)
A3	29 (18.1)
Fibrosis stage	
F0	21 (13.1)
F1	51 (31.9)
F2	37 (23.1)
F3	25 (15.6)
F4	26 (16.3)

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; WFA⁺-M2BP, wisteria floribunda agglutinin positive Mac-2-binding protein; APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4.

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according to necroinflammation grading. (Fig 1A) The median (25th- 75th percentile) serum WFA⁺-M2BP levels were 0.75 COI (0.46–1.32) of A0, 1.47 COI (1.14–2.26) of A1, 0.92 COI (0.42–1.63) of A2, and 2.57 COI (1.36–5.88) of A3, respectively (p for linear trend <0.001).

There was a significant correlation between serum WFA⁺-M2BP level and fibrosis stages. (Fig 1B) The median (25th- 75th percentile) serum WFA⁺-M2BP levels were 0.63 COI (0.29–1.06) of F0, 0.64 COI (0.38–1.34) of F1, 1.36 COI (0.84–1.80) of F2, 1.65 COI (1.28–3.04) of F3, and 2.70 COI (0.94–6.17) of F4, respectively (p for linear trend <0.001). There was no significant difference of serum WFA⁺-M2BP level according to fibrosis staging when stratified by the clinical profiles such as gender, age, HBeAg, HBV DNA level, and ALT level.

Comparison of the diagnostic performance of WFA⁺-M2BP, FIB-4, and APRI for liver fibrosis

The diagnostic performance of WFA⁺-M2BP, FIB-4, and APRI in the assessment of fibrosis stage was compared. The accuracy of serum WFA⁺-M2BP level for each fibrosis stage was 0.706 of ≥F1 (0.596–0.816, p = 0.002), 0.780 of ≥F2 (0.709–0.851, p <0.001), 0.785 of ≥F3 (0.707–0.864, p <0.001), and 0.769 of F4 (0.660–0.878, p <0.001), respectively. Significant diagnostic accuracy was observed for FIB-4 index in assessing fibrosis stages. The significant diagnostic accuracy of APRI index was only observed in assessing fibrosis stage ≥F3 (0.638, 0.547–0.728, p = 0.005). (Fig 2)

Based on Youden’s index from the AUROC, the optimal cutoff value of serum WFA⁺-M2BP level was determined. The optimal cutoff value was for F1 was 0.960, yielding the sensitivity of 61.2%, specificity of 76.2%, positive predictive value (PPV) of 94.4%, negative predictive value (NPV) of 22.9%, and accuracy of 63.1%, respectively. The optimal cutoff value was 1.345 for F2, yielding the sensitivity of 65.9%, specificity of 80.6%, PPV of 80.6%, NPV of 65.9%, and accuracy of 72.5%, respectively. The optimal cutoff values for F3 and F4 were 1.535, and 1.665, respectively. The sensitivity, specificity, PPV, and NPV were shown in Table 2.

The factors associated with advanced fibrosis were further analyzed. In univariate analysis, age (p <0.001), platelet (p <0.001), ALT (p = 0.015), and WFA⁺-M2BP level (p <0.001) were factors associated with significant fibrosis. The multivariate analysis adjusted above parameters revealed that age (OR 1.05, 95% CI: 1.010–1.092, p = 0.014), platelet count (OR: 0.99, 95% CI: 0.980–0.998, p = 0.013), and WFA⁺-M2BP level (OR: 1.97, 95% CI: 1.299–2.984, p = 0.001) were independently associated factors (Table 3).

WFA⁺-M2BP combined with age, and platelet to predict advanced liver fibrosis

We further aimed to develop a predictive model for advanced fibrosis. The prior analysis revealed age, platelet, and WFA⁺-M2BP level were the independent factors associated with

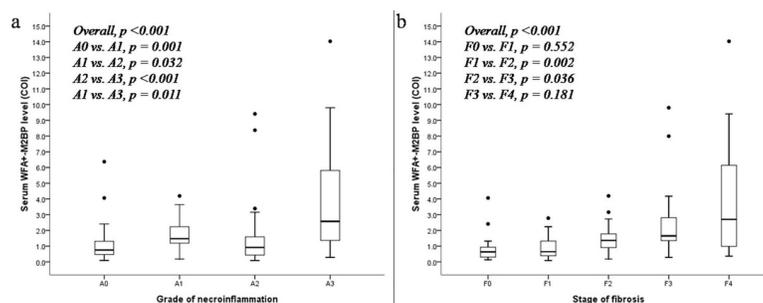


Fig 1. The correlations between serum WFA⁺-M2BP level and necroinflammation grade (a) and fibrosis stage (b).

