RESEARCH ARTICLE

M2BPGi as a potential diagnostic tool of cirrhosis in Chinese patients with Hepatitis B virus infection

Bin Wei¹ | Shu Feng¹ | Enqiang Chen² | Dongdong Li¹ | Tingting Wang¹ | Yu Gou¹ | Tingting Yang¹ | Dongmei Zhang² | Chuanmin Tao¹ | Hong Tang²

¹Department of Laboratory Medicine/
Clinical Research Center of Laboratory
Medicine, West China Hospital, Sichuan
University, Chengdu, Sichuan Province, China

²Center of Infectious Diseases, West China
Hospital, Sichuan University, Chengdu,
Sichuan Province, China

Correspondence

Chuanmin Tao, Department of Laboratory
Medicine/Clinical Research Center of
Laboratory Medicine, West China Hospital,
Sichuan University, Chengdu, Sichuan
Province, China.

and

Hong Tang, Center of Infectious Diseases,
West China Hospital, Sichuan University,
Chengdu, Sichuan Province, China.

Emails: taochuanmin@sina.com; htang6198@
hotmail.com

Background: M2BPGi is a novel serum glycomarker of liver fibrosis. In this study, we aimed to evaluate the efficacy of M2BPGi for predicting liver fibrosis and disease progression in Chinese hepatitis B virus (HBV) infected patients.

Methods: We enrolled 228 HBV infected patients with different status of liver fibrosis diagnosed using FibroScan. We analyzed the diagnostic accuracy of M2BPGi, and compared it with AST-to-platelet ratio (APRI), FIB-4 index, AST to ALT ratio (AAR), and RDW to platelet ratio (RPR). We performed receiver operating characteristics curve (ROC) to evaluate the diagnostic performance of M2BPGi for significant fibrosis and cirrhosis.

Results: Median M2BPGi values in each fibrosis stage were: 0.88 cut-off index (COI) in F0-1, 1.165 in F2-3, and 1.92 in F4 ($P < .01$), respectively. For $F \geq 2$, the sensitivity, specificity, accuracy of M2BPGi were 72.28%, 73.23%, 66.67%, while 55.07%, 93.71%, 82.02% for $F \geq 4$. For predicting significant fibrosis ($\geq F2$), M2BPGi showed comparable performance to FIB4 index ($P < .01$), APRI ($P < .01$) and RPR ($P < .01$) with area under the ROC curve (AUC) of 0.788. M2BPGi was superior to other surrogate markers for diagnosing cirrhosis (F4) with the highest AUC of 0.811 ($P < .01$).

Conclusions: M2BPGi levels increased with the progression of liver fibrosis in HBV infected patients. M2BPGi can be served as a potential glycomarker to assess the stage of liver fibrosis, especially for the diagnosis of cirrhosis.

KEYWORDS

China, hepatitis B virus, liver fibrosis, M2BPGi

1 | INTRODUCTION

Hepatitis B virus infection is a major public health problem worldwide. Roughly 30% of the world's population show serological evidence of current or past HBV infection.¹ In China, the prevalence of HBsAg for 1-59 years was 7.18% in 2006, turning China from a high endemic area of HBV into an intermediate endemic area.² Liver fibrosis is the common consequence of chronic hepatitis B virus infection, which can lead to the formation of cirrhosis. Accurate assessment of liver fibrosis is especially essential for the management of patients with

liver cirrhosis (LC) which is accompanied by distortion of the hepatic vasculature.³ This distortion results in increased resistance to portal blood flow and hence in portal hypertension and in hepatic synthetic dysfunction.⁴ Liver biopsy is considered to be the gold standard for diagnosis of cirrhosis and staging of fibrosis.⁵ However, it has many limitations, such as sampling error, subjectivity in reporting, high costs, the risks of bleeding and pneumothorax, discomfort to the patient, and the need for training and infrastructure in low- and middle-income countries (LMICs).⁶ As a result, many non-invasive methods such as serum markers and transient elastography have been reported.⁷⁻¹⁰ More and more studies suggest that FibroScan exhibited better diagnostic performance in patients with normal serum bilirubin and