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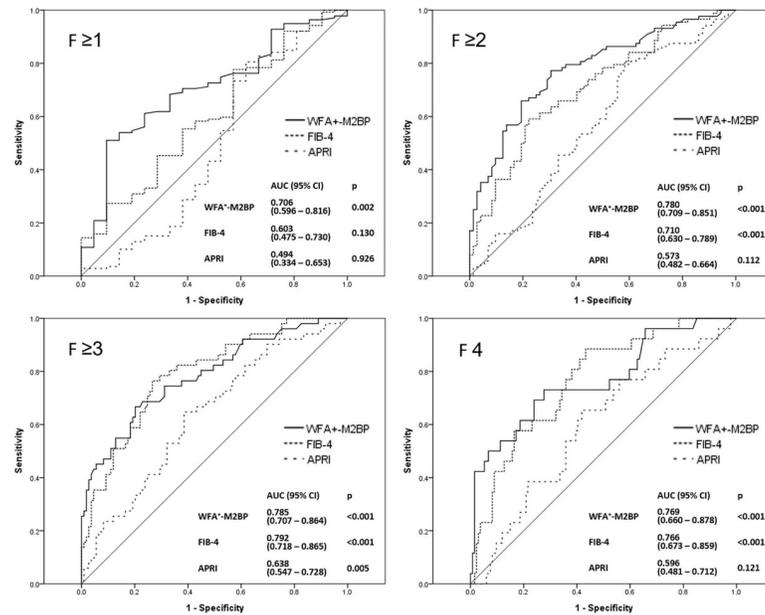


Fig 2. The diagnostic ability of WFA⁺-M2BP, FIB-4, and APRI in the assessment of fibrosis stage.

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severe fibrosis. The optimal cutoff value of the independent factors was determined as age of 40 years, platelet of $165 \times 10^3/\mu\text{l}$, and WFA⁺-M2BP level of 1.535 COI by Youden’s index from the AUROC. The comparison of diagnostic performance of age, platelet, WFA⁺-M2BP level, a combination of the threeparameters, APRI, APRI plus Age, FIB-4, and FIB-4 plus Age score was shown as Table 4. Comparing to each parameter, the combination model showed a better performance in terms of specificity, positive predictive value, and accuracy.

Discussion

In addition to ultrasound-based methods, several serum biomarkers have been vigorously developing and validating for assessing the degree of fibrosis in the past decade. WFA⁺-M2BP measurement is a newly-developed biomarker which has been validated for disease severity assessment in chronic liver diseases such as chronic hepatitis C and non-alcoholic fatty liver disease. In the present study, we aimed to explore the performance of serum WFA⁺-M2BP level in the prediction of liver fibrosis in CHB patients. Our results demonstrated that serum WFA⁺-M2BP is competent in evaluating disease severity in CHB. We also demonstrated a model of combined age, platelet, and WFA⁺-M2BP to accurately predict advanced liver fibrosis. Our results thus provided the evidence for clinical application of combined non-invasive tools in this aspect.

Table 2. Diagnostic performance and optimal cutoff of serum WFA⁺-M2BP level for assessing each fibrosis stage.

	AUC	Cutoff	p	Sensitivity	Specificity	PPV	NPV	Accuracy
F ≥ 1	0.706	0.960	0.002	61.2%	76.2%	94.4%	22.9%	63.1%
F ≥ 2	0.780	1.345	<0.001	65.9%	80.6%	80.6%	65.9%	72.5%
F ≥ 3	0.785	1.535	<0.001	66.7%	79.8%	60.7%	83.7%	75.6%
F 4	0.769	1.665	<0.001	69.2%	76.1%	36.0%	92.7%	75.0%

AUC, area under the receiver operating characteristics curve; PPV, positive predictive value; NPV, negative predictive value.

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Table 3. Uni- and multivariate analysis of factors associated with severe liver fibrosis.

	Severe fibrosis (≥ F3)	Non-Severe fibrosis (< F3)	p	Adjusted		
	N = 51	N = 109		OR	95% CI	p
Age, years	52.0 (42.0, 57.0)	37.0 (28.5, 45.5)	<0.001	1.05	1.010–1.092	0.014
Male gender	40 (78.4)	83 (76.1)	0.842			
platelet, x10 ³ /μl	137.0 (99.0, 179.0)	207.0 (163.0, 232.0)	<0.001	0.99	0.980–0.998	0.013
AST, U/L	87.0 (52.0, 140.0)	76.0 (54.0, 148.5)	0.895			
ALT, U/L	100.0 (58.0, 224.0)	141.0 (92.5, 245.5)	0.028	1.00	0.997–1.001	0.213
WFA ⁺ -M2BP, COI	2.11 (1.20, 4.59)	0.86 (0.44, 1.47)	<0.001	1.97	1.299–2.984	0.001
Positive HBeAg	23 (45.1)	33 (30.3)	0.077			
HBV DNA, log ₁₀ IU/ml	6.3 (4.9, 7.4)	5.7 (3.8, 7.3)	0.152			

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; WFA⁺-M2BP, wisteria floribunda agglutinin positive Mac-2-binding protein; APRI, aspartate aminotransferase-to-platelet ratio; FIB-4, Fibrosis-4.

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The main pathogenic mechanism of hepatocarcinogenesis is underlying necroinflammation and its subsequent fibrogenesis. Therefore, severity estimation of liver fibrosis is important for treatment efficacy, prognosis, HCC surveillance and determining the optimal treatment strategies. Liver core biopsy is much hindered by the potential complications, intra-observer variability, sampling errors and patients' willingness.[25–27] The investigation of non-invasive methods is a feasible way in a clinical setting. WFA⁺-M2BP is a newly developed serum biomarker for liver fibrosis assessment. M2BP is a glycoprotein which secreted from many cells, but mainly from hepatocytes. Fluctuations of M2BP in the sera increase following the progression of liver fibrosis. The diagnostic accuracy and clinical manifestation of WFA⁺-M2BP had been well reported in patients with chronic hepatitis C, non-alcoholic fatty liver disease, and in the prediction of HCC development or recurrence.[18,20,21,28–31]

Liver fibrosis stage should be taken into consideration regarding the initiation of antiviral therapy among CHB patients according to current regional guidelines. The performance of WFA⁺-M2BP in the diagnosis of liver fibrosis in CHB patients had been reported in previous studies. In most of the studies, WFA⁺-M2BP was reported to have similar diagnostic efficacy with other biomarkers in distinguishing each fibrosis stage.[32–34] Our results echoed the previous study conducted by Zou et al showing that WFA⁺-M2BP as a useful marker to assess liver fibrosis in CHB patients.[35] We also developed a model combined WFA⁺-M2BP, age, and platelet to better predict advanced fibrosis which was not reported in the previous studies. Although the sensitivity of the combination model was only 51.0%, the combination model still could distinguish patients who were really not advanced fibrosis because of the high

Table 4. Diagnostic performance of Age, Platelet, WFA⁺-M2BP, and the combination for assessing advanced liver fibrosis.

	AUC	p	Sensitivity	Specificity	PPV	NPV	Accuracy
Age >40 years	0.741	<0.001	80.4%	67.9%	53.9%	88.1%	71.9%
Platelet <165x 10 ³ /μl	0.739	<0.001	72.5%	75.2%	57.8%	85.4%	74.4%
WFA ⁺ -M2BP ≥1.535COI	0.785	<0.001	66.7%	79.8%	60.7%	83.7%	75.6%
Combination of Age, Platelet, and WFA ⁺ -M2BP	0.732	<0.001	51.0%	95.4%	83.9%	80.6%	81.3%
APRI ≥2.0	0.577	0.065	41.2%	74.3%	42.9%	73.0%	63.8%
APRI ≥2.0 plus Age >40 years	0.622	0.013	37.3%	87.2%	57.6%	74.8%	71.3%
FIB-4 ≥3.25	0.691	<0.001	51.0%	87.2%	65.0%	79.2%	75.6%
FIB-4 ≥3.25 plus Age >40 years	0.695	<0.001	49.0%	89.9%	69.4%	79.0%	76.9%

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specificity of the combination model. The result also showed a better diagnostic performance than APRI, FIB-4, or their combination index. To our knowledge, the present study was the first study addressing the combination of WFA⁺-M2BP and other markers to accurately predict liver fibrosis. In the future, a prospective, larger scale of study is needed for further validation of the novel findings of the current study.

The elastography, such as Fibroscan, which measured the liver fibrosis directly had been well developed. It would be a more direct and meaningful comparison to comparing WFA⁺-M2BP against Fibroscan. However, we did not perform the comparison because of the unavailable equipment at the time of data collection. To differentiate advanced fibrosis, FIB-4 ≥ 3.25 and APRI ≥ 2 were well studied and accepted values. We compared the diagnostic performance of the combination model with the established FIB-4, APRI, and with age combination model. The results showed a better performance using the WFA⁺-M2BP combination model. The WFA⁺-M2BP itself had shown a better predictive value than FIB-4 and APRI. That might explain why the WFA⁺-M2BP combination model was better than FIB-4 or APRI combination model. Although that, a further large scale study remains needed to confirm the results.