Co-first author: Bin Wei and Shu Feng contribute equally to this work.

alanine aminotransferase (ALT) level, and is superior to other noninvasive methods for the diagnosis of liver fibrosis and cirrhosis in patients with HBV infection.^{11–14}

Recently, glycoproteomic biomarker screening studies show that Wisteria floribunda agglutinin-positive Mac-2 Binding Protein (WFA⁺-M2BP) is a novel serum marker of liver fibrosis.^{15–17} This marker was initially described as WFA-binding M2BP or WFA⁺-M2BP in earlier studies, but renamed as M2BPGi upon commercialization of the diagnostic kit in collaboration with a commercial company.¹⁶ M2BP was primarily detected in the tissue culture fluid of the human breast cancer cell line CG-5 using monoclonal antibody SP-2 by Iacobelli S et al.¹⁸ in 1986. Furthermore, M2BP is a ligand of Mac-2 that is associated with the antiviral host defense and highly expressed in activated macrophage-lineage cells, including Kupffer cells.^{19,20} It contains multibranched and sialylated N-glycans, and WFA is considered to recognize the GalNAc residue of N-glycans and O-glycans or the clustered LacNAc (Gal-GlcNAc) structure. Alterations in M2BP may be caused by changes in N-glycosylation, during the progression of liver disease and fibrosis. Additionally, M2BP also interacts with galectin-3, β -1 integrins, collagens, and fibronectin and is associated with cell–cell and cell–extracellular matrix adhesion.^{21–23} In several early researches, M2BP was reported to modulate tumor cell adhesion and supply as a prognostic and therapeutic target, potential tumor biomarker in cancer immunotherapy.^{24–26} Nevertheless, the relevance of M2BPGi with liver fibrosis and cirrhosis has received increased attention in recent years.^{27,28} It has been demonstrated that M2BPGi can reflect the stage of fibrosis in patients with primary biliary cirrhosis,^{29,30} autoimmune hepatitis,³¹ non-alcoholic fatty liver disease³² and chronic hepatitis C,³³ and can serve as a predictor for the development of hepatocellular carcinoma in hepatitis C patients.^{34,35} However, few studies have concentrated on patients suffered from hepatitis B virus infection. Therefore, we aim to confirm the efficacy of serum M2BPGi values for assessing the stage of fibrosis in Chinese patients with hepatitis B virus infection.

2 | METHODS

2.1 | Patients

Two hundred and sixty-three HBV infected patients with normal bilirubin during the period from June 2015 to January 2016 were randomly selected in West China Hospital of Sichuan University. Exclusion criteria of the patients were as follows: (i) patients with hepatitis C virus (HCV) or human immunodeficiency virus (HIV) infection; (ii) patients with history of autoimmune hepatitis, primary biliary cirrhosis, alcoholic fatty liver disease or idiopathic portal hypertension; (iii) patients with incomplete biochemistry and blood routine tests results. The diagnosis of HBV infection is based on HBsAg positive detected by electrochemiluminescence immunoassay (ECLIA).³⁶ Among them, 35 patients were excluded because of increased serum alanine aminotransferase (ALT) level ($\geq 2 \times$ the upper limit of normal [ULN]) to keep the accuracy of Fibroscan to assess the degree of fibrosis. The ULN of ALT is 40 IU/L for both men and women. Therefore, 228 patients, of which the characteristics are summarized in Table 1, were enrolled in