In conclusion, serum WFA⁺-M2BP level was a useful non-invasive marker for liver fibrosis assessment. The combination of age, platelet, and WFA⁺-M2BP provided a better model to determine advanced liver fibrosis in CHB patients.

Supporting information

S1 File. Dataset of all enrolled patients.
(XLSX)

Author Contributions

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Writing – review & editing: Jee-Fu Huang, Jyh-Jou Chen.

References

1. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, et al. (2006) A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 354: 1001–1010. <https://doi.org/10.1056/NEJMoa051285> PMID: 16525137
2. Lin TM, Chen CJ, Lu SN, Chang AS, Chang YC, et al. (1991) Hepatitis B virus e antigen and primary hepatocellular carcinoma. *Anticancer Res* 11: 2063–2065. PMID: 1663719
3. Chen CJ, Yang HI, Su J, Jen CL, You SL, et al. (2006) Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 295: 65–73. PMID: 16391218

4. Lin D, Yang HI, Nguyen N, Hoang J, Kim Y, et al. (2016) Reduction of chronic hepatitis B-related hepatocellular carcinoma with anti-viral therapy, including low risk patients. *Aliment Pharmacol Ther* 44: 846–855. <https://doi.org/10.1111/apt.13774> PMID: 27549411
5. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, et al. (2013) Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. *Hepatology* 58: 98–107. <https://doi.org/10.1002/hep.26180> PMID: 23213040
6. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, et al. (2016) Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int* 10: 1–98.
7. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, et al. (2016) AASLD guidelines for treatment of chronic hepatitis B. *Hepatology* 63: 261–283. <https://doi.org/10.1002/hep.28156> PMID: 26566064
8. Liaw YF (2009) Natural history of chronic hepatitis B virus infection and long-term outcome under treatment. *Liver Int* 29 Suppl 1: 100–107.
9. Germani G, Hytiroglou P, Fotiadu A, Burroughs AK, Dhillon AP (2011) Assessment of fibrosis and cirrhosis in liver biopsies: an update. *Semin Liver Dis* 31: 82–90. <https://doi.org/10.1055/s-0031-1272836> PMID: 21344353
10. Bravo AA, Sheth SG, Chopra S (2001) Liver biopsy. *N Engl J Med* 344: 495–500. <https://doi.org/10.1056/NEJM200102153440706> PMID: 11172192
11. Castera L (2015) Noninvasive Assessment of Liver Fibrosis. *Dig Dis* 33: 498–503. <https://doi.org/10.1159/000374097> PMID: 26159265
12. Lin ZH, Xin YN, Dong QJ, Wang Q, Jiang XJ, et al. (2011) Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology* 53: 726–736. <https://doi.org/10.1002/hep.24105> PMID: 21319189
13. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, et al. (2006) Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 43: 1317–1325. <https://doi.org/10.1002/hep.21178> PMID: 16729309
14. Kuno A, Ikehara Y, Tanaka Y, Ito K, Matsuda A, et al. (2013) A serum "sweet-doughnut" protein facilitates fibrosis evaluation and therapy assessment in patients with viral hepatitis. *Sci Rep* 3: 1065. <https://doi.org/10.1038/srep01065> PMID: 23323209
15. Kuno A, Sato T, Shimazaki H, Unno S, Saitou K, et al. (2013) Reconstruction of a robust glycodiagnostic agent supported by multiple lectin-assisted glycan profiling. *Proteomics Clin Appl* 7: 642–647. <https://doi.org/10.1002/prca.201300010> PMID: 23640794
16. Yamasaki K, Tateyama M, Abiru S, Komori A, Nagaoka S, et al. (2014) Elevated serum levels of Wisteria floribunda agglutinin-positive human Mac-2 binding protein predict the development of hepatocellular carcinoma in hepatitis C patients. *Hepatology* 60: 1563–1570. <https://doi.org/10.1002/hep.27305> PMID: 25042054
17. Abe M, Miyake T, Kuno A, Imai Y, Sawai Y, et al. (2015) Association between Wisteria floribunda agglutinin-positive Mac-2 binding protein and the fibrosis stage of non-alcoholic fatty liver disease. *J Gastroenterol* 50: 776–784. <https://doi.org/10.1007/s00535-014-1007-2> PMID: 25326152
18. Ura K, Furusyo N, Ogawa E, Hayashi T, Mukae H, et al. (2016) 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. *Aliment Pharmacol Ther* 43: 114–124. <https://doi.org/10.1111/apt.13431> PMID: 26503582
19. Xu H, Kong W, Liu L, Chi X, Wang X, et al. (2017) Accuracy of M2BPGi, compared with Fibro Scan(R), in analysis of liver fibrosis in patients with hepatitis C. *BMC Gastroenterol* 17: 62. <https://doi.org/10.1186/s12876-017-0618-5> PMID: 28486931
20. Sasaki R, Yamasaki K, Abiru S, Komori A, Nagaoka S, et al. (2015) 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* 10: e0129053. <https://doi.org/10.1371/journal.pone.0129053> PMID: 26070204
21. Heo JY, Kim SU, Kim BK, Park JY, Kim DY, et al. (2016) Use of Wisteria Floribunda Agglutinin-Positive Human Mac-2 Binding Protein in Assessing Risk of Hepatocellular Carcinoma Due to Hepatitis B Virus. *Medicine (Baltimore)* 95: e3328.
22. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, et al. (2003) A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 38: 518–526. <https://doi.org/10.1053/jhep.2003.50346> PMID: 12883497
23. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, et al. (2007) FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 46: 32–36. <https://doi.org/10.1002/hep.21669> PMID: 17567829

24. (1994) Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. *Hepatology* 20: 15–20. PMID: [8020885](#)
25. Akay S, Karasu Z, Noyan A, Pala S, Musoglu A, et al. (2007) Liver biopsy: is the pain for real or is it only the fear of it? *Dig Dis Sci* 52: 579–581. <https://doi.org/10.1007/s10620-006-9493-6> PMID: [17211704](#)
26. Huang JF, Hsieh MY, Dai CY, Hou NJ, Lee LP, et al. (2007) The incidence and risks of liver biopsy in non-cirrhotic patients: An evaluation of 3806 biopsies. *Gut* 56: 736–737.
27. Myers RP, Fong A, Shaheen AA (2008) Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. *Liver Int* 28: 705–712. <https://doi.org/10.1111/j.1478-3231.2008.01691.x> PMID: [18433397](#)
28. Nishikawa H, Takata R, Enomoto H, Yoh K, Kishino K, et al. (2017) Proposal of a predictive model for advanced fibrosis containing *Wisteria floribunda* agglutinin-positive Mac-2-binding protein in chronic hepatitis C. *Hepatol Res* 47: E74–E84. <https://doi.org/10.1111/hepr.12724> PMID: [27075409](#)
29. Hasegawa K, Takata R, Nishikawa H, Enomoto H, Ishii A, et al. (2016) Impact of *Wisteria floribunda* Agglutinin-Positive Mac-2-Binding Protein in Patients with Hepatitis C Virus-Related Compensated Liver Cirrhosis. *Int J Mol Sci* 17.
30. Sato S, Genda T, Ichida T, Amano N, Sato S, et al. (2016) Prediction of Hepatocellular Carcinoma Development after Hepatitis C Virus Eradication Using Serum *Wisteria floribunda* Agglutinin-Positive Mac-2-Binding Protein. *Int J Mol Sci* 17.
31. Toyoda H, Kumada T, Tada T, Kaneoka Y, Maeda A, et al. (2016) Serum WFA⁺-M2BP levels as a prognostic factor in patients with early hepatocellular carcinoma undergoing curative resection. *Liver Int* 36: 293–301. <https://doi.org/10.1111/liv.12907> PMID: [26134114](#)
32. Jekarl DW, Choi H, Lee S, Kwon JH, Lee SW, et al. (2018) Diagnosis of Liver Fibrosis With *Wisteria floribunda* Agglutinin-Positive Mac-2 Binding Protein (WFA-M2BP) Among Chronic Hepatitis B Patients. *Ann Lab Med* 38: 348–354. <https://doi.org/10.3343/alm.2018.38.4.348> PMID: [29611385](#)
33. Nishikawa H, Hasegawa K, Ishii A, Takata R, Enomoto H, et al. (2016) A proposed predictive model for advanced fibrosis in patients with chronic hepatitis B and its validation. *Medicine (Baltimore)* 95: e4679.
34. Xiao G, Yang J, Yan L (2015) Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: a systemic review and meta-analysis. *Hepatology* 61: 292–302. <https://doi.org/10.1002/hep.27382> PMID: [25132233](#)
35. Zou X, Zhu MY, Yu DM, Li W, Zhang DH, et al. (2017) Serum WFA⁺-M2BP levels for evaluation of early stages of liver fibrosis in patients with chronic hepatitis B virus infection. *Liver Int* 37: 35–44. <https://doi.org/10.1111/liv.13188> PMID: [27300763](#)