TABLE 1 Baseline characteristics of 228 patients

Demographics	Value
Patient, n	228
Male gender, n (%)	157 (68.86)
Age, years	43 (18–71)
Laboratory data at entry	
Total bilirubin, $\mu\text{mol/L}$	15.6 (4.5–27.9)
ALT, IU/L	31 (7–75)
AST, IU/L	31 (14–138)
Total protein, g/L	76.9 (60.2–91.6)
Albumin, g/L	46.1 (31.4–54.1)
Globulin, g/L	30.6 (18.5–51.3)
A/G	1.51 (0.65–2.5)
ALP, IU/L	76.5 (33–244)
GGT, IU/L	24 (7–327)
RBC, $10^{12}/\text{L}$	4.85 (3.51–7.69)
Hemoglobin, g/L	150 (78–199)
Platelet count, $10^9/\text{L}$	124 (27–423)
Fibrosis markers	
M2BPGi, COI	1.08 (0.22–11.41)
APRI	0.65 (0.16–5.80)
FIB-4 index	2.06 (0.49–25.10)
AAR	1.05 (0.46–3.44)
RPR	0.11 (0.04–0.59)
FibroScan, kpa	8.15 (3.2–46.4)
Fibrosis stage, n (%)	
F0–1	127 (55.7)
F2–3	32 (14.04)
F4	69 (30.29)

ALT, alanine aminotransferase; AST, aspartate amino transferase; A/G, albumin/globulin; ALP, alkaline phosphatase; GGT, γ -glutamyl transpeptidase; RBC, red blood cell; APRI, AST-to-platelet ratio; AAR, AST to ALT ratio; RPR, RDW to platelet ratio.

this study, ultimately. This study was approved by the Ethics Review Committee of West China hospital, and consistent with the guidelines of the Helsinki Declaration.

2.2 | FibroScan

In our research, we used FibroScan (Echosens, Paris, France) as gold standard to diagnose liver fibrosis. FibroScan was performed by physicians or trained technicians with experience of at least 50 transient elastography procedures. Only results with at least 10 valid measurements, a 60% success rate, an interquartile range of less than 30% of the median elasticity, and a Body Mass Index (BMI) $< 30 \text{ kg/m}^2$ were considered reliable.³⁷ According to the present Chinese guideline of chronic hepatitis B (CHB),³⁶ recommendations for clinical application of transient elastography,³⁸ the product manual of FibroScan, and a Chinese multicenter research,³⁹ we employed the test algorithm

FIGURE 1 Test algorithm and the association between the cut-off values of FibroScan and METAVIR fibrosis stage

indicated in Figure 1. Meanwhile, we defined significant fibrosis as a fibrosis score of 2 or more ($\geq F2$), and cirrhosis as fibrosis score of 4 (F4) on the basis of the METAVIR score (F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; F4, cirrhosis).⁴⁰

2.3 | Laboratory testing

At the time of blood withdrawal, all patients underwent FibroScan. Biochemistry, blood routine tests, viral serum test and serum HBV DNA were performed in compliance with the automated procedures of the clinical laboratories. Serum samples of all patients were collected and stored at -20°C . Additional surrogate blood indices of liver fibrosis in our study included aspartate amino transferase (AST)-to-platelet ratio (APRI), FIB-4 index, AST to ALT ratio (AAR), and red cell distribution width (RDW) to platelet ratio (RPR). The calculation formulae were as follows: $\text{APRI} = [\text{AST}(\text{U/L}) / \text{UNL} \times 100] / \text{platelet count}(10^9/\text{L})$, where UNL is the upper limit of normal of the AST level⁴¹; $\text{FIB-4} = \text{Age}(\text{years}) \times \text{AST}[\text{U/L}] / [\text{platelet count}(10^9/\text{L}) \times \text{ALT}[\text{U/L}]^{1/2}]$ ⁴²; $\text{AAR} = \text{AST} / \text{ALT}$; $\text{RPR} = \text{RDW}(\%) / \text{platelet count}(10^9/\text{L})$.⁹

2.4 | Measurement of M2BPGi

The serum M2BPGi level was measured based on a lectin-antibody sandwich immunoassay using a fully automatic HISCL-5000 immunoanalyzer (Sysmex Corporation, Hyogo, Japan).¹⁵ Glycosylated M2BP was captured by WFA immobilized on magnetic beads. Then, the bound product was assayed with an antihuman M2BP monoclonal antibody linked to alkaline phosphatase (ALP- α M2BP). The lot number

of HISCL[®] M2BPGi assay kit and HISCL[®] M2BPGi control is BS0012 and BX457600, respectively. The measured values of M2BPGi conjugated to WFA were indexed with the obtained values using the following equation:

$$\text{Cutoff index (COI)} = ([\text{M2BPGi}]_{\text{sample}} - [\text{M2BPGi}]_{\text{NC}}) / ([\text{M2BPGi}]_{\text{PC}} - [\text{M2BPGi}]_{\text{NC}})$$

Where $[\text{M2BPGi}]_{\text{sample}}$ is the M2BPGi count of serum sample, PC is positive control, and NC is negative control. The positive control was used as a calibration solution preliminarily standardized to yield a COI value of 1.0.¹⁷

2.5 | Statistical analysis

Data analysis was done using SPSS 19.0 (SPSS Inc., Chicago, IL). All the data were analyzed anonymously. Categorical variables were expressed as percentage, while continuous variables were expressed as median and range. The Spearman rank correlation coefficient test was used for the correlation of continuous variables. To identify significant independent factors affecting FibroScan and serum M2BPGi level, variables that reached the $P < 0.05$ level in correlation analysis were entered into multiple liner regression model. Box plot was used to indicate the correlation between M2BPGi level and liver fibrosis stage. Kruskal-Wallis test was carried out to compare the COI values of M2BPGi stratified by the fibrosis stage. Receiver operating characteristics curve (ROC) analysis was performed to evaluate the diagnostic performance of M2BPGi for different degrees of liver fibrosis and cirrhosis. Value of $P \leq 0.01$ was regarded as statistically significant.

3 | RESULTS

3.1 | Patient characteristics

The baseline characteristics of the 228 patients are summarized in Table 1. Their ages ranged from 18 to 71 years old (median=43) with a male predominance (68.86%). The median values for FibroScan, M2BPGi, APRI, FIB-4 index, AAR, and RPR were 8.15, 1.08, 0.65, 2.06, 1.05, and 0.11, respectively. Simultaneously, 127 patients (55.7%) had fibrosis grade F0-1, 32 (14.04%) were F2-3, and 69 (30.29%) were F4. Among the 69 patients had cirrhosis, 68 patients (98.55%) were in Child class A, 1 (1.45%) was in Child class B.

3.2 | Factors associated with FibroScan

FibroScan values was significantly, positively correlated with age and the levels of total bilirubin, ALT, AST, globulin, alkaline phosphatase (ALP), γ -glutamyl transpeptidase (GGT), APRI, FIB-4 index, RPR. It was negatively correlated with albumin, A/G, RBC and platelet count. Furthermore, APRI ($r = .588$, $P < .01$), GGT ($r = .585$, $P < .01$) and M2BPGi ($r = .574$, $P < .01$) exhibited the top three highest coefficient correlation level. GGT (0.396, $P < .01$) and M2BPGi (0.379, $P < .01$) were the two most significant factors which are associated with FibroScan level according to a multiple linear regression analysis.

3.3 | Factors associated with M2BPGi

M2BPGi levels were positively correlated with age and the levels of total bilirubin, ALT, AST, globulin, A/G, ALP, GGT. It was negatively correlated with albumin, A/G, RBC, hemoglobin, platelet count. Our results showed that the independent variables of total bilirubin, albumin, globulin, A/G, ALP, hemoglobin, platelet count remained in the final equation (Table 2).

3.4 | M2BPGi value and fibrosis stage

Serum M2BPGi values for each fibrosis stage were exhibited in Figure 2. Serum M2BPGi values ranged from 0.22 to 11.41 (COI). The M2BPGi values in patients with stages F0-1, F2-3 and F4 had median COIs of 0.88, 1.165 and 1.92, respectively. According to Kruskal-Wallis test, the COI values stratified by the fibrosis stage were significantly different ($P<.01$). There were significant differences between fibrosis stages F0-1 and F2-3 ($P<.01$), F2-3 and F4 ($P<.01$), F0-1 and F4 ($P<.01$).

We analyzed the proportions of the patients with different M2BPGi levels stratified by the fibrosis stage (Figure 3). The proportion of F0-1 was 76 cases (77.55%) in M2BPGi <1 ($n=98$), 49 cases (53.26%) in M2BPGi ≥ 1 and <2 ($n=92$), 1 case (5.88%) in M2BPGi ≥ 2 and <4 ($n=17$), 1 case (4.76%) in M2BPGi ≥ 4 ($n=21$). Conversely, the proportion of patients with F4 was 11 cases (11.22%) in M2BPGi <1 ($n=98$), 26 cases (28.26%) in M2BPGi ≥ 1 and <2 ($n=92$), 12 cases (70.59%) in M2BPGi ≥ 2 and <4 ($n=17$), 20 cases (95.24%) in M2BPGi ≥ 4 ($n=21$).

TABLE 2 Factors associated with M2BPGi in patients with HBV infection as identified by correlation analysis and multiple liner regression analysis

Variables	Correlation analysis		Liner regression analysis	
	<i>r</i>	<i>P</i> value	Standardized coefficient β	<i>P</i> value
Age	.319	$<.01$	-	-
Laboratory data			-	-
Total bilirubin	.167	.012	0.170	$<.01$
ALT	.198	$<.01$	-	-
AST	.539	$<.01$	-	-
Total protein	.112	.092		
Albumin	-.384	$<.01$	-0.519	$<.01$
Globulin	.391	$<.01$	0.708	$<.01$
A/G	-.432	$<.01$	0.640	$<.01$
ALP	.408	$<.01$	0.191	$<.01$
GGT	.438	$<.01$	-	-
RBC	-.276	$<.01$	-	-
Hemoglobin	-.248	$<.01$	-0.126	.012
Platelet count	-.458	$<.01$	-0.194	$<.01$

ALT, alanine aminotransferase; AST, aspartate amino transferase; A/G, albumin/globulin; ALP, alkaline phosphatase; GGT, γ -glutamyl transpeptidase; RBC, red blood cell.

FIGURE 2 Box plots of M2BPGi level according to each fibrosis stage (Kruskal-Wallis test: $P<.01$). the tops and bottoms of the boxes represent the first and third quartiles, respectively, with the height of the box represents the interquartile range, covering 50% of the values. The line through the middle of each box represents the median. Outliers are either represented by (o) (=mild) or (*) (=extreme)

3.5 | Correlations between M2BPGi and other indicators

The correlation coefficient between M2BPGi and APRI was 0.587 ($P<.01$), while 0.594 between M2BPGi and FIB-4 index ($P<.01$), 0.232 between M2BPGi and AAR ($P<.01$), 0.483 between M2BPGi and RPR ($P<.01$), respectively.

3.6 | Diagnostic performance of M2BPGi for significant fibrosis and cirrhosis and compared with other indicators

Receiver operating characteristic curve analysis was performed to evaluate the diagnostic accuracy of M2BPGi for significant fibrosis ($\geq F2$) (Figure 4A) and cirrhosis ($\geq F4$) (Figure 4B). The area under the ROC curve for the diagnosis of types of fibrosis F2 and F4 with serum M2BPGi values were 0.788 and 0.811, respectively. Compared with other surrogate markers, the serum M2BPGi yielded the highest AUC of cirrhosis ($\geq F4$) (AUC=0.811, $P<.01$) and was significantly superior to APRI (AUC=0.809, $P<.01$), FIB-4 index (AUC=0.799, $P<.01$), AAR (AUC=0.560, $P=.151$) and RPR (AUC=0.786, $P<.01$). For diagnosis of significant fibrosis ($\geq F2$), M2BPGi (AUC=0.788, $P<.01$) was comparable to FIB-4 index (AUC=0.820, $P<.01$), APRI (AUC=0.817, $P<.01$) and RPR (AUC=0.799, $P<.01$), but significantly superior to AAR (AUC=0.585, $P=.027$). The optimal cutoff values were 1.12 COI for $F\geq 2$, 1.825 COI for $F\geq 4$. The sensitivity of M2BPGi for significant fibrosis and cirrhosis was 72.28% and 55.07%, while the specificity was 73.23% and 93.71%. The accuracy of M2BPGi for significant fibrosis and cirrhosis was 66.67% and 82.02%. Simultaneously, the PPV and NPV for $F\geq 2$ and $F\geq 4$ were 68.22%/76.86% and 79.17%/82.78%, respectively.

FIGURE 3 Proportions of patients with different M2BPGi levels stratified by the fibrosis stage

FIGURE 4 Receiver-operating characteristic of M2BPGi, APRI, FIB-4 index, AAR, and RPR for the estimation of (A) significant fibrosis ($\geq F2$), (B) cirrhosis (F4)

4 | DISCUSSION

In this study, we evaluated serum M2BPGi for assessing liver fibrosis stages in Chinese patients with HBV infection. M2BPGi is a significant factor closely associated with FibroScan liver stiffness measurements. This study demonstrated M2BPGi levels in HBV infected patients were increased with the progression of fibrosis stage, which is in consistent with several previous studies.^{27,34} Based on our research, we found that M2BPGi yielded higher diagnostic accuracy in cirrhosis than significant fibrosis. Meanwhile, the performance of M2BPGi was superior to other surrogate serum markers such as APRI, FIB-4 index, AAR, and RPR for diagnosing cirrhosis, indicating that M2BPGi is an accurate and reliable surrogate marker for patient with HBV infection.

M2BP is a kind of secretory glycoprotein and secreted from many cell types, including hepatocytes. It has been shown to modulate many

processes, particularly those related to cell adhesion. M2BP also interacts with a number of fibrosis-associated extracellular proteins, such as collagens IV-VI, fibronectin, and nidogen.²¹ Kuno A et al.¹⁵ applied a fully automated sandwich immunoassay for direct quantitation of serum M2BPGi with a measurement time of only 17 minutes. Using this method, a series of researches revealed that serum M2BPGi value was useful for assessing the stage of liver fibrosis in patients with non-alcoholic fatty liver disease (NAFLD),³² primary biliary cirrhosis (PBC),²⁹ autoimmune hepatitis (AIH)³¹ and Hepatitis C.³⁴ According to Toshima T et al.,²⁷ M2BPGi is superior in patients with hepatitis C than in those with hepatitis B for measurements of liver stiffness. Meanwhile, Heo JY et al.⁴³ reported that M2BPGi had better diagnostic accuracy in advanced fibrosis or cirrhosis for patients with chronic hepatitis B. In contrast, Zou X et al.⁴⁴ found that M2BPGi was an accurate serum indicator for assessing early stages of liver fibrosis

in patients with chronic hepatitis B virus infection. As a consequence, the role of M2BPGi in patients with HBV infection has not been elucidated sufficiently especially in Chinese hepatitis B patients.

Compared with FIB-4, APRI, RPR and AAR, M2BPGi is simply to be carried out and can directly reflect the stage of fibrosis without complex calculations and extra tests, which can save masses of time for clinical examination and costs for patients. Considering the invasiveness and heterogeneous distribution of fibrosis in the liver of liver biopsy, the reliability of FibroScan can be affected by operator experience and ascite, M2BPGi may be an alternative non-invasive serum marker for assessing liver fibrosis. Firstly, according to Xiao GQ et al.¹⁰ APRI and FIB-4 index can identify hepatitis B-related fibrosis with a moderate sensitivity and accuracy. M2BPGi assay was positively correlated with FIB4 index and APRI in this research. The correlation coefficients between M2BPGi and APRI ($r=.587$, $P<.01$), FIB-4 index ($r=.594$, $P<.01$) were slightly higher than Hanai T et al.²⁸ reported: $r=.43$ ($P<.01$) in FIB4 index and $r=.42$ ($P<.01$) in APRI. Secondly, several previous researches have demonstrated that the FibroScan exhibited a considerable good diagnostic performance when bilirubin and ALT is within normal level.^{13,14} Thus, we used it as an imperfect golden standard. In our results, M2BPGi is a significant influencing factor closely associated with FibroScan level which can also be affected by total bilirubin, albumin, GGT, platelet count. The correlation coefficient between ALT, total bilirubin, AST, total protein, albumin, ALP, GGT, platelet count and M2BPGi is similar to Zou X et al.⁴⁴ Umemura T et al.²⁹ and Ura K et al.³³ Meanwhile, M2BPGi level can be influenced by total bilirubin, albumin, globulin, A/G, ALP, hemoglobin, platelet count.

M2BPGi has a good diagnosis capacity for assessing the stage of liver fibrosis, especially for cirrhosis. In the process of viral hepatitis management, it is important to identify significant liver fibrosis and cirrhosis. Fibrotic change is closely correlated with hepatocarcinogenesis in patients with viral hepatitis, and the annual incidence of hepatocellular carcinoma in patients with different stage of fibrosis is varying.⁴⁵ According to previous studies, M2BPGi suggested better diagnosis performance for identifying cirrhosis than significant fibrosis in patients with chronic hepatitis C, non-alcoholic fatty liver disease and autoimmune hepatitis.^{15,27,32} In our study, M2BPGi had a considerably high AUC value in identifying cirrhosis (AUC=0.811, 95%CI 0.735-0.860), but slightly low AUC value in significant fibrosis (AUC=0.788, 95%CI 0.728-0.848), indicating that M2BPGi is a better serum marker to assess cirrhosis than significant fibrosis in patients with HBV infection, which was in agreement with Heo JY et al.⁴³ (AUC=0.688 for grade F $\geq$ 2 and AUC=0.704 for grade F $\geq$ 4), but somewhat distinct from Toshima T et al.²⁷ (AUC=0.837 for grade F $\geq$ 2 and AUC=0.795 for grade F $\geq$ 4) whose patients were mainly suffered from hepatitis C virus infection. The discrepancy of our results with Toshima T et al.²⁷ may be explained by different mechanisms to promote hepatic fibrosis between HBV and HCV,⁴⁶ as well as the variance of ethnic groups between China and Japan. Simultaneously, we found comparable optimal COI values, sensitivities, NPVs, and higher PPVs in Chinese patients with HBV infection than Japanese patients with various chronic liver diseases.²⁷ In view of the results, exclusion of cirrhosis could be identified with a high NPV of 82.78% at serum M2BPGi value less than

1.825. Compared with M2BPGi, other four serum biomarkers showed significantly lower AUCs for diagnosing cirrhosis. Therefore, we believe that serum M2BPGi can be an accurate and reliable surrogate marker to assess cirrhosis in Chinese HBV infected patients.

This study has several limitations. Firstly, we applied FibroScan instead of liver biopsy as the gold standard to evaluate the degree of liver fibrosis. The different diagnosis performance between FibroScan and liver biopsy may influence our results. Another limitation is the small sample size of HBV infections, and our results can only represent a small group of individuals with normal bilirubin and ALT level. Moreover, our research just concentrated on the diagnosis performance of this serum marker. The predictive values of serum M2BPGi for HCC progression among hepatitis B patients have not yet been clearly elucidated, indicating a direction for future research. Finally, healthy control is warranted in our further studies.

In conclusion, M2BPGi is closely associated with fibrosis stages and can predict the severity of fibrosing liver. It showed a good correlation with other four serum biomarkers. The ROC curve indicated that M2BPGi has a considerable high diagnostic value particularly in cirrhosis. As a result, we concluded that M2BPGi can be served as a potential glyco-biomarker to assess the stage of liver fibrosis, especially for the diagnosis of cirrhosis.

ACKNOWLEDGMENT

The authors would like to acknowledge the Sysmex company workers to provide HISCL[®] M2BPGi assay kit. We also acknowledge other colleagues who took part in the routine detection but do not become authors.

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How to cite this article: Wei B, Feng S, Chen E, et al. M2BPGi as a potential diagnostic tool of cirrhosis in Chinese patients with Hepatitis B virus infection. *J Clin Lab Anal*. 2018;32:e22261. <https://doi.org/10.1002/jcla.22261>